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TOXIGENIC POTENTIAL OF *Alternaria porri* ON ONION (*Allium cepa* L.)

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ABSTRACT

Onion (*Allium cepa* L.) is the important vegetable crop. Under favourable condition onion is attacked by various diseases. Among these diseases, purple blotch caused by *Alternaria porri* is one of the predominant disease of onion. Toxigenic potential of *Alternaria porri* was assessed against seed germination, seedling growth of onion and antibacterial activity against some phytopathogenic bacteria. Diseased samples were collected from the Department of Horticulture, Chilli and Vegetable Research Unit, Dr. P.D.K.V., Akola and farmers field at village Shivapur, Taluka-Barshitakali, District-Akola during the year 2015, resortal for isolation and *Alternaria porri* was observed as a predominant fungus in the diseased samples collected from different locations. Isolation frequency of *Alternaria porri* was observed in the range of 45 to 60 per cent. The seed germination, seedling growth of onion were adversely affected due to the culture filtrates of *Alternaria porri*. The metabolited produced by *Alternaria porri* inhibited the growth of *Bacillus subtilis* and was found ineffective against *Psudomonas fluorescens*, *Xanthomonas axonopodis* pv. *Citri* and *Erwinia caratovora*.

(Key words: *Alternaria porri,* purple blotch, *Bacillus* subtilis, *Psudomonas fluorescens*)

INTRODUCTION

Onion (*Allium cepa* L.) is an important bulb crop of India belonging to family Alliaceae. It is one of the most important winter vegetable crop of India. It is a nutritious vegetable and contains a good amount of Vitamin A and C, rich source of minerals (calcium, magnese and iron) and dietary fibres. It is cool season crop which thrives best in relative cool and moist climate. Purple blotch disease, which is caused by *Alternaria porri* (Ellis) Cif., is one of the most destructive disease restricted to the genus *Allium* and widespread in many regions of the world (Cramer,2000). Purple blotch disease of onion cause significant reduction in foliar production (Utikar and Padule,1980) and bulb yield (Gupta and Pathak, 1988). The disease is more severe on seed crop as compared to bulb crop sometimes causing a 100% loss of onion seed production. (Singh *et al.*,1992). Among these, purple blotch caused by '*Alternaria porri'* is one of the major disease of onion. The name purple blotch for this disease was proposed by Nolla (1927). He named the causal organism as *Alternaria allil* which was later amended to *Alternaria porri*. Ajrekar (1920) made first report on leaf spot and blight disease on onion in Bombay and attributed it to *Alternaria* spp. The losses about 50-100% due to purple blotch of onion have been reported by Shahanaz *et al.* (2007). The pathogen *Alternaria porri* destructs the leaf tissue which hinder the stimulus for bulb initiation and delay in bulbing and maturation. Sever attack on flowering, onion can completely girdle flower stalks with necrotic tissue, causing their collapse and total loss of seed production capacity (Agale *et al.*, 2014). Germination as well as seedling growth (radical and plumule elongation) were adversely effected up to 80% when the seed were treated with fungal culture filtrate (Madhavi *et al.*, 2012). The influence of environment on incidence of disease was studied by some workers from different part of countries and reported that high rainfall and high humidity favoured the disease development. *Alternaria porri* on onion occurred following a long period relative humidity > 90% or dew deposition and temperature ranges between 20-25^{0}C (Gupta and Pathak, 1986; Everts and Lacy, 1996).

MATERIALS AND METHODS

Collection of diseased samples

Onion leaves infected with Alternaria purple blotch were collected from the Department of Horticulture, Chilli and Vegetable Research Unit, Dr. P.D.K.V., Akola and farmers field at village Shivapur, Taluka-Barshitakali, District-Akola during the year 2015. Based on symptoms, microscopic examination of diseased samples association of the pathogen as *Alternaria porri* was recorded.

Glassware, plasticware and other materials

Petri plates, glass petri dishes, conical flasks, test tubes, blotter paper and roll paper towel were used in the present studies.

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Preparation of potato dextrose agar

Potato dextrose agar (PDA) medium was used for isolation and maintenance of cultures.

The medium was prepared with following ingredients

Peeled potatoes	-	200 gm
Dextrose	-	20 gm
Agar agar	-	20 gm
Distilled water	-	1000 ml

Disinfection / sterilization of laboratory materials

To detect the fungi on leaves, the plates were washed with cleaning powder under running water, dried and then disinfected with denatured spirit. However, glass plates were sterilized in hot air oven at 180^0 C for 1 hr. before use.

Isolation of pathogen by tissue isolation method

Infected leaf samples were cut into small pieces with sterilized blade and disinfected with sodium hypochloride (0.2%) solution for two minutes. Pieces were washed with three changes of sterilized distilled water and bits after dried on sterilized filter paper and around flame of spirit lamp were placed on solidified PDA medium in plate. Each plate contained five bits. The plates were incubated at room temperature (28 ± 2^0C). All these operations were carried out aseptically. The plates were examined regularly. Colonies were developed around the each bit were examined and sub cultured. Based on morphological characters and published literature the fungus was identified as *Alternaria porri*. The pure culture was transferred on PDA slants and maintained for further studies.

Purification and maintenance of fungal culture

Culture was purified by following hyphal tip method (Vincent, 1947) and culture obtained was maintained on potato dextrose agar (PDA) medium slants at room temperature by adopting subsequent sub culturing at periodical, regular intervals. Seven days old culture was used for further studies.

Pathogenicity test by spray inoculation method

Pathogenicity of fungus was proved by Inoculation of seventeen days old onion seedlings. The fungal suspension was prepared (4×10^4 spores ml^{-1} of water) from seven days old culture and used for inoculation. The seedlings were inoculated by automizing them with fungal suspension. The inoculated and un inoculated seedlings were covered with polyethylene bag for 48 hrs to provide humidity and favourable condition for disease development. Irrigated the seedlings daily to maintain moisture and watched for appearance of disease symptoms on leaves. Chlorotic oval eye shaped lesions were observed after three days of inoculation and full grown purple oval shaped lesion observed after ten days of inoculation. Further the lesion coalesced and spread rapidly on leaf blade and affected leaves showed drying from tip downward after twenty one days of inoculation. Re-isolation were made from infected part of inoculated leaves, yielded the same pathogen, whereas control plants remained healthy.

