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TRENDING MICROBIOLOGICALS AND THEIR ROLE TO ENHANCE GROWTH AND ESSENTIAL OIL CONTENT OF DILL (*ANETHUM GRAVEOLENS*)

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Keywords:

Anethum graveolens, Dill, Essential oil, Carvone, Mycorrhiza, *Sebacina vermifera*

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ABSTRACT: A greenhouse and field experiment was conducted to investigate the effects of Azotobacter *(Azotobacter chroococcum)*, Phosphate solubilizing bacteria (*Pseudomonas fluorescens)*, and Arbuscular mycorrhiza-like fungi (*Sebacinca vermifera*) on seed germination, physiology, and essential oil production of dill (*Anethum graveolens*). The experimentation was comprised of a randomized complete block design with six replicates of each treatment. Evaluation of germination was done using parameters; germination percentage, mean germination time, germination index, T_{50} of germination, seedling vigour, and vigour index. Physiological responses include the parameters for total length, total fresh weight, and total dry weight of the plant. For physiological evaluation, harvesting of plants was done a total of four times at an interval of 30 days *i.e*. on 30, 60, 90, 120 days after transplantation. Essential oil analysis was done by hydrodistillation followed by Gas chromatography-Mass spectrometry. All the data collected were statistically analyzed using the GraphPad Prism v6 software package. Results showed that all treatments negatively affected the emergence behavior of seeds, whereas physiology of plants and essential oil production by *Sebacina vermifera* were significantly higher than other treatments. More specific to essential oil, treatments not only affected the total yield of essential oil but also affected the most components of essential oil. This study is potentially representing an alternative way of promoting the growth and biosynthesis of essential oils in dill.

INTRODUCTION: *Anethum graveolens* L. (Dill) is an aromatic and herbaceous annual plant that belongs to the family Umbelliferae (Apiaceae). This plant is indigenous to the Mediterranean, central and southern Asia [1]. This plant has been used in condiments and medicinal purposes since Egyptian times [2].

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This plant has been used throughout Europe, Asia, and America in traditional medicine systems, including Ayurveda, Unani, and Chinese medicine system [2, 3].

This plant claims a wide range of ethnomedicinal uses like in stomachache, hemorrhoids, insomnia, flatulence, carminative, diuretic, soporific, galactagogue, cough, cold, flu, nosebleeds, abdominal pain, eye diseases, uterine pain, swelling of joints, and to heal drunkenness [2, 3]. According to Jana and Shekha wat (2010), [2] more than 56 Ayurvedic preparations include dill as an ingredient. Dried leaves of dill are used as a condiment and herbal tea, whereas fresh leaves are used in meats, fishes

sandwiches, and fish sauces. Dill seeds are used widely as spice and seasoning of various foods such as pickles, salads, and soups [4-6]. Dill seeds contain about 3.5% of essential oil (EO). Monoterpenes are the major constituents of EO, including d-carvone, d-limonene, and apiol, and responsible for its aroma and medicinal effects [7-9]. EO from dill used in food industries and perfumery [6]. EO components are mainly responsible for the medical use of dill. Numerous scientific studies has been conducted using dill extracts, suggesting it as a potential medicinal candidate as antimicrobial, antihyperlipidemic, antihypercholesterolemic, mucosal protective, antisecretory, anti-ulcer, anti-oxidant, spasmolytic, anti-inflammatory, analgesic, anticancer, antidiabetic, insecticidal, diuretic, and menstrual cycle regulator [2, 10, 11]. Worldwide cultivation wide array of medicinal active constituent and large scale perfumery utilization of dill make this plant as a suitable candidate for growth and EO enhancement studies whether it is genetically based or obligatory mutualistic symbiosis based. Azotobacter (*Azotobacter chroococcum),* Phosphate solubilizing bacteria (*Pseudomonas fluorescens),* and Arbuscular mycorrhizal (AM)-like fungi (*Sebacinca vermifera*) has been largely used to enhance the growth of a plant and alter the phytochemical productions. This microorganisms *viz. A. chroococcum* [12, 13], *P. fluorescens* [14, 15,] and *S. vermifera* [16, 17] are well known for their beneficial effects on plants. Therefore, it is interesting to investigate the comparative effects of tested microorganisms on germination, growth, and EO production of dill.

MATERIALS AND METHODS:

Microorganisms and Plant: The culture of *S. vermifera* was kindly gifted by Prof. Virendra Swarup Bisaria, department of Biochemical Engineering and Biotechnology, Indian Institute of Technology-Delhi, India. The culture of *S. vermifera* was incubated on a modified Kaefer-agar medium for 10 days at 30 ± 1 °C and pH 6.5; after that, the slants we restored at 4 °C[18]. Further cultivation was done in 100 ml of modified Kaefer liquid medium in 500 ml Erlenmeyer flasks at 30 ± 1 °C on a gyratory shaker rotating at 200 rpm [19-20]. Fungal culture (8 day old) was then mixed with 1 ml carboxy methylcellulose (CMC) followed by mixing of 75 g of sterilized talcum powder in 25 ml of CMC mixed culture [21]. The formulation was then filled in airtight polythene bags and stored at normal room temperature until further experimentation. Azotobacter (*A. chroococcum*) and Phosphate Solubilizing Bacteria (*P. fluorescens*) were marketed biofertilizers, procured form Ganesh Agro Service Centre, and belongs to local market of Moga, Punjab, India. Seeds of dill (*Anethum graveolens*) were collected from Punjab Agriculture University, Ludhiana, India, during the rabi season. Seeds were surface sterilized using 70% v/v alcohol, followed by treatment with 0.01% Bavistin. Seeds were kept 24 h in sterilized water for soaking before experimentation.

