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Antimicrobial, antibiofilm and antioxidant activities of *Citrullus colocynthis* fruit extracts

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ABSTRACT

According to its beneficial characteristics, the desert medicinal plant Citrullus colocynthis L. is commonly used in traditional medicine. The objective of this study is to investigate the antioxidant, anti-hemolysis, antimicrobial, and antibiofilm activities of Citrullus colocynthis L. fruit extracts, and their content on phenolic compounds. Butanol and ethyl acetate extracts contain a high content of polyphenols (222.72 and 260.61 µg GA/mg Extract) and flavonoids (73.03 and 77.7µg CAT/mg E) compared to hydro-methanol and chloroform extracts. Ferulic acid, rutin, and numerous other unidentified substances were identified in ethyl acetate and butanol extracts, according to RP-HPLCPDA analysis. These extracts exhibited the highest antioxidant activity in DPPH assay (65.09 µg/mL <IC_{50}>89.29 µg/mL), FRAP (99.7 µg/mL < $A_{0.5}$ >119.37 µg/mL), CUPRAC (19.41µg/mL < IC_{50}>26.63 µg/mL) and ABTS (77.43 µg/mL < IC_{50}> 86.87 µg/mL). Compared to the other extracts, the butanol extract revealed the best protective effect from hemolysis in red blood cells against AAPH-induced oxidative stress (IC_{50}=19.11 µg/mL). In addition, Gram-positive and -negative bacteria were more sensitive to butanol and ethyl acetate extracts, mainly Staphylococcus aureus ATCC 6538 and MicrococcusluteusATCC9341 with zone inhibition diameters ranging between 11 mm and 13 mm, and MIC values between 0,312 mg/mL and 0,625 mg/mL. These extracts exhibited an interesting anti-adhesion activity (35%-58%) against Staphylococcus aureus, Listeria monocytogenes, and Escherichia coli, which indicates their likely antibiofilm effect. Extracts had no specific antifungal activity against Candida albicans According to the obtained results colocynthis, a potential therapeutic herb can be used to treat infectious diseases and oxidative stress.

Keywords **:** Antibacterial, antibiofilm**,** antioxidant activity*, Citrullus colocynthis*, polyphenols

INTRODUCTION

The main cause of many chronic and neurodegenerative diseases, including cancer, diabetes, cardiovascular disease, Alzheimer, and autoimmune diseases, is free radical production, which antioxidants quickly neutralize **(**Thèriault *et al*., 2006**)**. Several scientific studies have been performed to reveal the therapeutic effects of phytochemicals on health by determining their impact on oxidative stress, and assessing their potential to increase enzymatic antioxidants, reduce peroxides, scavenge free radicals, and chelate transition metals (Li *et al*., 2009). A vast number of medicinal plants used in phytotherapy that have been evaluated for their antibacterial, antioxidant, and anti-inflammatory effects represent an inexhaustible source of natural antioxidants (Brewer, 2011, El-Kadi *et al.,* 2021).

Many species of the Cucurbitaceae family, including *Citrullus colocynthis*, are traditionally used as anti-diabetic remedies. Having smooth, and spherical fruits that are mottled green when young

and yellowish when mature, colocynth is a creeping annual herb that is native to India, West Asia, tropical Africa, and the Mediterranean region (Gurudeeban *et al*., 2010; Banjo *et al.,* 2021). In Asian and African countries, the fruits are traditionally used to treat infectious diseases, inflammation, ulcers, hepatitis, kidney illnesses, and diabetes (Azzi *et al*., 2012; Banjo *et al*., 2021). The anti-diabetic and antioxidant activities of *C. colocynthis* extracts have been established in many *in vivo* and *in vitro* research (Nmila *et al*., 2000; Kumar *et al*., 2008; Benariba *et al*., 2009; Benariba *et al.,* 2012; Benariba *et al.,* 2013a; Ckekroun *et al*., 2015; Ckekroun *et al*., 2017; Shahzadi *et al.,* 2022). The present study's main objective is to identify the polyphenols of the hydromethanolic extract, and its fractions ethyl acetate and butanol, prepared from *Citrullus colocynthis* fruits that were collected in Tlemcen, in the west of Algeria, and to investigate their antimicrobial, antioxidant, and antibiofilm activities.

MATERIALS AND METHODS

Plant materials

Citrullus colocynthis fruit was harvested in Naâma, in western Algeria. Its authenticity was confirmed by Tlemcen University's Laboratory for Ecology and Management of Natural Ecosystems. Once the fruits were cleaned and dried at room temperature, they were crushed into small pieces and kept for further extraction.

Preparation of extracts

For 48 hours at room temperature, 10 grams of crushed fruit were macerated in 100 mL of a water-methanol mixture (20/80 V/V). A vacuum concentration process was performed on the resultant solution after it was filtered. To obtain the dry hydromethanol extract, a portion of this extract was evaporated until it was completely dry. In a liquid-liquid funnel extraction, the remaining hydromethanol was fractionated by chloroform, ethyl acetate, and n-butanol. Ethyl acetate and butanol extracts were obtained by evaporating organic phases under a vacuum.

Total polyphenols content

The Folin-Ciocalteu method was used to determine the total polyphenol content in colocynthis fruit extracts according to Vermerris and Nicholson (2006). At various concentrations, 100 µL of samples or the standard (gallic acid) were combined with 2 mL of freshly prepared Na_2CO_3 (2%) solution. The mixture was incubated at room temperature for 5 min before being added to 100 µL of Folin-Ciocalteu reagent (0.2 N). At 700 nm optical density measurement was performed, and the results are expressed as mg of gallic acid equivalents per gram of extract (mg GAE/g).

Total flavonoid content

$AlCl_3$ reagent was used to measure flavonoid content according to Ardestani and Yazdanparast (2007). 500 µL of samples or catechin (standard) were combined with 150 µL of $NaNO_2$ solution (15%), and 2 mL of distilled water. A subsequent incubation at room temperature for 6 min was followed by the addition of 150 µL of $AlCl_3$ (10%) and 2 mL of NaOH (4%). Following a second 15-minute incubation at room temperature, distilled water was added to the total volume to adjust it to 5 mL, and optical density was determined at 510 nm. As a result, the data were reported as mg of dry extract/g catechin equivalent (mg Cat eq/mg).

High-performance liquid chromatography (HPLC-DAD) analysis of phenolic compounds

RP-HPLC-PDA was applied to separate and identify the phenolic component of the butanol and the ethyl acetate extracts *of C. colocynthis*. This analysis was conducted on an Eclipse ODS Hypersil C18 column and a Perkin Elmar Flexar system with a binate pump transmission method (150 mm x 4.6 m). Each sample was injected at a flow rate of 1 mL/min using 20 µl. A binary solvent system containing acetic acid 2% (A) and acetonitrile (B) formed the mobile phase. A 10% B (Acetonitrile) gradient was applied for 5 minutes, followed by 10% B for 25 minutes, followed by 15% from 90% to 100% B, followed by 15 minutes of equilibration. Filters of 0.22 m Millipore were used before the HPLC injection of samples and mobile phases. For component analysis, the chromatograms of extracts obtained at 280 nm were chosen (El-Haci *et al*., 2020; Adjdir *et al*., 2021a; Adjdir *et al.,* 2021b).

Antioxidant activity

Free radical scavenging activity: DPPH assay

C. colocynthis extracts were investigated for their ability to scavenge free radicals via the DPPH assay according to Ataoui *et al.* (2005). 1.95 mL of DPPH was incubated with 50 µL of each extract for 30 minutes, after which the absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and ascorbic acid were used as standard antioxidant molecules. The results are expressed as percent DPPH inhibition, and the IC50 value was calculated from the linear regression curve.

$$\text{Free radical scavenging activity (\%)} = \frac{A\ control - A\ sample}{A\ control} \times 100$$

Ferric Reducing power assay

The iron-reducing capacity of *C. colocynthis* extract was evaluated according to the method described by Karagözler *et al.* (2008). 1 mL of each extract was combined with 2.5 mL phosphate buffer (0.05 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). After standing at 50°C for 20 min, the mixture was cooled down to room temperature, and the absorbance of the samples was measured at 700 nm. Then, 2.5 mL of trichloroacetic acid solution (10%) was added to the medium and centrifuged at 3000rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL $FeCl_3$ (1%) solution to measure the absorbance at 700 nm. The results are presented as A0.5 (sample concentration in an absorbance range of 0.5).

Cupric reducing antioxidant capacity (CUPRAC)

96-well microplates were filled with 40 µL of extracts at various concentrations, all of which were combined with 50 µL of $CuCl_2$ (10 mM). Following the addition of 60 µL of ammonium acetate buffer (1M, pH 7.0) and 50 µL of neocuproin solution (7.5 mM), the medium was then filled to a final volume of 200 µL. Following 60 min of incubation, absorbance was measured at 450 nm (Apak *et al*., 2004).

ABTS cation radical scavenging assay

The $ABTS^+$ radical is produced by combining ABTS 7 mM (2,2'-azino-bis(3-ethylbenzothia zoline-6-sulfonic acid) and potassium persulfate (2.45 mM) and incubated in the dark at room temperature for 12 hours. Then, 160 µL of the $ABTS^+$ solution (with an absorbance of 0.700-0.025 at 734 nm) was mixed with 40 µL of the extracts, and the absorbance was measured at 734 nm after 10 minutes of incubation (Re *et al*., 1999; Dong *et al*., 2015). The trapping capacity of $ABTS^+$ was determined according to the following formula:

$$ABTS^+ \text{ scavenging effect (\%)} = \frac{A\ control - A\ sample}{A\ control} \times 100$$

Total Antioxidant Capacity

According to the process prescribed by Prieto *et al.* (1999), 0.2 mL of various concentrations of extract of *C. colocynthis* were mixed with 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate to determine their total antioxidant capacity (TAC). Following 90 minutes of incubation at 95°C, each tube's absorbance was measured at 695 nm. Gallic acid milligram equivalents per gram of extract (mg GAE/gE) were used to express the total antioxidant ability. The antioxidant activity was expressed in milligram equivalents of gallic acid per gram of extract (mg GAE/gE).

AAPH (2,2'-Azobis-2-amidinopropane dihydrochloride) induced hemolysis in erythrocytes

Using AAPH as a peroxyl radical generator, the capacity of *C. colocynthis* fruit extracts to prevent free radical-induced hemolysis was assessed according to Xiaoping Yuan *et al.* (2005).

Preparation of erythrocytes

Blood was obtained at the city hospital's blood transfusion service from healthy volunteers. In a

laboratory, erythrocytes were removed from the plasma by centrifugation at 3000g for 5 minutes at 4°C, and the pellet was then washed three times with a cold (4°C) solution of phosphate-buffered (10 mM sodium phosphate, 125 mM NaCl, pH=7.4). After each step, the washed erythrocytes recovered from the pellet were then carefully taken from the supernatant, and at the end, a final concentration of 5% (v/v) in PBS was used to resuspend the washed erythrocytes that were recovered from the pellet.

Erythrocyte hemolysis assay

During the hemolysis test, 100 µL of the erythrocyte suspension is mixed with 100 µL of extracts (20-100 µg/mL), and the mixture is then incubated at room temperature for 15 min. Incubation was followed by adding 200 µL of AAPH solution (100 mmol/L) and the tubes were incubated for 3 h tubes at 37°C; then, the medium was diluted with 8 mL of PBS before centrifugation at 2000g for 10 minutes. The optical density of the resulting supernatant was measured at 540 nm. The results were expressed as a hemolysis percentage according to the formula as follows:

$$\text{Hemolysis inhibition (\%)} = \frac{A\ control - A\ sample}{A\ control} \times 100$$

Antimicrobial activity

The antimicrobial activity of *Citrullus colocynthis* fruit extracts was evaluated on bacterial and fungal pathogenic strains of American Type Culture Collection (ATCC) using microdilution and disk diffusion methods. Among the gram-negative bacterial strains investigated were *Citrobacter freundii* ATCC 8090, *Acinetobacter baumanii* ATCC 19606, *Enterobacter cloacae* ATCC 1304, *Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 13311, *Proteus mirabilis* ATCC 35659, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853. Six Gram-positive bacterial strains were also tested, *Bacillus cereus* ATCC 25921, *Enterococcus faecalis* ATCC 49452, *Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* ATCC 9341, *Listeria monocytogenes* ATCC 15313, *Bacillus subtilis* ATCC 6633, as well as *Candida albicans* reference strains are used ATCC 26790, ATCC 10231, and IP 444. Extracts with significant antibacterial effects were tested for antibiofilm activity using an anti-adhesion bioassay against *Staphylococcus aureus*, *Listeria monocytogenes,* and *E. coli*.

Disk diffusion method

According to CLSI (2012), disk diffusion tests were conducted. 1 mL of each bacterial suspension was prepared from a suspension containing10^8 CFU/mL (0.5 McFarland standard; OD = 0.08-0.10 λ = 625 nm), then was cast onto the solid medium Mueller-Hinton agar. Discs of sterile filter paper (6 mm) containing 10 µL of colocynth extracts (512µg/mL) were puted on the agar surface. Then, Under adequate cultivation conditions, Petri Plates were incubated for 24 hours. the antibacterial action of each extract corresponded to the lowest concentration that create a zone of inhibition around the disk, refred to the positive control we used gentamicin. All experiments were carried out in triple.

Minimum Inhibitory Concentration (MIC)

The microdilution method using Borth medium (CLSI, 2009) was applied to extracts that showed antibacterial activity according to the previous method. 100 µL of bacterial suspensions containing 10^6 CFU (0.08-0.13/ λ =625 nm) were mixed with 100 µL of extracts (0.02-10 mg/mL) using 96-well microplate. In the control, only culture medium and bacterial suspension were used. The MIC is the lowest concentration of extracts that will completely inhibit microbial growth within 24 hours of incubation of the microplate at 37°C (no turbidity after 24 hours).

Antibiofilm activity: Bacterial adhesion assay

Adhesion is a necessary step in the formation of a biofilm (Guo *et al*., 2021). *Citrullus colocynthis* fruit extracts were used to test their anti-adhesion properties using an adhesion assay on a polystyrene microplate as reported by Agarwal *et al.* (2011) and Aissaoui *et al.* (2021). 100 µL of each concentration of extracts (0.0156 to 2 mg/mL) was mixed in 96-well microplates containing 10^8 CFU/mL of the bacterial strains. Cells can attach to a surface after 3 hours of incubation at 37°C without shaking. After incubation, To completely remove the medium as well as non-adherent bacteria, each well was rinsed three times with 200 µL of distilled water. Afterward, the adherent cells were stained for 1min

at room temperature with crystal violet (0.5%) and fixed for 30 minutes in methanol. Then, 200 µL of ethanol-acetone (80/20%) decolorization solution was added to each well for 15 minutes after excess crystal violet staining was removed with distilled water. Inhibition of bacterial adhesion expressed in percentage was achieved by the absorbance measurement of adherent cells at 595 nm.

$$anti\text{-}adhesion\ effect\ (\%) = \frac{A\ control - A\ sample}{A\ control} \times 100$$

Statistical analysis

The results of independent experiments that were carried out in trials are reported in means ± SEM. The mean differences were determined using the Student test "*t*" and a $P \leq 0.05$ is statistically significant.

RESULTS AND DISCUSSION

Polyphenol and flavonoid content

The polyphenol and flavonoid content in *Citrullus colocynthis* fruit extracts are reported in Table 1. The highest levels of total polyphenols, 260.61 µg eq AG/mg E and 77.7 µg eq Cat/mg E, and flavonoids 222.72 µg eq AG/mg E and 73.03 µg eq Cat/mg E were observed in the butanol and ethyl acetate extracts. These results are in line with Chekroun *et al.* (2015) study which reported that in the butanol extract of C. colocynthis fruit extract a total polyphenols and flavonoids content ranged between 221.85 µg eq AG/mg E and 61.20 µg eq Cat/mg E, respectively. However, Kumar *et al.* (2008) demonstrated a high total polyphenols and flavonoids content in the methanolic extract of *C. colocynthis* fruit which is in the order of 740 mg eq AG/100 g dry extract and 130 mg eq Cat/100 g dry extract, respectively. The content of phenolic compounds in a plant is related to the season of harvest, and the used parts of the plant. Current studies revealed a high level of polyphenols and flavonoids in aqueous and organic extracts in the fruit and the seeds than in the root of *C. colocynthis* extracts (Benariba *et al.,* 2013b; Al-Nabli *et al.,* 2022).

Phenolic compound identification

C. colocynthis extracts contained both ferulic acid and rutin in the ethyl acetate and butanol extracts based on the results of RP and HPLC-PDA chromatographic analysis (Figure 1). In addition, butanol extract contains more unidentified components than ethyl acetate extract. In contrast to previous analyses that have been published in the literature, this analysis is the first to identify phenolic compounds from *C. colocynthis* harvested in Algeria. The reverse phase HPLC identification of *C. colocynthis* from the Pakistani flora revealed the presence of phenolic acids including, ferulic, vanillic, p-coumaric, gallic, and p-hydroxybenzoic acids, as well as flavonoid, catechin, myricetin, and quercetin (Hussain *et al*., 2013).

Antioxidant Activity

The results of the antioxidant activity of *C. colocynthis* extracts expressed in IC_{50} values are reported in Table 2. With an IC_{50} of 65.09 µg/mL for butanol and 89.29 µg/mL for ethyl acetate, respectively, the butanol extract significantly increased the ability to scavenge DPPH. Furthermore, these two extracts demonstrated a substantial impact on the ABTS assay (65.09 IC50 > 89.26 µg/mL), FRAP (99.7 µg/mL A0.5 > 119.37 µg/mL), and CUPRAC (19.41 µg/mL A0.5 > 26.63 µg/mL), except for the total antioxidant capacity assay, where the chloroform extract. In addition, the chloroform extract demonstrated a significant value of 47.6 µg GAA/mg in comparison to the remaining extracts. Our results are in line with the results of Chekroun *et al*. (2015) which documented that the butanol extract of *C. colocynthis* fruit had a significant ability to scavenge DPPH (IC_{50} = 61 µg/mL) and moderate ferric-reducing activity. However, according to Kumar *et al.* (2008) and Benariba *et al.* (2013b), the fruit and seed extracts in methanolic and ethyl acetate showed DPPH scavenging action at high concentrations (0.350 mg/mL < IC_{50} > 2500 mg/mL). No results are published regarding the antioxidant power of colocynth using the ABTS and CURAC tests. According to the bibliography, flavonoids have an anti-oxidant effect due to their ability to transfer their hydroxyl groups to neutralize free radicals and produce FLO•, an effect primarily attributed to the 3',4'-orthodihydroxy group on the B ring, the 4-carbonyl group on the C ring, and the 5-OH and 3-OH groups on the C ring (Chira *et al.,* 2008; Benariba *et al.,* 2013b El Kadi *et al*., 2021).

Table 1: Total polyphenols content in *Citrullus colocynthis* fruit extracts

	Total polyphenol (µg GA/mg E)	Total flavonoid (µg CAT/mg E)
Hydro-methanol	203.94 ± 8.97	60.33 ± 2.52
Chloroform	117.27 ± 5.68	42.4 ± 1.44
EthylAcetate	222.72 ± 4.54	73.03 ± 2.05
Butanol	260.61 ± 13.88	77.7 ± 3.01

Table 2: *Citrullus colocynthis* fruit extracts have antioxidant activity expressed as IC_{50} and $A_{0.5}$.

	DPPH (IC_{50}µg/mL)	ABTS (IC_{50}µg/mL)	FRAP ($A_{0.5}$µg/mL)	CUPRAC ($A_{0.5}$µg/mL)	TAC (µg GAA/mg)
Hydromethanol	147.41±2.21	NT	130.75±2.35	-	103.54±2.67
Chloroform	274.28±13.51	159.12±2.1	277.58±7.92	57.69±2.98	47.6±1.38
Ethyl acetate	89.29±1.41***	86.87±1.26***	119.37±9.73**	26.63±3.43***	114.89±3.67
Butanol	65.09± 8.4**	77.43±3.36**	99.7±5.46***	19.41±1.94***	123.97±5.45
BHA[a]	1.61±0.04	1.81±0.10	14.53±5.76	3.64±0.19	-
BHT[a]	5.32±0.13	1.29±0.30	54.17±1.76	9.62±0.87	-
Ascorbic acid[a]	1.26±0.01	-	19.16±3.47	-	-

IC_{50} and $A_{0.5}$ values expressed asmeans ± SD (n=3)p <0.01; ***p <0.001**
([a]) standard molecule BHA: Butylated Hydroxyanisol.
(-) Not tested.

Table 3: Effect of *Citrullus colocynthis* fruit extracts on AAPH-induced hemolysis

	Hemolysis inhibition (%)				IC_{50} (µg/mL)
	20µg/mL	40 µg/mL	80 µg/mL	100 µg/mL	
Butanol	51.21±2.69	52.13±3.88	53.66±0.47	56.21±2.11	19.11±0.5***
Ethyl acetate	41.83±1.91	43±2.09	45.71±3.07	50.21±1.46	99.64±2.92
Hydro-methanol	35.88±1.38	37.42±0.95	37.46±3.44	41.63±8.06	>100
Quercetin[a]	53±1.39	53.87±0.55	57.37±0.83	58.75±0.63	18.27±0.2
Ascorbic acid[a]	59.5±0.57	59.88±0.69	64.5±0.81	68.63±0.51	16.32±0.05

[a] standard molecule: Quercetin, Acid ascorbic.
IC_{50}value expressed as means ± SD (n=3).
*****p <0.001; ***significance is compared to the positive control (quercetin).**

Effect of *C. colocynthis* extracts on AAPH-induced hemolysis

Upon incubation of red blood cell suspension with AAPH as a peroxyl radical generator, lipids and proteins in the cell membrane are oxidized, which leads to hemolysis (Sekiya *et al.,* 2005; Ilavenil *et al.,* 2011). The effect of colocynth extracts and antioxidant molecules (ascorbic acid and quercetin) on the inhibition of AAPH-induced hemolysis is reported in Table 3. A dose-dependent inhibition of hemolysis was observed with the investigated extracts, as well as the butanol extract showed the most protective effects with an IC_{50} of 19.11 µg/mL, which is equivalent to ascorbic acid (IC_{50} = µ16.32 g/mL) and quercetin (IC_{50} = 18.27 µg/mL). However, the IC_{50} observed in the hydromethanol and ethyl acetate extracts are close to 100 µg/mL. Since no studies on the protective impact of colocynth extracts on red blood cells against AAPH-induced hemolysis have been published, we have no way to compare our results to the existing research. Polyphenols and flavonoids present in butanol extract are thought to play an active role in its anti-hemolytic properties, which have already shown in the bibliography their ability to scavenge the radical species that cause lipid peroxidation and hemolysis of red blood cells (Sekiya *et al*., 2005). The liposolubility of flavonoids, allows them to bind to phospholipids

Table 4: Antimicrobial activity of *Citrullus colocynthis* fruit extracts determined by microdilution and disk diffusion method

		Diameter of inhibition zone (mm)						MIC (mg/mL)					
		Hydromethanol	Chloroform	Ethyl acetate	Butanol	Gentamicin	Amphotericin B	Hydromethanol	Chloroform	Ethyl acetate	Butanol	Gentamicin	Amphotericin B
Gram-positive	***E. coli* ATCC 8739**	6±0	6±0	8±0.4	8±0.6	22	-	2.5	-	1.25	1.25	0.32	-
	***K. pneumoniae* ATCC 700603**	6±0	6±0	9±0.6	7±0	19	-	2.5	-	1.25	0.625	4.16	-
	***P. aeruginosa* ATCC 27853**	6±0	6±0	6±0	7±0	12	-	2.5	-	2.5	1.25	0.78	-
	***A. baumanni* ATCC 19606**	9±0.6	6±0	10±0.6	11±0.7	35	-	2.5	-	1.25	0.625	0.78	-
	***C. freundii* ATCC 8090**	6±00	6±0	6±0	7±0	18	-	1.25	-	1.25	1.25	0.19	-
	***P. mirabilis* ATCC 35659**	9±0.9	7±0.4	11±0.6	11±0.9	25	-	5	-	1.25	0.625	0.19	-
	***S. typhimirium* ATCC 13311**	6±0	6±0	6±0	6±0	22	-	2.5	-	2.5	2.5	0.65	-
	***E. cloacae* ATCC 13047**	7±0.4	6±0	7±0	9±0.8	21	-	1.25	-	2.5	1.25	2.6	-
Gram-negative	***S. aureus* ATCC 6538**	10±0.8	7±0.7	11±0.8	13±0.9	32	-	1.25	-	0.625	0.312	0.19	-
	***B. cereus* ATCC 25921**	6±0	6±0	6±0	6±0	20	-	5	-	2.5	1.25	0.19	-
	***B. subtilis* ATCC 6633**	10±0.6	7±0.4	9±0.6	10±0.7	22	-	1.25	-	1.25	0.625	5.20	-
	***E. faecalis* ATCC 49452**	7±0.8	6±0	6±0	7±0.4	21	-	2.5	-	1.25	0.625	0.78	-
	***L. monocytogenes* ATCC 15313**	8±0.6	7±0.4	10±0.4	9±0.6	22	-	2.5	-	2.5	1.25	2.21	-
	***M. luteus* ATCC9341**	11±0.4	8±0.6	12±0.8	13±0.9	22	-	1.25	-	0.625	0.625	0.12	-
Yeast	***C. albicans* ATCC 10231**	11±0.8	6±0	12±0.9	15±0.8	-	32	≥50	-	50	25	-	4
	***C. albicans* IP444**	8±0.6	6±0	10±0.6	10±0.6	-	30	25	-	25	25	-	8
	***C. albicans* ATCC26790**	7±0	6±0	13±0.8	8±4	-	30	≥50	-	50	25	-	2

Data are expressed as mean ± SE values (n = 3) ; (-) Not tested.

and membrane proteins by hydrogen bonds, and react as antioxidants against free radicals produced at the red blood cell membrane, which reflects the ability of polyphenols to prevent red blood cell hemolysis (Blasa *et al.,* 2007; Paiva-Martins *et al.,* 2009; Ramchoun *et al.,* 2015).

Antimicrobial activity

Table 4 illustrates the antibacterial effect of *C colocynthis* fruit extracts. Several extracts were observed to be effective against Gram-positive and negative bacteria, but the butanol extract exhibited

Table 5: Bacterial antiadhesion activity (%) of *Citrullus colocynthis* fruit extracts

		Extract concentrations (mg/mL)							
		0.0156	**0.031**	**0.0625**	**0.125**	**0.25**	**0.5**	**1**	**2**
***S. aureus* ATCC 6538**	**Butanol**	10.97± 0.12	16.52± 0.74	25.59± 0.68	32.18± 0.99	46.36± 0.73	50.80± 0.74	51.17± 0.62	51.29± 0.67
	Ethyl acetate	8.75± 0.12	12.95± 0.25	19.3± 0.92	28.98± 0.62	35.08± 0.55	41.62± 0.55	41.8± 0.36	43.09± 0.31
	Hydro-methanol	8.08± 0.67	12.15± 0.06	14.72± 0.3	22.5± 0.55	28.91± 0.55	34.59± 0.18	36.31± 0.55	37.05± 1.04
***L. monocytogenes* ATCC 15313**	**Butanol**	10.73± 0.37	13.35± 0.3	18.48± 0.42	25.49± 0.37	31.4± 0.67	39.51± 0.97	48.84± 0.3	49.39± 0.12
	Ethyl acetate	12.68± 0.12	13.05± 0.12	18.9± 0.37	29.39± 0.85	32.93± 0.85	37.74± 0.43	41.46± 0.24	41.52± 0.19
	Hydro-methanol	7.87± 0.43	10.98± 0.24	15.49± 1.34	19.7± 0.55	27.13± 1.15	33.23± 0.55	34.63± 0.12	35.18± 0.3
***E. coli* ATCC 8739**	**Butanol**	13.66± 0.18	15.86± 0.95	19.59± 0.3	28.64± 0.12	34.62± 0.77	41.72± 1.24	41.89± 1.18	41.95± 1.0
	Ethyl acetate	8.46± 0.06	16.09± 0.83	16.57± 0.11	23.25± 0.53	27.81± 0.82	33.73± 0.36	36.27± 0.3	36.80± 0.35
	Hydro-methanol	9.4± 0.3	12.6± 0.3	15.08± 0.41	18.05± 1	20.65± 0.41	28.93± 0.89	33.25± 1.53	32.25± 0.65

the highest activity. In the disk diffusion method, inhibition zones were determined to have diameters between 7 and 13 mm, and the diameters were detected mostly against *Proteus mirabilis* ATCC 35659, *Staphylococcus aureus* ATCC 6538, and *Micrococcus luteus* ATCC 9341. Also, the butanol extract exhibited the highest MIC values against *Klebsiella pneumoniae* ATCC 700603, *Acinetobacter baumanii* ATCC 19606, and *Proteus mirabilis* ATCC 35659, ranging from 0.312 to 1.25 mg/mL. *Micrococcus luteus* ATCC 9341 and *Staphylococcus aureus* ATCC 6538 were the most sensitive strains to butanol (MIC = 0.312 mg/mL) and ethyl acetate extracts (MIC = 0.625 mg/mL). For antifungal activity, ethyl acetate and butanol extracts showed low activity against Candida albicans strains, with inhibition zone diameters ranging from 10-15 mm and MIC values between 25-50 mg/mL (Table 4), compared to the control antifungal molecule, amphotericin B (32 mm, 2-8 mg/mL).

According to Shahraki-Mojahede *et al.* (2021), *P. aeruginosa* was more sensitive to the ethyl acetate extract than the methanol extract of *C. colocynthis*, MIC = 0.62 mg/mL and 1.25 mg/mL, respectively. Furthermore, the aqueous and methanolic extracts of colocynth leaves showed low antibacterial and antifungal effects (Gurudeeban *et al*., 2010). Whereas, the aqueous and organic extracts of different parts from *C. colocynthis* (roots, stems, leaves, fruits, and seeds) inhibit the growth of the following strains, *E. coli* (MIC = 0.006 mg/ml), *C. albicans, C. kreusei, C. glabrata,* and *C. parapsilosis* (Merzouk *et al.,* 2011; Al-Snafi, 2016). In addition, organic extracts from fresh leaves of *C. colocynthis* ethanol, chloroform, and petroleum ether are more active against *Escherichia coli*, *Proteus vulgaris*, and *Staphylococcus aureus*, and slightly active against *Klebsiella pneumoniae* and *Salmonella typhi* (Paul, 2008). In the present study, the antibacterial activity of butanol and ethyl acetate extracts could be explained by the high content of polyphenols and flavonoids in these extracts. According to the literature, Gram-positive bacteria are more sensitive to phenolic compounds compared to Gram-negative bacteria; this sensitivity is related to the absence of the protective hydrophobic lipopolysaccharides of the bacterial outer membrane (Pitchamuthu *et al.,* 2012; Mezni *et al.,* 2015). In addition, polyphenols inhibit the production of nucleic acids, cell walls, and energy, as they can directly affect microbial metabolism by inhibiting the activity of some crucial enzymes, including RNA polymerase, alcohol

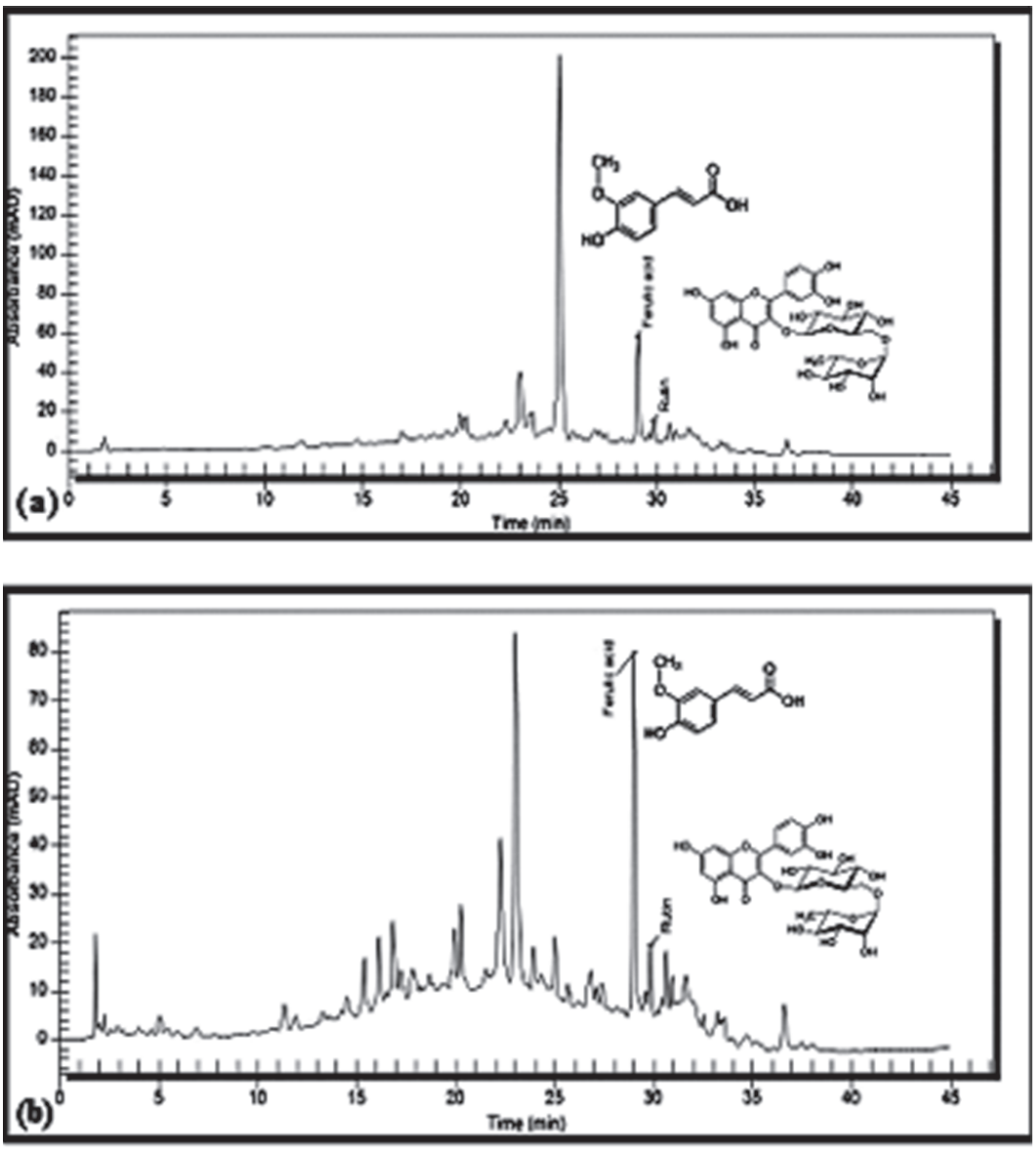

Fig. 1: *Citrullus colocynthis* fruit extracts' high-performance liquid chromatography profile (at 280 nm). (a) ethyl acetate extract (b) n-butanol extract

dehydrogenase, thioredoxin reductase, urease, and dihydrofolate reductase (Simões *et al.,* 2012; Khameneh *et al.,* 2019; Slobodníková *et al.,* 2016; Prakash *et al*., 2020; Almi *et al.,* 2022).

Our study is the first to investigate *Citrullus colocynthis*' anti-bacterial adhesion activity on polystyrene microplates as an informative test on its antibiofilm activity. The results (Table 5) revealed that ethyl acetate, butanol, and hydromethanol extracts significantly reduced the adhesion of *L. monocytogenes* ATCC 15313, *E. coli* ATCC 8739, and *S. aureus* ATCC 6538 at an inhibition rate ranging between 28% and 50%. These findings demonstrate the ability of colocynth to prevent the development of biofilm on the plastic surface. According to Satyavani *et al.* (2011) *Citrullus colocynthis* silver nanoparticles inhibited significantly biofilm-forming bacteria including *P. aeruginosa* (8 mm), *E. coli* (10.1 mm), *P. vulgaris* (9 mm), *V. paraheamolyticus* (10.1 mm), and *L. monocytogens* was the most inhibited (8 mm). The antibiofilm activity of our extracts can be linked to their polyphenol and flavonoid content. In the literature, several flavonoids and phenolic acids

such as quercetin, rutin, catechin, gallic, ferulic, and caffeic acids inhibit biofilm formation by *Listeria monocytogenes*. The anti-biofilm effect of polyphenols lies in their ability to inhibit the synthesis of extracellular polymeric substances, initial adhesion, cell motility, and modification of the physicochemical characteristics of bacteria, all of which are crucial steps in the formation of biofilms (Vazquez-Armenta *et al*., 2018). Polyphenols, without affecting bacterial growth, can inhibit biofilm formation by modifying regulation mechanisms such as quorum sensing or other bacterial systems (Slobodníková *et al*., 2016).

CONCLUSION

In conclusion, phenolic compounds such as ferulic acid, rutin, and other unidentified compounds identified in *C. colocynthis* fruit extracts, might promise the potent antioxidant and antimicrobial properties of these extracts, and their ability to inhibit bacterial adhesion to a plastic surface, which is subsequently the initial stage of biofilm development. *C. colocynthis* may have several benefits, including the potential to aid in the treatment of diseases related to oxidative stress and the prevention of diseases caused by oxidative stress. It can also be used as an antimicrobial source for the treatment of bacterial infections.

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Evaluation of some rooting substrates and cutting types in propagation of Fig (*Ficus carica*)

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ABSTRACT

The cultivar (collected from Middle-East countries known as Egyptian common fig) showed cultivation potentials while cultivated by amateur gardeners in Bangladesh and thereby further studies are needed to explore its suitable propagation method for successful commercial cultivation in Bangladesh. Seven rooting substrates and two types of cuttings (semi-hard and hard wood) were used from Egyptian Common fig (Ficus carica) where garden soil was used as control substrate. The experiments were conducted in autumn 2018 and spring 2019 following two factor factorial Completely Randomized Design with three replications obtaining five propagules in each replication. In comparison to the control, quick bud and leaf initiation (about 8 days earlier in autumn and 3 to 4 days earlier in spring) was recorded from semi hard wood cuttings in coco-peat. Delayed bud and leaf initiation than control was observed in hard wood cuttings while sand was used as rooting substrate (about 4 and 1 day delayed in autumn and 8 and 7 days delayed in spring). Irrespective of the season, higher average length of bud, root and shoot; more number of bud, leaf, root and shoot than control (1.14 to 2.56-fold) were recorded in semi-hard wood cuttings in coco-peat. The research revealed that fig can be propagated by semi hard wood cuttings using coco-peat as rooting substrate in spring or autumn in Bangladesh.

Keywords: Hardwood, propagation, rooting substrates, season, semi hard wood, survivability

INTRODUCTION

Fig (*Ficus carica*) is a Mediterranean deciduous and subtropical fruit belonging to the family Moraceae. It is rich in both nutritional and medicinal value (Soni *et al.*, 2014). In Bangladesh three species of fig as cluster fig (*Ficus racemosa*), hairy fig (*Ficus hispida*) and creeping fig (*Ficus pumila.*) grow in wild and consumed solely by wild animals and birds. Local people sometimes use *Ficus hispida* as wild vegetable and *Ficus racemosa* as medicinal fruit but *Ficus carica* is not grown here. Recently some varieties of *Ficus carica* have been collected by amateur gardeners and these varieties showed some cultivation potentials (Mehraj *et al*., 2013).

The introduction of fig in Bangladesh could contribute to increase the fruit diversity to the consumers for minimizing the gap between the need and supply of fruit (Siddique *et al*., 2010). But no attempt has been taken for mass and managed cultivation of fig in this country, though the soil and climatic conditions of Bangladesh are suitable for the cultivation of fig (Mehraj *et al*., 2013). This may be due to unavailability of suitable variety or lack of knowledge of cultivation procedure. To introduce a new fruit crop in an area, it is imperative to standardize its cultivation procedure and consequently availability of propagation material to the farmers for its cultivation.To prescribe a suitable propagation technique for fig, an investigation was made on the effect of different rooting substrate and cutting types on successful propagation of fig (*Ficus carica*).

MATERIALS AND METHODS

Experimental site and season

The experiment was conducted at Rangemari village under Batiaghata upazilla, Khulna, Bangladesh in autumn (Mid-August to Mid-October 2018) and spring (Mid-February to Mid-April 2019) (Banglapedia, 2021).Weather data (BMD, 2018 and 2019) of the experimental site during this periodhas been shown in Table 1.

Table 1: Weather conditions of study area during autumn (Mid- August to Mid-October) and spring (Mid-February to Mid-April) 2018-19 (BMD, 2018 and 2019)

Season	Monthly average max. temperature (°C)	Monthly average min. temperature (°C)	Monthly average relative humidity (%)	Monthly average rainfall (mm)
Autumn (Mid -August to Mid-October, 2018)	33.4	25.73	82	88.76
Spring (Mid-February to Mid-April, 2019)	31.67	20.5	73.33	114

Experimental materials

Cuttings from a 'Common fig' cultivar from Egypt (*Ficus carica)* (Wikimedia, 2020) and locally available rooting substrates, *viz*., garden soil, coco-peat, sand and vermicompost (VC) were used as experimental materials. Two types of cuttings viz. hardwood cuttings (dormant mature firm stems, which does not bend easily) and semi hardwood cuttings (partially mature wood which is reasonably firm and the leaves are of mature size)were taken as described by Ibironke (2013).

Preparation of the experimental materials

The cuttings were made with 4-5 nodes having a length of 12-15 cm. The leaves were removed from the cuttings and were trimmed to the required length by removing the terminal portions just above a bud. The proximal ends of the cuttings were given a slanting cut to expose maximum surface area for effective rooting. Cuttings were washed properly followed by soaking in detergent water for 15 minutes (Reddy *et al*., 2008). The cutting tool was dipped in a mixture of one part bleach and nine parts of water to prevent disease transmission (Ibironke, 2013). Before use, coco-peat was soaked well by water. Then excess water was removed by pressing firmly and kept hanging in a bag for two hours. All the rooting substances except vermicompost were sundried for natural sterilization.

Placement of cuttings in rooting substrates

Cuttings were planted on August 14, 2018 and February 14, 2019 in an inclined position (angle of 45°) in 4" x 3" plastic pots. A single cutting was placed in each pot and five pots with cutting were considered as a replication for a treatment. Watering was done as per requirement and other necessary measures were taken to ensure better care of the cuttings.