Preparation of culture filtrates of *Alternaria porri*

To study the phytotoxic effect of fungal culture filtrates on seed germination and seedling growth of onion, the fungus was cultured on Czapex-dox broth for 30 days. At the day of incubation, the fungal culture filtrates were collected under aseptic conditions and tested for phytotoxicity against seed germination and seedling growth of onion. To assess the effect of culture filtrates of *Alternaria porri* on seed germination and seedlings growth of onion, roll towel method was used.

Preparation of fungal metabolites produced by *Alternaria porri*

Alternaria porri was cultured on yeast extract sucrose medium (YES) for 30 days at room temperature as stationary culture. At the day of incubation, the fungal culture mat was separated by filtration through Whatman No.1 filter paper and the filtrates were collected. The culture filtrates was extracted with diethyl ether. The extract was evaporated to dryness on water bath avoiding excess heat. The residues were dissolved in 1.0 ml of diethyl ether, transferred to glass vial and preserved for testing of antibacterial activity against some selected phytopathogenic bacteria.

RESULTS AND DISCUSSION

Table 1. Association of *Alternaria porri* and other fungi recorded in collected diseased samples

Sr. No.	Locations	No. of bits used for isolation	No. of bits yielded fungi	Fungi obtained (No. of bits)		Occurrence of fungi (%)		
				Alternaria porri	other fungi	*Alternaria porri*	Other fungi	Name of other Fungi
1.	Shivapur Department of	30	24	18	6	60	24	*Fusarium* spp.
2.	Horticulture, Dr. P. D. K.V., Akola Chilli and Vegetable	25	20	10	7	40	28	*Rhizoctonia bataticola* *Curuvularia lunata*
3.	Research Unit, Dr. P. D. K.V., Akola	20	15	9	6	45	30	

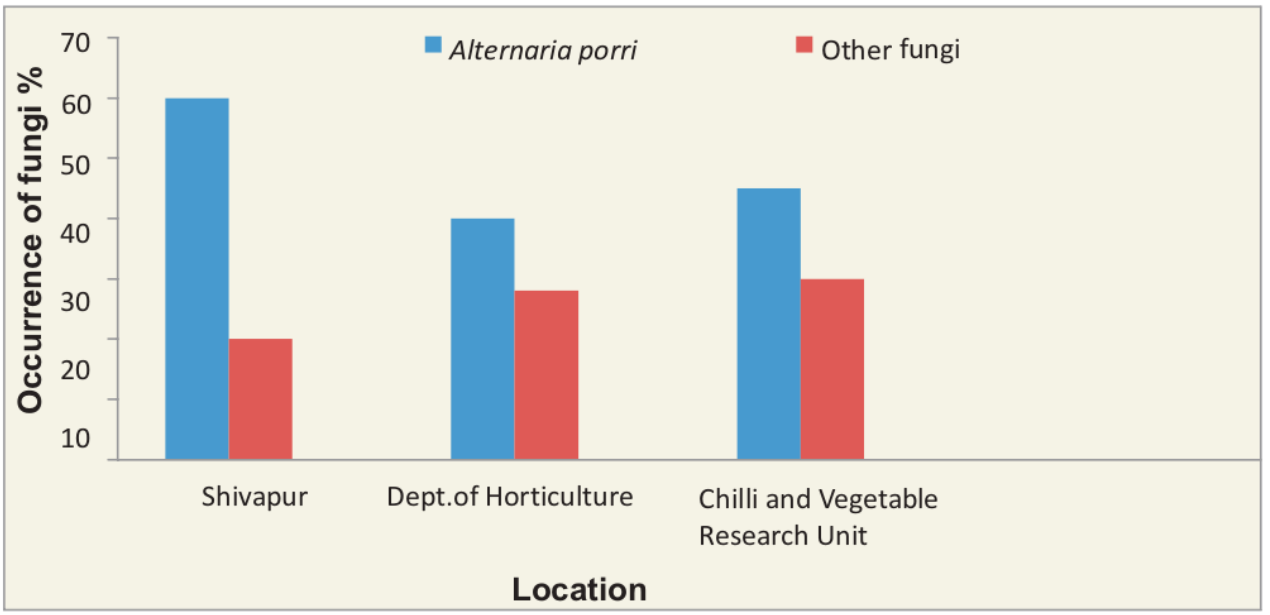

Fig.1. Association of *Alternaria porri* and other fungi in diseased samples

The data regarding frequencies of isolation are given in Table 1. It is seen that the majority of bits yielded the fungus *Alternaria porri* in the range of 40-60 per cent. Hence, *Alternaria porri* was observed to be predominant fungus in collected diseased samples. However, the association frequency of other fungi was recorded between the range of 24-30%. Wadile (2011) and Umbarakar (2013) have reported the association frequency of *Alternaria carthami* and *Alternaria brassicicola* in the range of 40-64% and 33-40% in the sunflower and cauliflower respectively.

Pathogenicity test

The diseased symptoms were noticed within 7 to 21 days on the tip of the older leaves as small, whitish, sunken, oval shaped lesion which later on became elliptical or oblong, which brown to purple at the centre and surrounded by a light brown area. Further the lesions coalesce and spread rapidly on leaf blade and affected leaves showed complete drying from tip downward within 21 day. These leaves were collected and used for reisolation yielded same pathogen, identity, confirmed and it is evident that the *Alternaria porri* was found pathogenic. Agale *et al.* (2012), Pandotra (1964) and Sekar *et al.* (2017) established the pathogenicityof *Alternaria porri* by following Koch's postulate.

Symptoms of *Alternaria porri* in naturally infected field

Brown lesions with reddish-purple margins were noticed. The purple blotch symptoms development starts from the older leaves as small, whitish, sunken, oval shaped lesion which later on became elliptical or oblong, brown to purple at the centre and surrounded by a light brown area. Further the lesion coalesce and spread rapidly on leaf blade and effected leaves showed drying from tip downward. Madhavi *et al.* (2012) and Agale *et al.* (2014) observed the similar symptoms of purple blotch of onion on various parts of the plants viz., leaves, stem, seeds, flower, bulb etc.