Experimental Conditions: Experimentation was performed under greenhouse conditions (in pots) and field conditions. Soils used in both conditions were different, and a sample of soils was tested for chemical reserve prior to the experimentations. The physical properties and chemical reserves are given in **Table 1.** The soil used for greenhouse conditions was autoclaved for 1 h in cotton bags and cooled at room temperature. The sterilization was repeated for 3 times after 24 h on consecutive days. The experimentation under greenhouse condition was conducted in controlled environmental conditions maintained at 25 ± 2 °C, 16 h light / 8 h dark with light intensity 1,000 Lux and relative humidity 70%. The field study was conducted in the agricultural farms using Randomized Complete Block Design (RCBD). The soil was made porous by several ploughings and disking to facilitate healthy root development. Population size was 100 for each replicate of an observational group. All experimentation was done in six replicates, and the sample size was also six from each replicates [22]. A complete topography of field experimentation and sowing scheme is given in **Fig. 1, 2.**

TABLE 1: PHYSICAL AND CHEMICAL PROPERTIES OF SOIL

Sample	pH	Electrical Conductivity (mmho/cm)	C (%)	P (kg/ha)	K (kg/ha)	Fe (kg/ha)	Zn (kg/ha)	Cu (kg/ha)	Mn (kg/ha)	N (%)
Pot	6.7	0.63	0.66	42.25	370.65	13.54	4.89	3.51	9.59	0.08
Field	7.4	0.59	0.48	32.37	311.35	12.26	4.10	1.53	7.36	0.06

Note: C-Carbon, P-Phosphorus, K-Potash, Fe-Ferrous, Zn-Zinc, Cu-Copper, Mn- Manganese,N-Nitrogen

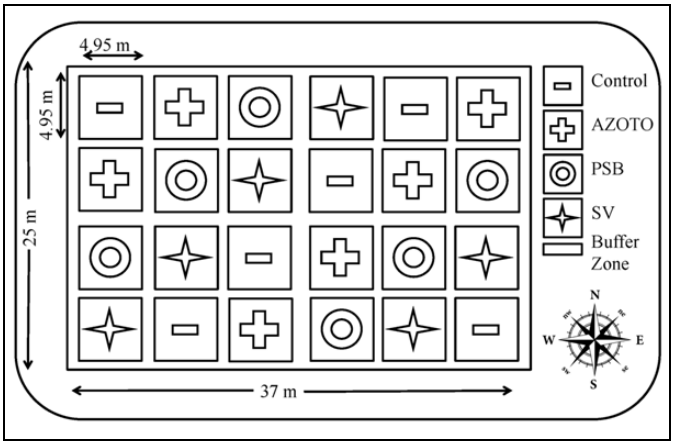

FIG. 1: TOPOLOGY OF FIELD EXPERIMENTS (RANDOMIZED COMPLETE BLOCK DESIGN)

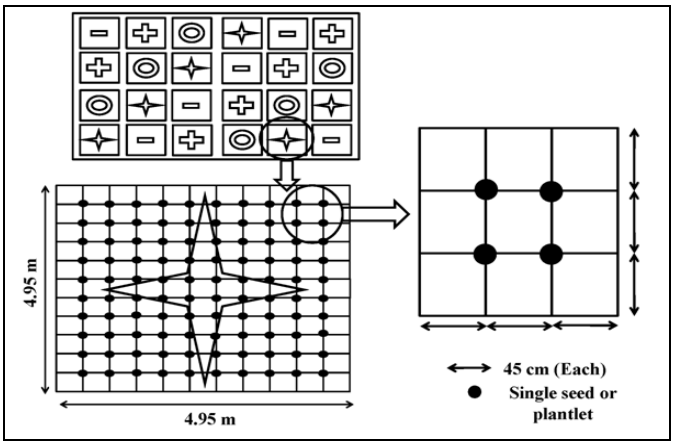

FIG. 2: SEEDS/PLANTLETS SOWING SCHEME

Treatments: There were four study groups *viz*. control (CON), Azotobacter (AZOTO), Phosphate solubilizing bacteria (PSB) and *S. vermifera* (SV). CON group received same treatment without any microbial strain, AZOTO, PSB, and SV groups received dual treatment with different schemes. Treatment of seeds with AZOTO, PSB, and SV was carried out using paste of 1 kg biomass in 1 L of water for seeds sufficient for 1 acre (approx. 10 Kg). The paste was applied uniformly on the surface of seeds and dried in the shade before sowing in pots/fields.

The treatment of plantlets (for physiological studies) was carried by root dipping in the paste of same composition used for seed treatment and sown immediately in pots/field. Soil treatment was carried by induction of 1 kg biomass in 19 kg of vermicompost and mixed with soil in the ratio of 60:40 (soil: treated vermicompost), whereas in the field, treated vermicompost was spread as 60 kg treated vermicompost per acre. For standing plants treatment was carried out by drilling/drenching method after 8 weeks using treated vermicompost [22].

Germination Studies: The pot and field studies were composed of 100 seeds per replicate of each treatment group. Seeds in pot were uniformly sown as 10 seeds per pot, in 2 kg capacity polythene pots, where first 3/4th part of treated soil was filled then the soil was moistened with water. After placing seeds uniformly 1/4th reaming part of soil was filled such that each seed were 2 cm below the soil surface. In field seeds were sown in plot containing 10 rows and 10 columns, and seeds were interspaced at 45 cm to next seed **Fig. 2.** There was a buffering zone surrounding the plots, which was a reservoir from contamination and a platform for handling and maintenance activities. All seeds of the field were covered 2 cm below the soil surface. The soil was moistened with a light spray of water. The soil was covered with straw to avoid certain dryness, and water was supplied daily to avoid drought stress. Germination behavior was observed regularly after 24 h and a seed was considered germinated when radical was visible on surface of soil. Germination study both under greenhouse and field conditions were observed until the germination was constant for 3 days and evaluation was comprised of following parameters:

- **a.** Germination percentage [23]
- **b.** Mean germination time [24,25]
- **c.** Germination index [26]
- **d.** T_{50} of germination [27-29]
- **e.** Seed vigour [30]
- **f.** Vigour index [31]

Physiological Studies: The young and healthy 30 days old plantlets of similar size and in the same development stage were taken for the physiological study. Under both green house and field studies the population of plants was maintained at 100 for each replicate of respective treatment groups. Under green house condition each pot of 10 kg capacity was containing single plantlet whereas under field conditions plantlets were sown in plot containing 10 rows and 10 columns and plantlets were interspaced at 45 cm to next plantlets **Fig. 2.** Soils of both conditions were irrigated regularly to avoid drought stress.