Design of experiment and treatments

Two factor factorial Completely Randomized Design (CRD) was followed with four replications for the treatments containing five propagules in each replication. The two factors were as treatment and the seasons (S_1= Autumn and S_2= Spring) of propagation. The all possible combinations of cutting types and rooting substrates were considered as treatments in the study. The treatments were as follows -

T_0 = Semi hard wood cutting (SWC) planted in garden soil (Control for SWC), T_1 = Hardwood cutting (HWC) planted in garden soil (Control for HWC), T_2 = SWC planted in garden soil with 25% vermicompost (VC), T_3 = HWC planted in garden soil with 25% VC, T_4= SWC planted in garden soil with 50% VC, T_5 = HWC planted in garden soil with 50% VC, T_6 = SWC planted in garden soil with 75% VC, T_7 = HWC planted in garden soil with 75% VC, T_8 = SWC planted in 100% VC, T_9 = HWC planted in 100% VC, T_{10} = SWC planted in sand, T_{11} = HWC planted in sand, T_{12} = SWC planted in coco-peat and T_{13} = HWC planted in coco-peat

Nutritional status of the used substrates

All rooting substrates used for the present study was analyzed in the laboratory of Soil Resource Development Institute, Daulatpur, Khulna under the Ministry of Agriculture, Peoples Republic of Bangladesh. The nutritional status of the substrates has been shown in Table 2.

Table 2: Nutritional status of the rooting substrates

Sl. no.	Sample	pH	Salinity (DSm^{-1})	Organic matter (%)	Organic Carbon (%)	Total Nitrogen (%)	Phosphorus µgm gm^{-1} soil	Potassium mleq. gm^{-1} soil	Sulphur µgm gm^{-1} soil	Zinc µgm gm^{-1} soil or %	Boron µgm gm^{-1} soil
1	GS	8	1.6	4.90	NT	0.178	103.65	0.55	87.37	6.28	1.05
2	Sand	7.5	0.8	2.93	NT	0.120	14.02	0.08	55.75	1.19	0.16
3	GS 50% + VC 50%	6.9	5.2	6.95	NT	0.181	160.01	0.94	105.70	7.31	2.23
4	GS 25% + VC 75%	6.7	6.5	8.31	NT	0.183	161.68	1.16	122.85	7.40	2.33
5	GS 75% + VC 25%	7.0	3.8	5.50	NT	0.179	162.15	0.78	85.92	7.35	2.39
6	VC 100%	6.1	NT	NT	17.6	1.1	0.6	1.2	0.5	<0.1%	NT
7	CP	6.0	NT	NT	22.5	0.5	0.5	0.8	0.5	<0.1%	NT

GS = Garden soil, VC = Vermicompost, CP = Coco-peat, NT = Not tested

Observations recorded

Data were collected on growth and morphological characteristics like days to bud and leaf initiation (observations made regularly); number of buds, leaves,; length of buds, leaves, roots and shoots and leaf width were measured in millimeter using simple measuring scale (starting from one month of planting, observations made weekly for six weeks and final data that recorded at eighth week were analyzed). Length of buds was measured from the base on the stem to the tip and leaf length from the base of the petiole to the leaf apex along the midrib. The most spacious part of leaf blades were considered as leaf width. After 12 week of planting the cuttings, two of them were pulled out from each replication to count the number of roots and measure the length from stem-root junction to the root tip. Data on survivability of cutting were also recorded during this time. The height from the collar to the tip of the highest leaf was considered as shoot length.

Statistical analysis

Collected data were subject to two way analysis of variance (ANOVA) by Statistical Tool for Agricultural Research (STAR) (IRRI, 2013). The effects of various treatments and their interactions were assessed within ANOVA and the level of significance was tested by Least Significant Differences (Fisher's LSD) following significant ($P \leq 0.01$) F test. The assumptions on normality of data and homogeneity of variance were checked to ensure the validity of analysis.

RESULTS AND DISCUSSION

Days required for bud and leaf initiation

Irrespective of the season, significant ($P \leq 0.01$) variation in days required for bud and leaf initiation was observed due to different combinations of rooting substrates and cutting types (Table 3). Early bud and leaf initiation was recorded in semi hard wood cuttings planted in coco-peat (T_{12}) (for bud initiation- 5.5 days earlier; and for leaf initiation- 6.33 days earlier than control). However, semi hard wood cuttings planted in garden soil with 50% vermicompost (T_4) also showed early bud emergence and leaf initiation in comparison to the control (for bud initiation- 5 days earlier; and for leaf initiation- 4.33 days earlier than control, T_0).

Table 3: Days required for bud and leaf initiation in fig (*Ficus carica*) cuttings according to the rooting substrates and cutting types in two seasons (autumn and spring)

Treatments	Days to bud initiation			Days to leaf initiation		
	S_1	S_2	Season mean	S_1	S_2	Season mean
T_0	12.00 bc	7.00 fg	9.5 C	17.67 ab	10.33 de	14.00 B
T_1	9.67 cd	9.00 ef	9.17 CD	14.00 b-d	11.67 c-e	12.84 BC
T_2	11.33 bc	10.00 de	10.67 BC	17.67 ab	14.33 a-c	16.00 AB
T_3	12.67 b	11.00 c-e	11.84 BC	19.33 a	16.33 ab	17.83 A
T_4	5.33 e	3.67 h	4.50 F	11.00 d	8.33 ef	9.67 C
T_5	7.33 de	6.00 gh	6.67 D-F	12.67 cd	10.67 de	11.67 BC
T_6	14.00 ab	10.67 c-e	12.34 B	20.00 a	15.33 ab	17.67 A
T_7	13.33 b	11.67 c-e	12.50 B	18.67 a	15.67 ab	17.17 AB
T_8	13.00 b	13.00 a-c	13.00 B	18.33 ab	17.00 ab	17.67 A
T_9	11.33 bc	14.67 ab	13.00 B	20.00 a	17.67 a	18.84 A
T_{10}	13.00 b	12.00 b-d	12.50 B	15.67 a-c	13.67 b-d	14.67 B
T_{11}	16.33 a	15.67 a	16.00 A	18.67 a	17.67 a	18.17 A
T_{12}	4.67 e	3.33 h	4.00F	9.67 d	5.67 f	7.67 C
T_{13}	6.67 e	5.33 gh	6.00EF	11.33 cd	8.33 ef	9.83 C
Treatment mean	10.76A	9.08B		16.05A	13.05B	

LSD ($P \leq 0.01$) for days to bud initiation: to compare treatment at each level of season= 2.69; to compare season at each level of treatment= 1.54; for days to leaf initiation: to compare treatment at each level of season = 3.49; to compare season at each level of treatment = 2.00; n = 3 replications.* Means with the same lower-case or upper-case letter in rows or columns are not significantly different at $P \leq 0.01$ by the Least Significant Difference (LSD) Test.S_1= Autumn and S_2= Spring; T_0 = Semi hard wood cutting (SWC) planted in garden soil (Control for SWC), T_1 = Hardwood cutting (HWC) planted in garden soil (Control for HWC), T_2 = SWC planted in garden soil with 25% vermicompost (VC), T_3 = HWC planted in garden soil with 25% VC, T_4= SWC planted in garden soil with 50% VC, T_5 = HWC planted in garden soil with 50% VC, T_6= SWC planted in garden soil with 75% VC, T_7= HWC planted in garden soil with 75% VC, T_8= SWC planted in 100% VC, T_9= HWC planted in 100% VC, T_{10}= SWC planted in sand, T_{11} = HWC planted in sand, T_{12} = SWC planted in coco-peat and T_{13}= HWC planted in coco-peat.

More or less similar observation was recorded in the hard wood cuttings planted in garden soil with 50% vermicompost (T_5). On the other hand, much delayed bud (6.83 days) and leaf (5.33 days) initiation than control (T_1) was found when hard wood cuttings were planted in sand only (T_{11}). The spring season significantly ($P \leq 0.01$) enhanced both of the bud and leaf initiation (1.68 days and 3.00 days earlier respectively).

Cutting is the main method used for the fig tree propagation. Growth factors of cuttings are directly influenced by factors like cutting type and rooting media (Antunes *et al*., 2003, Magesa *et al*., 2018). Coco-peat is a medium with small sized particle which ensures high moisture retention. It has a suitable range of pH (6.0 to 6.7) for supporting the cuttings to sprout early (Awang *et al*., 2009). In the current study, earliest bud and leaf initiation was observed in semi hard wood cuttings planted in coco-peat and garden soil with 50% vermicompost.

The result is in conformity with Sharath and Bhoomika (2018) who reported that vermicompost could be a definitive source of plant growth regulators produced by interactions between microorganisms and earthworms, which could contribute significantly to enhance plant growth. Similar result was reported by Verma *et al*. (2017) while they worked on marjorum and oregano to observe the effect of vermicompost on vegetative propagation. On the other hand, most delayed bud and leaf formation was recorded in the current study

Table 4: Number of buds, leaves and roots obtained from different types of cuttings and rooting substrates in two seasons of propagation of fig (*Ficus carica*)

Treatments	No. of bud			No. of leaf			Number of root		
	S_1	S_2	Season mean	S_1	S_2	Season mean	S_1	S_2	Season mean
T_0	1.33	2.00	1.67 CD	3.33 b	3.67 d-f	3.5 BC	32.67 c-e	32.67 cd	32.67F-H
T_1	1.00	1.00	1.00 D	3.67 b	3.33 d-f	3.5 BC	24.33 ef	24.33d-f	24.33H
T_2	1.33	2.33	1.83 B-D	3.33 b	5.67 b-d	4.33 B	30.33de	31.00 c-e	31.00F-H
T_3	1.33	1.67	1.50 CD	3.00 b	3.00 d-f	3.00BC	30.33 de	30.33 c-e	30.33GH
T_4	3.00	3.00	3.00 AB	4.33 ab	7.00 bc	4.5 B	64.67 a	71.33 a	68.00B
T_5	2.00	1.67	1.83 B-D	4.00 b	5.00 c-e	4.5 B	41.67 bc	56.67 b	49.17D
T_6	1.33	1.67	1.50 CD	3.00 b	4.67 c-f	3.84 BC	37.00 cd	37.00 c	37.00EF
T_7	1.33	1.33	1.33 CD	3.67 b	3.67 d-f	4.17 B	29.33 de	29.33 c-e	29.33H
T_8	1.00	1.00	1.00 D	3.33 b	3.33 d-f	3.33 BC	17.67 fg	21.67 ef	19.67I
T_9	1.33	1.00	1.17 CD	2.33 b	2.33 ef	2.33 C	13.00 g	16.33 f	14.67I
T_{10}	1.33	1.67	1.50 CD	2.67 b	3.33 d-f	3.00 BC	13.33 g	17.33 f	15.33I
T_{11}	1.00	1.00	1.00 D	2.00 b	2.00 f	2.00 C	11.67 g	15.67 f	13.67I
T_{12}	3.33	3.33	3.33 A	7.00 a	12.00 a	9.5 A	71.67 a	81.33 a	76.5A
T_{13}	2.33	2.33	2.33 A-C	3.33 b	8.00 b	5.67 B	50.33 b	60.67 b	55.5C
Treatment mean	1.62NS	1.7 NS		3.50NS	4.79NS		33.48NS	37.55 NS	

LSD ($P \leq 0.01$) for bud no: to compare treatment at each level of season = 1.25; to compare season at each level of treatment = NS; for leaf no: to compare treatment at each level of season = 2.85, to compare season at each level of treatment = 1.63; for root no: to compare treatment at each level of season = 10.67, to compare season at each level of treatment = 6.10; n = 3 replications.* Means with the same lower-case or upper-case letter in rows or columns are not significantly different at $P \leq 0.01$ by the Least Significant Difference (LSD) Test.S_1= Autumn and S_2= Spring; T_0 = Semi hard wood cutting (SWC) planted in garden soil (Control for SWC), T_1 = Hardwood cutting (HWC) planted in garden soil (Control for HWC), T_2 = SWC planted in garden soil with 25% vermicompost (VC), T_3 = HWC planted in garden soil with 25% VC, T_4= SWC planted in garden soil with 50% VC, T_5 = HWC planted in garden soil with 50% VC, T_6 = SWC planted in garden soil with 75% VC, T_7 = HWC planted in garden soil with 75% VC, T_8 = SWC planted in 100% VC, T_9 = HWC planted in 100% VC, T_{10} = SWC planted in sand, T_{11} = HWC planted in sand, T_{12} = SWC planted in coco-peat and T_{13} = HWC planted in coco-peat

Table 5: Effect of the combination of cutting types and rooting substrates on bud length, leaf length and leaf width in sprouted cutting of fig (*Ficus carica*)

Treatments	Bud length (mm)			Leaf length (mm)			Leaf width (mm)		
	S_1	S_2	Season mean	S_1	S_2	Season mean	S_1	S_2	Season mean
T_0	4.00	4.33	4.17A-D	30.00	33.67	31.83 CD	31.00	31.33	31.17 D-F
T_1	4.00	4.47	4.23A-D	33.00	38.00	35.50 C	31.67	35.33	33.50 DE
T_2	4.33	4.60	4.47A-D	33.33	36.67	35.00 C	33.33	34.67	34.00 DE
T_3	4.33	4.73	4.53A-D	34.00	35.33	34.67 C	33.67	33.67	33.67 DE
T_4	4.00	4.50	4.25 A-D	48.00	55.67	51.83 AB	47.00	54.00	50.50 B
T_5	4.00	4.27	4.13 B-D	44.33	50.33	47.33 B	44.00	48.00	46.00 BC
T_6	4.00	4.33	4.17A-D	27.67	33.67	30.67 CD	29.00	32.00	30.50 D-F
T_7	4.67	4.87	4.77A-C	34.67	39.33	37.00 C	36.00	36.67	36.33 CD
T_8	3.67	4.00	3.83B-D	21.00	28.67	24.83 D	22.00	26.33	24.17 EF
T_9	3.33	3.67	3.50CD	21.67	26.67	24.17 D	20.00	24.33	22.17 F
T_{10}	4.33	4.67	4.50 A-D	28.67	33.67	31.17 CD	25.00	30.00	27.50 D-F
T_{11}	3.00	3.17	3.08D	29.67	34.00	31.83 CD	29.00	32.33	30.67 D-F
T_{12}	5.33	6.07	5.70 A	59.00	62.67	60.83 A	59.67	61.67	60.67 A
T_{13}	5.00	5.23	5.12 AB	48.00	53.33	50.67 B	45.67	52.67	49.17 B
Treatment mean	4.14NS	4.49NS		35.22 B	40.12 A		34.79 B	38.07 A	

LSD ($P \leq 0.01$) for bud length: to compare treatment at each level of season = 1.55; to compare season at each level of treatment = 0.33; for leaf length: to compare treatment at each level of season = 9.12, to compare season at each level of treatment = 1.97; for leaf width: to compare treatment at each level of season= 10.05, to compare season at each level of treatment = 2.20; n = 3 replications.

* Means with the same lower-case or upper-case letter in rows or columns are not significantly different at $P \leq 0.01$ by the Least Significant Difference (LSD) Test.

S_1= Autumn and S_2= Spring; T_0 = Semi hard wood cutting (SWC) planted in garden soil (Control for SWC), T_1 = Hardwood cutting (HWC) planted in garden soil (Control for HWC), T_2 = SWC planted in garden soil with 25% vermicompost (VC), T_3 = HWC planted in garden soil with 25% VC, T_4= SWC planted in garden soil with 50% VC, T_5 = HWC planted in garden soil with 50% VC, T_6= SWC planted in garden soil with 75% VC, T_7 = HWC planted in garden soil with 75% VC, T_8 = SWC planted in 100% VC, T_9= HWC planted in 100% VC, T_{10}= SWC planted in sand, T_{11} = HWC planted in sand, T_{12} = SWC planted in coco-peat and T_{13} = HWC planted in coco-peat

Table 6: Root and shoot length of the fig (*Ficus carica*) cuttings planted in different rooting substrates

Treatments	Root length (mm)			Shoot length (mm)		
	S_1	S_2	Season mean	S_1	S_2	Season mean
T_0	27.00 cd	27.00 cd	27.00 DE	52.33	60.67	56.50 D
T_1	20.67 de	20.67 cd	20.67 EF	42.33	49.00	45.67 E
T_2	27.00 cd	27.00 cd	27.00 DE	56.00	62.33	59.17 D
T_3	24.33cd	24.67 cd	24.50 E	43.00	47.67	45.34 E
T_4	37.67 b	66.00 ab	51.84 B	93.33	98.67	96.00 B
T_5	29.67 c	61.67 ab	45.67C	80.33	89.33	84.83 C
T_6	30.00 c	30.00 c	30.00 D	44.00	52.00	48.00 E
T_7	27.33cd	27.33 cd	27.33DE	33.33	43.33	38.33 F
T_8	10.67 f	17.33 d	14.00 F	34.67	39.33	37.00 F
T_9	13.67 ef	16.33 d	15.00 F	32.00	36.67	34.33F
T_{10}	12.67 f	23.33 cd	18.00 F	26.67	28.00	27.33G
T_{11}	10.67 f	21.67 cd	16.17F	23.33	24.67	24.00 G
T_{12}	48.67 a	71.00 a	59.84 A	98.33	107.33	102.83 A
T_{13}	37.67 b	57.67 b	47.67C	85.67	90.67	88.17 C
Treatment mean	25.55 B	35.55 A		53.2B	59.26 A	

LSD ($P \leq 0.01$) for root length: to compare treatment at each level of season = 7.24, to compare season at each level of treatment = 4.14; for total shoot length: to compare treatment at each level of season = 5.14, to compare season at each level of treatment = 1.11; n = 3 replications.

* Means with the same lower-case or upper-case letter in rows or columns are not significantly different at $P \leq 0.01$ by the Least Significant Difference (LSD) Test.S_1= Autumn and S_2= SpringT_0 = Semi hard wood cutting (SWC) planted in garden soil (Control for SWC), T_1 = Hardwood cutting (HWC) planted in garden soil (Control for HWC), T_2 = SWC planted in garden soil with 25% vermicompost (VC), T_3 = HWC planted in garden soil with 25% VC, T_4= SWC planted in garden soil with 50% VC, T_5 = HWC planted in garden soil with 50% VC, T_6 = SWC planted in garden soil with 75% VC, T_7 = HWC planted in garden soil with 75% VC, T_8 = SWC planted in 100% VC, T_9 = HWC planted in 100% VC, T_{10} = SWC planted in sand, T_{11} = HWC planted in sand, T_{12} = SWC planted in coco-peat and T_{13} = HWC planted in coco-peat

in sand irrespective of cutting types and seasons. Similar findings were reported by Manila *et al*. (2017) where the lowest percentage of sprouted cuttings of pomegranate (*Punica granatum*) was recorded when planted in sand only.

Number of buds, leaves and roots

In comparison to the control, 1.66, 6.00 and 43.83 more buds, leaves and roots respectively were recorded from semi hard wood cuttings planted in coco-peat (T_{12}) which is statistically similar to semi hard wood cuttings planted in 50% vermicompost with garden soil (T_4). In case of leaf number the result was followed by hard wood cuttings planted in coco-peat (T_{13}) (5.67), semi hard wood cuttings planted in 50% vermicompost with garden soil (T_4)(4.5) and 50% vermicompost with garden soil (T_5) (4.5). However, semi hard wood cuttings planted in 50% vermicompost with garden soil (T_4) produced 35.33 more roots than control. The effect of seasonal variation was not significant for the number of bud, leaf and root (Table 4). Similar findings was reported by Nawarathna *et al*. (2020) while they studied the rooting and survivability performance of different cutting types of *Momordica dioica*in different rooting substrates.

Semi hard wood cuttings contain shorter internodes that mean more number of nodes in the same length compared to hard wood cuttings. Nodes reserve food which accelerates the growth of plants. So, more nodes imply possibility of more buds and leaves and consequently more roots. Similar findings were described by Alikhani *et al*. (2011). Coco-peat produced highest number of

Table 7: Survivability of rooted fig (*Ficus carica*) cuttings in respect of cutting types and rooting substrates in two seasons

Season	Cutting types	Substrates						
		Sb_0	Sb_1	Sb_2	Sb_3	Sb_4	Sb_5	Sb_6
Season I	SWC	+++++	+++++	+++++	+++	++	-	+++++
(Autumn)	HWC	+++++	+++++	+++++	++	-	-	+++++
Season 2	SWC	+++++	+++++	+++++	+++++	++	++	+++++
(Spring)	HWC	+++++	+++++	+++++	+++++	++	++	+++++

Single '+' sign = 20% survived cuttings, '-' = Cuttings not survivedSWC= Semi hard wood cutting and HWC= Hardwood cutting, Sb_0 = Garden soil (Control), Sb_1 = Garden soil with 25% vermicompost, Sb_2 = Garden soil with 50% vermicompost, Sb_3 = Garden soil with 75% vermicompost, Sb_4 = 100% vermicompost, Sb_5 = Sand and Sb_6 = Coco-peat

buds, leaves and roots and garden soil with 50% vermicompost produced second highest as these are the substrates which increase soil porosity as well as soil aeration. Similar findings were reported by Shamsuddin *et al*. (2021). On the contrary, low nutrient content and low water retention capacity of sand inhibited number of buds, leaves and roots. Torkashvanda and Shadparvar (2012) demonstrated the similar results from their study conducted on the effect of rooting substrates on China rose.

Bud Length, Leaf Length and Leaf Width

Semi hard or hard wood cuttings planted in coco-peat (T_{12} or T_{13}) showed statistically similar longest bud (5.70 and 5.12 mm respectively). However, these treatments were significantly different from others. In case of leaf length and leaf width statistical similarity was observed between $T_{4,}$ T_{12} and T_{13} and dissimilarity between control and other treatments (Table 5).

Longer and wider leaves were observed in T_{12} (29 mm longer and 29.5 mm wider than control) followed by T_4 (20 mm longer and 19.33 mm wider than control). Minimum length of bud (3 mm) and leaf (31.83 mm) was observed in T_{11} while the minimum leaf width was recorded in T_9 (22.17 mm) (Table 5).

In consideration to the seasons, the spring enhanced the length of bud and leaf and width of leaf, though no significant variation was observed for bud length in these seasons. The variation in the quality of the root and shoot characteristics by using various rooting substrates can be accredited to the direct consequence of the medium on the basal portion of the cutting (Hwang and Jeong, 2007). Coco-peat plays a vital role in spreading the canopy and increasing the leaf area through improving the physical and chemical properties of soil (Awang *et al*., 2009) which is in compliance with the results from the current study.

Length of root and shoot

The longest roots and shoots were recorded in semi hard wood cuttings planted in coco-peat (T_{12}) (32.84 mm and 46.33 mm longer than control) followed by semi hard wood cutting planted in the mixture of garden soil and 50% vermicompost (T_4) ; though, statistically similar root length was observed from the treatmentsT_5 and T_{13} (Table 6). The cuttings showed statistically significant ($P \leq 0.01$) variations for shoot length while they were planted in different rooting media. Irrespective of the cutting types, shortest length of root and shoot was found in the cuttings planted in sand only (T_{10} and T_{11}) (root length from 16.17 to 18.00 mm; shoot length from 24.00 to 27.33 mm). Length of root and shoot was found to be influenced by the seasonal variations having longer roots and shoots in spring (9.55 mm and 6.03 mm longer root and shoot respectively in spring than autumn).Sand is very poor in nutrient content and vermicompost adds slow releasing nutrients to soil and these become available through microbial activities in combination with soil.

Swarts *et al*. (2018) also reported significantly increased rooting percentage, rooting quality, budding leaves and survival percentage in heel cuttings of *Lobostemon fruticosus* (L) planted in coco peat during spring.

Survivability of rooted cuttings

During autumn none of the rooted hard wood cuttings survived while they were planted in substrates containing only vermicompost (Sb_4) or sand (Sb_5). However, none of the rooted semi hard wood cuttings survived in sand (Sb_4). On the other hand, minimum decease of the rooted semi hard or hard wood cuttings was recorded during spring from any of the rooting media (Table 7). Though irrespective of the cutting types, a lower rate of survivability (40%) was recorded while only vermicompost (Sb_4) and sand (Sb_5) were used as media in spring. On the other hand, a moderate rate of survivability (40 to 60%) was recorded in autumn while the cuttings were planted in garden soil with 75% vermicompost (Sb_3).

Similar results were documented by Blouin *et al*. (2019) where they observed improved soil functioning with the addition of vermicompost and its maximum positive effect on plant growth when they used 30% to 50% vermicompost of the total soil volume. They also stated that the best original material to be used for vermicompost production was cattle manure which was same as in the present experiment.

Initial vigor of plantlets has great impact on survivability and further growth of the plant (Via and Lande, 1985). More number and large size of leaves and roots enhance the percentage of survivability which has been reflected from the current study.

Similar findings were delineated by Lakshanthi and Seran (2019), Sudarjat *et al*. (2018) and Dahale *et al*. (2018).The phenotypic expression of a plant is the sum of its genotype and the interaction of genotype and environment (Via and Lande, 1985).

All of growth and morphological characters of the studied plant materials showed a negative trend regarding the increment of the amount of applied vermicompost over 50%. This may be due increased availability of nutrients in the rooting substrates as displayed in Table 2. Wilson (1988) stated that increased nutrient level sometime limits plant growth. These may also be the cause of low survivability rate of saplings grown in vermicompost in the current study (Table 7).

Again, all of the parameters did comparatively well in spring than autumn. The optimum monthly average temperature (26.09˚C) and monthly average relative humidity (73.33%) during spring favored vigorous growth, development and survivability of fig (*Ficus carica)* cuttings.

Findings by Nava *et al*. (2014) support the result obtained from current study. Similar result was also reported by Siddique *et al*. (2010) when investigation was done on some minor fruit cuttings.

CONCLUSION

The current study was conducted to observe the effect of cutting types and rooting substrates on the performance of cutting during autumn and spring. Two factor factorial Completely Randomized Design (CRD) was followed with four replications for the treatments containing five propagules in each replication. From the study it was observed that semi hard wood cuttings performed better in respect of growth parameters when they were planted in coco-peat and 50% vermicompost with garden soil. In these two rooting substrates hard wood cuttings also performed good but sand was observed as worst media for all types of cuttings. Both Autumn and Spring seasons are suitable for propagation of fig (*Ficus carica*) in Bangladesh but Spring is better in respect of all growth, morphological characteristics and survivability due to its moderate temperature and low relative humidity.

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Impact of wastewater on lemongrass (*Cymbopogon flexuosus*) essential oil yield and quality

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ABSTRACT

Lemongrass (Cymbopogon flexuosus) essential oil yield and quality were evaluated in a field experiment conducted at ICAR-Indian Agricultural Research Institute (IARI) to evaluate the effects of groundwater (GW), untreated wastewater (WW), constructed wetland treated wastewater (TWW) and groundwater in conjunction with untreated wastewater in cyclic mode (CW) in the main plots and three levels of fertilizers in subplots viz. control (no NPK fertilizer application)(N_0), the recommended dose of NPK fertilizers – the amount of NPK added through irrigation water (N_1) and recommended dose of NPK fertilizers (N_2) during 2018-2019.The trial was arranged in a split-plot design with three replications.The total oil yield from three harvests was the maximum (546 L ha^{-1}) in the case of wastewater irrigation and the minimum in the case of groundwater (476 L ha^{-1}). Irrigation with untreated wastewater resulted in significant increase of 11 and 16% in essential oil yield compared to treated wastewater and groundwater irrigation, respectively. The total oil yield from alternative irrigation with wastewater and groundwater was equal to that obtained in the case of irrigation with treated or untreated wastewater. The neral (neral is an isomer of citral i.e. the major compound in the lemongrass oil) and geranial contents obtained from groundwater and treated wastewater irrigation were 11-14% higher compared to alternative irrigations with untreated sewage and groundwater. Overall results indicated that the wastewater can be used for growing lemongrass aromatic crop to produce essential oils without causing any reduction in quantity and quality.

Keywords: Aromatic, citral, essential oil yield, sewage, wastewater

INTRODUCTION

With increasing population, irrigation water demand is expected to increase from 688 BCM in 2010 to 1072 by 2050. It will result in a reduction of the fresh water availability for agricultural use predicted from 83% in 1998 to 67% by 2050, (Gupta and Deshpande, 2004). Therefore, alternate water sources for agriculture is the need of hour. It is expected that by the year 2051, urban wastewater generation might cross 120,000 MLD and that rural India would also generate 50,000 MLD in view of water supply designs for community supplies in rural areas (Bhardwaj, 2005). The concentration of nutrients in wastewater irrigation by 1,000 m^3 per hectare was found to vary considerably: 4-24 kg phosphorus, 16-62 kg total nitrogen, 2-69 kg potassium, 27-182 kg sodium, 9-110 kg magnesium and 18-208 kg calcium (Qadir, 2011).

The use of wastewater for irrigation is relatively safe and considered to be a low-cost wastewater disposal strategy. This technology involves the conservation of water, water supply supplementation for irrigation and the use of nutrients present in the wastewater for productive purposes (Lopez *et al*., 2006). Cultivation of crops with non-edible economic parts like aromatic grasses, cut flowers, etc. has been proposed as a remunerative and a viable option for preventing pollutants entry in food chain (Lal *et al*., 2008a, b). Lemongrass (*Cymbopogon flexuosus*), being a perennial aromatic sedge and leading to huge biomass production, is widely cultivated for its essential oil (Zheljazkov *et al*., 2011). The essential oil is distilled from the foliar leaves of the lemongrass. The herbage on an average contains 0.2- 0.4% oil and the oil yield is 100-120 kg/ha/year. Oil of lemongrass is a viscous liquid, yellow to dark yellow or dark amber in colour turning red with age. Lemongrass oil is widely being used in cosmetics, perfumes, soaps, detergents and insect repellents. India being the largest producer (300–350 tonnes $annum^{-1}$) of lemongrass oil, exports 80%

of it (National Horticulture Board, Govt. of India, 2005). The lemongrass plant is hardy and flourishes in a wide variety of soil ranging from rich loam type of soils to poor laterite. There has been a growing gap in the global production and demand of lemongrass oil (3900 metric tonnes; Barbosa *et al.*, 2008). Hence, to meet the demand of this industrial crop, expansion of its production to wastewater irrigated lands seems to be a sustainable option. The yield of lemongrass may also be affected by nutrients, salts, pathogens, heavy metals and other pollutants present in wastewater. The magnitude of variation in lemongrass essential oil quality and yield may also be affected by the extent of wastewater treatment or when wastewater is used in conjunction with good quality groundwater. The information on the relative essential oil yield and quality of lemongrass produced with treated and untreated wastewater is not adequate. Therefore, a study was formulated to know the effects of wastewater both treated and untreated on essential oil yield and quality of lemongrass.

MATERIALS AND METHODS

Study site

The study was carried out in the experimental field having latitude of 28°38'21.3" N and longitude of 77°08'56.5" E at an elevation of 230 m above mean sea level located near to a sewage drain covering an area of 150 m^2 inside the ICAR-Indian Agricultural Research Institute (IARI) (Fig. 1).The climate of the study area is semi-arid monsoonal subtropical type. The mean annual temperature (°C) of the study area is 24°C and the mean annual rainfall is 769 mm.

Experimental wastewater treatment framework

The treatment system consisted of wetland in form of 18-treatment cells (or mesocosms) using 500 litre syntax tanks with vertical sub-surface flow (VSSF). Each mesocosm had a 0.20 m base layer which was made of fine gravel with 38% porosity and having a diameter of around 1.5cm overlain by a center layer 0.30 m high consisting of coarse sand. All the mesocosms were painted white to avoid excessive heating. The mesocosms were connected to the main (sewage water) influent discharge line maintaining the maximum hydraulic water head of 16.3 cm. The untreated wastewater was made to pass through a screen and was then collected in a sump (2.36 x 0.68 x 0.762 m^3) before pumping it into the individual mesocosms.

Only 12-mesocosms were then used for planting four replicates of three emergent macrophytes *viz*. *Typha latifolia* (Cattail), *Phragmites karka* (Reed), *Acorus calamus* (Vachh). The remaining 6-mesocosms were left un-vegetated. Then before the commencement of the experiment, plants in the vegetated mesocosms were allowed to grow and multiply with periodic application of wastewater as a source of nutrients in order to form a dense stand.

Intermittent flooding was done in all mesocosms up to a maximum depth of 16.3 cm with the wastewater during the experimental period (August 2018 to April 2019), thrice in a month. The treated effluent from each mesocosm was collected and stored treatment wise in 500 litre capacity tanks.

Micro-plot experimental field

The experimental field consisted of 36 micro-plots, each of size 1.8 × 1.5m. Root slips of lemon grass (*Cymbopogon flexuosus*)- var. Krishna were transplanted (15 slips per plot) at a spacing of 60 x 30cm on 6th September 2017. The crop was established for first year with uniform inputs using groundwater for irrigation. From September 2018 onwards, the crop was fertilized and irrigated as per treatment. Split plot design was used for experiment with three replications. A total number of 12 treatments were given which consisted of combinations of; (A) Different types of irrigation water in main-plots, *viz.* (i) groundwater (GW), (ii) untreated wastewater (WW), (iii) constructed wetland treated wastewater (TWW) and (iv) groundwater in conjunction with untreated wastewater in cyclic mode (CW) (B) three levels of fertilizers in subplots *viz.* (i) N_0: control (no NPK fertilizer application) (ii) N_1: It is the amount of N, P and K fertilizers added after subtracting the present amount of N, P and K in wastewater used for irrigation from the recommended doses of N, P and K (iii) N_2: It is the amount of N, P and K fertilizers added as per the recommended doses. Groundwater, treated wastewater and untreated wastewater used for irrigation were marginally saline and neutral in reaction. The N, P, K contents in untreated wastewater were significantly higher

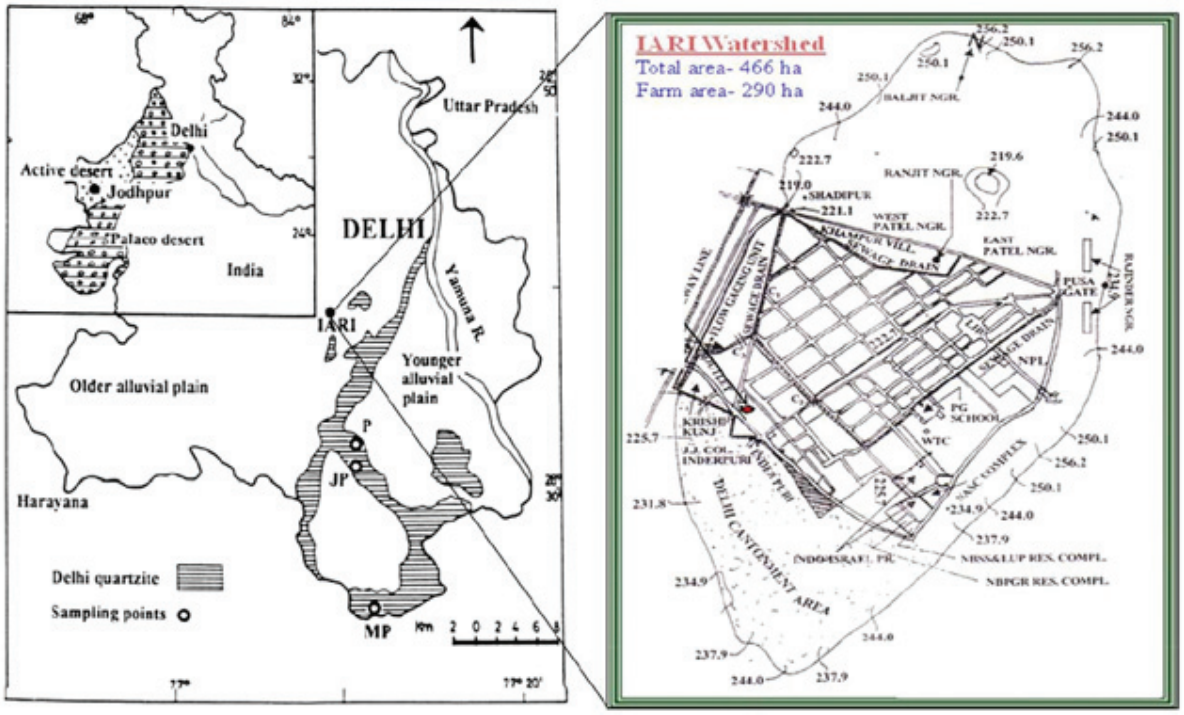

Fig.1 : Project site located within IARI micro-watershed

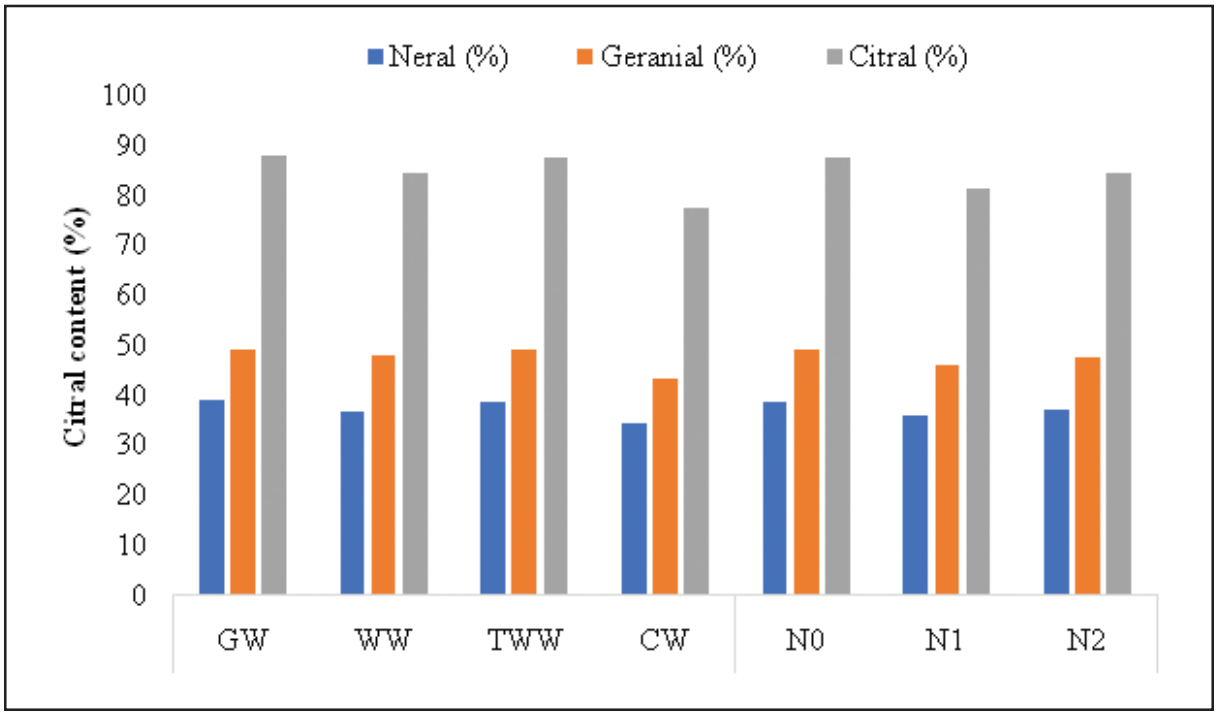

Fig. 2: Citral content (%) in lemongrass essential oil

GW: Groundwater, WW: Untreated wastewater, TWW: Treated wastewater, CW: Conjunctive water, N_0: Control, N_1: Adjusted doses of NPK fertilizers, N_2: Recommended dose of NPK fertilizers

and 2.3, 3.7 and 2.7 times more than the constructed wetland treated water (7.5, 1.06 and 5.4 mg L^{-1}), respectively. The fertilizers N, P_2O_5 and K_2O had recommended doses of 150, 60 and 60 kg ha^{-1}. One fourth of N and total P and K were applied as basal dose while the rest of N was top-dressed in three equal splits at 30 and 60 and 90 days after imposition of the treatments. Considering the irrigation frequency of once in 20 days, total 10 irrigations were applied from September to April.

The depth of irrigation was kept 5 cm which required 135 litres of water per irrigation in each subplot. For application of treated wastewater (TWW), 34 litres of treated wastewater obtained separately from mesocosms planted with *Typha latifolia* (Cattail), *Phragmites karka* (Reed), *Acorus calamus* (Vachh) and without vegetation were collected, mixed and applied with the help of 20 litre capacity buckets. In case of conjunctive wastewater irrigation, groundwater and untreated wastewater were applied alternately in a cyclic mode beginning with groundwater irrigation. Hand weeding was also carried out intermittently during the research duration.

Herbage and essential oil yield

During the study period, from each plot the crop was harvested about 10 cm above the ground three times on 15th November 2018, 26th February 2019 and 29th April 2019. The plot wise harvested biomass was weighed and converted into herbage yield per hectare.

To determine essential oil content, a known weight of fresh herbs W_1 (about 250 g) was taken and subjected to steam distillation using Clevenger apparatus and recorded the volume of oil (ml) from the Clevenger burette. The essential oil content (%) on fresh herbage basis was found as given below (equation 1):

$$Oil\ content\ (\%) = \frac{Volume\ of\ oil\ (ml)}{W_1\ (g)} \times 100 \quad ..(1)$$

The cut wise essential oil yield was calculated as follows (equation 2):

$$Oil\ yield\ (litre\ ha^{-1}) = \frac{Volume\ of\ oil\ (ml) \times 1000 \times herbage\ yield\ (Mg\ ha^{-1})}{W_1\ (g)} \quad ....(2)$$

The cut wise essential oil yields obtained were summed to find essential oil yields per hectare from all the three cuts.

Composition of essential oil

Atomic absorption spectrophotometer was used for the analysis of Zn, Cu, Fe, Mn, Cr, Ni, Pb and Cd contents in the lemongrass essential oil samples. The essential oil collected in the vial from the Clevenger burette was added with a pinch of sodium sulphate to remove water from the oil if any. Further the oil was filtered and the samples were diluted by hexane (5 micro Litres oil in 2 mL hexane) for composition analysis by using Gas Chromatography - Mass Spectrometry (GC-MS). A HP-5MS column (30 m × 0.25 mm;/0.25 m, Thermo Co., USA) was used for GC-MS analysis, which was directly connected to a triple axis mass spectrometer (Thermo Fisher, USA). The injection volume was 1 micro litre with flow mode in split control. Helium (High purity, New Delhi, India) was used as carrier gas at a head pressure of 10 psi and gas flow was set at 1 ml min^{-1}. Below describes the GC-MS condition: Initially, the oven temperature was held at 40°C for 1 minute. Thereafter, the temperature was allowed to rise at a rate of 3°C min^{-1} until it reached to 120°C and was held for 2 minutes. The temperature was again increased at a rate of 5°C min^{-1} up to 220°C and was held for 1 minute. Then finally, the temperature was raised to 280°C at a rate of 4°C min^{-1}. Total time taken was 65 minutes. The MS acquisition parameters were as follows: ion source 180°C, electron ionization 70 eV, full scan mode (50–550 mass units), transfer line temperature 280°C, solvent delay 3 min, and E.M voltage 1376. The ionization energy was 70 eV with a scan time of 1 s and mass range of 50–550 AMU. Compounds were identified by matching their mass spectra. NIST (National Institute of Standards and Technologies) Mass Spectra Library was used as a reference for identifying the essential components.

Statistical analysis

Each parameter data was subjected to a two-way ANOVA analysis (Gomez and Gomez, 1984) with separation of means. All tests of significance were performed at the level of 5% probability.