Plate 1. Pathogenecity test

Plate 2. Symptoms of leaf blight and purple blotch of onion caused by *Alternaria porri*

Phytotoxicity of culture filtrates of *Alternaria porri* on seed germination and seedlings growth of onion

To study the phytotoxic effect of culture filtrate on seed germination and seedling growth of onion the presoaked seeds in fungal culture filtrates were placed on moistened roll towel paper. Germination and seedling growth were adversely affected by 78% and 42.84% with reduction in seedling vigour index (44.77) when the seeds were treated with culture filtrates of *Alternaria porri*. Results on the same line have been reported by Madhavi *et al*. (2012). They reported that the germination as well as seedling growth were adversely affected upto 80% when the seeds were treated with culture filtrates of *Alternaria porri*. The inhibition or root length of onion seedling (80%) and chlorotic spot (5.00 mm) on onion leaves after three days due to sixteen days old culture filtrates of *Alternaria porri* with pH 8 was also shown by Khare and Goswami (1996). Suemitsu *et al.* (1995) observed that poriitoxin produce by *Alternaria porri* had an inhibitory effects on the seedling growth of stone leek and lettuce. The phytotoxic compounds consist of metaboliteds phyrotoxin sulfonic acid with an isoindoline skeleton. Shirurkar *et al*. (2014) reported the same results, who reported that culture filtartes of *Aspergillus niger* adversely affected the seed germination of local and African tall by 75% and Amber by 60% with the reduction in vigour index 372, 720 and 1408 in the varieties of maize respectively.

Table 2. Effect of culture filtrates of *Alternaria porri* on seed germination and seedlings growth of onion

Sr. No.	Seed germination (%)	Seedling length (cm)	Seedling Vigour Index
1. Treated	22.00	2.03	44.77
2. Untreated	88.00	4.75	380

Effect of fungal metabolites of *Alternaria porri* on bacterial pathogens

The metabolites produced by *Alternaria porri* were screened for antibacterial potential against some phytopathogenic bacteria using seeded plate technique. The metabolites adversely affected the growth (17.4 mm) of *Bacillus subtilis*. However, the metabolites were found ineffective against *Psudomonas fluorescens*, *Xanthomonas axonopodis* pv. *citri* and *Erwinia caratovora.* The present results are similar with the findings of Madhavi *et al.* (2012). They reported the metabolites produced by *Alternaria porri* adversely affected the growth of *Bacillus subtilis* (240 mm^2), *Escherichia coli* (235 mm^2) and *Psudomonas aeruginosa* (120 mm^2) but ineffective against *Psudomonas fluorescens* and *Xanthomonas axonopodis* pv.*citri*.

Table 3. Antibacterial activity of metabolites produced by *Alternaria porri* against some phytopathogenic bacteria

Bacteria	Zone of inhibition (mm)
Bacillus subtilis	17.4
Psudomonas fluorescens	00
Xanthomonas axonopodis pv. *citri*	00
Erwinia caratovora	00

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EFFECT OF TIME OF SOWING AND ROW SPACING ON GROWTH, YIELD AND ECONOMICS OF SOYBEAN (*Glycine max* L.)

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ABSTRACT

A field experiment was conducted during *kharif* season of 2020 at the College Farm, College of Agriculture, N.A.U, Bharuch to study the effect of time of sowing and row spacing on growth, yield and economics of soybean (*Glycine max* L.). The results revealed that sowing of soybean at onset of monsoon increased growth (plant height, number of branches plant^{-1}), yield attributes (number of pods plant^{-1}, number of seed pod^{-1}) and seed yield (1764 kg ha^{-1}) and stover yield (3431 kg ha^{-1}) as well as highest net realization (₹ 65,393 ha^{-1}) and BCR (2.89). Sowing of soybean at 60 cm row spacing recorded significantly higher growth and yield attributes, seed yield (1741 kg ha^{-1}) and straw yield (3353 kg ha^{-1}) as well as highest net realization (₹ 65,477 ha^{-1}) and BCR (3.06).

(Key words : Soybean, time of sowing, row spacing, growth, yield and economics)

INTRODUCTION

Soybean [*Glycine max* (L.)] is a well known oilseed and pulse crop. It is the richest and cheapest source of high quality proteins, minerals, vitamins and fats. Soybean is called as miracle "Golden bean" of 21st century. It is a boon for malnourished world because it is high nutritive and energy rich monocarp legume with protein (40 %), oil (20 %) and high level of essential amino acid like lysine (5 %), minerals (4 %), phospholipids (2 %) and the vitamins (thiamine and riboflavin). Soybean accounts for 54 per cent of global oilseed production. In India the area under soybean crop was 119.98 million ha with 118.89 million MT of total production in 2021 and an average productivity was 991 kg ha^{-1} (Anonymous, 2021). In Gujarat, it is cultivated in about 2.24 lakh hectares with an annual production of 2.27 lakh tonnes and average productivity of 1015 kg ha^{-1} (Anonymous, 2021). Sowing date plays a significant role in determining growth, development and yield of soybean. Sowing crop at optimum time increases the yield due to suitable environment at all the growth stages of the crop. Spacing is one of the important parameter, which ultimately affected nutrients uptake, growth and yield of plant. The increase or decrease of row spacing and plant population has definite pattern in relation to the yield. Therefore, it is necessary to study effect of time of sowing and row spacing on growth, yield and economics of soybean.

MATERIALS AND METHODS

The field experiment was conducted during the *kharif* season of the year 2020 at College Farm, College of Agriculture, Navsari Agricultural University, Bharuch, Gujarat. The experimental soil was clay in texture, medium in available nitrogen (256 kg ha^{-1}), low in available phosphorus (25 kg ha^{-1}), high in available potassium (340 kg ha^{-1}), low in available sulphur (7 mg kg^{-1}) and slightly alkaline in reaction (pH 7.50). Total nine treatment combinations consisting of three levels of time of sowing (T_1: Onset of monsoon, T_2: One week after T_1 and T_3: One week after T_2), and three levels of row spacing (S1: 30 cm, S2: 45 cm and S3: 60 cm) were evaluated in factorial randomized block design with three replications. Five plants were selected randomly from each net plot and tagged for recording growth parameters i.e. plant height (cm) and branches plant^{-1} at harvest and yield attributing parameters i.e. number of pods plant^{-1}, number of seeds pod^{-1} and test weight. The data on seed and straw yield was recorded from the net plot and converted on a hectare basis. Economics was also calculated, i.e. total cost of production, net realization and B:C ratio. Collected data were analyzed as per the method suggested by Panse and Sukhatme (1973).

