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Study of the effect of consortia of PGPR on the growth of *Trichosanthes cucumerina* in a hydroponic system

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Abstract

Hydroponics is the method of growing plants using soil-less cultivation systems. Plant growth-promoting bacteria are the rhizosphere bacteria that help plant growth and suppress plant diseases. In this study, we have isolated PGPR from the hydroponic systems and tested the efficacy of a consortium on the growth of *Trichosanthes cucumerina.* Ten organisms were isolated from a pre-set hydroponic system, of which three were selected based on their plant growth-promoting abilities. The isolated strains were identified as *Chryseobacterium jejuense*, *Pseudomonas oryzihabitans* and *Sphingomonas paucimobilis.* These isolates produced high levels of indole-3-acetic acid as well as other plant growth-promoting factors such as cellulase, pectinase, and siderophore production. All three isolates showed biofilm formation and growth in nutrient solutions with high EC values, indicating their ability to adhere to plant root surfaces and survive in nutrient solutions to promote plant growth. A consortium of these organisms used in the deep-water hydroponic system of *T. cucumerina* showed a significant increase in the number of leaves and root mass compared to control plants. Since these PGPR isolates exhibited multiple traits beneficial to the host plant, it has opened new possibilities for commercial application of these isolates in the hydroponic systems.

Key words: Hydroponics, plant growth-promoting bacteria, biofilm, *Trichosanthes cucumerina*

Introduction

Microbial flora on plant roots makes up the rhizomicrobiome of the plant. It is well known that the composition and activity of the rhizomicrobiome are well regulated by the plant and that the flora comprises many bacteria that aid in enhancing plant growth. Some of the direct benefits of this microflora include nitrogen fixation, phytohormone production, nutrient acquisition and assimilation viz-phosphate solubilization and iron sequestration. The indirect benefits include the production of hydrolytic enzymes and secondary metabolites that are often active against a broad spectrum of phytopathogens and also the production of various compounds that modulate stress responses of the plant and induce defence mechanisms (Backer *et al.,* 2018). Such bacteria that colonize on the root surface or are found in the rhizosphere or are associated with it are known as plant growth-promoting rhizobacteria (PGPR) (Souza *et al.,* 2015). Some PGPR belonging to the genera *Azospirillum, Azotobacter, Bacillus, Pseudomonas, Paenibacillus, Rhizobium, Klebsiella, Enterobacter* are used as biofertilizers for enhancing the productivity of crops (Dhayalan *et al.,* 2021). Kumari *et al.* (2019) have reported the application of PGPR as biostimulants, biocontrol agents and as agents that could enhance phytoremediation. A recent meta-analysis of studies from 2010-2020 provides evidence that microbial inoculants can enhance agricultural productivity and nutritional quality and can be used either alone or in combination with reduced amounts of agrochemicals to promote sustainable agriculture (Li *et al.,* 2022). Vasseur-Coronado *et al.* (2021) have suggested steps for screening and selection of PGPR having traits useful for their future development as commercial plant biostimulants.

The ever-increasing world population, deteriorating soil qualities and the new world's demand for agricultural products free of hazardous chemicals have forced humankind to look for alternative cultivation techniques with controlled environmental conditions. Hydroponics is one such widely and frequently used technique for growing plants without soil, providing for a considerable degree of control of the elemental as well as microbial environment surrounding the root. It also has the advantage of productivity throughout the year and needs less space. Crops grow faster in hydroponics and yield is much higher leading to more production from the same amount of space (Solanki *et al.,* 2017; Ohta, 2017). Since the global market for hydroponic systems is estimated at $9.5 billion in 2020 and is predicted to reach $16.6 billion by 2025 (Azizoglu *et al.,* 2021), good knowledge of microorganisms associated with hydroponics, especially the beneficial ones, would help the researchers obtain deeper insights to enhance the growth of the plants and in turn, help agriculturists enhance productivity.

Aini *et al.* (2019), Kalozoumis *et al.* (2021), Aini *et al.* (2019), and Rahmoune *et al.* (2017) have illustrated the impact of PGPR on hydroponically cultivated plants such as tomatoes, lettuce, and cherry tomatoes.

Snake gourd (*T. cucumerina* L.), a member of the Cucurbitaceae family is a tropical or subtropical herbaceous annual climber with perennial rootstock, raised for its strikingly long greenish-white fruit used as a popular vegetable in India and Africa. The fruit contains a rich variety of nutrients, vitamins A, B, C and minerals like manganese, magnesium, calcium, iron, potassium and iodine. It also has significant levels of dietary fiber, a low amount of calories and high levels of protein. (Kannan and Arulmozhiselvan, 2019). Both root and fruit are considered to be cathartic. The fruit is used as an anthelmintic agent. The seeds have nutritional properties (Okonwu and Muonewu, 2019) and

are used for stomach disorders and are also considered antifebrile and anthelmintic agents (Saboo *et al.,* 2012). To meet the demands of this vegetable and to grow it in controlled environments, hydroponic cultivation is considered to be a viable option. The current work was aimed at isolating PGPR from pre cultivated hydroponic systems of *Trichosanthes cucumerina* and testing the efficacy of a consortium on its growth.

Materials and methods

Isolation of organisms from hydroponics system: Hydroponic cultivation of *T. cucumerina* was set up with a deep tank method at the greenhouse of Ramnarain Ruia Autonomous College. The nutrient solution was sampled at different stages of the plant's growth *viz.* plantlet stage, vegetative, flowering, and fruiting stages of the plant. EC (electrical conductivity) of the nutrient solution was measured using an EC meter at each stage of sampling. The samples were isolated on glucose yeast extract agar (GYEA) plates to study microbial characteristics and pure cultures were then preserved on GYEA slants.

Effect of high EC concentration on isolates from the hydroponic system: Nutrient solution FloraGro, FloraMicro and FloraBloom of General Hydroponics was used in the hydroponic system that was the source of the said microbes. The nutrient solution was prepared as per directions given by the company according to the growing stages of the plant with EC 800 to 1982 microsiemen. All the collected isolates were checked for their ability to grow at high EC. The isolates were inoculated into GYE medium with EC range from [861- 1982] μScm^{-1}. 200µL of sterile GYE medium of varying EC's was inoculated with 50µL of the culture's isolates (OD=0.1) in 96 well microtiter plates and incubated at room temperature (RT) for 24 hours. Absorbance was measured at 490 nm using an ELISA plate reader (Bio-Rad).

Indole Acetic Acid (IAA) production: Detection and estimation of IAA produced by organisms from the hydroponic system by the tryptophan-dependent pathway as well as the tryptophan-independent pathway was carried out. For detecting the IAA produced by the tryptophan-dependent pathway, isolates were grown in King's B medium which was supplemented with L-tryptophan and incubated in total darkness under shaker conditions at 25 ºC for 3 days (Gao *et al.,* 2019); for detecting IAA produced by the tryptophan-independent pathway, the isolates were grown in yeast malt dextrose broth (YMD broth) and incubated at 28 ºC for 4 days. After incubation, the supernatants were used for the detection of IAA (Mohite, 2013).

IAA produced was detected by using Salkowski's reagent; 0.5 mL of Salkowski's reagent was added to 2 mL of the culture supernatant and incubated at RT for 30 minutes under dark conditions, and absorbance was measured at 530 nm (Gang *et al.,* 2019).

Biofilm formation: The overnight grown culture of isolates in the GYE medium was diluted (1:40) into a fresh medium in microtiter plates for biofilm assay. The planktonic cells were removed and the biofilms were washed and stained using 0.1% crystal violet. Stained biofilms were washed and quantified by adding 125 µL of 30% acetic acid in water and measuring absorbance at 550 nm (Vyas *et al.,* 2018).

Biosurfactant production: 50 mL of distilled water was added to Petri dishes followed by the addition of 100 µL of crude oil to the surface of the water. Then, 10 µL of the culture filtrates was put on the crude oil surface. The diameter of the clear zone on the oil surface indicates biosurfactant production (Shah *et al.,* 2016).

Chitinase production: The isolates were spot inoculated on a chitinase detection medium and incubated at 30ºC for 7 days. The development of halo zones around the colony indicates the production of chitinase (Muniroh *et al.,* 2019).

Cellulase production: 5 µL of overnight grown culture was spot inoculated on carboxymethyl cellulose (CMC) agar and incubated at 28ºC for 48 hours. After incubation, the plate was flooded with Gram's iodine for 3-5 minutes. Colonies showing a zone of clearance were identified as cellulase producers (Muniroh *et al.,* 2019).

Pectinase production: 5 µL of the overnight grown culture was spot inoculated on modified Czapek Dox agar medium and incubated at 28ºC for 3-5 days. Pectin utilization was qualitatively detected by visualizing halo zones around the colonies after flooding the plate with Iodine- Potassium iodide solution (Priya *et al.,* 2014).

Siderophore production : Siderophore production was qualitatively determined by Chrome Azurol S (CAS) plate assay (Modi *et al.,* 2017).

Antibiotic susceptibility: Antibiotic susceptibility was detected using Muller-Hinton agar plates and the following antibiotics piperacillin, amikacin, chloramphenicol, tetracycline, gentamycin, imipenem, norfloxacin, methicillin, erythromycin, penicillin, clindamycin, and trimethoprim were used.

Identification of PGPR obtained from the hydroponic system: From the three isolates, two isolates were identified using 16S rRNA sequencing at Saffron Life Sciences, Mumbai. The PCR amplicon was sequenced using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. The 16S rRNA sequence was used to carry out BLAST with the database of NCBI GenBank database. The third isolate was identified using ViTek system at Sunflower Laboratory & Diagnostic Centre, Mumbai.

Determining the effect of PGPR consortia on plant growth in a hydroponic system: Seeds of *T. cucumerina* were rinsed in 95% ethanol and submerged in the aqueous solution of sodium hypochlorite (2.5%). Seeds were then rinsed six times in sterile water and soaked in the consortium culture medium overnight for coating with PGPR. Seeds were then placed in coco-peat for germination (Paradiso *et al.,* 2017). A deep-water hydroponic system was set up to grow the plants.

Statistical analysis: The quantitative assays were conducted in triplicate, and Microsoft Excel was employed for the statistical analysis of the data.

Results and discussion

Isolation of organisms from a pre-set hydroponics system and confirmation of their tolerance to high EC: The organisms were isolated from the nutrient solution at each stage of plant growth, up to the fruiting stage. The isolates were cultured and maintained in a GYEA medium. Ten representative isolates were selected for further studies. Since the EC of nutrient solution used in hydroponics increased with the growth stage of plants, it was essential to determine the tolerance of isolates towards high EC of nutrient solution. The selected isolates tolerated the entire range of EC used in the nutrient medium (up to EC 1982),

indicating their sustenance used in the hydroponic systems and in turn, promoting plant growth during all the stages.

IAA production: Qualitative analysis of isolates for IAA production using Salkowski's reagent indicated that out of the ten isolates, eight isolates produced IAA; but in varying amounts. Out of these, *C. jejuense*, *P. oryzihabitans,* (identified by 16S rRNA sequencing) and *S. paucimobilis* (identified by ViTek system) were selected for further studies as they were the highest IAA producers, by the tryptophan-dependent (Table 1) as well as tryptophan independent (Table 2) pathway.

Table 1. Amount of IAA produced by selected isolates tryptophan dependent pathway

Isolate	Amount of IAA produced (µg/mL)
Chryseobacterium jejuense	6.27±0.25
Pseudomonas oryzihabitans	5.98±0.13
Sphingomonas paucimobilis	5.70±0.22

Table 2. Amount of IAA produced by selected isolates tryptophan independent pathway

Isolate	Amount of IAA produced (µg/ mL)
Chryseobacterium jejuense	6.35± 0.18
Pseudomonas oryzihabitans	4.70± 0.13
Sphingomonas paucimobilis	4.38 ± 0.17

Biofilm formation: All three isolates were capable of biofilm formation as tested by the crystal violet binding assay (Table 3), indicating that they could colonize on root surface effectively. Among the three isolates, *P. oryzihabitans* showed higher biofilm-forming capacity.

Table 3. Amount of Biofilm produced by PGPR isolates

Isolate	Amount of biofilm produced
Chryseobacterium jejuense	0.48± 0.11
Sphingomonas paucimobilis	0.34 ±0.02
Pseudomonas oryzihabitans	0.69 ±0.22

Detection of other PGPR markers: The three isolates were tested for other PGPR markers. Table 4 gives the results for the same. Briefly, the isolates selected showed cellulase, pectinase and siderophore production. Cellulases break down cellulose, found in plant cell walls, into sugars, making them available for plant uptake and serving as a source of energy for microbes. Pectinase promotes plant growth indirectly by increasing the plant's nutrient, water and mineral uptake without causing cell collapse. In a hydroponics system siderophores produced by PGPR help in making iron and other micronutrients readily available to the plants. The isolates were negative for chitinase and biosurfactant activity. Chitinase and biosurfactants (Mishra *et al.,* 2020) exhibit biocontrol activity by inhibiting phytopathogens.

Table 4. Detection of PGPR markers by the isolates from the hydroponic system

Activity	Isolates		
	Chryseobacterium jejuense	*Pseudomonas oryzihabitans*	*Sphingomonas paucimobilis*
Chitinase	-	-	-
Biosurfactants	-	-	-
Cellulase	++++	+	++
Pectinase	++++	+++	+
Siderophore	++	+++	+++

[Key: "+" = very low producer, "++" = low producer, "+++" = medium producer; "++++" = strong producer "-" = no activity]

Determination of antibiotic susceptibility of the isolates selected from the hydroponic system: An antibiotic susceptibility test was carried out for all three isolates. All the isolates were susceptible to all the antibiotics used. The purpose of performing this test was to ensure that the organisms are not of the multiple drug-resistant (MDR) type and are safe to use as a potential biofertilizer.

Determination of efficiency of consortia of PGPR isolates on plant growth in the hydroponic system

Deep-water system setup: The deep-water system was installed in the greenhouse of Ramnarain Ruia Autonomous College, Mumbai. Two systems were set up, one was a test (inoculated) system and the other was a control (uninoculated) system. Both the systems were monitored for a month for EC and growth of test as compared to the control. Vacheron *et al.* (2013) have suggested that the effect of PGPR on plant growth can be studied *In vitro* by monitoring the effects on plants, like the number and/or length of lateral roots, stimulation of root hair elongation, uptake of minerals and water, and thus the growth of the whole plant. Table 5 shows the weekly comparison of the plants set up with and without the consortium.

Table 5. Comparison of plant growth in the hydroponic system with and without PGPR consortium

Week	Treatment	EC	Height (cm)	No of leaves	Comparative root density
1	Control	1110 µScm^{-1}	25	6	+
	Test	1154 µScm^{-1}	12.5	4	+
2	Control	1119 µScm^{-1}	52	15	+
	Test	1164 µScm^{-1}	48	12	+
3	Control	1037 µScm^{-1}	95	31	+
	Test	1194 µScm^{-1}	94	35	+
4	Control	1548 µScm^{-1}	110	33	+
	Test	1621 µScm^{-1}	115	57	+++

By the end of week 2, the test system showed slower growth as compared to the control (Fig. 1). During week 3 there was a rapid increase in growth and by the end of week four, the test showed a significant increase in the number of leaves and root mass, indicating a positive effect on the plant due to the PGPR consortium. The results suggest that the PGPR organisms successfully adhered to the roots colonized and grew in response to root exudates till the end of week 2. During the next fortnight, they produced substantial amounts of plant growth promoters which led to increased plant growth.

PGPR-based inoculation technology has great potential for sustainable agriculture and is gaining popularity as a replacement for chemical fertilizers and chemical stimulants globally. The major challenges faced in this area at the laboratory and field level, however, are understanding the plant-specific microbiome interactions, especially their diversity and then making the right formulations (Kumari *et al.,* 2019). Gomez-Godínez *et al.* (2021) has stated that the success of biofertilizers depends on edaphic and environmental conditions, plant genotype, autochthonous microbiota, organic matter content and soil pH. These limitations, however, are easily overcome by the use of soilless cultivation systems. Hydroponic cultivation technologies have several advantages as it is easy to control growth conditions, such as temperature, flow velocity and volume of water, nutrients, relative humidity, and lighting duration. Also, plants can be

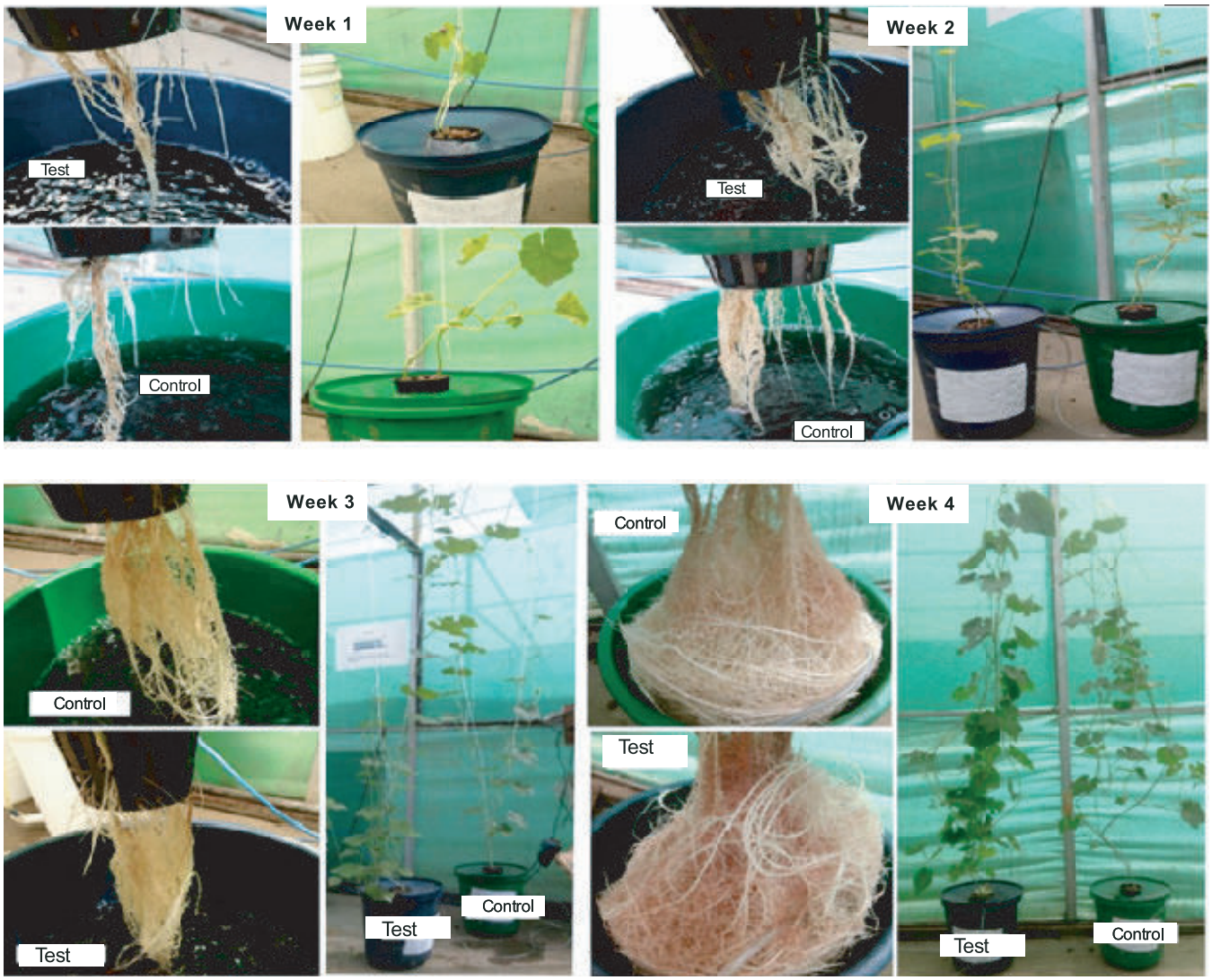

Fig. 1. Root and shoot growth in deepwater system of snake gourd during week 1 to week 4

cultivated year-round and cultivation is less labor-intensive (Lee and Lee, 2015). The deep-water culture system of hydroponics was developed to grow plants with roots constantly suspended in water. Such systems are already in use for the cultivation of cucumber and radish. (Lee and Lee, 2015). Although the root colonization efficiency of PGPR is closely associated with microbial competition and survival in the soil, in hydroponic systems, several PGPR bacteria have been identified that play a significant role.

This project was carried out to gain deeper insights into the microbiological flora of the hydroponic system, and the effect of a PGPR consortium on the growth of *T. cucumerina,* a vegetable plant popular in Central and Southern India. Isolates obtained, namely *C. jejuense*, *P. oryzihabitans* and *S. paucimobilis* were found to be potential PGPR because they produced several plant growth-promoting factors such as IAA, siderophores, cellulase, pectinase, isolates are also biofilm formers and grow readily into nutrient solution with high EC values. Previous studies on the vegetable plants like cauliflower, tomato, and bitter melon have reported the use of above-mentioned PGPRs for enhanced plant growth (Kushwaha *et al.,* 2013, Abdeljalil *et al.,* 2016, Singh *et al.,* 2017 and Khan *et al.,* 2018). In this study, a consortium of these organisms showed a visible increase in plant growth in the hydroponic system. This proved their potential as broad spectrum PGPR and has opened new possibilities for commercial application of these isolates in environmentally sound and sustainable, yet controlled agricultural practices like hydroponic systems.

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Optimizing phalsa (cv. Local) growth, flowering, and yield parameters through round-the-year pruning and fertilizer management

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Abstract

An experiment was conducted to study the effect of round-the-year pruning and fertilizer doses on phalsa's growth, flowering and yield parameters during 2020-21 and 2021-22 at Horticulture Research Farm, Anand Agricultural University, Anand. The experiment was laid out in a completely randomized design (Factorial) with two factors, eighteen treatment combinations, and three repetitions. The first factor was pruning time (1st week of January (Control), 1st week of March, 1st week of May, 1st week of July, 1st week of September and 1st week of November) and the second factor was fertilizer doses (100:50:50 g NPK/plant (Control), 200:75:75 g NPK/plant, 300:100:100 g NPK/plant). The results revealed that pruning in 1st week of May recorded minimum days for sprouting new shoots after pruning. Pruning in 1st week of March resulted in the maximum number of sprouted shoots per cane, length of the shoot at harvest, weight of fruit per plant, fruit yield and minimum days taken for flowering, fruit set and first picking after pruning. A fertilizer dose of 300:100:100 g NPK/plant recorded minimum days for sprouting of new shoots, maximum number of sprouted shoots per cane and length of shoot at harvest. The shortest duration for flowering, fruit set, and initial harvest was observed using a fertilizer dose of 100:50:50 g NPK per plant (Control). The application of 200:75:75 g NPK per plant was most effective for maximum fruit weight and overall yield of phalsa. Furthermore, this fertiliser dose significantly boosted phalsa yields when combined with pruning during the first week of March.

Key words: Round-the-year pruning, fertilizer, growth, flowering, yield, pruning time, new shoots, days taken for flowering, first picking

Introduction

Phalsa, belonging to the genus *Grewia* of the family Malvaceae, is a native fruit of India and is now widely cultivated in arid parts of tropical and subtropical regions. Commercial cultivation of phalsa is practiced in Punjab, Haryana, Rajasthan, Uttar Pradesh and Madhya Pradesh. Apart from these states, it is also grown on a small scale in Gujarat, Bihar, Maharashtra, Andhra Pradesh and West Bengal. It is widely grown for its sweet and sour acidic fruit, sold on the market throughout summer. Fruits include 10-11 % sugar, 2.0-2.5 % acid and 50-60 % juice.

Pruning is an important practice for phalsa; optimum pruning height gives a good crop. Phalsa bears fruit on the current season's growth and frequent annual pruning is required for good yield by cutting the old growth and enhancing the new growth. Plants can be pruned during December or January when they are dormant. The phalsa plants pruned 100 cm from ground level during late winter result in more vegetative growth and high yield (Aziz *et al.,* 2018).

As phalsa is a heavy feeder crop, nutrition given to it shows a significant effect on the various growth flowering and fruit set parameters (Hayes, 1957). The crop is borne on new growth, and applying fertilizers and manures will encourage vegetative growth, resulting in better fruit yield. Plant nutrition management has a stronger influence on flowering, fruit set, fruit size, the amount of vegetative growth, and other plant traits. N influences fruit development, yield, and quality more than P and K. Lower nitrogen doses lead to lower vegetative growth and yield, whereas too high dose of N results in fruit mortality (Bindra and Chauhan 1974). Phosphorus and potassium application increases the amount of sugars in the fruits.

Generally, the phalsa plant is pruned in winter (December-January) to get the yield in summer (April-May). Although, pruning in different months can give yield in summer, rainy and winter season. Pruning throughout the year in phalsa can produce fruit in the off-season, which may fetch a higher price on the market, and farmers might harvest two to three crops each year, so increasing their income. Application of fertilizers play an important role in phalsa's growth, flowering, yield, and quality. The phalsa's fertilizer dose varies according to the soil types, environment and climate. At present, there is an ad hoc dose of fertilizers in phalsa. So, this experiment was conducted to study the effect of round-the-year pruning and fertilizer doses on phalsa growth, flowering and yield parameters.

Material and methods

The present investigation was carried out at Horticulture Research farm, Anand Agricultural University, Anand in the years 2020-21 and 2021-22 at two years old phalsa plants having uniform vigour. The soil of the experiment field is loamy sand with soil pH (7.30), available N (233.35 kg/ha), available P (42.57 kg/ha) and Available K (265.45 kg/ha). The experiment was laid out in

a completely randomized design (Factorial) with two factors, eighteen treatment combinations, and three repetitions. First factor was pruning time (P_1= 1st week of January (Control), P_2= 1st week of March, P_3= 1st week of May, P_4= 1st week of July, P_5= 1st week of September and P_6= 1st week of November) and second factor was fertilizer doses (F_1= 100:50:50 g NPK/plant (Control), F_2= 200:75:75 g NPK/plant, F_3= 300:100:100 g NPK/plant).

Phalsa plants were pruned out at first week of particular month at height of 1 m from ground level as per the treatments with the help of sharp secateurs. Well prepared vermicompost @ 5 kg per plant and chemical fertilizers were applied as per the treatments at the time of pruning. Nitrogen was applied in the form of urea and diammonium phosphate (DAP), phosphorous was applied in the form of diammonium phosphate (DAP) and potassium was applied in the form of muriate of potash (MOP). The manures and fertilizers were applied in rings around the plants and were mixed into the soil.

Variables recorded included days to sprouting new shoots after pruning, sprouted shoots per cane, shoot length at harvest (cm), days to flowering/fruit set/first picking after pruning, fruit weight per plant (kg), and yield (kg/ha).

Result and discussion

Growth parameters: The data regarding effect of round the year pruning and fertilizer doses on growth parameters of phalsa are presented in Table 1. There was a significant difference among treatments for days to sprouting new shoots, number of sprouted shoots per cane and length of shoot at harvest. Pruning time P_3 (1st week of May) recorded significantly minimum number of days to sprouting of new shoots after pruning (6.33 and 6.11) in the years 2020-21 and 2021-22 which was at par with P_4 (1st week of July) in both the years. The significantly maximum number of sprouted shoots per cane (16.37 and 17.20) was found in pruning time P_1 [1st week of January (Control)] in the years 2020-21 and 2021-22 which was found at par with P_2 (1st week of March) in the year 2021-22. The highest length of shoot at harvest (139.56 and 139.64 cm) was recorded with pruning time P_1 [1st week of January (Control)] in the years 2020-21 and 2021-22 which was found at par with pruning time P_2 (1st week of March) in both the years. Due to the optimal environmental conditions, *i.e.*, high temperature and optimal humidity for the plant, summer pruning resulted in more plant growth than winter pruning. During the growth phase, plants grow more rapidly when temperatures are relatively high (Khodorova and Boitel-Conti 2013). These findings are in accordance with Aziz *et al.* (2018) and Mahida *et al.* (2022) in phalsa and Kumar *et al.* (2014a) in ber.

Among the fertilizer doses treatments, treatment F_3 (300:100:100 g NPK/plant) registered significantly minimum number of days to sprouting of new shoots after pruning (7.00 and 6.89), maximum number of sprouted shoots per cane (17.36 and 16.62) and highest length of shoot at harvest (134.47 and 134.42 cm) in both the years 2020-21 and 2021-22. The application of nitrogen enhances the production of the necessary protoplasm and amino acids required for the building of plant tissue and plant proteins (Chu 2021), whereas phosphorous enhance cell division and cell elongation (Kavanova *et al.,* 2006) which directly influences the growth of the plant. Similar observations were also made by Saravanan *et al.* (2013), Gill *et al.* (2015), Gochar *et al.* (2017), Ahmad *et al.* (2019) and Fareed *et al.* (2021) in phalsa.

Table 1. Effect of round the year pruning and fertilizer doses on growth parameters of phalsa

Treatment	Days to sprouting new shoots		No. of sprouted shoot per cane		Length of shoot at harvest (cm)	
	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22
Pruning time						
P_1	9.22	8.56	16.37	17.20	139.56	139.64
P_2	7.11	7.56	14.27	16.06	138.87	137.39
P_3	6.33	6.11	14.08	13.77	125.13	125.86
P_4	6.67	6.78	12.49	12.69	123.97	124.16
P_5	7.44	7.78	11.59	10.23	122.09	119.78
P_6	11.00	9.89	13.32	12.42	102.81	102.86
SE(m)±	0.26	0.26	0.53	0.42	0.92	1.12
CD at 5 %	0.76	0.74	1.51	1.19	2.64	3.22
Fertilizer doses						
F_1	8.94	8.72	10.46	10.93	115.66	113.76
F_2	7.94	7.72	13.24	13.64	126.08	126.67
F_3	7.00	6.89	17.36	16.62	134.47	134.42
SE(m)±	0.19	0.18	0.37	0.29	0.65	0.79
CD at 5 %	0.54	0.52	1.07	0.84	1.87	2.28

Flowering parameters: The data regarding effect of round the year pruning and fertilizer doses on flowering parameters of phalsa are presented in Table 2. The pruning time and fertilizer doses treatments had a significant effect on days taken to flowering after pruning, days taken to fruit set after pruning and days taken to first picking after pruning in phalsa. The treatment pruning in 1st week of March (P_2) recorded significantly minimum number of days taken to flowering after pruning (31.11 and 32.11), days taken to fruit set after pruning (55.56 and 56.44) and days taken to first picking after pruning (90.44 and 91.67) in the both years of 2020-21 and 2021-22. With reference to data, it was observed that pruning in summer season recorded minimum number of days taken to flowering after pruning, days taken to fruit set after pruning and days taken to first picking after pruning while these parameters recorded maximum number of days in winter pruning. Early vegetative growth in summer season pruned plants as well as ideal climatic conditions such as high temperature and optimal humidity resulted in the early flowering in summer season. This early flowering resulted in early fruit set and early picking in summer season in phalsa. Similar results were found by Aziz *et al.* (2018) and Mahida *et al.* (2022) in phalsa, Sharif *et al.* (2018) in ber and Widyastuti *et al.* (2019) in guava.

In terms of treatment fertilizer doses, treatment F_1 [100:50:50 g NPK/plant (Control)] registered significantly minimum number of days taken to flowering after pruning (37.11 and 36.39), days taken to fruit set after pruning (64.28 and 63.33) and days taken to first picking after pruning (99.50 and 100.78) in the both years of 2020-21 and 2021-22. It has been observed that increasing nitrogen doses caused a delay in flowering, which in turn delayed fruit set and the first picking of phalsa. Higher nitrogen levels resulted in a low C: N ratio, which encouraged more vegetative growth and delayed reproductive growth in plants. Similar results were found by Kumar *et al.* (2014b), Gill *et al.* (2015) and Gocher *et al.* (2017) and in phalsa.