Plants were uprooted periodically at an interval of 30 days *i.e.* 30, 60, 90 and 120 DAT (Day after transplantation) for physiological evaluations. Evaluation was done using following parameters:

a. Total plant length (root + shoot)

b. Total fresh weight of plant (root + shoot)

c. Total dry weight of the plant (root + shoot)

Colonization Studies: The plants were harvested, four time at and interval of 30 days. Collected roots were washed thoroughly under slow stream of water to remove the adhering soil particles. Small segments of roots (1.0 cm approximately) were heated in 10 % KOH for 15 min. After washing with water 3-5 times, the root segments were neutralized by treating with 1 N HCl for 2-3 min 32. After washing with water, root segments were then stained with 0.02 % Trypan blue overnight and were de-stained with 50 % lacto-glycerol for1-2 h prior to the observation under a light microscope. Root colonization was assessed using the following formula 33:

Percentage colonization = Number of root segments colonized / Number of total segments examined × 100

Phytochemical Analysis:

Oil extraction: Sample of seeds were collected from well-grown healthy plants of each group and dried at room temperature. Dried samples were grounded in a blender. The extractions of grounded seeds were done by hydrodistillation using a Clevenger apparatus for 4 h. The oil samples were collected in airtight container and stored at 4 °C until further analysis.

Gas Chromatography-Mass Spectrometry: GC-MS analyses were carried out on a gas chromatograph Thermo trace 1300 GC coupled to a Thermo TSQ 8000 mass spectrometer with electron impact ionization method. Ion source temperature was 230 °C. A TG-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) was used. The column temperature was programmed to rise from 50 °C (2 min) to 280 °C at the rate of 10 °C / min. The S/SL injector's temperature was maintained at 250 °C, and the injection volume was 1.0 µL. The MS transfer line temperature was maintained at 280 °C. The carrier gas was helium with a flow rate 1 mL/min, and mass range was 50-500 m/z.

EO components were identified by comparison spectral data to those from mass spectra stored in the National Institute of Standards and Technology (NIST) spectral library. The percentage peak area of corresponding component was taken as content concentration without using correction factors [34].

Statistical Analysis: All experiments were performed in six replicates (Sample size was 6 from each replicate). The data were expressed as mean ± SD (standard deviation). Data from germination studies, a yield of EO and composition of various EO components, was analyzed using one-way - analysis of variance (ANOVA) followed by Tukey's post hoc test to compare means at the significance level $p<0.05$. Data from physiological studies were analyzed using two-way - ANOVA, followed by Bonferroni post-test for multiple comparison of means at the significance level $p<0.05$. All statistical analysis was performed using the GraphPad Prism v6 software package.

RESULTS:

Colonization Studies: The presence of fungal hyphae confirm the positive colonized association between the tested plant and *S. vermifera* under both conditions, *i.e*., pots under greenhouse and field conditions. Further, there was a variation in colonization percentage under pots and field conditions.

Colonization percentage was found lowest at 30DAT (Pot-25.65%; Field-15.25%), which gradually increased at 60 DAT (Pot-46.31%; Field-26.12%) and 90 DAT (Pot-70.32%; Field-40.38%). The highest colonization percentage was recorded at 120 DAT (Pot-85.40%; Field-78.31%) under both greenhouse and field conditions. Colonization responses are given as **Table 2.**

TABLE 2: COLONIZATION PERCENTAGE OF SEBACINA VERMIFERA WITH DILL ROOTS AT DIFFERENT TIME PERIODS IN POT AND FIELD TRIALS

Harvesting	POT	Field
30 DAT	25.65±0.67	15.25±0.31
60 DAT	46.31±1.12	26.12±0.79
90 DAT	70.32±2.18	40.38±1.26
120 DAT	85.40±2.79	78.31±2.26

Root colonization (%) of *Sebacina vermifera* treated plants: data expressed as mean ± SD of six replicates. CON- Normal control; SV- *S. vermifera*; DAT- Day after transplantation
Note: (-): not detected

Germination Studies:

Germination Studies Under Greenhouse Conditions: Emergence studies on dill seeds were observed for 25 days from the day of sowing of seeds. The statistical analysis revealed significant positive effects of all three treatments for all the germination traits on tested plant species, *i.e*., dill **Table 3.** It was observed that all three treatments enhanced the emergence trait and positively decreased mean germination time. Among all groups, the SV group showed better emergence performance with respect to all germination traits. In comparison to CON, SV here increased germination percentage, germination index, seedling vigour, and vigour index by 38.46%, 265%, 22.58%, and 69.73%, respectively. PSB enhanced the same traits by 28.85%, 258.86%, 16.13%, and 49.63%, respectively. Similarly, AZOTO enhanced these factors by 19.23%, 31%, 6.45%, and 26.92%, respectively. On the other hand, SV, PSB, and AZOTOpositivelydecreased mean germination time by19.40%, 18.35%, and 9.02%, respectively. Similarly, T_{50} germination was positively decreased by 14.29%, 12.63%, and 8% for SV, PSB, and AZOTO, respectively **Table 3**.

TABLES 3: EFFECTS OF VARIOUS TREATMENTS ON DILLSEEDS UNDER POT TRIALS

Parameters	CON	AZOTO	PSB	SV
Germination percentage	52.00±2.00	62.00±1.53^{a}	67.00±1.53^{a,b}	72.00±1.53^{a,b,c}
Mean germination time	16.29±0.61	14.82±0.67^{a}	13.30±0.50^{a,b}	13.13±0.30^{a,b}
Germination index	7.00±1.00	9.17±1.04^{a}	25.12±1.12^{a,b}	25.55±1.10^{a,b}
T_{50} germination	17.50±0.46	16.10±0.35^{a}	15.29±0.71^{a}	15.00±0.60^{a,b}
Seedling vigour (mm)	62.00±2.00	66.00±1.00^{a}	72.00±1.00^{a,b}	76.00±2.00^{a,b,c}
Vigour index (mm)	3224.00±228.01	4092.00±161.53^{a}	4824.00±175.63^{a,,b}	5472.00±257.79^{a,b,c}

Emergence response of seeds to various treatments in pots trials: data expressed as mean ± SD of six replicates. Superscripts with different letters (a-c) within the same row represent significance level as pa<0.05 v/s Con; pb<0.05 v/s AZOTO; pc<0.05 PSB; CON- Normal control; AZOTO- Azotobacter; PSB- Phosphate solubilizing bacteria; SV- *S. vermifera* formulation

Germination Studies Under Field Conditions: Similar, to pot trials, under the field, all treatment groups exerted a positive effect on the germination performance of dill seeds. Overall emergence of seeds under field conditions, including treated and non treated groups, were slightly changed **Table 4**. When compared to CON, SV increased the germination percentage, germination index, seedling vigour, and vigour index by 44.68%, 167.41%, 37.86%, and 99.46%, respectively. For PSB, the same factors were increased by 40.43%, 162.74%, 22.33%, and 71.78%, respectively. Similarly, AZOTO increased the same factors by 14.89%, 152.55%, 8.74%, and 24.93%, respectively. Simultaneously, SV, PSB, and AZOTO positively decreased the mean germination time by 20.10%, 14.92%, and 13.42%, respectively. Similarly, T_{50} germination was positively decreased by 11.69%, 6.68%, and 4.78% for SV, PSB, and AZOTO, respectively **Table 4**.