RESULTS AND DISCUSSION

Effect of time of sowing

Growth parameters

Significantly the highest plant height of 77.12 cm and 81.78 cm was observed at 60 DAS and at harvest, respectively and number of branches plant^{-1} of 6.24 and 6.36 at 60 DAS and at harvest, respectively with treatment T_1 (Onset of monsoon). While no significant difference in plant height was observed at 30 DAS due to time of sowing. Similar to this results Potbhare *et al.* (2020)

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reported that the plant height influenced by sowing dates recorded at 25 DAS was non-significant and at 50, 75 DAS and at harvest were found significant in *kharif* soybean. The soybean sown during 26th MW has recorded maximum plant height of 33.61, 45.20 and 47.16 cm at 50, 75 DAS and at harvest, respectively. However, it was at par with 27th MW at 25, 50, 75 DAS and at harvest and significantly superior over 25th and 28th MW. They also reported that number of branches plant^{-1} was significantly affected due to different sowing dates. Sowing of soybean at 26th MW produced maximum number of branches plant^{-1} of 12.63, 14.78 and 14.99 at 50, 75 DAS and harvest, respectively. It was followed by 27th MW and 25th MW. This might be due to congenial climatic condition for better germination and further growth and development of *kharif* soybean.

Yield attributes and yield

The significantly highest number of pods plant^{-1} (84.36), number of seeds pod^{-1} (2.76), seed yield (1764 kg ha^{-1}) and straw yield (3434 kg ha^{-1}) was recorded under treatment T_1 (Onset of monsoon). The test weight of soybean was not significantly influenced due to different time of sowing. Similar result reported by Potbhare *et al.* (2020). They reported that sowing taken on 26th MW had recorded significantly superior in number of pods plants^{-1} (31.50), number of seeds pod^{-1} (2.77) and hundred seed weight (11.88 g), however, it was found at par with 27th MW. They also reported that sowing taken on 26th MW had recorded highest seed yield (2470 kg ha^{-1}) and straw yield (3597 kg ha^{-1}) and found at par with 25th MW and 27th MW, however, it was significantly superior over 28th MW and recorded lowest seed yield (2009 kg ha^{-1}) and straw yield (2968 kg ha^{-1}). This might be due to adequate and increased availability of nutrients for development of more number of pods plant^{-1} and better seed filling with maintenance of better source-sink relationship. With delayed planting the growth period becomes short, while high temperature during flowering decreased the seed yield and yield components of soybean planted early.

Economics

The highest net return (₹ 65393 ha^{-1}) and benefit: cost ratio (2.89) was obtained under treatment T_1 (Onset of monsoon) followed by T_2 (One week after T_1) with net return of 53915 ₹ ha^{-1} with BCR 2.38. The lowest net realization (₹ 41315 ha^{-1}) and BCR (1.83) was obtained under treatment T_3 (One week after T_2). A similar economic benefit of soybean with time of sowing was reported by Potbhare *et al.* (2020). They recorded that sowing taken on 26th MW had recorded significantly superior in net monetary return (₹ 76719 ha^{-1}) and B;C ratio (3.12) over sowing carried out on 25th MW and 28th MW in soybean. Singh *et al.* (2014) also reported that net return (₹ 50418 ha^{-1}) and B:C ratio (3.65) were significantly higher in 5 June sowing than 25 June sowing, which were, however at par with 15 June sowing in soybean.

Effect of row spacing

Growth parameters

Significantly the highest plant height of 78.15 cm and 82.18 cm was observed at 60 DAS and at harvest, respectively and number of branches plant^{-1} of 6.10 and 6.23 at 60 DAS and at harvest, respectively with treatment T_1 (Onset of monsoon). While no significant difference in plant height was observed at 30 DAS due to time of sowing. This increase in plant height at wider row spacing might be due to fact that plant gets enough space for growth i.e. 60 cm row spacing showed a better row to row spacing for better plant height. While, higher number of branches plant^{-1} due to sufficient availability of sunlight and nutrient which increased plant growth and development. The present results are in cognizance with those of Mondal *et al.* (2014) in respect to branches plant^{-1}. They reported that the highest branches plant^{-1} (3.7) was observed at the wider spacing of 20 cm x 30 cm followed by spacing of 15 x 30 cm with same statistical rank. In contrast, the lowest branches plant^{-1} was recorded in the plant spacing of 5 cm x 30 cm in soybean crop. Sowjanya *et al.* (2017) also reported that significantly maximum plant height (50.11 cm) was recorded with the wider spacing of 45 cm x 15 cm which was statistically at par (49.23 cm) with spacing of 30 cm x 20 cm in gladiolus crop.

Yield attributes and yield

Significantly higher number of pods plant^{-1} (85.16), number of seeds pod^{-1} (2.84), seed yield (1741 kg ha^{-1}) and straw yield (3353 kg ha^{-1}) were recorded under treatment S_3 (60 cm). The test weight of soybean was not significantly influenced due to different time of sowing. This was possibly due to less competition between plants for nutrient, soil moisture, space and solar radiation etc. in wider spacing than closer spacing. This was due to the fact that at 60 cm row spacing the number of rows m^2 get decreased and as the row to row spacing is decreased the number of rows m^2 get increased hence it increased the plant population per m^2. Plants in close proximity have more competition as compared to wider spacing. Improved yield attributing characters such as test weight, seeds pod^{-1} and number of pods plant^{-1} was recorded at higher spacing ultimately increasing the seed yield and straw yield. This also confirms the results of Khade *et al.* (2017). They reported that the highest number of capsules plant^{-1} (42.7), seed yield (499 kg ha^{-1}) and straw yield (1495 kg ha^{-1}) were obtained in wider spacing of 45 cm x 20 cm, which was at par with spacing of 45 cm x 10 cm and significantly more over closer spacing of 30 cm x 10 cm and 30 cm x 20 cm in *kharif* sesame. Sanap *et al.* (2019) also reported that effect of drilling distance on number of grains panicle^{-1} was found to be significant in rice crop. However, drilling distance of 30 cm recorded higher number of grains panicle^{-1} which was significantly superior over drilling distance of 20 cm but was at par with 25 cm drilling distance.