RESULTS AND DISCUSSION

Herbage yield

The herbage yield of lemongrass was found to be varying from 20.61 to 24.75 Mg ha^{-1} in first cut, 18.27-21.96 Mg ha^{-1} in second cut and 15.48-17.84 Mg ha^{-1} in third cut (Table 1). The total herbage yield achieved from all the three cuts was the maximum (63.45 Mg ha^{-1}) when irrigated with untreated wastewater followed by conjunctive use of wastewater (59.85 Mg ha^{-1}), treated wastewater (58.95 Mg ha^{-1}) and the minimum in case of groundwater irrigation (55.26 Mg ha^{-1}). Compared to groundwater, irrigation with untreated wastewater resulted in a significant increase of 15% in the crop biomass. Soil irrigated with treated wastewater or alternatively with sewage and groundwater yielded statistically at par with untreated wastewater or groundwater irrigation.

Similarly, use of recommended or adjusted doses of N, P and K produced significantly higher total and cut wise crop biomass compared to control (no fertilizer application). The difference in crop biomass obtained with the application of recommended fertilizer doses and doses adjusted due to supply of nutrient from irrigation was not significant.

Content, yield and quality of lemongrass essential oil

The essential oil content and yield obtained from the fresh biomass of lemongrass in three harvesting are presented in Table 2 and 3. In case of first cut, oil content obtained by the herbage harvested from the differentially irrigated plots ranged from 0.75 to 0.81 % with the mean values varying from 0.77 to 0.78% only. Similar values of essential oil content were also recorded in case of second and third harvests. No significant differences were found in the oil content obtained from the differentially irrigated plots in all three cuts. Also, there were no significant differences noted in the oil content of all three cuttings with different nutrient dose treatments.

Mean values of essential oil yield obtained in the first cut varied from 184 to 212 L ha^{-1}. The maximum oil yield was obtained from crop growing in wastewater irrigated plots and the minimum in case of groundwater usage. Oil yield obtained from the crop irrigated with wastewater was 15% higher than that obtained with ground water irrigation. In case of second cut, essential oil yield ranged from 157 to 179 L ha^{-1}. Similar to the first cut, in second cut also untreated wastewater irrigation produced the maximum essential oil yield whereas the minimum (156.84 L ha^{-1}) in case of groundwater irrigation. In second cut, essential oil yield obtained with untreated wastewater irrigation was found significantly higher compared to groundwater and treated wastewater irrigation but at par with conjunctive wastewater use. Similar trend was observed in third cut also.

The total oil yield obtained from three harvests was the maximum (546 L ha^{-1}) in case of wastewater irrigation and the minimum in case of groundwater (476 L ha^{-1}). Irrigation with untreated wastewater resulted in significant increases of 11 and 16% in essential oil yield compared to treated wastewater and groundwater irrigation, respectively. Compared to no fertilizer application, application of recommended or adjusted dose of fertilizers resulted in significantly higher production of cut wise and total essential oil yield. No interaction effects in the herbage and oil yield were observed amongst all the treatments of the experimental study.

For testing the quality of lemongrass essential oil, the diluted samples were aspirated in atomic absorption spectrophotometer for the estimation of Zn, Cu, Mn, Fe, Cd, Cr, Ni and Pb. The results showed absence of heavy metals in the essential oil of lemongrass. Component analysis of the lemongrass oil by GC-MS resulted in a number of chemical compounds (Premathilake *et al*., 2018 and Matasyoh *et al*., 2007). Citral or 3, 7-dimethyl-2,6-octadienal (both E- and Z-isomer) was the major compound in the lemongrass oil.

Citral has two isomers- the E-isomer which is also known as geranial or citral A and the Z-isomer, which is also known as neral or citral B. It was observed that the maximum neral content was present in the oil extracted from the plants irrigated with groundwater and the minimum was found with conjunctive use of water (Fig. 2). The neral content obtained from groundwater usage and alternative irrigations with untreated sewage and groundwater was 14% higher than that of groundwater usage. The neral content obtained from groundwater and treated wastewater were almost the same. Geranial content in the essential oil obtained from groundwater, treated wastewater and untreated

Table 1: Herbage yield (Mg ha^{-1}) obtained from three cuts and their total from the plots irrigated with different types of irrigation water and nutrient levels.

Water quality	Herbage yield (Mg ha^{-1}))			
	First cut	Second cut	Third cut	Total
GW	20.6	18.3	15.5	55.2
WW	24.7	21.9	17.8	63.4
TWW	22.1	19.7	15.8	58.9
CW	22.8	20.2	17.2	59.8
CD (5%)	2.7	2.2	1.6	5.1
Nutrient Doses				
N_0	20.4	18.4	14.9	54.8
N_1	23.0	20.2	17.2	60.3
N_2	24.3	21.4	17.5	63.8
CD (5%)	1.98	1.35	1.35	2.79

GW: Groundwater, WW: Untreated wastewater, TWW: Treated wastewater, CW: Conjunctive water, N_0: Control, N_1: Adjusted doses of NPK fertilizers, N_2: Recommended dose of NPK fertilizers. Mg ha^{-1}: It is Megagram per hectare. Megagram is equal to 10^3 kg.

Table 2: Oil content (%) in above ground biomass of lemongrass

Water quality	Oil content (%)		
	First cut	Second cut	Third cut
GW	0.78	0.76	0.78
WW	0.78	0.75	0.78
TWW	0.78	0.72	0.77
CW	0.77	0.76	0.77
CD (5%)	NS	NS	NS
Nutrient Doses			
N_0	0.78	0.75	0.78
N_1	0.77	0.74	0.78
N_2	0.78	0.75	0.78
CD (5%)	NS	NS	NS

GW: Groundwater, WW: Untreated wastewater, TWW: Treated wastewater, CW: Conjunctive water, N_0: Control, N_1: Adjusted doses of NPK fertilizers, N_2: Recommended dose of NPK fertilizers

wastewater usage were more or less the same but 11 to 13% higher than in the cyclic mode of groundwater and untreated wastewater usage. Similarly, in case of nutrient treatments, maximum neral and geranial content was obtained with control followed by recommended doses and then N_1.

Citral (neral + geranial) content also followed the similar trend and was found the maximum in the oil extracted from plants irrigated with groundwater and from N_0 in case of nutrient dose treatments.

The changes in essential oil contents with changing quality of irrigation water were non-significant. Significant improvement in essential oil yield were mainly ascribed to the increase in herbage yield produced with untreated wastewater irrigation either alone or in conjunction with ground water. Increased soil organic carbon and improved soil fertility with the addition of substantial amount of essential plant nutrients supplied through sole or conjunctive use of untreated wastewater irrigation could have produced higher crop yields compared to ground water irrigation (Lopez *et al*., 2006; Lal *et al*., 2013). Compared to the groundwater irrigation an increase of 14-15% in total essential oil yield of lemongrass with sole use

Table 3: Oil yield (L ha^{-1}) in above ground biomass of lemongrass

Water quality	Oil yield (L ha^{-1})			
	First cut	Second cut	Third cut	Total
GW	184	157	135	476
WW	212	179	155	546
TWW	194	158	142	494
CW	193	171	145	508
CD (5%)	18	17	13	47
Nutrient Doses				
N_0	183	153	131	468
N_1	196	169	147	511
N_2	208	176	155	539
CD (5%)	14	11	12	29

GW: Groundwater, WW: Untreated wastewater, TWW: Treated wastewater, CW: Conjunctive water, N_0: Control, N_1: Adjusted doses of NPK fertilizers, N_2: Recommended dose of NPK fertilizers

of wastewater or in conjunctive mode with groundwater was also noticed by Lal *et al*. (2013). Higher yields of lemongrass essential oil with wastewater were also recorded by other workers (Singh, 1998 and Darvishi *et al*., 2010). Compared to sole use of untreated wastewater, half amount of essential plant nutrients was supplied through wastewater irrigation in case of conjunctive use of wastewater. Non-significant difference in herbage yield of lemongrass irrigated solely with wastewater or in combination with groundwater indicated that amount of essential plant nutrients added were sufficient enough for meeting crop requirement (Anwar *et al*., 2010). Nutrients contained in wastewater get recycled when used for irrigation, thus not only saving fertilizers but also improving soil fertility. Sum of amount of nutrients supplied through irrigation and adjusted amount of nutrient added through fertilizer equals to the recommended nutrient doses, which would have been sufficient enough to meet the crop nutrient requirement.Therefore, the differences in yield obtained with adjusted and recommended nutrient doses were not found significant. Kumar *et al*. (2017) also observed that application of 100 % recommended dose of nitrogen along with groundwater irrigation produced herb and oil yield of mentha statistically at par with that obtained by applying 50% recommended dose of nitrogen under wastewater irrigation. Nutrients supplied through irrigation along with application of recommended nutrient doses through fertilizer would have resulted in overdoses of nutrients. Overdoses of nutrients added did not result in significant increase in plant nutrient content or not utilized for photosynthate formation. Simultaneous accumulations of pollutants in soil can lead to environmental issues when overdosing of nutrients along with its low use efficiency takes place and later may lead to leaching in groundwater.

CONCLUSIONS

The overall experimental evidences show the potential of cultivating the aromatic lemongrass with untreated wastewater which will protect the fresh water reserves. The total oil yield from three harvests was the maximum in case of untreated wastewater irrigation and the minimum in case of groundwater. Irrigation with untreated wastewater resulted in significant increases of 11 and 16% in essential oil compared to treated wastewater and groundwater irrigation, respectively. Compared to no fertilizer application, application of recommended or adjusted dose of nutrients (recommended nutrient doses - doses supplied by wastewater) resulted in significantly higher total herbage and essential oil yield. Lemongrass essential oil mainly contained citral in which neral accounted for 34-39% and geranial 43-49%. The neral and geranial contents obtained from groundwater and treated wastewater irrigation were 11-14% higher compared to alternative irrigations with untreated sewage and groundwater.

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Qualitative analysis of various ginger (*Zingiber officinale* Rosc.) genotypes suitable in Nagaland

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ABSTRACT

Ginger is a perennial tropical herb, the economic part being the rhizome used mostly as a spice all over the world. In Nagaland, ginger is cultivated under rainfed condition and it is the main cash crop supporting the livelihood and improving the economic level among the ginger growers. The investigation was done to assess the performance of different ginger genotypes for qualitative parameters at experimental farm of School of Agricultural Sciences and Rural Development, Medziphema campus, Nagaland University during the year 2021-2022. Seventeen genotypes were collected from different districts of Nagaland, namely Jalukie, Pherema, Medziphema, Chumoukedima and planted for the evaluation and qualitative performance of genotypes suitable in Nagaland condition. The genotype 'BGG-8' performed better for qualitative parameters such as oil content (2.83%), oleoresin content (6.59%) followed by 'CBG-1' having oil content (2.57%), oleoresin content (5.21%), fibre content (4.77%) and 'CBG-4' having oil content (2.47%), oleoresin content (4.83%).

***Keywords*:** Genotypes, ginger, performance**,** qualitative

INTRODUCTION

Ginger is valued as a medicinal crop and has been used as a spice for over 2000 years (Bartley and Jacobs, 2000). It has a significant role in our national economy. The scientific name of ginger is *Zingiber officinale* Roscoe belonging to the family Zingiberaceae under the order Zingiberales. Ginger possesses a refreshing aroma along with strong taste which makes it an important ingredient in the world food processing industry (Sarwar and Butt, 2016). Dry gingers are used in the manufacturing of non-alcoholic beverages and food products (Afzal *et al.,* 2001). The ginger rhizome is one of the most common constituents of diets worldwide and is reported to possess antioxidants, anti-inflammatory, antiseptic and carminative properties. (Sekiwa *et al*., 2000). The aroma of ginger is due to the presence of more than 70 constituents present in the volatile oil of rhizome. The aroma and flavor of fresh ginger will be different from dry ginger because some of the volatile oils are lost by evaporation during drying (Hazarika and Kakoti, 2013). India accounts for 30% production of ginger in the global market (Sial and Tarai, 2017).

Many ginger cultivars are grown in North Eastern region. In India 70% of the total ginger production is from the North-East. Ginger cultivars grown in North-East Region vary from each other in quality, quantity and productivity. In India, the North Eastern hills (NEH) region accounts for 49% of the area for ginger and 72% for production (Rahman *et al.,* 2009). Nagaland falls under sub-tropical and temperate climate where farmers cultivate ginger and use their own seed stock and as such they were not able to specify the variety they have grown and they named their cultivar according to their locality. The cultivars are Ungma, Yisimyong, Chanki from Mokokchang District; Longkhim, Shamtor from Tuensang District; Kedima, Tsemenyu, Jalukie from Kohima District (Kanjilal *et al.*, 1997). The objective of this experiment was to analyse the quality of ginger genotypes grown suitably in Nagaland.

MATERIALS AND METHODS

The field experiment was carried out at the Horticultural Farm of School of Agricultural Sciences and Rural Development (SASRD), Medziphema, Nagaland University during April 2021 to March 2022. The site is located at an altitude of 310 m above mean sea level with 20°45'43"N latitude and 95°53'04"East longitude. The climatic condition of the experimental site is sub-tropical, high humid and moderate temperature about 12 ° C to 32° C. The area receive medium to high rainfall (2000-3000mm) with relative humidity of 70% to 80%. The experimental plot soil was sandy loam and well drained. A total of seventeen ginger genotypes were collected from Nagaland and assessed for its qualitative traits**.** The experiment was laid out in Randomized Block Design (RBD) with seventeen treatments of ginger genotypes replicated three times. The healthy seed rhizomes weighing around 25-30 g were planted on a raised bed of size 1 m x 2 m at a spacing of 30 cm x 25 cm between rows and in between plants at a depth of 5-7 cm with the bud facing upward in the first week of April, 2021. Five plants in each plot were selected randomly and they were tagged for taking data on the qualitative traits viz. oil content, fibre content, oleoresin content and dry recovery. The rhizomes were carefully harvested with the help of khurpi and spade to minimize injury. The harvested rhizomes were kept separately for all plots and the tagged rhizomes were also kept separately for taking further observations**.** Fresh ginger oil was extracted by using modified Clevenger's method as mentioned in Official Methods of Analysis, Association of Official Analytical Chemists (A.O.A.C, 1976). 200 g of fresh ginger was ground using mortar and pestle. Then it was transferred to the apparatus and 500 ml of distilled water was added. It took 6 hours to extract the fresh ginger oil for each sample. Percentage of extracted oil was measured in the form of volatile oil (ml) per weight of sample used (g). The oil content percentage was calculated by volume of oil extracted in (ml) divided by the weight of sample used in (g) multiplied by 100. For determining the fibre content in rhizomes, Leibig's digestion apparatus was used. 2g of fine powdered ginger were put into a beaker and 200 ml of 1.25% H_2SO_4 was added. Then it was boiled for 30 minutes in the digestion bench. The contents of the beaker were then filtered using muslin cloth and the residue were washed thoroughly using distilled water till the pH neutralizes. After washing, the residue was transferred into the beaker and 200 ml of 1.25% NaOH was added. Again it was boiled for 30 minutes in the digestion bench and the contents were again filtered and washed. The residue was then transferred to the Gooch crucible which was already weighed and it was oven dried for 1 hour. Weight was taken after it was oven dried and the sample was kept in muffle furnace for 20 minutes to obtain the ash content. The crucible is cooled and the weight was taken. The fibre content was calculated by the loss in weight of sample divided by weight of sample taken multipled by 100 (George, 1961). Dry ginger oleoresin was extracted using "Soxhlet extraction" method. 10 g finely ground powdered ginger was put into titration pipette. 50 ml acetone was added and kept overnight. The extract was drained in the beaker which was already weighed after which 30 ml acetone was added and kept for 1hour. After that it was kept into the oven for 2 hours for the acetone to evaporate. The weighed was immediately taken after it was taken out of the oven. Percentage of non-volatile ether extract was calculated by the weight of non volatile extract (g) divided by the weight of sample taken for extraction (g) multiplied by 100 (Jensen, 2007). For estimating dry matter content in ginger, 100 g of freshly harvested cleaned ginger were weighed and were cut out into small slices and dried in the oven at 60° C for 24 hours. After drying the weighed was taken in weighing machine. The value was converted in percent dry recovery. Mean, range of variation, standard error of mean and critical difference for each qualitative characters were worked out by the method of analysis of variance using Randomized Block Design (Panse and Sukhatme,1967).

RESULTS AND DISCUSSION

The data in Table 1 and Figure 1 shows significant variations for oil content among the genotypes. The fresh ginger oil content varied between 1.33% to 2.83%. The highest percentage of oil content was recorded in BGG-8 (2.83%) followed by CBG-1 (2.57%) and CBG-4 (2.47%).The lowest oil content was recorded in

Table 1: Variability in quality attributes of different ginger genotypes

Genotypes	Oil content (%)	Fibre content (%)	Oleoresin content (%)	Dry recovery (%)
BGG-1	1.77	3.66	3.26	23.33
BGG-2	1.50	3.36	4.16	16.33
BGG-3	2.17	3.62	3.80	19.67
BGG-4	2.10	4.23	3.63	22.00
BGG-5	1.83	4.77	4.26	21.33
BGG-6	1.77	3.60	3.60	14.33
BGG-7	1.53	3.47	4.11	22.33
BGG-8	2.83	4.03	6.59	23.00
BGG-9	1.33	4.42	3.50	21.00
BGG-10	1.33	3.67	3.58	11.67
CBG-1	2.57	4.46	5.21	20.00
CBG-2	2.07	6.04	4.83	22.67
CBG-3	2.17	4.49	3.83	24.00
CBG-4	2.47	3.43	4.30	20.33
CBG-5	2.13	4.66	4.20	25.33
CBG-6	0.27	4.61	3.53	31.33
CBG-7	1.93	4.52	4.53	28.00
SEm±	**0.09**	**0.24**	**0.22**	**2.38**
CD(P=0.05)	**0.26**	**0.70**	**0.63**	**6.85**

BGG-9 (1.33%), BGG-10 (1.33%). The general mean for the oil content was 1.99±0.09. According to the findings of Gopalam and Ratnambal (1989) where they conducted a study to evaluate 9 ginger cultivars using gas chromatography for essential oil and found that the total essential oil yield ranged from 1.5 to 2.2%.

Table 1 and Figure 2 shows that there was a significant variation in the fibre content among the genotypes. The fibre content ranged between 3.36% - 6.04%. The highest fibre content was recorded in CBG-2 (6.04%) followed by BGG-5 (4.77%), CBG-5 (4.66%) and CBG-6 (4.61%). The lowest fibre content was recorded in BGG-2 (3.36%). The general mean for fibre content was 4.18±0.24. The findings were in conformity with Mohanty and Panda (1991) where they assessed high yielding mutant V1K1-3 ginger and observed that the fibre content ranged between 3.8% to 4.4% in the genotypes evaluated. Chandra and Govind (1999) also conducted a study on twenty-one indigenous and exotic genotypes under mid-hills of Meghalaya and recorded maximum fibre content in the genotype Khasi Local (7.6%).

Table 1 and Figure 3 shows that there was a notable difference in the oleoresin content among the seventeen ginger genotypes. The oleoresin content varied between 3.26% - 6.59%. The highest percentage of oleoresin content was recorded in BGG-8 (6.59%) followed by CBG-1 (5.21%), CBG-2 (4.83%). The lowest percentage was recorded in BGG-1 (3.26%). The general mean for the oleoresin content was 4.17±0.22. This was evident from the earlier results of Mohanty *et al.* (1990) where they conducted a study on ginger in with special reference to its varietal and cultural improvement and observe that the maximum oleoresin content was in the cultivar Suprabha was 8.9%. Sanwal *et al*. (2009) also conducted a study on eighteen ginger genotypes and found that the genotype Meghalaya Local contained the highest concentration of total gingerol.

Table 1 and Figure 4 shows that there was a significant difference in the dry recovery percentage among the ginger genotypes. The dry recovery percentage varied between 11.67% to 31.33%. The highest dry recovery rate was recorded in CBG-6 (31.33%) followed by CBG-7 (28.00%) and CBG-5 (25.33%). The lowest dry recovery percentage

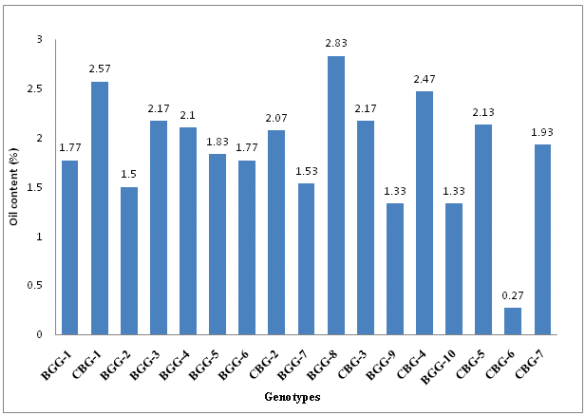

Fig. 1: Variability studies on ginger genotypes and landraces on oil content (%)

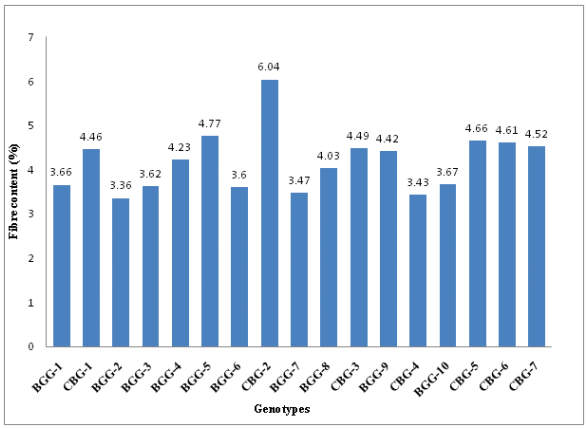

Fig. 2: Variability studies on ginger genotypes and landraces on fibre content (%)

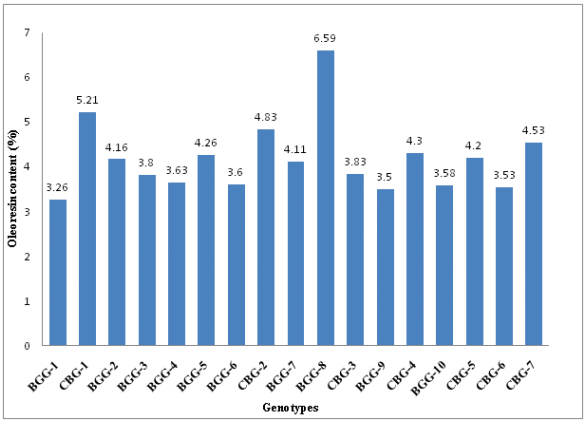

Fig. 3: Variability studies on ginger genotypes and landraces on oleoresin content (%)

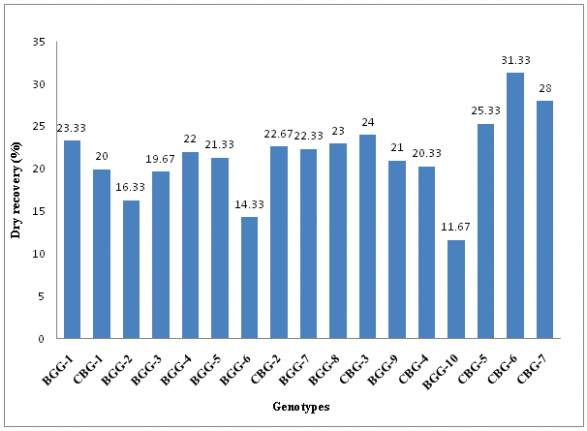

Fig. 4: Variability studies on ginger genotypes and landraces on dry recovery (%)

was recorded in BGG-10 (11.67%). The general mean for the dry recovery percentage was 21.57±2.38. The findings was at par with Bertila (2019) who observed that the dry recovery percentage ranged between 15.13% to 23.44% in the genotypes evaluated.

CONCLUSION

Among the seventeen ginger genotypes used for the evaluation, it was found that the genotype BGG-8 performed better for qualitative parameters such as oil content (2.83%), oleoresin content (9.50%) followed by CBG-1 having oil content (2.57%), oleoresin content (7.2%), fibre content (4.72%) and CBG-4 having oil content (2.47%), oleoresin content(6.50%). Genotypes BGG-8, CBG-1 and CBG-4 are suitable in Nagaland and can be recommended for growing for oil and oleoresin purpose.

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Effect of plant growth regulators on the performance of African marigold (*Tagetes erecta* L.) cv. Pusa Narangi Gainda

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ABSTRACT

Effect of time of application and plant growth regulators on the performance of African marigold (Tagetes erecta L.) cv. Pusa Narangi Gainda was investigated. During 2021–2022, a field experiment in RBD two factors with three replications was conducted at the Research Farm, Department of Horticulture, School of Agricultural Sciences and Rural Development (SASRD), Nagaland University, Medziphema.The eexperimental factors include two time of application such as (i) at 25 DAT, (ii) at 50 DAT, and seven sprays of PGRs such two doses of MH (1000 ppm and 2000 ppm), GA_3 (150 ppm and 250 ppm) and B-9 (50 ppm and 100 ppm) +and control. The results showed that the application of growth regulators at 25 days after transplanting (DAT) provided greater values for all of the vegetative and yield criteria. Amongst the plant growth regulators, it was found that spray of 250 ppm GA_3 increased plant height, plant spread, number of flowers per branch, number of flowers per plant, dry weight and fresh weight of single flower, dry weight and fresh weight of flower per plant and, flower yield per hectare except number of primary branches per plant and number of leaves per plant which was maximum with B-9 @ 100 ppm under study.

***Keywords*:** GA_3, growth regulators, *Tagetes erecta*, yield parameters

INTRODUCTION

There is a high demand of marigold for decorative purposes at different religious and social gatherings as loose flowers, garlands, and garden displays. In the United States, however, marigold powder and extract are the only approved colors in poultry feed. Yellow and orange color of marigold flower is due to the presence of lutein pigment hence marigold flower is added in poultry diet to increase the yellow color of egg yolks and broiler skin. Recently the marigold flower is the marketable source of lutein. Petals of marigold are luxuriant in esters of lutein fatty acids and lutein, representing more than 90% of the pigments identified in *Tagetes* plant (Becerraa *et al.,* 2020). Plant growth and yield are mostly affected by two main factors such as management and genetic factors. However, the plant growth is now regulated using PGRs and is considered as third furthermost important advanced technology to improve growth and flowering parameters in flowering plants (Kumar *et al.,* 2015). Plant growth regulators change plant physiological processes inside the plant that finally influenced plant growth and development. The main objectives of experiment were to see the effect of PGRs on the performance of *Tagetes erecta* cv. Pusa Narangi Gainda.

MATERIALS AND METHODS

The field trail was conducted during 2021-2022 at the instructional cum research farm of SASRD, Nagaland University (a central university), Medziphema, which is located at an altitude of 305 meters above sea level at latitude 25°45'43'N and longitude 93°53'04'E. The climatic conditions of the experimental site was typically a humid sub-tropical zone with an average temperature between 12.53^{0}C to 26.24^{0}C and average rainfall was 12.21 mm. Soil of trail site was sandy loam with a pH of 5.5, with organic carbon 1.5%, available NPK 298 kg ha^{-1}, 48.5 kg ha^{-1} and 90.4 kg ha^{-1}, respectively. A field trail with three replicates was performed in RBD two factors. Treatment consists of time of application (25 DAT and 50 DAT) and different PGRs (MH 1000 ppm and 2000 ppm, GA_3 150 ppm

and 250 ppm, B-9 50 ppm and 100 ppm) and was control. DAT mean days after transplanting of seedlings. Seeds of *Tagetes erecta* cv. Pusa Narangi Gainda was sown in flower pots on 12 November 2021. Soil mixture comprising of garden soil, sand and well-rotted farmyard manure in the ratio of 1:1:1 was mixed thoroughly and added into the pots, followed by sowing. Light irrigation was carried out immediately after sowing. 30-day-old plants were transplanted into the trail field. The size of the plot was kept at 1.6 m x 1.6 m and the spacing was kept at 40 cm x 40 cm. Besides the application of well-rotted farmyard manure @ 22 t/ha, fertilizers were used at a rate of 120 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5, and 100 kg ha^{-1} K_2O. Well-rotted farmyard manure was mixed well when preparing the bed. Nitrogen was applied in two divided doses i.e., 50 percent N at time of bed preparation and remaining 50 percent N was used at 45 DAT as top dressing in form of urea. Full dose of P_2O_5 and K_2O was used at the time of final bed preparation, P_2O_5 in form of SSP and K_2O in form of MOP. Good cultural practices were followed during the entire crop period. Observations on vegetative (at 60 DAT) and yield parameters were observed and analysed statistically using the analysis technique of variance with factorial RBD (Gomez and Gomez, 1985). The level of significance of t-test and F-test was kept at 5%. (P=0.05).

RESULTS AND DISCUSSION

Vegetative parameters

Plant height and plant spread

Time of application, and PGRs had a profound effect on the plant height, which is summarized in (Table 1); a higher plant height (49.57cm) was obtained when foliar sprays of PGRs at 25 DAT, followed by the application of PGRs at 50 DAT (47.55 cm). In case of PGRs, the data also showed that a highest plant height (59.43 cm) was noted with sprays of 250 ppm GA_3 (G_4) while, lowest (42.30 cm) plant height was noted with application of 2000 ppm MH (G_2). The interaction between time of application and PGRs was noted to be significant. Highest plant height (60.40 cm) was noted on application of 250 ppm GA_3 at 25 DAT while the lowest plant height (41.26 cm) was noted by spray of 2000 ppm MH at 50 DAT. The plant spread was not significantly influenced time of application, whereas PGRs significantly influenced as represented in (Table 1). With regards to time of application, maximum plant spread (50.98 cm) was registered when applied at 25 DAT whereas; least plant spread (49.73cm) was noted when application of PGRs at 50 DAT. A cursory glance of data revealed that greater plant spread (56.54cm) was noted with foliar application of 250 ppm GA_3 (G_4) however; least plant spread (44.57 cm) was registered with foliar sprays of 2000 ppm MH (G_2). The interaction amid time of application and PGRs also failed to markedly affect the plant spread of marigold. When the PGRs were applied at 25 DAT, growth and yield increased due to beneficial effect of PGRs and that was also noted by Kumar *et al.* (2020) in African marigold. The results indicated that application of PGRs at 25 days after planting showed maximum vegetative growth and yield. The GA_3 increased plant height because GA_3 persuaded the active cell division and cell elongation (Anuradha *et al.*, 2017)). Sheng *et al*. (2022) stated that gibberellins (GA_1,GA_3 and GA_4) regulates plant height especially GA_3 increased plant height. Comparable finding was stated by (Arti *et al*., 2019) in African marigold cv. Lemon Yellow. Comparable finding was stated by (Arti *et al*., 2019) in African marigold cv. Lemon Yellow.

Number of leaves per plant and primary branches per plant

Data showed that, the maximum number of leaves (133.60) were obtained when PGRs were applied at 25 DAT, whereas PGRs applied at 50 DAT noted the lowest number of leaves (132.56). Among the different PGRs, maximum number of leaves (138.63) was noted with spray of 100 ppm of B-9 (G_6) however, lowest number of leaves (127.87) was registered with control (G_0). Interaction effect of time of application and PGRs was significantly. A greater number of leaves (139.47) was noted by application of 100 ppm of B-9 (G_6) at 25 DAT, whilst; minimum number of leaves (127.67) was noted, with no application of PGRs (G_0) at 50 DAT (Table 1). Among the time of application, a greater number of primary branches per plant (11.51) were noted when applied at 25 DAT, whereas applying at 50 DAT noted minimum number of primary branches per plant (11.06). The maximum number of primary branches

Table 1: Effect of PGRs on the vegetative parameters of *Tagetes erecta*cv. Pusa Narangi Gainda

Treatments	Plant height (cm)	Plant spread (cm)	Number of leaves per plant	Number of primary branches per plant
T_1(25 DAT)	49.57	50.98	133.63	11.51
T_2(50 DAT)	47.55	49.73	132.58	11.06
SEm(±)	**0.44**	**0.49**	**0.20**	**0.10**
C.D at 5%	**1.29**	**NS**	**0.60**	**0.29**
G_0 (Control)	48.97	50.78	127.87	10.40
G_1(1000 ppm MH)	44.13	44.92	134.00	11.32
G_2(2000 ppm MH)	42.30	44.57	133.26	11.44
G_3 (150 ppm $GA_{3)}$	54.87	54.10	129.10	11.14
G_4(250 ppm $GA_{3)}$	59.43	56.54	132.38	11.20
G_5(50 ppm B-9)	45.60	50.20	136.50	11.52
G_6(100 ppm B-9)	44.63	51.35	138.63	11.96
SEm(±)	**0.62**	**0.26**	**0.11**	**0.05**
C.D at 5%	**0.69**	**0.77**	**0.32**	**0.15**
T_1G_0	49.46	51.68	128.07	10.60
T_1G_1	44.67	45.73	134.20	11.51
T_1G_2	43.33	45.10	133.60	11.61
T_1G_3	57.47	54.75	129.80	11.26
T_1G_4	60.40	57.33	132.56	11.37
T_1G_5	46.20	50.38	137.73	11.90
T_1G_6	45.47	51.86	139.47	12.29
T_2G_0	48.47	49.89	127.67	10.20
T_2G_1	43.60	44.11	133.80	11.12
T_2G_2	41.26	44.05	132.93	11.28
T_2G_3	52.26	53.45	128.40	11.02
T_2G_4	58.46	55.75	132.20	11.04
T_2G_5	45.00	50.01	135.27	11.13
T_2G_6	43.80	50.83	137.80	11.63
SEm (±)	**0.62**	**0.69**	**0.29**	**0.14**
C.D at 5%	**1.82**	**NS**	**0.84**	**NS**

T= Time of application, G= PGRs

per plant (11.96) was registered with foliar application of 100 ppm of B-9 (G_6) whereas; a lowest number of primary branches per plant (10.40) was registered in control (Table 1). Due to a decrease in stem growth and a rise in the number of branches per plant, B-9 and MH treatments produce a greater number of leaves and primary branches per plant. B-9 and MH are related to anti-auxins and perhaps also anti-gibberellins that have reduced the height of plant leading to an increase in the number of branches and the formation of a greater number of leaves. Comparable finding was noted by Mahalle *et al.* (2001) in *Chrysanthemum indicum*.

Yield parameters

Number of flowers per branch and flowers per plant

Analysis of data showed in Table 2 that time of application was insignificant, a greater number of flowers per branch (6.67) was noted when sprayed at 25 DAT and the minimum number of flowers per branch (6.49) was noted at 50 DAT. The different PGRs had a marked influence on the

Table 2: Effect of PGRs on the yield parameters of *Tagetes erecta* cv. Pusa Narangi Gainda

Treatments	Number of of flowers per branch	Number of flowers per plant	Fresh weight of a single flower (g)	Fresh weight of flower per plant (g)	Dry weight of a single flower (g)	Dry weight of flower per plant (g)	Estimated flower yield per hectare (q)
T_1(25 DAT)	6.67	76.44	7.62	592.37	1.18	93.12	511.96
T_2 (50 DAT)	6.49	72.08	7.43	545.70	1.06	79.42	471.34
SEm(±)	**0.06**	**1.60**	**0.08**	**12.09**	**0.08**	**5.44**	**10.37**
C.D at 5%	**NS**	**NS**	**NS**	**35.33**	**NS**	**NS**	**30.30**
G_0(Control)	5.22	54.24	6.26	339.87	0.75	40.76	294.58
G_1(1000ppm MH)	6.18	68.88	7.11	490.06	0.90	61.62	423.28
G_2(2000ppm MH)	6.03	67.65	7.20	495.25	0.93	63.12	427.76
G_3(150 ppm $GA_{3)}$	7.97	89.63	8.25	739.40	1.40	125.23	638.70
G_4(250 ppm $GA_{3)}$	8.11	92.15	9.24	851.61	1.56	143.78	735.60
G_5(50 ppm B-9)	6.35	73.16	7.27	527.56	1.08	79.50	455.66
G_6(100 ppm B-9)	6.20	74.10	7.31	539.49	1.21	89.87	465.98
SEm(±)	**0.03**	**0.85**	**0.11**	**6.46**	**0.04**	**2.91**	**5.54**
C.D at 5%	**0.10**	**2.49**	**NS**	**18.89**	**NS**	**8.50**	**16.19**
Interaction (TxG)							
T_1G_0	5.23	55.49	6.33	351.46	0.83	46.20	305.64
T_1G_1	6.23	70.16	7.16	502.33	0.96	67.50	433.90
T_1G_2	6.10	69.42	7.31	513.57	0.95	66.29	443.60
T_1G_3	8.10	91.74	8.40	770.28	1.47	134.43	665.36
T_1G_4	8.28	94.02	9.31	875.80	1.59	149.20	756.50
T_1G_5	6.5	77.34	7.38	565.60	1.20	92.239	488.53
T_1G_6	6.26	76.85	7.40	567.53	1.25	95.96	490.20
T_2G_0	5.20	52.97	6.20	328.27	0.67	35.36	283.52
T_2G_1	6.13	67.59	7.06	477.79	0.83	55.74	412.66
T_2G_2	5.97	65.88	7.10	476.92	0.91	59.94	411.93
T_2G_3	7.84	87.51	8.10	708.52	1.33	116.03	612.03
T_2G_4	7.94	90.28	9.16	827.42	1.53	138.35	714.70
T_2G_5	6.20	68.97	7.16	489.51	0.96	66.76	422.80
T_2G_6	6.13	71.35	7.23	511.45	1.16	83.77	441.76
SEm (±)	**0.03**	**2.26**	**0.12**	**17.10**	**0.11**	**7.69**	**14.66**
C.D at 5%	**0.10**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

T- Time of application, G- PGRs

number of flowers per branch, a greater number of flowers per branch (8.11) was registered with application of 250 ppm GA_3(G_4) while minimum number of flowers per branch (5.22) was noted with control (G_0). Interaction effect between time of application and PGRs was significantly registered. More number of flowers per branch (8.28) was noted by foliar application of 250 ppm GA_3 at 25 DAT while the minimum number of flowers per branch (5.20) was registered with control at 50 DAT (Table 2). Highest number of flowers per plant (76.44) was noted with application at 25 DAT and lowest number of flowers per plant (72.08) at 50 DAT. Maximum number of flowers per plant (92.15) was registered with spray of 250 ppm GA_3 (G_4) whereas, lesser number of flowers per plant (54.24) was noted by control (G_0). The interaction effect between the time of application and PGRs was shown significant difference for number of flowers per branch and failed to reach the level of significance for number of flowers per plant as per the data represented in (Table 2). A greater number

of flowers per branch by the application of GA_3 might be due to highest auxin activity in the floral buds (Sajid *et al.,* 2016). The gibberellins are well recognised for their promoter effects on cell division and cell elongation. B-9 is anti-auxin and possibly anti-gibberellin properties would have decreased flower size and numbers. (Murali *et al.*, 1988). Comparable findings were stated by Kanwar and Khandelwal, 2013; Kumar *et al*., 2014) in African marigold (*Tagetes erecta* L.). Increase in number of flowers per plant lends support from previous discussion on the flowering parameter such as number of flowers per branch. Comparable finding was stated by Sathappan (2018) in *Tagetes erecta*.

Fresh weight of a single flower and fresh weight of flower per plant

Time of application was found significant; the highest fresh weight of a single flower (7.62 g) was noted on spray of PGRs at 25 DAT than fresh weight of a single flower (7.43 g) obtained on spray of PGRs at 50 DAT. Further analysis of data also showed that maximum fresh weight of a single flower (9.24 g) was registered by application of 250 ppm GA_3 (G_4) and lowest fresh weight of a single flower (6.26 g) was noted by control (G_0). Interaction effect between time of application and the PGRs failed to produce a significant impact on fresh weight of flower (Table 2). Time of application was registered insignificant, with a greater fresh weight of flower per plant (592.37 g) was noted when sprayed at 25 DAT while lowest fresh weight of flower per plant (545.70 g) when PGRs were sprayed at 50 DAT. As regard to the influence of PGRs, determined fresh weight of flower per plant (851.61 g) was noted with foliar application of 250 ppm GA_3 (G_4). While, least fresh weight of flower per plant (339.87 g) was noted with control (G_0). Interaction effect between time of application and PGRs was insignificant (Table 2). The increment in fresh weight of a single flower with GA_3 as it improves the cell division and enlargement, enhancement of protein synthesis as well as a high dry matter accumulation Dalal *et al.* (2009). Increase in weight of single flower due to GA_3 application was also stated by Swaroop *et al.* (2007) in marigold. Comparable findings were also noted by Mishra (2017) in *Tagetes erecta*. The number of flowers per primary branch, the number of flowers per plant, and the fresh weight of a single flower are all factors that support the previous discussion of flowering parameters. Comparable conclusions were noted by Badge *et al*. (2015; Kalaimani *et al.* (2017) in *Tagetes erecta*.

Dry weight of a single flower and dry weight of flower per plant

A cursory glance of data revealed that time of application was not significant, highest dry weight of a single flower (1.18 g) was noted on a spray of plant growth regulators at 25 DAT compared to dry weight of a single flower (1.06 g) found on a spray of PGRs at 50 DAT. Among the different PGRs, maximum dry weight of a single flower (1.56 g) was noted on spray of 250 ppm GA_3 (G_4) while, least dry weight of a single flower (0.75 g) was registered on control (G_0). Interaction effect between time of application and the PGRs failed to exert any significant effect (Table 2). Among the time of application, highest dry weight of flower per plant (93.12 g) was noted with application at 25 DAT and lowest dry weight of flower per plant (79.42 g) at 50 DAT. Highest dry weight of flower per plant (143.78 g) noted on application of 250 ppm GA_3 (G_4) whereas, least dry weight of flower per plant (40.76 g) was recorded with control (G_0). Interaction effect between PGRs and time of application failed to exert any significant effect (Table 2). The maximum dry weight of a single flower of marigold with GA_3 application is associated with increased cell division and enlargement which promotes the synthesis of protein as well as high dry matter accumulation (Dalal *et al.,* 2009). Hence, the growth promoting compounds might have a positive effect on weight of flowers. The Comparable conclusions were observed by (Palei *et al.,* 2016; Narute *et al*., 2020) in *Tagetes erecta*. Increase in dry weight of flower per plant lends support from previous discussion on the flowering parameters viz., number of flowers per plant and weight of single flower. Comparable finding was stated by Palei *et al.* (2016) in *Tagetes erecta*.

Estimated flower yield per hectare

The effect of time of application was registered to be significant. The greatest estimated flower yield

per hectare (511.96 q) was noted by application at 25 DAT and minimum estimated flower yield per hectare (471.34 q) was recorded at 50 DAT. In case of PGRs, maximum estimated flower yield per hectare (735.60 q) was registered with spray of 250 ppm GA_3 (G_4) while, least estimated flower yield per hectare (294.58 q) was noted with control (G_0). The interaction effect between time of application and PGRs was non-significantly registered. This increase in flower yield per hectare is supported by prior data on the yield parameters, such as number of flowers per branch, number of flowers per plant, weight of single flower and weight of flower per plant. Comparable findings were stated by Arti *et al*. (2019) in *Tagetes erecta* L. cv. Lemon Yellow.