TABLES 4: EFFECTS OF VARIOUS TREATMENTS ON DILL SEEDS UNDER FIELD TRIALS

Parameters	CON	AZOTO	PSB	SV
Germination percentage	47.00±1.53	54.00±1.53^{a}	66.00±1.00^{a,b}	68.00±1.53^{a,b}
Mean germination time	16.62±0.45	14.39±0.72^{a}	14.14±0.40^{a}	13.28±0.65^{a,b}
Germination index	9.42±0.47	23.79±0.75^{a}	24.75±0.43^{a,b}	25.19±0.58^{a,b}
T_{50} germination	17.36±0.50	16.53±0.74	16.20±0.50^{a}	15.33±0.50^{a,b}
Seedling vigour (mm)	51.50±1.00	56.00±1.53^{a}	63.00±1.00^{a,b}	71.00±1.53^{a,b,c}
Vigour index (mm)	2420.50±124.72	3024.00±165.01^{a}	4158.00±129.00^{a,b}	4828.00±210.58^{a,b,c}

Emergence response of seeds to various treatments in field trials: data expressed as mean ± SD of six replicates. Superscripts with different letters (a-c) within the same row represent significance level as p^{a}<0.05 v/s CON; p^{b}<0.05 v/s AZOTO; p^{c}<0.05 PSB; CON- Normal control; AZOTO- Azotobacter; PSB- Phosphate solubilizing bacteria; SV- *S. vermifera* formulation

Physiological Studies:

Physiological Response Under Green House Conditions: Plants were evaluated four times during the experimentation, at an interval of 30 days *i.e*., 30, 60, 90, and 120 DAT to understand the effects of treatments on the physiological performance of plants. All treatments significantly enhanced the physiology of plants in terms of the total length of the plant, total fresh weight of the plant, total dry weight **Fig. 3, 4**. Of all three treatments, the SV performed extremely well and consistently with an increment in the total length of

the plant (range from 29.19% to 159.20%), total fresh weight (range from 58.39% to 226.67%), and total dry weight (range from 62.79% to 357.89%). The enhancement was found significantly higher when compared to all other groups, including treated and non-treated. Here, PSB was recorded as the second performer to enhance the total length of plant (range from 16.02% to 105.79%), total fresh weight (range from 29.64% to 147.22%) and total dry weight (range from 33.69% to 231.58%) and enhancements by PSB were significantly higher when compared to CON and AZOTO groups.

AZOTO was recorded as the third performer, which significantly enhanced total length of a plant (range from 7.56% to 50.89%), total fresh weight (range from 16.01% to 58.89%), and total dry weight (range from 18.38%to 92.11%) **Fig. 4.**

FIG. 3: PLANTS UNDER POT TRIALS AT 120 DAT (A) CONTROL (B) AZOTOBACTER (C) PHOSPHATE SOLUBILIZING BACTERIA (D) *S. VERMIFERA*