Yield parameters: The data regarding effect of round the year pruning and fertilizer doses on yield parameters of phalsa are presented in Table 3. There was a significant difference among treatments for weight of fruit per plant and fruit yield. The study found that the treatment involving pruning in the first week of

Table 2. Effect of round the year pruning and fertilizer doses on flowering parameters of phalsa

Treatment	Days taken to flowering		Days taken to fruit set		Days taken to first picking	
	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22
Pruning time						
P_1	37.33	37.44	65.56	64.78	95.00	95.11
P_2	31.11	32.11	55.56	56.44	90.44	91.67
P_3	35.00	34.33	58.56	58.22	101.33	103.78
P_4	42.78	42.11	72.78	72.11	107.56	107.00
P_5	42.33	41.44	70.44	69.67	106.56	108.89
P_6	45.44	44.33	76.56	75.11	109.44	108.78
SE(m)±	0.26	0.25	0.20	0.24	0.19	0.31
CD at 5 %	0.76	0.72	0.58	0.68	0.55	0.90
Fertilizer doses						
F_1	37.11	36.39	64.28	63.33	99.50	100.78
F_2	39.06	38.94	66.56	66.06	101.67	102.44
F_3	40.83	40.56	68.89	68.78	104.00	104.39
SE(m)±	0.19	0.18	0.14	0.17	0.14	0.22
CD at 5 %	0.54	0.51	0.41	0.48	0.39	0.64

March (P_2) resulted in significantly higher fruit weight per plant (1.777 and 1.902 kg) and fruit yield (1974.25 and 2113.62 kg/ha) compared to other treatments in both 2020-21 and 2021-22. An increase in fruit weight per plant may be attributable to favourable growing conditions, increased vegetative growth, and better fruit growth during summer. The higher number of sprouting shoots per cane during summer also contributes to the higher yield. A decrease in productivity during the rainy season was mostly the result of high fruit drop owing to heavy rainfall. The study found that pruning phalsa plants throughout the year resulted in a consistent fruit yield year-round. This is consistent with previous studies on phalsa by Aziz *et al.* (2018) and Mahida *et al.* (2022), as well as a study on ber by Shukla *et al.* (2007).

The study found that treatment F_2 (200:75:75 g NPK per plant) resulted in significantly higher fruit weight per plant (1.777 and 1.902 kg) and fruit yield (1974.25 and 2113.62 kg/ha) during both 2020-21 and 2021-22, and was comparable to treatment F3 (300:100:100 g NPK per plant) in both years. Applying fertilizers increased the fruit yield of phalsa, with higher doses leading to even greater yields up to a certain point, after which the yield started to decline. Similar results were found in earlier studies on phalsa by Gill *et al.* (2015), Gochar *et al.* (2017), and Fareed *et al.* (2021), as well as in a study on guava by Baviskar *et al.* (2018).

Table 3. Effect of round-the-year pruning and fertilizer doses on yield parameters of phalsa

Treatment	Weight of fruit per plant (kg)		Fruit yield (kg/ha)	
	2020-21	2021-22	2020-21	2021-22
Pruning time				
P_1	1.669	1.771	1854.01	1967.33
P_2	1.777	1.902	1974.25	2113.62
P_3	0.264	0.262	293.06	290.96
P_4	0.212	0.226	236.03	251.21
P_5	1.363	1.459	1514.17	1620.58
P_6	1.179	1.193	1309.62	1325.79
SE(m)±	0.025	0.026	27.23	28.61
CD at 5 %	0.070	0.074	78.11	82.06
Fertilizer doses				
F_1	0.934	1.011	1038.11	1123.10
F_2	1.169	1.207	1298.20	1340.67
F_3	1.129	1.189	1254.26	1320.98
SE(m)±	0.017	0.018	19.26	20.23
CD at 5 %	0.050	0.052	55.23	58.03

This study found that pruning phalsa plants in the first week of March resulted in higher vegetative growth, early flowering, and higher yields. Higher doses of fertilizers (300:100:100 g NPK/plant) promoted early and vigorous vegetative growth, while lower doses (100:50:50 g NPK/plant) resulted in earlier flowering, fruit set, and picking. Pruning in the first week of March with fertilizer application of 200:75:75 g NPK/plant resulted in the highest fruit yield.

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Ex situ conservation of rare and threatened orchid: *Diplomeris hirsuta* (Lindl.)

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Abstract

The *Diplomeris hirsuta* Lindl. is a rare and endangered orchid species indigenous to India. This delicate species faces numerous threats, including landslides and road expansion, which have decreased its population and placed the species at risk of extinction. However, there is hope for this species, as the paper has reported the successful *ex situ* conservation of *D. hirsuta* plants. The researchers achieved this feat by creating a simulated natural habitat that closely mimicked the conditions of the plant's native environment. This simulated habitat provided the ideal environmental conditions necessary to sustain the plant's growth and development, including temperature, humidity, light, and soil composition. As a result, the live plants of *D. hirsuta* were successfully conserved. This study's *ex situ* conservation method is essential in preserving species' survival, especially when their natural habitats are at risk or destroyed. The success of this conservation effort provides a potential model for future conservation efforts of other threatened plant species, giving hope for the protection and preservation of rare and endangered plant species worldwide.

Key words: Conservation, *Diplomeris hirsuta*, *ex situ*, habitat, snow orchid, threatened, watering

Introduction

The Snow Orchid (*Diplomeris hirsuta* Lindl.) is a lithophytic orchid species found in India, Northern Myanmar, Southern China, Northern Vietnam, North-East Thailand, Bhutan, and Nepal (Iamwiriyakul and Kaewphung, 2008). "Diplomeris" is derived from the Greek word for "divided stigma," whereas "hirsuta" is derived from the Latin word for "hairy" (Singh, 2012). It is found in India (Fig. 1) in Sikkim, Arunachal Pradesh, West Bengal, and the Western Himalaya (Rao, 2010).

This orchid species has large ovate-oblong hairy leaves and produces single or occasionally two white flowers. It grows in cool and shady places on moist rocks near running water streams, along with other vegetation such as liverworts, ferns, and wild begonia. Unfortunately, due to anthropogenic activities such as the clearing of land for road widening, landslides, and scraping of retaining walls for beautification of roads, populations of this orchid are vulnerable to extinction. There are no records of conservation or successful rehabilitation of this species in *ex situ* environments (Behera *et al.,* 2012).

Ex situ conservation, the practice of conserving a species outside of its natural habitat, is a valuable strategy for orchid conservation. This strategy can be implemented by cultivating orchids in specialized facilities like botanical gardens, greenhouses, and other controlled environments.

In the case of *D. hirsuta*, there is a lack of information regarding its growth cycle, watering, and substrate requirements, which can be crucial for successful *ex situ* conservation and rehabilitation. In this study, we aimed to investigate the role of watering on *D. hirsuta* using live plants.

Understanding the watering requirements of endangered orchid species is crucial for their successful conservation and rehabilitation. To achieve this, *D. hirsuta* plants should be grown in a controlled environment and subjected to different watering regimes. Such an approach will help understand the significance of providing optimal watering conditions for the species' *ex situ* conservation and rehabilitation efforts. By ensuring optimal

Fig.1. Distribution of *Diplomeris hirsuta* (Lindl.) in Sikkim and West Bengal.

watering conditions, the survival and growth rates of this species can be improved, which will increase the chances of its successful conservation and reintroduction to its natural habitat. Therefore, understanding the watering requirements of endangered plant species, such as *D. hirsuta*, is essential to conserve and protect them for future generations.

The study involved growing live *D. hirsuta* plants in a controlled environment and subjecting them to different watering regimes. The growth, survival, and physiological parameters of the plants, such as leaf water potential, chlorophyll content, and photosynthetic rate, were monitored for uderstanding the optimal watering requirements for successful *ex situ* conservation and rehabilitation of *D. hirsuta*, and how do different watering regimes affect the growth, survival, and physiological parameters of this species

Materials and methods

Live plants of Diplomeris hirsuta were collected from its natural habitat in Assam Linzey, East Sikkim, during the year 2017-18. To create simulated natural habitat conditions, the collected plants were placed on bricks along with its associated vegetation, replicating the conditions found in nature (Fig. 2).

The experiment was conducted in the semi-automated Glasshouse at ICAR-National Research Centre for Orchids, Pakyong, Sikkim. The experiment followed a randomized block design with three treatments and seven replications. The treatments consisted of watering the live plants at different intervals, including T_1: watering in February, T_2: watering in March, and T_3: watering in April. The aim was to study the effect of watering on the growth and flowering of *D. hirsuta*.

Fig. 2. *Diplomeris hirsuta* in controlled condition (*ex situ* conservation)

Observations were recorded at different stages of plant growth, including days to plant emergence, leaf length, leaf width, number of leaves per plant, days to bud initiation, days taken for flowering from bud initiation, and days to flower withering.

Statistical analysis: This experiment was carried out in a completely randomised design (CRD) with ?? replicates per treatment. Statistical significance between mean values was evaluated using one-way ANOVA (Gomez and Gomez, 1984).

Results and discussion

Diplomeris is considered as an endangered and threatened orchid (Ayensu, 1986; Jalonen *et al.,* 2009). It is found growing in the rocks along the roadside. Habitat destruction, landslides and the widening of roads lead to this orchid extinction's vulnerability (Burman and Devdas, 2013). These orchids need artificial assistance to migrate or translocate from such hostile environments to new places where they can be conserved (Swarts and Dixon, 2009).

To successfully conserve Diplomers hirsuta in *ex situ* conditions, live plants were subjected to watering treatments to create natural habitat. The results indicated that during the first year, significant differences were observed for leaf length and leaf width among the treatments. Maximum leaf length and leaf width was recorded in T_1 (10.97 and 3.22cm) followed by T_2 (8.27cm and 2.53cm) and T_3 (7.78cm and 2.38cm). However, no significant difference was observed for number of leaves. Days to bud initiation and days to flower emergence were earlier in T_3 i.e April watering (35 days) followed by T_1 and T_2 (50 and 50.85 days, respectively). However, the plant emerged earlier in T_1 between 12-26th, May as compared to the other two treatments between 30th May-6th June and 17th June to 24th June, respectively. Significant differences were observed for days to flower emergence in all the treatments. However, T_1 and T_2 were at par. Minimum days for flowering were recorded by T_3 (67.28 days) followed by T_1 (80.28 days) and T_2 (83 days). For days to flower withering, all the treatments showed insignificant differences.

During the second year, similar observation was recorded for leaf length, width, and number of leaves. The leaf length and width were smaller than the previous year for all the treatments. Maximum leaf length and leaf width were recorded by T_1 (6.52cm and 1.85cm) followed by T_2 (5.13cm and 1.47cm) and T_3 (4.62cm and 1.38cm), respectively. Plant emergence was earlier in all the treatments as compared to the previous year, *i.e.,* in T_1 (5-15th May), T_2 (14-21st May) and T_3 (17-29th May). Though the plant emergence was earlier in T_1, the minimum days taken for bud initiation was recorded by T_3 (41.86) followed by T_2 (48.57) and T_1 (49.43). For days to flower opening, all treatments showed significant differences. T_2 significantly differed from T_1 and T_3 and recorded minimum days for flower opening (60.85). However, flower withering was observed earlier in T_3 (14.62 days) from flower opening (Table 1).

It was observed that the plant emergence was earlier during 2019

Table 1. Effect of watering treatment on growth and flowering of *Diplomeris hirsuta*

Treatment	First Year (2018)						Second Year (2019)					
	Leaf length (cm)	Leaf width (cm)	Number of leaves/ plant	Days to bud initiation	Days to flower emergence	Days to flower withering	Leaf length (cm)	Leaf width (cm)	Number of leaves/ plant	Days to bud emergence	Days to flower emergence	Days to flower withering
T1	10.97[a]	3.22[a]	2.14	50.00[a]	80.28 [a]	20.14	6.52[a]	1.85[a]	1.86	49.43[a]	74.43[a]	15.57[b]
T2	8.27[b]	2.53[b]	2.00	50.85[a]	83.00 [a]	21.14	5.13[b]	1.47[b]	2.00	48.57[a]	60.85[b]	28.72[a]
T3	7.78[b]	2.38[b]	2.00	35.00[b]	67.28 [b]	19.00	4.62[b]	1.38[b]	2.00	41.86[b]	72.43[a]	14.57[b]

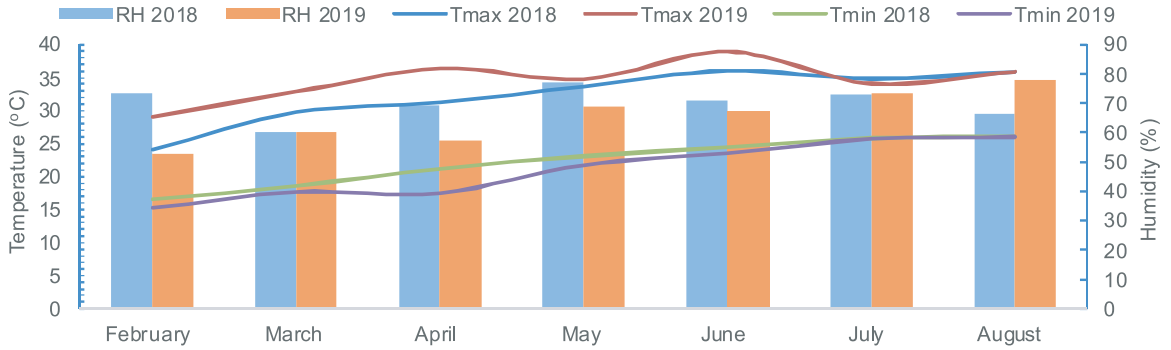

Fig. 3. Average maximum, minimum temperature (°C) and relative humidity (%) during peak growing season of *Diplomeris hirsuta* (Lindl.)

compared to 2018 which extended till last week of June, whereas all the plants of 2019 in all treatments emerged within May. It may be because the plant requires a warm and humid climate during its active growing stage and the temperature recorded during 2019 was higher than the previous year (Fig 3).

It was observed that all the plants emerged between May to June in both the years in all the treatments. Vegetative parameters such as leaf length and width were highest in T_1 during both the years followed by T_2 and T_3. This is because the plants remain dormant after flowering (Aug-Sept) and survives by a tuber and reappears in May-June with water availability and favorable climate. So, a continuous water supply from February led to good vegetative growth of the plants in T_1. However, T_3 plants were exposed to watering treatment for a short duration till its germination; hence, their leaf length and width were smaller than other treatments. Also, the flower size was smaller in T_3 than in T_1 and T_2. Pradhan (1974a); Pradhan (1974b) also reported that *D. hirsuta* could be grown by keeping the plants in a warmer area, protected from direct sunlight with good moisture during the growing season and keeping the media dry during winter. The peak flowering was during August for all treatments in both the years irrespective of treatments though there was some variation in number of days in different treatments. Water played an important role in the overall growth and survival of the plants.

Ex situ conservation efforts were undertaken to preserve this species, and the study found that watering treatments played a crucial role in maintaining natural habitat conditions and promoting growth and survival of the plants. The results showed that continuous water supply during the initial and active growing stage led to good vegetative growth and increased chances of survival. The study provides useful insights into the conservation of rare and endangered plant species and highlights the importance of *ex situ* conservation efforts in ensuring the survival of such species.

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Physical, nutritional, functional and thermal properties of muskmelon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* L.) seeds and flours

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Abstract

Food sustainability and waste reduction have gained considerable importance in recent years. Despite being rich in nutrients and functional characteristics, by-products like seeds from fruits remain underutilized. The decorticated seeds and seeds flour of muskmelon and watermelon were analyzed for their physical, nutritional, functional, and thermal properties to aid in designing systems for storage, processing, and incorporation as functional ingredients in food products. Standard methods estimated the seeds' dimensional, frictional, gravimetric, nutritional, and functional properties. The seeds' flour was defatted to assess thermal properties using the DSC (Differential Scanning Calorimetry). The seeds of muskmelon and watermelon were rich in proteins (29.21 %, 29.56 %) and fats (39.07 %, 44.31 %), respectively. Both the seeds' flour exhibited a similar range of porosity (68.8 %). The static coefficient of friction (0.78) was the highest for thermocol among all tested surfaces for both seeds. The foaming capacity (39.39 %) and oil absorption capacity (1.26 g/g) of muskmelon seeds flour were higher than watermelon seeds flour (36.36 % and 1.00 g/g, respectively). The thermal denaturation temperature of defatted watermelon seeds flour (66.4 °C) was higher than defatted muskmelon seeds flour (63.8 °C). Reports on these seeds' properties, especially the thermal properties of seed flour, are very scarce. This research work would aid in effectively utilizing seeds and their flours as functional ingredients in the food processing industry.

Key words: Dimensional, functional, gravimetric, muskmelon, thermal, watermelon

Abbreviations: θ- angle of repose**;** AMD- arithmetic mean diameter**;** ρ_b- bulk density (BD), gmL^{-1}; °C- degree celsius; DMMSF- defatted muskmelon seeds flour; DWMSF- defatted watermelon seeds flour; D- diameter; DSC- differential scanning calorimetry; ER- elongation ratio; EC- emulsifying capacity; FR- flakiness ratio; FC -foaming capacity; GMD- geometric mean diameter; h -height; L -length; µm- microns; MMS- muskmelon seeds; MMSF- muskmelon seeds flour; OAC- oil absorption capacity; P -porosity; PA- projected area; rpm- rotations per minute; S_a -seed surface area; φ -sphericity; µ- static coefficient of friction; S_v- surface volume; ρ_{td}-tapped density, gmL^{-1}; T-thickness; TSM- thousand seed mass; ρ_t- true density (TD), gmL^{-1}; v- volume; WAC- water absorption capacity; WMS- watermelon seeds; WMSF- watermelon seeds flour; w -weight; W -width.

Introduction

Muskmelon and watermelon are sweet, juicy fruits with excellent amounts of nutrients, polyphenols, carotenoids, and vitamin C (Deshmukh *et al.,* 2015; Gómez-García *et al.,* 2020). The world production of melon seed was recorded 934161 tonnes in 2020 (FAOSTAT 2020). Muskmelon seeds are cream-colored and oval-shaped, 10 mm in length (Saltveit, 2011). Watermelon seeds are black or dark brown, compressed, pyriform, and 6-10 mm in length (Galit *et al.,* 2019). The seeds of watermelon and muskmelon are generally discarded as waste has a negative impact on the environment. However, they are rich in nutritional constituents such as fat, protein, ash, fiber, carbohydrates, and minerals (de Melo *et al.,* 2000; Rekha *et al.,* 2016). The physical properties of these fruit seeds are important factors, which help design equipment for storage, conveying, sorting, sizing, sieving, oil extraction, drying, and packaging processes (Mansouri *et al.,* 2017: Zhang *et al.,* 2022). The functional properties of seed flour have an essential role in the processing, preparing, storing, quality, and sensory attributes of the food matrix used (Obi *et al.,* 2015; Sonawane *et al.,* 2016). The thermal properties of seed flour give an insight into protein denaturation temperature affecting the thermal stability of the proteins (Biswal *et al.,* 2021). Sustainability in the food industry is challenging, and efforts must be directed toward achieving these goals. Innovation in developing value-added products using by-products of food processing like seeds is crucial for reducing wastage. (Silva *et al.,* 2020). There is an increasing need to develop efficient machinery for achieving improved quality and quantity of seeds for better utilization in the food industry (Adekanye, 2014).

The present research aims to investigate the physical, nutritional, functional, and thermal properties (DSC) of muskmelon seeds (*Cucumis melo* L.) and watermelon seeds (*Citrullus lanatus* L.) for designing systems of handling, processing, and storage. The research will also bring opportunities for using seeds and their flours in value-added functional food products and edible packaging systems.

Materials and methods

Sample preparation: The decorticated seeds (1 kg each) of Muskmelon (*Cucumis melo* L.) and Watermelon (*Citrullus lanatus* L.) were procured from a local vendor at Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh. The procured seeds were cleaned, washed, and dried using a tray dryer (Macro Scientific Workers, MAC, ATC-222, India) at 45 ºC until a static weight was obtained. The seeds were ground in a mixer grinder (Model no. HMB50W3S-DBF, Techno Kart India Limited, India) and sieved to get seeds flour of uniform particle size using a sieve of mesh size 1000 µm for the analysis. The seeds and their respective flours were kept at 4 ºC in the refrigerator till further use. The samples were analyzed for physical, nutritional, and functional properties at ambient temperature (21±2°C). Defatting of seeds flour was done by stirring the solution of n-hexane: seeds flour in the ratio of 10:1 (v/w) using magnetic stirring on a hot plate (28 °C) for 4 h at 1100 rpm, followed by centrifugation in a Refrigerated Centrifuge (Sigma, Germany) at 6000 x g for 30 min (Devi *et al.,* 2019) to obtain defatted seeds flour for DSC analysis.

Chemicals and reagents: Chemicals and reagents of analytical grade such as n-hexane, toluene, sulfuric acid, sodium hydroxide, copper sulfate pentahydrate ($CuSO_4.5H_2O$), potassium sulphate, petroleum ether purchased from Shanghai Richem International Co., Ltd was used for this study.

Determination of physical properties

Seed length (L), width (W), thickness (T), arithmetic mean diameter (AMD), geometric mean diameter (GMD), and sphericity (φ): The L (mm), W (mm), and T (mm) were estimated using a random selection of seeds (n= 100) from each bulk seeds sample of muskmelon and watermelon using Vernier Calliper (Caliper series 1108-150, Germany; least count of 0.01 mm). The observations were taken in triplicates. The AMD, GMD, and φ of the seeds were computed using the following equations (Mansouri *et al.,* 2017):

$$AMD = \frac{L+W+T}{3} \quad (1)$$

$$GMD = \sqrt[3]{LWT} \quad (2)$$

$$\varphi = \frac{\sqrt[3]{LWT}}{L} \times 100 \quad (3)$$

Seed surface area (S_a) and seed volume (S_v): The S_a and S_v were calculated according to equations given by Bande *et al.* (2012).

The S_a (mm^2) was determined using equation 4

$$S_a = \pi(GMD)^2 \quad (4)$$

where GMD is the geometric mean diameter (mm).

The S_v (mm^3) was computed using the equation 5

$$S_v = \frac{\pi (GMD)^3}{6} \quad (5)$$

Flakiness ratio (FR), elongation ratio (ER), and projected area (PA): The FR and ER of MMS and WMS were determined using the equations (6) and (7), as reported by Mora *et al.* (2000).

$$FR = \frac{\text{Seed thickness (T)}}{\text{Seed width (W)}} \quad (6)$$

$$ER = \frac{\text{Seed length (L)}}{\text{Seed width (W)}} \quad (7)$$

PA is essential in determining aerodynamic properties. It was computed using equation (8) as given by Mansouri *et al.* (2017):

$$PA = \frac{\pi LW}{4} \quad (8)$$

Frictional properties

Angle of repose: It is defined as the angle to the horizontal surface on which a material is piled to form a cone. This was analyzed using Milani *et al.* (2007) method with some modifications. A cylinder with open ends of 3.8 cm diameter and 15 cm height was filled with seeds and kept on a flat surface. The cylinder was raised slowly, allowing seeds to form a natural slope. The height and diameter of the slope were measured to derive the angle of repose using the equation: -

$$\theta = \tan^{-1}\left(\frac{2h}{D}\right) \quad (9)$$

where θ = angle of repose; h = height of the cone (cm); and D = diameter of the cone (cm).

Static coefficient of friction: It indicates the friction between various surfaces and the seeds. It was obtained for four surfaces, *i.e.*, plywood, galvanized iron, glass, and thermocol, using the method by Obi *et al.* (2015) with some modifications. A topless and bottomless cylinder with dimensions 4.0 cm diameter and 7.2 cm height was filled with seeds and kept on the desired surface. The cylinder was raised using the screw until the seeds started sliding down. The angle at the time of sliding of seeds was observed, and the static coefficient of friction was calculated using the equation:

$$\mu = \tan \alpha \quad (10)$$

where, μ = static coefficient of friction; α = angle at which the seeds started sliding on the surface

Gravimetric properties

Thousand seed mass (TSM): TSM was determined through the method by Obi *et al.* (2015) with slight modification. 100 seeds of musk melon and watermelon were selected randomly from the whole sample. Their respective mass was measured using a digital weighing balance with an accuracy of 0.0001 g (Sartorius, BAS224S-CW, Germany). The mass of 100 seeds was multiplied by 10 to obtain a mass of thousand seeds.

Bulk density (BD), tapped density, true density (TD), and porosity: BD (ρ_b) is the ratio of the weight of the sample to the volume occupied by the poured sample, including the voids between flour particles in a container of known volume. The ratio of mass to volume was denoted as BD (gmL^{-1}) (Koocheki *et al.,* 2007). Tapped density (ρ_{td}) was observed according to the method by Khan *et al.* (2016). It is obtained after tapping the container containing the sample several times on the bench and recording the reduced volume due to settling the flour particles. The seeds flour was poured into a pre-weighed calibrated measuring cylinder of known volume, and the weight of the cylinder was recorded. The measuring cylinder was tapped multiple times on the bench, and a volume change was recorded. TD (ρ_t) is the ratio of the sample's weight to the sample's volume, excluding the volume of internal voids. The liquid displacement method was used to obtain the TD of the samples. Due to low surface tension, less absorbability, and low dissolution power, Toluene (C_7H_8) was used instead of water to fill the measuring cylinder. The mass ratio gave the TD of samples to true volume (Khan *et al.,* 2016). Porosity(ε)depends on the materials' BD and TD and differs with seed. It indicates the voids in the seeds flour (Bande *et al.,* 2012).

Porosity (ε) was calculated using ρ_b and ρ_t through the following equation (Bande *et al.,* 2012).

$$\varepsilon = \left(1 - \frac{\rho b}{\rho t}\right) \times 100 \quad (11)$$

Determination of nutritional properties

The seeds' moisture, ash, fat, protein, and crude fiber content were determined using AOAC (2012) methods. The moisture content was analyzed by the oven drying method at 105 °C for 2 h in a hot air oven (Macro Scientific Works Pvt Ltd., RM-SP-325, Delhi, India). Ash was determined by weighing the sample and then charring it on a hot plate, followed by incinerating it at 550°C for 4 h in the muffle furnace (Ocean Life Science Corporation, New Delhi, India). The final weight was measured, and the ash content was estimated. Protein content was analyzed according to the Kjeldahl method, and fat content was analyzed using the Soxhlet method. Carbohydrate content was determined by formula: 100 – (moisture % + ash % + fat % + protein % + crude fibre %) (Jacob *et al.,* 2015).

Determination of functional properties

Color: The colour of the seeds was observed using a portable Minolta Chroma Meter (Konica Minolta CR400, Japan) calibrated using standard white tile. The colour coordinates L*, a*, and b* values were determined to obtain the colour measurement of seeds. L* value represents the lightness with black for 0 to white for 100, and a* value represents red to green with positive values for red tints and negative values for the green tints. The b* value represents yellow to blue colors with positive values for yellowish tints and negative values for bluish tints (Mallek-Ayadi *et al.,* 2017). Chroma (C*) and hue angle (h°) were calculated according to equations (12) and (13) as mentioned by Coelho *et al.* (2015):

$$h = \tan^{-1}\left(\frac{b*}{a*}\right) \quad (12)$$

$$C* = \sqrt{(a*)^2 + (b*)^2} \quad (13)$$

Water absorption capacity (WAC): WAC of the samples was analyzed according to the method of Chawla *et al.* (2020) with minor modifications. 1 g of seed flour was mixed with 10 mL distilled water and centrifuged at 5000 x g for 5 min at 26° C. The supernatant was discarded, and the weight of tubes with pallets was re-recorded. WAC was represented as grams of water absorbed per gram of the seed flour using the equation 14.

$$WAC = \frac{Weight\ of\ water\ absorbed\ (g)}{Weight\ of\ the\ sample\ (g)} \quad (14)$$

Oil absorption capacity (OAC): OAC was analyzed according to the methodology of Chawla *et al.* (2020) with minor modifications. 1 g of seed flour was mixed with 10 mL sunflower oil and centrifuged at 5000 x g for 5 min at 26 °C. The weight of tubes with pallets was re-recorded after discarding the supernatant. OAC was represented as grams of oil absorbed per gram of seed flour and calculated using the equation (15):

$$OAC = \frac{Weight\ of\ oil\ absorbed\ (g)}{Weight\ of\ the\ sample\ (g)} \quad (15)$$

Foaming capacity (FC): 1 g of seed flour was mixed with 50 mL of distilled water and whipped using a Moulinex homogenizer (T 18D, Ika, Germany). The whipped samples were immediately poured into a graduated cylinder. FC was measured using the following equation 16 (Mallek-Ayadi *et al.,* 2019).

$$FC\% = \frac{VFAW - VFBW}{VFBW} \times 100 \quad (16)$$

Where, VFAW=Volume of foam after whipping (mL). VFBW= Volume of foam before whipping (mL)

Emulsifying capacity (EC): EC was analyzed according to the method given by Dimitry *et al.,* (2022). An emulsion was developed by mixing 1 g of seed flour with 10 mL distilled water and 10 mL sunflower oil for 1 min in a Moulinex homogenizer, followed by centrifugation at 2000 × g for 5 min. The EC was calculated using the following formula: Height of emulsified layer (mL)

$$EC\% = \frac{Height\ of\ emulsified\ layer\ (mL)}{Height\ of\ entire\ solution\ (mL)} \times 100 \quad (17)$$

Emulsion stability (ES): ES was analyzed by heating the emulsions of seeds flour to 80°C for 30 min in a water bath and centrifuged again at 2000 x g for 15 min (Dimitry *et al.,* 2022). ES was calculated according to the equation (18):

$$EC\% = \frac{Height\ of\ emulsified\ layer\ after\ heating\ (mL)}{Height\ of\ entire\ solution\ after\ heating\ (mL)} \times 100 \quad (18)$$

Thermal properties: The thermal property was determined using Differential scanning calorimetry DSC (DSC; NETZSCH, DSC 200F3 240-20-1056-L, Germany) as per the method by Sonawane *et al.* (2016) with some modifications. The instrument was calibrated with indium, and an empty pan was used as a reference. The samples were weighed (approximately 4-5 mg) and hermetically sealed in aluminum pans. The samples were further heated from 35°C up to 160°C under a nitrogen atmosphere at 5°C min^{-1}. The thermogram depicts heat flow rate as a function of temperature. The onset, peak, and endpoint temperatures were observed along with enthalpy.

Statistical analysis: The average values of triplicate readings are reported as results (Mean ± standard deviation) with significance differences (P<0.05). The data were analyzed through one-way ANOVA in SPSS Statistics software {version 28.0.0.0 (190), SPSS Inc. Chicago, IL, USA}.

Results and discussion

Dimensional properties: The dimensional properties of the MMS and WMS are depicted in Table 1. The L of MMS (10.47 mm) was higher than WMS (9.04 mm), but W (5.35 mm) and T (2.17 mm) were significantly (P<0.05) higher in WMS compared to MMS. AMD and GMD are directly dependent on the dimensions of the seeds. An increase in L, W, and T would increase the values of AMD and GMD (Bande *et al.,* 2012). The AMD and GMD of MMS (5.71 mm, 4.49 mm) and WMS (5.52 mm, 4.71 mm) had slight differences due to variation in respective dimensions. S_a is a function of GMD, so it is higher in WMS (69.85 mm^2) than in MMS (63.81 mm^2). These parameters are important in designing machines for sorting, grading, and sizing these seeds. The (φ) of MMS and WMS was 42.97 % and 52.23 %, respectively. The W of MMS (4.87 mm) and (φ) of MMS (42.97 %) was also similar to that reported by Alibas *et al.* (2012). FR of WMS (0.40) was higher than MMS (0.36). ER of MMS (2.15) was more significant than ER of WMS (1.68). Lower values (<1) of FR and higher values (>1) of ER for both seeds indicate the shape (flat oblong) of the seeds. The (φ) values and higher ER of both seeds indicate their tendency to slide rather than roll on a surface. These parameters could aid in designing these seeds'

conveyors, hoppers, and separators (Jafari *et al.,* 2011). The results of FR (0.36), ER (2.15) PA (40.04) of MMS is in line with the previous findings reported by Omobuwajoa *et al.* (1999) and Mansouri *et al.* (2017).