CONCLUSION

Out of the time of application, it can be inferred from the current research work that PGRs spraying at 25 DAT had a stronger impact on the vegetative and yield characteristics of of *Tagetes erecta* cv. Pusa Narangi Gainda. Among the PGRs, foliar spray with GA_3 @ 250 ppm was found to be the best treatment in most of the parameters of economic importance except number of leaves and number of primary branches, where B-9 @ 100 ppm exhibited better result. Since the above conclusions are made based on the result of a one-year investigation, a further study on a similar line would be required in order to give a more reliable recommendation.

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Beneficial microorganisms impacts rooting and the establishment of African marigold cuttings

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ABSTRACT

The investigation was done using bio-formulations containing beneficial microorganisms to assess the impact of beneficial microorganisms on the performance of African marigold cuttings during propagation and their establishment. Significant differences were observed for almost all parameters across different treatments. Observations have shown that the highest survival percentage (78.40%), number of branches per cutting (4.16), number of roots per cutting (34.46), the average length of roots per cutting (6.10 cm.), fresh weight of roots per cutting (0.25 g) and dry weight of root per cutting (0.11g) were recorded with the use of Trichoderma harzianum which gave the best results for all the parameters.

***Keywords*:** African marigold, beneficial microorganisms, cuttings.

INTRODUCTION

African marigold is a widely cultivated for its loose flower which isused in religious purposes and social gatherings. Marigold is one of the important commercial loose flower crops in India which ranks first in area and production among loose flowers (NHB, 2014-15). Karnataka, Tamil Nadu, Andhra Pradesh, West Bengal, and Maharashtra are significant marigold-producing states. They are rich sources of useful phytochemicals like terpenoids, flavonoids, carotenoids, and thiophenes. Marigold is the marketable source of lutein; petals of marigold are luxuriant in esters of lutein fatty acids and lutein, representing more than 90% of the pigments identified in Tagetes plant (Becerraa *et al*., 2020). Marigolds are typically multiplied by herbaceous tip cuttings and seeds. Propagation of African marigold *(Tegetes erecta* L.*)* through herbaceous tip cutting can give early flowering in addition to producing uniform and true to type plants. Beneficial microorganisms or plant growth promoting microorganisms (PGPMs) maintain key agroecological cycles fundamental for soil nutrient enrichment, crop nutrient improvement, plant tolerance to biotic and abiotic stresses, biocontrol of pests and diseases, and water uptake enhancement (Lobo *et al.,* 2019.The use of bio-formulations to boost crop output is currently given a lot of attention. The use of beneficial microbes in flower crops besides other horticultural crops, specially with respect to propagation and nursery raising is on the rise due to harmful effect of pesticides and chemical fertilizers on soil and environment. Considering the above points the investigation was carried out to study the impact of beneficial microorganisms on rooting and the establishment of African marigold cuttings.

MATERIALS AND METHODS

Experimental site

The experiment was conducted during 2021-2022 at the research cum instructional farm of School of Agricultural Sciences and Rural Development, Nagaland University, Medziphema, which is located at $25^0$45’43’’N latitude and $93^0$53’04’’ E longitude at an elevation of 305 m above mean sea level. The climatic condition of the experimental site is typically a sub-humid tropical region with high humidity and moderate

temperature (12-32°C), having high rainfall (2000-3000 mm) and RH of 70-80%. The mean temperature ranges from 21° C to 32° C during summer and rarely goes below 8°C during winter. The average rainfall varies between 200-250 cm from April to September and from October to March it remains virtually dry.

Experimental details

The experiment was laid out in a randomized block design with seven treatments and three replications. Treatment consists of water soaking (Control), PSB (Phosphate Solubilizing Bacteria), Jeevamrutha, microbial consortia (*Psuedomonas taiwanensis, Bacillus aryabhatai, Azotobacter tropicalis*), IEM (Indigenous Effective Micro-organisms), *Pseudomonas fluorescence* and *Trichoderma harzianum.* There were ten number of cuttings per replication. Jeevamritha and IEM were prepared in the department, whereas other bioformulations were procured from University of Agricultural Sciences, GKVK, Bengaluru, India.

Preparation of Jeevamrutha

Jeevamrutha is organic liquid manure which was prepared using the following ingredient: 1kg of cow dung and 1litre of cow urine was mixed properly with the help of wooden stick in a plastic drum. To this mixture 200 g jaggery, 200 g gram flour and 100 g forest soil was added and kept for 7 days for fermentation. These mixtures were stirred for about every 6-8 hours for better microbial growth.

Preparation of IEM (Indigenous Effective Micro-organisms)

Indigenous effective micro-organism was prepared in 4 steps by following standard procedure which Japanese farmers followed. Firstly, cooked rice was taken in a bamboo container and kept under a bamboo groove and covered with leaf litter. After 4 days microbial growth on this rice media was collected and named as IMO-1. IMO-1 was mixed with equal part of jaggery and kept for 2-3 days for microbial growth, which named as IMO-2. Then IMO-3 was prepared by mixing of IMO-2 with 1 part of soil, 2 part of rice bran, 1 part of jaggery and 1 part of bean cake and kept in an air tight container to encourage anaerobic microbial growth for about a week. Lastly, IMO-4 was prepared using 50 mg IMO-3 which was diluted in 200 ml of water.

Preparation of beneficial microorganisms solution

A 2% solution was made by dissolving 20 g of PSB/ microbial consortium / *Pseudomonas fluorescence / Trichoderma harzianum* in 1 litre of water. For Jeevamrutha/ IEM, 20 ml was mixed with 980 ml of water for preparation of 2% solution.

Preparation of cuttings

Herbaceous tip cuttings were prepared from matured mother plant about 5 cm long, which had three to four buds.

Rooting of cuttings

Prior to planting, the cuttings were treated with the solution containing beneficial microorganisms for 60 minutes. Treated cuttings were planted in the sand with polythene bags and the medium around the cuttings was pressed firmly. In a single polythene bag, a single cutting was planted. For better rooting, polythene bags were kept in the polyhouse.

Observations and data analysis

The data recorded were tabulated and subjected to statistical analysis by following the standard ANOVA method with a 5% level of significance described by Gomez and Gomez (2012).

RESULTS AND DISCUSSION

Survival of cutting (%)

The data pertaining to survival percentage of cutting is presented in Table 1. The survival percentage was significantly influenced by the effect of different treatments. Survival percentage varied from 53.50% to 78.40%. Among the treatments, *Trichoderma harzianum* showed the best results (78.40%) for the final survival percentage followed by microbial consortia and *Pseudomonas fluorescence* which recorded a value of 76.40% and 70.40% respectively. Similarly, the minimum survival percentage (53.50%) was recorded in control. *Trichoderma* kills several major harmful root fungi like *Pythium, Rhizoctonia, Fusarium*, and results in healthy root development by decreasing the attack of soil-born pathogens (Woo *et al*., 2014), which in turn results in increased survival of the cuttings. The results are in agreement with the earlier findings of Patil *et al.* (2001) in pomegranate.

Table 1: Impact of beneficial microorganisms on survival percentage, number of branches and root per cutting

Treatments	Survival percentage (%)	Number of branches per cutting	Number of root per cutting
T_1	53.50	2.56	15.50
T_2	63.40	2.63	27.56
T_3	67.40	2.66	28.40
T_4	76.40	3.16	32.43
T_5	67.50	2.76	29.50
T_6	70.40	2.95	30.40
T_7	78.40	4.16	34.46
SEm (±)	**0.07**	**0.09**	**0.06**
CD (5%)	**0.22**	**0.29**	**0.20**

Table 2: Impact of beneficial microorganisms on the average length of roots, fresh weight and dry weight of roots per cutting

Treatments	Average length of roots per cutting (cm)	Fresh weight of roots per cutting (g)	Dry weight of roots per cutting (g)
T_1	3.20	0.16	0.06
T_2	3.50	0.17	0.09
T_3	3.50	0.17	0.10
T_4	5.50	0.19	0.10
T_5	4.10	0.18	0.10
T_6	4.50	0.24	0.10
T_7	6.10	0.25	0.11
SEm (±)	**0.03**	**0.06**	**0.06**
CD (5%)	**0.12**	**0.19**	**0.19**

Number of branches per cutting

A perusal of the data given in Table1 revealed that the number of branches per cutting was significantly influenced by the effect of treatments. Number of branches per cutting varied from 2.56 to 4.16. Among the treatments studied, *Trichoderma harzianum* showed the best results (4.16) in terms of the number of branches per cutting followed by microbial consortia which recorded the value (3.16). However, the minimum number of branches per cutting (2.56) was recorded with control. The increased number of branches might be due to plant growth regulators like IAA and cytokinins which were released by *T. harzianum* resulting in the breaking of apical dominance and accelerating production of higher number of branches. These results are in agreement with the reports of Karishma *et al*. (2011) in chrysanthemum, Sunitha and Hunje (2010) in African marigold and Harshavardhan *et al.* (2017) in carnation.

Number of roots per cutting

The results presented in Table 1 shown a significant effect of the treatments on the number of roots per cutting. The number of roots per cutting varied from 15.50 to 34.46. Among the treatments studied, *Trichoderma harzianum* recorded the maximum number of roots per cutting (34.46) followed by microbial consortia which recorded a value of 32.4. However, the minimum number of roots per cutting (15.50) was recorded with control. The increased number of roots might be due to the increased synthesis of growth promoting substances associated with treatment of *Trichoderma harzianum*.

Average length of roots per cutting

The length of root per cutting was significantly influenced by the effect of treatments as shown in Table 2. It varied from 3.20 cm to 6.10 cm. Among the treatments studied, *Trichoderma harzianum* recorded the maximum length of roots per cutting (6.10 cm.) followed by microbial consortia which recorded the value (3.10cm). However, the minimum length of roots per cutting (3.20cm) was recorded with control. The faster rate of cell division in the root tips brought on by the use of *T. harzianum* may be the cause of the increased root growth. These findings were also reported by Brandler *et al*. (2017) in gerbera, Nosir (2016) in tuberose, Bhargava *et al*. (2015) in antirrhinum. *Trichoderma* spp have the ability to increase soil and nutrient accessibility for the roots, increase the solubility of insoluble substances, and increase the availability of micronutrients, all while promoting growth and yield (Li *et al*., 2015).

Fresh weight of roots per cutting

The effect of treatments on the fresh weight of roots was statistically significant. The fresh weight of the root per cutting was significantly influenced by the effect of treatments. Fresh weight of the root per cutting varied from 0.16 g to 0.25 g. Among the treatments studied, treatment with *Trichoderma harzianum* showed the best results (0.25 g) and it was statistically superior to other treatments while the minimum fresh weight of roots per cutting (0.16g) was recorded with control.

Dry weight of roots per cutting (g)

It is clear from the data presented in Table 2 that the dry weight of root per cutting was significantly influenced by the effect of treatments. The dry weight of the root per cutting varied from 0.06 g to 0.11 g. Among the treatments studied, treatment with *Trichoderma harzianum* showed the best results (0.11 g) and it was statistically superior to other treatments. However, the minimum dry weight of roots per cutting (0.06 g) was recorded with control. Increased fresh root weight brought on by *Trichoderma harzianum* can be attributed to longer shoot lengths with longer internodes and a maximum number of foliage, which in turn are responsible for increased photosynthesis and its subsequent transfer to the root system. This mechanism of enhanced fresh weight may be due to the production of growth-regulating substances by *Trichoderma harzianum*.

CONCLUSION

Based on the above findings, it can be concluded that *Trichoderma harzianum* could be recommended for treating cuttings of African marigold for better rooting, establishment and production of more number of healthy seedlings as it showed best results across all metrics like survival percentage (%), number of branches per cutting, number of roots per cutting, average root length (cm), fresh weight of root per cutting (g), and dry weight of root per cutting (g).

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Effect of different planting materials and chemicals on the fruit quality of Pomegranate (*Punica granatum* L.) cv. Bhagwa

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ABSTRACT

The quality parameters like total aril weight, percentage of aril weight, 100 aril weight, TSS, titrable acidity and anthocyanin content were seen in plants grown from tissue culture plants, followed, in that order, by grafts and air layers. Soil drenching with paclobutrazol (0.375 g of the active ingredient per metre of canopy diameter), foliar spray of nitrobenzene (2.0 ml/l) 60 days following the measures to induce stress and methyl jasmonate were the most effective treatments to increase the total aril weight (108.57 g, 122.73 g), percentage of aril weight (57.06%, 57.42%), 100 aril weight (28.77g, 32.86g), TSS (19.54 ^{0}B, 15.01 ^{0}B), titrable acidity (0.44%, 0.61%) and anthocyanin content (3.59 mg / 100g, 4.62 mg / 100 g) in ambe bahar and hastha bahar seasons. Soil drenching of paclobutrazol @ 0.365 g a.i. m^{-1} canopy diameter, foliar spray of nitrobenzene @ 2.0 ml litre^{-1} and methyl jasmonate @ 200 ppm litre^{-1} were most effective in improving fruit quality and anthocyanin content.

***Keywords*:** Methyl jasmonate, nitrobenzene, paclobutrazol, planting material, *Punica granatum*

INTRODUCTION

The pomegranate (*Punica granatum* L.), which is thought to be the oldest species of tree with edible fruit, is a significant fruit crop in terms of nutrition (Asghari *et al*. 2019). Pomegranates are becoming more and more common in arid and semi-arid areas because they are resilient, adaptable to a variety of climates, low care, produce well, and have a favourable cost:benefit ratio (Marathe *et al*., 2016). Pomegranate will withstand drought, salinity and able to grow in low fertile soils. This, combined with its perennial habit and some degree of salinity tolerance, makes pomegranate particularly suitable for better exploitation of soil moisture and marginal lands. Also, drier environments have superior fruit quality. The fruit is widely preferred by consumers and is in high demand even outside of the production regions. Pomegranate production in India is the highest in the world (Jadhav and Sharma, 2007). It is produced annually in 4.5 million tonnes on an area of 0.31 million hectares (National Horticultural Board, 2022).

Hardwood cuttings, air layering, grafting (grafted on 'Daru' rootstock) and tissue culture are all methods used to grow pomegranates. Tissue culture plantlets are frequently used to start orchards. It has not yet been thoroughly investigated how to get good quality fruits from plants produced from grafts and tissue culture plants.

When fruits are plucked too early, they may not ripen correctly and lose their flavour. Because it is a "non-climacteric" fruit crop, pomegranate fruits should be harvested as soon as they are fully developed on the plant itself (Pal and Babu, 2014; Gaikwad *et al*., 2014). Furthermore, it is preferable to avoid picking fruits too late because it reduces their shelf life and increases the prevalence of physiological disorders like Internal Breakdown. Because of this, it's essential to select pomegranate fruits at the right stage of development to guarantee exceptional quality and a longer shelf life. Although a great deal of research has been done on how to use chemicals like naphthalene acetic acid, gibberellic acid, salicylic acid, and ethrel to improve

quality with superior-quality fruits in pomegranate, the potential of a few other PGRs like methyl jasmonate, nitrobenzene, and paclobutrazol to obtain similar benefits in pomegranate and other fruit crops has not yet been fully explored. In light of this, the current study was conducted with the goal of better understanding how chemicals and plants raised through different propagation methods affect the quality of pomegranate "Bhagwa."

MATERIALS AND METHODS

The studies were carried out at the experimental farm of the Indian Institute of Horticultural Research in Hessaraghatta, Bengaluru, which is situated at 13°7 N and 77°29 E. The location is 890 metres above mean sea level. Pomegranate plants (cv. "Bhagwa") cultivated at a spacing of 5.0 m × 6.0 m and raised from tissue-cultured plantlets, grafts, and air layers made up the experimental material. The plants were five years old. The experiment was conducted during *ambe bahar* (January to February flowering) and *hastha bahar* (September to October flowering) during 2020-2021. Average maximum and minimum temperatures during the experimental period were 36.08°C and 25.43°C, respectively, with relative humidity and rainfall totalling 85.04% and 79.95 mm, respectively. The chemicals imposed were T_1: Nitrobenzene @ 1.0 ml / litre, T_2: Nitrobenzene @ 1.5 ml / litre, T_3: Nitrobenzene @ 2.0 ml / litre, T_4: Methyl Jasmonate (MeJA) @ 100 ppm, T_5: Methyl Jasmonate (MeJA) @ 150 ppm, T_6: Methyl Jasmonate (MeJA) @ 200 ppm, T_7: Soil drenching of paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 30 days after bahar treatment, T_8: Soil drenching of paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 45 days after bahar treatment, T_9: Soil drenching of paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment, T_{10}: Control (Water spray). The following regimen is used in the experiment to induce stress: two months of no watering, surface soil removal to expose the roots just a little, light pruning, and ethephon (marketed as Ethrel, 2.0 ml / litre mixed with 5.0 g / l diammonium phosphate) spraying to promote defoliation. This practice followed during both *ambe bahar* and *hastha bahar*. In order to encourage profuse flowering, irrigation was restarted right away after covering the exposed roots with soil mixed with farmyard manure. By drip irrigation, the suggested fertiliser doses were given. The same system was continued even after the monsoon season. To record total aril weight, percentage of aril weight, juice weight, 100 Aril weight, TSS, titrable acidity, and anthocyanin content, twelve trees (four per replication) were chosen for each treatment.

Total aril weight (g)

Each treatment's nine randomly chosen fruits were split into their individual arils, which were then weighed separately. The average aril weight was given as grams (g $fruit^{-1}$).

Percentage of aril weight

The percentage of aril weight was calculated by using the formula:

$$\frac{\text{Aril weight}}{\text{Fruit weight}} \times 100$$

Fruit Juice percentage

The juice percentage was calculated by using the formula:

$$\frac{\text{Juice weight}}{\text{Fruit weight}} \times 100$$

Hundred Aril weight (g)

By removing 100 arils from each of the nine randomly chosen fruits in each treatment, the weight of 100 arils was calculated, and the mean value was represented in grams.

Total soluble solids (^{0}B)

Using a hand-held refractometer (Carl-Zeiss), the total soluble solids content of the fruit was measured, and the mean result was given in ^{0}Brix. The refractometer's prism was cleaned after each reading using tissue paper and methanol, then dried before being rinsed with distilled water.

Titrable acidity of juice (%)

By using the titration method, juice acidity was determined (AOAC, 2000). A measuring cylinder was used to weigh 10 grams of juice, and distilled water was used to dilute the juice to a volume of 50 ml. This filtrate was titrated with 0.01N NaOH using Phenolphthalein as an indicator using 10 ml of the filtrate. Using a standard curve, acidity was

estimated as mg of citric acid equivalents per 100 g of fresh weight.

Anthocyanin content (mg 100g $^{-1}$)

The Srivastava and Kumar method (2003) was used to determine the anthocyanin content of arils. By combining 10 g of the sample with 10 ml of ethanolic HCL and then pouring the mixture into a 100 ml volumetric flask, anthocyanin was extracted from the sample. The volume was prepared, the solution was chilled to 4°C, and whatman No.1 filter paper was used to filter the mixture. At 520 nm, the filtrate's optical density was measured.
Anthocyanin content (mg $100g^{-1}$) =

$$\frac{\text{O.D.520} \times \text{Volume made up}}{\text{Weight of sample}} \times 100$$

RESULTS AND DISCUSSION

Total aril weight /fruit

Significant variations in total aril weight across the propagules were noted in *ambe bahar*, according to the findings (Table 1). The plants developed from grafts (grafted on 'Daru' rootstock) had the highest overall aril weight (108.57 g), and their arils were also noticeably wider than those of the trees raised from air layers. Regarding growth regulators, the maximum total aril weight was reported with nitrobenzene at 2.0 ml / litre plant^{-1} (106.54 g fruit^{-1}). The combination of nitrobenzene @ 2.0 ml / litre plant^{-1} and grafted plants showed noticeably the highest overall aril weight (130.93 g).

The grafted plants recorded the highest total aril weight during *hastha bahar* (122.73 g fruit^{-1}), which was considerably different from the air layer plants. Nitrobenzene application at 1.5 ml / litre plant^{-1} increased the overall aril weight among the compounds (134.27 g fruit^{-1}). Regarding total aril weight, there were no appreciable differences seen between the interactions of chemicals and propagules. Nonetheless, when foliar treated with nitrobenzene @ 2.0 ml / litre plant^{-1}, grafted plants produced fruits with a high total aril weight (142.03 g fruit^{-1}).

Nitrobenzene treatment increased the total aril weight in grafted plants in both growing seasons. That might be because nitrobenzene has auxin-like properties, which may have encouraged fruit cell division and cell elongation to increase the weight of the aril. The buildup of water, carbohydrates, and other soluble substances in higher amounts as a result of the translocation of metabolites towards the fruit may also be the cause of the increased cell size and intercellular gaps. According to Wetzstein *et al*. (2011), there is a strong link between pomegranate fruit weight and mean aril weight. As a result, fruits from grafted plants that were larger in size had mean arils that were heavier. The alleged observations in pomegranate cv. Bhagwa are in agreement with Vidya *et al*. (2016).

100 Aril weight (g)

In *ambe bahar*, there were noticeable differences in 100 Aril weight across the propagules, with grafted plants exhibiting the highest 100 Aril weight (28.77 g) (Table 1). Sixty days after bahar treatment, soil drenching with paclobutrazol at 0.375 g a.i. m^{-1} canopy diameter recorded the greatest 100 aril weight (31.81 g). With a foliar application of nitrobenzene @ 2.0 ml / litre plant^{-1}, the fruits of grafted plants recorded the greatest 100 aril weight (32.59 g).

During *hastha bahar*, grafted plants with higher 100 Aril weights (32.86 g) than the remainder of the propagules were observed. Considering the impact of chemicals, nitrobenzene foliar spray at 1.5 ml / litre plant^{-1} has been linked to an increase in 100 aril weight (33.86 g). With the treatment of nitrobenzene @ 2.0 ml / litre plant^{-1}, the grafted plants recorded the highest 100 aril weight (36.35 g).

In the current study, fruits from nitrobenzene-treated grafted plants showed high mean 100 aril weights over the two growing seasons. According to Yahya *et al*. (2017), fruiting characteristics including fruit weight, length, width, and volume as well as quality indicators like the total weight of the arils and the weight of 100 arils are directly proportional to one another. The current investigation takes into account these conclusions.

Juice weight (g)

The findings showed that the fruit juice weight (g) of *ambe bahar* varied significantly across the propagules, with the propagule tissue culture plants recording the greatest juice weight (86.58 g fruit^{-1}) (Table 2). The greatest juice weight was recorded

Table 1: Effect of different seasons and plant growth regulators on total aril weight and 100 aril weight in pomegranate cv. Bhagwa

	Total aril weight (g)/fruit								100 Aril weight (g)							
	Ambebahar				*Hasthabahar*				*Ambebahar*				*Hasthabahar*			
Propagules	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**
T_1, Nitrobenzene 1.0 ml / litre	102.00	92.60	59.82	**84.81**	113.55	99.62	94.14	**102.43**	26.04	23.92	25.10	**25.02**	29.86	31.34	29.41	**30.20**
T_2, Nitrobenzene 1.5ml / litre	102.31	100.43	60.84	**87.86**	137.25	122.52	98.74	**119.50**	28.66	25.07	22.14	**25.29**	30.44	32.70	30.14	**31.09**
T_3, Nitrobenzene 1.0 ml / litre	107.96	111.91	68.95	**96.27**	101.75	107.44	91.59	**100.26**	25.15	25.80	20.01	**23.65**	32.21	30.56	29.29	**30.68**
T_4, Methyl Jasmonate @ 100 ppm	102.63	117.12	66.88	**95.54**	120.46	133.46	126.84	**126.92**	26.66	31.67	22.94	**27.09**	33.46	33.86	33.07	**33.46**
T_5, Methyl Jasmonate @ 150 ppm	100.46	120.80	81.32	**99.16**	132.58	138.68	131.55	**134.27**	29.25	32.26	26.29	**29.27**	32.65	35.18	33.76	**33.86**
T_6, Methyl Jasmonate @ 200 ppm	105.07	130.93	83.63	**106.54**	132.01	142.03	124.73	**132.92**	28.47	32.59	27.22	**29.42**	30.46	36.35	34.29	**33.70**
T_7, Paclobutrazol soil drenching 30 days after bahar treatment	104.75	106.00	81.89	**97.54**	116.22	132.41	115.30	**121.31**	29.58	29.67	29.95	**29.73**	30.59	32.83	32.02	**31.81**
T_8, Paclobutrazol soil drenching 45 days after bahar treatment	106.89	110.13	88.41	**101.81**	110.95	121.84	112.95	**115.24**	31.52	30.92	31.81	**31.41**	30.93	32.43	30.81	**31.39**
T_9, Paclobutrazol soil drenching 60 days after bahar treatment	112.31	113.09	86.03	**103.81**	107.07	134.06	107.07	**116.07**	32.24	31.3	31.91	**31.81**	31.05	34.07	31.15	**32.09**
T_{10}, Control	95.35	82.73	57.23	**80.14**	91.57	95.29	86.32	**91.06**	23.19	24.55	19.55	**22.43**	28.51	29.26	28.32	**28.70**
Mean	**103.97 P**	**108.57 T**	**73.50 PXT**		**116.34 P**	**122.73 T**	**108.92 PXT**		**28.07 P**	**28.77 T**	**25.69 PXT**		**31.02 P**	**32.86 T**	**31.22 PXT**	
SE(m)	1.19	2.18	3.78		2.33	4.27	7.39		0.39	0.71	1.23		0.17	0.32	0.56	
C.D (5%)	3.39	6.19	10.73		6.63	12.12	N.S		1.10	2.01	3.49		0.50	0.92	1.59	

P_1 – Tissue culture plants, **P_2** – Grafted plants, **P_3** – Air layer plants

Table 2: Effect of different seasons and plant growth regulators on fruit juice weight and juice percentage in pomegranate cv. Bhagwa

	Fruit juice weight (g)								Juice percentage (%)							
	Ambebahar				*Hasthabahar*				*Ambebahar*				*Hasthabahar*			
Propagules	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**
T_1, Nitrobenzene 1.0 ml / litre	64.10	78.23	59.15	**67.16**	98.75	98.88	80.57	**92.73**	35.87	43.21	38.62	**39.23**	33.23	31.70	27.63	**30.85**
T_2, Nitrobenzene 1.5ml / litre	78.82	77.61	50.69	**69.04**	103.91	89.76	89.70	**94.45**	42.36	42.46	32.94	**39.25**	34.28	26.69	30.57	**30.51**
T_3, Nitrobenzene 1.0 ml / litre	84.23	83.38	58.12	**75.24**	105.43	110.58	89.56	**101.86**	44.69	45.00	35.92	**41.87**	34.61	34.05	30.05	**32.90**
T_4, Methyl Jasmonate @ 100 ppm	85.47	96.31	55.41	**79.06**	114.21	97.5	102.85	**104.85**	43.56	48.22	33.70	**41.83**	32.21	32.12	30.07	**31.46**
T_5, Methyl Jasmonate @ 150 ppm	92.03	99.62	70.90	**87.51**	147.04	100.93	116.79	**121.59**	46.43	49.33	38.36	**44.70**	40.76	34.59	33.56	**36.30**
T_6, Methyl Jasmonate @ 200 ppm	92.72	106.66	70.85	**90.08**	165.23	126.95	118.92	**137.03**	46.54	52.05	38.52	**45.70**	45.71	34.06	34.81	**38.19**
T_7, Paclobutrazol soil drenching 30 days after bahar treatment	100.34	79.11	81.06	**86.84**	132.7	101.51	103.19	**112.46**	50.08	41.31	42.82	**44.74**	38.31	33.37	30.69	**34.12**
T_8, Paclobutrazol soil drenching 45 days after bahar treatment	104.34	86.51	83.88	**91.57**	93.85	92.68	96.12	**94.22**	51.12	44.81	44.05	**46.66**	28.03	24.85	29.80	**27.56**
T_9, Paclobutrazol soil drenching 60 days after bahar treatment	105.41	88.8	87.95	**94.05**	114.03	110.51	104.11	**109.55**	50.96	45.31	45.81	**47.36**	35.61	27.05	33.20	**31.95**
T_{10}, Control	58.38	64.92	51.66	**58.32**	96.3	87.92	81.78	**88.67**	34.34	39.19	35.11	**36.21**	34.92	29.91	30.71	**31.84**
Mean	**86.58 P**	**86.11 T**	**66.97 PXT**		**117.14 P**	**101.72 T**	**98.36 PXT**		**44.60 P**	**45.09 T**	**38.58 PXT**		**35.77 P**	**30.84 T**	**31.11 PXT**	
SE(m)	1.50	2.74	4.75		2.42	4.43	7.67		0.62	1.13	1.96		0.71	1.30	2.25	
C.D (5%)	4.26	7.78	13.48		6.88	12.57	N.S		1.76	3.22	5.58		2.02	3.68	N.S	

P_1 – Tissue culture plants, **P_2** – Grafted plants, **P_3** – Air layer plants

Table 3: Effect of different seasons and plant growth regulators on total soluble solids and titrable acidity in pomegranate cv. Bhagwa

	Total soluble solids (°B)								Titrable acidity (%)							
	Ambebahar				*Hasthabahar*				*Ambebahar*				*Hasthabahar*			
Propagules	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**
T_1, Nitrobenzene 1.0 ml / litre	18.87	15.02	14.65	**16.18**	14.40	14.80	14.89	**14.69**	0.55	0.44	0.49	**0.49**	0.63	0.57	0.82	**0.67**
T_2, Nitrobenzene 1.5ml / litre	19.90	14.50	14.60	**16.33**	14.53	14.73	14.73	**14.66**	0.45	0.46	0.46	**0.46**	0.57	0.79	0.71	**0.69**
T_3, Nitrobenzene 2.0 ml / litre	19.91	14.94	14.60	**16.48**	15.40	15.66	15.46	**15.51**	0.39	0.42	0.46	**0.43**	0.52	0.85	0.78	**0.72**
T_4, Methyl Jasmonate @ 100 ppm	19.12	13.53	14.11	**15.59**	15.00	15.60	14.80	**15.13**	0.48	0.40	0.49	**0.46**	0.58	0.49	0.83	**0.63**
T_5, Methyl Jasmonate @ 150 ppm	19.46	13.44	14.41	**15.77**	15.46	15.26	16.00	**15.57**	0.41	0.40	0.51	**0.44**	0.68	0.82	0.86	**0.79**
T_6, Methyl Jasmonate @ 200 ppm	19.25	13.93	14.55	**15.91**	14.53	14.86	16.11	**15.17**	0.49	0.43	0.48	**0.46**	0.54	0.74	0.81	**0.70**
T_7, Paclobutrazol soil drenching 30 days after bahar treatment	20.37	14.67	14.73	**16.59**	14.86	14.46	14.71	**14.68**	0.42	0.47	0.50	**0.46**	0.64	0.96	0.85	**0.82**
T_8, Paclobutrazol soil drenching 45 days after bahar treatment	19.52	15.12	14.57	**16.40**	16.00	12.93	15.06	**14.66**	0.40	0.42	0.47	**0.43**	0.68	0.77	1.14	**0.87**
T_9, Paclobutrazol soil drenching 60 days after bahar treatment	20.44	15.05	15.07	**16.85**	15.86	15.20	15.06	**15.37**	0.45	0.43	0.50	**0.46**	0.55	0.87	0.80	**0.74**
T_{10}, Control	18.57	13.82	13.88	**15.42**	13.26	13.86	13.33	**13.48**	0.60	0.53	0.53	**0.55**	0.70	0.97	1.03	**0.90**
Mean	**19.54 P**	**14.40 T**	**14.52 PXT**		**14.93 P**	**14.74 T**	**15.01 PXT**		**0.46 P**	**0.44 T**	**0.49 PXT**		**0.61 P**	**0.78 T**	**0.86 PXT**	
SE(m)	0.12	0.23	0.39		0.18	0.33	0.58		0.01	0.02	0.04		0.03	0.05	0.10	
C.D (5%)	0.35	0.65	N.S		N.S	0.95	N.S		0.03	0.07	N.S		0.09	0.16	N.S	

P_1 – Tissue culture plants, **P_2** – Grafted plants, **P_3** – Air layer plants

Table 4: Effect of different seasons and plant growth regulators on anthocyanin content and percentage of aril weight in pomegranate cv. Bhagwa

	Anthocyanin content (mg 100 g^{-1})								Percentage of aril weight (%)							
	Ambebahar				*Hasthabahar*				*Ambebahar*				*Hasthabahar*			
Propagules	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**
T_1, Nitrobenzene 1.0 ml / litre	3.13	3.98	1.66	**2.92**	2.52	4.44	3.95	**3.64**	52.37	51.35	39.25	**49.24**	49.00	50.94	55.21	**51.72**
T_2, Nitrobenzene 1.5ml / litre	3.22	3.68	2.19	**3.03**	2.51	5.65	3.74	**3.96**	52.74	55.1	39.67	**49.96**	53.96	55.00	58.00	**55.65**
T_3, Nitrobenzene 1.0 ml / litre	3.96	4.27	2.62	**3.61**	2.88	8.41	4.48	**5.25**	57.3	60.51	42.63	**53.48**	54.49	57.46	55.82	**55.92**
T_4, Methyl Jasmonate @ 100 ppm	2.40	3.25	1.91	**2.52**	1.79	6.18	3.86	**3.94**	52.3	59.24	40.75	**50.76**	64.77	59.94	58.93	**61.21**
T_5, Methyl Jasmonate @ 150 ppm	2.69	3.64	1.85	**2.73**	2.68	4.25	3.23	**3.38**	52.27	59.93	44.45	**50.81**	61.74	62.45	58.68	**60.96**
T_6, Methyl Jasmonate @ 200 ppm	3.46	3.93	2.20	**3.19**	2.58	3.86	2.14	**2.86**	55.11	63.95	45.45	**54.04**	68.71	65.60	59.22	**64.51**
T_7, Paclobutrazol soil drenching 30 days after bahar treatment	2.85	3.76	2.13	**2.91**	2.63	4.40	3.59	**3.54**	54.22	55.4	43.28	**50.32**	56.06	56.38	53.93	**55.45**
T_8, Paclobutrazol soil drenching 45 days after bahar treatment	2.52	3.79	2.53	**2.95**	2.62	2.32	3.68	**2.87**	57.12	56.98	46.48	**51.94**	53.64	59.12	52.25	**55.00**
T_9, Paclobutrazol soil drenching 60 days after bahar treatment	2.50	3.34	2.43	**2.76**	2.56	4.89	3.68	**3.71**	59.11	57.92	44.81	**52.32**	51.93	58.43	52.66	**54.34**
T_{10}, Control	2.31	2.24	1.73	**2.09**	2.56	1.85	1.40	**1.93**	48.06	50.21	39.12	**49.48**	46.53	48.92	50.76	**48.74**
Mean	**2.90 P**	**3.59 T**	**2.12 PXT**		**2.53 P**	**4.62 T**	**3.37 PXT**		**54.06 P**	**57.06 T**	**42.59 PXT**		**56.08 P**	**57.42 T**	**55.54 PXT**	
SE(m)	0.04	0.07	0.12		0.09	0.17	0.30	0.04	0.67	1.22	2.12		0.57	1.05	1.83	
C.D (5%)	0.11	0.21	0.36		0.27	0.50	0.87	0.11	1.9	N.S	6.04		N.S	3.00	5.20	

P_1 – Tissue culture plants, **P_2** – Grafted plants, **P_3** – Air layer plants

by the chemical paclobutrazol soil drenching at 0.375 g a.i. m^{-1} canopy diameter 60 days after the bahar treatment (94.05 g $fruit^{-1}$). The impact of chemicals on juice weight varied greatly across the propagules, with nitrobenzene foliar spraying at a rate of 2.0 ml / litre per plant per grafted plant being implicated in improving the juice weight (106.66 g $fruit^{-1}$).

The results showed that fruit juice weight (g) varied significantly among the propagules during *hastha bahar*, with propagule tissue culture plants recording the greatest juice weight (110.26 g), which was significantly different from the juice weight of the other propagules. In comparison to the other chemicals used, nitrobenzene administration at 2.0 ml / litre $plant^{-1}$ as a foliar spray was associated with increasing juice weight (137.03 g). Chemical effects on juice weight did not differ significantly across propagules.

The rise in juice appears to be the result of water and glucose moving around in the arils. The percentage of seed had naturally decreased in proportion to the juice content as the water content of arils increased. These findings are consistent with pomegranate reports by Supe and Saitwal (2016).

Juice percentage (%)

The data in Table 2 for *ambe bahar* showed that the juice percentage (%) varied significantly among the propagules and was highest in the propagule-grafted plants (45.09%). Among the compounds, paclobutrazol soil drenching at 0.375 g a.i. m^{-1} canopy diameter increased juice percentage (47.36%) 60 days after bahar treatment. Chemicals had a considerably different impact on fruit juice percentage throughout the propagules, however nitrobenzene foliar spraying at 2.0 ml / litre $plant^{-1}$ on grafted plants increased fruit juice percentage (52.05%).

The data in Table 2 showed that during *hastha bahar*, the propagule juice percentage (%) varied significantly. The propagule tissue culture plants recorded the highest juice percentage (35.77%) and it differed significantly from the other propagules. Nitrobenzene @ 2.0 ml / litre $plant^{-1}$ was one chemical that had an additive impact on juice percentage (38.19%).

According to the observations made on the percentage of juice, the fruit's juice content grew as it ripened. The progressive decline in the percentage of seeds may be the cause of the increase in fruit juice content. These findings support the theory put forth by Vidya *et al*. (2016), who suggested that as a fruit matured, its juice content would rise and its seed content would fall. At the same time, during fruit maturity, peel and seeds percentage declined significantly, while the percentage of aril and juice (of whole fruit) increased significantly (Zarei *et al*., 2011).

Total soluble solids (^{0}B)

Total soluble solids (^{0}B) varied significantly among the propagules in the case of *ambe bahar*, and the fruits grown in tissue culture plants had the greatest TSS (19.54 ^{0}B), which was statistically different from the rest of the propagules (Table 3). Among the compounds, paclobutrazol at 0.375 g a.i. m^{-1} canopy diameter soil soaking increased total soluble solids 60 days after bahar treatment (16.85 ^{0}B). The soil drenched with paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment, which resulted in the fruits from tissue culture plants having the highest TSS (20.44 ^{0}B).

Total soluble solids (^{0}B) did not significantly vary among the propagules during *hastha bahar*, however the propagule air layer plants produced fruits with a high TSS content (15.01 ^{0}B). Among the compounds, nitrobenzene foliar spray at 1.5 ml / litre $plant^{-1}$ has been linked to increasing the TSS content of fruits (15.57 ^{0}B). Table 3 made it clear that there were no notable variances in how chemicals interacted with propagules for total soluble solids. Yet, when foliar sprayed with nitrobenzene @ 2.0 ml / litre $plant^{-1}$, the fruits produced by air layer plants recorded a higher TSS concentration of (16.11 ^{0}B).

Despite the fact that there was no discernible difference in TSS level, after two seasons of paclobutrazol application, the fruits of both tissue culture and air layer plants had high TSS content. Increased amounts of sucrose, starch, and sugar, as a result of decreased vegetative growth and the absence of other potentially competitively active growing sinks, can be used to explain this. This led to a greater allocation of nutrients to fruits (Abdel Rahim *et al*., 2011). Our results are consistent with those of Wani *et al*. (2007) in the Red Delicious apple variety.

Titrable acidity (%)

The data in Table 3 for *ambe bahar* showed that all propagules significantly affected the titrable acidity content, although the fruits grown from grafted plants had less titrable acidity (0.44%). The least titrable acidity (0.43%) was recorded by paclobutrazol soil soaking at 0.375 g a.i. m^{-1} canopy diameter 45 days after bahar treatment, out of all the chemicals. Regarding the effect of chemicals on titrable acidity across the propagules, significant changes were not visible. From tissue culture plants, however, foliar spraying with methyl jasmonate at 200 ppm / litre $plant^{-1}$ resulted in fruits with less acidity (0.39%).

The data in Table 3 demonstrate that all propagules were considerably affected by *hastha bahar* in terms of titrable acidity content, although fruits produced from tissue culture plants had less titrable acidity (0.61%). In comparison to other agents, nitrobenzene foliar spray at 1.0 ml / litre $plant^{-1}$ resulted in fruits with reduced titrable acidity (0.63%). Regarding the influence of chemicals on titrable acidity across the propagules, significant changes were not visible. Nevertheless, grafted plants that received a foliar spray of nitrobenzene at 1.0 ml / litre $plant^{-1}$ produced fruits with less titrable acidity (0.49%).

Increased total soluble solids, which may be the result of the rapid metabolic conversion of starch and pectin into soluble substances and the rapid translocation of sugars from leaves to growing fruits, may be the cause of the reduction in fruit juice acidity during both seasons. The current findings are consistent with those made by Ghosh *et al*. (2009) in the Ruby variety of pomegranate.

Anthocyanin content (mg / 100 g)

Table 4 clearly shows that all the propagules in the case of *ambe bahar* were significantly impacted in terms of anthocyanin content. The anthocyanin content in fruits grown from grafted plants was the highest (3.59 mg / 100g). Methyl jasmonate, one of the chemicals, had an incremental influence on the anthocyanin content of fruits (3.61 mg / 100g), and it differed significantly from the other chemicals used. When the grafted plants were foliar sprayed with methyl jasmonate at 200 ppm / litre $plant^{-1}$, the fruits they produced had the highest anthocyanin content (4.27 mg / 100 g).

The data in Table 4 show that all propagules were considerably impacted by *hastha bahar* in terms of anthocyanin concentration. The highest anthocyanin content (4.62 mg / 100 g) was found in the fruits produced by grafted plants, which was significantly higher than that of the other propagules. Methyl jasmonate, one of the compounds, had an incremental impact on the anthocyanin content of fruits when applied as a foliar spray at 200 ppm $litre^{-1}$ $plant^{-1}$ (5.25 mg / 100 g). When the grafted plants were foliar treated with methyl jasmonate at 200 ppm / litre $plant^{-1}$, the fruits they produced had the highest anthocyanin content (8.41 mg / 100 g).

Methyl jasmonate has been linked to increasing the amount of anthocyanin in fruits grown from grafted plants over two seasons. The principal source of anthocyanin products is sugar, particularly glucose. An increase in phenol concentration and simultaneous activation of the phenylalanine enzyme can be related to an increase in anthocyanin content. The expression of the gene pUFGluT linked to the anthocyanin expression in pomegranate juice may also contribute to the increase in anthocyanin content. Similar results were found in apple cv. Tsugaru by Satoru *et al*. (2002).

Percentage of aril weight (%)

Grafted plants in *ambe bahar* had the highest proportion of aril weight (57.06%), which was significantly higher than the rest of the propagules (Table 4). Nitrobenzene foliar spray, 2.0 ml / litre $plant^{-1}$, increased percentage of aril weight (54.04%). Nitrobenzene was sprayed over the leaves of grafted plants at a rate of 2.0 ml / litre per plant, which increased the percentage of aril weight to 63.95%.