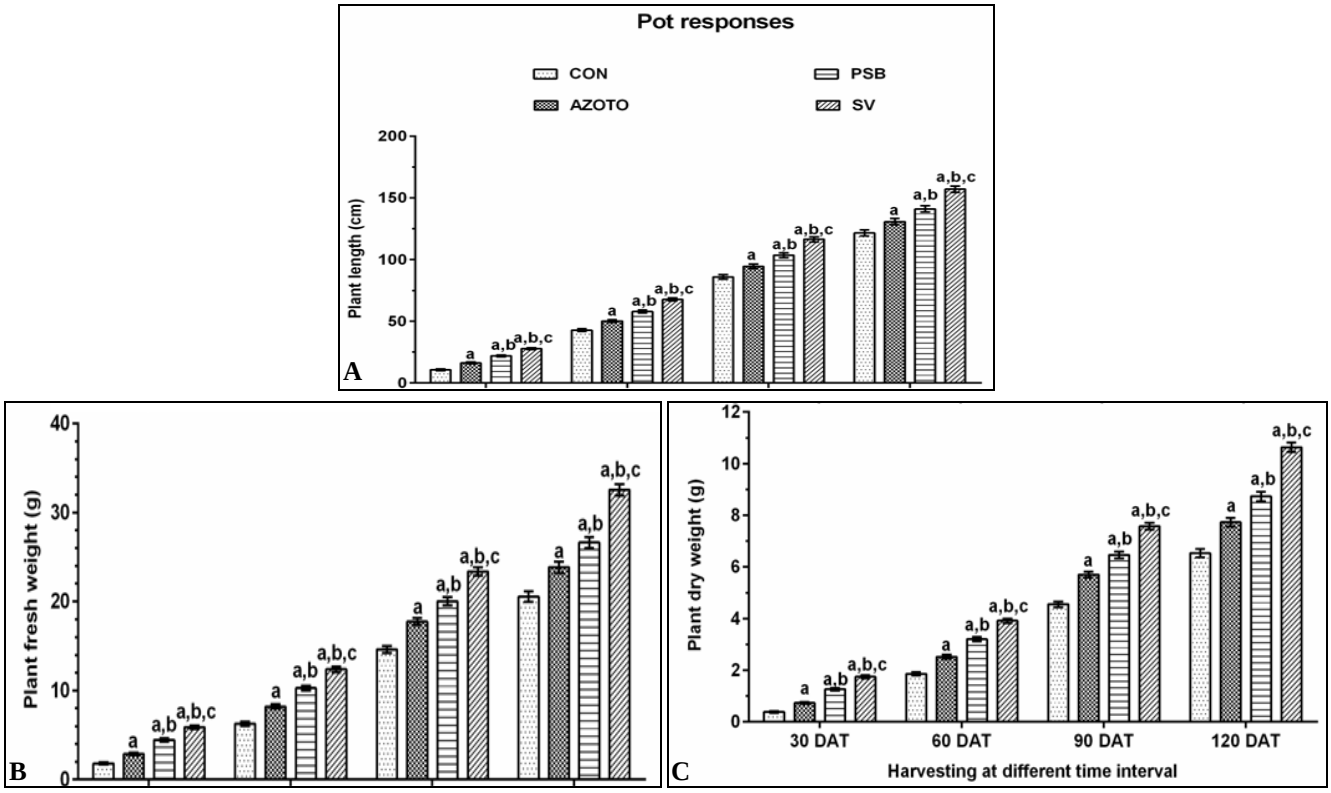

FIG. 4: EFFECT OF TREATMENTS ON (A) TOTAL LENGTH OF PLANTS (B) TOTAL FRESH WEIGHT OF PLANTS (C) TOTAL DRY WEIGHT OF PLANTS UNDER POT CONDITIONS; DATA EXPRESSED AS MEAN ± SD OF SIX REPLICATES. SUPERSCRIPTS WITH DIFFERENT LETTERS (a-c) WITHIN THE SAME HARVESTING GROUP REPRESENT SIGNIFICANCE LEVEL AS p^a<0.05 v/s CON; p^b<0.05 v/s AZOTO; p^c<0.05 v/s PSB; CON-NORMAL CONTROL; AZOTO- *AZOTOBACTER*; PSB- PHOSPHATE SOLUBILIZING BACTERIA; SV- *S. VERMIFERA*

Physiological Response Under Field Conditions: Overall physiology of plants including length, fresh weight, and dry weight of plant was found slightly higher under field conditions unlike plant grown under pot conditions **Fig. 5, 6**. Like pot trials under field trial, SV also performed extremely well and consistently with an increment in a total length of a plant (range from 24% to 153.86%), total fresh weight (range from 57.68% to 187.26%), and total dry weight (range from 62.22% to 326.67%).

The enhancement was found significantly higher when compared to all other groups, including treated and non-treated.

Here PSB was recorded as the second performer to enhance the total length of a plant (range from 14.39% to 101.30%), total fresh weight (range from 30.73% to 100.64%), and total dry weight (range from 31.77% to 176.67%) and enhancements by PSB were significantly higher when compared to CON and AZOTO groups.

AZOTO was recorded as the third performer, which significantly enhanced the total length of a plant (range from 6.32% to 46.98%), total fresh weight (range from 16.08% to 50.96%), and total dry weight (range from 15.23 to 83.33%) **Fig. 6.**

FIG. 5: PLANTS UNDER FIELD TRIALS AT 60 DAT (A) CONTROL (B) *AZOTOBACTER* (C) PHOSPHATE SOLUBILIZING BACTERIA (D) *S. VERMIFERA*