Frictional properties: Various factors such as the shape, size, and roughness of seeds affect the frictional properties of seeds. The results of the frictional properties are depicted in Table 2. The angle of repose (θ) for WMS (25.05°) was significantly (P<0.05) higher compared to MMS (20.93°). The (θ) generally depends on the moisture content (Al-Hashemi *et al.,* 2018). Due to the low moisture content of the seeds (<1%), (θ) was also low for both the seeds. The (μ) of thermocol (0.78, 0.78) was highest among all surfaces and lowest for glass (0.32, 0.34) for MMS and WMS, respectively. A higher (μ) could be attributed to the surface roughness, which is highest in thermocol, thereby reducing the sliding ability of the seeds. The (μ) plays a vital role in designing conveyors as friction is responsible for holding seeds on the conveyor without slippage or backward slide (Obi *et al.,* 2015). The (μ) of WMS for galvanized iron (0.40) and plywood (0.61) was near to that reported by Bande *et al.* (2012) and Adekanye *et al.* (2014).

Gravimetric properties: Gravimetric properties are listed in Table 3. Thousand seed mass for WMS (39.16 g) was significantly (P<0.05) higher than that for MMS (36.16 g) as they are larger. TSM of MMS (36.16 g) was near to that reported by Mansouri *et al.* (2017). BD and (ρ_{td}) are essential for determining the space required for designing seed hoppers, storage, and conveying facilities. BD and TD were the same for both the seeds flour (0.39 gmL^{-1}, 1.25 gmL^{-1}). BD of the seed flours (0.39 gmL^{-1}) was near to that reported by Olorode *et al.* (2014). The (ρ_{td}) of MMSF and WMSF was 0.84 gmL^{-1} and 0.75 gmL^{-1}, respectively. Porosity is essential in designing aeration facilities during storage and packing systems. High porosity results in better aeration and water vapor diffusion while drying (Rodríguez-Miranda *et al.,* 2016). The porosity of both seeds flour was in a similar range (68.8 %).

Table 3. Mean, standard deviation (SD), maximum and minimum values for gravimetric properties of seeds/seeds flour of musk melon and watermelon

Parameters	Muskmelon seed			Watermelon seed		
	Mean±SD	Max.	Min.	Mean±SD	Max.	Min.
Thousand seed mass (g)	36.16 ±0.76^{b}	37.0	35.5	39.16 ±0.57^{a}	39.5	38.5
Bulk density, BD (g mL^{-1})	0.39 ±0.01^{a}	0.40	0.38	0.39 ±0.01^{a}	0.40	0.38
Tapped density, (g mL^{-1})	0.84 ±0.03^{a}	0.88	0.81	0.75 ±0.07^{a}	0.83	0.68
True density, TD (g mL^{-1})	1.25 ±0.00^{a}	1.25	1.25	1.25 ±0.00^{a}	1.25	1.25
Porosity (%)	68.8 ±0.00^{a}	68.8	68.8	68.8 ±0.00^{a}	68.8	68.8

Note: Values are mean ± standard deviation, a maximum and minimum value of three replicates. The mean values within the same row with different superscripts have significant differences (P<0.05).

Nutritional properties: The nutritional compositions of the seeds are listed in Table 4. These values differ by region of production and variety. The moisture content of the MMS (2.57 %) and WMS (3.67 %) was low, which could help to increase the shelf life of seeds and reduces microbial spoilage. Ash content for MMS (4.23 %) was higher than that of WMS (3.69 %), which might be possible due to the considerable amount of minerals such as potassium, magnesium, calcium, and sodium (Shalaby *et al.,* 2020). A similar result has been reported by Sajjad *et al.* (2020)

Table 1. Mean, standard deviation (SD), maximum and minimum values for dimensional properties of seeds of muskmelon and watermelon

Parameters	Muskmelon seed			Watermelon seed		
	Mean±SD	Maximum	Minimum	Mean±SD	Maximum	Minimum
Length (mm)	10.47±0.77^{a}	11.26	9.72	9.04±0.50^{b}	9.43	8.47
Width (mm)	4.87±0.23^{b}	5.11	4.65	5.35±0.09^{a}	5.46	5.27
Thickness (mm)	1.79±0.36^{a}	2.19	1.47	2.17±0.07^{a}	2.25	2.12
Arithmetic mean diameter, AMD (mm)	5.71±0.33^{a}	6.10	5.52	5.52±0.20^{a}	5.28	5.64
Geometric mean diameter, GMD (mm)	4.49±0.39^{a}	4.93	4.14	4.71±0.14^{a}	4.83	4.55
Sphericity, φ (%)	42.97±2.92^{b}	45.39	39.72	52.23±1.67^{a}	53.78	50.45
Seed surface area, S_a (mm^2)	63.81±11.39^{a}	76.31	54.00	69.85±4.37^{a}	73.49	65.00
Seed volume, S_v (mm^3)	48.33±13.03^{a}	62.73	37.32	54.98±5.09^{a}	59.25	49.35
Flakiness ratio, FR	0.36±0.07^{a}	0.31	0.44	0.40±0.00^{a}	0.41	0.40
Elongation ratio, ER	2.15±0.21^{a}	2.31	1.90	1.68±0.07^{b}	1.76	1.60
Projected area, PA (mm^2)	40.04±2.63^{a}	38.10	43.04	38.02±2.58^{a}	39.52	35.03

Note: Values are mean ± standard deviation, a maximum and minimum value of three replicates. The mean values within the same row with different superscripts have significant differences (P<0.05).

Table 2. Mean, standard deviation (SD), maximum and minimum values for frictional properties of seeds of muskmelon and watermelon

Parameters	Muskmelon seed			Watermelon seed		
	Mean±SD	Maximum	Minimum	Mean±SD	Maximum	Minimum
Angle of repose (°)	20.93±0.76^{b}	21.80	20.35	25.05±2.14^{a}	27.42	23.26
Static coefficient of friction						
Galvanized iron	0.38±0.01^{a}	0.40	0.37	0.40±0.03	0.44	0.36
Plywood	0.62±0.00	0.62	0.62	0.61±0.00	0.62	0.62
Glass	0.32±0.10	0.32	0.32	0.34±0.00	0.34	0.34
Thermocol	0.78±0.00	0.78	0.78	0.78±0.01	0.80	0.78

Note: Values are mean ± standard deviation, a maximum and minimum value of three replicates. The mean values within the same row with different superscripts have significant differences (P<0.05).

and Siddeeg *et al.* (2014). The fat content of MMS (39.07 %) was lower than WMS (44.31 %). Overall, both seeds were high in fat, more than 30%. The fat content of MMS and WMS was found to be higher or close to available research literature (Bouazzaoui *et al.,* 2016; Mehra *et al.,* 2015; Shalaby *et al.,* 2020; Akusu *et al.,* 2015). The protein content of WMS (29.56 %) was slightly higher than MMS (29.21 %). The protein of MMS andWMS in the present study was near to some available data (Hu *et al.,* 2007; Omobolanle *et al.,* 2014; Marie *et al.,* 2015). However, the WMS was less in carbohydrates (13.83 %) than MMS (21.74 %). The crude fiber of WMS (4.73 %) was higher than that of MMS (3.16 %). A significant difference (P<0.05) was noticed among moisture, fat, crude fiber, and carbohydrates. Both seeds, rich in protein, fat, and minerals, could be incorporated into various food products to enhance their nutritional quality.

Table 4. Mean, standard deviation (SD), maximum and minimum values for nutritional composition of seeds of musk melon and watermelon

Parameters	Muskmelon seed			Watermelon seed		
	Mean ± SD	Max.	Min.	Mean ± SD	Max.	Min.
Moisture %	2.57 ±0.02^{b}	2.55	2.60	3.64 ±0.02^{a}	3.66	3.62
Ash %	4.23 ±0.02^{a}	4.25	4.21	3.69 ±0.46^{a}	4.21	3.32
Protein %	29.21 ±0.33^{a}	29.56	28.89	29.56 ±0.20^{a}	29.80	29.40
Fat %	39.07 ±0.18^{b}	39.21	38.86	44.31 ±0.80^{a}	44.91	43.39
Crude fibre %	3.16 ±0.25^{b}	3.40	2.90	4.73 ±0.05^{a}	4.80	4.70
Carbohydrates %	21.74 ±0.75^{a}	22.54	21.03	13.83 ±1.54^{b}	15.57	12.63

Note: Values are mean ± standard deviation, a maximum and minimum value of three replicates. The mean values within the same row with different superscripts have significant differences (P<0.05).

Functional properties: Functional properties are listed in Table 5. The colour parameters of MMSF and WMSF were in the range of 63.90- 76.54 (L*), 1.31-1.65 (a*), 17.81-21.14 (b*), 27.88-39.89 (ΔE), 17.86-21.18 (C*) and 85.33-86.28 (h). L* value of MMSF (76.18) is higher than WMSF (66.01), which indicates MMSF was whiter than WMSF. The b* value of MMSF (20.69) was higher than that of WMSF (18.34), which means MMSF has more yellowness than WMSF. The a* value indicates redness, slightly greater for MMSF (1.46) than WMSF (1.44). L*, b*, ΔE, and C* bear significant differences (P<0.05). The L* (76.18) and b* (20.69) values of MMSF were near to that reported by Siddeeg *et al.* (2014). ΔE, C*, and h were higher for MMSF (39.42, 20.74, and 85.96) than WMSF (29.63, 18.39, and 85.51), respectively. WAC indicates starch and protein's hydrophilic nature and gelation ability in flours (Rodríguez-Miranda *et al.,* 2016). WAC is an essential indicator of the ability of a protein to be incorporated with aqueous food formulations such as dough and custards in the bakery (Elaveniya *et al.,* 2014). WAC of WMSF (0.73 g/g) was higher than MMSF (0.20 g/g). However, the WAC reported for seeds flours of Apple (3.58 g/g) and Papaya (3.39 g/g) was higher than the MMSF and WMSF due to variation in the protein matrix (El-Safy *et al.,* 2012). OAC has an important role in retaining the flavor of food products (cakes, soups, and sausages) with imparting textural properties (Elaveniya *et al.,* 2014). OAC of MMSF (1.26 g/g) was higher than that of WMSF (1.00 g/g). Various factors such as protein concentration, water-oil interaction, and conformational features are responsible for variations in OAC among seeds (Sonawane *et al.,* 2016). WAC and OAC for these flours were in the range Chawla *et al.,* (2020) reported. EC of WMSF (49.20 %) was higher than that of MMSF (48.61 %). High EC is desirable in sausages and comminuted meat products. The proteins are surface-active agents and provide stability to the emulsion by applying electrostatic repulsion on the surface of the oil droplet (Mallek-Ayadi *et al.,* 2019). However, ES was lower for WMSF (40.99 %) than for MMSF (52.77 %), but both seeds' flour had appreciable ES with a significant difference (P<0.05). High ES could be due to high protein content and surface charge (Chawla *et al.,* 2020). EC of MMSF (48.61 %) was much higher than that reported by Mallek-Ayadi *et al.* (2019) and Siddeeg *et al.* (2014). EC % of both the seeds flour (48.61 %, 49.20 %) were found in a similar range reported by Chawla *et al.* (2020). EC (48.61 %) of MMSF was near that reported by Uduwerella *et al.* (2021). FC depends on the interfacial film conformed by flour proteins which maintain the air bubbles in suspension and decrease the coalescence rate (Rodríguez-Miranda *et al.,* 2016). FC of WMSF (44.24 %) was higher than that of MMSF (39.39 %). FC of MMSF (39.39 %) was much higher than that reported by Siddeeg *et al.* (2014).

Table 5. Mean, standard deviation (SD), maximum and minimum values for functional properties of seeds flour of musk melon and watermelon

Parameters	Muskmelon seed flour (MMSF)			Watermelon seed flour (WMSF)		
	Mean ± SD	Max.	Min.	Mean ± SD	Max.	Min.
L*	76.18 ±0.55^{a}	76.54	75.55	66.01 ±1.98^{b}	67.84	63.90
a*	1.46 ±0.17^{a}	1.65	1.31	1.44 ±0.11^{a}	1.54	1.32
b*	20.69 ±0.48^{a}	21.14	20.18	18.34 ±0.54^{b}	18.90	17.81
AE	39.42 ±0.61^{a}	39.89	38.72	29.63 ±1.67^{b}	31.21	27.88
C*	20.74 ±0.47^{a}	21.18	20.23	18.39 ±0.55^{b}	18.96	17.86
h	85.96 ±0.45^{a}	86.28	85.44	85.51 ±0.22^{a}	85.76	85.33
WAC (g/g)	0.20 ±0.00^{a}	0.20	0.00	0.73 ±0.41^{a}	1.20	0.40
OAC (g/g)	1.26 ±0.11^{a}	1.20	1.40	1.00 ±0.34^{a}	1.40	0.80
FC (%)	39.39 ±5.24^{a}	45.45	36.36	36.36 ±0.00^{a}	36.36	36.36
EC (%)	48.61 ±10.48^{a}	58.33	37.50	49.20 ±1.37^{a}	50.00	47.61
ES (%)	52.77 ±4.80^{a}	58.33	50.00	40.99 ±3.77^{b}	45.00	37.50

Note: Values are mean ± standard deviation, a maximum and minimum value of three replicates. The mean values within the same row with different superscripts have significant differences (P<0.05).

Thermal properties: DSC gives quantitative and qualitative analysis of physicochemical changes involving endothermic or exothermic processes (Sonawane *et al.,* 2016). The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpies (ΔH) for the DMMSF and DWMSF were (70°C, 57.9 °C), (63.8 °C, 66.4 °C), (75.9 °C, 87.5 °C) and (57.23 J/g, 25.53 J/g) respectively. Fig. 1 and Fig. 2 depict the DSC thermogram of DMMSF) and DWMSF. The thermal denaturation temperature of DWMSF (66.4 °C) was higher than that of DMMSF (63.8 °C). The peak temperature (T_p) is the denaturation temperature indicating the proteins' thermal stability (Siddeeg *et al.,* 2014). Higher denaturation temperature is due to the existence of nonpolar residues. The polar and nonpolar constituents in the

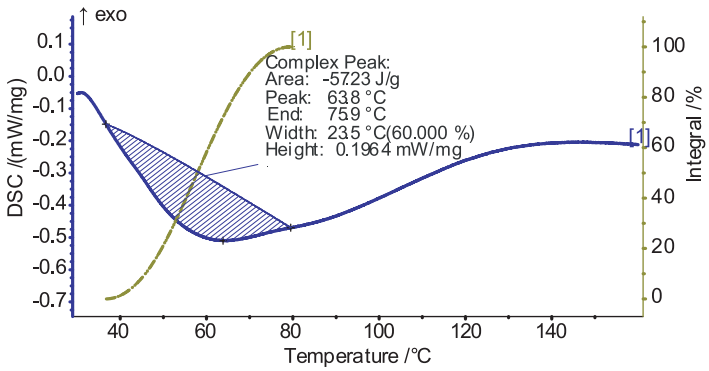

Fig. 1. DSC thermo gram of DMMSF

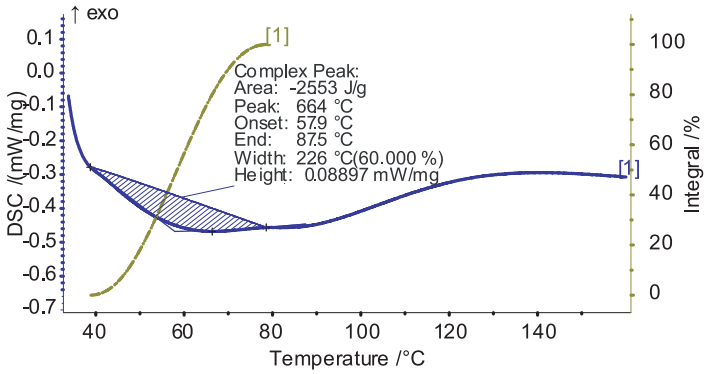

Fig. 2. DSC thermo gram of DWMSF

protein are responsible for thermal stability (Sonawane *et al.,* 2016).

The present study indicates that the MMS and WMS are rich sources of nutrients such as protein, fats, and minerals; therefore, they could be incorporated into various food products for nutritional enhancement. They also possess functional properties such as WAC, OAC, FC, EC, and high ES so that they could be successfully incorporated as functional food ingredients in the desired food industries such as bakery products, infant food formulations, and meat products. The data obtained for dimensional, gravimetric, and frictional properties would help design systems for the seed's storage, transportation, and processing units. The DSC analysis depicts the thermal degradation of the proteins in the seeds, indicating behavioral patterns during thermal processing. Overall, this study could be helpful in the food industry involved in handling and processing these seeds for inclusion as a value-added ingredient. Further studies regarding anti-nutritional factors and toxicity of these seeds are suggested to assure their safety for food incorporation.

Conflict of interest: The authors declare no conflict of interest.

Author contribution: **Manika Mehra**: Conceptualization, Methodology, Investigation, Resources, Formal analysis, Writing—original draft, Writing—review & editing, Visualization; **Ankur Ojha**: Visualization, Writing—review & editing, supervision; **Murlidhar Meghwal**: Writing—review & editing, supervision; **Komal Chauhan**: Writing—review & editing, supervision; **Sunil Pareek**: Writing—review & editing, supervision.

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Antifungal activity of *Citronella* essential oil against stem-end rot of mango

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Abstract

Stem-end rot caused by *Diplodia natalensis* is one of the significant postharvest diseases causing setbacks in the mango industry. Essential oil shows excellent potential as an alternative method in controlling postharvest diseases, which are considered safe and biodegradable with no residual effect. Hence, the study was conducted to determine the antifungal activity of *Citronella* essential oil against *Diplodia natalensis* (Pole Evans), to identify the effective concentration of *Citronella* essential oil to control *D. natalensis In vitro* and to evaluate the potential of *Citronella* oil as treatment against stem-end rot disease of mango fruit. Results showed that *Citronella* oil at 30% - 80% concentration exhibits fungistatic activity. At the same time, *Citronella* at 90% concentration showed fungicidal activity, which was most effective, showing complete inhibition of mycelial growth in the *In vitro* experiment. Furthermore, a significant reduction in fruit decay and percent fruit decay was noted with the 90% concentration of *Citronella* essential oil compared with the control in the *In vivo* experiment. However, no significant differences were observed between treatments regarding the length of exposure at 90% concentration of the essential oil. These results suggest that *Citronella* essential oil can potentially control stem-end rot in mango.

Key words: *Citronella* essential oil, antifungal activity, stem-end rot, *Diplodia natalensis*

Introduction

Mango (*Mangifera indica* L.) is famous for its excellent flavor, attractive fragrance and nutritional value, which plays an important role in the human diet. It contains macronutrients and micronutrients (Maldonado-Celis *et al.,* 2019). As one of the top ten fruit crops in the world, the annual production is estimated to be at approximately 54.83 million tons in 2020 (Statista, 2021). In 2021, the volume of mangoes produced in the Philippines amounted to approximately 741.7 thousand metric tons (Statista, 2022).

Despite these facts, the productivity of mango in the Philippines is affected by various postharvest diseases which reduce the fruit quality and cause severe losses. Among these postharvest diseases, stem-end rot caused by *Diplodia natalensis* (P. Evans) is one of mango's most important and serious problems. The most common method to control postharvest diseases is through fungicide application. However, using synthetic chemicals to control postharvest diseases can cause side effects on human health (Unnikrishnan and Nath, 2002). Moreover, continuous synthetic fungicide use may induce resistance development on postharvest pathogens (Dianz *et al.,* 2002). These suggest the need to use alternative control measures to reduce crop losses incurred by safe postharvest pathogens that pose no risk to humans and the environment.

One of the alternatives that can be utilized is essential oils. These are a complex mixture of plant volatile compounds such as terpenoids and phenolic compounds (Fokou *et al.,* 2020). These compounds are biodegradable, non-pollutant and possess no residual properties. Several studies are well documented on the effect of essential oil in postharvest diseases. For instance, Bosquez-Molina *et al.* (2010) reported that essential oil from thyme showed fungicidal effect against both *C. gloeosporioides* and *R. stolonifer* in papaya fruit. Pérez-Alfonso *et al.* (2012) also reported that thymol, carvacrol and the mixture opure essential oils have been proved effective against *Penicillium digitatum* and *Penicillium italicum*, in lemons. Essential oils from Rosewood (RO), thyme red (TR) and fennel (FO) exhibits inhibitory effect against *B. cinerea, M. nidicola and M. piriformis, P. expansum and Penicillium sp.*, and *R. stolonifer* in peach (Lin *et al.,* 2022). Essential oil from *Cymbopogon nardus* (L.) suppressed the growth of several species of *Aspergillus, Penicillium* and *Eurotium* (Nakahara *et al.,* 2013). Sangeetha *et al.* (2010) reported that *Citronella* oil extracted from *Cymbopogan citratus, C. martinii, and C. nardus* completely arrested the mycelial growth of *Lasiodiplodia theobromae and Colletotrichum musae* (crown rot disease). These reports suggest that essential oils such as *Citronella* has the potential to be an alternative control method against postharvest diseases like *Diplodia* stem-end rot. Hence, this study aimed to determine the antifungal activity of *Citronella* essential oil against *Diplodia natalensis* (Pole Evans), to identify the effective concentration of *Citronella* essential oil to control *D. natalensis In vitro*, and to evaluate the potential of *Citronella* oil as a treatment on mango fruits against *Diplodia* stem-end rot disease *In vivo*.

Materials and methods

Isolation and culture of *Diplodia natalensis*: Infected mango was collected and brought to the laboratory in the College of Engineering, ESSU, Salcedo, Eastern Samar for pathogen isolation. Several 3 mm tissue sections of the infected fruits showing typical symptoms of stem-end rot were cut and

disinfected with 1% sodium hypochlorite for one minute to remove the surface contaminants and rinsed three times with sterile water. It was blotted dry using sterile tissue paper. Dried tissue was placed aseptically in the surface of previously plated solidified potato dextrose agar (PDA) using flamed-sterilized forceps. The plates were incubated at room temperature for 24-48 hours in an inverted position. Typical growth of *D. natalensis* was transferred to a PDA slant to have a pure culture. The culture was allowed to grow and placed inside the refrigerator.

Procurement and preparation of *Citronella* essential oil: *Citronella* essential oil was purchased online. The oil was considered as 100% pure concentration. The concentrations of *Citronella* essential oil used are 20, 30, 40, 50, 60, 70, 80 and 90%. Tween 80 was added drop by drop and was mixed thoroughly with sterile stirrer until separate layers are no longer visible.

Standard control checks such as sterile distilled water (negative control check) and 40% Acetic acid (positive control check) was also provide.

***In vitro* vapor agar technique**: The effect of *Citronella* essential oil on fungal growth was determined by cutting several agar discs from the edge of an actively growing fungal culture with 5mm cork borer. One agar disc was placed at the center of each plated PDA and was allowed to stay for 6 hours under normal position before adding treatment. Two pieces of sterile filter paper were layered inside each dish's lid. One mL of *Citronella* oil at different concentrations was pipetted into the center of the filter paper. The lid was inverted and fitted into the lid at the bottom of the dish. The petri dish was placed upside down with the mycelium plug above the filter paper. Dishes were sealed with parafilm to reduce the vaporization of essential oil from the plate.

The petri dish was arranged in a completely randomized design (CRD) with ten (10) treatments: T1 = Negative control check (sterile distilled water), T2 = 20% *Citronella* essential oil, T3 = 30% *Citronella* essential oil, T4 = 40% *Citronella* essential oil, T5 = 50% *Citronella* essential oil, T6 = 60% *Citronella* essential oil, T7 = 70% *Citronella* essential oil, T8 = 80% *Citronella* essential oil, T9 = 90% *Citronella* essential oil, and T10= 40% Acetic acid (positive control check) and were replicated three (3) times. Cultures were then incubated at room temperature for 5 days and diameter of each mycelial colony was measured in mm using a ruler. Growth inhibition percentage was determined according to the formula (Fang *et al.,* 1994).

$$\text{Growth inhibition (\%)} = 100 - [[\ \frac{a}{b}\ ] \times 100]$$

Where: a = Mycelial diameter of the treatment, b = Mycelial diameter of negative control

***In vivo* fruit fumigation technique**: Mango fruits were inoculated at least two hours before the fumigation treatment with the mycelial disc of about 3mm in diameter. The most effective concentration of *Citronella* essential oil in the *In vitro* assay was used. The fumigation chamber was designed using a plastic container with secure cover. The effective concentration in the *In vitro* experiment was used. The beaker containing the 10 mL *Citronella* oil was placed in the other side of the chamber to avoid direct contact with the fruit. The lid was closed and sealed with impermeable tape to create airtight conditions. Moist cotton was placed inside the chamber to create humid conditions for ideal infection. Three mango fruits were placed inside per chamber. The fumigation chambers were arranged in a completely randomized design (CRD) with ten (10) treatments: T1 = water treatment (negative control check), T2 = 90% *Citronella* essential oil (1 hour exposure), T3 = 90% *Citronella* essential oil (2-hour exposure), T4 = 90% *Citronella* essential oil (4 hours exposure), T5= 90% *Citronella* essential oil (6 hours exposure), T6 = 90% *Citronella* essential oil (12 hours exposure), T7 = 90% *Citronella* essential oil (24 hours exposure), T8= 90% *Citronella* essential oil (48 hours exposure), T9= 40% Acetic acid (24hours exposure), and T10 = 40% Acetic acid (48hours exposure) and replicated three (3) times.

After the incubation, the fruits were taken out of the chamber and was further incubated inside a plastic bag with moist cotton for another 24hrs; then, it was exposed at room temperature. The daily observation was taken until 60% fruit decay was noted in control. The volume of the fruit was measured and recorded upon set-up termination. The fruit volume was determined using a water displacement method described by Curran (2004). The one-liter beaker was filled with water and the volume of the water was recorded. The entire mango fruit was carefully dipped inside the beaker and the volume of the water was recorded. The volume of the mango fruit was calculated by subtracting the volume of water alone from the volume of water with the fruit. Fruit portions that showed decay were cut out and removed, then the remaining part of the fruit was dipped inside the beaker and the volume of the water was recorded. Percent fruit decay was calculated as follows:

$$\text{Percent fruit decay (\%)} = [\frac{a - b}{a}]\ \times 100$$

Where: a = Total fruit volume, b = volume of the remaining fruit part

Statistical analysis: The collected data were analyzed using the Statistical Tool for Agricultural Research (STAR) software version 2.0.1. One-way analysis of variance (ANOVA) was used to determine the significant effects of the treatments. Tukey's Honest test was used to compare means at 5% level of significance.

Results and discussion

***In vitro* vapor agar technique**: Different concentrations of *Citronella* oil are tested for its vapor effect as a fumigant. It is clearly shown that essential oil has an inhibitory property compared with the control (sterile water) that reached its maximum growth at 5th day. On the other hand, it was observed that at the 5th day there was an increase in mycelial growth starting from the lowest concentration of *Citronella* oil (20%), followed by 30% at the 6th day, 40% at the 7th day and 50, 60 70 and 80% at the 8th day (Fig. 1). The mycelial growth keept increasing until the 12th day, however, no mycelial growth was noticed for 90% *Citronella* oil and 40% acetic acid up until the 12th day (Fig. 2). This suggests that concentration of *Citronella* at 90% exhibits full inhibitory effect while concentrations below 90% exhibit partial inhibitory effect.

The results revealed that after 5 days of fumigation, the highest mycelial diameter was observed in the treatments with sterile water, followed by 20% *Citronella* oil. Treatments with 30% to 90% *Citronella* oil had the lowest mycelial growth, highly comparable to the 40% acetic acid. However, seven days after the removal of *Citronella* oil, treatments were treated with sterile

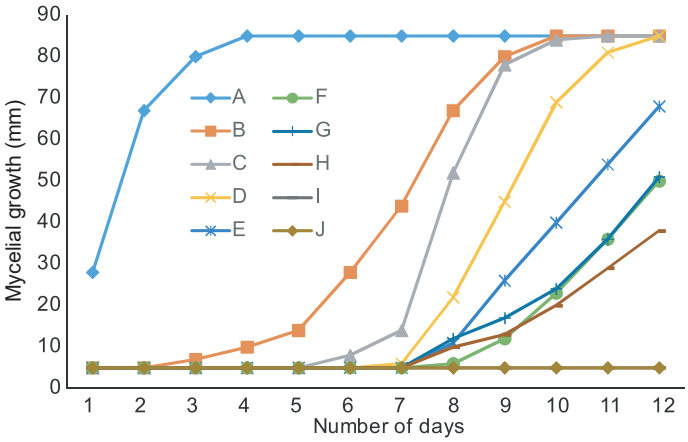

Fig 1. Mycelial growth (mm) of *D. natalensis* on vapor agar set-up as affected by the indicated treatments: A) Sterile water B) 20% *Citronella* oil, C) 30% *Citronella* oil, D) 40% *Citronella* oil, E) 50% *Citronella* oil, F) 60% *Citronella* oil, G) 70% *Citronella* oil, H) 80% *Citronella* oil, I) 90% *Citronella* oil, and J) 40% acetic acid.

water, 20, 30, 40 and 50% had the highest mycelial diameter, followed by 60%, 70% and 80% *Citronella* oil. Meanwhile, treatments treated with 90% *Citronella* oil and 40% acetic acid had the lowest mycelial diameter (Table 1).

Table 2 showed that *Citronella* oil at 30, 40, 50, 60, 70, 80 and 90% concentration and 40% acetic acid had the highest

Table 1. Mycelial mean diameter* (mm) of *Diplodia natalensis* as affected by different concentrations of *Citronella* essential oil after 5 days of fumigation and 7 days after the removal of essential oil in vapor agar set-up

Treatments	After 5 days of fumigation	7 days after removal of fumigant
Sterile water	85.00^{a}	85.00^{a}
20% *Citronella* oil	13.67^{b}	85.00^{a}
30% *Citronella* oil	5.00^{c}	85.00^{a}
40% *Citronella* oil	5.00^{c}	85.00^{a}
50% *Citronella* oil	5.00^{c}	67.67^{a}
60% *Citronella* oil	5.00^{c}	50.33^{ab}
70% *Citronella* oil	5.00^{c}	51.00^{ab}
80% *Citronella* oil	5.00^{c}	38.33^{ab}
90% *Citronella* oil	5.00^{c}	5.00^{b}
40% acetic acid	5.00^{c}	5.00^{b}

*Means in a column followed by the same letter are not significantly different at 5% level of significance based on Tukey's test.