The fruits grown from grafted plants had the highest proportion of aril weight (57.42%) during *hastha bahar*. Regarding the impact of chemicals on the percentage of aril weight, there have been observed to be significant variances. Nitrobenzene foliar spray at 2.0 ml / litre of plant per improved percentage of aril weight (64.51%). Nitrobenzene foliar spray applied to tissue culture plants at a rate of 2.0 ml / litre $plant^{-1}$ has a cumulative effect on the percentage of aril weight (68.71%).

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In vitro responses of raspberry plantlets to the culture media enriched with some plant sprout powders

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ABSTRACT

The growth and morphogenesis responses of cultured cell and plant tissues can be improved by addition of small amounts of some organic materials like plant sprout powders (PSP). A preliminary experiment was undertaken to examine the response of raspberry plantlets to media enriched with plant sprout powders derived from wheat, vetch and alfalfa plant species. The Murashige and Skoog (MS) medium supplemented with IBA (2.0 mg/l) and activated charcoal (200 mg/l) was exploited as optimized shoot proliferation for raspberry. Seeds of wheat, vetch or alfalfa were subjected to germination and when the rootlets attained 1 cm long, the sprouts were dried and ground to provide a homogenous powder. The shoot proliferation medium already optimized for raspberry was supplemented with 1.0 g/l of each wheat, vetch, or alfalfa sprout powders. The double-node cuttings of in vitro grown raspberries were inoculated on these media. Therefore, the growth responses of raspberries were measured in media with or without PSP. The results revealed the positive response of raspberry plantlets to the medium containing vetch sprout powder and most of the growth parameters including number of shoots, leaves, roots, and plantlet length were improved. Besides obvious apparent quality of in vitro cultures and better growth performance, the plantlets grown on media enriched with vetch sprout powder showed highest leaf number (6.41) and longer internodal length (1.89 mm). Therefore, the obtained data encourage the utilization of PSP in plant tissue culture media.

***Keywords*:** *In vitro* culture, plant sprout powder, raspberry**,** tissue culture

INTRODUCTION

Raspberry (*Rubus idaeus* L.) is a nutritious plant species that has been produced commercially in Europe and the US since the early 19th century and the *Rubus* genus is widely spread across the temperate zones (Pergolotti *et al*., 2023). The European varieties of red raspberry were introduced to the US and were crossed with the native species. Recently, interests have been provoked to raspberry because of its high nutritional value and its high contents of vitamin A, C, fibers, and antioxidant compounds (Barney *et al*., 2007). Since raspberry is perennial, its improved cultivars and elites should be preserved for both field planting and tissue culture. Shoot multiplication is extensively employed to propagate small fruits *in vitro* because it can produce disease-free plants in sterile conditions with a high propagation rate. Given the highest genetic diversity of raspberry cultivars that require a diverse set of nutrients, micropropagation of raspberry is laborious (Zawadzka and Orlikowska, 2006; Wu *et al*., 2009). So, a critical factor for viable propagation of this plant species is to have an optimal culture medium.

The growth response and *in vitro* morphogenesis of cultured plant tissues can be significantly improved by addition of small amounts of certain organic elements. Besides a natural source of carbon, organic additives may contain different natural vitamins, proteins, fiber, phenols, and also plant hormones (Khorsha *et al*., 2016). The viability of the culture of a plant's cells, tissue, or organ depends on various parameters, the most effective ones being the selection of nutritional compounds and growth regulators. A plant culture medium contains obligatory and arbitrary compound requirements, and such medium varies with cultivar, species, and explants; however, a culture

medium should contain all essential nutrients required by the plants at their optimal levels. In this sense, high production cost, which is partially associated with these compounds, is a disadvantage of plant tissue culture techniques.

High prices of chemicals have limited their extensive use in developing countries. So, the interest for the use of available highly nutritional compounds in tissue culture media is increasing. Researchers have focused on the effects of applying yeast and plant extracts in tissue culture media. Coconut milk stimulates cell division and is used as an additive in many laboratories (Hamdeni *et al*., 2022). There are numerous reports on health benefits as well as nutritional facts of plant sprouts. The nutritional and medicinal properties of sprouts are derived from their remarkable vitamin, minerals, and organic compounds content. They contain a significant amount of protein and dietary fiber as well as vitamin K, folate, pantothenic acid, niacin, thiamin, vitamin C, A and riboflavin. In case of minerals, they contain Mn, Cu, Zn, Mg, Ca and Fe. Many of these nutrient compounds increase dramatically as the sprout continues to develop. Hence, the present study aimed to explore the feasibility of application of different plant sprout powders (PSP), as an inexpensive and accessible organic source to enrich the plant tissue culture media in Raspberry.

MATERIALS AND METHODS

The present study was carried out in the plant tissue culture laboratory, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran based on a completely randomized design with four replications. Initially, a series of experiments was conducted to standardize the shoot proliferation medium of raspberry plantlets (Alizadeh *et al*., 2016). The MS (Murashige and Skoog, 1962) basal medium containing 2 mg/l IBA and 200 mg/l activated charcoal was found to be the optimum culture medium for shoot proliferation of raspberry. So, this standardized culture medium was supplemented with different doses of already ground, oven-dried wheat, vetch, or alfalfa sprout powders. In overall, five culture media were evaluated, namely: the standardized proliferation medium optimized for *in vitro* shoot proliferation of raspberry (MS), media supplemented with wheat (WSP), vetch (VSP) and alfalfa (ASP) sprout powders. Furthermore, to discriminate the role of MS ingredients and PSP effects, a medium containing only PSP but lacking MS salts was used as control (C).

To prepare PSP, a bulk seeds of wheat (*Triticum aestivum)*, vetch (*Vicia sativa*) and alfalfa (*Medicago sativa)* were prepared. The broken and defective seeds were excluded, and the healthy seeds were selected for germination. The seeds were thoroughly washed and were then soaked in water for 12 hours to swell. Then, the extra water was removed, and the seeds were wrapped between two pieces of wet cloths and were left at room temperature to germinate (5-7 days). When the rootlets were 1 cm long, they were taken away from the cloth wrappers and the sprouts were oven-dried at 55°C. This dry mass was, then, ground to provide a homogenous powder (PSP). The PSP were refrigerated in glass bottles prior to utilization in tissue culture media. The optimized MS medium for raspberry proliferation was supplemented with 1.0 g/l of each wheat, vetch, or alfalfa sprout powders. The double-node cuttings of *in vitro* grown raspberries that had been already propagated by consecutive reproduction in previous stages were prepared and inoculated on these media. Therefore, the growth responses of raspberries were measured in media with or without PSP.

Four weeks after inoculation, the samples were subjected to the measurement of fresh and dry weights, as well as some growth parameters such as visual assessment (apparent quality of the cultures (Fig.1)), chlorophyll content, plantlet length, the number of shoots, roots, and leaves, and internode length. Visual assessment of the cultures was performed as scoring ranged from 1-5 based on visual performance. The score 1 stands for least and 5 for highest visual status. The scoring was undertaken for at least 5 cultures and the average was used for analysis. Leaf area was measured with the Image J software package. The internode length and root length were measured with a caliper and ruler respectively. Also, chlorophyll content was measured with the method described in Barnes *et al*. (1992). In their method, the dimethyl sulphoxide (DMSO) is utilized as solvent to extract pigments. The 500 mg leaf tissues were collected and cut into small pieces. These were poured into the test tubes,

and then 5 ml DMSO solution was added to the tubes, then it was placed in an oven at a temperature of 75 degrees Celsius for three hours. Then 1 ml of the solution was transferred to another tube and 2 ml fresh DMSO was added to the samples. Then, the optical absorption of the solution was read using a spectrophotometer at wavelengths of 480, 510, 645 and 663 nm. Pure DMSO was used as a blank. The amount of chlorophyll a, b, total chlorophyll and carotenoid was calculated using the related formula.

Furthermore, the *in vitro* grown leaves were scanned (Fig. 2) and their area were measured by J Image software. The data were analyzed by the SAS software package and the mean data were compared by LSD test at the $p < 0.01$ and $p < 0.05$ levels.

RESULTS AND DISCUSSION

The growth response and *in vitro* morphogenesis of cultured plant cells and tissues can be improved by addition of small amounts of certain organic elements. The PSP is a natural source of carbon, and it may contain several vitamins, phenols, fiber, hormones and also proteins. Hence, their addition to the media may have a positive role (Alizadeh *et al.,* 2016). The supplementation of PSP had desirable impacts on growth traits of raspberries under *in vitro* culture conditions. The analysis of variance showed that the PSP treatments had significant impacts on apparent quality, chlorophyll content, root number and plantlets fresh or dry weights at the $p < 0.01$ level and on leaf number, internode length, and leaf area at the $p < 0.05$ level. However, PSP had no significant effects on number of raspberry shoots (Table 1). In a general vision, it can be observed that the plantlets grown on media supplemented with PSP have better visual quality (Fig.1), more broad and attractive leaves (Fig. 2). The VSP enriched media gave more vigorous cultures as compared to other treatments.

The commercial tissue culture technique is influenced by several factors including the right selection of plant species, physical environment, and chemicals for the culture medium (Puchooa and Ramburn, 2004). The concentration and type of carbon sources added to the culture medium are effective in the success of tissue culture. Sugars are essential as a source of energy and also, to hold the osmotic potential of the culture medium (Lipavská and Konrádová, 2004). Carbohydrates have various functions in tissue culture, including energy supply to *in vitro* plants, especially at the early stages of the tissue culture cycle when the photosynthesis rate is still low, cell growth when the cells are exposed to radiation that is outside of photosynthetically active range, and the generation of osmotic pressure; it also has certain morphogenetic impacts in some cases (Al-Khateeb, 2008; Anwar *et al*., 2005). Carbon sources can have simple and/or complex sugars (Akter *et al*., 2007). Most cultures rely on a carbohydrate source as long as they are prepared for adaptation.

Chlorophyll and carotenoid are two major photosynthetic pigments. The frequency of these pigments would be critical especially when the tissue culture raised plantlets are taken out to *ex vitro* (soil) conditions. The leaves with high density pigments may increase the survival percentage during hardening stage or *ex-vitro* transfer. The wider leaves with high chlorophyll content contribute to higher photosynthetic rate in these plantlets. Such alterations mitigate transplanting shock during hardening stage. The application of seaweed extract as growth stimulator to some plants (Fornes *et al*., 2002; Vernieri *et al*., 2005) enhanced chlorophyll content and photosynthesis rate of the leaves. Khorsha *et al.* (2016) reported that the application of apricot gum as an organic compound to the culture medium of *Stevia*, a medicinal plant, increased chlorophyll content versus control 28 days after inoculation. In the present study (Table 2), the media supplemented with PSP had chlorophyll content comparable to MS media. However, the highest chlorophyll content (5.78 mg/g FW) was recorded in plantlets grown on MS medium but it was not significantly different with PSP enriched media. This observation was corroborating with Khorsha *et al*. (2016).

There are numerous reports on the use of inexpensive and convenient organic resources for the rooting of plants in the tissue culture medium, such as for beet molasses, sugarcane juice (Buah *et al*., 2011), and date palm syrup (Al-Khateeb, 2008). The application of apricot gum to the grapevine and Stevia culture media increased the number and length of the roots (Khorsha *et al.,* 2016). The rooting in raspberry explants was

Table 1: Analysis of variance for the effect of plant sprout powder (PSP) on the *in vitro* performance of raspberry.

Sources of variations	df	Visual assessment	Chlorophyll	Shoot no.	Root no.	Leaf no.	Fresh weight	Dry weight	Root length	Plantlet length	Internode length	Leaf area
Treatment	4	0.4**	4.52**	0.78ns	1.53**	5.14*	0.41**	0.04**	3.87**	0.24*	0.17*	0.19*
Error	10	0.05	0.31	0.28	0.006	1.03	0.01	0.001	0.04	0.06	0.03	0.03
CV		13.26	11.73	27.06	16.41	21.69	12.51	10.26	26.9	14.5	11.03	24.9

**: significance at the $p < 0.01$ level; *: significance at the $p < 0.05$ level; ns: non-significance.

observed only in MS and VSP supplemented media. The highest number of roots (1.52) was recorded in VSP, while the highest root length (2.48 cm) was realized in MS treatment (Table 3). The existence of long roots may not be essential for plantlets under *in vitro* conditions because they have easy access to water and nutrients under *in vitro* conditions. Even explants with no roots are capable of nutrient absorption from medium. However, longer roots with several small and diffuse hairy roots would be significant for *ex vitro* transfer phase (Alizadeh *et al*., 2010). Therefore, they provide the plantlets higher area for the uptake of water and nutrients and reduced plantlet loss during acclimation.

Different organic additives and sugars were applied as carbon sources to the growth and propagation of PLBs of *Dendrobium noble* plants, which were supplemented with banana homogenate, tomato homogenate, and coconut milk at different rates. The results showed that banana and tomato homogenates were effective in the proliferation of orchids and that the coconut milk was shown to be the best organic additive for the proliferation of PLBs so that it increased fresh weight by four times in only 4 weeks when compared to the initial weight. The highest growth in PLBs was recorded for glucose, fructose, and sucrose, 0.94 ± 0.55, 9.1 ± 0.82, and 6.51 ± 0.52 g, respectively. Galactose, mannitol, and sorbitol were desirable for increasing the growth of PLBs. They introduced coconut milk as the best organic additive and glucose as the best carbohydrate source for the propagation of orchid PLBs. Some other organic additives such as coconut milk, banana homogenate, potato pulp and juice, honey, date pulp syrup, corn pulp, papaya pulp, and beef extract have also been utilized in plant tissue culture studies (Murdad *et al*., 2010). Organic additives were reported to contribute to the production of PLBs, shoots and leaves (Akter *et al*., 2007), increased the size of somatic embryos (Al-Khateeb, 2008), and help the growth and develop of seeds and regeneration (Tawaro *et al*., 2008). In an experiment undertaken by Puchooa and Ramburn (2004), it was found that the increase in fresh and dry weights of explants was lower in culture media containing carrot juice as compared to media supplemented with cytokinin and auxin. However, with the increase in carrot juice concentration, the fresh and

Table 2: The effect of plant sprout powder (PSP) on some *in vitro* parameters of raspberries

Treatments	Visual assessment	Chlorophyll mg/g FW	Leaf no.	Leaf area cm^2	Shoot no.	Shoot length cm	Internode length(cm)
MS*	2.00a	5.78a	4.00b	1.12a	1.47b	2.04a	1.51bc
VSP	2.04a	5.07a	6.41a	0.82a	2.64a	1.90a	1.89a
ASP	1.85a	5.18a	5.24ab	0.73ab	2.27ab	1.80a	1.74ab
WSP	1.90a	5.21a	4.90ab	0.81a	2.06ab	1.74a	1.61ab
C	1.15b	2.64b	2.94c	0.41b	1.47b	1.29b	1.26c

*MS: Murashige and Skoog medium; VSP, ASP, WSP: Vetch, Alfalfa and wheat sprout powder respectively; C: control. The means indicated by the same letter in each column are not statistically different ($P < 0.01$).

Table 3: Mean comparison for the effect of plant sprout powders on raspberry plantlets

Treatments	Root length(cm)	Root no.	Fresh weight (g)	Dry weight (g)
MS*	2.48a	1.00ab	0.67b	0.31b
VSP	1.45b	1.52a	1.24a	0.47a
ASP	0.00c	0.00c	0.59b	0.26b
WSP	0.00c	0.00c	1.14a	0.43a
C	0.00c	0.00c	0.37c	0.17c

*MS: Murashige and Skoog medium; VSP, ASP, WSP: Vetch, Alfalfa and wheat sprout powder respectively; C: control. The means indicated by the same letter in each column are not statistically different ($P < 0.01$).

dry weights were also increased. In our experiment, we found higher fresh and dry weights of *in vitro* grown raspberry plantlets in media supplemented with either wheat or vetch sprout powders (Table 3).

The usefulness of organic additives in plant tissue culture media was also highlighted in some other articles. For example, the effect of organic additives on the proliferation of orchids in half-strength MS culture medium enriched with local banana homogenate, tomato homogenate, and immature coconut milk showed that the type and concentration of organic additives influenced the proliferation response of PLBs (Nambiar *et al*., 2012). The addition of organic substances to culture media not only acts as an organic carbon source but they also contain natural vitamins, phenols, fibers, hormones, and proteins (Gnasekaran *et al*., 2010).

Different basal media are commercially exploited for tissue culture of plants and they have diverse effects on the growth and proliferation of plants depending on their macro and microelement levels. In addition to standard compounds, organic acids and a wide range of natural extracts are also applied randomly in the culture of specific species. Whenever the compounds supplemented to these basal media did not have the appropriate results, the researchers used some other compounds such as coconut milk, malt extract, tomato juice, yeast extract, or orange juice, which gave them optimal results (Molnár *et al*., 2011). Singh and Kaur (2011) revealed that the application of malt extract did not cause significant differences in proliferation percentage, but they obtained the longest branches as compared to the plants exposed to cytokinin treatments.

Khorsha *et al.* (2016) reported that the number of shoots and intermodal length of grapevines plantlets were influenced in optimized medium enriched with apricot gum. They observed highest number of micro-shoots and longer internodes in media supplemented with 4 g/l apricot gum. In another report by Khorsha (2014) the highest number of shoots in stevia plantlets was observed in medium enriched with apricot gum at the rate of 6 g/l. The raspberry tissue culture media enriched with PSP had considerable influence on shoot proliferation (Table 2). The highest number of shoots (2.64) and long internodes (1.89 cm) were obtained in VSP supplemented media. These results are consistent with Khorsha *et al.* (2016).

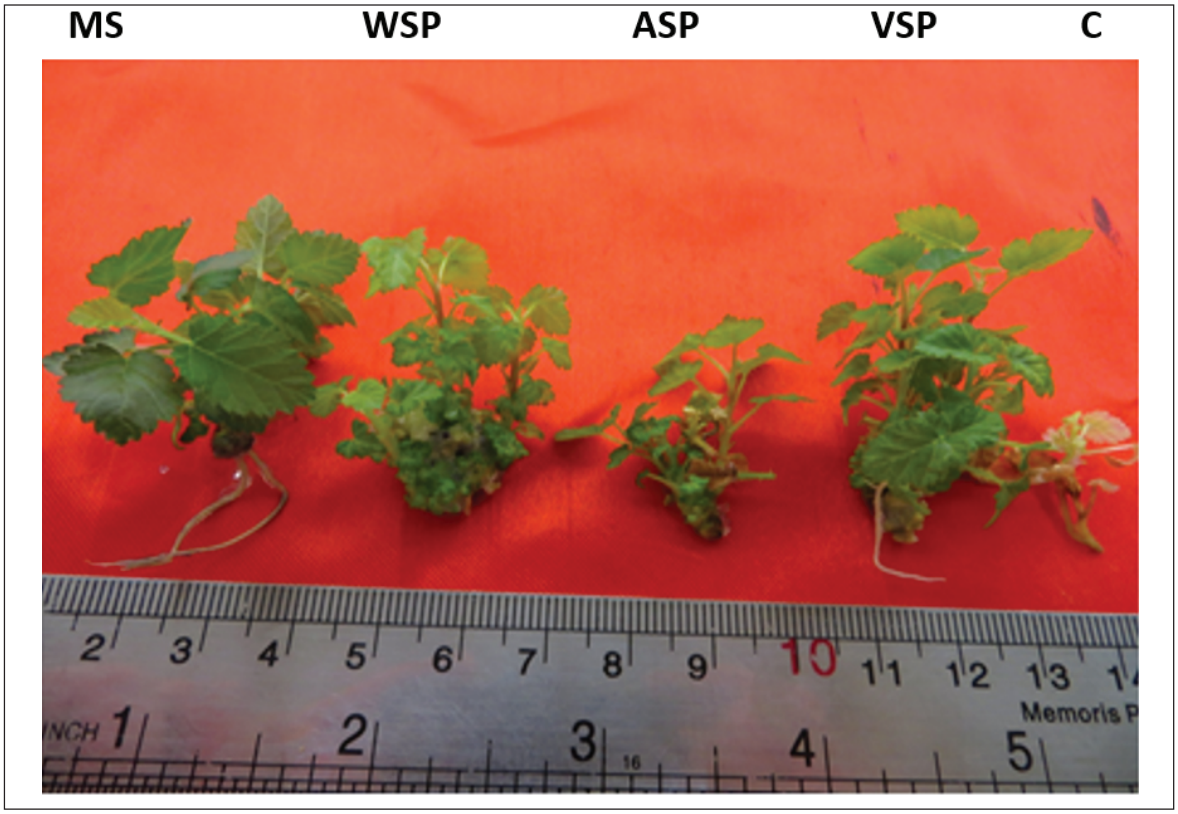

Fig. 1 : The morphology of *in vitro* grown raspberry plantlets as affected by media enriched with plant sprout powders. MS: Murashige and Skoog medium; VSP, ASP, WSP: Vetch, Alfalfa and wheat sprout powder respectively; C: control.

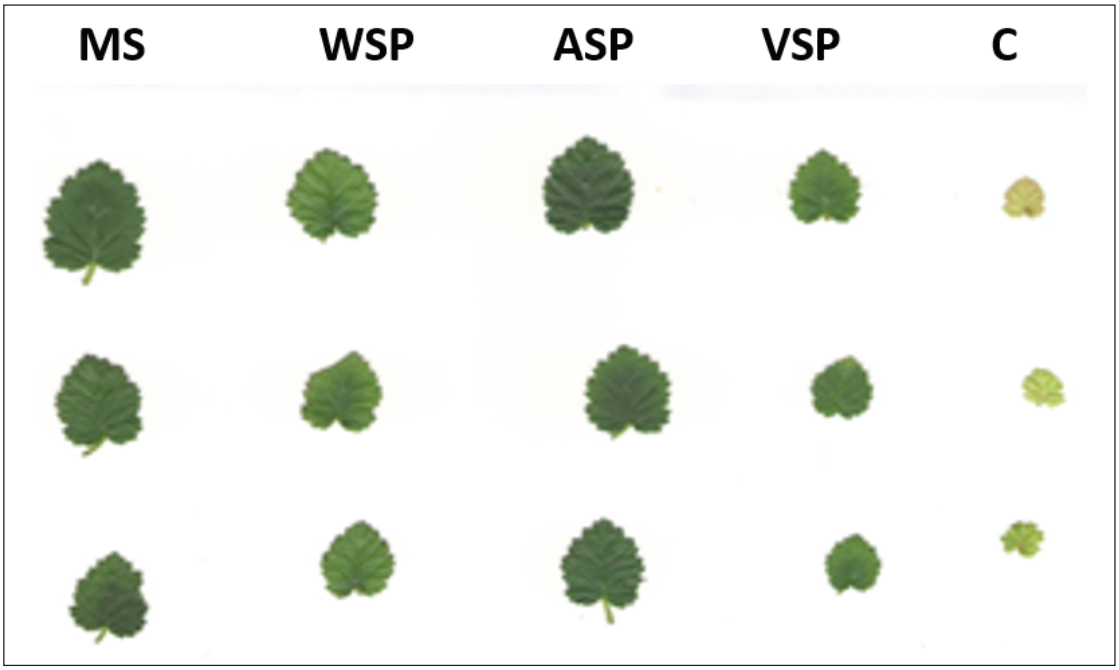

Fig. 2: Samples of *in vitro* raspberry leaves scanned with a scanner. MS: Murashige and Skoog medium; VSP, ASP, WSP: Vetch, Alfalfa and wheat sprout powder respectively; C: control.

CONCLUSION

In conclusion it can be stated that, PSP have nutritious constituents that can be used as a substitute or supplement to tissue culture media. Furthermore, addition of VSP may improve the growth parameters of *in vitro* raspberry plantlets and its utilization in tissue culture media may be encouraged. However, though demonstration of positive effects of PSP-enriched media in our study, recommendation for application of such ingredients as a constant part of media culture or in commercial laboratories, needs to further complementary experiments. Furthermore, it is recommended to examine the PSP derived from other seeds such as barley, pea and corn to find out the rate of *in vitro* growth and proliferation of plants.

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Economics in production of large cardamom (*Amomum subulatum* roxb.) in Sankhuwasabha, Nepal

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ABSTRACT

The study was conducted to assess the economics and minimum support price of Large Cardamom in the Sankhuwasabha district. A field survey using a structured questionnaire with 100 simple random households was done to collect the information regarding cost and return of cardamom production. The secondary data related to cost of production, minimum support price, marketing system and the channel was reviewed from Journal, Articles, Books and Newspaper. Data entry and analysis was done by using Microsoft Excel (2007). The study found that Benefit-cost ratio and net profit per hectare of Large cardamom was 1.33 and NPR 2,46,765 respectively with payback period of 4.89 years. The minimum support price of Large Cardamom in the study area was calculated to be NPR 720 per kg. The main reason for choosing Cardamom farming was higher income than other crops. Major risks associated with this enterprise were indirect marketing channel from Nepal to other countries, unorganized market and monopoly in abroad market. Large cardamom farming can be developed as an important livelihood source of mountain people in the eastern Himalayan region of Nepal.

***Keywords*:** Large Cardamom, production, profitability

INTRODUCTION

Cardamom (*Amomum subulatum* roxb) is spices crops that represent Nepal in the global market and ranks in the terms of production and exports (Kandel, 2019). *A. subulatum* is only grown in three eastern Himalayan countries namely Nepal, India and Bhutan (Sharma *et al*., 2009). Cardamom is the world's oldest spice and third most expensive spice followed by saffron and vanilla (Tangjang and Sharma, 2018). Large Cardamom is high-value crop with low volume which has high export value (Bhandari and Bhandari, 2018). Cardamom fruit is capsule which contains 8.5% moisture, protein 6%, volatile oil 2.8%, crude fiber 22%, starch 43.2%, ether extract 5.3%, and alcohol extract 7% (Shankaracharya *et al*.,1990). Gopal *et al*.(2012) reported that cardamom seed is diuretic, antidote for snake and scorpion venom, stimulant, stomachic, alexipharmic and astringent in properties. Kalauni and Joshi (2020) reported that 80% of total Cardamom in Nepal is produced in districts of Koshi province i.e. Illam, Taplejung, Sankhuwasabha, Dhankuta, Bhojpur, Panchthar and Tehrathum. Bumble bees and honey bees are most frequent visitors of Large cardamom flowers (Gaira *et al*., 2016) and these pollinators have a economic role in the production of Large Cardamon. The fruits mature during the third year after the plantation and harvesting is carried out during August to November-December. The production of Large Cardamom in Nepal in the year 2019/20 was 7954 Mt and was planted in15,055 ha (MoALD,2021) area. Thapa (2016) stated that minimum support price (MSP) is a form of market intervention by the government that ensures farmers a guarantee price as well as an assured market for their produce. The marketing channel of Large Cardamom is farmers to wholesaler to trading Centre in Birtamod to Exported to India and third Countries (Baniya *et al*., 2019). Kattel *et al.* (2020) reported that 90% of Nepalese Cardamoms is exported to Indian market. Sankhuwasabha district is one of the major leading Cardamom producing districts of Nepal because of the favorable climatic environment and better average precipitation in the district.

Despite the very good prospect of quality Cardamom in the Sub Himalayan of Nepal, the

average productivity at present is in declining trend Sharma *et al*. (2016). During 2014, the large Cardamom price was at a peak point that hit a high of NPR. 2500 per kg which went down to NPR. 750 per kg in 2019/20 (Gautam & Prasain, 2020). A study was carried out to know whether the cardamom farmers in Sankhuwasabha are benefitted or not and to know whether they can really be sustained in their livelihood or not.

METHODS AND METHODOLOGY

Selection of research site

The study was conducted in Sankhuwasabha district of Koshi province of Nepal. Cardamom cultivation area lies between 1000 m to 3000m above mean sea level.

Sample size, sampling procedure and selection of the respondents

A total of 100 households were selected to meet a goal for the survey in the study area. Large Cardamom farming households were surveyed and a sample was selected based on a simple random sampling method. Farmers cultivating Cardamom was selected.

Source of data

Primary data

The primary data were collected from the farmers of the study area by face-to-face interaction and key informant interview (KII) with a structured questionnaire to get the information regarding the economics and marketing of Cardamom production. The field survey was conducted during March to May, 2021.

Sources of secondary data

The secondary data related to Cardamom yield was collected from different organizations and institutions related to agronomy such as Krishi diary, Ministry of Agriculture and Livestock Development (MoALD), Central Bureau of Statistics (CBS). The data relating to the cost of production were collected from different journals published in national and international journals.

Methods and techniques of data analysis

The collected data was checked for accuracy and after collection of necessary information, it was entered in MS excel 2007. Same software was used for analysis. Estimation of Benefit-cost ratio, payback period and analysis of minimum support price was done.

Benefit-Cost Ratio : Total Revenue/Total Expenditure

Payback period= Number of year before full recovery+ (Absolute value of last negative cumulative cash flow / Cash flow in the year of first positive cumulative cash flow)

RESULTS AND DISCUSSION

Reason for choosing Cardamom cultivation with ranks

The survey in the area stated that majority of respondents choose Cardamom farming due to higher income than other crop, followed by easy marketing etc. The details about reason for choosing Cardamom cultivation is mentioned below in Table1:

Cost of Large Cardamom production

The cost for Cardamom production was estimated using production cost data received from field survey and secondary data from agribusiness promotion and marketing development directorate. Therefore, from base year to third year, an actual expense was collected through Focus group discussion. For the cost items from fourth year onwards 10% addition in production cost of previous year was added.

Cost of production of Large Cardamom has been found NPR.160797/ha in the 1st year which reduced to 44377 /ha in the 2nd year due to cutting off of cost of sapling and planting *Alnus spp.* and manuring cost. In the 3rd and 4thyear cost again increased to NPR.88353 and NPR. 94543/ ha respectively. The detail calculation is given in Table 2.

Financial Analysis of Large Cardamom

Financial analysis of the Large Cardamom cultivation as enterprise had been made to understand the profitability of the project. The study found that, Benefit-Cost Ratio (BCR) of Cardamom was 1.33 with the payback period of 4.89 year. The financial analysis revealed that the enterprise is profitable.

$$\text{Benefit} - \text{Cost Ratio}: \frac{\text{Total Revenue}}{\text{Total Expenditure}} = \frac{979073}{732310} = 1.33$$

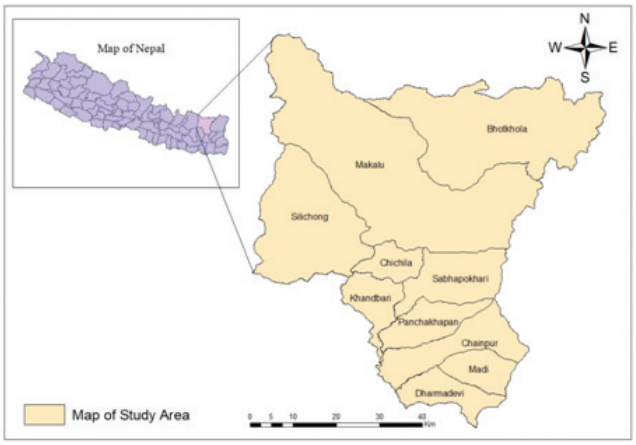

Fig. 1: Map of Nepal showing the study district, Sankhuwasabha

Table 1: Reason for choosing Cardamom cultivation in Sankhuwasabha district

Reasons	Index Value	Rank
Higher income than other crop	0.885	I
Easy Marketing	0.689	II
Easier than other crop cultivation	0.604	III
Marginal Land can be utilized	0.544	IV
Following the tradition	0.480	V

Table 2: Details of cost estimation for Large Cardamom production (per ha)

Description		Base year			Year 1	Year 2	Year 3
Particular	Unit	Quantity	Rate	Total	Total	Total	Total
Variable Cost							
Large Cardamom	D	166	550	91300	40700	42350	43000
sapling	No.	6119	10	61190	0	0	0
Alnus Sapling	No.	463	10	4630	0	0	0
Labor cost for Harvesting/Drying	D		550	0	0	29700	32450
Wood for Drying	NPR			0	0	4210	5000
Human labor for curing	NPR		1000	0	0	7000	9000
Transportation cost	NPR		1416	0	0	1416	1416
Total Variable Cost	**157120**	**40700**	**84676**	**90866**			
Fixed Cost							
Land tax	NPR		555	555	555	555	555
Water tax	NPR		443	443	443	443	443
Repair and maintenance	NPR		679	679	679	679	679
Miscellaneous	NPR		2000	2000	2000	2000	2000
Total fixed cost			**3677**	**3677**	**3677**	**3677**	
Total cost(1+2)			**160797**	**44377**	**88353**	**94543**	

Source: Field Survey, 2021Note: (D means Days, No. means No. of Labor, NPR means Nepalese Currency)

Table 3: Financial Analysis of Large Cardamom (per ha)

Year	Total expenditure (NPR)	Total income (NPR)	Net benefit (NPR)	Cumulative cash flow
Base Year	160797	0	(160797)	(160797)
Year 1	44377	0	(44377)	(205174)
Year 2	88353	0	(88353)	(293527)
Year 3	94543	250475	155932	(137595)
Year 4	104000	257400	153400	15805
Year 5	114400	249200	134800	150605
Year 6	125840	222000	96160	246765
Total	**732310**	**979073**	**246765**	

Source: Field Survey, 2021

Table 4: Analysis of Minimum Support Price (NPR.720/kg) from initial year of income

Year	Total expenditure (NPR)	Total income (NPR)	Net benefit (NPR)	Cumulative cash flow
Investment year	160797	0	(160797)	(160797)
Year 1	44377	0	(44377)	(205174)
Year 2	88353	0	(88353)	(293527)
Year 3	94543	167760	73217	(220310)
Year 4	104000	224640	120640	(99670)
Year 5	114400	256320	141920	42250
Year 6	125840	266400	140560	182810
Total	**732310**	**915120**	**182810**	**-**

Source: Field Survey, 2021
BCR = Total Income / Total Expenditure = 915120 / 732310 = 1.24
PBP = 5 + (99670/141920) = 5+0.70= 5.70 years

Payback period= No. of year before full recovery + (Absolute value of last negative cumulative cash flow / Cash flow in the year of first positive cumulative cash flow)

$$= 4 + \frac{137595}{153400} = 4 + 0.89 = 4.89 \text{ years.}$$

Calculation of Minimum Support Price

MSP per kg Cardamom =

$$\frac{\text{Cost of production} + 25\% \text{ cost of production}}{\text{Total yield}}$$

$$= \frac{732310 + 25\% \; of \; 732310}{1271} = 915387 / 1271 = 720$$

Analysis of Minimum Support Price

The Minimum support price per kg of large Cardamom is NPR720/ kg. It makes BCR 1.24 having payback 5.70 years in the study area.

CONCLUSION

In study area, it was found that the total cost of production of Cardamom per hectare as NPR.732310 (Base year to 6th year) with total income (NPR.979075) per hectare. The Benefit Cost Ratio of Cardamom was found to be 1.33 with payback period of 4.89 year. Based on the analysis of cost of production and financial analysis of the enterprise, we can conclude that the enterprise is profitable and feasible in the research area. Also

farmers should adopt the modern techniques to reduce the production cost rather than using man labour.

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Mutagenic effect on seed germination, seedling growth and seedling survival of Bael (*Aegle marmelos* Correa.)

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ABSTRACT

There is no named variety or cultivar in bael like other fruit crops. Mostly local selections having desirable traits are utilized for planting. Therefore, its breeding is crucial for creating new improved varieties. An effective method for generating variety and choosing superior plants appears to be mutation breeding. The current study was conducted in July 2022 at the Uttar Banga Krishi Viswavidyalaya at Pundibari Faculty of Horticulture Pomology and Post-Harvest Technology Laboratory and Teaching Farm. 100 numbers of Bael (Aegle marmelos Correa.) seeds treated in different concentration of EMS (0.25%, 0.50%, 0.75% and 1.00%) and Colchicine (0.25%, 0.50%, 0.75% and 1.00%) along with control with 4 replications following randomized block design. The study indicated that the percentage of seed germination was decreased with increasing concentrations and doses, when compared to the control. On the basis of the percentage of germination of seeds, the LD_{50} (Lethal dosage) value was calculated. The 50% decrease in seed germination was seen in both the 0.75% of EMS (T_4) and 1.00% in Colchicine (T_5) treatments, and regarded as the LD_{50} value for both. The maximum shoot length, root length, seedling length and seedling survival % were observed in 0.25 % EMS (T_2) whereas, root length and seedling survival % in 0.25% of colchicines (T_2) but shoot length, total seedling length and numbers of leaves in 0.75% (T_3). The lowest were observed in 1.00% of Ethyl Methane Sulfonate and Colchicine.

***Keywords*:** Bael, colchicine, EMS, lethal dose, mutagen, seedling survival

INTRODUCTION

Bael (*Aegle marmelos* Correa.), significant crop of Rutaceae family with high medicinal value. Plant is robust by nature, has strong nutritional content, and is well-suited for processing (Kundu and Ghosh., 2017). In addition to the fruit, the entire plant, including the leaves, wood, roots, and bark, is utilized in ayurveda medicine and for other purposes (Sharath *et al.,* 2016). The locally available genotypes of bael need to be identified and evaluate to develop a good cultivar. The high degree of variability with desirable quantity and qualitative characters are useful tools to identify new genotypes. Although bael is rarely grown in systematic orchards, it is typically grown in parks, backyards of houses, gardens and temples. Recently it has been produced for commercial orchards, making it one of India's underutilized fruits.

Genetic advancement by induced mutation in the agricultural sector, has a wide range of applications to create new varieties with better traits that are resistant/tolerant to disease, insects, drought, salinity, heat, and pests. When compared to other chemical mutagens, EMS and Colchicine significantly increase the variability of plant materials. The frequency and saturation of mutations may be controlled by altering the dosage of the mutagenic agent (Menda *et al*., 2004). Mutagen cause varied length fragments with sudden insertions or deletions (Kim *et al*., 2006). EMS and Colchicine also improved plant morphological characteristics (Vikhe and Nehul, 2020). Ethyl Methane Sulphonate (EMS) is a mutagenic and carcinogenic organic compound that is thought to be the most potent and effective mutagen as it replaces nucleotide by abnormal base pairing (Waungh *et al.*, 2006). Colchicines is an efficient polyploidy mutagen to create variability by doubling the chromosome number (Liu and Guan, 2006). Colchicine, at a concentration of 0.1%, was found to accelerate the emergence of early seedlings, improve plant morphological traits, and increase the generation of total chlorophyll and total carbohydrates in Jamun leaves (Barman *et al*., 2014). Considering high degree of mutagenic effect of EMS and Colchicine, these chemicals were used

to know their LD_{50} value and the seedling behavior in bael for creating new genotype/s suitable for *Terai* region of West Bengal, India.

MATERIALS AND METHODS

The present investigation was carried out during July 2022 in the Laboratory and instructional Farm of Pomology and Post-Harvest Technology, Faculty of Horticulture at Uttar Banga Krishi Vishwavidyalaya, Pundibari. Mature fruits were collected from the trees which grown at the university farm. Seeds were extracted from ripe fruits, and washed thoroughly with running water. Immediately after extraction, the seeds of bael were treated with different doses of EMS (0.25%, 0.50%, 0.75% and 1.00%) and Colchicine (0.25%, 0.50%, 0.75% and 1.00%) along with control with 4 replications for studying the impact on seed germination. For EMS treatment, 100 numbers of healthy seeds were treated with 0.25%, 0.50%, 0.75% and 1.0% freshly prepared EMS solution in 0.1 M phosphate buffer maintaining pH-7.0 for 8 hours. Whereas for colchicine treatments, 100 numbers of healthy seeds were treated with 0.25%, 0.50%, 0.75% and 1.0% freshly prepared aqueous colchicine solution for 8 hours. After 8 hours the EMS and Colchicine treated seeds were washed thoroughly for 1 hour in running water to eliminate the residual effect of the chemicals. After that, the seeds were sown immediately in the black polythene bag containing a single seed per bag for germination. The treatments were arranged in randomized block design with four replications. 100 numbers of seeds were treated for each replication of each treatment. Single seed was sown in each polythene bag. Four seedlings per replication were randomly selected for shoot length (cm), root length (cm), total seedling length (cm), number of leaves and seedling survival at 60 days after seed sowing. The germination percentage, seedlings survival percentage, shoot length, root length and total seedling length were calculated as follows:

$$\text{Germination Percentage} = \frac{\text{Total number of emerged seedling}}{\text{Total number of planted seeds}} \times 100$$

$$\text{Seedling survival percentage} = \frac{\text{Total number of germinated seed}}{\text{Total number of seeds sown}} \times 100$$

Shoot length (cm) = Shoot length was calculated by measuring the shoot length with the help of a scale from the cut-base of soil line to the shoot tip of 4 plants in each replication and average value was calculated

Root length (cm) = Length of root was measured with a scale from the cut-base to the tip of taproot of 4 plants in each replication and average value was calculated.

Total seedling length (cm) =Total seedling length was calculated by measuring the full length of the seedling (shoot length + root length) after uprooting 60 days after germination.

RESULTS AND DISCUSSION

Germination percentage (%)

The data presented in Table 1 showed that seed germination was highest in control (untreated seeds) 91.00%. Regarding effect of EMS and Colchicine on seed germination, it was observed that germination percentage was decreased with the increase of concentrations irrespective of mutagens. Highest germination of 75.25 per cent was noted with EMS 0.25% concentration and lowest of 39.75 per cent with 1.00 % EMS. Similar mutagenic effect of EMS was observed by Singh *et al*. (2021) in short day onion. In case of Colchicine, highest germination was with 0.25 per cent (83.50%) and lowest with 1.0% (50.25%). LD_{50} (Lethal dosage) was for EMS was 0.75 % while 1.00 per cent was for Colchicine. From this investigation it was assumed that Colchicine was better mutagen than EMS for getting higher seedlings populations in bael. The preceding studies by El-Latif *et al.* (2018) in Papaya provided support for the findings. Interruptions at the molecular material may be responsible for the decreased seed germination with greater doses/concentrations of the mutagens. Similar findings were reported by Kumar and Mishra (2004) in where of germination was reduced with increasing concentrations of physical and chemical mutagen.