Table 1. Effect of time of sowing and row spacing on growth parameters, yield attributes, yield and economics of

Treatments	Plant height (cm)			Number of branches plant^{-1}		Number of pods plant^{-1}	Number of seeds Pod^{-1}	Test weight (g)	Seed yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Total cost of production (Rs.)
	30 DAS	60 DAS	At harvest	60 DAS	At harvest						
(A) Time of sowing (T)											
T_1: Onset of monsoon	19.02	77.12	81.78	6.24	6.36	84.36	2.76	10.37	1764	3431	22619
T_2 : One week after T_1	18.05	72.27	75.84	5.83	5.97	81.53	2.36	10.02	1531	3053	22619
T_3 : One week after T_2	17.14	52.84	57.28	4.98	5.27	74.38	2.05	9.87	1279	2501	22619
S Em ±	0.52	1.63	1.84	0.16	0.17	1.79	0.07	0.25	38.2	70.1	-
C D at 5 %	-	4.90	5.51	0.49	0.51	5.39	0.21	-	115	210	-
(B) Row spacing (S)											
S_1: 30 cm	16.95	50.68	55.61	4.97	6.18	72.86	1.95	9.77	1312	2520	23865
S_2: 45 cm	18.40	73.40	77.12	5.98	5.20	82.25	2.38	10.18	1521	3112	22619
S_3: 60 cm	18.86	78.15	82.18	6.10	6.23	85.16	2.84	10.31	1741	3353	21373
S Em ±	0.52	1.63	1.84	0.16	0.17	1.79	0.07	0.25	38.2	70.1	-
C D at 5 %	-	4.90	5.51	0.49	0.51	5.39	0.21	-	115	210	-
Interaction (TXS)											
S Em ±	0.91	2.83	3.18	0.28	0.29	3.11	0.12	0.44	66.2	121.4	-
C D at 5 %		-			-	-	-	-	-	-	-

Economics

Higher net realization of 65477 ₹ ha^{-1} with higher BCR 3.06 were obtained under treatment S_3 (60 cm row spacing) followed by 53575 ₹ ha^{-1} with BCR of 2.37 under treatment S_2 (45 cm row spacing). However, the lowest net realization of 41571 ₹ ha^{-1} with BCR 1.74 was obtained under treatment S_1 (30 cm row spacing). This result was supported by Khade *et al*. (2017). They reported that wider spacing of 45 cm x 20 cm recorded significantly higher net monetary return over closer spacing of 30 cm x 10 cm and 30 cm x 20 cm but it was at par with 45 cm x 10 cm. They also recorded maximum B:C ratio (4.7) under wider spacing of 45 cm x 20 cm.

Finally it is inferred that for getting potential yield and economic from soybean grown under rainfed condition could be obtained by sowing of soybean during onset of monsoon along with spacing of 60 cm.

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EFFECT OF DIFFERENT NUTRIENT SOLUTIONS ON ROOTING OF *Acmella calva* (DC.) R. K. JANSEN IN HYDROPONIC SYSTEM

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ABSTRACT

Experiment was conducted to evaluate the important medicinal plant *Acmella calva* (DC.) R.K. Jansen for the rooting ability of shoots treated with Indole-3-butyric acid (IBA) using hydroponic system at Green House Centre, Herbal Garden, PG and Research Department of Botany, Holy Cross College (autonomous), Tiruchirappalli-02, Tamil Nadu, India, during the year 2020. The IBA treated shoots were cultured in different concentrations of MS medium and modified Hoagland medium for rooting. A maximum of 100% response was in 1/6, 1/8, 1/10 strength of MS medium and 1/2 strength of modified Hoagland medium. Number of roots were many (16 roots/shoot) in 1/10 strength of MS medium, and 1/2 modified Hoagland medium. An average length of 3.2 cm (1/10 strength of MS medium) and 3.0 cm (1/2 modified Hoagland medium) were identified. Hence, this is the first evident report on successful optimal growth condition developed through hydroponic system (soilless nutrient condition) for the growth of an important medicinal herb *Acmella calva* (DC.) R.K. Jansen. The report proves as an alternative system of growth of traditional medicinal plant in hydroponics, which ultimately aids in conservation of the plant at low cost and less time. Eventually the study also provides an effective reproducible method of farming to encounter the downsides of farmers and uplift their economic status in the country.

(Key words: *Acmella calva*, hydroponic, IBA treatment, MS medium, modified Hoagland medium, rooting)

INTRODUCTION

In India, agriculture sector has been considered as the back bone of the country and plays vital role in the livelihood of farmers even throughout the world. Farmers typically utilize cultivable land as growing media to develop their agricultural products, fronting several threats due to urbanisation, climate change and high cost of fertilizers and pesticides. The plants grown with high use of pesticides create several health problems to the consumers leading to various diseases and disorders. This has to be considered as a major obstacle that must be solved and need to develop an alternative method to assist farmers to get a high yield of disease-free crop. The hydroponic technology is a suitable and conventional method adopted for the cultivation of crops in the soilless media. This system involves simple growing method, where water is used as a substrate supplemented with essential nutrients. This technique involves no need for heavy machinery and man power, also weeds and diseases can be controlled without spraying chemicals. In recent years, this technique has been created a great impact in the agriculture technology to accomplish modern farming on a large scale to elevate the socioeconomic status of farmers in the country (Prayoga and Putra, 2020).

The herb *Acmella calva* (DC.) R.K. Jansen is a rare medicinal plant belongs to the family Asteraceae. The decoction of the root is used as a laxative. The plant parts are used for toothache, throat problems and paralysis of the tongue, rash, dysentery and rheumatism. The leaf decoction was used as both in rheumatism or as a long for scabies and psoriasis (Kurian, 2007). Leaves are chewed to relieve toothache and infections of throat and gums. Tincture made from flower head was reported as a substitute for tincture of pyrethrum to treat inflammation of jaw-bones and caries. The extract of fresh flower tops is effective against anopheles mosquito larvae. It increases the flow of saliva and is useful in fever especially during summer (Prajapati *et al.,* 2003). Hydroponics offers an opportunity to provide optimal conditions for plant growth with higher yields as compared to open field condition for conservation of this valuable medicinal plant. Hydroponic technique can be used anywhere in all agro-climatic zones and those plants have higher nutritional value and tastes more than field growing crops (Singh and Singh, 2013).

The two chief merits of the soilless cultivation of plants are, A) much higher crop yields and B) the hydroponics can be used in places where ordinary agriculture or gardening is impossible. Other advantages

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include faster growth combined with relative freedom from soil diseases; consistent crops and the quality of plants are excellent. Plants grown in hydroponics are not inferior to naturally reared ones in point of favour and their vitamin (van Kooten *et al.,* 2004). The cost of production in hydroponics is also less over conventional methods. Therefore, hydroponics system was considered as a potential alternative for rooting of *A. calva*. No report on hydroponics-based rooting of *A. calva* has been reported so far. The present study aims at the rooting ability of economically important medicinal plant *A. calva* through hydroponics.