Table 2. Growth inhibition* (%) of *Diplodia natalensis* as affected by different concentrations of *Citronella* essential oil after 5 days of fumigation and 7 days after the removal of essential oil in vapor agar set-up

Treatments	After 5 days of fumigation	7 days after the removal of fumigant
Sterile water	0.00^{c}	0.00^{b}
20% *Citronella* oil	83.92^{b}	0.00^{b}
30% *Citronella* oil	94.12^{a}	0.00^{b}
40% *Citronella* oil	94.12^{a}	0.00^{b}
50% *Citronella* oil	94.12^{a}	20.39^{b}
60% *Citronella* oil	94.12^{a}	40.78^{ab}
70% *Citronella* oil	94.12^{a}	40.00^{ab}
80% *Citronella* oil	94.12^{a}	54.90^{ab}
90% *Citronella* oil	94.12^{a}	94.12^{a}
40% acetic acid	94.12^{a}	94.12^{a}

*Means in a column followed by the same letter are not significantly different at 5% level of significance based on Tukey's test.

percent growth inhibition compared with sterile water and 20% *Citronella* oil that had the lowest percent growth inhibition after 5 days of fumigation. On the other hand, 7 days after removing the fumigant, it was observed that 90% *Citronella* oil and 40% acetic acid had the highest percent growth inhibition. The lowest percent growth inhibition was noted in treatments treated with sterile water, 20% to 40% *Citronella* oil. Results indicate that 20-80% *Citronella* oil only exhibits a fungistatic effect due to the increase in mycelial growth upon its removal after five days of fumigation. This result was in agreement with the result of Inouye *et al.* (2000) wherein there is a regrowth of the hyphae after removal of the vapor. This result suggests that essential oil's inhibitory property is highly influenced by the dosage and length of exposure (Banihashemi and Abiyardi, 2011).

On the other hand, *Citronella* oil at 90% showed fungicidal effect, same as the 40% acetic acid, showing complete inhibition of mycelial growth even at seven days after the removal of the essential oil (Fig. 2). According to Hu *et al.* (2017) fungal growth inhibition is related to the disruption of fungal cell endomembrane system including the plasma membrane and mitochondria, specifically *i.e.*, the inhibition of ergosterol synthesis, mitochondrial ATPase, malate dehydrogenase, and succinate dehydrogenase activities due to its exposure to essential oil. Tyagi and Malik (2010) also added that the volatility of the

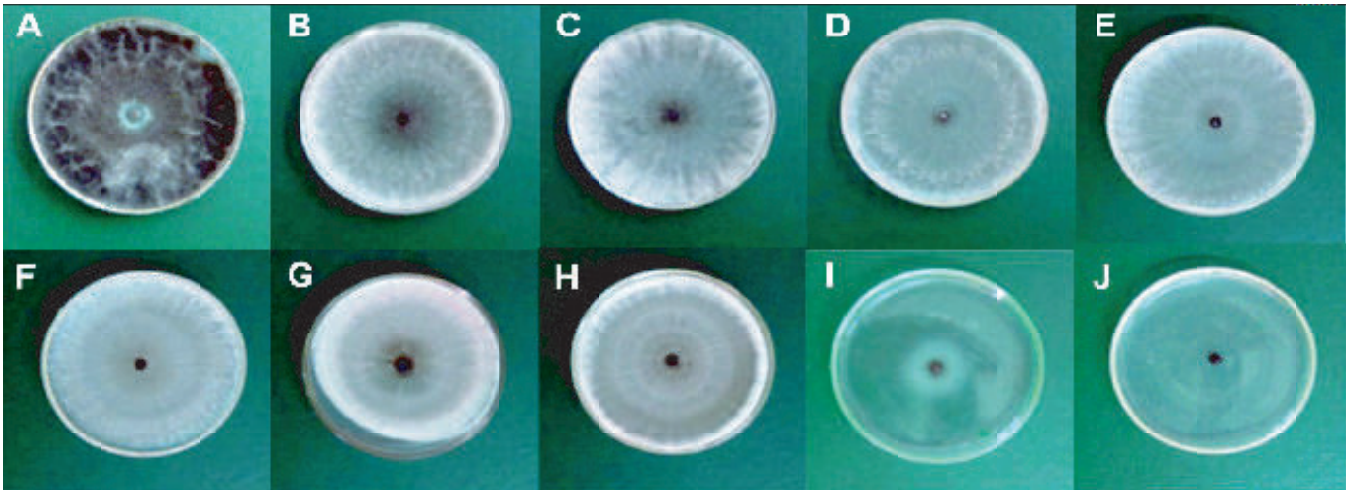

Fig 2. Mycelial growth (mm) of *Diplodia natalensis* as affected by different treatments as follows: A) Sterile water B) 20% *Citronella* oil, C) 30% *Citronella* oil, D) 40% *Citronella* oil, E) 50% *Citronella* oil, F) 60% *Citronella* oil, G) 70% *Citronella* oil, H) 80% *Citronella* oil, I) 90% *Citronella* oil, and J) 40% acetic acid.

essential oil is dominant in vapor phase assay. Furthermore, it is also reported that vapor action of essential oil inhibited filamentous fungi life-cycle through disruption on conidial germination and mycelium growth sporulation (Reyes-Jurado *et al.,* 2020).

***In vivo* fruit fumigation technique**: The inhibitory activity of *Citronella* oil at 90% concentration was assessed as a fumigant against mango stem-end rot. The fruit was inoculated with mycelial disc and were fumigated at different durations. Significant differences were observed between the treatments. Table 3 shows treatments treated with sterile water (control) had the highest fruit decay and percent fruit decay. The lowest fruit decay and percent fruit decay was observed in mango treated with 40% acetic acid at 24 hrs and 48 hrs of exposure. Meanwhile, mango treated with *Citronella* at 90% concentration had lower volume of fruit decay and percent fruit decay than mango treated with sterile water (control). On the other hand, no significant differences were observed between mangoes treated with 90% *Citronella* oil at the different duration of exposure based on the volume of fruit decay and percent fruit decay.

Table 3. Volume of Fruit decay and percent fruit decay as affected by the length of exposure to the treatments at 5th day observation

Treatments	Fruit decay* (cm^3)	Percent fruit decay (%)*
Sterile water	86.59^{a}	54.58^{a}
90% *Citronella* oil (1 hr)	37.52^{bc}	27.64^{bc}
90% *Citronella* oil (2 hrs)	43.30^{b}	32.64^{b}
90% *Citronella* oil (4 hrs)	43.30^{b}	34.76^{b}
90% *Citronella* oil (6 hrs)	40.41^{bc}	29.17^{bc}
90% *Citronella* oil (12 hrs)	37.52^{bc}	26.59^{bc}
90% *Citronella* oil (24 hrs)	43.30^{b}	30.44^{b}
90% *Citronella* oil (48 hrs)	28.86^{bc}	19.61^{bc}
40% acetic acid (24 hrs)	17.32^{cd}	13.06^{cd}
40% acetic acid (48 hrs)	0.00^{d}	0.00^{d}

*Means in a column followed by the same letter are not significantly different at 5% level of significance based on Tukey's test.

Furthermore, it is also observed that exposure to acetic acid (40%) significantly reduced fungal growth compared with other treatments *In vivo*. However, too much exposure of the mango to acetic acid (40%) at 48 hrs resulted to phytotoxicity compared with mango at 24 hrs of exposure (Fig. 3). These results suggest that the fumigation of *Citronella* essential oil is effective in reducing the fungal growth of *D. natalensis* compared with control (sterile water) in the treated mangoes *In vivo*. Other reports also indicated that essential oil as fumigants *In vivo* significantly reduced the growth of postharvest pathogens (Elshafie *et al.,* 2015; Santoro *et al.,* 2018). Throughout this study, it was observed that the efficacy of 90% *Citronella* essential oil was reduced in the *In vivo* experiment compared with *In vitro* experiment with complete mycelial growth inhibition. Hu *et al.* (2017) stated that fungal growth inhibition was related directly to the interaction of the fungal mycelia and essential oil. This is true in the *In vitro* set-up, wherein the mycelium is directly exposed to the essential oil vapor. Inouye *et al.* (2000) found that the predominant absorption of essential oil was observed in the fungal mycelia compared with in agar medium. This is highly due to mycelia's lipophilic nature concerning essential oil's volatile properties. On the other hand, the mycelial growth of *D. natalensis* in the mango fruit was not directly exposed to the essential oil under the fumigation technique since mycelial growth was predominant underneath the mango peel rather than the surface. Thus, this may suggest why stem-end rot of mango was not completely inhibited in the *In vivo* experiment compared with the *In vitro* experiment.

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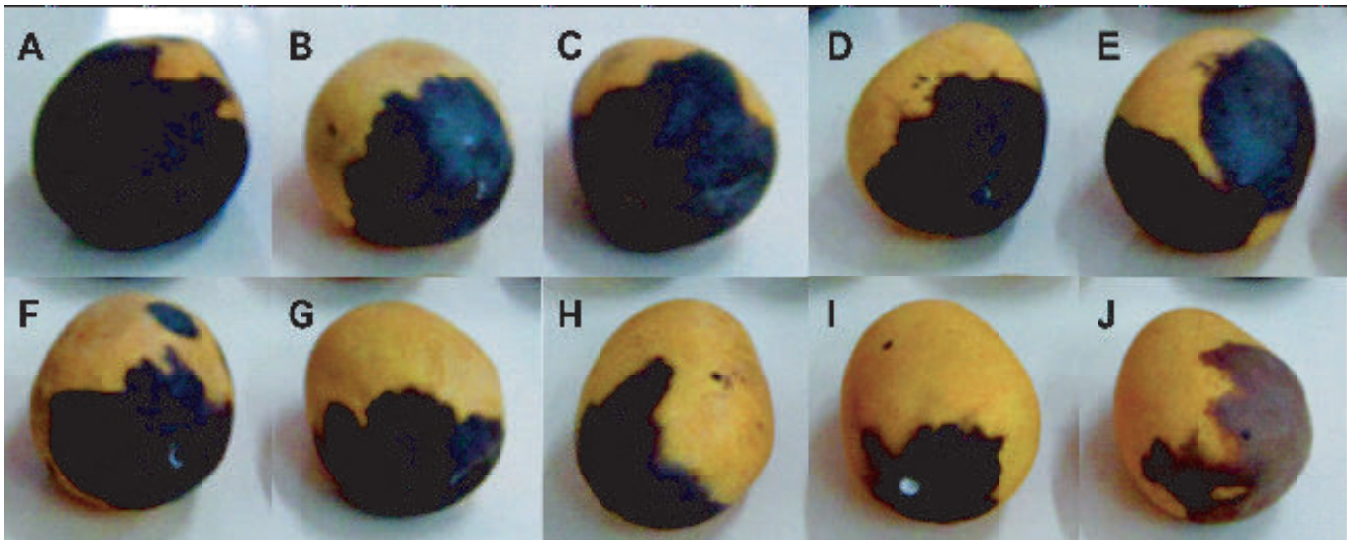

Fig 3. Stem-end rot infection on mango fruit as affected different treatments with varying time of exposure of *Citronella* essential oil as follows: A) sterile water, B) 90% *Citronella* oil (1 hr.), C) 90% *Citronella* oil (2 hrs.), D) 90% *Citronella* oil (4 hrs.), E) 90% *Citronella* oil (6 hrs.), F) 90% *Citronella* oil (12 hrs.), G) 90% *Citronella* oil (24 hrs.), H) 90% *Citronella* oil (48 hrs.), I) 40% acetic acid (24 hrs.) and J)

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Development and characterization of intraspecific hybrids of low chill peach [*Prunus persica* (L.) Batsch]

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Abstract

An experiment was conducted to broaden the range of early-ripening peach cultivars through hybridization. Low chill peach cultivars, specifically Shan-i-Punjab and Tropic Sweet, were selected as female parents and crossed with Florda Prince, Flordaglo, and Prabhat. Among the crosses, the highest fruit set (72.63%) was observed in Shan-i-Punjab x Florda Prince, while the lowest fruit set was recorded in Tropic Sweet × Flordaglo (18.87%). Despite the lower fruit set in Tropic Sweet crosses, they exhibited a significantly higher fruit retention percentage and a lower fruit drop rate than those involving the Shan-i-Punjab cultivar. Following ripening, seeds were extracted from the fruits and subjected to stratification at low temperatures until radicle emergence occurred. The stratification duration for the hybrid seedlings ranged from 76.00 days in Shan-i-Punjab × Florda Prince to 88.33 days in Tropic Sweet × Flordaglo. After sowing the seeds in the field, the highest seed germination percentage of 90.43 was recorded in Tropic Sweet × Florda Prince, which did not show a significant difference from Tropic Sweet × Flordaglo (88.94%), followed by Tropic Sweet × Prabhat (85.11%). Regarding seedling growth, Tropic Sweet × Flordaglo exhibited the maximum seedling height of 36.03 cm, while minimal variations were observed among different crosses regarding petiole length, leaf area, and internodal length.

Key words: Hybridization, morphological characterization, F_1 hybrids, peach [*Prunus persica* (L.) Batsch]

Introduction

Peach (*Prunus persica* L.) is a significant fruit crop belonging to the Rosaceae family, valued for its fresh and canned fruits. Originating in China, its cultivation dates back at least 4000 years. With successful breeding efforts, peach has adapted to various sub-tropical regions worldwide due to its wider climatic adaptability. It is a diploid plant with a relatively small genome of 5.9 × 108 bp or 0.61 pg in the diploid nucleus, with a haploid size of 300 Mb (Baird *et al.,* 1994). This genome size is approximately twice that of the Arabidopsis genome. Peach is globally recognized as an important fruit and is highly genetically characterized, making it a model species for the Rosaceae family (Monet and Bassi, 2008; Arus *et al.,* 2012).

In India, peach cultivation is limited to the warm temperate and sub-tropical regions of Jammu and Kashmir, Himachal Pradesh, Punjab, Haryana, Uttarakhand, parts of Uttar Pradesh, Tamil Nadu, and the North Eastern states. Peach occupies an area of 18,000 ha in India, producing 111,000 MT (NHB, 2021). China leads the world in peach production, accounting for approximately 58% of the total global production, followed by Italy, Spain, and the USA. The cultivation area for peach is expanding rapidly in the subtropical regions of northern India due to the availability of suitable cultivars and higher returns per unit area. Breeding efforts have led to the development of low-chilling peach cultivars, enabling their cultivation from temperate to sub-tropical regions worldwide.

Initially, peach breeding goals focused primarily on improving external fruit quality, post-harvest life, disease and pest resistance, and a greater range of fruit maturities and types (Byrne, 2005). However, as consumer standards have increased, there is now a growing emphasis on improving fruit eating quality, including nutritional composition. Numerous peach breeding programs are currently striving to enhance fruit quality and productivity within locally adapted germplasm (Monet and Bassi, 2008; Byrne *et al.,* 2012). Important tree and fruit quality parameters are often interconnected, as complex genetic and physiological factors govern them. Traits related to plant growth, architecture, yield, blooming, and harvesting time are typically influenced by multiple genes (Dirlewanger *et al.,* 1999). Fruit size, for example, is a polygenic trait with low to moderate heritability, influenced by environmental conditions, plant nutrition, and cultural practices (Souza *et al.,* 1998).

Significant advancements have been made in the genus Prunus over the past century, utilizing traditional methods of genetic improvement such as crossing, selection, evaluation of superior lines, and *in vitro* propagation of new cultivars (Hancock *et al.,* 2008; Okie and Hancock, 2008; Iezzoni, 2008). These traditional breeding methods have resulted in the development and commercialization of highly productive, good quality, and resistant cultivars to both biotic and abiotic conditions. Therefore, the current breeding program aims to expand the range of early-ripening peach cultivars characterized by low chilling requirements, summer stratification, and controlled germination conditions to recover peach hybrid seedlings.

Materials and methods

The hybridization programme was started in low chill peach cultivars, *viz.* Shan-i-Punjab (♀) × Florda Prince (♂), Shan-i-Punjab (♀) × Flordaglo (♂), Shan-i-Punjab (♀) × Prabhat (♂), Tropic Sweet (♀) × Florda Prince (♂), Tropic Sweet (♀) × Flordaglo (♂) and Tropic Sweet (♀) × Prabhat (♂) at Fruit

Research Farm, Department of Fruit Science, Punjab Agricultural University, Ludhiana. Branches with unopened blossom at popcorn stage were selected and were emasculated to prevent self pollination. All the opened flowers and undeveloped buds were removed. The flower buds of the female parent were emasculated at balloon stage in the morning (9-11 am) and pollinated with camels hair brush during the day (11.30 am to 2.00 pm) on the same day. Emasculated flowers were pollinated either with fresh pollen (cultivars in which the flowering period coincided) or stored pollen (cultivars in which flowering periods did not coincide). The anthers from the male parents were collected at balloon stage and dehisced in a silica gel desiccator. The pollen was collected in 10 mL vials and stored at 5 °C till use. The pollens were applied to stigmas with camel hair brush and pollinated flowers were not bagged or protected because emasculated flowers do not attract pollinators (Monet and Bassi, 2008). Data on fruit set, fruit retention and fruit drop was recorded. When fruits ripened they were harvested separately and seeds were excised from the fruits in the laboratory. Seeds were kept in media containing cocopeat, vermiculite and perlite (2:1:1) and was moistened with Bavistin to avoid the fungal diseases. Seeds were kept at 4±2°C until the maximum seeds showed radicle emergence. After that they were sown in pot trays and kept in growth chamber initially but when the seeds showed germination they were transferred in polyhouse. Data regarding days taken for stratification was recorded when the seeds kept for stratification started radicle emergence. After germination in field, seedlings were evaluated for total seed germination percentage, days taken for seed germination, seedling height, intermodal length, petiole length and other leaf characters such as leaf area, shape, arrangements, margins and presence of nectaries. The experiment was laid out as randomized block design (RBD) with three replications. The data were analyzed using SAS v 9.0.0 software and means were compared using Duncan's Multiple Range Test (DMRT).

Table 1. The parentage of the cultivars used for hybridization

Cultivar	Parentage
Shan-i-Punjab	(Southland x Jewel) F_2 x June Gold
Florda Prince	Fla.2-7 x Fla. 13-72 (Maravilha)
Flordaglo	Sundowner nectarine x Maravilha peach
Prabhat	Sharbati x Flordasun
Tropic Sweet	Fla. 46-95 x Fla. A5-107 (Keygold nectarine)

Results and discussion

Maximum mean fruit set was observed in Shan-i-Punjab × Florda Prince (72.63 %), however the differences in fruit set was non significant in crosses Shan-i-Punjab × Florda Prince, Shan-i-Punjab × Flordaglo and Shan-i-Punjab × Prabhat (Fig.1). Minimum mean fruit set was recorded in Tropic Sweet × Flordaglo (18.87 %). It has been observed that those crosses where Shan-i-Punjab was taken as a female parent showed higher fruit set, whereas the crosses made with Tropic Sweet as a female exhibited less than 25 % fruit set during both the years. The low fruit set might be due to the temperature fluctuations; thus, Tropic Sweet plants failed to undergo dormancy and showed staggered flowering. Hesse (1975) found that initial fruit set in peach crosses can vary from 10% to 90% and this information is consistent with our data showing less fruit set in Tropic Sweet crosses compared to Shan-i-Punjab crosses. Eroglu *et al.,* (2016) reported 78.27% and 73.10% fruit set for two years in different peach crosses.

Although in Tropic Sweet crosses, fruit set was lower but they showed significantly higher percentage of fruit retention and lower percentage of fruit drop than the crosses made with Shan-i-Punjab cultivar (Fig. 1). The highest fruit retention was recorded in Tropic Sweet × Florda Prince crosses (74.12 %) followed by Tropic Sweet × Prabhat (70.41 %) and minimum in Shan-i-Punjab × Flordaglo (22.99 %). As far as fruit drop is concerned, it was maximum in Shan-i-Punjab × Flordaglo (76.99 %) and Shan-i-Punjab × Florda Prince (75.80 %) followed by Shan-i-Punjab × Prabhat (69.05 %) whereas, minimum fruit drop was recorded in Tropic Sweet × Florda Prince (25.84 %). Among Tropic Sweet crosses maximum fruit drop was observed in Tropic Sweet × Flordaglo (34.08 %) followed by Tropic Sweet × Prabhat (29.57 %) and minimum in Tropic Sweet × Florda Prince (25.84 %).

Peach seeds require stratification (low chilling treatment) for

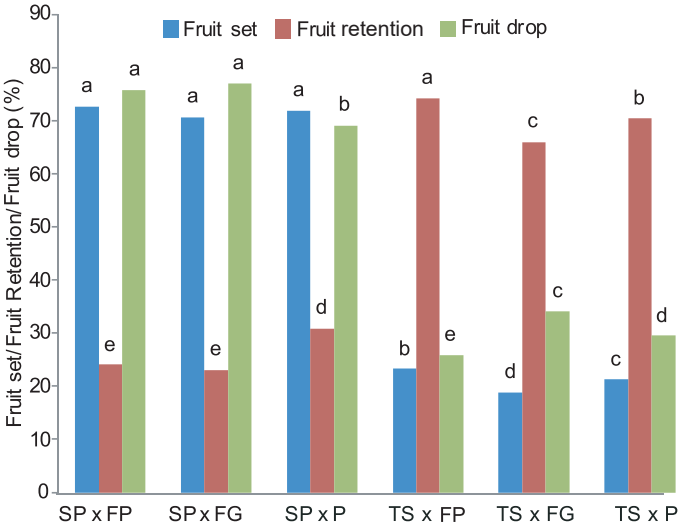

Fig. 1. Fruit set, fruit retention and fruit drop in crosses made between low chill peach cultivars

SP x F*P*=Shan-i-Punjab x Florda Prince, SP x FG=Shan-i-Punjab x Flordaglo, SP x *P*=Shan-i-Punjab x , Prabhat, TS x F*P*=Tropic Sweet x Florda Prince, TS x FG=Tropic Sweet x Flordaglo, TS x P =Tropic Sweet x Prabhat

germination. F_1 seeds of Tropic Sweet × Flordaglo took maximum days for stratification (88.33), whereas F_1 seeds obtained from those crosses where Shan-i-Punjab was taken as a female took slightly lesser time for stratification and minimum days taken for stratification was recorded in Shan-i-Punjab × Florda Prince (76.00) and data was recorded when the seeds kept for stratification showed 100 per cent radicle emergence (Fig. 2). Seeds from all the crosses has taken more than 75 days for radicle emergence (Fig 3). Biggs, (1966) demonstrated that seeds from different cultivars differed due to the duration of chilling needed for stratification. Bruckner *et al.,* (2012) also found the strong effect of embryo genotype on the chilling requirement of the seeds. Stratification is used to break embryo dormancy and found that stratification treatment of 10 weeks increased the per cent germination over 3 weeks stratification (Mendez, 2005). In present studies seeds without endocarp were kept for stratification at 4°C until the seeds showed radicle emergence and seeds obtained from all crosses took 76 to 88 days for radicle emergence and this is in accordance with the results of Eroglu *et al.,* (2016). They stratified the seeds of different peach crosses without endocarp at 4-5°C for 40 to 90 days and reported differential response of the crosses. After the chilling requirement was fulfilled seeds were sown in field and

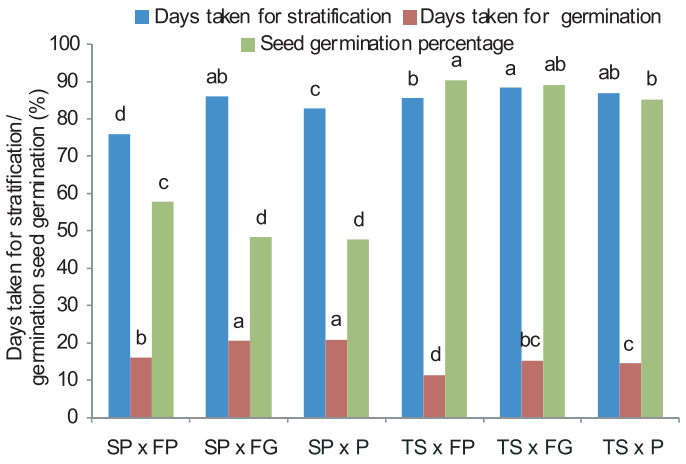

Fig. 2. Days taken for stratification, days taken for germination and germination percentage of hybrid seeds

SP x F*P*=Shan-i-Punjab x Florda Prince, SP x FG=Shan-i-Punjab x Flordaglo, SP x *P*=Shan-i-Punjab x , Prabhat, TS x F*P*=Tropic Sweet x Florda Prince, TS x FG=Tropic Sweet x Flordaglo, TS x P =Tropic Sweet x Prabhat

the data of days taken for germination was taken after the seeds started germination in the field. Among all the crosses made, Tropic Sweet × Florda Prince seeds germinated in minimum days (11.33 days) and F_1 seeds of Shan-i-Punjab × Prabhat and Shan-i-Punjab × Flordaglo crosses took maximum mean days for germination (20.73 days and 20.50 days, respectively). In comparison to Shan-i-Punjab, seeds obtained from Tropic Sweet crosses took lesser time for germination after sowing in the field because of longer fruit development period, matured seeds and more dry matter in the seeds. After 30 days of sowing total seed germination was observed and mean maximum seed germination 90.43% was recorded in F_1 seeds of Tropic Sweet × Florda Prince which was statistically at par with the seed germination of Tropic Sweet × Flordaglo (88.94 %) followed by Tropic Sweet × Prabhat (85.11%). F_1 seeds of Shan-i-Punjab × Florda Prince crosses showed 57.73% germination whereas minimum seed germination was recorded in Shan-i-Punjab × Prabhat (47.76 %) and Shan-i-Punjab × Flordaglo (48.41%). In Tropic Sweet hybrid seeds, very quick germination was observed and more than 70% seeds germinated even after 10 days of sowing but F_1 hybrids obtained from Shan-i-Punjab showed very less percentage of seed germination. This may be due to the immature embryo of Shan-i-Punjab crosses because of short fruit development period (FDP, days from flowering to harvest) and embryo of these seeds have little reserve and unable to achieve maximum dry weight thus they are too weak to germinate, whereas Tropic Sweet cultivar took more time for ripening, thus embryo was matured and showed higher germination. Bacon and Byrne (1995) reported up to 85% seed germination from genotypes with fruit development period of more than 105 days and seeds stratified without endocarp has increased the germination rate and shorten the length of germination duration (Tukey and Carlson, 1945).

The seedling height, internodal length, leaf area, and petiole length of the hybrid seedlings were recorded after six months of growth (Table 2). The maximum seedling height of 36.03 cm was observed in the F_1 seeds of Tropic Sweet × Flordaglo, which did not show a significant difference from the seedling height of Tropic Sweet × Florda Prince and Tropic Sweet × Prabhat crosses (35.59 cm and 35.87 cm, respectively). The seedlings of Shan-i-Punjab × Flordaglo and Shan-i-Punjab × Prabhat crosses had the minimum mean seedling height of 27.00 cm and 27.20 cm, respectively. This variation in seedling height can be attributed to the different genotypes used in the crosses.

In terms of internodal length, the minimal difference was observed among the hybrid seedlings of all crosses, with the maximum observed in Tropic Sweet × Flordaglo and Tropic Sweet × Prabhat (1.60 cm). No significant difference was observed in the internodal length of Shan-i-Punjab × Florda Prince and Shan-i-Punjab × Flordaglo hybrid seedlings. The maximum leaf area was observed in Shan-i-Punjab × Florda Prince (20.16 cm²), which was statistically similar to the leaf area of Tropic Sweet × Prabhat (19.79 cm²), while the minimum leaf area was found in Shan-i-Punjab × Flordaglo and Shan-i-Punjab × Prabhat (18.35 cm² and 18.40 cm², respectively). Similar variations in leaf area were also reported by Ahmed Emad-Eldin *et al.* (2012) in their study on F_1 hybrid seedlings. Wang *et al.* (2006) mentioned that leaf characteristics, such as leaf area, petiole length, and petiole thickness, are genetically inherited and can vary among different varieties. There was very little variation observed among the hybrids for petiole length, with most crosses having a petiole length of 0.65 cm.

The leaf shape and leaf arrangement of all hybrids (Table 3) did not show any significant variation. All hybrids exhibited a consistent leaf shape, specifically lanceolate, and an alternate

Table 2. Seedling height, intermodal length, leaf area and petiole length of hybrid seedling

Parents	Seedling height	Internodal length	Leaf area	Petiole length
Shan-i-Punjab x Florda Prince	30.34b*	1.41c	20.16a*	0.65a*
Shan-i-Punjab x Flordaglo	27.00c	1.38c	18.35d	0.61ab
Shan-i-Punjab x Prabhat	27.20c	1.32d	18.40d	0.56b
Tropic Sweet x Florda Prince	35.59a	1.53b	18.78cd	0.65a
Tropic Sweet x Flordaglo	36.03a	1.60a	19.21bc	0.65a
Tropic Sweet x Prabhat	35.87a	1.60a	19.79ab	0.65a
LSD $_{0.05}$	0.76	0.03	0.60	0.06

*Values with the same letters are not significantly different according to Fisher's LSD test at 5% level of significance.

Table 3. Leaf characters and presence of nectaries in hybrid seedlings

Parents/Crosses	Leaf shape	Leaf arrangement	Leaf margins	Nectaries
Shan-i-Punjab x Florda Prince	LAN*	ALT	SS	AB
Shan-i-Punjab x Flordaglo	LAN	ALT	SS	AB
Shan-i-Punjab x Prabhat	LAN	ALT	SS	AB
Tropic Sweet x Florda Prince	LAN	ALT	SDS	AB
Tropic Sweet x Flordaglo	LAN	ALT	SS	AB
Tropic Sweet x Prabhat	LAN	ALT	SS	AB

*LAN= Lanceolate, ALT= Alternate, AB= Absent, SS= Shallow Serrate, SDS=Shallow and Deep Serrate

leaf arrangement. These results are consistent with the findings of Chalak *et al.* (2006), who reported a lanceolate leaf shape for all peach accessions. Similarly, no notable differences were observed in leaf margins among the hybrids. Shallow serrate leaf margins were observed in all hybrids, except for Tropic Sweet x Florda Prince, where both shallow and deep serrate leaf margins were observed. Furthermore, nectaries were absent in all hybrids.

The results of the present study indicate that using Shan-i-Punjab as the female parent in crosses results in higher fruit set and fruit drop rates, with relatively low fruit retention. In contrast, when Tropic Sweet is used as the female parent, crosses display significantly higher fruit retention and lower fruit drop percentages. Furthermore, Tropic Sweet crosses demonstrate a higher seed germination percentage of F1 seeds, likely due to their longer growth period compared to Shan-i-Punjab.

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Influence of 4-CPA and GA_3 on physiological, biochemical and yield attributes of tomato under high-temperature conditions

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Abstract

The present research investigated the impact of plant growth regulators in mitigating the effects of heat stress in tomato (*Solanum lycopersicum* L.) genotype LST-6 and cultivar Punjab Varkha Bahar-4. In north India, the temperature in the summer season ranges between 25-45 ºC and temperature above 28 ºC leads to heat stress in plants and negatively affects the reproductive stage of plants. Considering this, we subjected the plants to varying concentrations of GA_3 (10, 20, 30 µg/mL) and 4-CPA (15, 45, 75 µg/mL). GA_3 application took place three weeks after transplanting, while 4-CPA was administered during the anthesis stage. We recorded observations from both control and treated plants, with a 10-day gap between each spray treatment. The application of plant growth regulators (PGRs) enhanced the plants' ability to withstand high temperatures by improving photosynthetic efficiency, as evidenced by increased chlorophyll and carotenoid levels in the leaves. The level of different biochemical constituents (total protein, starch, total soluble sugars, phenol and proline content) also increased in PGRs treated plants. Application of GA_3 and 4-CPA also enhanced the membrane thermostability and reduced lipid peroxidation. The PGRs treated plants exhibited increased plant height, leaf area, pollen viability, fruit set, number of fruits per plant and fruit weight, ultimately improving yield. GA_3 and 4-CPA application also increased the total soluble solids, lycopene content and titratable acidity in tomato fruits. Thus, overall improvement was observed with the application of PGRs; however, 75µg /mL 4-CPA was most effective in imparting thermo tolerance.