Seedling growth

The maximum shoot length (29.38 cm), root length (24.58 cm) and seedling length (53.95 cm)

Table 1: Effects of EMS and Colchicine on seed germination and seedling behavior of bael

Treatment details	Germination percentage (%)	Shoot length (cm)	Root length (cm)	Total seedling length (cm)	Number of leaves	Seedling survival percentage (%)
Control	91.00	27.70	22.70	50.40	8.50	86.00
0.25% EMS	75.25	29.38	24.58	53.95	7.50	70.75
0.50% EMS	61.50	23.70	17.80	41.50	6.50	55.00
0.75% EMS	50.50	20.48	13.58	34.05	6.75	42.00
1.00% EMS	39.75	18.80	13.48	32.27	5.00	30.50
0.25% Colchicine	83.50	22.10	25.28	47.38	6.25	74.25
0.50% Colchicine	75.25	27.83	21.43	49.25	9.50	61.50
0.75% Colchicine	61.00	22.30	16.70	39.00	7.50	52.75
1.00% Colchicine	50.25	21.83	16.63	38.45	6.50	40.50
CD (0.05%)	**3.90**	**3.60**	**4.79**	**6.48**	**1.66**	**4.73**
SEm (±)	**1.36**	**1.26**	**1.67**	**2.26**	**0.58**	**1.65**

in 0.25% EMS treated plants (Table 1). It was decreased along with a similar rise in EMS dosages. The minimum shoot length (18.80 cm), root length (13.48 cm) and seedling length (32.27 cm) in 1.00% EMS. Singh *et al.* (2022) reported that higher dose of mutagen inhibits plant height in Mosambi. The highest shoot length (27.83 cm), root length (21.43 cm) and seedling length (49.25 cm) was observed in 0.50% Colchicine while the lowest shoot length (21.83 cm), root length (16.63 cm) and seedling length (38.45 cm) was noted in 1.00% Colchicine. When compared to EMS, Colchicine caused a smaller reduction in seedling height. Similar findings were reported by various researchers for sunflower (Jayakumar and Selvaraj, 2003) and gladiolus for colchicine (Manzoor *et al*., 2018). The root and shoot length were reduced with increasing concentration of mutagen was indicative of the inhibitory impact of mutagens on seedling length. The activation of growth hormone, such as auxin, and an increase in cell division rates were theorized to be the causes of these stimulations by EMS and Colchicine treatments (Zaka *et al*., 2004).

Number of leaves

From the Table 1 it was observed that the minimum numbers of leaves were obtained in 1.00% EMS (5.00), whereas the maximum leaves was recorded in 0.50% colchicine (9.50).

Seedling survival percentage (%)

After 60 days of germination the survival percentage was maximum (86.00%) in control followed by 74.25% in 0.25 % Colchicine and 70.75% in 0.25% EMS whereas the minimum was reported in 1.00% EMS (30.50%) (Table 1). With a rise in the concentration of both mutagens, a progressive decline in plant survival was seen. It was maximum in Colchicine at 0.50% (74.25%) and lowest in Colchicine at 1.00% (40.50%). Auti (2005), Dhanavel *et al*. (2008), Kavithamni *et al.* (2008), Potdukhe and Narkhede (2002) found decreased survival rates as a result of mutagenesis treatments in different crops.

CONCLUSION

From the investigation it was concluded that the 50 per cent seed germination rate was observed in EMS (T_4) at 0.75% and Colchicine at 1.00%. The development of seedlings and the percentage of seeds that germinate were suppressed when mutagen concentrations/doses increased. As the concentration or dose of the mutagens increased, the survival rate significantly decreased. Almost every mutagenesis treatment reduced the length of the shoots, the roots, the entire seedling, and the number of leaves per seedling.

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Effect of seed treatments on seed germination and seedling growth of Indian Olive (*Elaeocarpus floribundus*)

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ABSTRACT

Indian olive (Elaeocarpus floribundus) is propagated by seeds. Although germination and seedling growth is a major concern in this crop as seeds remain dormant for long time. There are various methods like scarification, stratification, chemical treatment, biological and irradiation etc. for breaking the seed dormancy of Indian olive. However there is very few scientific reports regarding overcoming the dormancy of Indian olive seeds. Keeping this view, Indian olive seeds has been collected and stored for 2 months followed by seed treatment. Seeds were treated with seven different types of chemicals (HCl @ 5%, H_2SO_4 @5%, GA_3@500 ppm, soaking in water, Benzyl adenine@100 ppm, thiourea@1% and KNO_3 0.5%) and planted in poly bags filled with growing media (sand: garden soil: FYM=1:1:1). Maximum germination was recorded in the seeds treated with Benzyl Adenine@100 ppm followed by H_2SO_4@5%, whereas highest speed of germination was noticed in H_2SO_4 @5% followed by Benzyl Adenine @100 ppm. The maximum average height of the seedlings (12.26 cm) and average number of leaves (6.86) was recorded in Benzyl Adenine treatment @100 ppm followed by H_2SO_4@5%. Biggest leaf size and greater seedling girth was also observed under Benzyl Adenine treatment @100 ppm. Seedling survival was also better under BA @100ppm treatment.

***Key wards*:** Indian olive, seed treatments, seed germination and seedling growth.

INTRODUCTION

Indian olive (*Elaeocarpus floribundus*) belongs to the family Elaeocarpaceae. The tree is found in eastern Himalayas up to 3,000 ft and in the evergreen forests of North Kanara and western coast down to Travancore (Bhowmick, 2011). The fruit is somewhat similar to the fruit shape of olive, so it also known as Indian olive or 'Jalpai' in Bengali. The olive fruits are famous for medicinal property possessing an array of bioactive phytochemicals (Sircar and Mandal, 2017). It is a tropical and subtropical, evergreen, medium size, hard seeded fruit crop. Seed is the most natural resource of plant reproduction, preservation of genetic viability, transportation and propagation of flora. Indian Olive is generally propagated by seeds but seed germination rate is very low due to seed dormancy. Large scale plantation in agro forestry, social forestry and home garden is limited due to poor seed germination and deferred nursery establishment. Seed dormancy is a major concern in germination due to hard seed coat. To overcome dormancy of Indian olive seeds different pre-sowing chemical treatments is commonly followed (Basavaraj and Prabhuling, 2020). It may be overcome either by pre-treatment of seed by scarification, stratification etc. However, out of these methods seed treatment is the easiest way to enhance seed germination by breaking the dormancy and then seed treatment can ensure success in seed germination. Considering the importance of seed treatment, the present experiment has been carried out to study the effect of chemical pretreatment on germination and seedling growth of Indian olive.

MATERIALS AND METHODS

The experiment was conducted at Horticulture farm, Department of Horticulture and Post-Harvest Technology, Palli Siksha Bhavana, Visva-Bharti, Sriniketan, West Bengal during the year 2022. Mature fruits of *Elaeocarpus floribundus* were

collected from a single plant from New-alluvial zone, Habra-1 block under district North 24 Parganas, West Bengal. Pulps of the fully mature ripe fruits were removed by scraping and seeds were then properly washed. Washed seeds have been dried under shed for 7 days and stored for 2 months in perforated polythene packet under ambient condition. After 2 months of storage, the seeds were soaked in different treatment solutions, then washed in plain water and then placed in petri-dish with moist filter paper under laboratory condition for 24 hours during January, 2022. Different treatment were i) HCl @ 5% for 6 hours then washing and again soaking in normal water for 12 hours (T_1), ii) H_2SO_4 @ 5% for 6 hours then washing and again soaking in normal water for 12 hours (T_2), iii) GA_3 500ppm for 24 hours (T_3), iv) KNO_3 @ 5% for 24 hours (T_4), v) Benzyl Ademine (BA) @ 100ppm for 12 hours (T_5), vi) Thio-urea @ 1% for 24 hours (T_6) and vii) control untreated (T_7). Treated seeds were sown immediately in polybags (12cm x 10 cm) containing media comprising of garden soil, sand and FYM at a ratio of 1:1:1 at a depth of 5 cm. The experiment was laid out in completely randomized design (C.R.D.) with seven treatments and all the treatments were composed of three replications each with 50 seeds. All the polybags were then kept under shade. Light irrigation was given with rose can after sowing. Regular watering to the poly bags has been done with rose can at an interval of 7 days to ensure good germination.

Regular weeding and application of pesticide and fungicide have been carried out to get healthy seedling. The count of germinated seedlings was taken at an interval of one day after 70 days of seed sowing and up to when germination ended (120 days after sowing). In the present experiment data with respect to different plant growth parameters has been recorded at 150 days after seed sowing. Observations like seedling height, number of leaves per seedling, chlorophyll content of leaves, leaf length and leaf width were taken. SPAD (Soil Plant Analysis Development) chlorophyll meter was used to measure leaf chlorophyll content. The percentage of germination was calculated on the basis of total numbers of seeds sown and total numbers of germinated seeds.

RESULTS AND DISCUSSION

Seed Germination

Significant variation was observed in the seed germination as effected by pre sowing chemical treatments of Indian olive seeds. The highest germination percentage (56.69) was observed in the seeds treated with Benzyl Adenine (BA)@100ppm (T_5). The most used frequently cytokinins are N-(Phenyl methyl)-7H-purin-6-amine (benzyladenine; 6-Benzyladenine or 6-Benzylaminopurine or BAP) and kinetin (Kn) or 6-furfurylaminopurine. The cytokinins regulate growth and effect on germination rate in a variety of ways in different plants (El-Ghamery and Mousa, 2017; Graeber *et al*., 2012). They are active in all germination cycles (Chiwocha *et al*., 2005; Nikolic *et al*., 2006; Riefler *et al*., 2006). The germination percentage was 56.69% which signifies that the chemical was able to break seed germination to a great extent as compared to other chemicals. The lowest germination was observed in seeds treated with GA_3 @ 500ppm (T_3) which showed germination percentage of 13.32%. The findings of the present experiment corroborate with the findings of Parab *et al.* (2017) where pre sowing papaya seed treatment with Benzyl Adenine showed better result.

Days to first germination

Seeds treated with H_2SO_4 (T_2) took significantly minimum time (73.27 days) to start germination while KNO_3 (T_4) treatment of seeds took maximum time (84.62 days) to start the germination. Treatment of Indian olive seeds with HCl (T_1) as well as GA_3 (T_3) also improved the earliness of germination (74.13 and 74.47 days respectively) in the present experiment. On contrary little late germination (82.19 days) was observed in control treatment (T_7). Perhaps the stratification effect of HCl over the hard seed coat of Indian olive triggered the quick germination. But late germination in case of KNO_3 treatment may be due some inhibitory effect of nitrate ions in the solution (Graeber *et al*., 2012).

Germination duration

Shortest span of germination (16.28 days) of Indian olive seeds was observed under thio-urea

Table 1: Effect of seed chemical treatment on germination and seedling growth in *Elaeocarpus floribundus*

Treatments	Germination (%)	Days to 1st germination	Germination duration	Seedling height (cm)	No of leaves per plant	Chlorophyll content (spad)	Leaf length (cm)	Leaf width (cm)
T_1	23.37	74.13	34.24	11.52	5.38	39.48	7.53	2.56
T_2	40.16	73.27	16.72	10.68	6.86	54.73	7.18	2.84
T_3	13.32	74.47	34.19	6.72	3.47	39.46	4.14	1.54
T_4	30.16	84.62	32.41	10.16	4.19	48.92	7.02	2.71
T_5	56.69	78.38	22.13	12.26	5.12	87.13	7.38	2.83
T_6	20.14	78.67	16.28	9.72	4.85	37.14	7.19	3.06
T_7	13.34	82.19	25.17	8.39	4.54	95.65	6.52	2.64
SE±M	0.71	2.6	1.2	0.5	0.3	4.5	0.27	0.4
$CD_{(0.05)}$	2.13	8.1	3.3	1.45	0.8	13.6	0.8	1.1

T_1: HCl @ 5% for 6 hours then washing and again soaking in normal water for 12 hours

T_2: H_2SO_4 @5% for 6 hours then washing and again soaking in normal water for 12 hours

T_3: GA_3 500ppm for 24 hours

T_4: KNO_3 @ 5% for 24 hours

T_5: Benzyl Ademine (BA) @ 100ppm for 12 hours

T_6: Thio-urea @ 1% for 24 hours and

T_7: Control (untreated)

treatment (T_6) which was closely followed by H_2SO_4 treatment of seeds (T_2) which required 16.72 days. On contrary wide span of germination (34.24) was observed under HCl treatment (T_1) which was statistically *at par* with that of GA3 treatment (T_3) (34.19 days). Benzyl adenine treatment also reduced the duration of germination (22.13 days) of Indian olive seeds in the present experiment.

Plant height and number of leaf per plant

The highest plant height (12.26 cm) was observed in the seeds treated with Benzyl Adenine (BA) @100ppm (T_5) which was followed by treatment with HCL (T_1) and H_2SO_4 (T_2) it may be due to the long duration of plant growth under these two treatments as HCl and H_2SO_4 has showed faster germination while BA has showed little bit late germination. Due to the maximum time of plant growth the plant height of T_1 and T_2 statistically similar with the plant height of T_5. Results were also observed with seeds treated with HCl @ 0.5% (T_1) showing height of 11.5 cm. Lowest plant height was observed in seeds treated with GA_3 @ 500ppm(T_3) showing height of 6.72cm. In the case of pre sowing treatment of Maringa seeds highest seedling growth was observed in Benzyl adenine100 mgl^{-1} (El Dayem *el al.,* 2021). Number of leaves per plant varied from 3.47 (T_3) to 6.86 (T_2).

Leaf chlorophyll content

In the case of leaf chlorophyll content of the present experiment as the control treatment exhibited the maximum chlorophyll content and in the other treatments chlorophyll content was lower. Thus, the result indicates that all the chemical pre-treatments have some inhibitory effect on chlorophyll development during the successive development of seedlings. The leaf chlorophyll content of Indian olive seedling has been recorded in Data every 3 days after 1 month of germination. Highest chlorophyll content (95.65 SPAD unit) was found from control treatment (T_7) which was statistically similar with T_5 (87.13 SPAD unit). Lowest Chlorophyll content was found in seeds treated with Thiourea @ 1% (T_6) showing chlorophyll content of 37.16 SPAD unit.

Leaf length and width

A significant variation was observed between leaf length and leaf width of the Indian olive seedlings in the present experiment. Leaf length of the seedlings varies between 4.14cm (T_3) to 7.53cm (T_1). Leaf length of T_5 (7.38 cm) was at par with T_1. Leaf width was between 1.54 cm (T_3) to 3.06 cm (T_6). Leaf width of T_5 (2.83 cm) was statistically similar with T_6.

CONCLUSION

From the findings of the present experiment, it can be concluded that for better germination of Indian olive seeds the seed treatment with Benzyl Adenine @100 ppm for 12 hours was most effective as well as in breaking the dormancy. Seed treatment with H_2SO_4 @ 5% can also improve seed germination of Indian olive significantly. Benzyl adenine also increases plant height compared to the other treatments.

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Exploration and collection of different germplasm accessions of Oregano (*Origanum vulgare* L.) from the Kashmir Himalayas

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ABSTRACT

The collection of accessions of Origanum vulgare is an important resource for its conservation and utilization. To explore and collection of the different accessions of Origanum vulgare, an extensive survey was conducted across the Kashmir Himalaya. These exploration trips were concerted in North, Central and South zones of Kashmir Himalaya. The germplasm collections were conducted from late march to mid-May. This extensive survey yielded a total of nineteen accessions from different places; of which 17 accessions were from wild origins and two accessions were from cultivated sources. The germplasms were submitted to the National Bureau of Plant Genetic Resources (NBPGR) gene bank for their preservation and allotment of IC numbers. All the live plants of all the accessions were maintained in experimental plots at the Faculty of Forestry, SKUAST-K. These accessions can be used to maintain the species' genetic diversity, improvement of culinary, medicinal, and ornamental qualities, and to ensure its long-term survival.

***Keywords*:** Collection, germplasm accession, Kashmir**,** NBPGR, *Origanum vulgare*

INTRODUCTION

Organum is a large and diverse genus of plants belonging to the family Lamiaceae. This genus includes 49 taxa belonging to 10 sections (Ietswaart, 1980). Several species including *Origanum vulgare* L. are rich in essential oils and are commonly known as Oregano (Skoulaand Harborne, 2002). It is a perennial herb that grows to a height of 1.5 m and is distributed in the Mediterranean region, the Middle East, China and South Asia (Kokkini, 2002).The plant has been introduced to many other parts of the world and is now found in temperate and subtropical regions worldwide. The herb of oregano is widely consumed and traded as a culinary spice globally (Kaefer and Milner, 2008). It has been established that the herb contains a large diversity of secondary metabolites particularly thymol, carvacrol sometimes linalool (Lukas *et al*., 2008; Nurzynska-Wierdak *et al.,* 2012; Mastro *et al.,* 2017, Machado *et al.,* 2023) which are regarded as the signature class of compounds for recognizing the quality oregano. And due to these compounds, it is used for centuries as a traditional medicinal plant for curing myriad diseases across the globe. The same has been established through numerous clinical trials that a herb possesses a potential pharmacological activity especially, anti-microbial, anti-fungal, anti-oxidant and expectorant (Cleff *et al.,* 2010; Senderski, 2014; Brđanin *et al.,* 2015; Brondani *et al.,* 2018; Campos *et al.,* 2022).

In India*, Origanum vulgare* subsp. vulgare (common oregano) is spread abundantly in the central, north and northeastern Himalayan region (Sarin, *et al.,* 1991) and is the only representative of *Origanum vulgare* in India (Chishti *et al.,* 2013). It is recognized with different names depending

upon regional dialect, in Kashmiri, it's famous as (van babber) in Persian (marzanjosh) in Arabic (*za'atar)* in Hindi (ban tulsi). The herb of *Origanum* is very famous among the tribal communities of Kashmir Himalaya (Atta, 2017). Exploration and collection of accessions/populations of *Origanum vulgare* is an important step in preserving the genetic diversity of the species. Leto and Salamone, (1997) collected 214 biotypes from 24 sites in the Mediterranean and were maintained for ex-situ conditions. The biotypes varied in morphology thus exhibiting great diversity. In a similar study, 70 accessions of *Origanum vulgare* were collected and results after their establishment showed variability in their agro-morphic traits (Mastro, 1997). Likewise, the exploration and collection of Origanum accessions from Europe (Raduõienë *et al.,* 2005; Azizi *et al.,* 2012; Sivicka *et al.,* 2013; Kosakowska and Czupa, 2018; Myagkikh *et al.,* 2020; Weglarz *et al*., 2020), and Iran (Morshedloo *et al.,* 2018) have been done for the appraisal of quality evaluation based on their morphological traits, production potential and essential oil characterization by isolating the elite lines from collected germplasm. Nevertheless, such research has been subject to only a few studies in India (Chauhan *et al.,* 2013, Raina and Negi, 2014; Manivel *et al*., 2019). And thus our present investigation is the first step towards identifying and collection of Oregano biotypes from different geographical locales of Kashmir Himalaya to make a base for the future improvement of this species for its commercial exploitations.

MATERIALS AND METHODS

The Collection assignment for germplasm accessions of oregano was meticulously planned to collect large populations from the wild as well as the cultivated origins in the whole Kashmir valley. For this purpose, an extensive preliminary survey was conducted across the Kashmir Himalaya during 2019-20 and during the survey, nineteen sites (Fig. 1.) were identified out of which 17-sites were from the wild origin and 2-sites were from cultivated sources. During the collection following sites were explored from the wild; Uri(chakra), Tral(buchoo), Mawar, Dachigam, Bamanhaar, Astanmarg, Zuthan, Sanzipora, Kiterdij, Urpash, Lar, Naranag, Yusmarg, Izmarg, Markoot, Khilanmarg, Tulail (safeed-aab) while the accessions from cultivated sources were procured from CSIR-IIIM Kashmir at their two sub-stations (Yarikha, Tangmarg and Bonera, Pulwama) (Table 1) The collected accessions were submitted to the NBPGR, New Delhi to store in the national gene bank and national idendity IC numbers were obtained. Also, while collection the soil samples were collected which were later analyzed for pH, EC and soil texture class with the aid of *Mridaparikshak* (mini soil testing kit developed by ICAR-Indian Institute of Soil Science, Bhopal)

RESULTS AND DISCUSSION

Biotypes/accessions are distinct varieties of a species that are adapted to specific environmental conditions. Exploration and collection can help us

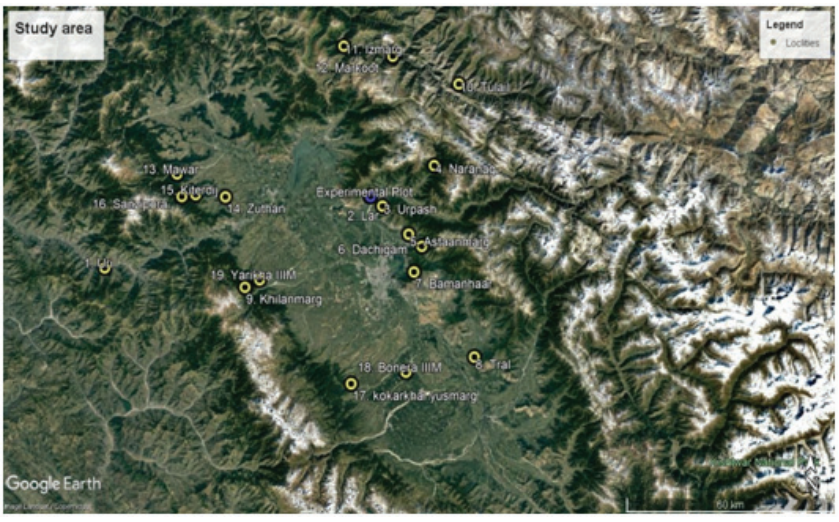

Fig.1: Map of collection sites of (wild & cultivated) accessions of *Origanum vulgare* L.

Table 1: Passport data sheet of *Origanum vulgare* accessions collected from different ecological niches of Kashmir Himalaya

S.No	Precinct	Collector No.	IC-No.	Type of material	Collecting site/ acquisition source	Frequency	Sampling method	Habitat
1.	Uri (Chakra)	UA/SAG/706	IC-0644413	Cuttings	Wild	Abundant	Random	Forest
2.	Tral (Buchoo)	UA/SAG/707	IC-0644414	Cuttings	Wild	Frequent	Random	Orchard
3.	Mawar	UA/SAG/717	IC-0644423	Cuttings	Wild	Frequent	Random	Forest
4.	Bamanhaar	UA/SAG/703	IC-0644410	Cuttings	Wild	Abundant	Random	Orchard
5.	Pulwama(Bonera-IIIM)	UA/SAG/710	IC-0644417	Cuttings	Cultivated	-	Random	Cultivated
6.	Dachigam	UA/SAG/709	IC-0644416	Cuttings	Wild	Frequent	Random	Forest
7.	Zuthan	UA/SAG/716	IC-0644422	Cuttings	Wild	Frequent	Random	Forest
8.	Sanzipora	UA/SAG/718	IC-0644424	Cuttings	Wild	Occasional	Bulk	Forest
9.	Kiterdij	UA/SAG/719	IC-0644425	Cuttings	Wild	Occasional	Bulk	Forest
10.	Urpash	UA/SAG/702	IC-0644409	Cuttings	Wild	Abundant	Random	Orchard
11.	Lar hills	UA/SAG/07	IC-063889	Cuttings	Wild	Frequent	Random	Forest
12.	Yusmarg (Kokarkhal)	UA/SAG/704	IC-0644411	Cuttings	Wild	Occasional	Bulk	Orchard
13.	Tangmarg(yarikha-IIIM)	UA/SAG/08	IC-063890	Cuttings	Cultivate	-	Bulk	Cultivated
14.	Naranag	UA/SAG/708	IC-0644415	Cuttings	Wild	Abundant	Random	Forest
15.	Astaanmarg	UA/SAG/705	IC-0644412	Cuttings	Wild	Occasional	Bulk	Roadside
16.	Izmarg	UA/SAG/711	IC-0644418	Cuttings	Wild	Abundant	Random	Meadow
17.	Markoot	UA/SAG/712	IC-0644419	Cuttings	Wild	Frequent	Random	Forest
18.	Khilanmarg	UA/SAG/715	IC-0644421	Cuttings	Wild	Frequent	Bulk	Meadow
19.	Tulail(safeed-aab)	UA/SAG/AS/713	IC-0644420	Cuttings	Wild	Abundant	Random	Forest

Table 2: The geographical localization & soil Physiochemical attributes of different sites of investigated populations

S.No	IC-No.	Geo-coordinates			Physiochemical analysis of soil		
		Latitude	Longitude	Altitude (m)	Soil pH	Soil E.C	Soil texture
1.	IC-0644413	34^{0}05′.94″	73^{0}57′.49″	1237(m)	7.03±0.04	1.64±0.03	Clay loamy
2.	IC-0644414	33^{0}54′.41″	75^{0}05′.67″	1609(m)	6.35±0.03	1.18±0.05	Loamy
3.	IC-0644423	34^{0}12′.10″	74^{0}07′.44″	1635(m)	6.36±0.13	0.79±0.02	Loamy
4.	IC-0644410	34^{0}06′.31″	74^{0}53′.80″	1655(m)	6.51±0.15	0.82±0.01	Loamy
5.	IC-0644417*	34^{0}50′.92″	74^{0}53′.17″	1701(m)	6.86±0.09	1.61±0.06	Loamy
6.	IC-0644416	34^{0}15′.36″	74^{0}92′.17″	1709(m)	6.24±0.96	1.92±0.04	Loamy
7.	IC-0644422	34^{0}18′.10″	74^{0}16′.10″	1724(m)	6.27±0.03	1.33±0.07	Loamy
8.	IC-0644424	34^{0}21′.70″	74^{0}11′.23″	1731(m)	6.37±0.06	0.94±0.09	Loamy
9.	IC-0644425	34^{0}17′.36″	74^{0}11′.37″	1784(m)	6.93±0.07	1.04±0.05	Loamy
10.	IC-0644409	34^{0}14′.98″	74^{0}48′.29″	1827(m)	6.91±0.05	0.85±0.11	Loamy
11.	IC-063889	34^{0}16′.72″	75^{0}46′.37″	1927(m)	6.94±0.11	0.08±0.17	Loamy
12.	IC-0644411	33^{0}50′.39″	74^{0}45′.57″	2087(m)	6.36±0.07	0.43±0.02	Loamy
13.	IC-063890*	34^{0}04′.51″	74^{0}25′.43″	2119(m)	6.67±0.05	0.91±0.05	Loamy
14.	IC-0644415	34^{0}21′.13″	74^{0}58′.53″	2281(m)	7.03±0.04	1.17±0.08	Loamy
15.	IC-0644412	34^{0}11′.21″	74^{0}45′.57″	2290(m)	6.73±0.01	0.07±0.01	Clay loamy
16.	IC-0644418	34^{0}39′.28″	74^{0}40′.54″	2358(m)	6.55±0.05	1.3±0.1	Loamy
17.	IC-0644419	34^{0}37′.45″	74^{0}50′.43″	2466(m)	6.79±0.09	1.41±0.11	Loamy
18.	IC-0644421	34^{0}04′.75″	74^{0}38′.53″	2532(m)	6.96±0.04	1.27±0.03	Clay loamy
19.	IC-0644420	34^{0}33′.40″	75^{0}01′.43″	3171(m)	7.01±0.02	0.14±0.03	Loamy

Note: *represent cultivated accessions

identify new sources of genes that can be used to breed new varieties of oregano with improved traits, such as increased resistance to pests or diseases, or improved flavor. The observations made from the data about the geo-coordinates and soil analysis collected from the different ecological niches in Kashmir Himalayas, (Table 2.) revealed that the *Origanum vulgare* L. (Oregano) finds its habitual zone in an elevation between 1237-3171m in the clay loamy- loamy soils having pH ranging from 5.9- 7.3 The plants of oregano usually occurs in the places which are; open, previously cleared forest patches, semi-dry meadows, forest edges, wastelands, elevated bunds of horticulture lands etc. Also, it was observed that oregano thrives well in the extreme competition of surrounding species. The places facing the southern or southeastern slope receiving more sunlight for a longer time duration were witnessed with abundant growth of *Origanum vulgare* than the north-facing slopes where growth was limited. Our results are in line with studies made earlier in the temperate Himalayas (Jan *et al*., 2018; Tewari *et al*., 2015) and also in the Mediterranean region (Skoula and Harborne, 2002; Meyers, 2005, Bonfanti *et al*., 2012) which suggests that *Origanum vulgare* L. can adapt itself to diverse environmental conditions.

CONCLUSION

Exploration and collection of biotypes of *Origanum vulgare* is an important step in preserving the genetic diversity of the species. By identifying and conserving the biotypes of the species, we can ensure that the species will continue to be available for future generations. Moreover, exploration and collection can be used to identify new sources of genes that can be used to breed improved varieties of oregano. In the present study, a substantial number of oregano germplasm accessions (19) were collected from the Kashmir Himalaya and conserved.

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Influence of organic manures and fruit coatings on biochemical parameters of papaya cv. Arka Prabhat

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ABSTRACT

A study was done to determine how long papaya could be stored by using recommended dose of nitrogen supplied with the organic manures viz., FYM, Vermicompost, Neem cake and sheep manure alone and in combinations and the harvested fruits are coated with aloe gel and bee wax. Among the treatment combinations, ascorbic acid content was observed highest in plants treated with 100% recommended dose of nitrogen (RDN) with sheep manure 63.37 mg $100g^{-1}$and coated with aloe gel 53.18 mg $100g^{-1}$ whereas minimum 37.82 mg $100g^{-1}$ in fruits of plants applied with 100% recommended dose of fertilizers(RDF) and in uncoated fruits 48.49 mg $100g^{-1}$ on 12^{th} day of storage. The highest total soluble solids of 12.77°Brix recorded in fruits plants which were treated by RDN 100% sheep manure and in gel coated fruits with 12.46 °Brix whereas minimum of 10.84°Brix in fruits of plants applied with 100% recommended dose of fertilizers(RDF) and in uncoated fruits (11.75°Brix) on 12^{th} day of storage. The fruits of plants treated with vermicompost had the greatest titrable acidity 0.115%. and in fruits coated with aloe (0.097 %) and the lowest was noticed in fruits of plants supplied with complete dose of recommended inorganic fertilizersof 0.077% and in uncoated fruits 0.087% on 12^{th} day of storage. The total sugars of 9.03% was noticed highest in fruit plants treated by neem cake RDN100% and in gel coated fruits(8.34%) and lowest in fruits applied with 50% RDN FYM + Neem cake 50% RDN (7.72%) and in uncoated fruits7.93% on 12^{th} day of storage respectively.

***Keywords*:** Chemical composition, bio-coating, organic manures, papaya, storage

INTRODUCTION

Papaya is one of the important fruit crops of tropical and subtropical regions of the world. It is one of the few fruit crops that flowers and fruits throughout the year giving early (9-10 months after planting) and high yields of about 100 tones per hectare. Papaya is rich in minerals like potassium followed by sodium, phosphorous, zinc, calcium, iron and vitamins *viz*., vitamin A, C, B1 and B2, niacin and in fiber content. It has digestive enzyme papain which aids in digestion and cures many digestive related problems. It is rich in antioxidants, antimicrobial, ant carminative, and immunological properties (Farhan *et al.,*2014). However, the ripe fruits are highly perishable and cannot be stored for a longer duration. The perishability of papaya fruit was caused due to weight loss, moisture loss, softening of the flesh and prone to microbes. Since ancient times, an edible coat is used to prevent perishability of produce from deterioration by delaying dehydration, reduce the respiration, improve the texture quality and reduces microbial growth. The weak cell wall integrity of papaya directly contributes to their lower shelf life when compared to other tropical fruits. Some edible coatings, packing materials, and value addition can extend the shelf life of papaya. Despite a good papaya production in India, there is no primary processing units at the farm or wholesale/retailer levels. These are sold without proper packaging right away after harvesting. There is a lot of demand for the processed products and these can be stored for longer time than the whole fruit as a benefit to escape from perishability nature of papaya.

Due to the poor shelf life and inadequate postharvest processing, a considerable amount of papaya fruit will lose before reaching the market in underdeveloped nations. (Emana and Gebremedhin, 2007). According to estimates, papaya post-harvest losses totaled 25.49%, with 1.66% of those losses occurring in the field, 4.12%

in transit, 8.22% in the market, and 11.49% in retail (Gajanana *et al*., 2010). So the temperature and relative humidity must be controlled during storage which is chief cause of fruit and deterioration during storage. Aloe gel is safe and environmentally friendly substitute for synthetic preservatives like sulphur dioxide. It forms a protective coat against oxygen and moisture in the air, inhibits the activity in case of microorganisms which leads to food-borne diseases by its varied antibacterial and antifungal commixture, and is tasteless, colourless, and odourless (Aney *et al.,* 2020). Considering beneficial effect of manures and fertilizers and poor shelf life of mature papaya fruits, a study was conducted with an objective to know the effect of integrated nutrient management and bio-coating on change in fruit quality during storage of papaya.

MATERIALS AND METHODS

The current study was done in the year 2015–16 at the campus field of the College of Horticulture in Venkataramannagudem, Andhra Pradesh. The field trial used a Randomised Block Design (RBD) with three replications of each of the eight treatments. Each replication's allocation of treatments was random. The Indian Institute of Horticultural Research in Bengaluru provided papaya seeds of cv. Arka Prabhat. The seeds were sown in 25 x 15 cm polybags that contained a mixture of FYM, sand, and red soil at the ratio of 1:1:1 and they were routinely watered. The 45-day-old, healthy, uniform, and disease-free seedlings were chosen and planted in the pits with a spacing of 2 m 2 m distance. The necessary amounts of organic manures were estimated equivalent to recommended dose of Nitrogen (150 g/plant). The organic manures used in the experiment were FYM (Nitrogen 0.5 %), vermicompost (Nitrogen 3.0 %), neem cake (Nitrogen 5.2 %), and sheep manure (Nitrogen 3.0 %). Organic manures were applied at four splits *viz.,* one at base application and the other three at intervals of 60 days. Total quantity of inorganic nitrogen fertilizer was applied in four splits; first application as a basal dose and other 3 split applications at 2 months intervals from the first split. Four separate doses of nitrogen fertilizer, one as the basal dose, were used to apply the total dose. The treatments were comprised of T_1 - FYM 100% Recommended dose of Nitrogen (RDN), T_2- Vermicompost 100% RDN, T_3 - Neem cake 100% RDN, T_4 - Sheep manure 100% RDN, T_5- FYM 50% RDN + Vermicompost 50% RDN, T_6- FYM 50% RDN + Neem cake 50% RDN, T_7- FYM 50% RDN + Sheep manure 50% RDN and T_8- 100 % Recommended dose of fertilizer (RDF).

For storage study the matured papaya fruits showing two yellow streaks were harvested from the above field treatment papaya plants applied with different organic manures separately. The harvested fruits were washed in running tap water thoroughly and air dried at room temperature. After drying for about 6 hours**,** the fruits were coated with commercially available aloe gel and bee wax. There was three coating treatments viz., aloe get at 2% (C1), bee wax at 2% (C2) and uncoated (C3). The fruits were dipped in respective coating solutions for a period of fifteen minutes and then allowed to drain and form a thin film over the fruit surface. The coated and uncoated fruits were weighed and stored in ambient conditions for different storage studies. The biochemical parameters, ascorbic acid, Total Soluble Solids, acidity and Total Sugars were analyzed at 4 days intervals and the laboratory study was employed is Factorial CRD with two number of replication and in each replication there were 20 number of fruits in each replication.

Following bio-chemical studies were taken:

i. Estimation of ascorbic acid

Ascorbic acid content (mg $100g^{-1}$) has been observed by extracting 10 grams of papaya pulp which do blend along metaphosphoric acid (3% HPO_3) and volume was compose to 100 ml with HPO_3 (3%). The total volume was shacked well and filtered by Whatman No.1 filter paper. 10 ml aliquot filtrated solution was titrated against 2,6-dichlorophenol-indophenol dye till light pink colour noticed (AOAC, 1965). The ascorbic acid content was estimated by using the following formulae and stated in milli gram per 100 gram.

$$\text{Ascorbic acid } \left(\text{mg } 100g^{-1}\right) = \frac{\text{Titre value x Dye factor x Volume made up x 100}}{\text{Volume of the sample taken x Volume of aliquot taken}}$$

ii. Estimation of TSS

The TSS was decisive by using ERMA hand refractrometer through place a drop of juice on prism of refractrometer and observes the coexistence of shadow of the sample with the reading on the scale and expressed as ^{O}Brix. Before taking the reading, the refractrometer was tested for its error with distilled water, corrected accordingly and TSS content was recorded as per the procedure of Ranganna (1986).

iii. Estimation of acidity

Ten grams of papaya pulp was extracted, grinded and transferred to flask and volume was making up to 100 ml by using distilled water. The extract was filtered by whatman No.1 filter paper. An aliquot of 10 ml was transferred to conical flask, add 2-3 drops of phenolphthalein indicator and titrate against 0.1 N NaOH until pink colour was observed, which carries on for 15 seconds and was considered as end point as per the procedure given by (Ranganna, 1986). The titrable acidity has been calculated by the below formulae and given in percentage.

$$\text{Titrable acidity } (\%) = \frac{\text{Titre value} \times \text{ Normality of NaOH } \times 0.0064 \times 100}{\text{Volume of aliquot taken } (\text{ml})}$$

iv. Estimation of total sugars

The total sugars were resolute as procedure given by Lane and Eyon method (AOAC, 1965). A 50 ml lead free filtrated extract was taken in a 100 ml volumetric flask, added 5 ml of 50% HCl to it, mixed toughly and then wait for 24 hours at room temperature. Acid has been neutralized by NaOH and use a drop of phenolphthalein indicator till pink colour persist for few seconds. The volume has been make up to 100 ml with distilled water. Total sugars are evaluated by taking solution into a burette and titrated against a mixture of standard Fehling's solution of A and B (1:1) by make use of methylene blue as indicator till the brick red colour precipitate was formed. The per cent total sugar was obtained by using the below formula:

$$\text{Totalsugars } (\%) = \frac{\text{Factor x Volume made up x 100}}{\text{Titer value x Weight of the sample}}$$

RESULTS AND DISCUSSION

Effect on ascorbic acid

The data on ascorbic acid content of papaya fruits was influenced by the applied nutrients through different organic manures and inorganic fertilizers and application of bio-coatings to fruits were presented in Figure 1. On 1st day of storage, the highest ascorbic acid of 69.65 mg 100g^{-1} has been recorded in fruit of plants supplied by sheep manure 100% RDN and lowest of 48.52 mg 100g^{-1} in fruit of plants supplied by 100% inorganic RDF.

On fourth day of storage, ascorbic acid levels ranged from a minimum of 44.37 mg 100g^{-1} in fruit of plants supplied by 100% RDF to a highest of 68.13 mg 100g^{-1} in fruit of plants supplied by 100% RDN of sheep dung. On the fourth day of storage, the ascorbic acid concentration of the fruits coated with aloe gel reached a highest of 58.07 mg 100g^{-1} and a minimum of 56.12 mg 100g^{-1} for the uncoated fruits. On the fourth day of storage, the ascorbic acid content in the interactions ranged from a minimum of 44.00 mg 100g-^{1} in fruits of plants supplied by 100% RDF without coating to a highest of 68.70 mg 100g^{-1} in fruits of plants supplied by sheep manure 100% RDN coated with gel.

The highest ascorbic acid of 66.06 mg 100g^{-1} was observed in fruits of plants supplied by sheep manure and minimum of 41.38 mg 100g^{-1} in fruits of plants supplied with 100% RDF on 8th day of storage. The fruits coated with aloe gel showed maximum ascorbic acid of 56.08 mg 100g^{-1} and minimum of 52.50 mg 100g^{-1} for uncoated fruits on 8th day of storage. Among the interactions, ascorbic acid was maximum of 67.33 mg 100g^{-1} in fruits obtained from plants supplied by sheep manure 100% RDN coated with gel and minimum of 40.25 mg 100g^{-1} in fruits of plants supplied by 100% RDF uncoated on 8th day of storage.

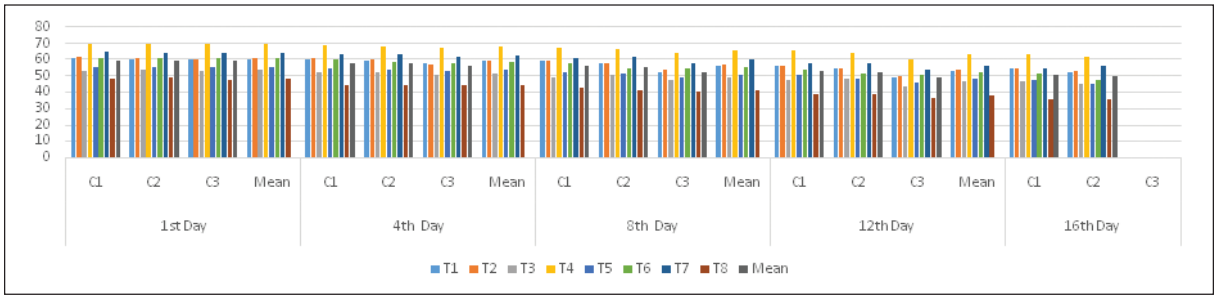

Fig. 1 : Effect of nutrient management and fruit coatings on ascorbic acid content (mg/100g) of papaya fruits at different storage dates

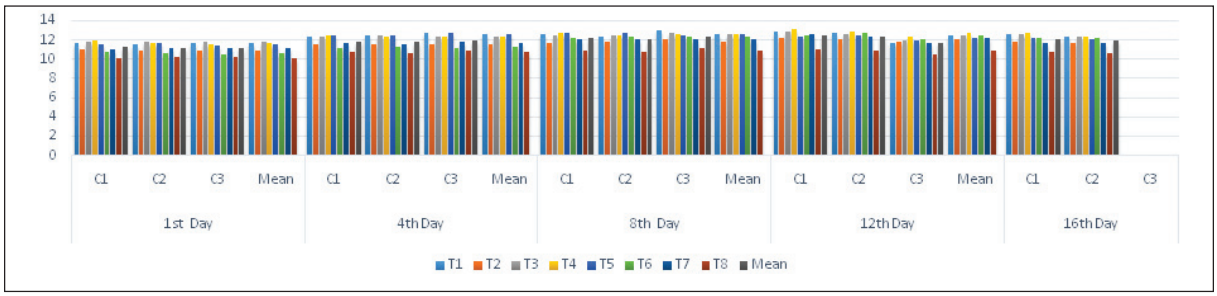

Fig. 2 : Effect of nutrient management and fruit coatings on TSS content (^{0} Brix) of papaya fruits at different storage dates

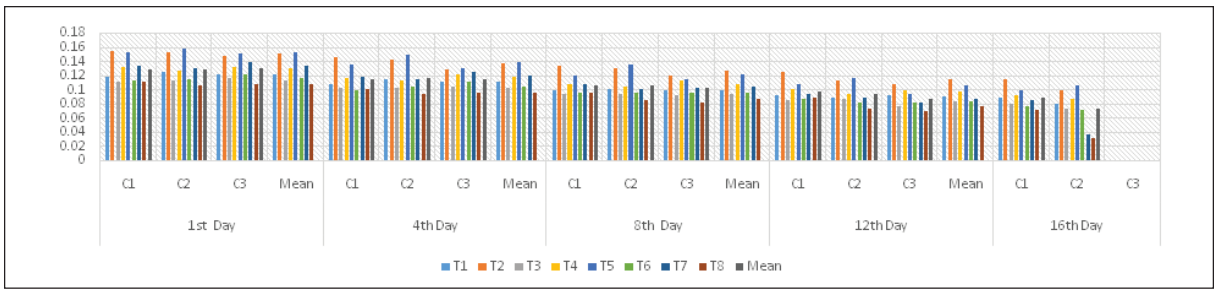

Fig. 3: Effect of nutrient management and fruit coatings on acidity content (%) of papaya fruits at different storage dates

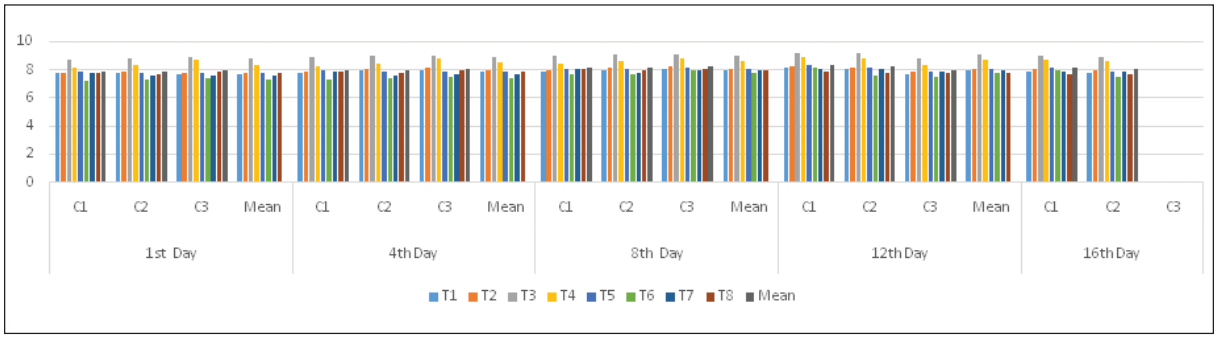

Fig. 4 : Influence of organic manures and biocoatings on total sugars (%) of papaya

On 12th day of storage, highest ascorbic acid of 63.37 mg $100g^{-1}$ has been observed in fruits of plants supplied by sheep manure 100% RDN and minimum of 37.82 mg $100g^{-1}$has been observed in fruits of plants supplied by 100% RDF. The fruits coated with aloe gel showed highest ascorbic acid of 53.18 mg $100g^{-1}$ and minimum of 48.94 mg $100g^{-1}$ for uncoated fruits on 12th day of storage. Among the interactions, ascorbic acid content was maximum of 65.42 mg $100g^{-1}$ in fruits of plants supplied by sheep manure 100% RDN coated with aloe gel and minimum of 36.41 mg $100g^{-1}$ in fruits of plants supplied by 100% RDF uncoated on 12th day of storage.