MATERIALS AND METHODS

Plant material collection

The healthy and uniform size of about 10 cm long shoots of *A. calva* were collected from Herbal Garden of PG and Research Department of Botany, Holy Cross College (Autonomous), Tiruchirappalli, Tamil Nadu and washed in running tap water.

Treatments in hydroponic system

The basal cut ends of shoots were provided dipping treatment for 3 hrs. with the growth regulator IBA solution (2.0 mg l^{-1}). Control was maintained without IBA treatment by using distilled water. The IBA (2.0 mg l^{-1}) treated explants were inserted in the hole made in thermocole sheets (10 mm) and placed in the nutrient solution of test tube. Two experiments were conducted simultaneously. In the first experiment, the cut ends of the planting materials along with the thermocole sheets were dipped to a depth of 1.0 cm and sub-irrigated in 20 ml of nutrient solution containing different strengths such as 1/2, 1/4, 1/6, 1/8 and 1/10 of MS (Murashige and Skoog) medium (without adding vitamins and amino acids) (Murashige and Skoog, 1962). In second experiment, the plants were sub-irrigated in different strengths (1/2, 1/4, 1/6, 1/8 and 1/10) of modified Hoagland medium (Gibeaut *et al.,* 1997). The culture tubes were incubated under room temperature for root initiation. The nutrient solution was replaced at 3 days interval with same concentration for further growth of roots.

Experimental design and data analysis

Each treatment had 10 replicates and the experiment was repeated thrice. Data were recorded as percentage of rooting, number and length of roots after 21 days of culture.

RESULTS AND DISCUSSION

Preliminary rooting studies were carried out for the stem cuttings of *A. calva* by culturing in distilled water (control) and treated with IBA. Rooting was found better in IBA treatment when compared to the control (distilled water without IBA). The roots were initiated after 7 days of treatment.

The 100 % response was observed in the control. The number of roots recorded in IBA (2.0 mg l^{-1}) were 15 / shoot (Table 1) in water. In the beginning, the plants from both the control and IBA treated were green and healthy in appearance. But in later stage, the shoots were dropping and the leaves were found yellowish due to loss of chlorophyll and subsequently it led to the death of plants.

Root initiation of *A. calva* in hydroponics containing both MS medium and modified Hoagland medium were experimented. Before introducing the plants into the media, the cut shoot end of *A. calva* was treated in IBA (2.0 mg l^{-1}). After three days of inoculation, the root initiation was observed in plants in both the medias. The plants in MS medium with the strength of 1/6, 1/8, 1/10 showed 100% response (Fig. 1c, 1d and1e). Whereas, rooting was 100% in 1/2 strength of modified Hoagland medium and 90% response was observed in 1/6 strength.

Regarding number of roots, in 1/10 strength of MS medium 16 roots/shoot were observed and the same number of roots were recorded in 1/2 strength of modified Hoagland medium (Fig.1f). The average length of root was 3.2 cm and 3.0 cm respectively. The response was 20% in ¼ strength MS medium containing only 2 roots/shoot (Fig. 1b). The plants were dark green and healthy in appearance. After 15 days, the root system was well developed. The increase in the number of lateral roots indicate that MS medium and modified Hoagland medium enhanced the formation of lateral roots (Fig. 1).

In the present study, the hormone IBA (2.0 mg l^{-1}) played an important role in rooting of *A. calva* shoots on hydroponics with different strengths of MS medium. Similar results were observed by Jeyachandran *et al.* (2004) in *Anicochilos carnosa* where IBA treated MS medium revealed better shooting at ¼ strength. Also, Sahni and Gupta (2002) reported development of multiple shoots in ½ strength MS medium with 14.7µM of IBA treated *Acacia catechu*. Whereas, the propagation of softwood cuttings of *Pistacia vera* was achieved by Al Barazi and Schwabe (1982) after the treatments of different concentrations (upto 45000 rpm) of IBA. The stem cutting of *Rubia cordifolia* (L.) treated with the combination of IBA and NAA at 2000 rpm each auxin produced high number of roots (24.82 roots/ cutting). Combination of NAA and kinetin were needed for rooting of *Arachis* species (Laxmi and Giri, 2003) and 2,4-D and kinetin *in Boerhaavia diffusa*. A study on relationship of soil fertility and leaf nutrient status in Litchi. It was reported that, nutrients such as N, P, K, Ca and C in leaf is positively correlated to the fertility status of the soil (Kumar *et al.,* 2021). But the present study provides a strong proof that hydroponic system with different strengths of MS medium showed greater response in rooting, shooting and overall development of plantlet. Thus, ensuring that the growth of *A. calva* in hydroponic system revealed better growth and development in the quality of the plant than in soil system.

Of the different media used, the MS medium was more effective in root initiation than the modified Hoagland

medium. In the present study the low strength (1/10) of MS medium produced rooting. Our results are in accordance with the report of Karthic and Seshadri (2009), they reported that *Gymnema sylvestre* stem cuttings produced roots under hydroponic system with 1/10 MS salts supplemented with different IBA concentration. In contrast to the present study, the works of Rudra and Jawarkar (2002) in *Punica granatum*, Sahni and Gupta (2002) in *Acacia catechu*, Benniamin *et al.* (2004) in *Cratarva magna* have reported that, the half strength MS liquid medium was proved to be best for hydroponic system. However, in the present findings, the percentage of response was low in low concentration of modified Hoagland medium. When the concentration is lowered, it does not support the growth of root system in nutrient medium. Deficiencies of the essential element resulted in the abnormalities of plants like chlorosis and necrosis. This essentiality of particular mineral could be known by using artificial methods of solution culture reported by Verma (2009). Whereas, the increasing concentration (1.0 – 2.0X) of modified Hoagland medium resulted in maximum growth in *Saliva splendens* was observed by Kang and van Iersel (2004). In contrary to the present findings, a report by Aseafa and Wagari (2021) on organic vermicompost, supplemented with NPS greatly improved the soil fertility and nutrient content of the soil for the growth of *Glycine max* with high yield capacity. But, the nutrient supplemented hydroponic medium developed in the present study breaks the importance of external addition of fertilizers and role of other soil parameters for the growth and yield of the plant. The concentrations selected for the study was found to be beneficial in the rooting *A. calva*. The strength of MS medium such as 1/6, 1/8, 1/10 and 1/2 strength of modified Hoagland medium were proved to be effective in the rooting of *A. calva*.