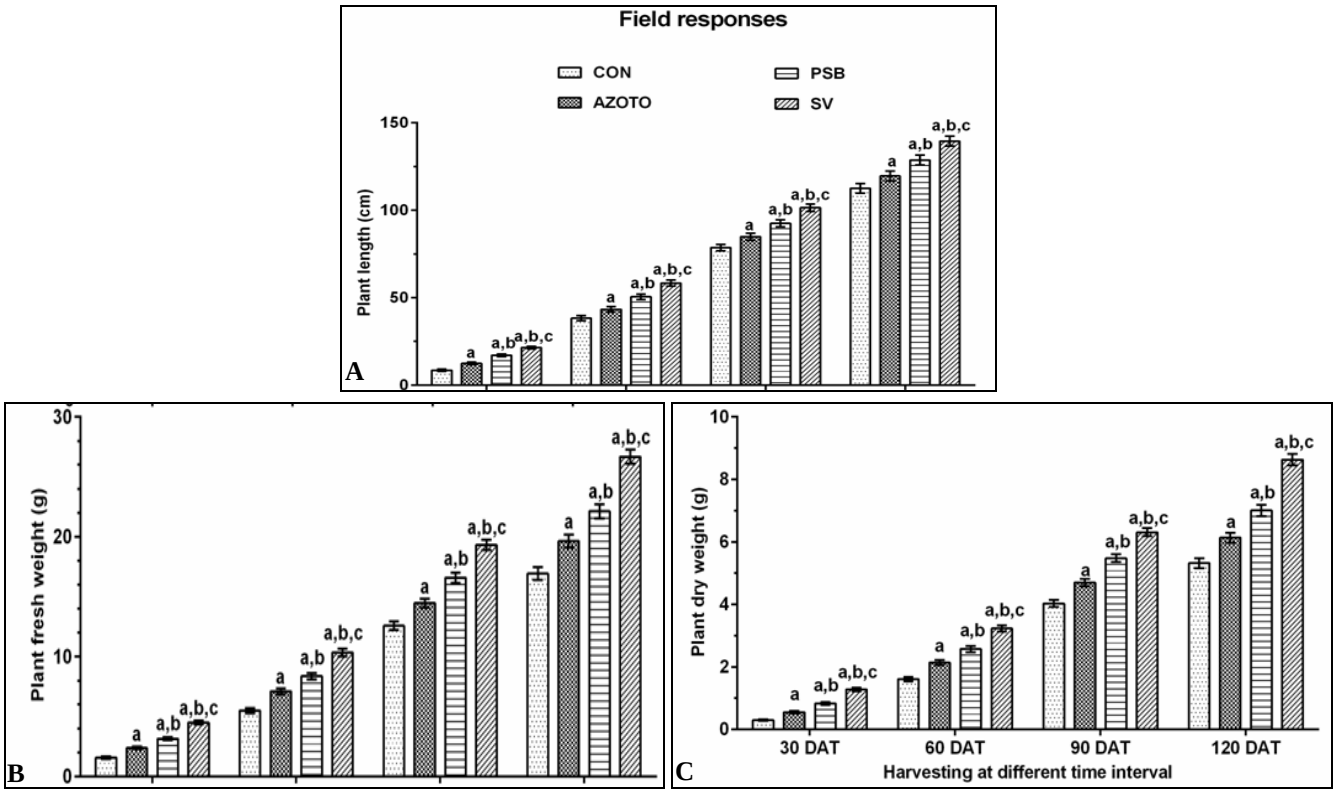

FIG. 6: EFFECT OF TREATMENTS ON (A) TOTAL LENGTH OF PLANTS (B) TOTAL FRESH WEIGHT OF PLANTS (C) TOTAL DRY WEIGHT OF PLANTS UNDER FIELD CONDITIONS; DATA EXPRESSED AS MEAN ± SD OF SIX REPLICATES. SUPERSCRIPTS WITH DIFFERENT LETTERS (A-C) WITHIN THE SAME HARVESTING GROUP REPRESENT SIGNIFICANCE LEVEL AS P^A<0.05 V/S CON; P^B<0.05 V/S AZOTO; P^C<0.05 *V/S* PSB; CON- NORMAL CONTROL; AZOTO- *AZOTOBACTER*; PSB- PHOSPHATE SOLUBILIZING BACTERIA; SV- *S. VERMIFERA*

Phytochemical Analysis:

Yield of Essential Oils: EO yield was significantly influenced by treatments *viz*. AZOTO, PSB, and SV. Like physiological enhancements, SV also prominently enhanced the yield of EO efficiently under both conditions (pot & field). SV treated group significantly increased EO yield (pot 2.90%; field 2.30%) in comparison to CON, AZOTO, and PSB. Here PSB (pot 1.90%; field 1.80%) and AZOTO (pot 1.80%; field 1.40%) were also recorded with significantly enhanced yield, whereas, CON (pot 1.40%; field 1.20%) group was recorded with the lowest yield **Fig. 7**. Chemical composition of EO of dill: GC-MS analysis of EO of dill seed of greenhouse (in pots) condition resulted in the identification of 16 chemical constituents **Table 5** adding up to99.99 to 100% of total area. The major constituents of EO were limonene (25.19-33.18%), carvone (20.57-30.95%), apiol (12.79-26.07%) followed by cis-

dihydrocarvon (3.59-24.44%), trans- dihydro-carvone (8.17-12.94%). Different treatments exerted relatively different effects significantly. Limonene was the maximum elevated compound of EO in comparison to all other compounds and reached to highest in the AZOTO (33.18%) group, followed by PSB (30.55%) and SV (26.33) whereas, CON (25.19) group was recorded with the lowest percentage of limonene. Carvone was the second, elevated compound reached to its maximum (30.95%) with SV treatment followed by PSB (28.38%) and AZOTO (23.91%) while the CON group restricted the Carvone to 20.57% only. The third elevated compound has Apiol recorded maximum with SV again (26.07%) followed by PSB (13.07%) and CON (12.79%) while it was absent in the AZOTO group. It was observed that cis-dihydrocarbon and trans-dihydrocarvone, p-cymene, limonene oxide-cis were the compounds recorded highest in CON, whereas down-regulated by all treatments. α-pinene, o-cymene, limonene, ϒ-terpinene, linalool, carvone, anethole, carvacrol, myristicin, and apiol were upregulated by other treatments. In contrast to individual components, the effect of various treatments is summarized in **Table 5**.

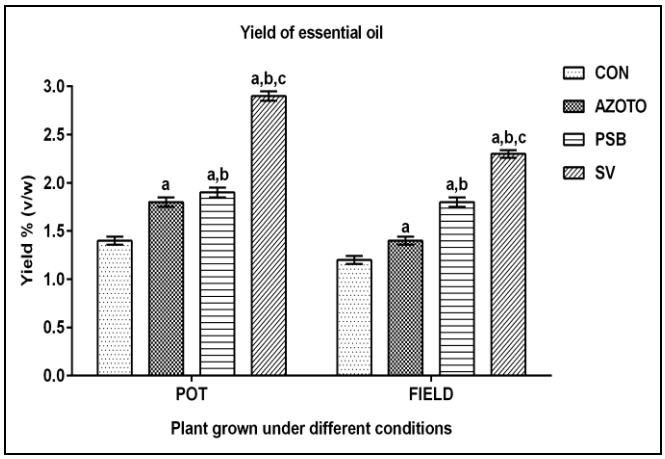

FIG. 7: EFFECT OF TREATMENTS ON YIELD OF EO OF PLANT SEEDS UNDER POT AND FIELD CONDITIONS: DATA EXPRESSED AS MEAN ± SD OF SIX REPLICATES. SUPERSCRIPTS WITH DIFFERENT LETTERS (A-C) WITHIN THE SAME CONDITION GROUP REPRESENT SIGNIFICANCE LEVEL AS P^A<0.05 V/S CON; P^B<0.05 V/S AZOTO; P^C<0.05 V/S PSB; CON- NORMAL CONTROL; AZOTO- *AZOTOBACTER*; PSB- PHOSPHATE SOLUBILIZING BACTERIA; SV- *S. VERMIFERA*

TABLE 5: EFFECT OF DIFFERENT TREATMENTS ON COMPOSITION OF VARIOUS EO COMPONENTS OF DILLSEEDS GROWN IN POTS UNDER GREENHOUSE CONDITIONS

S. no.	RT	Components	Pot			
			CON	AZOTO	PSB	SV
1	7.81	α-Pinene	0.46±0.10	0.51±0.11	-	-
2	8.13	1 ,3,8-p-Menthatriene	0.90±0.04	0.87±0.10	0.62±0.03^{a,b}	1.34±0.06^{a,b,c}
3	8.5	o-Cymene	-	1.67±0.13	0.69±0.03	-
4	8.74	Limonene	25.19±0.75	33.18±0.59^{a}	30.55±0.56^{a,b}	26.33±0.03^{a,b,c}
5	9.3	ϒ-Terpinene	-	0.86±0.04	0.36±0.02	-
6	9.85	P-Cymene	0.75±0.05	0.67±0.04^{a}	0.45±0.02^{a,b}	-
7	10.09	Linalool	-	-	-	0.53±0.02
8	10.87	Limonene oxide,c*is*	0.51±0.02	0.51±0.10	-	-
9	12.18	*cis*-Dihydrocarvone	24.44±0.53	11.80±0.55^{a}	14.50±0.94^{a,b}	3.59±0.29^{a,b,c}
10	12.31	*trans*-Dihydrocarvone	12.94±0.09	8.17±0.57^{a}	10.47±0.62^{a,b}	11.20±0.36^{a,b}
11	12.67	Neodihydrocarveol	1.14±0.02	-	-	-
12	13.1	Carvone	20.57±0.81	23.91±0.58^{a}	28.38±0.53^{a,b}	30.95±0.49^{a,b,c}
13	13.82	Anethole	0.31±0.07	1.04±0.14^{a}	0.32±0.02^{b}	-
14	14.06	Carvacrol	-	0.37±0.13	0.58±0.02	-
15	18.08	Myristicin	-	16.42±1.03	-	-
16	19.96	Apiol	12.79±0.03	-	13.07±0.59	26.07±0.85^{a,c}