In the present research, the maximum ascorbic acid of 69.65, 68.13, 66.06 and 63.37 mg $100g^{-1}$ was recorded in fruits of plants supplied by sheep manure 100% RDN on 1st, 4th, 8th and 12th day of storage respectively. The fruits coated with aloe gel showed maximum ascorbic acid content of 44.75, 42.45 and 37.82 mg $100g^{-1}$ on 4th, 8th and 12th day of storage respectively. Ascorbic acid is a crucial indicator of the fruit's nutrient content and is very susceptible to oxidative deterioration (Veltman *et al.,* 2000) compared to other nutrients during food processing and storage. The increased metabolic processes brought about by the balanced nutrient availability contributed to the improved fruit quality using organic and inorganic manures (Singh *et al*., 2004). The higher uptake of potassium in plants supplied by sheep manure 100% RDN might resulted in higher content of ascorbic acid. Similar result of improved fruit quality with organic manures compared to RDF were given by Ravishankar *et al.* (2010) in papaya and Singh and Sharma (2006) in apple was reported.

Higher ascorbic acid levels in the Aloe gel-treated fruits than in the control fruit could be attributed to decreased cytochrome oxidase, ascorbic acid oxidase, and peroxidase activity during respiration. Aloe vera gel coating's reduced oxygen permeability, which decreased the activity of degrading enzymes and prevented ascorbic acid oxidation, the loss of ascorbic acid content in coated orange fruits was smaller. as reported by Adetunji *et al.*(2012).

Effect on TSS

The data concern to TSS of papaya fruits has been influenced by organic manures and biocoatings was given in Figure 2. The significant differences were observed with the supply of organic manures and bio-coatings at different days of storage. Interactions were found to be non-significant at 4th day, 8th day and 12th day of storage.

The lowest total soluble solids of 10.16°Brix has been observed in fruits of plants supplied by 100% RDF on 1st day of storage and the highest of 11.81°Brix were recorded in fruits of plants supplied by neem cake 100% RDN. The fruits coated with aloe gel showed highest TSS of 11.24°Brix and minimum of 11.17°Brix in uncoated fruits on 1st day of storage., the minimum of 10.09°Brix was observed in fruits of plants supplied by 100% RDN coated with aloe gel and highest total soluble solids of 11.91°Brix were recorded in fruits of plants supplied with sheep manure 100% RDN coated with gel which was on par and uncoated fruits of plants supplied by neem cake 100% RDN (11.82°Brix) and with the fruits of plants supplied by neem cake 100% RDN coated with aloe gel (11.83°Brix) among the interactions.

The maximum total soluble solids of 12.59 °Brix were recorded in fruits of plants supplied with 50% nitrogen by Farm yard manure in combination with vermicompost 50% RDN which is on par with the fruits of plants supplied by FYM 100% RDN (12.55°Brix) and lowest of 10.78°Brix in fruits of plants supplied by 100% RDF on 4th day of storage. The uncoated fruits showed maximum TSS of 11.96°Brix and fruits coated with bee wax had minimum of 11.85°Brix on 4th day of storage.

On 8th day of storage, maximum total soluble solids of 12.64°Brix were recorded fruits of plants supplied with 50% nitrogen by Farm yard manure in combination with vermicompost 50% RDN which was on par with the fruits of plants applied with FYM 100% RDN (12.62°Brix) and neem cake 100% RDN (12.56°Brix) and minimum of 10.78°Brix was recorded in fruits of plants applied with 100% RDF. The uncoated fruits showed maximum TSS of 12.31°Brix and fruits coated with bee wax had minimum of 12.14°Brix on 8th day of storage.

The maximum total soluble solids of 12.77°Brixwas observed fruits of plants applied with 100% recommended dose of nitrogen with sheep manure and minimum of 10.84°Brix in fruits of 100% inorganic fertilizers as per

recommendation on 12th day of storage. The fruits coated with aloe gel showed maximum total soluble solids of 12.46°Brix and minimum of 11.75°Brix for uncoated fruits on 12th day of storage.

Present study, it was recorded that fruits obtained from organic manure treated plants particularly with sheep manure 100% RDN recorded higher total soluble solids in the fruit, may be due to release of plant hormones and easy nutrient uptake. The peak in TSS initially may be due to increase of transpiration from fruit surface and decrease at later stage is due to rapid utilization of soluble solids during respiration. These results were in consistent with findings of Babu Ratan (2006). The increased fruit quality is due to increased nutrient availability and better uptake of nutrient from the soil. These results were in conformity with the findings of Singh and Sharma (2006) and Aditi *et al*. (2020).

Further, the increase in TSS of coated fruits may be due to less transpiration and by creation of modified atmosphere. This might have led to slow down in the carbohydrate metabolism and delayed starch hydrolysis. The present findings infers that the coated fruits had delayed increase in TSS compared to quick increase in uncoated fruits which were similar with findings of Adetunji *et al*. (2014) in cucumber, Kumar and Bhatnagar (2014) and Ochiki Sophia *et al*. (2015) in mango.

Effect on acidity

The titrable acidity of papaya fruits as persuaded by application of different organic manures and inorganic fertilizers and bio-coatings to fruits (Figure 3) indicated significant differences in nutrient management, bio-coatings and their interactions on 4th day, 8th day and 12th day of storage respectively.

On 1st day of storage, the highest titrable acidity content of 0.153% was recorded in fruits of plants supplied with 50% nitrogen by Farm yard manure in combination with vermicompost 50% RDN which is equipollent with vermicompost 100% RDN (0.151%) and lowest of 0.108% in fruits of 100% RDF. Among the interactions, the maximum titrable acidity of 0.158% was recorded in the fruits of plants supplied by 50% nitrogen by Farm yard manure in combination with vermicompost 50% RDN coated with bee wax which is equipollent with the vermicompost 100% RDN fruits coated with aloe gel (0.154%) and minimum of 0.105% in fruits of plants supplied by 100% RDF coated with bee wax on 1st day of storage.

On 4th day of storage, the highest titrable acidity of 0.139% is observed in fruits of plants supplied by 50% nitrogen by Farm yard manure in combination with vermicompost 50% RDN which is equipollent with vermicompost 100% RDN (0.137%) and least 0.096% in fruits of plants applied with 100% RDF. Among the interactions, the highest titrable acidity content of 0.148% was recorded in the fruits of plants supplied by 50% nitrogen by Farm yard manure in combination with vermicompost 50% RDN coated with bee wax and minimum of 0.094% in fruits of plants supplied by 100% RDF coated with bee wax on 4th day of storage.

On 8th day of storage, the highest titrable acidity content of 0.127% was observed in fruits of plants supplied by vermicompost 100% RDN which was on par with fruits of plants supplied by50% nitrogen by Farm yard manure in combination with vermicompost 50% RDN(0.122%) and minimum (0.087%) in fruits of plants supplied by 100% RDF. The fruits coated with aloe gel showed maximum titrable acidity of 0.106% which was on par with fruits coated with bee wax (0.105%) and minimum of 0.102% in uncoated fruits on 8th day of storage. Among the interactions, the highest titrable acidity content of 0.135% was observed in fruits of plants supplied with 50% nitrogen by Farm yard manure in combination with vermicompost 50% RDN coated with bee wax which is equipollent with the vermicompost 100% RDN fruits coated with aloe gel (0.133%) and vermicompost 100% RDN fruits coated with bee wax (0.129%) and lowest 0.082% in fruits of plants supplied by 100% RDF uncoated fruits 8th day of storage.

On 12th day of storage, the highest titrable acidity content of 0.115% was recorded in fruits of plants supplied by nitrogen in the form of vermicompost 100 per cent and minimum of 0.077% in fruits of applied with 100% RDF. The fruits coated with aloe gel showed maximum titrable acidity content of 0.097% and minimum of 0.087% in uncoated fruits on 12th day of storage. Among the interactions, the highest titrable acidity content of 0.125% was recorded in the fruits of

plants supplied by vermicompost 100% RDN coated with aloe gel and lowest of 0.070% in fruits of plants supplied by 100% RDF uncoated fruits.

In the present investigation, the highest titrable acidity content (0.151, 0.137, 0.127 and 0.115%) was recorded in fruits of plants supplied by vermicompost 100% RDN on 1st day, 4th day, 8th day and 12th day of storage respectively. The fruits coated with aloe gel showed maximum titrable acidity content (0.106% and 0.097%) on 8th day and 12th day of storage. The titrable acidity content of fruits was decreased significantly from 1stday to 12th day of storage in all the treatments. The acidity in fruits is an important factor in determining maturity. Titrable acidity gives the total or potential acidity, rather than indicating the number of free protons in any particular sample. It is a measure of all aggregate acids and sum of all volatile and fixed acids. In the present study, the titrable acidity of the papaya fruits was declined significantly over the storage period. There was a gradual decline in the titrable acidity content of fruits with the storage period. This might be due to the conversion of organic acids into simple sugars and their utilization during respiration.

The decrease in acidity in uncoated fruits is high when compared to coated fruits may be due to higher rate of respiration and greater reduction in acidity in uncoated fruits compared to coated fruits with aloe gel. The lesses rate of respiration in coated fruits may be due to due to lesser use of organic acids. These findings were also in conformity with the work carried out by Vahdat *et al*. (2010) in strawberry, Arowora *et al.* (2013) in oranges and Jawadul *et al*. (2014) in their review report of alovera gel coatings.

Effect on total sugars

The data on total sugars of papaya fruits as effected by the nutrient management and fruit coatings were given in Figure 4 and revealed significant differences in nutrient management and bio-coatings and interactions.

The highest total sugars of 8.78% was observed in nitrogen supplied plants with neem cake alone and lowest of 7.26% fruits of plants supplied with 50% nitrogen by Farm yard manure in combination with neem cake 50% RDN. The uncoated fruits recorded maximum total sugars of 7.93% and minimum of 7.85% in fruits coated with aloe gel on 1st day of storage. Among the interactions, highest sugars (8.87%) was recorded in the in nitrogen supplied plants with neem cake alone and without coationg which was on par with nitrogen supplied plants with neem cake alone coated with bee wax (8.82%) and lowest of 7.20% in plants treated with nitrogen half dose with FYM and half through neem cake coated with aloe gel on 1st day of storage.

On 4th day of storage, the highest total sugars of 8.92% was observed in nitrogen supplied plants with neem cake alone and lowest of 7.38 % fruits of plants supplied with 50% nitrogen by Farm yard manure in combination with neem cake 50% RDN. The uncoated fruits recorded highest total sugars of 8.08% and lowest of 7.96% in fruits coated with aloe gel on 4th day of storage. Among the interactions, highest total sugars of 8.97% was observed in the fruits of plants supplied with nitrogen in the form of neem cake alone and uncoated and lowest of 7.31% in fruits of plants supplied with 50% nitrogen by Farm yard manure in combination with vermicompost 50% RDNRDN coated with aloe gel on 4th day of storage.

On 8th day of storage, the highest total sugars of 9.00was observed in nitrogen supplied plants with neem cake alone and lowest of 7.77 % fruits of plants supplied with 50% nitrogen by Farm yard manure in combination with neem cake 50% RDN. The uncoated fruits recorded highest total sugars of 8.25% and minimum of 8.11% in fruits coated with aloe gel on 8th day of storage.

The highest total sugars of 9.03% was observed in of plants treated with nitrogen in the form of neem cake alone and lowest (7.72%) in fruits of plants supplied with 50% nitrogen by Farm yard manure in combination with neem cake 50%.The highest total sugar of 8.34% was recorded in fruits coated with aloe gel and lowest of 7.93 in fruits without coating on 12th day of storage. The highest total sugars (9.16%) was recorded in fruits coated with aloe gel from the plants where nitrogen is supplied in the form of neem cake alone and minimum (7.46%) was observed in fruits of plants applied with nitrogen half dose with FYM and other half with sheep manure on 12th day of storage.

In the present study, the highest total sugars of 8.78, 8.92, 9.00 and 9.03% were recorded in fruits

of plants where nitrogen is supplied through neem cake on all days of storage. The conversion of cell wall material by hydrolysis of polysaccharides takes place initially and hence initially there will be increase in total sugars and due to respiration decreases at later period of storage. Dutta Ray *et al.* (2014) also reported that plants applied with neem cake had higher total sugars.

In the present investigation, the coated fruits had significantly more sugars content than without coating may be due to less exchange of gases from the fruit surface into the atmosphere during storage of fruits. The higher total sugars with aloe gel coatings might be due to decrease in rate of respiration and eventually catabolism of solids including sugars and organic acids. Aloe gel coated fruits reported highest total sugars content in strawberry (Vahdat *et al*., 2010) and waxing in pineapple (Huigang *et al.,* 2011) and Dikki *et al.* (2010) in papaya.

CONCLUSION

The highest biochemical compounds were observed during storage in the plants in which nitrogen is supplied through organic sheep manure, vermicompost and neem cake and fruits coated with aloe gel and bee wax when compared to plants grown with inorganic fertilizers alone and uncoated fruits. The shelf can be easily increased by the application of organic manures in the field and by using edible fruit coatings after harvest in papaya cv. Arka Prabhat.

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Effect of types of rootstocks and their age on performance of cleft grafting of sweet orange (*Citrus sinensis*) cv. BARI Malta-1

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ABSTRACT

A suitable rootstock as well as rootstock age is predominating factor for production of quality planting materials (saplings). This study was conducted to find out a suitable rootstock as well as age of rootstock for successful grafting of BARI Malta-1. The experiment was conducted at pomology division of Horticulture Research Center, BARI. The factorial experiment consists of two types of rootstocks viz., R_1: Rangpur lime and R_2: Rough lemon and nine different ages of the rootstocks viz., 5th, 6th, 7th, 8th, 9th, 10th, 11th,12th and 13th months. Thus eighteen treatment combinations were used for this study. Healthy, diseases free and ideal scion of sweet orange was grafted on to rootstocks following cleft method. The results showed that the treatment combination R_2T_3 (Sweet orange scion grafted on 7th months old rough lemon rootstock) was performed better among the all combinations as it gave the highest results in respect of plant height (64.42 cm), canopy volume (0.042 cm^3), number of leaves per plant (61.00), stem fresh weight (32.38 g) and total chlorophyll (1.44 mg/g tissue). The treatment combination R_2T_3 also showed 80% graft survivability after one year of grafting. These results suggest that sweet orange cv. BARI Malta-1 should be grafted on 7th months old rough lemon rootstock in cleft grafting method for achieving better planting materials.

***Keywords*:** *Cleft grafting, graft morphology, graft physiology, rootstock age, rootstock type, sweet orange*

INTRODUCTION

In Bangladesh sweet orange (*Citrus sinensis*) is commonly known as Malta. Sweet orange is getting popular day by day in Bangladesh and farmers are very interested to cultivate it. Bangladesh Agricultural Research Institute developed two sweet orange varieties and they are BARI Malta-1 and BARI Malta-2. BARI Malta-1 is high yielding and getting popular among the farmers since it can grow almost all over the country. It is medium size and about 146 g of weight, number of fruit per plant is 300-400, yield is 20 tons/hectare, very juicy (33.7%) and sweet (TSS 7.8% and total acid 0.36%). External appearance of fruit is smooth, round in shape and skin is very thin. One of the most important factors contributing towards high productivity of sweet orange is quality planting material. In Bangladesh, mainly seed propagation is practiced which takes long duration to get maturity and fruiting as well as attributed to low fruit qualities due to high genetic variations. For heterozygosity of fruit crops, usage of vegetative multiplied planting material is the scientific way (Ghosh and Bera, 2015). Propagation by cleft grafting is the easiest method in sweet orange in Bangladesh. In fruit crops rootstocks have been utilizing for long period to defend against soil born diseases and pests (Ranpise and Ahire, 2016). As an ancient practice, grafting delivers several agronomical benefits to citrus crops. Using of a proper rootstock can have significant developments for the scion such as decline of the juvenility period, excessive yield, better fruit quality, uniform plant architecture, guard against pests and diseases, and appropriate tolerance to abiotic stress factors (Balfagón *et al.,* 2022). Various factors influence the success and survivability of grafts viz. time of grafting operation, varieties, maturity and age of scion and rootstock, growing condition of grafts, methods of grafting etc. For a graft union to be effective, it is required that temperature states in

the times of cellular activity, callus construction in addition to during healing should be supportive. The grafting operation normally carried out while the temperatures are appropriate for cambial action and there is the high humidity in the surrounding area of the cambial zone of the graft joint. Effective balance recognized between grafted scions with leaves and rootstocks' roots are very much required for boosting assimilates partitioning plus free transmission of water plus necessary nutrient element (Perez-alfocea *et al*., 2010). Nevertheless, leaf chlorophyll also found to have considerable impact on the propagation accomplishment as well as plant persistence. Better root and scion composition improved the biomass of root, length of root, root: shoot ratio as well as whole biomass accumulation in Khasi mandarin (Deshmukh *et al*., 2017). Thus, the variations in the level of morpho-physiological features of scion leaves of grafts as sources are determined by the rootstocks age and type of rootstocks. Therefore, detection of suitable age of different rootstocks along with appropriate morphological and anatomical features is essential to know its effect on development of scion (Zoric *et al.,* 2012), multiplication success and plant persistence. Grafting of different aged rootstocks can satisfy the need of grafts to be united at whichever period of the year and thus we can do year round grafting to fulfill the high demand of sweet orange saplings. Leaf and shoot attributes, root growth and morphology study is applicable for development and tuning of multiplication procedures. Information related to the effect of age of rootstock at scion physiology and root morphology of grafted plants are not sufficient for sweet orange. There is immense scope of employment and income generation through year round production and supply of quality planting materials of sweet orange as well as other citrus fruits. Keeping in view all these points, the present study was under taken to investigate the grafting performance of different aged citrus rootstocks on sweet orange.

MATERIALS AND METHODS

The investigation was conducted at the Fruit Research Farm of the Pomology Division of Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh during the period from October, 2016 to November, 2018; which is located at 23°59'02'' N latitude and 90°24'38'' E longitude with an altitude of 15 m above sea level with silt loam to silty clay loam in soil texture with the pH varying from 4.5 to 7.2 (FRG, 2012), average temperature and humidity was 26° and 85% respectively during the experiment. The experiment consisted of two factors with eighteen treatment combinations which were as follows; Factor A: Types of rootstocks; R_1: Rangpur lime (*Citrus limonia* Osbeck) rootstock and R_2: Rough lemon (*Citrus jambhiri* Lush) rootstock. Factor B: Different ages of rootstocks (T_1: Five months old rootstock, T_2: Six months old rootstock, T_3: Seven months old rootstock, T_4: Eight months old rootstock, T_5: Nine months old rootstock, T_6: Ten months old rootstock, T_7: Eleven months old rootstock, T_8: Twelve months old rootstock, T_9: Thirteen months old rootstock). The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. There were eighteen treatment combinations in each replication. For each treatment combination grafting operations had performed on 10 rootstocks, so for one replication 180 and total 540 number of grafting work was performed. The treatment combination was randomly assigned. Seeds of each rootstock collected from ripen fruits and immediately sown on 10th October 2016 in germination tray separately and after germination when seedlings achieved two to three leaves stage they were transferred to polybag (15 cm x 10 cm) respectively. After five months of when these rootstocks were grown up, then among them healthy, vigorous, pest and disease free, uniform in size and growth were selected for first cleft grafting work for the experiment and it continued every month till the thirteen-month age of the rootstock according to the treatment combinations. After wrapping the graft union, the scion along with the union portion was covered with a polythene cap and kept in the grafting chamber where temperature can be controlled to protect the scion from the loss of moisture through transpiration. Proper care and management of the grafts were taken to maintain their good health. All the data on different parameters were recorded at one year age of grafted sapling except days to bud breaking and graft success.

The methodology used to take data in respect of days to bud breaking, percentage of graft success was calculated by using the following formula: Percentage of graft success = Number of successful grafts × 100/Total number of grafted rootstock), graft survivability percentage was measured by using the following formula:

Graft survivability (%) = Total number of successful grafts - Total number death of grafts after success) × 100/ Total number of successful grafts} (Chakma *et al.,* 2013), number of leaves per plant, leaf area (m^3), leaf fresh weight (g), leaf turgid weight (g), leaf dry weight (g),

Relative water content = {RWC of fresh leaves was calculated using the following formula: RWC (%) = (leaf fresh weight - leaf dry weight) × 100/ (leaf turgid weight - leaf dry weight)} (Deshmukh *et al.,* 2017).

Chlorophyll a, chlorophyll b and total chlorophyll content of leaves was determined according to the method described by Witham *et al*. (1971), the amount of chlorophyll was determined by using these following formul as (Arnon, 1949);

C_a=0.0127 D_{663}- 0.00269 D_{645}, C_b=0.0229 D_{645} - 0.00468 D_{663} and C = C_a + C_b, Where D=Density values at the respective wavelengths as obtained on the spectrophotometer, C_a=Chlorophyll a, C_b=Chlorophyll b, C=Total chlorophyll. Chlorophyll content was expressed in mg/g tissue.

Plant height (m), rootstock diameter (mm), scion diameter (mm), stem fresh weight (g), stem dry weight (g), root volume (cm^3), root fresh weight (g), root dry weight (g), root: shoot ratio (The root to shoot ratio was computed by using following formula; Root: Shoot = Root dry weight/Shoot dry weight where, After weighing fresh weight, each sample was kept in paper envelop separately according to the treatment combination and placed in an electrical oven for 72 hours in 65°C. Weight of fresh and oven dried samples was recorded by an electrical balance in g. Collected data were analyzed by following statistical package software R.

RESULTS AND DISCUSSION

The data presented in Table 1 indicated that combination of rootstocks and rootstock age had significant effect on days to bud break of sweet orange grafts. The longest (25 days) days were required to bud break for R_2T_4 (Sweet orange scion grafted on eight months old rough lemon rootstock) and followed (24 days) by R_1T_5 (Sweet orange scion grafted on nine months old Rangpur lime rootstock). The shortest (12 days) days were required for R_1T_2 (Sweet orange scion grafted on six months old Rangpur lime rootstock), R_2T_1 (Sweet orange scion grafted on five months old rough lemon rootstock) and R_2T_2 (Sweet orange scion grafted on six months old rough lemon rootstock) to bud break (Table 1).

Combined effect on sweet orange graft success was maximum (100.00%) when it was R_1T_2 (Sweet orange scion grafted on six months old Rangpur lime rootstock) and it was statistically significant from other combined effect. R_1T_3 (Sweet orange scion grafted on seven months old Rangpur lime rootstock) R_1T_8 (Sweet orange scion grafted on twelve months old Rangpur lime rootstock), R_2T_2 (Sweet orange scion grafted on six months old rough lemon rootstock), R_2T_6 (Sweet orange scion grafted on ten months old rough lemon rootstock), R_2T_7 (Sweet orange scion grafted on eleven months old rough lemon rootstock) showed the second highest (96.67%) integration effect on graft success. The proper rootstock age with greater sugars and adequate C: N ratio should have added to the greater graft success percentage. The prompt failure in success of graft in different treatments can be as a result of lack of vigorous sprouts, physiological state of the rootstock as well as weakened sap flow which eventually affected the growth of graft union establishment. Minimum (63.33%) combined effect showed by R_1T_1 (Sweet orange scion grafted on five months old Rangpur lime rootstock) on graft success (Table 1).

Rootstocks and rootstock ages also had no significant combined effect on graft survivability of sweet orange grafts. Maximum (93.33%) graft survivability of sweet orange graft found when it was grafted on six months old rough lemon rootstock (R_2T_2) which was followed (90%) by R_1T_8 (Sweet orange grafted on twelve months Rangpur lime rootstock) but minimum (56.67%) was recorded at R_1T_1 (Sweet orange scion grafted on five months old Rangpur lime rootstock) and preceded (70%) by R_2T_9 (Sweet orange scion

Table 1: Combined effect of rootstock and rootstock ages on days to bud break, graft success, graft survivability and plant height

Treatment		Days to bud break (days)	Graft success (%)	Graft survivability (%)	Plant height (after one year of grafting)(cm)
Rootstock	**Age**				
R_1	T_1	15.00^{d}	63.33^{d}	56.67	48.33
	T_2	12.00^{e}	100.00^{a}	86.67	58.58
	T_3	14.00^{d}	96.67^{ab}	86.67	60.17
	T_4	20.00^{c}	86.67^{abc}	73.33	54.08
	T_5	24.00^{a}	83.33^{bc}	73.33	63.00
	T_6	22.00^{b}	83.33^{bc}	73.33	52.18
	T_7	20.00^{c}	90.00^{abc}	83.33	49.70
	T_8	22.00^{b}	96.67^{ab}	90.00	49.00
	T_9	22.00^{b}	86.67^{abc}	76.67	49.42
R_2	T_1	12.00^{e}	86.67^{abc}	73.33	56.39
	T_2	12.00^{e}	96.67^{ab}	93.33	58.75
	T_3	15.00^{d}	90.00^{abc}	80.00	64.42
	T_4	25.00^{a}	95.00^{ab}	80.00	56.13
	T_5	21.00^{bc}	86.67^{abc}	73.33	49.50
	T_6	20.00^{c}	96.67^{ab}	86.67	48.83
	T_7	15.00^{d}	96.67^{ab}	86.67	54.42
	T_8	22.00^{b}	86.67^{abc}	80.00	53.39
	T_9	22.00^{b}	80.00^{c}	70.00	48.17
LSD(0.05)		**1.10**	**13.45**	**13.87**	**11.94**
Level of significance		*****	*****	**NS**	**NS**
CV%		**5.02**	**9.11**	**10.57**	**13.29**

*=significant at 5% level of probability, NS=Not Significant

Here, R_1=Rangpur lime rootstock, R_2=Rough lemon rootstock, T_1=Five months old rootstock, T_2=Six months old rootstock, T_3=Seven months old rootstock, T_4=Eight months old rootstock, T_5=Nine months old rootstock, T_6=Ten months old rootstock, T_7=Eleven months old rootstock, T_8=Twelve months old rootstock and T_9=Thirteen months old rootstock

grafted on thirteen months old rough lemon rootstock) (Table 1).

Combination of rootstocks and rootstock age had no significant effect on plant height of sweet orange grafts after one year of grafting. The tallest (64.42 cm) plant height was recorded for the effect R_2T_3 (Sweet orange scion grafted on seven months old rough lemon rootstock) and followed (63 cm) by R_1T_5 (Sweet orange scion grafted on nine months old Rangpur lime rootstock). The shortest (48.17 cm) height of plant was recorded for R_2T_9 (Sweet orange scion grafted on thirteen months old Rangpur lime rootstock) and preceded by (48.33 cm) R_1T_1 (Sweet orange scion grafted on five months old Rangpur lime rootstock) and R_2T_6 (Sweet orange scion grafted on ten months old rough lemon rootstock) (Table 1).

Combined effect on sweet orange rootstock diameter was significantly dissimilar. Maximum (9.37 mm) rootstock diameter was recorded when it was R_1T_3 (Sweet orange scion grafted on seven months old Rangpur lime rootstock) and followed (9.29 mm) by R_1T_9 (Sweet orange scion grafted on thirteen months old Rangpur lime rootstock). Minimum (4.9 mm) combined effect showed by R_1T_4 (Sweet orange scion grafted on eight months old Rangpur lime rootstock) for rootstock diameter and preceded (6.64 mm) by R_1T_1 (Sweet orange scion grafted on five months old Rangpur lime rootstock) (Table 2).

Table 2: Combined effect of rootstock and rootstock ages on rootstock diameter, scion diameter, number of leaves per plant, canopy volume, leaf area and relative water content

Treatment		Rootstock diameter (mm)	Scion diameter (mm)	Number of leaves/ plant	Canopy volume (m^3)	Leaf area (cm^2)	RWC (%)
Rootstock	Age						
R_1	T_1	6.64^{e}	6.06^{ef}	23.67	0.019	15.31^{cdefg}	85.86^{abcd}
	T_2	8.04^{abcd}	7.00^{abcdef}	50.67	0.029	19.95^{abc}	92.49^{abc}
	T_3	9.37^{a}	8.27^{ab}	51.67	0.038	24.27^{a}	84.88^{bcd}
	T_4	4.90^{f}	3.69^{g}	44.00	0.023	18.98^{abcde}	90.79^{abcd}
	T_5	8.80^{abc}	8.34^{ab}	44.00	0.038	15.41^{cdefg}	89.25^{abcd}
	T_6	8.68^{abc}	6.58^{cdef}	41.33	0.022	14.55^{defg}	69.48^{e}
	T_7	8.10^{abcd}	6.20^{def}	31.00	0.021	17.25^{bcdefg}	89.47^{abcd}
	T_8	7.55^{cde}	5.90^{f}	27.67	0.014	12.82^{g}	99.15^{a}
	T_9	9.29^{ab}	8.54^{a}	22.00	0.021	18.30^{bcdef}	97.21^{ab}
R_2	T_1	7.34^{de}	6.42^{def}	33.67	0.031	20.26^{abc}	78.35^{de}
	T_2	8.86^{abc}	7.58^{abcde}	54.00	0.033	18.41^{bcdef}	89.66^{abcd}
	T_3	8.91^{ab}	7.70^{abcd}	61.00	0.042	15.72^{cdefg}	95.61^{ab}
	T_4	7.98^{bcde}	6.73^{bcdef}	33.00	0.023	22.28^{ab}	80.73^{cde}
	T_5	7.98^{bcde}	8.43^{a}	44.00	0.023	13.76^{efg}	91.15^{abcd}
	T_6	8.72^{abc}	6.63^{cdef}	38.67	0.025	19.75^{abcd}	92.40^{abc}
	T_7	8.30^{abcd}	8.15^{abc}	36.67	0.031	18.29^{bcdef}	87.57^{abcd}
	T_8	8.04^{abcd}	6.31^{def}	22.00	0.020	13.25^{fg}	93.46^{abc}
	T_9	7.22^{de}	6.07^{ef}	25.33	0.021	18.33^{bcdef}	90.58^{abcd}
LSD(0.05)		**1.34**	**1.63**	**18.25**	**0.02**	**5.37**	**13.73**
Level of significance		*	*	NS	NS	*	*
CV%		**10.00**	**14.19**	**28.93**	**36.53**	**18.38**	**9.31**

* = significant at 5% level of probability, NS=Not Significant

Rootstocks and rootstock age collectively had significant effect on scion diameter. The widest (8.54 mm) scion diameter of sweet orange occurred when it was grafted on thirteen months old Rangpur lime rootstock (R_1T_9) which was statistically similar (8.4) to R_2T_5 (Sweet orange grafted on nine months old rough lemon rootstock) but the narrowest (3.69 mm) was for R_1T_4 (Sweet orange scion grafted on eight months old Rangpur lime rootstock) and preceded (5.9 mm) by R_1T_8 (Sweet orange scion grafted on twelve months old Rangpur lime rootstock) (Table 2). Perhaps cause of quick and tough development of unification between the rootstock and scion, consecutively inducing bigger absorption of nutrient elements by developed shoots.

Rootstocks and rootstock age collectively had no significant effect on number of leaves per plant (Table 2). the highest (61.00) number of leaves per plant of sweet orange occurred when it was grafted on seven months old rough lemon rootstock (R_2T_3) and followed (54.00) by R_2T_2 (Sweet orange grafted on six months old rough lemon rootstock) but the lowest (22.00) number of leaves per plant of Sweet orange was for R_2T_8 (Sweet orange scion grafted on twelve months old rough lemon rootstock) and R_1T_9 (Sweet orange scion grafted on thirteen months old Rangpur lime rootstock) preceded by (23.67) R_1T_1 (Sweet orange scion grafted on five month old Rangpur lime).

There were no statistical dissimilarities among the treatment combinations related to canopy volumes of sweet orange saplings as they were grafted on different ages' Rangpur lime and rough lemon of respectively. Combined effect R_2T_3 (Sweet orange scion grafted on seven months old rough lemon rootstock) showed the biggest (0.042 cm^3) canopy volume of sweet orange and followed (0.038 cm^3) by R_1T_3 and R_1T_5 but the smallest (0.014 cm^3) canopy volume was for R_1T_8 (Sweet orange

scion grafted on twelve months old Rangpur lime rootstock) (Table 2).

Remarkable variation was observed among the combined effect on sweet orange grafts' leaf area. The largest (24.27 cm^2) leaf area was produced by R_1T_3 (Sweet orange scion grafted on seven months old Rangpur lime rootstock) and followed (22.28 cm^2) by R_2T_4 (Sweet orange scion grafted on eight months old rough lemon rootstock) while the smallest (12.82 cm^2) leaf area was produced by R_1T_8 (Sweet orange scion grafted on twelve months old Rangpur lime rootstock) (Table 2). Larger leaf area may be due to satisfactory stock-scion collaboration which is succeeded through better root shoot indicating system and good scion physiology and thus upper shoot biomass gathering (Ali *et al*.,1996).

Combination of Rootstocks and rootstock age had significant effect on relative water content (RWC) of leaves of sweet orange grafts. The highest (99.15%) relative water content was recorded for the effect R_1T_8 (Sweet orange scion grafted on twelve months old Rangpur lime rootstock) and followed (97.21%) by R_1T_9 (Sweet orange scion grafted on thirteen months old Rangpur lime rootstock). The lowest (69.48%) relative water content of leaves of plant was recorded for R_1T_6 (Sweet orange scion grafted on ten months old Rangpur lime rootstock) and preceded (78.35%) by R_2T_1 (Sweet orange scion grafted on five months old rough lemon rootstock) (Table 2). Better water holding capacity of the grafts in different treatments may be due to improved water use efficacy which has straight relation with total vigor and biomass collection (Passioura, 1986).

Combination of Rootstocks and rootstock age had no significant effect on stem fresh weight of sweet orange grafts. The highest (32.38 g) stem fresh weight was recorded for the effect R_2T_3 (Sweet orange scion grafted on seven months old rough lemon rootstock) and followed (31.36 g) by R_2T_5 (Sweet orange scion grafted on nine months old rough lemon rootstock). The lowest (11.78 g) stem fresh weight of plant was recorded for R_1T_1 (Sweet orange scion grafted on five months old Rangpur lime rootstock) and preceded (14.79 g) by R_1T_8 (Sweet orange scion grafted on twelve months old Rangpur lime rootstock) (Table 3).

Rootstocks and rootstock age had no significant effect on root volume of sweet orange graft. The maximum (30.00 cm^3) root volume of sweet orange graft found when it was grafted on ten months old rough lemon rootstock (R_2T_6) which was followed (25.67 cm^3) by R_2T_3 (Sweet orange grafted on seven months old rough lemon rootstock) but the minimum (12.33 cm^3) was for R_1T_1 (Sweet orange scion grafted on five months old Rangpur lime rootstock) and preceded (13.33 cm^3) by R_1T_2 (Sweet orange scion grafted on six months old Rangpur lime rootstock) (Table 3).

Rootstocks and rootstock age had no significant combined effect on root fresh weight of sweet orange graft. The largest (28.58 g) root fresh weight of sweet orange graft found when it was grafted on nine months old rough lemon rootstock (R_2T_5) which was followed (25.29 g) by R_2T_3 (Sweet orange grafted on seven months old rough lemon rootstock) but the smallest (9.42 g) was recorded at R_1T_1 (Sweet orange scion grafted on five months old Rangpur lime rootstock) and preceded (10.45 g) by R_1T_2 (Sweet orange scion grafted on six months old Rangpur lime rootstock) (Table 3).

No significant variation was observed among the combined effect on root: shoot ratio of sweet orange grafts. Maximum (1.05) root to shoot ratio was recorded at R_2T_9 (Sweet orange scion grafted on thirteen months old rough lemon rootstock) and followed (0.94) by R_1T_9 (Sweet orange scion grafted on thirteen months old Rangpur lime rootstock) while minimum (0.45) root to shoot was recorded at R_1T_2 (Sweet orange scion grafted on six months old Rangpur lime rootstock) and preceded (0.55) by R_1T_5 (Sweet orange scion grafted on nine months old Rangpur lime rootstock) (Table 3).

Combined effect on total chlorophyll of sweet orange grafts was significantly dissimilar. The highest (1.44 mg/g tissue) total chlorophyll was recorded in treatment combination of R_1T_4 (Sweet orange scion grafted on eight months old Rangpur lime rootstock), R_2T_3 (Sweet orange scion grafted on seven months old rough lemon rootstock) and R_2T_4 (Sweet orange scion grafted on eight months old rough lemon rootstock) while it was statistically similar (1.43 mg/g tissue) to R_2T_9 (Sweet orange scion grafted on thirteen months old rough lemon rootstock). The lowest (1.12 mg/g tissue) total

Table 3: Combined effect of rootstock and rootstock ages on stem fresh weight, root volume, root fresh weight, root: shoot ratio and total chlorophyll

Treatment		Stem fresh	Root volume	Root fresh	Root: Shoot	Total chlorophyll
Rootstock	Age	weight(g)	(cm^3)	weight(g)	ratio	(mg/g tissue)
R_1	T_1	11.78	12.33	9.42	0.70	1.41^{ab}
	T_2	19.37	13.33	10.45	0.45	1.41^{ab}
	T_3	28.98	25.00	22.96	0.63	1.40^{ab}
	T_4	19.14	19.67	16.55	0.59	1.44^{a}
	T_5	24.25	25.00	20.79	0.55	1.37^{abc}
	T_6	19.44	21.67	18.16	0.64	1.42^{ab}
	T_7	18.42	17.33	17.62	0.74	1.39^{abc}
	T_8	14.79	15.00	16.04	0.72	1.33^{bc}
	T_9	18.79	23.33	22.15	0.94	1.37^{abc}
R_2	T_1	19.18	17.00	14.37	0.64	1.40^{ab}
	T_2	26.95	19.67	22.42	0.64	1.36^{abc}
	T_3	32.38	25.67	25.29	0.60	1.44^{a}
	T_4	22.07	21.33	19.63	0.58	1.44^{a}
	T_5	31.36	20.00	28.58	0.65	1.30^{c}
	T_6	26.96	30.00	24.57	0.71	1.39^{abc}
	T_7	19.21	18.00	15.58	0.67	1.35^{abc}
	T_8	18.76	22.67	18.93	0.72	1.12^{d}
	T_9	15.19	19.67	17.24	1.05	1.43^{a}
LSD(0.05)		**7.37**	**8.65**	**9.84**	**0.26**	**0.099**
Level of significance		**NS**	**NS**	**NS**	**NS**	*****
CV%		**20.66**	**25.58**	**31.32**	**22.96**	**4.34**

* = significant at 5% level of probability, NS=Not Significant

chlorophyll was recorded by R_2T_8 (Sweet orange scion grafted on twelve months old rough lemon rootstock) and preceded by (1.30 mg/g tissue) by R_2T_5 (Sweet orange scion grafted on nine months old rough lemon rootstock) (Table 3). The greater chlorophyll content observed in the leaves of different treatments certified to the better stock-scion collaboration lead to improved capture and assimilation of light, consumption of water and nutrient elements which overall enriched the leaves of grafted scion's photosynthesis ability and biomass making (Deshmukh *et al*., 2017).

CONCLUSION

From above outcomes it was found that R_2T_3 (Sweet orange scion grafted on seven months old Rough lemon rootstock) was preferable for achieving better planting materials, among the combined effects as it gave the highest results in respect of plant height, number of leaves per plant, stem fresh weight and total chlorophyll. R_2T_3 (Sweet orange scion grafted on seven months old Rough lemon rootstock) showed also 80% graft survivability after one year of grafting.

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SHORT COMMUNICATION

Optimization of IBA dose for rooting in fig (*Ficus carica* L.) cuttings

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ABSTRACT

Present investigation was carried out to optimize the dose of rooting hormone, Indole-3-butyric acid (IBA) for rooting of fig cv. Brown Turkey cuttings under arid irrigated zone of Punjab. The hard wood cuttings were collected during January and treated with different concentrations of IBA (0, 100 ppm, 1000 ppm, 2000 ppm, 3000 ppm). The results of investigation indicated that the treatment of IBA @ 1000 ppm induced maximum cutting success (68.6%), number of buds sprouted per cutting (2.4), number of leaves (11.3), shoot length (37 cm), fresh weight of shoots (36 g) and dry weight of shoots (11.7 g). Also, the maximum number of roots per cutting (69.5), fresh weight of roots (4.9 g) and dry weight of roots (2.4 g) was recorded under the same treatment after 180 days of planting. It is concluded that treatment of cuttings with IBA @ 1000 ppm for five minutes was helpful in rapid vegetative propagation of fig crop in the arid part of the Punjab state.