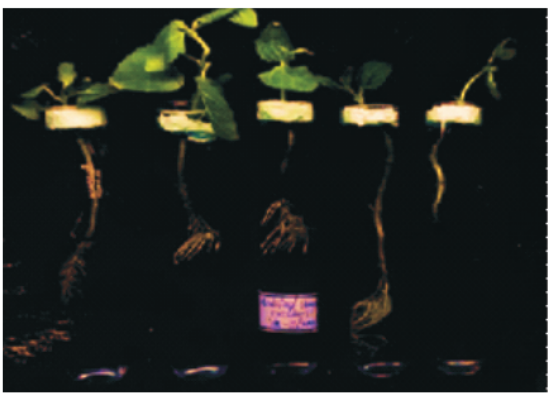
Hence, it is proved that the soilless system of cropping (hydroponic system) is very effective for large scale production and conservation of an important medicinal plant *Acmella calva*. The study also helps to avoid major problems faced by farmers like excessive usage of lands, fertilizers, pesticides, manure and search of different types of soil specific to each plant variety, thus providing an effective method with use of small space for the development of nutritionally effective traditional medicinal plants, ultimately encouraging the economic status of the farmers. This hydroponic system based growth of plant is cost-effective, produce high yield in less time would significantly help agronomists to improve the economic welfare of the country to promote, conserve and preserve this important medicinally valuable plant with pharmaceutical importance for the future world.

The inclusive results of the present experimentation evidenced the surplus rooting of *Acmella calva* developed hydroponically using different nutrient medium. *A. calva* is a traditionally important medicinal plant used to treat various diseases is less noticed in medicinal field and it is imperative to conserve the plant for near future. Beside the general advantages of this culture system, such as IBA treated plants in mineral nutrition and access to root system development, test tubes and plant-holders were also designed for flexibility, easy setting up and low maintenance of the culture, so that this hydroponic design will be suitable for many experimental purposes.

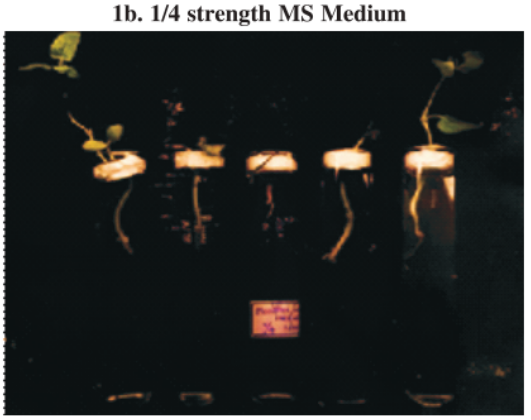
Soilless agriculture or hydroponic system is much preferred in our country due to its various advantages in the agronomy. In the present study, the optimum condition maintained for the growth of experimental plant *A.calva,* is authentically validated for its growth in hydroponic agriculture. This reproducible protocol could be a better alternative for the cultivation of traditional medicinal crops and other plants in the soilless medium and provide a ray of hope for the vulnerable farmers to battle against their socioeconomic condition in the society. Further studies on mass cultivation of *A. calva* will be carried out in hydroponics as future aspect.