Data expressed as mean ± SD of three replicates: Superscripts with different letters (a-c) within the same row represent significance level asp^a<0.05 v/s CON; p^b<0.05 v/s AZOTO; p^c<0.05 v/s PSB; CON- Normal control; AZOTO- Azotobacter; PSB- Phosphate solubilizing bacteria; SV- *S. vermifera* formulation Note: (-): not detected

Overall field response in contrast to individual compounds was found slightly different, unlike pot responses. GC-MS analysis of EO of dill seed under field conditions also resulted in the identification of 16 chemical constituents **Table 6** adding up to 99.99 to 100% of the total area. The major constituents of EO were limonene (23.40-32.12%), carvone (19.40-29.47%), apiol (10.49-25.21%) followed by *cis*-dihydrocarvone (3.57-23.36%), *trans*-dihydrocarvone (7.26-11.27%). Different treatments exerted relatively different effects significantly. Limonene was the maximum

elevated compound of EO in comparison to all other compounds and reached to highest in AZOTO (32.12%) group, followed by PSB (28.90%) and SV (25.93), whereas, CON (23.40) group was recorded with the lowest percentage of limonene. Carvone was the second, elevated compound reached its maximum (29.47%) with SV treatment followed by PSB (26.57%) and AZOTO (21.52%) while CON group restricted carvone to 19.40% only. The third elevated compound was apiol recorded maximum with SV again (25.21%) followed by PSB (12.87%) and CON (10.49%) while it was absent in the AZOTO group. It was observed that *cis*- dihydrocarvone and *trans*-dihydrocarvone, p-cymene, limonene oxide-*cis* were the compound recorded highest in the CON group, whereas down-regulated by all treatments. α-pinene, o-cymene, limonene, ϒ-terpinene, linalool, carvone, anethole, carvacrol, myristicin, and apiol were upregulated by other treatments. In contrast to individual components, the effect of various treatments is summarized in **Table 6**.

TABLE 6: EFFECT OF DIFFERENT TREATMENTS ON COMPOSITION OF VARIOUS EO COMPONENTS OF DILLSEEDS GROWN UNDER FIELD CONDITIONS

S. no.	RT	Components	Field			
			CON	AZOTO	PSB	SV
1	7.81	α-Pinene	1.37±0.08	2.38±0.09	-	-
2	8.13	1 ,3,8-p-Menthatriene	2.71±0.06	2.78±0.12	1.60±0.05[a,b]	2.26±0.08[a,b,c]
3	8.5	o-Cymene	-	2.43±0.11	1.66±0.01	-
4	8.74	Limonene	23.40±0.77	32.12±0.61[a]	28.90±0.58[a,b]	25.93±0.05[a,b,c]
5	9.3	ϒ-Terpinene	-	1.62±0.02	0.34±0.02	-
6	9.85	P-Cymene	1.91±0.07	1.62±0.06[a]	0.70±0.04[a,b]	-
7	10.09	Linalool	-	-	-	2.48±0.04
8	10.87	Limonene oxide,*cis*	1.48±0.04	0.42±0.12	-	-
9	12.18	*cis*-Dihydrocarvone	23.36±0.51	10.38±0.53[a]	14.11±0.92[a,b]	3.57±0.27[a,b,c]
10	12.31	*trans*-Dihydrocarvone	11.27±0.11	7.26±0.59[a]	10.38±0.64[a,b]	11.08±0.38[b]
11	12.67	Neodihydrocarveol	3.09±0.04	-	-	-
12	13.1	Carvone	19.40±0.83	21.52±0.60[a]	26.57±0.55[a,b]	29.47±0.51[a,b,c]
13	13.82	Anethole	1.51±0.05	1.13±0.12[a]	1.31±0.02[a,b]	-
14	14.06	Carvacrol	-	1.28±0.15	1.56±0.04	-
15	18.08	Myristicin	-	15.05±1.01	-	-
16	19.96	Apiol	10.49±0.05	-	12.87±0.61[a]	25.21±0.87[a,c]

Data expressed as mean ± SD of three replicates: Superscripts with different letters (a-c) within the same row represent significance level asp^a<0.05 v/s CON; p^b<0.05 v/s AZOTO; p^c<0.05 *v/s* PSB; CON- Normal control; AZOTO- Azotobacter; PSB- Phosphate solubilizing bacteria; SV- *S. vermifera* formulation Note: (-): not detected

DISCUSSION: The evaluation of colonization percentage is the key indicator for the symbiotic association between AM fungi and tested plant[34]. The present work established the active symbiotic relation between *S. vermifera* and *A. graveolens.* These observations are in line with previous studies that demonstrated the successful establishment of an association between *S. vermifera* and a wide range of plant species like *Trigonella Foenumgraecum* [21], *Coriandrum sativum* [22], *Foeniculum vulgare* [35], *Nicotiana attenuate* [36], *Thymus vulgaris* [37], Brassicaceae plants[38], *Panicum virgatum* [39, 40] and *Oryza sativa* [17]. All tested microbes have positively influenced germination traits. Similar positive effects of *A. chroococcum* was observed on germination of *Dodonaea viscose*seeds [41] similarly *P. fluorescens* was recorded with a positive effect on germination of *Vicia faba* [42]; contrary to this, a negative effect of *A. chroococcum* was observed on germination of *H. vulgare*seed [43] also *P. fluorescens*was recorded with a negative effect on germination of *Triticum turgidum* [44] and *Ambrosia artemisiifolia* seeds (weed plant) [45]. Although the underlying mechanisms behind the stimulatory and inhibitory effects of selected microbes are still not exposed but it is hypothesized that enhanced germination is the due response of phytohormones and other complex mixture of biologically active compounds and growth-promoting metabolites that are modulated by *A. chroococcum* [46-48], *P. fluorescens* [49-50]. Similarly, fungal inoculant (*S. vermifera*)was recorded with a positive influence on the germination of dill seeds. These observations are supported by previous studies on *N. attenuate*, *P. virgatum, Cynorkis purpurea* [16, 51]. In context to *S. vermifera*, it is conceivable that the performance of *S. vermifera* dependent on planting species,