***Keywords*:** Cutting success, fig, IBA, propagation, rooting

INTRODUCTION

Fig (*Ficus carica* L.) is an important fruit crop of Moraceae family. Fig crop is mainly cultivated in Maharashtra, Karnataka, Andhra Pradesh, Gujarat, Uttar Pradesh and Tamilnadu. Nowaday, the demand of this crop is increasing due to its nutritional value and hardy nature of plant (Nandi *et al.,* 2018). The figs are consumed as fresh, dried, preserved, candied, canned and also used for jam making (Caetano *et al.,* 2017). Though, the crop possesses huge market potential, still area under its cultivation is limited. Its economic potential of cultivation has not been completely realized and is considered as an underutilized fruit crop in Punjab. Due to late arrival of monsoon rains in this part, it is also a potential area for fig cultivation.The main reason for low area under this potential fruit crop seems to be the unavailability of elite planting material of superior genotypes. In recent, the Punjab Agricultural University has recommended the cultivation of a promising fig variety 'Brown Turkey' for cultivation in Punjab state (Anonymous, 2021). Fig is generally propagated through hardwood cuttings collected during dormant period (December-January). There are various factors which determine the success of rooting in fig (Boliani *et al.,* 2019). Among these the local environment and use of growth regulators (auxins) exert profound influence in rooting of different crops (Kumar *et al.,* 2015).The optimum dose for root induction may also vary according to crop and cultivar (Ludwig-Muller, 2000). The present study was planned to optimize the dose of indole-3-butyric acid (IBA) for treating the hardwood cuttings to produce rooted plants in fig cv. Brown Turkey under South-Western region of Punjab.

The experiment was performed at the fruit nursery, Regional Research Station, Abohar, Punjab Agricultural University, Punjab. The experiment was carried out during January-July, 2022. The cuttings of 20 cm length and 2.5 cm diameter, were prepared from last season growth of 7 years old fig plants var. 'Brown Turkey' during 1st week of January.The experiment was laid out in completely randomized design (CRD) with five treatments and four replications. Fifteen hardwood cuttings were used per replication. Cuttings were treated with four different concentrations of indole-3- butyric acid (IBA) *i.e.,* 100 ppm, 1000 ppm, 2000 ppm and 3000 ppm and water (control) for a period of 5 minutes.The treated cuttings were planted into plastic bags filled with a potting mixture of soil,

Table 1: Effect of IBA concentrations on cutting success and leaf, shoot and root parameters in fig cv. Brown Turkey

Treatment details	Days taken for sprouting	Cutting success (%)	Number of buds sprouted / cutting	Number of leaves	Shoot length (cm)	Number of roots/ cutting	Root length (cm)
T_1 (IBA @ 100 ppm)	29.7^{c}	45.2^{d}	2.3	8.5^{b}	25.0^{c}	44.5^{c}	34.9^{ab}
T_2 (IBA @ 1000 ppm)	26.7^{d}	68.6^{a}	2.4	11.3^{a}	37.0^{a}	69.5^{a}	42.1^{a}
T_3 (IBA @ 2000 ppm)	30.3^{bc}	60.2^{b}	2.1	9.0^{b}	32.9^{ab}	56.0^{b}	37.8^{ab}
T_4 (IBA @ 3000 ppm)	32.8^{ab}	55.1^{c}	1.9	8.8^{b}	29.8^{b}	52.0^{b}	32.5^{b}
T_5 (control)	34.0^{a}	26.4^{e}	2.1	8.3^{b}	21.4^{c}	33.5^{d}	24.3^{c}
C.D. (*P*=0.05)	**2.9**	**1.5**	**-**	**1.7**	**4.3**	**6.9**	**7.7**
C.V.	**6.2**	**1.9**	**16.1**	**12.3**	**9.7**	**7.2**	**12.4**
ANOVA	**s**	**s**	**ns**	**s**	**s**	**s**	**s**

* s: Significant; ns: Non-significant

sand and FYM (1:1:1 v/v proportion) by keeping at least three buds outside above the potting mixture. The cuttings were watered at alternate days. Weeding was done as and when required. The data was taken on days to bud sprout initiation in different treatments, percent cutting success and average of buds sprouted/cuttings was recorded after 60 days of treatment. Further, per cutting, the data on shoot and root growth parameters including total number of leaves, shoot length, number of roots, root length, fresh weight of root, fresh weight of shoot, dry weight of shoot and dry weight of root were recorded after 180 days of treatment. For shoot and root length, the data was taken on longest shoot or root per cutting. During the experiment, average temperature and relative humidity were in range 10.1-28.5°C and 47.1-70.5%, respectively. All the data were analysed using OPSTAT (Sheoran *et al.,* 1998) and discussed at $P < 0.05$ for significance of difference between their mean values.

The results showed that IBA had significant effect on days to sprout initiation and cutting success ($P<0.05$). Among, the different treatments, earliest sprouting (26.7 days) was recorded in cuttings treated with IBA @ 1000 ppm (T_2) as shown in Table 1. Sprout initiation was not advanced with any further increase in concentration of IBA. There was a significant increase in the percentage of rooted cuttings with the use of IBA over control (Table 1). The maximum cutting success (68.6%) was recorded with IBA @ 1000 ppm followed by IBA @ 2000 ppm (T_3), respectively. The data also showed that an increase in IBA concentration over 1000 ppm progressively decreased the cutting success. This might be due to the sensitivity of cuttings to higher concentrations of auxin. These results are in accordance with Ghosh *et al*. (2017) who reported more mortality and less survival percentage of phalsa cuttings with use of higher concentrations of auxin. No significant effect of IBA was seen on number of buds sprouted per cutting but significant effect was recorded for number of leaves/ cutting ($P<0.05$). Comparatively, higher number of leaves (11.3) was recorded in T_2 (IBA @ 1000 ppm) over the other treatments. Similarly, maximum shoot length (37 cm, 32.9 cm) was recorded for cuttings treated with IBA @1000 ppm (T_2) followed by IBA @ 2000 ppm (T_3), respectively, which were statistically on par. More number of leaves in these cuttings possibly reflects their comparatively better photosynthesis that resulted in better growth of shoots. Further increase in IBA concentration did not influence the shoot length ($P<0.05$) (Table 1). Significantly higher number of roots per cutting (69.5) was also observed in cuttings treated with IBA @ 1000 ppm (T_2). The effect of IBA treatments also reflected clearly on root length of the cuttings. The IBA treated cuttings had significantly lengthier roots compared to untreated control. The significantly longer roots (42.1 cm, 37.8 cm) were recorded in cuttings treated with IBA @ 1000 ppm (T_2) followed by IBA @ 2000 ppm (T_3),

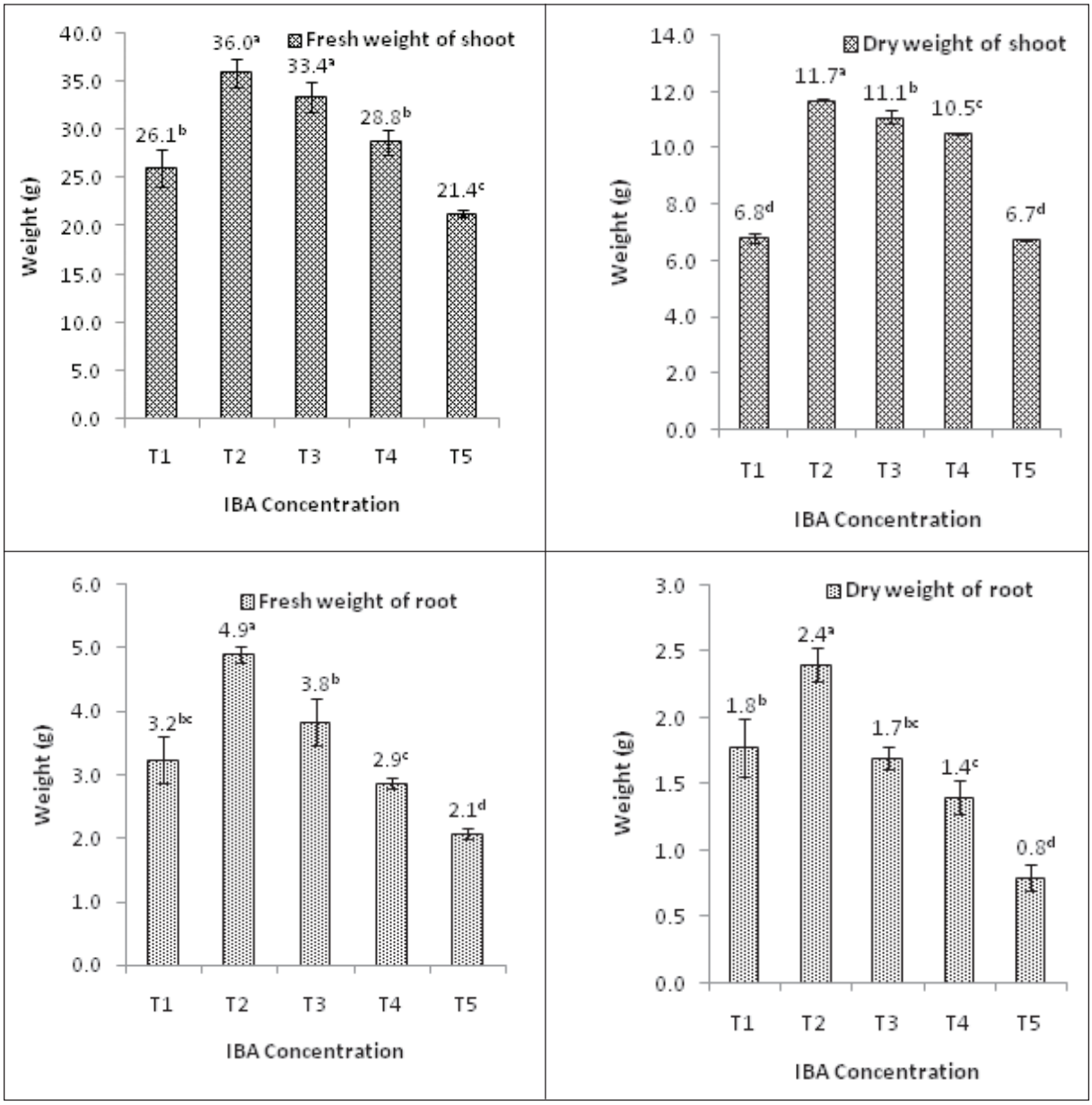

Fig. 1: Effect of IBA concentrations on fresh and dry weight of shoot and root in fig cv. Brown Turkey (Vertical bars indicate ± SE mean)

respectively (Table 1), which were statistically at par. The results are in conformity with Kumari *et al.* (2020) who suggested that number of roots per cutting is intensified by auxin through polysaccharides hydrolysis which provides energy for root formation. Higher concentrations of auxin can cause damage to the cuttings base. Optimum concentration of auxin varies with crop and cultivars and possesses an inhibitory effect at higher concentrations (Cerveny and Gibson,2005). Significant differences among treatments were observed for fresh and dry weight of shoots as well roots ($P<0.05$) (Figure 1). The optimum concentration of auxin also helps in translocation of carbohydrates and nitrogenous substances to the base of cuttings, that promotes accelerated cell division and cell elongation (Singh *et al.,*2015). The maximum mean fresh weight (36.0 g, 33.4 g) and dry weight (11.7 g, 11.1 g) of shoots was found with IBA @ 1000 ppm followed by IBA @ 2000 ppm, respectively. Similarly, higher fresh and dry weight of roots was recorded in cuttings treated

with IBA @ 1000 ppm (Figure 1). The increase in shoot and root biomass with use of auxins is consistent with the earlier findings of Thota *et al*. (2012), Kaur and Kaur (2017) and Kumari *et al.* (2020) in fig crop.

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SHORT COMMUNICATION

Ethnobotanical study of anti-inflammatory medicinal plants in the region of El Bayadh (Western Algeria)

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ABSTRACT

This ethnobotanical study aimed to document the traditional use of anti-inflammatory medicinal plants by the inhabitants of El Bayadh province in Algeria. Data was collected using a questionnaire consisting of a series of questions, which yielded information on the use of plants by the local population. The study identified 100 medicinal plant species belonging to 50 families, with Lamiaceae being the most commonly used family. Results showed that women use roots more frequently than men, and infusions are the preferred method of preparation for medicinal remedies. The most commonly treated ailments were related to digestive system disorders, followed by sarcoidosis, Buerger's disease, Lupus skin inflammation, and other skin disorders. The Floweraceae family was found to be the most commonly used family.

***Keywords*:** Anti-inflammatory, El Bayadh, ethnobotanical study, traditional use

INTRODUCTION

Plants have been used for medicinal purposes for centuries, and they contain bioactive compounds with various therapeutic properties, such as anti-inflammatory, antioxidant, antimicrobial, and anticancer activities. Alkaloids, phenolic acids, terpenes, vitamins, and minerals are among the bioactive compounds found in medicinal plants, which possess anti-inflammatory properties. Additionally, the use of medicinal plants for treating inflammatory diseases has gained popularity due to their perceived safety and natural origins (Atik Bekkara *et al*., 2007).

Inflammation is a necessary physiological response to injury, infection, or tissue damage. However, chronic inflammation can lead to various diseases, including cancer, arthritis, and cardiovascular diseases. Therefore, the evaluation of phytotherapeutic properties, such as anti-inflammatory activity, is essential and valuable, especially for plants that are commonly used in traditional medicine. These plants represent a vast source of biologically active substances (Boudjida and Sahnoun, 2017).

Ethnobotanical studies are commonly used to identify plants used in traditional medicine for various ailments, including inflammation. El Bayadh, a region in Western Algeria, has a rich diversity of plant species used in traditional medicine (Hcds, 2017). Therefore, this study aims to identify anti-inflammatory plants in the region using ethnobotanical methods and to evaluate their potential as sources of new drugs for treating inflammatory diseases.

Presentation of the study area

The wilaya of EL Bayadh, located in southwestern Algeria, plays a crucial role in the region's Great Steppe Fields. Bordered by several wilayas, including Saida, Tairet, Laghouat, Ghardaïa, SidiBelAbbès, Naàma, Bechar, and Adrar, it covers a vast area of 71697 km². In 2008, the wilaya's population was estimated at 228,624, a significant increase from 168,789 in 1998, with numerous communities exceeding 10,000 inhabitants. The bone-dry environment of the district is characterized by a significant temperature difference, making it a unique and challenging habitat for plant life (ANAT, 2003).

Investigation process

For information assortment, an ethnobotanical review was completed during the long periods of March and April 2022 utilizing a survey sheet, including explicit inquiries concerning the source and the restorative plant utilized. The overviews endured just about 10 to 20 min. The examination began at first by reaching the different nearby friendly entertainers, who have a nearby association with restorative plants, the most perceived, regarded, and experienced locally. During each interview, we gathered all the data on the respondent and the restorative plants utilized by him. Subsequently, from the factors examined, specifically age, orientation, level of instruction, and family circumstances. Information gathered for every medicinal plant incorporates normal neighbourhood name, utilizes, part(s) utilized, method of arrangement, state of the plant, portion utilized, utilization of the plant, sicknesses.

Analyses of ethnobotanical data

In statistical analysis, we utilized Microsoft Office Excel rendition 2010 for measurable examination. Examines of differences and means were performed for every variable. Through the ethnobotanical study completed among the number of inhabitants in the districts, it just so happens, that there is a variety of practices, with respects to the species and parts utilized. As well as an assortment of data concerning individuals studied; Gender, age bunch, family circumstances, level of study, State of the plants, Parts utilized, Mode of planning, Dose utilized, Use of the plant, and Diseases treated by the plant.

Profile of the respondent

Membership sex

In the locale examined, all kinds of people practice conventional medication. Notwithstanding, the female sex prevails with a level of 53%. Besides, this rate is just 47% for the male sex (Fig. 1). This makes sense in the way that ladies are more worried about phytotherapeutic treatment and the planning of plant-based recipes. The outcomes acquired in the locale show that ladies hold more conventional phytotherapeutic information than men. The outcomes got through this ethnobotanical study uncover that the female sex prevails with a level of 53%. Also, this rate is just 47% in the male sex (Fig. 1). This makes sense of the way that ladies are more worried about phytotherapeutic treatment and the readiness of vegetable-based recipes, for themselves as well as for the entire family. The outcomes acquired by Benkhnigue (2011) El Hafian *et al.* (2014), Adouane (2016), Fah *et al*. (2013) (Morocco) and in the districts of Aurès (Algeria); in MechraâBelKsiri (Beskra) and Kabylia (Lahsissene, 2009) show that ladies hold more conventional phytotherapeutic information than men. Accordingly, Aribi (2013) likewise finds in an ethnobotanical investigation of restorative plants in the Jijel locale that it is ladies (68%) who have more information on therapeutic species contrasted with men (32%).

Age class

The utilization of restorative plants at the level of the area examined is far and wide among all age bunches (Fig. 2). The age gatherings of 20 to 30 years (8.7%) don't utilize the conventional medication for their clinical wellbeing. Nonetheless, individuals in the age bunch 30 to 50 (30.1%) and age 50 to 80 (61.2%). These qualities affirm the outcomes got in different chips away at the utilization of restorative plants, which show that the old realize conventional home grown medication all around contrasted with other age gatherings, correspondingly, the apathy toward Phytotherapy among individuals in the age gathering of 20 to 30 years is made sense of by the question, especially of youngsters who tend not to trust a lot in this customary medication. The outcomes got through this ethnobotanical study uncover that most of the respondents are beyond fifty years old, which makes sense why these old individuals have more information about the utilization of restorative plants contrasted with youngsters. These qualities affirm the outcomes got in different examinations on the utilization of restorative plants (Benlamdini *et al*., 2014; Orch *et al.,* 2015; Aribi (2013) ; Ait, 2015) which successfully show that the old realize customary home grown medication all around contrasted with other age gatherings, likewise, the indifference toward natural medication among individuals in the age gathering of 21 to 30 years makes sense by doubt, especially of youngsters who tend not to trust a lot in this conventional medication.

Family situation

The utilization of restorative plants by married people addresses 67.33%. Then again, single individuals address just 32.67%. This is made sense by the way that wedded individuals are dependable as guardians for giving first helpful consideration to the entire family, subsequently diminishing the material costs expected by the specialist and drug specialist.

The outcomes got are affirmed by other ethnobotanical studies done by El Hafian *et al*. (2014) (Beskra), the last option shows that 70% of clients of restorative plants are hitched individuals. We can presume that restorative plants are utilized considerably more by wedded individuals than by single individuals because of multiple factors; family encounters exhibit at times the shortcoming of present-day medication to treat basic everyday pathology, and the symptoms of specific medicines, especially in youngsters. Yet in addition, this distinction could be because of the monetary means; today, current medication has turned into a significant weight on little families.

Academic level

Out of the relative multitude of clients of conventional medication, illiterate people rule with a level of 43.7%. This level of purpose is not insignificant among individuals with an essential level (22.67%). While scholastics utilize restorative plants less with a level of 33.63% (Fig. 3). The outcomes got in an investigation discovered that 43.7 that is certified in specific uneducated individuals who utilize the restorative plants by a nonsensical method of individuals utilize the restorative plants are unskilled. In this review, we can see that the various degrees of investigation of the populace are keen on customary medication. As per El Hilah *et al*. (2016) (Beskra), restorative plants can be hazardous when utilized unknowingly, and this is affirmed by a few unskilled individuals who unreasonably utilize therapeutic plants, different uneducated people can't exactly comprehend the verbal directions communicated by cultivators and healers. This high pace of ignorance among clients of restorative plants is a genuine hindrance to neighbourhood improvement. The outcomes acquired by Fah *et al.* (2013) (Western Morocco), demonstrate that famous information is right now held by a couple of individuals, among which there is a high pace of ignorance. Benlamdini *et al.* (2014) in a review at the Eastern High Chartbook (Beskra) level see that 41% of individuals utilizing restorative plants are uneducated, 26% have an essential level, 24% have an optional level and 9% are college graduates. Comparably, Orch *et al*. (2015), in an ethnobotanical investigation of restorative plants utilized in the locale of Izarène (northern Morocco), see that 75% of individuals reviewed were unskilled or had an elementary school level. Baba Aissa (1999) finds that in the Jijel District most clients of restorative plants are ignorant (52%).

Plant condition

44% of the plants are utilized new, they are fundamentally utilized in the planning of mother tinctures, poultices, and soups. Then again, 56% is utilized in dried structure, they comprise the premise of natural teas, powders, and concentrates (Fig. 4). In this review, we can see that 44% of plants are utilized new versus 56% are utilized in dried structure, comparable outcomes tracked down in Morocco (Ait Ouakrouch , 2015; El Hilah *et al*., 2016) .

Parts used

The Fig. 5 demonstrates that different plant organs are involved in the populace for the fulfillment of their remedial necessities. In the review region, the leaves comprise the most utilized part with a level of 39%, trailed by the stems (14%), the entire plant (37%), and the seeds (10%). This distinction in extents in the plant parts involved is legitimate by the fluctuation in the convergence of the dynamic standards in each plant organ or even every species. This distinction in extents in the plant parts involved is legitimate by the changeability in the centralization of the dynamic standards in each plant organ or even every species. The predominance of the leaves is legitimate by the way that they are the site of most of the phytochemical responses and repository of the natural matter which gets from them, the leaves supplies most of the alkaloids (Bouallala *et al*., 2014 ; Chehma *et al*., 2005). The leaves give most of the alkaloids. The significance of natural products is because of the convergences of their unpleasant, glucidic or fragrant substances related to specific shades which

give them a trademark shading. The utilization of blossoms is because of their extravagance in natural ointments. The equivalent is valid for roots and seeds plentiful in sugars and nutrients (Babba Aissa, 1999).

Method of preparation

Different remedial practices are utilized by the neighbourhood individuals for the treatment. The mode most applied in the district is infusion (44.33%) trailed by a decoction (18.67%), maceration (15.79%), powder (11.54%) poultice (9.67%) (Fig. 6). Clients are continuously searching for the least difficult technique to plan phytomedicines, which affirms the strength of the imbuement mode for our situation. To work with the organization of the dynamic fixing, a few remedial practices are utilized, to be specific decoction, and mixture. The best utilization of a plant is what might protect every one of its properties while permitting the extraction and digestion of the dynamic fixings (Dextreit, 1984). The implantation is the technique for an arrangement that saves the plant's dynamic standards (Moatti *et al.,* 1983). The decoction warms the body and cleans the plant to drop the poisonous impact of specific recipes, however, it can obliterate specific dynamic standards of the species utilized. Moreover, natural meds have antagonistic impacts when polished mistakenly by patients. Accordingly, conventional medication should be drilled with care and unmistakable boundaries and measures.

As per Salhi *et al*. (2010), clients are continuously searching for the easiest technique to get ready phytomedicines, which affirms the predominance of the imbuement mode for our situation. Crafted by Chehma and Djebbar (2005) (Algerian Sahara, instance of Ouargla) and El Hilah *et al.* (2016) (Morocco) record that the imbuement mode is the prevailing and address paces of (half), (20.45%) and (72.50%) separately. The rest (33%) is ready in another mode; grinding, steam distillation, maceration, mixing, omen, and cauterization.

Rates (dose) used

76% of medicinal plants announced are utilized with unknown portions. The portion is as yet irregular, which shows itself in antagonistic wellbeing impacts at times. Therapeutic plants are utilized with unknown portions; 9% per squeeze, 11% per spoonful, and 4% per small bunch (Fig. 7). The portion stays unsure, which is appeared by destructive consequences for well-being in specific cases, since it is said: "no substance is harming itself, the portion makes the toxin". This outcome is reliable with different outcomes got somewhere else by different creators Benkhnigue *et al.* (2011) who showed that 85.12% of therapeutic plants are utilized with undefined portions; 8.8% per squeeze, 26.20% per scoop, and 50.12% per small bunch 11.56%.

Use of the plant

The outcomes acquired through this ethnobotanical study uncover that most of the respondents utilized 70% restorative plants, 15% consumable, 12% beauty care products, and 3% harmful (Fig. 8). The outcomes got are affirmed by other ethnobotanical studies completed by El Hafian *et al.* (2014) Beskra. These show that 73% restorative and 17% consumable, 12% corrective and 3% for grain assets, and 5% for unrefined components for industry and artworks. 12% for beauty care products and fragrant items, and finally (1%) therapeutic plants are fancy and developed for improvement purposes.

Inflammatory diseases treated by medicinal plants

Ethnobotanical analysis has identified several diseases treated by medicinal plants as illustrated in fig. 9. We observed that the most treated pathologies are: Ulcerative colitis (24%) and Crohn's disease (18%), followed by the diseases Sarcoidosis (16), Buerger's disease (14) %) and chronic lupus erythematosus (13%), then the other diseases which present by small percentages Inflammation of the tonsils (10%), and Eczema (5%). In general, the results obtained show that the most treated symptoms are inflammatory diseases. The dominance of inflammation is confirmed by several other authors. Indeed, Chesham *et al*., (2005) showed that the most widely treated symptoms are chronic inflammation representing respectively 26% and 24%. %. These same results were found by Tari *et al.* (2012) in the province of Settat (Beskra), who showed that most species are used in the care of inflammatory diseases.

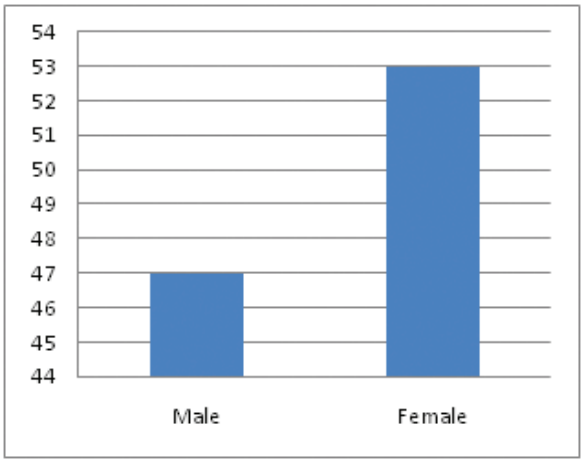

Fig. 1: Use of medicinal plants by gender

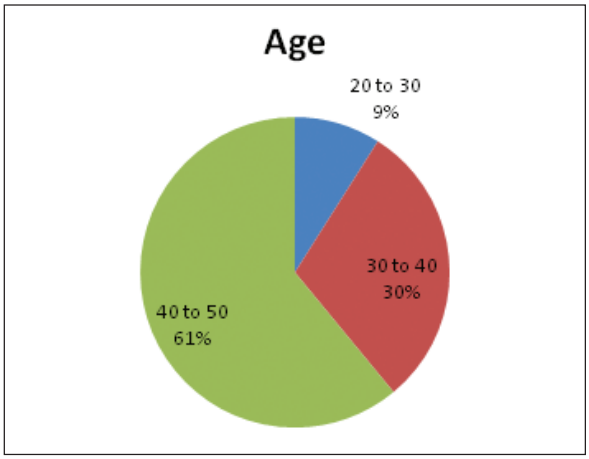

Fig. 2: Use of medicinal plants according to age of respondent

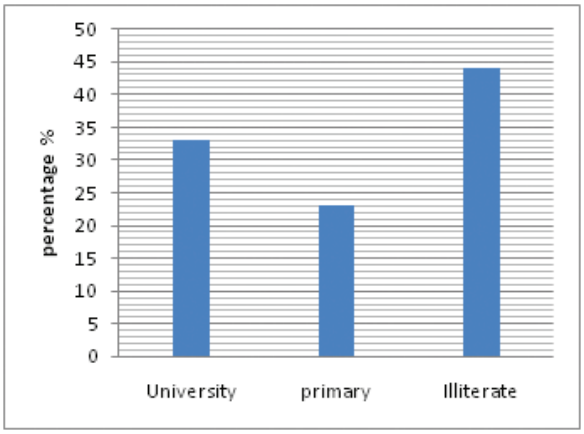

Fig. 3: Use of plants according to level of education

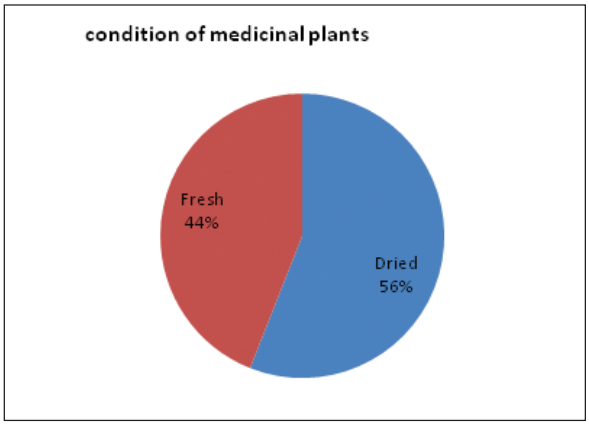

Fig. 4: Use of medicinal plants according to their condition

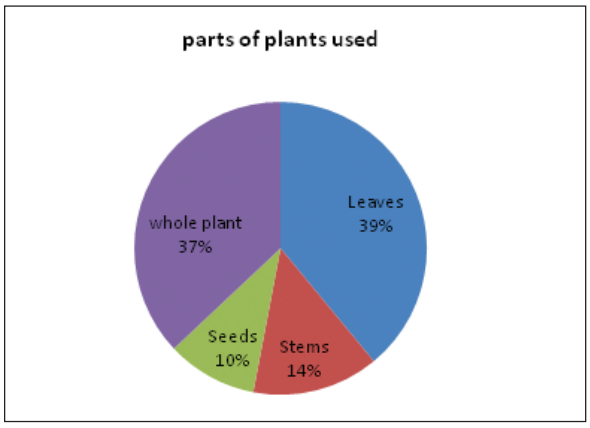

Fig. 5: Different parts of medicinal plants used

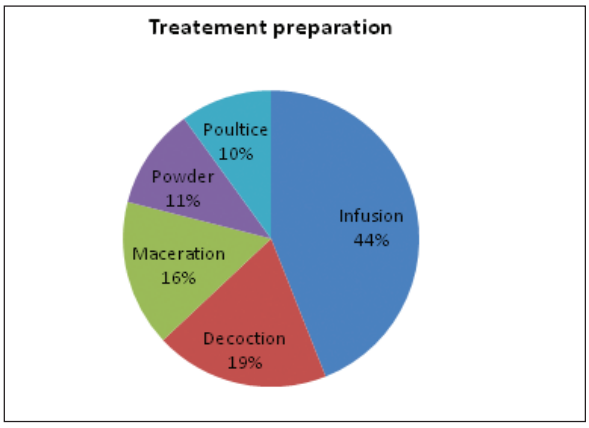

Fig. 6: Different treatment preparation methods

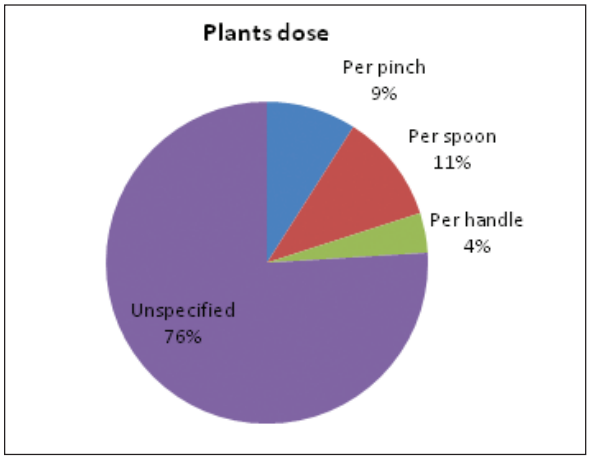

Fig. 7: Use of plants according to dose

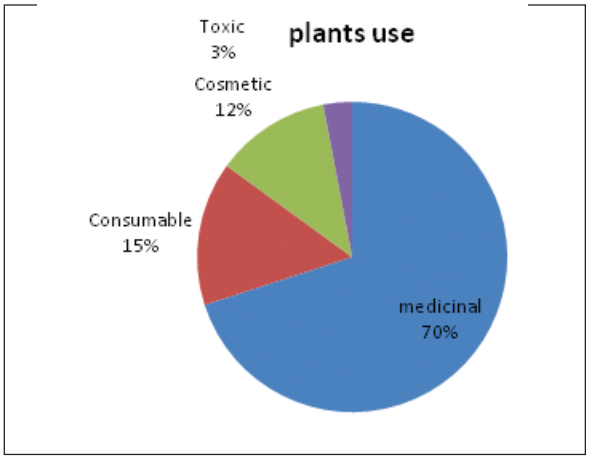

Fig. 8: Use of the plant

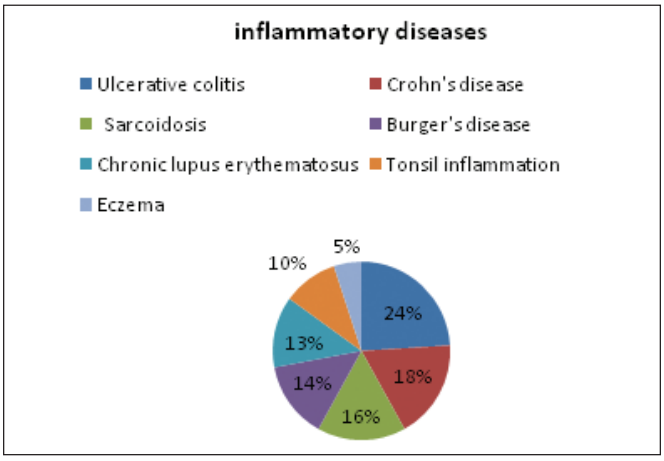

Fig. 9: Pie chart represents inflammatory diseases treated by medicinal plants in the study area

El Rhaffari and Zaid (2002), find in a similar study that in Tafilalet (South-East of Morocco) plants are used for the treatment of the main dysfunctions as follows: Hemorrhagic proctocolitis (19.3%), dermato -cosmetology (14%), Sarcoidosis (9%), osteo -articular affections (7%), metabolism and secretion (4.9%). Several species are used against several diseases, such as thyme, mugwort, and rosemary, they are used against chronic lupus erythematosus, respiratory and skin diseases, which explains the particular pressure exerted on these plants in the region studied.

According to the most used plant

During our ethnobotanical study, we figured out how to recognize a sum of 36 restorative plants with their remedial purposes. Among the species that are better utilized, some end up being all the more as often as possible referred to. This vouches for their extraordinary handiness being taken care of by conventional medication around here. Among the species referenced are Lemon verbena (*Aloysia citrodora*) 20.95%, Lavanders (*Lavandula angustifolia*)17.26%, Eucalyptus (*Eucalyptus globulus*) 15.18%, Ocean purslane (*Atriplex halimus* L) 12.69%, white mugwort (*Artemisia absinthium*)11.55 %, Harmal (*Peganum harmala* L.) 5.8%.

From every one of the outcomes acquired, we have accumulated the restorative plants most utilized by the nearby populace. The vast majority of the plants developing are *Lavandula angustifolia*, *Lemon verbena,* Eucalyptus, *Ocean purslane,* Rosemary, white mugwort, Harmal , in the review region. The recurrence of Lemon verbena is the most noteworthy, this demonstrates that rosemary is the restorative plant most utilized by the nearby populace considered, trailed by Eucalyptus.

Table 1: Lists of the species identified in the study region

Nbr	Family	Scientific nomenclature	Common arabic name
1	Apiaceae	*Carum carvi*	كروية
		Foeniculum vulgare	بسباس
		Cuminum cyminum	كمون
		Pimpinella anisum	حبة الحلاوة
		Thapsia garganica	بونافع درياس
		Petroselinum cripum	معدنوس
2	Amaranthaceae	*Artiplix halimus*	قطف
		Arthrophytum scoparium	رمت
		Spinacia oleracea	سلك
3	Asteraceae	*Artemisia absinthium*	شيح
		Matricaria chamomilla	بابونج
		Matricania pathenium	أقحوات
		Artemisia campestris	دقوفت
		Silybum marianum	شوك الجمل
		Ocimum basilicum	هباغ
		Helianthus annus	دوار الشمس
		Launaea resedifolia	روقيام
		Anvillea radiata	نقد
		Cynaraca rdunculus	خرشوف

Contd.

Contd. Table 1

4	Lauraceae	*Laurus nobilis*	رند
		Cinnamom umverum	قرفة
5	Lamiaceae	*Lavandula angustifolia*	خزامة
		Mentha pulegium	فليو
		Mentha spicata	نعناع
		Thymus vulgaris	زعتر
		Salvia rosmarius	الأزير
		Sauge officinale	مرامية
6	Zingiberaceae	*Zingiber officinale*	زنجبيل
		Curcuma longa	كركم
		Elettaria cardamomum	الهيل
		Alpinia purapurate	اللبنية
7	Cactaceae	*Cereus repandus*	الصبار
		Opuntia ficus indica	هندي
8	Caryrophyllaceae	*Syzygium aromaticum*	قرنفل
9	Cupressaceae	*Juniperus communis*	عرعار
10	Cucurbitaceae	*Citrullus colocynthis*	هندهل
		Colocynthis vulgaris	هدج
		Ecballium elaterium	فقوس الحمير
11	Crocoideae	*Crocus sativus*	زعفران

Contd.

Contd. Table 1

12	Mytaceae	*Callistemom viminalis*	فراش الزجاج
		Eucalyptus globulus	كاليبتوس
		Syzygium aromaticum	طب
13	Malvaceae	*Tiliato mentosa*	زيزفون
		Malva sylvestris	خبيز
		Hibiscus sabdariffa	كركدية
14	Nitrariaceae	*Peganum hermala*	حرمل
		Lnula helenium	غسن
15	Poaceae	*Zea mays*	بشنة
		Stipa tenacissima	حلفاء
		Avena sativa	الشوفان
		Hordeum vulgare	الشعير
		Triticum vulgare	قمح
16	Fabaceae	*Retama raetam*	رتم
		Glycyr rhizaglabra	عرق سوس
		Tetrapleura tetraptera	خروب
		Cassia angustifolia	سنا مكي
		Lupinus luteus	ترمس
		Astragalus armatus	لقداد
		Trigonella foenum-graecum	حلبة
17	Rosaceae	*Tormentilla offcinalis*	لنجبار
		Crataegus azarolus	زعرور
		Punus myrtifolia	ورك الخوخ
		Rosa acicularis	ورد

Contd.

Contd. Table 1

18	Rhumnaceae	*Ziziphus lotus*	سدرة
19	Rutaceae	*Ruta montana*	فيجل
20	Rubiaceae	*Rubia tinctorum*	فوة
		Trachyspermum ammi	نوخة
21	Ranunculaceae	*Nigeria sativa*	حبة البركة
22	Vebenaceae	*Aloysio citriodora*	لوزية
23	Araliaceae	*Panax ginseng*	جنسينغ
24	Amaryllidaceae	*Allium vineale*	ثوم
		Alchemilla vulgaris	خميلة
25	Piperaceae	*Piper nigrum*	فلفل أسود
26	Solanaceae	*Capsi cumannum*	فليفلة حرفية
		Capsicum frutescens	فلفل حار
27	Salicaceae	*Salix alba*	صفصاف
28	Astereneae	*Petroselinum cripum*	بكرونس
29	Brassicaceae	*Lepidium sativum*	حب الرشاد
30	Padaliaceae	*Sesamum indicum*	جلجلان
31	Leguminoseae	*Glycine max*	فول الصوجا
32	Urticaceae	*Urtica dioica*	حريق
		Paretaria Judaica	حشيشة الجراح
33	Anacardiceae	*Rhus glauca*	سماك
34	Apiaceae	*Apium graveolens*	كرافس
35	Lilaceae	*Oncostema peruviana*	بسباس بروفي
36	Geraniaceae	*Pelargonisum graveolens*	عطرشة
37	Ginkgoaceae	*Ginkgo biloba*	جنكة

Contd.

Contd. Table 1

38	Droseraceae	*Droderaro tundifolia*	فراش الندى
39	Ephedraceae	*Ephedra foeminea*	علندة
40	Tamaricaceae	*Tamarix gallica*	طرفية
41	Thymeliaceae	*Thymelaea hirsuta*	مثنان
42	Oléaceae	*Olea europaea*	زيتون
43	Oxalidaceae	*Oxalis pes-caprae*	حميضة
44	Papavéraceae	*Papaver rhoeas*	بوغار عون
45	Lythraceae	*Lawsonia inermis*	حنة
46	Euphorbiaceae	*Ricinus communis*	خروة
47	Plantaginaceae	*Plantago lanceolata*	ناردين
48	Apocynaceae	*Vinca major*	عنقية
49	Allaceae	*Aliumnae politanum*	الثوم الأبيض
50	Brassicaceae	*Capsella bursa-pastoris*	حندوق حلقي

The low recurrence for restorative plants less utilized by the neighbourhood populace can be made sense of by:

- Significant expense of specific restorative plants sold.
- The harmfulness of specific species which makes the populace extremely careful about these plants.
- By and large the species that have a high recurrence of purpose are considered by clients as seeds and nursery vegetables different plants are utilized as flavours, spices, or toppings.

According to the botanical families

Furthermore, the plant recognizable proof showed that among the 50 recorded families, those most addressed are the Asteraceae with 10 species or 17%, trailed by the Lamiaceae and the Apiaceae with the Fabaceae 6 species or 12.15%, the Poaceae (5 species 10.4%) Zingiberaceae and (4 species or 9.8%) Amaranthaceae with 3 species each (4.83%), Nitrariaceae and Rubiaceae with two species. These families alone hold 41 species or 20.72% of all the outnumber. While different families are each addressed by just a single species.

Altogether, 100 restorative plants have been distinguished, they are isolated into 50 families. The most addressed families are Asteraceae with 10 species or 17%, trailed by Lamiaceae and Apiaceae with Fabaceae 6 species or 12.15%, Poaceae (5 species 10.4%) Zingiberaceae and (4 species or 9.8%) the Amaranthaceae with 3 species each (for example 4.83%), the Nitrariaceae and Rubiaceae with two species. the other excess families are addressed by three plants all things considered (Table 1). The predominance of the Asteraceae family is made sense of by the biological elements which favour the turn of events and transformation of most of the species in the area of El_Bayadh.

The diversity of species for ethnobotanical use

At the end of the survey, 100 species are identified in the 4 sites sampled mainly in the region of El_Bayadh and which are divided into 50 botanical families (Table 1).

CONCLUSION

The use of traditional herbal medicine remains a popular choice among populations who trust in its effectiveness and cannot afford modern medication. Herbal medicine plays a crucial role in the modern medical field and provides a database through ethnobotanical studies. Studies show that older women possess more knowledge about the use of medicinal plants compared to younger generations, and few academics resort to traditional herbal medicine. The region of El Bayadh utilizes 100 species of medicinal plants, with the families Asteraceae, Lamiaceae, Apiaceae, Fabaceae, and Poaceae being the most popular. It would be interesting to invest in the most commonly used plants in the region to analyze the active molecules that could provide a solution for certain pathologies that cannot be cured by chemical medicine.

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