Key words: *Solanum lycopersicum*, heat stress, GA_3, 4-chlorophenoxyacetic acid (4-CPA), thermotolerance, yield

Introduction

Tomato, member of family Solanaceae, is an important crop in India because its production ranks second globally. About 11% of the world's total tomato crop comes from India (Pavani *et al.,* 2020). It is grown as a vegetable crop and a cash crop. Temperatures beyond the ideal threshold cause heat stress in tomato plants, which prefer temperatures between 20 and 28 °C for growth. Heat stress is caused by the summer season temperature range of 25-45 °C in north India. One of the main abiotic factors that affect tomato plants' output is heat stress, which has a negative impact on physiological and biochemical processes (Abdalla *et al.,* 2020). Heat stress slows down plant development, leaf area, biomass, production of flower buds, fruit formation *etc.* The reproductive stage of plant is more vulnerable to heat stress than vegetative stage (Carmody *et al.,* 2020). The high-temperature stress mainly reduces the yield by reducing pollen viability, fruit set (%) and fruit size. The main non-reproductive processes negatively impacted by high temperatures include photosynthesis, and enzyme activity, especially those engaged in food metabolism, cell membrane stability, *etc.* (Bita and Gerats, 2013).

The primary impact of heat stress on photosynthesis is the inhibition of crucial enzymatic activity, disruption of lipid chains, and elevation of electrolyte loss. The reduction in plant reserves resulting from the limitation of photosynthesis leads to a decline in the sweetness of fruits and vegetables. High temperature also reduces nutrient uptake, negatively affecting biochemical processes (Giri *et al.,* 2017). Heat stress leads to oxidative stress, which results in the formation of reactive oxygen species (ROS) like O_2^-, OH^- *etc.* ROS denatures the proteins, breaks lipid chain (which increases membrane fluidity) and breaks the strands of DNA, ultimately resulting in cell death. To counteract the effects of high temperatures, plants have developed a variety of defenses, including the production of heat shock proteins and antioxidant enzymes, as well as the closing of stomata to limit water loss (Das and Roychoudhury, 2014). The antioxidant enzymes such as glutathione reductase, peroxidase, and superoxide dismutase assist plants in reducing oxidative stress. Ahmed *et al.* (2021) claimed that some PGRs can be useful for conferring thermotolerance to plants. Auxins at low concentrations reduce fruit abscission by promoting tissue attachment by enhancing differentiation and development of vascular bundles. Auxins also increase flowering, cell division *etc.* (Pramanik *et al.,* 2018). Also, GA_3 application promotes stem elongation, promote flowering, increases fruit set %, enhances fruit weight, *etc.* (Ahmed *et al.,* 2022). However, there is a dearth of thermo-tolerant varieties in tomato. It becomes very difficult for farmers to fulfil market demand for tomato during the summer when the temperature reaches 40-45°C, especially in North Indian plains. Considering the information mentioned above, employing plant growth regulators (PGRs) to safeguard tomato plants against the adverse effects of heat stress is plausible. Therefore, the current study was conducted to examine the effects of 4-CPA and GA_3 treatments on the tolerance of tomato plants to temperature stress and, consequently, their overall yield.

Materials and methods

Plant material, growth conditions and application of plant growth regulators (4-CPA and GA_3): The crop was exposed to heat stress under natural conditions when the temperature raised upto 36 °C (Table-1). This experiment was conducted on tomato genotype LST-6 and compared with a heat-tolerant cultivar Punjab Varkha Bahar-4 (PVB-4), at Vegetable Research Farm and laboratories of the Department of Botany, Punjab Agricultural University, Ludhiana. For the experiment, sowing was done in first fortnight of January 2021 and transplanting was done during last week of February 2021. All the field management practices were followed according to the Package of Practices for cultivation of Vegetables, Punjab Agricultural University, Ludhiana. The foliar application of GA_3 (10, 20 and 30 µg/mL) was done after 3 weeks of transplanting *i.e.* at 21 DAT and 4-CPA (15, 45 and 75 µg/mL was done at anthesis stage *i.e.* at 24 DAT. There were 3 replications per treatment and the treatments were arranged in randomized block design. Observations were recorded at an interval of 10 days after spray treatment and the data presented in tables depict the mean values. The data was analysed using SAS software.

Table 1. Temperature and relative humidity from January to June (2021)

Month (2021)	Temperature (˚C)		Relative Humidity(%)	
	Maximum	Minimum	Morning	Evening
January	16.9	7.1	94	65
February	23.8	10.2	93	54
March	29.5	14.9	82	37
April	34.2	16.9	59	20
May	36.3	22.6	57	32
June	36.3	25.3	68	42

Determination of morpho-physiological parameters: Morpho-physiological parameters included plant height, leaf area, membrane thermo stability, total chlorophyll, and carotenoid content. Plant height, leaf length and breadth were measured using scale. Membrane thermo stability was measured by dipping the third leaf of plant in 15 mL distilled water and conductivity was measured after 20 h. Then samples were boiled for 20 minutes and then again conductivity was measured after cooling.

Membrane thermo stability = 100 - electrolyte leakage (%), where

$$\text{Electrolyte leakage} = \frac{\text{CAB-CBB}}{\text{CAB}} \times 100$$

CAB=Conductivity after boiling, CBB=Conductivity before boiling

Leaf chlorophyll and carotenoid content were measured by dipping 0.05g of fresh leaf sample in DMSO followed by heating in incubator at 65 °C for 4 h. After cooling, the absorbance was read at 480 nm, 645 nm and 665 nm. The total chlorophyll and carotenoid content was calculated using following equations
Total Chlorophyll content: $[20.2 \times A_{645} + 8.02 \times A_{665}]$

Total Carotenoid content: $(A_{480}) + 0.114 (A_{665}) - 0.638 (A_{645})$

Determination of biochemical parameters: It included total proteins, total phenols, total soluble sugars, starch, proline content and lipid peroxidation in leaves. Procedure given by Lowry *et al.* (1951) was followed to measure total protein content and was expressed in mg/g dw. Phenol content was determined by following the method given by Swain and Hills (1959) and absorbance was recorded at 630 nm. Total soluble sugars content was measured in mg /g by following the method given by Dubois *et al.* (1956). Starch content was estimated according to the procedure given by McCready *et al.* (1985) and was expressed in mg /g dw. Proline content (mg /g FW) was estimated according to Bates *et al.* (1973) and Lipid peroxidation was measured as malondialdehyde (MDA content) by following the method described by Dhindsa and Matowe (1981).

Determination of quality and yield attributes: Number of fruits per plant was calculated by adding all the number of fruits harvested in entire season from a plant. For pollen viability, anthers were crushed to get pollen grains and then a drop of aceto-carmine stain was added to pollen grains and then slide was observed under microscope. Only darkly stained pollens were considered as viable. Total soluble solids were measured by placing few drops of tomato juice on hand reflectometer. Lycopene content was measured by mixing 2 g of tomato puree with a solution containing hexane, acetone and ethanol in ratio 2:1:1 followed by addition of 0.05% (w/v) butylatedhydroxytoluene. After 15 minutes, distilled water was added and the setup was left undisturbed for the separation of polar and non -polar layer. Hexane layer was used to measure absorbance at 530 nm.

Lycopene (mg/kg) = A503 * 171.7/W

Where, W = Exact weight of tomato added, in grams

Titratable acidity was measured by titrating 10g of tomato puree (diluted with 50 mL deionised water) with 0.1N NaOH to pH8.

$$\text{Titratable acidity} = \frac{\text{NaOH (mL) used x 014878}}{\text{weight of aliqout}} \times 100$$

For calculating fruit set %, 10 flowers in each replication of each treatment were tagged and there fruits were harvested and its percentage was calculated accordingly. Average fruit weight of harvested fruits was measured in grams. Total yield per plant was measured by adding the total weight of fruits harvested form single plant and it was expressed as g/plant.

Results

Effect of plant growth regulators on morpho-physiological traits: Application of growth regulators (4-CPA and GA_3) positively affected various morpho-physiological parameters in genotype LST-6 as well as cultivar PVB-4. 75 µg/mL 4-CPA and

Table 2. Effect of plant growth regulators on morpho-physiological parameters in Punjab Varkha Bahar-4

Treatment	Plant height (cm)	Leaf area (cm^2)	Membrane thermostability in leaves (%)	Chlorophyll content(mg/gm FW)	Carotenoid content(mg/gm FW)
Conc. of GA_3					
10 µg/mL	56.75^{a}	3.75^{jih}	82^{bac}	2.06^{def}	0.12^{ab}
20 µg/mL	61.96^{a}	4.80^{gdfce}	85^{bac}	2.13^{de}	0.13^{ab}
30 µg/mL	66.53^{a}	6.88^{b}	91.8^{a}	3.23^{b}	0.15^{ab}
Conc. of 4-CPA					
15 µg/mL	61.66^{a}	4.73^{gdfceh}	84.4^{bac}	2.04^{dgef}	0.13^{ab}
45 µg/mL	62.13^{a}	5.55^{dc}	88.2^{ba}	2.33^{d}	0.14^{ab}
75 µg/mL	68.05^{a}	7.15^{a}	93.8^{a}	3.82^{a}	0.16^{a}
Control	55.08^{a}	3.03^{jlk}	75.8^{bdac}	1.46^{gh}	0.11^{b}

Values represented by same letters are not significantly different as per Tukey's test ($P<0.001$). Values are the mean values of different stages

Table 3. Effect of plant growth regulators on morpho-physiological parameters in LST-6

Treatments	Plant height (cm)	Leaf area (cm^2)	Membrane Thermostability in leaves (%)	Chlorophyll Content(mg/gm FW)	Carotenoid content(mg/gm FW)
Conc.of GA_3 (µg/mL)					
10	49.86^{a}	3.96^{gjfih}	63.0^{dc}	1.67^{gef}	0.11^{b}
20	50.29^{a}	4.97^{dfce}	70.2^{bdac}	1.83^{dgef}	0.12^{ab}
30	62.17^{a}	5.16^{dce}	76.6^{bdac}	1.93^{dgef}	0.14^{ab}
Conc. of 4-CPA (µg/mL)					
15	56.26^{a}	3.83^{gjih}	67.6^{bdc}	1.72^{gef}	0.12^{ab}
45	58.19^{a}	4.04^{gjfieh}	73.4^{bdac}	1.83^{dgef}	0.13^{ab}
75	62.90^{a}	5.51^{dc}	82.6^{bdac}	2.27de	0.15^{ab}
Control	56.29^{a}	2.28^{l}	59.4^{d}	0.93^{h}	0.10^{b}

Values represented by same letters are not significantly different as per Tukey's test ($P<0.001$). Values are the mean values of different stages

30 µg/mL GA_3 application were most effective (Table 2 and 3). The maximum mean plant height and leaf area was observed in 75 µg/mL 4-CPA treated LST-6 plants and it was 7.56 % and 27.97 % more than in PVB-4 plants. The increase in plant height and leaf area may be attributed to the role of auxins and gibberellins in cell division and cell elongation.

According to Bhattarai *et al.* (2022), application of GA_3 promotes cell division and enlargement in sub-apical meristem. Foliar application of gibberellins leads to taller plants as it stimulates the cell elongation. Along with this it also increases cell permeability which enhances the flow of water and soluble substances within the cell, thus it increases the cell size. Similar results of increase in plant height and leaf area with the application of auxins and GA_3 were stated by Ahmed *et al.* (2022) in tomato. Application of PGRs helped in reducing the electrolyte leakage by increasing the stability of cell membranes in leaves. In LST-6, the recorded maximum mean membrane stability was 93.8 % (75 µg/mL 4-CPA) followed by 91.8 % (30 µg/mL GA_3). While in PVB-4 it was 82.6 % (75 µg/mL 4-CPA) and 76.6 % (30 µg/mL GA_3). Increased membrane stability in PGR treated sunflower plants has been reported by Zayed *et al.* (2017). The application of PGRs also increased chlorophyll, carotenoid, and photosynthetic efficiency in leaves. The leaves of LST-6 possessed higher chlorophyll (3.82 mg/g FW) and carotenoid content (0.16 mg/g FW) in leaves (which ultimately also increased the photosynthetic efficiency in leaves) as compared to PVB-4. The role of auxins in increasing photosynthetic efficiency by increasing the activity of enzymes involved in dark reactions is well established (Khatoon *et al.,* 2020). According to Guo *et al.* (2022) application of GA_3 reduced electrolyte leakage and enhanced the synthesis of antioxidants in chloroplasts which helped in the scavenging of ROS and reduced oxidative damage.

Effect of plant growth regulators on biochemical parameters: Foliar application of PGRs resulted in increased biochemical constituents in leaves *viz.,* total soluble sugars, total starch, total soluble proteins, total phenols and proline content. Among the different concentration of GA_3 and 4-CPA, 30µg/mL GA_3 and 75µg/mL 4-CPA was most effective in all aspects. The genotype LST-6 possessed maximum mean total soluble sugars (96.93 mg/g dw), total soluble proteins (15.20 mg/g dw), total phenol (3.14 mg/g dw), and proline content (0.92 mg/g dw) in leaves with the treatment of 75 µg/mL 4-CPA. However, the maximum mean total starch (233.37 mg/g dw) was observed in 75 µg/mL 4-CPA-treated plants of PVB-4. The application of PGRs reduced the lipid peroxidation in leaves, which was measured in terms of MDA content, thereby stabilizing the membrane. The recorded lipid peroxidation in leaves with the treatment of 75 µg/mL 4-CPA in LST-6 was 0.3 nmol/g FW and in PVB-4 was 0.48 nmol/g FW.

The results obtained in our study are concomitant with those obtained by Muthulakshmi and Pandiyarajan (2013), who also reported an increase in total soluble sugars in *Catharanthusroseus,* following the application of PGRs. An increase in protein content in the leaves of mung bean has been reported with the application of auxins and GA_3 (Islam *et al.,* 2021). The increase in total soluble proteins, total soluble sugars and starch content may be attributed to increased metabolic activity with the application of PGRs. According to Sharma *et al.* (2019) phenols play key role in cell division, hormonal regulation, photosynthesis and nutrient assimilation. Under abiotic stress conditions, the concentration of polyphenols increased, which helped the plant cope with stressful conditions and the application of PGRs also enhanced phenol content in marigold (Sardoei and Shahdadneghad, 2014). The increase in proline content with the application of GA_3 and auxins in tomato and garden peas was observed by Guo *et al.* (2022) and Sergiev *et al.* (2018). According to Harsh *et al.* (2016), plants tend to accumulate more proline content which helps to stabilize enzymes, maintain membrane integrity and scavenge ROS under abiotic stress conditions. Application of PGRs increased the membrane stability and antioxidant activity and reduced the lipid peroxidation in *Phoenix dactylifera,* as reported by Khan *et al.* (2020)

Effect of plant growth regulators on yield and quality attributes: Foliar application of plant growth regulators (PGRs) significantly impacted the yield and quality attributes of LST-6 and PVB-4 tomato cultivars, as demonstrated in Tables 4 and 5. The application of 30 µg/mL gibberellic acid (GA_3) and 75 µg/mL 4-chlorophenoxyacetic acid (4-CPA) resulted in comparable outcomes in terms of yield and quality attributes for both cultivars.

The maximum mean number of fruits per plant was observed in LST-6 when treated with 75 µg/mL 4-CPA. However, PVB-4 displayed superior performance in several key attributes when treated with 30 µg/mL GA_3 and 75 µg/mL 4-CPA. Specifically, PVB-4 exhibited the maximum mean pollen viability, total soluble solids, lycopene content, titratable acidity, fruit set percentage, fruit weight, and overall yield with the application of 30 µg/mL GA_3 and 75 µg/mL 4-CPA.

In the case of LST-6, applying 30 µg/mL GA_3 and 75 µg/mL 4-CPA also led to notable improvements in various quality attributes. However, the values obtained for LST-6 were statistically comparable to those observed in PVB-4, indicating that both cultivars responded similarly to the PGR application.

The increased number of fruits, fruit set %, fruit weight and yield with GA_3 treatment could be due to enhanced assimilate accumulation in treated plants (Ujjwal *et al.,* 2018). The application of auxins is also known to reduce the abscission of flowers and fruits as it supresses the ABA signalling pathway (main cause for senescence) in tomato. Dalai *et al. (*2015) also reported that applying GA_3 and auxins in cucumber improved

Table 4. Effect of plant growth regulators on yield and quality attributes in Punjab Varkha Bahar-4

Treatment	No. of fruits per plant	Pollen viability(%)	Total soluble solids (˚brix)	Lycopene content (mg/100g)	Titratable acidity%	Fruit set %	Fruit weight (g)	Yield (g/plant)
Conc.of GA3 (µg/mL)								
10	17ij	74.51ji	3.88a	3.48b	0.52ebdacf	41.4gh	59g	1010gfieh
20	19ih	80.42gefd	3.85a	3.78b	0.55bdac	53.33e	61ef	1160gfdeh
30	24ced	83.64cbd	3.94a	3.76b	0.57ba	62.66cb	62ed	1500bdac
Conc. of 4-CPA (µg/mL)								
15	20hg	81.55cefd	3.90a	3.61b	0.53ebdac	63.33cb	57h	1150gfdeh
45	22feg	82.92cebd	3.92a	3.74b	0.56bac	65.00b	60gf	1330bdec
75	26cb	84.05cb	3.98a	3.81b	0.63a	70a	64cb	1670ba
Control	15kj	73.13ji	3.90a	3.41b	0.50ebdagcf	35j	54i	820kijh

Values represented by same letters are not significantly different as per Tukey's test (P<0.001). Values are the mean values of different stages of development.

Table 5. Effect of plant growth regulators on yield and quality attributes in LST-6

Treatment	No. of fruits per plant	Pollen viability (%)	Total soluble solids (˚brix)	Lycopene content (mg/100g)	Titratable acidity%	Fruit set %	Fruit weight (g)	Yield (g/plant)
Conc.of GA3 (µg/mL)								
10	13kl	76.58jih	3.36a	3.29b	0.40ebdgcf	39.11ih	45mn	590kj
20	16j	98.68a	3.46a	3.34b	0.42ebdgcf	44.42gf	45.98mL	740kij
30	26cb	48.42m	3.5a	3.65b	0.48ebdagcf	58.79d	47.58k	1280fdec
Conc. of 4-CPA (µg/mL)								
15	20.66fhg	52.18l	3.3a	3.5b	0.46ebdagcf	37.49ji	46.89kl	960gfih
45	25.41cbd	56.27k	3.26a	3.24b	0.44ebdagcf	50.5e	47kl	1040gfieh
75	29.71a	77.53gih	3.54a	3.33b	0.51ebdgcf	62.67cb	49.3j	1460bdac
Control	12l	59.52k	3.2a	3.11b	0.38ebdgcf	22.62k	44a	530k

plant metabolic activity, positively influencing the reproductive state. Thus, all these factors can be considered as major reasons for the increased number of fruits per plant, fruit set, fruit weight and yield in tomato plants with the application of GA_3 and 4-CPA. According to Gelmesa *et al. (*2013) application of GA_3 and auxins resulted in an increased rate of assimilate export from leaves, fruit carbon metabolism, and increased protein, carbohydrate, potassium, sugar, starch, phosphorous and organic acids in tissue because of increased activities within kerb cycle and all this ultimately resulted in increased total soluble solids, titratable acidity (%) and lycopene content in tomato. Ali *et al.* (2022) also reported increased total soluble solids by applying GA_3 and 4- CPA.

The application of PGRs helped confer tolerance to tomato plants against elevated temperature mainly by increasing membrane stability, proline content, total phenols, improving photosynthetic efficiency, and reducing lipid peroxidation. PGRs also increased the plant height, leaf area, total soluble sugars, total soluble proteins, pollen viability, fruit set %, fruit weight, total soluble solids, titratable acidity, and lycopene content, ultimately the yield per plant. Both the varieties *i.e.,* LST-6 and PVB-4 exhibited almost similar effects of plant growth regulators at different concentrations. While LST-6 yielded less than PVB-4 in control plants, the outcomes were statistically comparable with the use of plant growth regulators. There was a notable increase of 63.69% in LST-6 yield and 50.8% in PVB-4 yield compared to their respective control groups. The application of 75 µg/mL 4-CPA followed by 30 µg/mL GA_3 was found to be the most effective method for enhancing thermotolerance.

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Distribution of nutrients and their indexing in major mango-supporting soils of different agro-climatic zones of Karnataka and its impact on yield

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Abstract

Knowledge of the spatial distribution of soil nutrients is essential for site-specific nutrient management, which forms an effective strategy in precision agriculture. As mango is one of southern Karnataka's most important horticultural crops, the present study was conducted on 108 mango orchards under different agro-climatic zones to assess spatial nutrient variability for nutrient management. The soils of the study areas were acidic and non-saline. Nitrogen (N) deficiency was found in 84.26 percent area, whereas phosphorus (P) and potassium (K) contents were medium in most soils. Sulphur was sufficient, and calcium and magnesium contents varied with agro-climatic zones. The micronutrient (Zn, Fe, Mn) status was sufficient, except for copper and boron. The nutrient index for nitrogen was low, while phosphorus and potassium were low to medium. It is inferred that agro-management should include proper nitrogen fertilization, FYM, and boron throughout the growing cycle for better yield and quality.

Key words: Mango-supporting soils, spatial distribution of nutrients, nutrient index, nutrient management

Introduction

The spatial and temporal variation of surface soil nutrients affects crop yield, land use management and the environment (Zhang *et al.,* 2014). Accurate estimation and proper knowledge of factors impacting the distribution of soil nutrients are quite important for soil and crop management and economic return. Soil fertility is the inherent character that signifies the availability of nutrient elements for plant growth (Sarkar *et al.,* 2002; Dinesh *et al.,* 2020). Landscape diversity, topographic features, and diverse climatic variability affect nutrient variability (Menezes-de-Souza *et al.,* 2006). Crop productivity was low in large areas due to poor water availability and multi-nutrient deficiencies in semi-arid conditions.

The mango tree originates from the Indo-Burma region and thrives across tropical, sub-tropical, and semi-arid zones with diverse soil types (Rajan, 2012). Among Indian states, Karnataka ranks as the third-largest mango producer. Notably, the eastern dry zone (EDZ) and southern dry zone (SDZ) within southern Karnataka play a pivotal role in mango production, forming the prominent mango cultivation area of the state. Due to its intensive nutrient requirements, mango orchards deplete substantial amounts of nutrients, leading to a phenomenon known as nutrient mining. Focus on micronutrient deficiency is even more important than the primary nutrients, as these are essential for the quality of nutrients, plant metabolism, and physiology (Durán *et al.,* 2004). For sustaining yield potential of mango, knowledge of spatial nutrient distribution with its proper management is essentially required, but limited research effort was made earlier to identify the spatial extent of deficiency of major, secondary and micronutrients for this SAT climate. In the present study, farm-level information from 108 orchards around major mango-growing taluks was done with the objectives: (i) to assess the status of soil pH, EC, OC, available primary, secondary and micronutrients and factors affecting its availability and (ii) to study the spatial variability with critical nutrient deficiency sites with specific nutrient management.

Material and methods

Study area: Major mango-supporting soils of southern Karnataka were chosen based on proportional contribution to total area, production and productivity along with traditional mango-growing belts, potential and new orchards areas. Our study comprised fifteen major mango-growing taluks under six major agro-climatic zones of southern Karnataka with various soil types under diverse climatic settings (Table 1). From the extreme west, Sorab, under the hilly zone (Fig. 1) to Srinivasapura, under the Eastern dry zone, covers around 400 km of southern peninsular plateau. Elevations varied from 597 to 936 m with varied landform characteristics. Climate varied from sub-humid tropics in the HZ to semi-arid tropics in the EDZ. SDZ could be seen with varied rainfall of 691.1 to 1459.3 mm and PET of 1318.24 to 1887.58 mm. The soils having their parental legacy with archaean granite and gneissic and their mineral make-up, along with varied mineralogy, greatly influence and impact the availability of essential plant nutrients.

Sampling and soil analysis: A total of 108 composite surface soil samples (0-15 cm) were collected from different representative mango orchards from each taluk. Four samples were collected from each orchard using the grab sampler and scoop method and mixed to get the representative composite sample. Collected

Table 1. Climatic and landforms information of major mango-supporting taluks

ACZ	Taluks	Landforms	Elevation (m above MSL)	Rainfall (mm)	Temperature (°C)	Potential Evapo transpiration (mm)	Length of dry Period (days)	Texture of soils	Soil sub-group
HZ	Sorab	Hilly	597	1459.3	25.27	1318.24	166	sc	Rhodic Kandiustalfs
	Tarikere	Hilly	725	928.9	24.56	1408.18	150	c	Rhodic Paleustalfs
NTZ	Channagiri	Plateau	647	808.4	25.00	1509.16	160	scl	Typic Rhodustalfs
STZ	Hunsur	Plateau	792	833.9	23.41	1453.96	133	sc	Typic Rhodustalfs
CDZ	Holalkere	Upland	794	691.1	26.94	1551.80	172	sc	Typic Rhodustalfs
SDZ	Magadi	Midland	925	913.0	24.73	1484.26	135	sl	Dystric Haplustepts
	Ramanagara	Midland	749	899.4	25.31	1568.52	140	c	AquerticHaplustalfs
	Nagamangala	Upland	841	797.0	24.84	1472.53	135	sl	Typic Haplustepts
EDZ	Gubbi	Upland	877	812.2	24.34	1522.80	151	scl	KanhaplicRhodustults
	Tumkur	Upland	812	921.9	24.30	1513.70	147	c	Rhodic Kandiustults
	Chintamani	Upland	859	734.1	24.87	1617.28	160	c	Rhodic Kandiustalfs
	Srivasapura	Upland	837	759.4	24.87	1571.26	140	scl	Kandic Paleustalfs
	Mulabaghilu	Midland	802	812.8	24.87	1399.76	130	sc	Aquic Haplustalfs
	Hoskote	Plateau	906	808.4	24.87	1513.66	143	c	Kandic Paleustalfs
	BangaloreNorth	Upland	936	963.1	25.00	1887.58	145	sc	Rhodic Kandiustults

HZ- Hilly zone; STZ-Southern Transition zone; CDZ-Central Dry Zone; SDZ-Southern Dry Zone; EDZ-Eastern Dry Zone

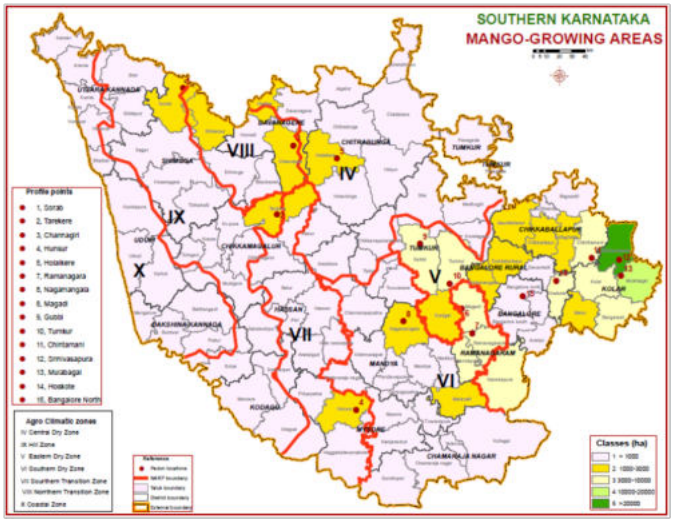

Fig.1 Major mango-growing areas and concentration of orchards of southern Karnataka

samples were dried at room temperature and sieved through 2 mm sieve. Soil pH was measured using pH meter by inserting in the supernatant of 1:2.5 soil into water. The standard dichromate oxidation method determined soil organic carbon content (Walkley and Black, 1934). The alkaline potassium permanganate method assessed available nitrogen using the Kjeldahl apparatus (Subbiah and Asija, 1956). Phosphorus was determined by Olsen's method (Olsen and Sommers,1982; Kumar and Maiti, 2015) using UV–visible spectrophotometer. Available K, Ca and Mg were determined by extraction with neutral normal ammonium acetate and the filtered extract was estimated using atomic adsorption spectrophotometer (AAS) (Page *et al.,* 1982). Soils were extracted with $CaCl_2$ to determine the available S, which was measured by a spectrophotometer at 420 nm (Black, 1965). Cationic micronutrients Fe, Mn, Cu and Zn were determined using AAS by extracting the soils with DTPA extractant (Lindsay and Norvell, 1978). Hot water soluble B was extracted with the method described by Gupta (1967).

Statistical analysis and calculation of nutrient index: Nutrient index of each agro-climatic zone was determined to compare fertility with soil, as per the procedure introduced by Parker *et al.* (1951). Test results of the composite samples for pH, OC, available N, P_2O_5 and K_2O were presented as box plots with standard nutrient ratings (Fig.3). After rating, the composite sample's nutrient index was calculated as per following equation and classified into low, medium and high according to the nutrient index categories (Abah and Petja, 2015; Parker *et al.,* 1951).

$$\text{Nutrient index} = \frac{\begin{array}{c}(1 \times \text{number of samples rated low}) + \\ (2 \times \text{number of samples rated medium} + \\ (3 \times \text{number of sample rated high})\end{array}}{\text{Total number of samples}} \quad (1)$$

Multiple correlation analyses along with descriptive statistics were done using SPSS version 20. Principal component analysis (PCA) was done using R software, where principal soil nutrient properties were displayed by biplot analysis. Principal nutrient variables were selected based on component loading values >0.8 and correlated with the yield of specific taluks to make a proper nutrient management plan for the selected sites.

Results and discussion

Spatial variability measured soil properties: The soils were extremely acid (pH 4.02) to slightly alkaline (pH 7.78) (Table 2). Orchard soils of Sorab from HZ, Gubbi, Tumkur from EDZ were strongly acid in reaction, whereas soils of Holalkere, Magadi and Nagamangala from SDZ were slightly alkaline (Table 3).

The electrical conductivity of soils varied from 0.02 to 0.47 dSm^{-1} (mean 0.07 dS m^{-1}). Organic carbon in the surface ranged from low (0.27 %) to high (1.33 %), with a mean of 0.84 percent. The surface soils had relatively high organic matter content except in Nagamangala and Mulabaghilu (Table 3). Our study supports the earlier findings of a wide range of pH, EC and OM content, which might be ascribed to varied soils and climatic diversity (Reddy *et al.,* 1996; Satyavathi and Reddy, 2004; Shukla *et al.,* 2018).