application procedures, and experimental designs and conditions under which experiments are to be performed. The observation of Maighal *et al.* (2016) [52] also supports our observation and suggests that two factors, "Mycorrhiza" and "seed species," are responsible for seed viability and hence germination. In the contrary, Lendzemo *et al.* (2007) [53] explain that inhibitory action of AMF may be the result of the formation of unwanted metabolites, inhibitions of required metabolites, and negative niche effects. The overall emergence responses in pot under greenhouse conditions were found better in comparison to the field because of controlled environmental conditions, while under field conditions, external unfavorable conditions largely inhibit the metabolic processes and proliferation of embryonic tissues and negatively altered the emergence. Morphological evaluation represents the growth-enhancing features of tested microbes. The same observation was also made previously for *A. chroococcum* [12, 13], *P. fluorescens* [14, 15] and *S. vermifera* [16, 17].

In addition, a prominently enhanced fresh weight and dry weight of fungi inoculated *T. vulgaris* was recorded by Dolatabadi *et al.* (2011b) [37]. Similarly, *S. vermifera* inoculated plants have been recorded with enhanced Plant length, enhanced fresh weight, dry weight, enhanced root system, and number of nodes [38, 54]. Previous works hypothesized that increased plant growth due to AMF inoculation could be attributed to ea earlier expression of developmentally regulated genes [55] and phytohormonal involvements [56]. Eventually, *A. chroococcum, Pseudomonas* species, and AM-like fungi (*P. indica*- closely related to *S. vermifera*) has been demonstrated for phytohormonal modulator effects [56-60]. Moreover, improved plant growth may be ascribed to higher nutrient uptakes, especially nitrogen and phosphorus. Nitrogen and phosphorus largely contribute to biomass allocation, accumulation, and prominent growth of plants, an excellent yield of seeds, and EO production [61].

It is well known that *A. chroococcum* specifically fixes nitrogen to soil [62, 63,] and *Pseudomonas* genera are well known as phosphate solubilizing bacteria, which fixes non-utilizable phosphate to utilizable forms to plant [64] whereas, *S. vermifera* not restricted to a selected nutrient but has the potential to fix and efflux wide range of macro-and micro-nutrients in addition to Nitrogen and Phosphorus [16, 40]. Indeed, S. vermifera has contributed to the exploration of large soil volume, enhanced nutrient uptake, and mass accumulation more than other treatments. In support, elemental analysis of CHN from a dry matter of plants confirms enhanced nutrient uptake has contributed to mass accumulation (Data is not shown here). The inoculation of microbes also altered the yield of EO and contributed to the better quantity and quality of components. A similar investigation has demonstrated that *A. chroococcum* stimulates the production of EO in *Cymbopogon martini* and *F. vulgare* [65, 66]. Also, *P. fluorescens* has investigated to increase the EO in *Ocimum basilicum* and *Origanum majorana* [67, 68]. Prior investigations demonstrated that *S. vermifera* significantly increased the yield of EO in *Coriandrum Sativum* [22], *Foeniculum vulgare* [35], *T. vulgaris* [37].

Although the underlying mechanism behind the increment in EO is still to disclose however it may attribute to defensive response [68, 69], morphological traits [70, 71], up-regulation of biosynthetic genes [71-73] and P availability [74-78]. It is speculated that *S. vermifera* may have associated with aforesaid factors and indeed the higher efflux of N and P may have stimulated isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) synthesis better than other microbes resulted in better yield of EO in treated plants. In context to major constituent of EO already some studies demonstrated that *A. chroococcum* had increased the content of Anethole in *F. vulgare*[66], *A. chroococcum* has increased the content of (+) pulegone and (-) menthone in *Mentha piperita* [79], Similarly *S. vermifera* increased the content of linalool, anethole and thymol in *C. sativum*, *F. vulgare* and *T. vulgaris* respectively [22, 35, 37].

Similarly, *S. vermifera* enhanced the production of podophyllotoxin and its 6-methoxy derivative in *Linum album.* Additionally, precursor and elicitor of fungal origins are also in prevalence to enhance secondary metabolites in plant cell culture [83-85]. Enhancement in commercially important secondary metabolites *i.e.,* artemisinin and withaferin-a, were already achieved efficiently in cell culture technique facilitated with elicitor of fungal origins [86-87].

Although the underlying mechanism(s) for enhancement in major components of EO is still hidden however it is hypothesized that upregulation of biosynthetic genes, modulation of phyto-hormones, enhanced physiology, enhanced defensive response higher influx of N and P may enhance the particular class of secondary metabolites. This hypothesis is well supported by Baldi *et al.* (2008) [80] stated that *S. vermifera* could enhance the secondary metabolite production by activating the defensive pathways. Baldi *et al.* (2010) [18] also demonstrated that elicitor of fungal origin could stimulate the biosynthetic pathway, which resulted in enhanced secondary metabolites in cell culture; further, it was supported by Ammonia-lyase (PAL) activity, which is a short limiting step to synthesize secondary metabolite of lignin origin (Farkya *et al.* 2010) [82]. It is worth mentioning that the overall physiological and biochemical responses were slightly higher under field trials, whereas they were low in pots trials. It is tempting to speculate that the undisturbed core, higher fertility, and presence of indigenous microflora were responsible for better performance of plants under field conditions while the soil of pots was sterilized with disturbed core might be the reason for underperformance of plants.

CONCLUSION: Although AZOTO and PSB could extend their contribution towards better performance of dill plant but *S. vermifera*implied the greater magnitude for multifaceted benefits for the dill plant in all aspects (except germination) and support the growth of plants, stimulate the defensive mechanisms, maintain the health and vitality of plant, enhance the yield of EO and hence the secondary metabolites. By extrapolating the overall results and considering the efficacy, we promote*S. vermifera*as a potential alternate to achieving eco-friendly and sustainable agricultural practices in general and in particular to dill.

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