Figure 1. Effect of Hydroponics on rooting of A. calva

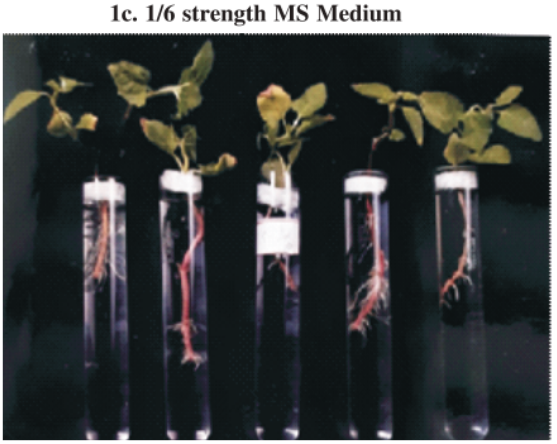
1a. 1/2 strength MS Medium



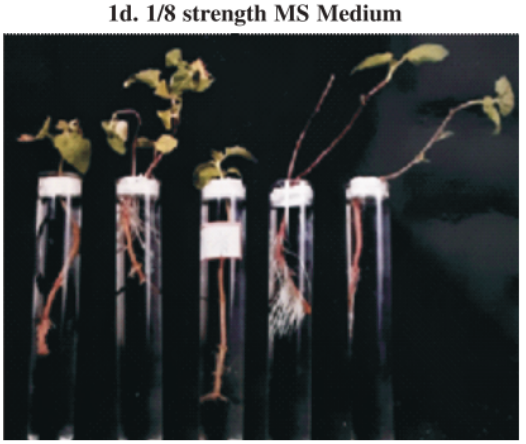
1b. 1/4 strength MS Medium



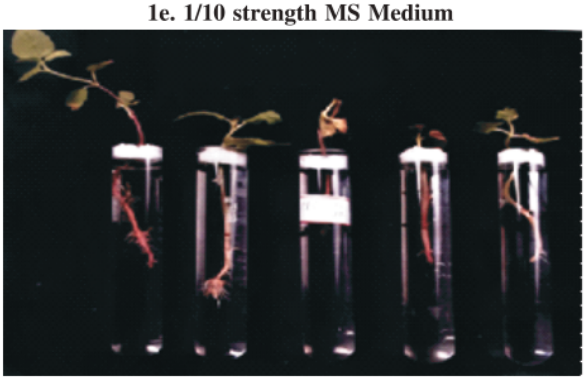
1c. 1/6 strength MS Medium



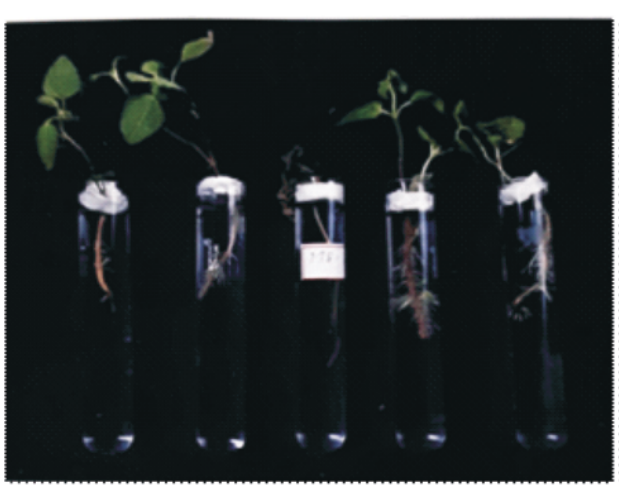
1d. 1/8 strength MS Medium



1e. 1/10 strength MS Medium



1f. 1/2 strength modified Hoagland Medium



1g. strength modifiedd Hoagland Medium

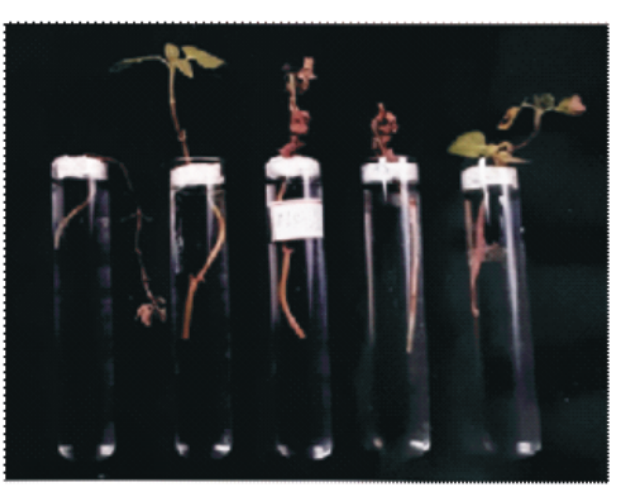


Table 1. Effect of different medium at various concentrations on rooting of *A.calva*

Sr. No.	Growth Medium	Different Concentrations	No.of roots	Root length (cm)	% of response
1.	MS Medium	1/2 strength	6	1.50	80
		1/4 strength	2	1.25	20
		1/6 strength	11	2.88	100
		1/8 strength	9	2.12	100
		1/10 strength	16	3.20	100
2.	Modified Hoagland Medium	1/2 strength	16	2.21	100
		1/4 strength	3	1.73	70
		1/6 strength	5	3.62	90
		1/8 strength	4	2.87	70
		1/10 strength	2	1.84	40
3.	IBA Treated	2.0 mg l^{-1}	15	4.21	90
4.	Control	Sterile distilled water	10	3.34	100

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DETERMINATION OF ANTIBACTERIAL POTENTIAL OF MELGHAT HONEY SAMPLES

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ABSTRACT

Honey is a natural product used for the treatment of many diseases due to its anti-microbial, anti-oxidantal, and anti-inflammatory potential. Melghat, a tribal region of Maharashtra, is full of a variety of forests and honey is a prime jungle product, basically collected by the tribal people from the hives of wild bees. In the present study, the antibacterial potential of the Melghat honey samples from four different locations was evaluated by using the disc diffusion technique. The samples were collected and examined in 2019. The antibacterial potential of the Melghat honey samples was detected against six species of bacteria, three from each gram-positive (*Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus)* and gram-negative (*Escherichia coli*, *Salmonella typhi,* and *Pseudomonas fluorescens)*. Except for bacteria *E. coli,* all of the honey samples tested positive against all of the bacterial species tested. Surprisingly, no honey sample showed its activity against the bacteria *E. coli*. The results showed that the Melghat honey has good potential against bacteria and therefore must have tremendous therapeutic properties. It is concluded that there is a greater need for research to explore the antimicrobial properties of Melghat honey.

(Key words: Melghat, Honey, Antibacterial, Chikhaldara, Amravati)

INTRODUCTION

Due to the development of resistance of microorganisms to recent drugs, there is a need to use natural materials against microbes. Honey is one of these materials, that has the potential to work against the pathogens in various diseases. The ancient Indian medicinal therapy "Ayurveda" has been using honey as a prime ingredient in medicines against many diseases (Boussaid *et al*., 2018). It was reported very firstly in 1982 that the honey shows antimicrobial properties. To date, there are numerous research papers published showing the antimicrobial capacity of honey. Manuka honey showed its activity against more than 60 species of bacteria. Malaysian honey and Egyptian honey, as well as other varieties of honey throughout the world, including India, have shown their potential against a variety of microbial as well as viral species. Many mechanisms have been proposed to justify the reason for the antibacterial activities demonstrated by honey, though it is not entirely unspoken to this day. Some of the factors which are supposed to be accountable for the antimicrobial potential of honey are; generation of hydrogen peroxide; high concentration of sugars; presence of phenolic compounds; presence of compounds that are proteinaceous over and above some physical characteristics such as low pH of honey, its low water activity due to less moisture content, the osmolarity of honey, the presence of the enzymes, as well as the presence of some other unidentified compounds (Khan *et al*., 2018; Kalidasan *et al*., 2017; Ismail, 2017; Mandal and Mandal, 2011).

As reported by Bhoyar *et al*. (2018), Melghat is a region in Amravati district and spread over Dharni and Chikhaldara tehsils. It is a forest area predominantly inhabited by tribal people. Melghat honey is extensively used by the tribal people as food and medicine and is produced from the different nectaries of an assortment of flowers. It is assumed that the honey from Melghat must possess antimicrobial potential. The potential of honey against some selected bacterial species was determined here in this study by applying the standard methods.

MATERIALS AND METHODS

Honey samples were collected from four different locations in Melghat forest and designated as H_1, H_2, H_3, and H_4. The antibacterial potential of these samples was detected against six species of bacteria, three from each gram-positive (*Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus)* and gram-negative (*Escherichia coli*, *Salmonella typhi,* and *Pseudomonas fluorescens)*. The samples were examined for the detection of antibacterial potential by the disc diffusion method (Anonymous, 2011; Tendencia, 2004). The CLAIRO COMBI, i.e. the combined microbial sensitivity discs, were made by using the sterile and special grade filter paper, were used. The paper possessed fourteen projected arms. The tip of each projection arm was carefully soaked into the stipulated amount of chemotherapeutic sample. The discs are found to be very useful for the evaluation of the *in vitro* potential of the materials, which may show their activities as agents

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