In all the orchards, N content was low with a mean value of 220.11 kg ha^{-1} (Table 2), whereas P content was high and showed wide variation (mean 72.20 kg ha^{-1}), but deficiency of P was found in Sorab, Holalkere and Magadi (Table 3). The higher available P in a few soil samples might be due to application of a large quantity

Table 2. Descriptive statistics of the area

	Minimum	Maximum	Mean	Median	Std. deviatiom	CV (%)	Skewness
pH	4.02	7.78					
EC (dS m^{-1})	0.02	0.47	0.07	0.05	0.07	100.00	2.98
OC (%)	0.27	1.33	0.84	0.85	0.20	23.81	-0.06
N (kg ha^{-1})	109.76	369.34	220.11	225.79	57.54	26.14	0.02
P (kg ha^{-1})	2.39	303.01	72.20	60.95	60.05	83.17	1.88
K (kg ha^{-1})	40.32	490.56	158.63	145.60	1.44	0.91	1.44
S (mg kg^{-1}	2.57	121.90	42.29	30.12	30.12	71.22	0.95
Ca (mg kg^{-1})	57.05	2521.75	404.39	282.00	344.98	85.31	2.79
Mg (mg kg^{-1})	8.80	271.30	74.86	68.30	49.98	66.76	1.35
Cu (mg kg^{-1})	0.36	5.60	1.95	1.66	1.09	55.90	1.01
Fe (mg kg^{-1})	4.42	221.76	33.22	22.62	31.41	94.55	3.09
Mn (mg kg^{-1})	3.12	124.52	35.54	29.42	20.98	59.03	1.79
Zn (mg kg^{-1})	0.26	4.84	0.99	0.73	0.81	81.82	2.29
B (mg kg^{-1})	0.19	1.13	0.59	0.56	0.23	38.98	0.42

of phosphatic fertilisers. Available potassium was medium in the area (mean 158.63 kg ha^{-1}), with the highest content in Holalkere taluk (Table 3). Calcium was found to be sufficient in soils with an average of 404.39 mg kg^{-1}, but magnesium was deficient, with a mean of 74.86 mg kg^{-1}. In the Eastern dry zone, both Ca and Mg were poor in surface soils. Sulphur was sufficient in the entire area, averaging 42.29 mg kg^{-1}. Average values of available Fe, Mn, Cu, Zn and B were 33.22, 35.54, 1.95, 0.99, and 0.59 mg $kg^{-1,}$ respectively. The deficiency of Cu in some sites might have been aggravated due to the complexation of Cu in the soils, having relatively higher organic carbon. The spatial diversity of cationic micronutrients supports the findings of Shukla *et al.* (2016). Soil properties exhibited low (only for available K_2O) to medium variability, with 100 percent of CV values indicating low, moderate and high degree variability, respectively (Nielsen and Bouma, 1985). Moderate variability of micronutrients supports the study of Shukla *et al.* (2016) of India's Trans-Gangetic Plain and Shivalik Himalayan region. Higher spatial variability of cationic micronutrient content was due to the diversity of weathering regimes and pedogenic processes (Bowen 1979;). Comparatively low soil pH variability and OC status and primary nutrients status are seen in the study areas.

Relationships among soil properties and yield: The correlation between the soil properties and available plant nutrients is shown in Table 4. Soil pH and EC significantly correlate with the availability of plant nutrients. Significant positive relationships (P<0.01) with K, S, Ca, Mg and Zn have been observed in strongly acid soils of Gubbi and Tumkur taluks, as these nutrients were deficient in these soils. A significant negative correlation of pH with P_2O_5, Fe and Mn supports the observations of Shukla *et al. (*2018) in the semi-arid Deccan plateau, which showed reduced solubility of Fe, Mn and Cu with increased alkalinity. Electrical conductivity showed significant positive relation (P<0.01) with K_2O, S and Zn due to the high solubility product of these nutrients. N's availability in surface soils was highly dependent on OC content in soils, as a highly significant correlation between N and organic carbon with a value of r=0.55 (P<0.01) was observed. The deficiency of N in the overall area might be the effect of the medium to low OC content of these semi-arid tropical areas. Organic carbon, the key component of soil organic matter, influences availability of primary and secondary and micronutrients (Tisdale *et al.,* 1985). There was a positive interaction with K_2O, S and Zn found for the mango orchards. Positive correlation among the cationic micronutrients, Cu vs. Fe, Cu vs Mn, and Fe vs Mn (P<0.01) indicates similar sets

Table 3. Spatial distribution of average primary, secondary and micronutrient content in major mango growing soils of Southern Karnataka

Taluks	pH	EC	OC	N	P_2O_5	K_2O	Ca	Mg	S	Fe	Mn	Cu	Zn	B
		dS m-1	%	Kg ha^{-1}			mg kg^{-1}							
Sorab	5.35cdef	0.04cd	1.11a	255.7ab	21.5c	101.7c	291.1b	130a	31.7bc	109a	79.6a	3.37a	0.87bc	0.64ab
Tarikere	5.88bcde	0.07bcd	0.84bcd	266.1a	40.5bc	146.7c	473.8ab	110.3ab	38.9bc	46.5b	41.9bc	2.55ab	0.71c	0.64ab
Channagiri	5.13ef	0.04d	0.77bcd	241.3abc	118ab	117.7c	461.3ab	102ab	29.9bc	46.7b	38.0bc	2.21bc	0.47c	0.56abc
Hunsur	5.81bcde	0.05bcd	0.85bc	201.2bcd	64.1abc	125.9c	370ab	100.4ab	54.1bc	34bcd	25.0c	1.22def	0.89bc	0.68ab
Holalkere	6.58ab	0.09bcd	0.73cd	247abc	17.6c	286.7a	723.8a	97.7ab	37.6bc	14.8de	38.8bc	2.4abc	1.37bc	0.44bc
Magadi	6.34bc	0.09bcd	0.89bc	186.9cd	23.1c	176.7bc	608.6a	88.2ab	61.6ab	19.8cde	53.1ab	2.77ab	0.75c	0.64ab
Ramanagara	5.78bcde	0.06bcd	0.74cd	197.8cd	133.5a	137.5c	610.3a	86.1ab	46.6bc	39.1bc	31.8bc	2.14bc	1.07bc	0.75a
Nagamangala	7.17a	0.11bc	0.62d	223.2abcd	66.6abc	236.8ab	522.4ab	76.5ab	67.8ab	14.3de	14.0c	0.99ef	1.39bc	0.48bc
Gubbi	5.19def	0.04d	0.75bcd	200.6cd	75.7abc	134.4c	501ab	69.5ab	22.7bc	22.3cde	24.9c	0.90f	0.52c	0.39c
Tumkur	4.7f	0.04d	0.86bc	185.7cd	106.8abc	141.4c	181.9b	47.5ab	23.5bc	27.8bcde	27.8bc	2.07bcd	0.79bc	0.51bc
Chintamani	5.06ef	0.06bcd	0.87bc	214.8abcd	127.5ab	188.2abc	134.4b	45.3b	26.6bc	17.5cde	28bc	1.4cdef	0.95bc	0.46bc
Srinivasapura	5.84bcde	0.11b	0.98ab	233.1abc	38.2bc	227.7ab	295.5b	40.5b	41.6bc	16.5de	42.2bc	1.95bcde	0.88bc	0.62ab
Mulabagilu	5.89bcde	0.03d	0.77bcd	225.6abc	64.8abc	114.7c	150.2b	39.2b	45bc	43.9bc	14.6c	1.06ef	0.57c	0.62abc
Hoskote	6.01bcd	0.22a	0.93abc	270.5a	63.3abc	157.9bc	320.6ab	26.1b	101.2a	12.8de	31.9bc	2.2bc	2.89a	0.5bc
Bangalore N.	6.02bcd	0.17a	0.84bcd	155.2d	89abc	155.2bc	150.5b	25.5b	5.9c	0.5e	20.9c	1.02ef	2.08ab	0.79a

(Values in column bearing different subscript are significantly different at P=0.05 level)

Table 4. Correlation among fertility parameters

	pH	EC	OC	N	P	K	S	Ca	Mg	Cu	Fe	Mn	Zn	B
pH	1.00													
EC	0.35**	1.00												
OC	0.09	0.17	1.00											
N	0.17	0.19	0.55**	1.00										
P	-0.21*	-0.04	-0.14	-0.10	1.00									
K	0.40**	0.33**	0.00	0.04	-0.02	1.00								
S	0.51**	0.64**	0.10	0.10	-0.16	0.29**	1.00							
Ca	0.41**	0.16	0.08	0.22*	-0.13	-0.01	0.22*	1.00						
Mg	0.33**	0.09	-0.08	0.11	-0.13	-0.05	0.23*	0.80**	1.00					
Cu	-0.08	0.08	0.20	0.18	-0.16	-0.14	-0.03	0.30**	0.39**	1.00				
Fe	-0.36**	-0.24*	0.17	0.21*	0.06	-0.23*	-0.27**	-0.13	-0.05	0.34**	1.00			
Mn	-0.21*	-0.02	0.23	0.07	-0.26**	-0.12	-0.17	0.10	0.18	0.68**	0.48**	1.00		
Zn	0.31**	0.56**	0.14	0.14	0.02	0.27**	0.50**	0.08	-0.02	0.13	-0.12	0.03	1.00	
B	0.04	-0.03	0.12	0.00	0.01	-0.15	0.09	-0.08	-0.04	0.13	0.08	0.07	-0.06	1.00
Yield	-0.04	-0.07	0.06	0.04	-0.17	-0.21*	-0.07	0.08	0.20	0.18	0.35**	0.22*	-0.03	0.02

of factors that influence the distribution of these micronutrients (Behera and Shukla, 2013; Shukla *et al.,* 2018).

Surface soil properties were taken for the principal component analysis (PCA) and presented as a biplot in Fig. 4. PC1 explains 22.8 percent variability in soil properties, showing high loading values with pH, EC and available S. Whereas PC2 explains 18.5 percent variability of soil properties mainly focused on cationic micronutrients with high loading values of Cu, Mn and Fe. Principal Component Analysis aggregated soil fertility properties into components explaining most spatial variabilities. Similar to the findings of Shukla *et al. (*2018), biplot analysis of PC1 and PC2 revealed two prominent groups of soil properties, in which soil reaction constituted one group and micronutrients created another. These fertility components significantly influenced yield limiting characteristics of mango throughout the area (Table 4). Among the principal soil nutrients, Fe and Mn have a significant positive correlation (r=0.35, $P<0.01$ and r=0.22, $P<0.05$) with yield. The availability of micronutrients in red ferruginous soils influences the most towards sustainable mango production in these traditional mango-growing areas.

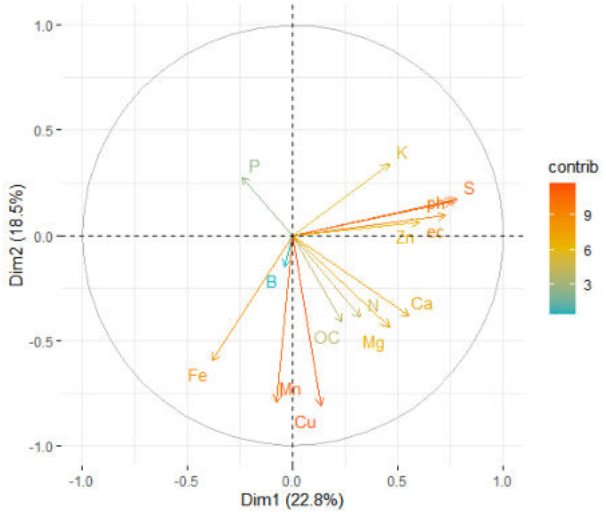

Fig 2. Principal component analysis (PCA) of nutrient parameters. Dim 1 and Dim 2 in biplot represents principal component 1 (PC1) and principal component 2 (PC2), respectively)

Spatial variability in nutrient index: The nutrient index (NI) of the major mango-supporting areas of southern Karnataka was determined through the nutrient rating status of the major taluks, using Eq. 1. Nutrient ratings of the areas were prepared based on the rating chart set by Ravikumar and Somashekar (2013) and Muhr *et al.* (1965). Nutrient indices of the major agro-climatic zones are presented in Fig. 3 and categories with low, medium and high nutrient status were followed by the limits set by Ravikumar and Somashekar (2013). For the entire area, pH was rated low by following the nutrient rating chart, whereas only the central dry zone has a medium index value for pH. Sixty-seven percent of orchard sites were rated low and 33 percent were rated medium for pH. Spatial variation of OC can be found from low to high. The nutrient Index is rated high for all agro-climatic zones for OC, except the southern dry zone. It was found to be low in 3.7 percent sites, medium in 28.7 percent sites and 65.03 percent areas were found to be high in OC. High OC in surface soils is due to litter deposition of perennial mango orchards. Though considerable variation was noticed in the concentration of available N among sampling sites, all the sites were rated low except in CDZ. It was low in 84.26 percent of sites, whereas 15.74 percent sites were medium in N. The variability was high for available P, with 12.96 percent of sites rated low, 28.71 percent of areas rated medium and 58.33 percent areas rated high. EDZ is very much important as per the production of mango. High available P and medium K played a very important role for maintaining mango supply from these mango-growing belts of southern Karnataka. Around 56.48 percent of areas had medium K availability.

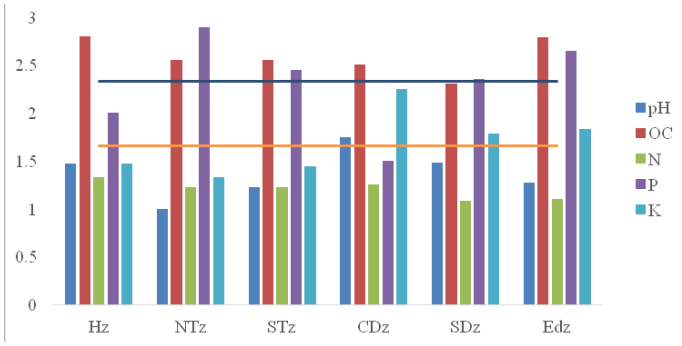

Fig 3. Nutrient index of different agro-climatic zones

Nutrient management: Sustainable crop production needs a proper knowledge of soil and climatic constraints, a proper management technique, and employing contemporary field practices. The productivity potential of soils can be improved by properly understanding soils and their potentials and constraints (Karthika *et al.,* 2022) and, in turn, suitable agro-interventions. The knowledge of variability in soils gives an idea about yield-limiting factors. Acidic soil reaction was one of the major limitations for the sites under Channagiri, Tumkur and Gubbi as the ideal pH for mango production is 5.5 to 7.0 (Sys *et al.,* 1993; Naidu *et al.,* 2006, Singh *et al.,* 2008). As these soils have their genesis from acidic granitic parent material, the surface and sub-surface acidity of some of the sites (dominantly from eastern dry zone) was due to geogenic factors than crop management practices. Application of N fertilizer along with FYM in the package of practices (POP) can tackle this problem. Nitrogen deficiency is a serious problem for the study area. Earlier researchers (Sahrawat *et al.,* 2010; Vasu *et al.,* 2017) also reported N deficiency in soils of similar climatic belts in India.Split doses of nitrogenous fertilizers and proper biomass management may help alleviate the N problem. Cu, Zn and B were deficient in large and hence application of Cu and B through fertigation and/or soil application should be carried out to alleviate the deficiencies.

Nutrient management with proper spatial nutrient distribution knowledge is needed to enhance the productivity of mango raised on varied soils and landforms. Our present investigation showed the climate zone of spatial variability under southern Karnataka. Nitrogen deficiency was the major limiting factor in nearly all the sites, along with copper and boron in large tracts. Integrated nutrient management with a special focus on these nutrients through different sources would enhance mango production and fruit quality.

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Effects of discarded cheese-whey amended substrate on growth and flowering of different snapdragon cultivars (*Antirrhinum majus* L.)

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Abstract

The recycling of organic waste as a feedstock for the plant use emerges to be an eco-friendly process for the production of various fruit, vegetable, and ornamental crops. Cheese-whey has very short shelf life, therefore, in the present study the wasted cheese-whey is used as organic material to observe its effect on ornamental plant. In this study, seven commercial cultivars of snapdragon (Magic Carpet, Antiquity Sunset, Day and Night, Chuckles, Illumination, Madame Butterfly, and Twilight) were planted in pots containing either (1) 1:3:1 ratio of sand, peat-based compost, and perlite substrate (control), or (2) 1:3:1 ratio of sand, peat-based compost, and perlite substrate, which was amended by adding 200 mL per pot cheese-whey. These pots were placed in a glasshouse, under ambient environment. The layout of the experiment was two-factorial completely randomized design with six replicates. All plant growth and flowering parameters were significantly ($P \leq 0.05$) affected by substrates. Snapdragon cultivars grown in cheese-whey amended substrate displayed maximum plant height, number of leaf per plant, leaf area, leaf fresh weight, leaf dry weight, plant fresh weight, plant dry weight, specific leaf weight, and leaf area ratio. Comparing the cultivars, it was observed that the cultivar Day and Night had maximum days to flowering, plant height, number of leaf per plant, leaf area, leaf fresh weight, leaf dry weight, plant fresh weight, plant dry weight, specific leaf weight, and leaf area ratio. The interaction of the both factors indicated that all snapdragon cultivars had significantly promising results when grown in cheese-whey amended substrate compared to control plants.

Key words: Snapdragon, *Antirrhinum majus* L., cheese-whey, substrate, growth, flowering

Introduction

In the process of global economic growth and development, agriculture has played a strategic part (Weatherspoon *et al.,* 2001). However, the rapid increase in agricultural productivity has put more pressure on the environment's natural resources (Pawlak and Kołodziejczak, 2020). The massive amount of waste material produced by the intensive crop cultivation is one of its major issues. The majority of this material is biomass waste. This kind of residue emerges into a resource with significant potential for the extraction of high-value by-products (Duque-Acevedo *et al.,* 2020). Waste biomass has great recycling potential due to abundancy and having no harmful effects on environment. Recycling of organic waste as a feedstock for plants is an eco-friendly method of cultivating various fruit, vegetable, and ornamental crops. The re-use of such waste material also supports the economic development in sustainable way (Munir *et al.,* 2020; Thomson *et al.,* 2022). The value of organic waste and other residual materials from bio-based enterprises and private residences is increasing. The wasted materials that were once a strain on the economy have become valuable resources (Klitkou *et al.,* 2019).

The significance of dairy industry to the rural agricultural economy cannot be overstated. The dairy products such as milk, milk powder, butter, and cheese are widely consumed and enjoyed all over the world. When it comes to processing, fermenting, and consuming dairy products, there has been a staggering amount of ingenuity and innovation over the last couple of millennia. The global dairy industry was valued at ca. 871 billion US dollars in 2021, and by 2026, it is expected to have increased to ca. 1,128 billion (Jaganmai and Jinka, 2017, Shahbandeh, 2022). The growing demand for dairy products across the globe leads to the expansion of the dairy sector and an increase in waste production. It is estimated that ca. 20% of dairy products are wasted (Askew, 2022). Cheese-whey, dairy sludges, milk and milk products residue, and wastewater are the main wastes generated by dairy industries. This waste contain both organic and inorganic contents and have significant nutrient concentrations, as well as biological and chemical oxygen demands. They can also contain a variety of chemicals, both acid and alkaline, and sterilizing agents. Pollution due to the waste of dairy industry affects the air, soil and water quality (De Jesus *et al.,* 2015; Raghunath *et al.,* 2016; Ahmad *et al.,* 2019; Sar *et al.,* 2022. It is therefore needed to take initiatives not only to reduce dairy waste but to recycle and utilize the wasted material, which can lead to enhanced sustainability, including reduced environmental impacts and cost savings (Eriksson *et al.,* 2014).

One of the largest reserves of food protein is found in whey, the liquid byproduct of the production of cheese, casein, and yoghurt. It includes 80–90% of the total volume of milk and about 50% of the nutrients found in the original milk (soluble protein, lactose, vitamins, and minerals). Sweet whey is a by-product of making hard, semi-hard, or soft cheese with rennet casein, whereas acid whey is produced by using mineral-acid precipitated casein (Smithers, 2008; Yadav *et al.,* 2015). The application of whey at

different concentrations (25-100%) on tomato, okra, corn, and potatoes significantly increased the vegetative growth, yield and fruit quality (Sharratt *et al.,* 1962; Al-Mughrabi, 2007; Pane *et al.,* 2012; Abed *et al.,* 2016; Mahmood *et al.,* 2020). Application of whey improved the soil structure and trace elements (Al, Fe, B, Cu, Zn, Mn, and Cr) concentration (Robbins and Lehrsch, 2020). Cheese-whey can improve the physical condition of sodic soils or those susceptible to erosion by increasing their aggregate stability (Lehrsch and Robbins, 1996). This study sought to investigate the impact of wasted cheese-whey amended substrate on the growth and flowering of ornamental snapdragon cultivars and to compare it with the traditional substrate. The outcome of the study identified the recycling of one of the dairy wasted materials in order to reduce its negative pollutant effects.

Materials and methods

The glasshouse experiment was conducted at the Date Palm Research Center of Excellence, Training and Research Station, King Faisal University, Al-Ahsa, Saudi Arabia during 2020 and 2021 (Latitude 25° 16' 7.068'' N and Longitude 49° 42' 27.522'' E). Seeds of seven snapdragon cultivars Magic Carpet, Antiquity Sunset, Day and Night, Chuckles, Illumination, Madame Butterfly, and Twilight were obtained from Sutton Seeds, Devon, England. Seeds were sown into 84 cells seed plug trays containing peat-based compost. Seed trays were placed in an environment-controlled growth chamber (Microclima 1000, Snijders Scientific B.V. Tilburg, Holland) at 23 ± 2°C temperature providing 70 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) using white LED lights installed at one meter tray height with a 16 h d^{-1} photoperiod.

After 75% seed germination, plants were transplanted into 10 cm plastic pots. These pots were divided into two groups based on different substrates: (1) Pots were filled in with 1:3:1 ratio of sand, peat-based compost, and perlite (control), and (2) Pots were filled in with 1:3:1 ratio of sand, peat-based compost, and perlite, however, this substrate was amended by adding 200 mL per pot cheese-whey. These pots were then transferred in a glasshouse, under ambient environment. The temperature, relative humidity, and light intensity within the glasshouse was recorded every 5 seconds using a HOBO Analog/Temp/RH/Light data logger (MX1104, Onset Computer Corporation, MA, USA).

The experiment was laid out on two-factorial completely randomized design (CRD) having six replications for each treatment. The first factor was seven commercial cultivars of snapdragon (Magic Carpet, Antiquity Sunset, Day and Night, Chuckles, Illumination, Madame Butterfly, and Twilight) and the second factor was two types of substrates (control and cheese-whey). The wasted cheese-whey was obtained from the local superstore and was analyzed in the Biochemistry laboratory, King Faisal University, Saudi Arabia. The cheese-whey analysis result indicated that it had 9% protein, 65% lactose, 0.4% fats, 333 mg L^{-1} total nitrogen, 57 mg L^{-1} available nitrogen, 98 mg L^{-1} total phosphorus, 341 mg L^{-1} total potassium, 54 mg L^{-1} total magnesium, 251 mg L^{-1} calcium, 483 mg L^{-1} sodium, 45 mg L^{-1} sulphur, 0.15 mg L^{-1} lead, 0.01 mg L^{-1} nickel, < 0.01 mg L^{-1} cadmium, 0.05 mg L^{-1} chromium, < 0.01 mg L^{-1} arsenic, < 0.01 mg L^{-1} mercury, 3.32 mg L^{-1} zinc, 0.91 mg L^{-1} copper, 0.02 mg L^{-1} molybdenum, 0.02 mg L^{-1} selenium, 4.8 pH, 4.14 mmhos electrical conductivity, 1.24% dry matter (AOAC, 2016).

Plant nutrients were given in the form of a water soluble fertilizer 15:15:15. To avoid *Pythium*, water was applied manually every two or three days as required. Plants in each treatment were observed daily until flower opening (corolla fully opened). The following parameters were recorded during the study according to AOAC standard methods (AOAC, 2016): days to flowering, plant height, number of leaf per plant, leaf area, leaf fresh weight, leaf dry weight, plant fresh weight, plant dry weight, specific leaf area, specific leaf weight, leaf weight ratio, leaf area ratio, net assimilation rate, and relative growth rate. The collected data were statistically analyzed according to Gomez and Gomez (1984), using Statistical Analysis Software, Release 9.4 (SAS Institute, North Carolina, USA), and the Duncan Multiple Range Test (DMRT) was applied to determine the least significance difference between the treatment means (Waller and Duncan, 1969). The two-way factorial completely randomized design layout is as below:

	Factor-A (Cultivars)	Factor-B (Substrates)
1	Magic Carpet	(1) Control (2) Cheese-whey
2	Antiquity Sunset	(1) Control (2) Cheese-whey
3	Day and Night	(1) Control (2) Cheese-whey
4	Chuckles	(1) Control (2) Cheese-whey
5	Illumination	(1) Control (2) Cheese-whey
6	Madame Butterfly	(1) Control (2) Cheese-whey
7	Twilight	(1) Control (2) Cheese-whey

Results and discussion

Data presented in Table 1 revealed that there was a significant ($P \leq 0.05$) difference among means of different snapdragon cultivars regarding days to flowering, plant height, number of leaf per plant, leaf area, leaf fresh weight, leaf dry weight, plant fresh weight, plant dry weight, specific leaf area, specific leaf weight, leaf weight ratio, leaf area ratio, net assimilation rate, and relative growth rate. Maximum days to flowering (104.50 days), plant height (55.95 cm), number of leaf per plant (30.25), leaf area (203.22 cm^2), leaf fresh weight (9.11 g), leaf dry weight (1.16 g), plant fresh weight (51.97 g), and plant dry weight (5.20 g) were recorded in cultivar Day and Night. Cultivar Illumination (103.58 days) was statistically at par with cultivar Day and Night regarding days to flowering parameter, however, it had highest specific leaf area (289.43 cm^2 g^{-1}) followed by cultivar Magic Carpet (282.07 cm^2 g^{-1}). Similarly, cultivar Madame Butterfly (54.08 cm) was statistically alike with cultivar Day and Night regarding plant height parameter. The specific leaf weight was highest in cultivars Antiquity Sunset and Day and Night (0.0058 g cm^{-2}). The leaf weight ratio was statistically similar but maximum in cultivars Day and Night (0.130), Antiquity Sunset (0.126), Twilight (0.124), and Madame Butterfly (0.121). Similarly, leaf area ratio (45.08) was highest in cultivar Twilight, whereas cultivar Magic Carpet had maximum net assimilation rate (0.0163 g g^{-1} d^{-1}) and relative growth rate (0.326 g cm^{-2} d^{-1}).

The comparative analysis between the control and cheese-whey amended substrate indicated that apart from days to flowering there was a significant ($P \leq 0.05$) difference regarding plant height, number of leaf per plant, leaf area, leaf fresh weight, leaf dry weight, plant fresh weight, plant dry weight, specific leaf area, specific leaf weight, leaf weight ratio, leaf area ratio, net assimilation rate, and relative growth rate attributes (Table 1). Snapdragon plants growing in cheese-whey amended substrates had higher plant height (43.87 cm), number of leaf per plant (25.40), leaf area (151.53 cm^2), leaf fresh weight (5.82 g),

Table 1. Effect of cheese-whey amended substrate on the days to flowering (DF), plant height (PH), number of leaf per plant (L/P), leaf area (LA), leaf fresh weight (LFW), leaf dry weight (LDW), plant fresh weight (PFW), plant dry weight (PDW), specific leaf area (SLA), specific leaf weight (SLW), leaf weight ratio (LWR), leaf area ratio (LAR), net assimilation rate (NAR), and relative growth rate (RGR) of seven commercial cultivars of snapdragon

Treatments	DF (days)	pH (cm)	L/P	LA (cm^2)	LFW (g)	LDW (g)	PFW (g)	PDW (g)	SLA ($cm^2\ g^{-1}$)	SLW ($g\ cm^{-2}$)	LWR	LAR	NAR ($g\ g^{-1}\ d^{-1}$)	RGR ($g\ cm^{-2}\ d^{-1}$)
A. Cultivars														
MAG	84.25^{D}	17.52^{E}	16.08^{F}	64.78^{D}	1.89^{E}	0.24^{E}	18.52^{E}	2.32^{E}	282.07^{AB}	0.0036^{B}	0.063^{B}	28.60^{C}	0.0163^{A}	0.326^{A}
ANT	99.75^{B}	34.66^{D}	19.08^{E}	102.26^{C}	3.36^{D}	0.59^{C}	26.71^{D}	2.67^{D}	180.56^{D}	0.0058^{A}	0.126^{A}	38.36^{B}	0.0099^{B}	0.249^{B}
DN	104.50^{A}	55.95^{A}	30.25^{A}	203.22^{A}	9.11^{A}	1.16^{A}	51.97^{A}	5.20^{A}	176.49^{D}	0.0058^{A}	0.130^{A}	39.68^{AB}	0.0051^{D}	0.124^{C}
CHK	93.00^{C}	47.75^{B}	24.08^{C}	137.87^{B}	4.42^{C}	0.54^{CD}	40.24^{C}	4.02^{C}	262.34^{B}	0.0039^{C}	0.076^{B}	34.33^{BC}	0.0076^{C}	0.163^{C}
ILL	103.58^{A}	45.33^{C}	22.08^{D}	139.31^{B}	4.45^{C}	0.48^{D}	38.19^{C}	3.82^{C}	289.43^{A}	0.0034^{C}	0.073^{B}	36.49^{B}	0.0074^{C}	0.171^{C}
MAD	100.67^{B}	54.08^{A}	28.25^{B}	196.40^{A}	8.01^{B}	0.95^{B}	49.41^{B}	4.94^{B}	212.73^{C}	0.0048^{B}	0.121^{A}	39.91^{AB}	0.0052^{D}	0.132^{C}
TWL	100.75^{B}	35.00^{D}	19.91^{E}	110.45^{C}	3.44^{D}	0.57^{CD}	24.73^{D}	2.47^{DE}	198.85^{CD}	0.0051^{B}	0.124^{A}	45.08^{A}	0.0093^{B}	0.270^{AB}
LSD	2.30*	2.09*	1.55	16.84*	0.76*	0.09*	2.34*	0.25*	26.91*	0.0006*	0.035*	5.82*	0.0008*	0.056*
B. Substrates														
Control	98.38^{A}	39.07^{B}	20.23^{B}	121.12^{B}	4.08^{B}	0.53^{B}	33.54^{B}	3.41^{B}	248.14^{A}	0.0042^{B}	0.153^{A}	35.19^{B}	0.0098^{A}	0.329^{A}
Cheese-whey	97.76^{A}	43.87^{A}	25.40^{A}	151.53^{A}	5.82^{A}	0.76^{A}	37.82^{A}	3.85^{A}	209.71^{B}	0.0051^{A}	0.051^{B}	39.81^{A}	0.0075^{B}	0.081^{B}
LSD	1.23^{NS}	1.11*	0.83*	9.00*	0.41*	0.04*	1.25*	0.13*	14.38*	0.0003*	0.018*	3.11*	0.0004*	0.030*
C. Cultivar x Substrate Interaction														
MAG × Control	83.67^{H}	15.46^{I}	12.50^{I}	50.33^{H}	1.24^{G}	0.15^{I}	16.20^{J}	2.04^{I}	319.09^{A}	0.0031^{K}	0.081^{BD}	25.52^{E}	0.0199^{A}	0.506^{A}
MAG × Cheese-whey	84.83^{H}	19.58^{H}	19.66^{G}	79.22^{G}	2.54^{F}	0.32^{H}	20.85^{I}	2.61^{GH}	245.05^{CD}	0.0041^{GJ}	0.046^{D}	31.68^{DE}	0.0127^{B}	0.146^{DE}
ANT × Control	100.83^{CE}	32.15^{G}	17.16^{H}	92.06^{FG}	2.73^{F}	0.48^{FG}	24.76^{H}	2.47^{H}	196.14^{EG}	0.0053^{BE}	0.197^{A}	37.31^{BD}	0.0109^{C}	0.406^{B}
ANT × Cheese-whey	98.67^{E}	37.18^{F}	21.00^{FG}	112.47^{EF}	3.98^{E}	0.70^{CE}	28.67^{G}	2.86^{G}	164.98^{G}	0.0063^{A}	0.055^{D}	39.41^{BD}	0.0089^{D}	0.093^{EF}
DN × Control	105.67^{A}	52.89^{B}	27.83^{C}	185.50^{B}	7.89^{C}	1.01^{B}	48.86^{BC}	4.89^{BC}	185.63^{FG}	0.0055^{AD}	0.207^{A}	38.09^{BD}	0.0054^{FG}	0.205^{CD}
DN × Cheese-whey	103.33^{AC}	59.01^{A}	32.66^{A}	220.94^{A}	10.33^{A}	1.32^{A}	55.09^{A}	5.51^{A}	167.36^{G}	0.0061^{AB}	0.053^{D}	41.27^{B}	0.0047^{G}	0.044^{F}
CHK × Control	94.67^{F}	45.33^{DE}	21.66^{FG}	124.15^{E}	3.62^{EF}	0.44^{GH}	38.19^{EF}	3.82^{EF}	283.87^{AB}	0.0036^{IK}	0.116^{B}	32.74^{CE}	0.0083^{D}	0.262^{C}
CHK × Cheese-whey	91.33^{G}	50.16^{BC}	26.50^{CD}	151.59^{CD}	5.22^{D}	0.63^{DE}	42.30^{D}	4.23^{D}	240.82^{CD}	0.0043^{FI}	0.036^{D}	35.92^{BD}	0.0068^{E}	0.064^{F}
ILL × Control	104.83^{AB}	43.16^{E}	19.66^{G}	123.17^{E}	3.59^{EF}	0.39^{GH}	36.36^{F}	3.64^{F}	311.42^{A}	0.0032^{JK}	0.109^{BC}	33.94^{BD}	0.0081^{D}	0.275^{C}
ILL × Cheese-whey	102.33^{BD}	47.50^{CD}	24.50^{DE}	155.45^{C}	5.31^{D}	0.58^{EF}	40.02^{DE}	4.00^{DE}	267.43^{BC}	0.0037^{HK}	0.037^{D}	39.03^{BD}	0.0066^{EF}	0.068^{EF}
MAD × Control	99.67^{DE}	51.83^{B}	26.16^{CD}	181.01^{B}	6.96^{C}	0.82^{C}	47.36^{C}	4.74^{C}	224.27^{DE}	0.0045^{EH}	0.174^{A}	38.38^{BD}	0.0055^{FG}	0.212^{CD}
MAD × Cheese-whey	101.67^{BE}	56.33^{A}	30.33^{B}	211.80^{A}	9.07^{B}	1.07^{B}	51.47^{B}	5.14^{B}	201.20^{EG}	0.0051^{CF}	0.067^{BD}	41.45^{B}	0.0048^{G}	0.053^{F}
TWL × Control	99.33^{DE}	32.66^{G}	16.66^{H}	91.64^{FG}	2.56^{F}	0.42^{GH}	23.09^{HI}	2.31^{HI}	216.57^{DF}	0.0046^{DG}	0.186^{A}	40.31^{BC}	0.0110^{C}	0.438^{AB}
TWL × Cheese-whey	102.17^{BD}	37.33^{F}	23.16^{EF}	129.25^{DE}	4.32^{DE}	0.71^{CD}	26.36^{GH}	2.63^{GH}	181.13^{FG}	0.0055^{AC}	0.061^{CD}	49.85^{A}	0.0080^{D}	0.102^{EF}
LSD	3.25*	2.96*	2.20*	23.81*	1.08*	0.13*	3.32*	0.35*	38.05*	0.0008*	0.049*	8.23*	0.0011*	0.079*

Means showing common letter(s) in a column are non-significant statistically at 5% probability using Duncan Multiple Range Test.

leaf dry weight (0.76 g), plant fresh weight (37.82 g), plant dry weight (3.85 g), specific leaf weight (0.0051 g cm^{-2}), and leaf area ratio (39.81), however, specific leaf area (248.14 $cm^2\ g^{-1}$), leaf weight ratio (0.153), net assimilation rate (0.0098 g $g^{-1}\ d^{-1}$), and relative growth rate (0.329 g $cm^{-2}\ d^{-1}$) were higher in control plants.

The interaction between the cultivars of snapdragon and substrates showed that all plant growth and flowering attributes were significantly different at 5% level of probability (Table 1). Maximum days to flowering was recorded in snapdragon cultivar Day and Night grown in control (105.67 days) and cheese-whey (103.33 days) substrates whereas minimum days to flowering were counted in Magic Carpet in control (83.69 days) and cheese-whey (84.83 days) substrates. Maximum plant height (59.01 cm), number of leaf per plant (32.66), leaf area (220.94 cm^2), leaf fresh weight (10.33 g), leaf dry weight (1.32 g), plant fresh weight (55.09 g), and plant dry weight (5.51 g) were calculated in cultivar Day and Night grown in cheese-whey amended substrate. Snapdragon cultivar Madame Butterfly grown in cheese-whey amended substrate was statistically at par with cultivar Day and Night regarding plant height (56.33 cm) and leaf area (211.80 cm^2) traits. Cultivar Magic Carpet raised in control substrate had highest specific leaf area (319.09 $cm^2\ g^{-1}$), net assimilation rate (0.0199 g $g^{-1}\ d^{-1}$), and relative growth rate (0.506 g $cm^{-2}\ d^{-1}$). The specific leaf area (311.42 $cm^2\ g^{-1}$) of cultivar Illumination grown in the similar substrate was statistically alike with Magic Carpet. However, specific leaf weight parameter was maximum (0.0063 g cm^{-2}) in cultivar Antiquity Sunset followed by cultivar Day and Night (0.0061 g cm^{-2}) grown

in the cheese-whey substrate. Four snapdragon cultivars grown in control substrate, Antiquity Sunset (0.197), Day and Night (0.207), Madame Butterfly (0.174), and Twilight (0.186) statistically behaved alike regarding leaf weight ratio parameter. Data regarding leaf area ratio was significantly increased when snapdragon cultivars were grown in cheese-whey amended substrate, however, it was higher in cultivar Twilight (49.85).

The combined data of seven snapdragon cultivars and two substrates were analyzed to find out the correlation among various plant growth attributes using Spearman's method (Fig. 1). A highly significant positive correlation was found among plant height, number of leaf per plant, leaf area, leaf fresh weight, leaf dry weight, plant fresh weight, and plant dry weight attributes. Correlation coefficient of plant height with number of leaf per plant, leaf area, leaf fresh weight, leaf dry weight, plant fresh weight, and plant dry weight ranged from 0.82 to 0.96. It was 0.90 to 0.96 when number of leaf per plant was correlated with plant height, leaf area, leaf fresh weight, leaf dry weight, plant fresh weight, and plant dry weight. Leaf area was significantly increased with the increased in plant height (0.95), number of leaf per plant (0.97), leaf fresh weight (0.98), leaf dry weight (0.92), plant fresh weight (0.97), and plant dry weight (0.96) attributes. The correlation coefficient ranges of leaf fresh weight, leaf dry weight, plant fresh weight, and plant dry weight within the above group of attributes were 0.88−0.98, 0.82−0.96, 0.84−0.99, and 0.82−99, respectively. However, specific leaf area, net assimilation rate, and relative growth rate parameters were significantly negative correlated to days to flowering, plant height, number of leaf per plant, leaf area, leaf fresh weight, leaf dry weight, plant fresh weight, plant dry weight, specific leaf weight, and leaf area ratio. Similar negative trend was found when specific leaf weight and leaf area ratio correlated with specific leaf area, net assimilation rate, and relative growth rate traits.

The findings of this study revealed that the cheese-whey amended substrate did not influence time of flowering. However, the difference in the said parameter was cultivar dependent. Snapdragon is categorized as facultative long day plant (Baloch *et al.,* 2012) and the floral initiation is responsive to photoperiod (Munir *et al.,* 2017) and light intensity (Munir *et al.,* 2004). Therefore, it is assumed that the amended substrate did not affect floral time and it was the cultivars' photo-sensitive response to the prevailing light condition that is why they can be grouped into early (Magic Carpet), mid (Chuckles), and late (Antiquity Sunset, Day and Night, Illumination, Madame Butterfly, and Twilight) flowering cultivars (Adams *et al.,* 2003).

The results obtained from the present study suggested that the substrate amended with cheese-whey significantly increased plant growth and development attributes compared to control in snapdragon. However, the difference among cultivar regarding these parameters was due to the difference in genotypes. Desirable plant height can be acquired through biological, physical, and chemicals methods (Baloch *et al.,* 2013; Munir and Alhajhoj, 2017; Demir and Çelikel, 2019). The positive influence of leaf mold substrate was reported in snapdragon cultivar Orchid Rocket that significantly enhanced plant height, number of leaves, stem, leaf, and plant fresh, and dry weight (Naz *et al.,* 2013). Similarly, Rainbow and Wilson (1998) reported that the substrate from green waste significantly enhanced growth of snapdragon, stock and tomato plants. In the present study, number of leaf per plant, leaf area, fresh and dry weights of leaf and plant, specific leaf weight, and leaf area ratio were significantly higher when plants were grown in cheese-whey amended substrate. It could be due to the maximum nutrient availability in the cheese-whey amended substrate, therefore, plants utilized much of the nutrients to produce maximum assimilates. This assumption appears logical while looking at the plant fresh and dry weight data. It is reported that the whey application improves the physical soil properties and provides essential micro-nutrients to the plant that enhance plant growth and development (Lehrsch and Robbins, 1996; Robbins and Lehrsch, 2020). Similarly, the vigor (height and weight) of wheat, soybean, and broccoli plants was significantly enhanced by the application of whey (Grosu *et al.,* 2012).

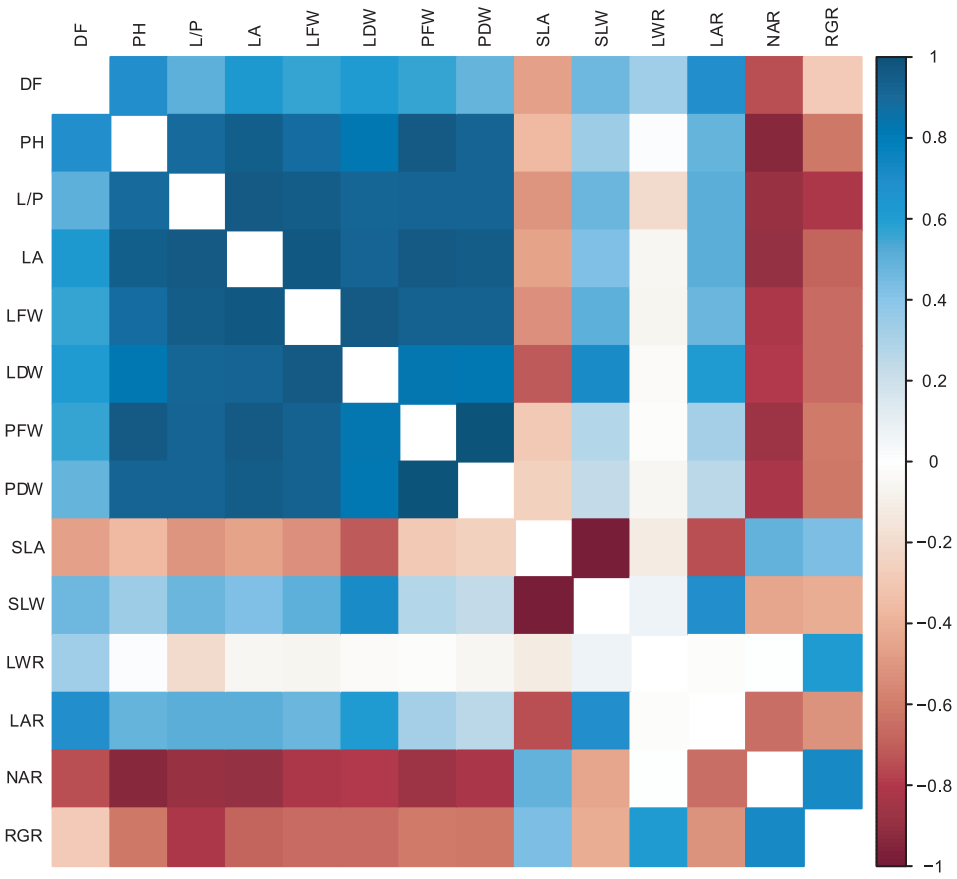

Fig. 1. Correlation test among various growth attributes in the seven snapdragon cultivars and two substrates. A total of 84 biological replicates were averaged. DF, days to flowering (days), PH, plant height (cm), L/P, number of leaf per plant, LA, leaf area (cm^2), LFW, leaf fresh weight (g), LDW, leaf dry weight (g), PFW, plant fresh weight (g), PDW, plant dry weight (g), SLA, specific leaf area ($cm^2\ g^{-1}$), SLW, specific leaf weight ($g\ cm^{-2}$), LWR, leaf weight ratio, LAR, leaf area ratio, NAR, net assimilation rate ($g\ g^{-1}\ d^{-1}$), and RGR, relative growth rate ($g\ cm^{-2}\ d^{-1}$)

It is concluded that cheese-whey can be recycled into useful soil conditioner to improve the properties of substrate that subsequently enhance plant growth and development traits.

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Increased relative humidity in the dry season during stomata opening promotes growth, leaf area, and biomass of CAM orchid: *Dendrobium* Sonia 'Earsakul'

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Abstract

Dendrobium cut-flower producers commonly employ sprinkler systems with high water consumption. Our study aimed to identify a more water-efficient irrigation method for orchids. Specifically, we investigated the optimal timing of water application during a day in the dry season, intending to minimize water usage. The research used a 3x2x2 completely randomized factorial design, factoring in the times of the day for irrigation (dawn, morning, and evening), the type of sprinkler head (standard or large vs. mini), and the duration of irrigation (6 minutes vs. 4 minutes). The study revealed that adjusting these factors could reduce the standard water volume used by 30 to 60% without negatively affecting the orchids' growth or flower quality. Over five months of testing various irrigation techniques, metrics such as the height of the front pseudobulb, leaf count on the front pseudobulb, total leaf number per plant, pseudobulb count, and inflorescence quality (like length, number of flowers, and vase life) remained consistent across different methods. A notable discovery was that irrigating at either dawn or evening using a standard-sized sprinkler led to higher fresh and dry leaf weights and a greater leaf area than morning irrigation. Impressively, these results were observed even when the irrigation time was reduced to just 4 minutes, a 30% reduction from typical water usage. In summary, our research suggests that during the dry season, *Dendrobium* orchid growers can potentially reduce irrigation water usage by 30% without sacrificing the growth or quality of their plants.

Keywords: Cut-flower, inflorescence, leaf area, pseudobulb, vase life, water use

Introduction

Orchid cut-flower production is a leading floral commodity in Southeast Asia, particularly in Thailand, which stands out as a major exporter. In 2021, Thailand's export value for this commodity reached approximately 45 million USD, as the Office of Agriculture Economics reported in 2022. Among the various orchids, *Dendrobium* is the primary cultivar used in Thailand's cut-flower industry. Intriguingly, *Dendrobium* is a Crassulacean acid metabolism (CAM) epiphyte, a type of photosynthetic plant. Notably, CAM plants require less water. Yet, they exhibit the highest water use efficiency compared to other photosynthetic processes. This efficiency is attributed to their unique nocturnal CO_2 fixation pattern (Jindamol *et al.,* 2019; Nobel and Jordan, 1983).

CAM photosynthesis behaviour can be divided into four phases. Phase I is when light is absent, CO_2 is fixed by the PEP carboxylase (PEPC) enzyme, and phase II is the CO_2 fixation peak during the dark-light transition due to PEP carboxylase and Rubisco enzymes fixing CO_2. Phase III is when a light reaction happens while stomata are closing, and phase IV usually happens if favourable conditions occur during the light-dark transition (Smith and Lüttge, 1985). Traditional irrigation for orchid production starts from morning until noon, during phase III when the stomata are closed. This encouraged our hypothesis that if we change the irrigation time of the day to increase relative humidity (RH) during open plant stomata, which are Phase II and IV, at dawn and evening, respectively, plants will show better growth and yield quality and quantity. We found that increased RH can promote plant growth, yield, leaf area, and photosynthetic capacity in many plants. For example, CAM orchid, *Doritaenopsis* 'NewCandy' had higher fresh and dry weight, leaf size, and photosynthetic efficiency under higher RH (Jeon *et al.,* 2006). In addition, growth, biomass, leaf area index, CO_2 fixation rate, and yield of tomatoes (*Solanum lycopersicum* L.) were significantly higher when providing micro-fog to increase RH inside the greenhouse (Zhang *et al.,* 2015).

Under global warming and climate change, water scarcity is one of the significant problems. The agriculture sector accounts for the most extensive freshwater consumer, around 92 %, in the South East Asia region (Aquastat, 2014). Currently, cultivation practice for *Dendrobium* production in Thailand is based on the farmer's experience to decide when and how much to irrigate by climate observations. However, the CAM epiphyte orchid requires less water than other plant types (Winter and Smith, 2012). Hence,

the current cultivation method showed overuse, inefficient water consumption, and caused run-off water from agricultural fields to contaminate the environment. Therefore, finding a way to minimise water use for the orchid producer will benefit farmers in terms of cost reduction and indirectly benefit the community by having a higher freshwater resource availability during the dry season and reducing water resource contamination from the agricultural run-off.

In this article, we represented the growth, physiological response, and inflorescence quality of *Dendrobium* at different irrigation times of the day, in addition, the reduction of irrigation water used by another type of irrigation head and irrigation duration for the long term during the dry season to summer monsoon in Thailand.

Materials and methods

Plants cultivation and treatment application: Young *Dendrobium* Sonia 'Earsakul' propagated by meri-cloning was transferred to 23x35 cm coconut husk block, four plants for each block, in February 2021. The culture at Horticultural Research Field 1 under the lath house has sunlight filtration of about 50 % and a PAR value of approximately 678 µmol s^{-1} m^{-1} at midday, Horticulture Department, Kasetsart University Kamphaeng Saen Campus, Nakorn Pathom province. Ten months after the transplant, different irrigation water systems were applied continuously for five months (from January-May 2022). January-March was the dry season, and the total rainfall was less than 45 mm (Fig. 2). April-May was the early period of the rainy season. The total precipitation was 76 to 128 mm (Fig. 2). The experiment was designed as a Factorial in CRD, which had 3x2x2 levels of treatment comprising the period of giving the irrigation (dawn 4.00 to 5.00, morning 8.00 to 9.00, and evening 18.00 to 19.00), type of irrigation head (big-PVC sprinkler head (600 litre/hour), mini sprinkler head (200 litre/hour)), duration of giving the irrigation (average water volume given by farmer, 6 minutes), decrease given time (minimise water volume, 4 minutes). We collected irrigation water volume from eight *Dendrobium* cut-flower production farms and found that the standard average water volume was 310 mL per one growing media per time. Hence, each treatment received a water volume, as shown in Table 1.

Table 1. Treatment combination and irrigation water volume for each treatment per one growing media per time

Time of the day	Sprinkler type	Irrigation duration (min)	Average irrigation volume per growing media (mL)	Compared with control (%)
Morning	Big	6	310±12	100
Morning	Big	4	220±38	71
Morning	Mini	6	177±35	60
Morning	Mini	4	136±44	43
Evening	Big	6	310±12	100
Evening	Big	4	220±38	71
Evening	Mini	6	177±35	60
Evening	Mini	4	136±44	43
Dawn	Big	6	310±12	100
Dawn	Big	4	220±38	71
Dawn	Mini	6	177±35	60
Dawn	Mini	4	136±44	43

The irrigation system was controlled using an electric solenoid valve and a Sonoff smart Wi-Fi switch to control the irrigation water's timing and duration. The average relative humidity during the first three months ranged from 47 to 94 %; in the last two months, it was 51 to 90 % (Fig. 1a, b). The average temperature was 22 to 36 °C and 24 to 36 °C during the first three months and last two months, respectively, Fig. 1 c, d). An arrow indicates the different irrigation timing. The rainfall accumulation during the first three months was 10.6 to 39.8 mm. The last two months were the summer monsoon season with high rainfall from 76.4 to 128.7 mm (Fig. 1e). Each treatment contains four replications, each containing four growing media (16 plants) accounting for 0.5 cm^2 of growing area.

Physical and physiological assessment: The physical responses, the height, number, and diameter of the front pseudobulb and leaf number were measured 3 and 5 months after treatment. Carbon exchange rate (CER), transpiration rate (Tr), and stomatal conductance (g_s) were measured five months after giving different irrigation treatments using four plants per treatment by a portable photosynthesis system (Licor-6400XT; Licor Inc.; Lincoln, NE, USA) on the 3rd leaf of the front pseudobulb. The measurement was done at dusk-dawn transition (04.00 to 07.00), where the peak of CO_2 fixation happens under fixed CO_2 air concentration at 600 ppm, leaf temperature at 25 °C, and relative humidity at 72 to 75 % conditions. After that, whole plants of each treatment were harvested and immediately brought to the laboratory for physiological assessment. First, leaf and pseudobulb were separated and fresh weight, then leaf area of the leaves was measured using leaf area meter LI-3000 (LI-COR Lincoln, Nebraska, USA). Next, the 3rd leaf from the front pseudobulb position was punched into a circle shape by a cork borer (1 cm^2 diameter) and used for chlorophyll and carotenoid extraction in acetone (80 %) (Lichtenthaler and Buschmann, 2001). Then, leaf and pseudobulb tissue were dried using Thermotec 2000 hot air oven at 70 °C for 7 to 10 days, and water content was calculated (Jin *et al.,* 2017). The dry sample was ground by cross beater mill Retsch SK100 ("Retsch Co.", Germany) until fine powder and 0.05 g were used for total non-structure carbohydrate analysis. The extraction procedure followed Smith *et al.* (1964) by using 0.2 N H_2SO_4. The TNC determination followed Nelson (1944) for photometry determination using glucose as a standard using a UV-VIS spectrophotometer (T80 UV/VIS spectrophotometer, PG Instrument Ltd., UK).

Inflorescence quality assessment*:* Inflorescence quality assessment was done by randomly harvesting 20 inflorescences per treatment, at 50% bloom in the inflorescence stage. After cutting, the inflorescence length was measured, and the number of flowers was counted. The length and width of the lowest flower were measured. After that, inflorescences were brought to the laboratory for vase life evaluation. Vase life evaluation was done under 27 ± 2°C and 12 hours of lights on condition. Inflorescence stems were cut again under water to prevent air bubbles in a vascular bundle and transferred to a test tube containing distilled water. The end of vase life was determined by 50% of floret dropped or wilted (Ketsa *et al.,* 1995). The number of inflorescences was collected throughout the experiment.

Statistical analysis: Statistical analyses were performed using the one-way variance analysis or independent sample t-test. In addition, mean differences were evaluated by using Duncan's new multiple range test at $P < 0.05$ significance level.

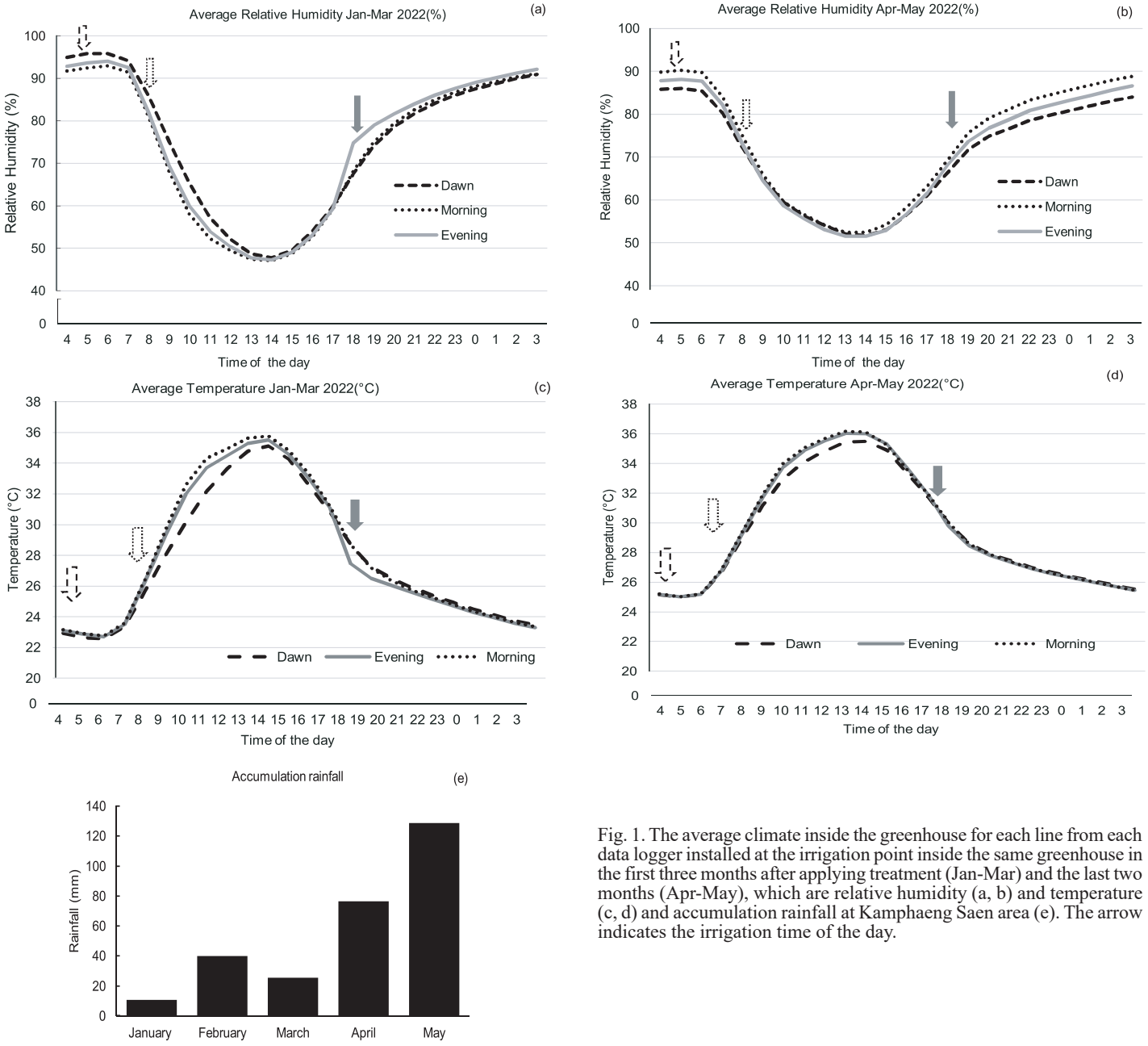

Fig. 1. The average climate inside the greenhouse for each line from each data logger installed at the irrigation point inside the same greenhouse in the first three months after applying treatment (Jan-Mar) and the last two months (Apr-May), which are relative humidity (a, b) and temperature (c, d) and accumulation rainfall at Kamphaeng Saen area (e). The arrow indicates the irrigation time of the day.

Table 2. Physical change after giving the different irrigation treatments for 3 and 5 months

Factor	Front pseudobulb height (cm)		Front pseudobulb diameter (cm)		Pseudobulb number per plant		Leaf number per plant	
	3 months	5 months	3 months	5 months	3 months	5 months	3 months	5 months
Factor 1 Time of the day (A)								
Dawn	31.7±0.7a	33.7±0.8	1.45±0.01	1.4±0.03	3.8±0.14	4.4±0.1b	12.3±0.7	12.5±0.7
Morning	30.7±0.7b	34.0±1.1	1.5±0.03	1.4±0.06	3.7±0.11	4.9 ±0.2a	11.1±0.5	11.1±0.7
Evening	32.4±0.4a	34.7±0.6	1.5±0.01	1.4±0.03	3.9±0.07	4.7±0.2ab	12.3±0.3	11.7±0.5
Factor 2 Type of sprinkler head (B)								
Big	32.1±0.4a	34.8±0.6	1.5±0.02	1.5±0.02	3.9±0.09	4.8±0.1	12.3±0.5	12.3±0.6
Mini	31.1±0.6b	33.4±0.7	1.4±0.02	1.4±0.04	3.7±0.08	4.6±0.2	11.5±0.4	11.2±0.4
Factor 3 Duration (C)								
Normal (6 min)	31.5±0.5	34.5±0.1	1.5±0.02	1.5±0.03	3.9±0.11	4.6±0.1 b	12.1±0.5	12.1±0.6
Short (4 min)	31.7±0.7	33.8±0.5	1.5±0.02	1.4±0.02	3.7±0.05	5.0±0.2 a	11.7±0.5	11.5±0.4
F-test/T-test								
A	*	ns	ns	ns	ns	**	ns	ns
B	*	ns	ns	ns	ns	ns	ns	ns
C	ns	ns	ns	ns	ns	*	ns	ns

Note: The data are means ±SE. Different letters indicate significant differences at $P < 0.05$ between treatments within the same factor.

Results

Physical response to different irrigation treatments: After applying different irrigation treatments for 3 and 5 months, the eyes barely detected the physical difference between each treatment. We found a statistically different front pseudobulb height at another irrigation time and type of sprinkler head at three months. Giving water at dawn and evening showed 31.7 and 32.4 cm height, respectively, while it was only 30.7 cm in the morning. The big-head sprinkler measured 32.1 cm, whereas the mini-sprinkler measured 31.1 cm height (Table 2). However, height was not statistically different in 5 months, varying from 33.7 to 34.7 cm.

In contrast, we found significantly higher pseudobulb number when irrigated in the morning, with a shorter time of five months. Irrigation in the morning had an average of 4.9 pseudobulbs, while in the evening and dawn, had 4.7 and 4.4 pseudobulbs, respectively (Table 2). Giving shorter irrigation time had five pseudobulbs per plant while only 4.6 pseudobulbs per plant were recored if water was given at regular timing. The diameter of the front pseudobulb and leave number per plant were not statistically different among irrigation treatments 3 and 5 months after the treatment (Table 2).

Physiological response to different irrigation treatments: Big-headed sprinkler irrigation had a significantly higher CER than the mini sprinkler, which was 6.81 and 5.62 µmol CO_2 $m^{-2}s^{-1}$, respectively (Table 3). However, the different periods of the day and the irrigation duration did not show any statistical differences, ranging from 6.1 to 6.45 µmol CO_2 $m^{-2}s^{-1}$. The g_s and Tr were also not significantly different between each irrigation treatment, ranging from 0.081 to 0.092 mol H_2O $m^{-2}s^{-1}$ and 0.63 to 0.69 mmol H_2O $m^{-2}s^{-1}$, respectively (Table 3). Although photosynthetic activity was different, pigment content in the leaves, chlorophyll a, b, total chlorophyll, and carotenoid were not significantly different among treatments (data not shown). Interestingly, irrigation at dawn showed significantly higher fresh leaf weight (FLW), dry leaf weight (DLW), and leaf surface area, followed by evening and morning irrigation. The FLW was 76.12, 67.72, and 56.55 grams, the DLW were 7.24, 6.56, and 5.59 grams, and the leaf area was 576.95, 525.28, and 435.37 cm^2 in the dawn, evening, and morning irrigation time, respectively (Table 4). The FLW was also significantly higher using a big headspring than a mini sprinkler, 72.35 and 61.24, respectively. However, different irrigation times and levels did not affect the water content of plants, which was between 88.09 and 88.92 % (Table 4).

Table 3. CO_2 exchange rate (CER), stomatal conductance (g_s), and transpiration rate (Tr) at five months after different irrigation treatments

Factor	CO_2 exchange rate (µmol CO_2 $m^{-2}s^{-1}$)	Stomatal conductance (mol H_2O $m^{-2}s^{-1}$)	Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)
Factor 1 Time of the day (A)			
Dawn	6.11±0.31	0.084±0.005	0.67 ±0.04
Morning	6.10±0.42	0.091±0.006	0.68±0.04
Evening	6.45±0.32	0.083±0.006	0.63±0.04
Factor 2 Type of sprinkler head (B)			
Big	6.81±0.20 a	0.091±0.004	0.69±0.03
Mini	5.62±0.31 b	0.082±0.005	0.63±0.04
Factor 3 Duration (C)			
Normal (6 min)	6.23±0.27	0.081±0.005	0.64±0.04
Short (4 min)	6.20±0.30	0.092±0.004	0.68±0.03
F-test/T-test			
A	ns	ns	ns
B	*	ns	ns
C	ns	ns	ns

Note: The data are means ±SE. Different letters indicate significant differences at $P < 0.05$ between treatments within the same factor.

Table 4. Fresh leaf weight (FLW), dry leaf weight (DLW), leaf area, and whole plant water content (WC) at five months after different irrigation treatments

Factor	Fresh leaf weight (g)	dry leaf weight (g)	Leave area (cm^2)	Whole plant water content (%)
Factor 1 Time of the day (A)				
Dawn	76.12±5.7 a	7.24±0.52 a	576.95±45.7 a	88.82±0.37
Morning	56.55±3.8 b	5.59±0.36 b	435.37±32.3 b	88.61±0.46
Evening	67.72±5.2 ab	6.56±0.49 ab	525.28±42.3 ab	88.10±0.52
Factor 2 Type of sprinkler head (B)				
Big	72.35±4.37 a	6.92±0.4	553.03±35.2	88.92±0.28
Mini	61.24± 4.04 b	6.01±0.4	472.04±32.5	88.09±0.43
Factor 3 Duration (C)				
Normal (6 min)	66.75±4.56	6.43±0.42	513.36±1.29	88.39±0.36
Short (4 min)	66.85±4.16	6.50±0.38	511.71±1.09	88.63±0.38
F-test/T-test				
A	*	*	*	ns
B	*	ns	ns	ns
C	ns	ns	ns	ns

Note: The data are means ±SE. Different letters indicate significant differences at $P < 0.05$ between treatment within the same factor.

Considering the total non-structure carbohydrate (TNC) in plants after receiving different irrigation treatments, we found that the shorter irrigation caused a significantly higher accumulation of TNC than normal irrigation time, which had 4.99 and 4.34 mg glucose equivalent/g DW, respectively (Table 5). In contrast, other irrigation methods showed no statistical difference between 4.45 and 4.70 mg glucose equivalent/g DW.

Table 5. Total non-structure carbohydrate (TNC) in the whole plant after receiving different irrigation treatments for five months

Factors	Total non-structure carbohydrate (TNC) (mg Glucose equivalent/gDW)
Factor 1 Time of the day (A)	
Dawn	4.45 ±0.14
Morning	4.70 ±0.26
Evening	4.74 ±0.20
Factor 2 Type of sprinkler head (B)	
Big	4.60 ±0.16
Mini	4.67 ±0.17
Factor 3 Duration (C)	
Normal (6 min)	4.34 ±0.17b
Short (4 min)	4.99 ±0.14a
F-test/T-test	
A	ns
B	ns
C	*

Note: The data are means ±SE. Different letters indicate significant differences at $P < 0.05$ between treatments within the same factor.

Inflorescence quality to different irrigation treatments: After five months of different irrigation treatments, the quality of the inflorescences did not differ statistically from each other. The inflorescence lengths ranged from 33.84 to 35.64 cm, while the diameter of the flowers ranged from 0.47 to 0.49 cm (Table 6). The number of flowers for each inflorescence ranged from 8 to 8.63. In addition, the total yield and lifespan were not statistically different in providing irrigation at a different time of day or duration, with a yield from 17 to 31.5 inflorescences per 0.5 m^2 and vase life around 11.88 to12.66 days after harvest (Table 6).

Table 6. Inflorescence quality assessment by inflorescence length, flower diameter, flower number per inflorescence, yield and vase life after cut at five months after the different irrigation treatments

Factor	Inflorescence length (cm)	Flower diameter (cm)	Flower number per inflorescence	Total inflorescence per 16 plant (0.5 m^2)	Vase life (day)
Factor 1 Time of the day (A)					
Dawn	34.57 ±0.93	0.49 ±0.008	8.00±0.19	17.25±3.0	12.30±0.43
Morning	35.26 ±1.00	0.48 ±0.007	8.63±0.20	17.0±2.9	12.31±0.50
Evening	34.39 ±0.96	0.48 ±0.011	8.34±0.22	22.0±3.0	12.20±0.40
Factor 2 Type of sprinkler head (B)					
Big	35.64 ±0.65	0.49±0.008	8.41±015	31.5±2.2	12.31±0.37
Mini	33.84 ±0.85	0.47±0.006	8.23±0.19	24.75±3.6	12.23±0.34
Factor 3 Duration (C)					
Normal (6 min)	34.92 ±0.63	0.49±0.006	8.42±0.16	28±2.7	11.88±0.38
Short (4 min)	34.56 ±0.91	0.48±0.008	8.22±0.19	28.25±3.2	12.66±0.32
F-test/T-test					
A	ns	ns	ns	ns	ns
B	ns	ns	ns	ns	ns
C	ns	ns	ns	ns	ns

Note: The data are means ±SE. Different letters indicate significant differences at $P < 0.05$ between treatments within the same factor.

Discussion

Conventional irrigation methods for orchid farmers usually start from 8.00 until the afternoon. However, we found that irrigation during the stomata opening of CAM plants (dawn and evening) can promote growth, leaf area, and dry matter (Table 4). Even though the CER at peak hour was not statistically different between different times of the day (Table 3), the whole night CER must be considered. The dawn irrigation can prolong higher RH around plants almost until midday (Fig. 1a), extending the opening of stomata when light is present at phase II (peak of CO_2 fixation), and also evening irrigation immediately decreases the temperature and increases RH. This caused a favourable condition for stomata opening (phase IV) and might increase CO_2 fixation time. In CAM, epiphytes like *Dendrobium* and *Tillandsia recurvate,* it was found that stomata respond directly to air humidity. Therefore, the increased air humidity promotes stomata opening and CO_2 fixation during the nighttime (Lange and Medina, 1979). Similarly reported in the CAM orchid, *Doritaenopsis* 'NewCandy', the acclimatization under high relative humidity conditions exhibited better physical development, which is leaf size, leaf area, and CER slightly higher at phase II and IV of CAM photosynthesis (Jeon *et al.,* 2006).

Adjusting the irrigation method using a smaller irrigation head and shorter duration can reduce water from up to 71% of normal practice (Table 1). Even though the irrigation water was reduced, it did not affect physical growth (Table 2,3,4) and inflorescence quality (Table 6) when given the treatment for the long term. We found that with shorter irrigation time, the pseudobulb number was statistically increased in five months while at three months it was not different (Table 2). The shorter irrigation time could cause mild drought stress to plants during the first three months and then recovery from drought by the rain during the summer monsoon (Fig. 1e). There was a report that drought promotes shoot budding after re-watering like it was reported in European beech seeding (Hájíčková *et al.,* 2017). The accumulation of TNCs also did not decrease as a result of water reduction. However, it was slightly higher in treatments that received lower water volume in shorter time irrigation (Table 5). It represents that the carbohydrate pool did not get depleted from the water reduction. As reported in *Averrhoa carambola*, the increase in TNC could be because of the mild drought that promotes carbohydrate accumulation (Wu *et al.,* 2017). The pseudobulb organ is the key organ acting as a reservoir during water deficit. It helps plants maintain water status and physiological activity during the first three months. Moreover, long-term water reduction did not affect the water status in long-term cultivation because the water content was not significantly different among treatments (Table 4). *Dendrobium* showed high tolerance to drought, even withholding water for a month, still maintaining relative water content in tissue higher than 95 % (Yang *et al.,* 2016).

Neither reduced irrigation water nor different irrigation times affected the quality and grade of inflorescence yield in a growing area of 0.5 cm^2 (Table 6). However, further observation is needed on a larger scale because irrigation in the evening and using a larger irrigation head resulted in a higher yield of inflorescence. While using the larger head yielded more than the mini sprinkler, the difference was not statistically significant. Additionally, we observed that the larger irrigation head resulted in a higher grade for longer inflorescences. This may be attributed to the larger sprinkler head's ability to cover a wider radius and deliver more water per minute, thereby maintaining a higher relative humidity compared to a mini sprinkler.

This study will be advantageous for orchid cut-flower producers as it provides reliable information that reducing water usage during irrigation, even in the dry season, will not have a long-term impact on growth and the quality and quantity of inflorescences. This benefits orchid producers by reducing costs and indirectly benefits the community by increasing the availability of freshwater during dry periods. Furthermore, these findings suggest that changing the irrigation schedule from the traditional morning time to dawn or evening can also enhance growth and increase the quantity of inflorescences. This could encourage farmers to adopt and integrate IoT technology into their farms, thereby addressing the shortage of human resources in the near future.

A reduction of approximately 30% in irrigation water for orchids can be achieved either by reducing the duration of irrigation or by changing the type of sprinkler head used. Our findings indicate that even during the dry

season, long-term cultivation of orchids is not significantly affected in terms of growth and the quality of inflorescences. Although slight differences were found between each method, a reduction in irrigation time was recommended because it was convenient and did not cost the farmer more. Furthermore, irrigation at dawn and evening showed better growth and yield than the conventional method, which is irrigation from morning until noon.

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Quantifying the effects of drought stress on cucumber genotypes differing in membrane integrity

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Abstract

Cucumber yield is profoundly influenced by soil moisture, with drought representing a pivotal factor. This study evaluated four cucumber lines (CBECS-37, CBECS-38, CBECS-19, and CBECS-7) within a split-plot experimental design comprising four replications. Irrigation occurred once every seven days, spanning from sowing to the flowering stage. Drought stress was imposed at two critical stages: from flower bud initiation to harvesting (withheld irrigation for 25 days) and from flowering to harvesting (withheld irrigation for 15 days). Morphological and physiological parameters, including plant height, primary branch count, days to first male and female flower appearance, total soluble solids (TSS), relative water content (RWC), chlorophyll content, leaf electrolyte leakage, and malondialdehyde, were assessed 15 days after drought stress. Results indicated greater membrane damage during the flower bud initiation to the harvesting stage (404.5%) compared to the flowering to the harvesting stage (304.6%). Thus, drought stress during flower bud initiation to harvesting was more critical. CBECS-7 demonstrated the highest tolerance to drought conditions, displaying superior outcomes in primary branches, plant height (20.6%), chlorophyll a (16.7%), chlorophyll b (53.4%), total chlorophyll (26.7%), and RWC (6.7%). CBECS-7 exhibited increased chlorophyll content, enhanced photosynthetic activity, robust vegetative growth, and prolific flower and fruit production. These findings establish CBECS-7 as a drought-tolerant line during flower bud initiation to harvesting. In conclusion, this study underscores the critical nature of the flower bud initiation to the harvesting stage and identifies CBECS-7 as a drought-tolerant cucumber line.

Key words: Cucumber, drought stress, lines, tolerance, TSS, plant height, chlorophyll

Introduction

Cucumber (*Cucumis sativus* L.) is a monoecious annual crop, ranking as the second most cultivated vegetable globally after watermelon. In Asia, it stands as the fourth most significant vegetable crop, offering vital vitamins and minerals, notably vitamin A. Cucumber is renowned for its cooling properties, preventing digestive issues and aiding in jaundice control (Nandkarni and Prakash, 1927). This low-calorie vegetable (15 calories/100g) is 95% water, an ideal hydrator, rich in antioxidants, potassium, vitamin K (Pandey *et al.,* 2020), vitamins 'B' and 'C,' plus minerals like calcium, phosphorus, iron, and potassium. Its antioxidants combat harmful free radicals, preventing diseases (Pandey *et al.,* 2020). Nutritionally, cucumber offers carbohydrates (3%), protein (1%), total fat (0.5%), and fiber (1%) per 100g (Nwofia *et al.,* 2015).

India cultivates cucumbers across 0.122 million ha, yielding 1.71 MT (Indiastat, 2022), with increased yield as the primary target (Devi *et al.,* 2022). However, abiotic stresses, especially drought challenge cucumber production, affecting plant growth and fruit quality (Chmielewska *et al.,* 2016; Aujla *et al.,* 2007). Cucumber's global importance, nutritional value, and resilience underscore its significance in agriculture.

The cucumber is a relatively shallow-rooted crop and it is susceptible to drought. Typically, cucumber requires much water, particularly at the fruiting stage (Swiader *et al.,* 2002). Reduced plant growth and yield under drought stress are associated with phytohormones and reactive oxygen species (ROS) signaling changes, plant hydraulic status, and osmotic adjustment (Khan *et al.,* 2015). To mitigate the damage from ROS, plants evolve enzymatic antioxidants like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), as well as non-enzymatic antioxidants like ascorbic acid and glutathione (Xu *et al.,* 2008).

Wahb-Allah *et al.* (2011) studied the impact of irrigation regime on tomato growth and yield and reported that tomato growth parameters and yield decreased significantly at a lower soil moisture level than its fully irrigated conditions. In muskmelon, RWC was found to be significantly associated with yield (Barzegar *et al.,* 2017). Similarly, Rehman *et al.* (2023) showed that chlorophyll content was significantly decreased in melon genotypes under drought stress conditions, and the highest decrease was observed in sensitive genotypes. Ansari *et al. (*2018) found in muskmelon that drought stress altered the activity of antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX).

Due to its osmotic effect, drought has been widely studied in many crops, but cucumber has not been extensively investigated

in relation to drought despite seedlings being more susceptible to water changes than mature plants.

This research aims to assess the impact of different irrigation regimes, specific cucumber lines, and their interplay on the growth and physiological aspects of cucumber genotypes.

Materials and methods

The field experiment was carried out during the summer of 2023 at the Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The details of the line are given in Table 1.

Table 1. List of genotypes and their place of collection

S.No	Lines	Place of collection
1.	CBECS-37	Namakkal, Tamil Nadu, India
2.	CBECS-38	Vadalur, Tamil Nadu, India
3.	CBECS-19	Namanasamuthiram, Tamil Nadu, India
4.	CBECS-7	Pirattiyur, Tamil Nadu, India

Crop husbandry: The main field was ploughed thrice with 5 tyre, 3 tires, and cultivator. Pits of 45 cm diameter and 30 cm depth were formed at a spacing of 1.5 × 1.5m. Three seeds per pit were sown. The cultural and management practices were followed as per the package of practices recommended by the crop production guide.

Drought stress imposition: The experiment was conducted in a split-plot design with four replications. The main plots were irrigation regimes (T_1: irrigated once in seven days; T_2: withholding irrigation for 25 days from flower initiation to harvest stage, and T_3: withholding irrigation for 15 days from flowering stage to harvest stage) and subplots were lines (CBECS-37, CBECS-38, CBECS-19, and CBECS-7). The crop was irrigated once in seven days from the sowing to the flowering stage. After that, drought stress was imposed by withholding water. Drought stress during flower initiation to the harvest stage was imposed by withholding irrigation water for 25 days from flower bud initiation. Similarly, drought stress was imposed during the flowering to harvesting stage for 15 days.

Morphological traits: The morphological traits like plant height and number of primary branches were recorded at maturity. However, the days to the first male and female flower appearance were recorded at two days intervals. The physiological traits namely relative water content (RWC), chlorophyll content, leaf electrolyte leakage and malondialdehyde (MDA) contents, were recorded in the third leaf from the top on 15th day after drought stress. TSS (^{o}Brix) was recorded in fruits at harvest.

Total l (^{o} Brix): A hand refractometer was used for assessing TSS.

Relative water content: The procedure given by Barrs and Weatherley (1962) was used to estimate the RWC:

$$RWC = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}}$$

Leaf electrolyte leakage: According to Liu *et al.* (2011) methodology, electrical conductivity was used to assess the stability of the cell membrane. The following formula was used for calculation:

$$\text{Electrolyte leakage (\%)} = \frac{\text{Initial electrical conductivity}}{\text{Final electrical conductivity}} \times 100$$

Chlorophyll content: The method given by Lichtenthaler and Wellburn (1983) was used to extract pigments from leaf tissue, including chlorophyll *a*, chlorophyll *b* and total chlorophyll content.

Malondialdehyde (MDA): The lipid peroxidation was determined by measuring the breakdown product MDA as suggested by Heath and Packer (1968). Using 0.1 % TCA, 0.5 g of leaf sample was extracted and centrifuged at 13,000 rpm for 20 min. After centrifugation, the supernatant was taken from 0.5 mL and added to 0.5 % TBA in 20 % TCA, which was later kept in a hot water bath for 30 mins at 95 ºC, then placed in a water bath to cool. The absorbance was calculated using the extinction value of 155 mM^{-1} cm^{-1} and estimated at 523 and 600 nm.

Statistical analysis: Data were assessed using R- program (Version 4.3.1) using the Agricolae package 1.2 -8. The Analysis of Variance was performed to determine the significance of the variance. Least significance difference (LSD) at 0.05 significance level was used to compare the difference between treatment means.

Result and discussion

Plant height: There was no significant (P<0.05) differences among the lines; however, the effect of irrigation regime and interaction of irrigation regimes and lines for plant height was significant (Table 2). Among the irrigation regime, drought stress during flower initiation to maturity decreased the plant height (96.9 %) over irrigated control. However, drought stress during flowering to maturity decreased plant height by (96.2 %). Among the lines, CBECS-7 had the highest plant height than other lines and the percentage increased over CBECS-37 (89.7 %), CBECS-38 (92.5 %), CBECS-19 (80.8 %). In interaction, the line CBECS-7 exposed to drought stress from flowering to harvest had the highest plant height (99.6 %) and CBECS–19 had the lowest plant height (99.5 %) during flowering to maturity. Drought stress has been shown to negatively impact the plant height of melon (Kusvuran, 2012) and wild barley genotypes have also been found to cause a 31 % decrease in the plant height. It might be due to the morpho-anatomical changes associated with tolerant genotypes under drought stress, which might helped the plants to complete their physiological and metabolic activities and eventually affect the growth rate.

Table 2. Plant height and number of primary branches of cucumber genotypes under different irrigation levels

Genotypes (G)	Plant height (cm)			Primary branches (plant^{-1})		
	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)
CBECS-37	182.50	175.25	174.25	3.50	2.50	2.50
CBECS-38	191.75	182.50	179.25	3.00	2.25	2.50
CBECS-19	161.00	155.00	161.25	3.25	2.50	2.25
CBECS-7	199.00	195.50	198.25	3.25	2.50	3.00
Mean	183.56	177.06	178.25	3.25	2.44	2.56
CD (0.05)						
T		NS			0.59**	
G		5.35**			NS	
T x G		NS			NS	

NS=Not Significant, **= Significance at P<0.05

Number of primary branches: There was no significant (P<0.05) differences among the lines and interaction of irrigation regimes and lines; however, the effect of irrigation regime was significant (P<0.05) (Table 2). Among the irrigation regime, drought stress during flower initiation to maturity decreased the primary branches (95.9 %) over irrigated control. However, drought stress during flowering to maturity increased primary branches by (96.2 %). Among the lines, CBECS-38 had the highest primary branches than other lines and the percentage increased over CBECS-37 (100 %), CBECS-19 (100 %), CBECS-7 (97.2 %). In interaction, the line CBECS-38 exposed to drought stress from flowering to harvest had the highest primary branches (109.3 %) and CBECS–19 had the lowest primary branches (100 %) during flowering to maturity. Parveen *et al.* (2019) observed that there was a maximum reduction in the number of branches in Kashi Amrit (14.33).

Days to first male and female flower appearance: Among the irrigation regimes, drought stress during flower initiation to maturity stage and flowering to maturity stage there was no significant difference in days to first male and female flower appearance over irrigated control. Lesser number of days to first male flower appearance was recorded in CBECS - 19 under both drought conditions. Among the lines, CBECS-37 and CBECS - 7 taken lesser number of days to first female flower appearance under drought stress condition. In interaction, there was no significant difference between the lines and irrigation regime. Early flowering had been affected by drought stress, which resulted in flower abscission and limited fertilisation (Lamin-Samu *et al.,* 2021) and drought stress increased the dropping of flowers and young fruits during flowering and fruit set stages, which has a significant negative impact on the final crop.

Table 3. Days to first male and female flower appearance under control and drought stress condition

Genotypes (G)	Days to first male flower		Days to first female flower			
	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)
CBECS-37	35.83	34.50	35.00	40.75	46.00	43.00
CBECS-38	35.00	35.17	34.67	40.25	47.50	41.00
CBECS-19	32.42	31.58	31.67	45.00	50.00	42.50
CBECS-7	34.92	32.75	32.75	41.00	47.25	40.50
Mean	34.54	36.17	33.83	41.75	47.69	41.75
CD (0.05)						
T		NS			2.28**	
G		0.76**			1.88**	
T at G		NS			NS	

NS=Not Significant, **= Significance at P<0.05

Total soluble solids (TSS): There was a significant differences among the irrigation regime, lines and interaction of irrigation regimes and lines for TSS (Table 4). Among the irrigation regimes, drought stress during flower initiation to maturity fruit set was hindered due to flower dropping. However, drought stress during flowering to maturity increased TSS by (115.5 %). Among the lines, CBECS-7 had the highest TSS than other lines and the percentage increased over CBECS-37 (84.2 %), CBECS-38 (83.4 %), CBECS-19 (95.4 %). In interaction, the line CBECS-7 exposed to drought stress from flowering to harvest had the highest TSS (112 %) over the control and CBECS-38 had the lowest TSS (84 %) over the control during flowering to maturity. Fruit TSS was increased during stress condition in tomato (Nora *et al.,* 2012). This phenomenon results from a rise in phloem sap concentration and a decrease in its flux, with the phloem flux being what causes the growth in tomato size (Guichard *et al.,* 2001). Meanwhile, a decrease in its flow combined with an increase in the concentration of sugar leads to smaller fruits with a higher dry matter content.

Relative water content: There was a significant differences among the irrigation regime, lines and interaction of irrigation regimes and lines for RWC (Table 4). Among the irrigation regimes, drought stress during flower initiation to maturity decreased the RWC (91 %) over irrigated control. However, drought stress during flowering to maturity decreased the RWC (80.9 %). Among the lines, CBECS-7 had the highest RWC over other lines and the percentage increased over CBECS-37 (82.3 %), CBECS-38 (85.8 %), CBECS-19 (84.3 %). In interaction, the line CBECS-7 exposed to drought stress from flowering to harvest had the highest RWC (87.08 %) over the control and CBECS-37 had the lowest RWC (77.6 %) over control during flowering to maturity. Patane *et al.* (2016) observed that in okra and tomato, the RWC was reduced, respectively with imposing drought tolerance. These findings suggest that these drought-resistant cucumber lines have a significant adaptation mechanism because they can maintain low leaf transpiration rates and a higher RWC, which leads to an osmotic adjustment by proline accumulation.

Table 4. TSS and RWC of cucumber genotypes under different irrigation levels

Genotypes (G)	TSS (°Brix)			RWC (%)		
	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)
CBECS-37	3.78	-	4.50	90.08	77.91	66.08
CBECS-38	3.75	-	4.45	87.08	72.88	56.28
CBECS-19	4.40	-	4.98	85.69	72.70	55.62
CBECS-7	4.63	-	5.20	88.95	73.92	63.33
Mean	4.14	-	4.78	87.95	74.35	60.33
CD (0.05)						
T	0.176**			4.30**		
G	0.120**			8.15**		
T at G	0.251**			NS		

NS=Not Significant, **= Significance at P<0.05 '- '= fruit was not developed.

Leaf electrolyte leakage (EL): There was significant differences among the irrigation regime, lines and interaction of irrigation regimes and lines for EL (Fig. 1). Among the irrigation regimes, drought stress during flower initiation to maturity had increased EL by (304.6 %) over irrigated control. However, drought stress during flowering to maturity increased EL by (404.5 %). Among the lines, CBECS-7 had the highest EL over other lines and the percentage increased over CBECS-37 (82.9 %), CBECS-38 (66.4 %), CBECS-19 (72.7 %). In interaction, the line CBECS-7 exposed to drought stress from flowering to harvest stage had the highest EL (384 %) and CBECS-38 had the lowest EL (352 %) during flowering to maturity over the control. A decrease in membrane integrity results in enhanced ion leakage, which is indicated by an increase in electrical conductivity. Plants that are subjected to stresses like drought have this trait by nature (Korkmaz *et al.,* 2007).

Chlorophyll content: There was significant differences among the irrigation regime, lines and interaction of irrigation regimes and lines for chlorophyll a, chlorophyll b and total chlorophyll content (Table 5). Among the irrigation regimes, drought stress during flower initiation to maturity decreased the chlorophyll a content (76.4 %) over irrigated control. However, drought stress during flowering to maturity decreased chlorophyll a content by (71.5 %). Among the lines, CBECS-7 had the highest chlorophyll

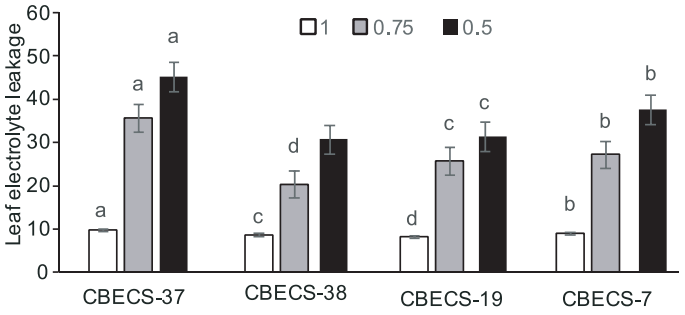

Fig. 1. Leaf electrolyte leakage of cucumber genotypes under different irrigation levels.□irrigated once in seven days; ▒ withholding irrigation for 25 days from flower initiation to harvest stage, and ■ withholding irrigation for 15 days from flowering stage to harvest stage

a content then other lines and the percentage increased over CBECS-37 (94.7 %), CBECS-38 (85.7 %), CBECS-19 (98.8 %). Among the genotype interaction, the line CBECS-7 had the highest chlorophyll a content (74.7 %) then other treatment during flowering to harvest and CBECS-38 had the lowest chlorophyll a content (59.1 %) drought stress during flowering to maturity. Among the irrigation regimes, drought stress during flower initiation to maturity decreased the chlorophyll b content (90.5 %) over irrigated control. However, drought stress during flowering to maturity decreased chlorophyll b content (77.8 %). Among the lines, CBECS-7 had the highest chlorophyll b content then other lines and the percentage increased over CBECS-37 (91.3 %), CBECS-38 (90.8 %), CBECS-19 (40.6 %). Among the genotype interaction, the line CBECS- 7 had the highest chlorophyll b content (90.4 %) then other treatment during flowering to harvest and the line CBECS-19 had the lowest chlorophyll b content (47.7 %), drought stress during flowering to maturity. Among the irrigation regimes, drought stress during flower initiation to maturity decreased the total chlorophyll content (85.6 %) over irrigated control. However, drought stress during flowering to maturity decreased total chlorophyll content (82.1 %). Among the lines, CBECS-7 had the highest total chlorophyll content over other lines and the percentage increased over CBECS-37 (83 %), CBECS-38 (75.8 %), CBECS-19 (74 %). Among the genotype interaction, the line CBECS- 7 had the highest total chlorophyll content (92.7 %) than other treatments during flower initiation to maturity and the line CBECS-38 had the lowest total chlorophyll content (76.5 %), drought stress during flowering to maturity. This decreases may be due to oxidative stress, decreased chlorophyll synthesis and increased chlorophyll breakdown (Kumar and Singh, 1996). Asharaf and Mahmood (1990) reported drought stress would lower chlorophyll b concentrations more than chlorophyll a and under water stress conditions, plants' total chlorophyll content was reduced.

Malondialdehyde (MDA): There was significant differences among the irrigation regime, lines and interaction of irrigation regimes and lines for MDA (Fig. 2). Among the irrigation regimes, drought stress during flower initiation to maturity had increased the MDA (109.8 %) over irrigated control. However, drought stress during flowering to maturity increased MDA by 120.7 %. Among the lines, CBECS-37 had the highest MDA over other lines and the percentage increased over CBECS-38 (85.8 %), CBECS-19 (85.9 %), CBECS-7 (89.9 %). Among the genotype interaction, line CBECS- 37 had the highest MDA (141.6 %) than other treatments during flowering to maturity and line CBECS-19 had the lowest MDA (114.9 %), drought stress during flower initiation to maturity. In this study, MDA was increased under drought stress. Numerous investigations showed that there was a link between electrolyte leakage and MDA. MDA, a secondary oxidative product, is formed when the accumulated ROS degrades the polyunsaturated lipids in the cell membranes (Ayala *et al.,* 2014).

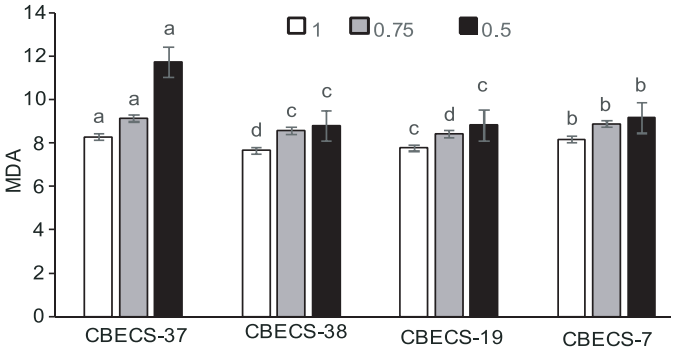

Fig. 2. Malondialdehyde activity of cucumber genotypes under different irrigation levels. □irrigated once in seven days; ▒ withholding irrigation for 25 days from flower initiation to harvest stage, and ■ withholding irrigation for 15 days from flowering stage to harvest stage.

Overall, based on this study, it could be stated that advances in biotechnology, such as genetic engineering and gene editing techniques like CRISPR-Cas9, offer the potential to enhance drought tolerance in crops. The CRISPR/Cas9 system is a cutting-edge technology that enhances crop improvement by producing high-yielding, high-quality resistant plants to biotic and abiotic stresses. However, challenges include selecting genes for mutations, genome sequencing, and delivering CRISPR-Cas agents into plant cells. Creating a universal and effective genetic transformation and regeneration system for vegetable crops remains challenging due to the need for precise alterations and genome sequencing. Identifying and modifying specific genes responsible for drought response is possible, thereby creating more resilient and water-efficient plant varieties. A multidisciplinary approach involving genetics, biochemistry, biotechnology, physiology, plant breeding, and crop science will be appropriate to evolve superior drought-resistant genotypes. Breeding programs focused on developing climate-resilient crop varieties could lead to the cultivation of drought-tolerant

Table 5. Chlorophyll a, Chlorophyll b, Total chlorophyll of cucumber genotypes under different irrigation levels

Genotypes (G)	Chlorophyll a (mg g^{-1})			Chlorophyll b (mg g^{-1})			Total chlorophyll (mg g^{-1})		
	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)	T_1 (100 %)	T_2 (75 %)	T_3 (50 %)
G_1 - CBECS-37	1.07	0.92	0.86	0.61	0.54	0.49	1.86	1.46	1.36
G_2 - CBECS-38	1.15	0.75	0.68	0.60	0.55	0.47	1.65	1.37	1.27
G_3 - CBECS-19	1.19	0.92	0.86	0.30	0.28	0.14	1.54	1.35	1.30
G_4 - CBECS-7	1.19	0.93	0.89	0.63	0.60	0.57	1.97	1.84	1.80
Mean	1.15	0.88	0.82	0.54	0.49	0.42	1.76	1.51	1.43
CD (0.05									
T	0.01**			0.01**			0.01**		
G	0.01**			0.01**			0.01**		
T at G	0.02**			0.02**			0.02**		

**= Significance at $P<0.05$

plants that can thrive under changing environmental conditions. Drought tolerance is a complex trait influenced by multiple genes and environmental factors. Developing plants with high drought tolerance involves a deep understanding of the genetic and physiological mechanisms underlying this trait.

The results of the present study indicate that the drought exerted different effects on cucumber lines under different levels of drought conditions. It may be concluded that CBECS-7 shows maximum tolerance to imposed drought conditions with better results in plant height, TSS, chlorophyll a, chlorophyll b, total chlorophyll, leaf electrolyte leakage, RWC followed by CBECS-37 in MDA. Among the different genotypes evaluated, CBECS-7 was the best genotype for the drought conditions, as it showed maximum chlorophyll content, which increases photosynthetic activity and results in good vegetative growth with the maximum number of flowers and fruits.

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