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Evaluation of Liquid Bio-inoculants for Production of Plant Growth Promoting Hormones and their Effect on Growth of Amaranthus *(Amaranthus cruentus)*

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Abstract

An investigation was carried out to assess the ability of liquid bio-inoculants *viz*., *Azotobacter chroococcum, Bacillus megaterium, Frateuria aurentia, Psuedomonas fluorescens, Bacillus subtilis* and *Trichoderma viridae* for production of plant growth hormones and study their effect on growth of Amaranthus (*Amaranthus cruentus*). Bio-assay was conducted for production of GA, IAA and Cytokinin. It was noticed to be significantly highest in *Psuedomonas fluorescens* (4.75µg, 180.07 µg and 4.94 µg of GA, IAA and Cytokinin, respectively) and least was recorded in *Trichoderma viridae* (1.43 µg, 9.32 µg, 0.88 µg of GA, IAA and Cytokinin, respectively). The results of green house studies revealed that the plants inoculated with a consortia of *A. chroococcum* + *B. megaterium* + *F. aurentia* + *P. fluorescens* + *B. subtilis* + *T. viridae* recorded significantly highest plant height (8.82 cm, 29.00 cm and 40.87 cm), highest number of leaves (6.23, 9.13 and 12.57) at 15 DAS, 21DAS and 30 DAS, respectively, maximum root and shoot length (7.80 cm and 40.87 cm, respectively) as well as root and shoot fresh weight and dry weight at harvest.

Keywords: Liquid bio-inoculants, GA, IAA, cytokinin, *amaranthus cruentus*

Amaranthus (*Amaranthus* sp.) popularly known as chouli, is a nutritive and highly suitable crop for kitchen gardening and commercial cultivation. Rapid growth, quick rejuvenation after each harvesting and high yield of edible matter per unit area in limited time and the most unique benefits includes its ability to stimulate growth and reduce inflamation (Belanger *et al.,* 2004). Plant hormones are signal molecules acting as chemical messengers that control plant growth and development. Aside from their role in plant response to changes in environmental conditions, hormones are also the principal agents that regulate expression of the intrinsic genetic potential of plants. Numerous soil bacteria and fungi are also able to produce phytohormones. The commonly recognized classes of phytohormones are *viz.,* auxins, gibberellins, cytokinins, abscisic acid and ethylene (Gabriele Berg, 2009).

Liquid bio-inoculants are the promising and updated technology in spite of many advantages not only over agrochemicals but also carrier based biofertilizer several reasons major being the viability of organism. Shelf life is the first and foremost problem with carrier based inoculants and does not retain throughout the crop cycle. Liquid bio-inoculants on the other hand facilitate long survival of organism by providing the suitable growth medium which is sufficient for entire crop cycle (Uma Maheswari and Elakkiya, 2014). Keeping in view of the advantages of liquid bio-inoculants, this study aims to evaluate the plant growth hormones production by liquid bio-inoculants and its effect on growth of Amaranthus under greenhouse condition.

Material and Methods

The present investigation was conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, G.K.V.K, Bengaluru.

Estimation of phytohormone production by bio-inoculants under In-vitro condition: Bioassay for GA and IAA production by bioinoculants was done by using Starch agar halo test and Cucumber root elongation method, respectively (Loper and Schroth, 1986). Bioassay for cytokinins determined by using Cucumber cotyledon greening bioassay (Fletcher *et al*., 1982).

Determination of efficacy of bio-inoculants under green house condition: Experiment was conducted under green house conditions with the

application of bio-inoculants in different treatment combinations are given below. :

T_1- Control; T_2 - A.c + B.m + F.a, T_3 - A.c + B.m + F.a + B.s; T_4 - A.c + B.m + F.a + T.v; T_5 - A.c + B.m + F.a + T.v + P.f + B.s; T_6 - A.c + B.m + F.a + P.f; T_7 - A.c + B.m + F.a +T.v+ B.s; T_8 - A.c + B.m+ P.f + T.v

Note: A.c - *Azotobacter chroococcum;* B.m - *Bacillus megaterium;* F.a – *Frateuria aurantia;* P. f - *Pseudomonas fluorescens;* B.s- *Bacillus subtilis* and T.v -*Trichoderma viridae*.

Crop and statistical design: Amaranthus cruentus crop was taken up following Complete Randomised Design (CRD) and each treatment replicated thrice.

Plant height and Number of leaves per plant: Plant height was measured from the base of plant to the terminal growing point of the main stem at 7, 21 and 30 Days After Sowing (DAS). The average plant height was expressed in centimeters (cm). The leaves which were fully opened, matured and not senescent were counted for each plant and recorded as number of leaves per plant (5 plants/treatment) at 7, 21 and 30 DAS.

Shoot length and Root length: Five normal plants were selected randomly from each treatment at harvest. The shoot length was measured from collar region to the tip of the seedling with the help of a scale and the mean shoot length was expressed in centimeter (cm). Five normal randomly selected plants used for the measurement of root length. The root length measured from collar region to the tip of primary root with the help of a scale and the mean root length was expressed in cm.

Root and shoot dry weight (g): The root and shoot of the same five seedlings selected for measurement were kept in butter paper bag and dried in an oven maintained at 85 ± 2°C for 24 hours. After drying, the butter paper bags were removed and kept in desiccators for cooling. The weight of shoot and root was recorded and mean dry weight of seedlings was calculated and expressed in grams.

Results and Discussion

Production of Plant growth promoting hormones by bio-inoculants

*Production of Gibberellic Acid (*GA): The concentration of GA in the liquid bio-inoculants was determined by the starch agar halo test and presented in Table-I. The highest GA was recorded in *Pseudomonas fluorescens* (4.75 µg) followed by *Bacillus subtilis* (2.57 µg) and *Bacillus megaterium* (2.29 µg). The least was recorded with *Trichoderma viridae* (1.43 µg). Lenin and Jayanthi (2012) have also isolated and purified GA3 and GA like compounds from genera *Azotobacter*, *Bacillus* and *Pseudomonas* isolated from soil in the range of 6.45 to 7.10 µg

Table I

Bioassay for Plant growth hormones production by liquid inoculants

Liquid inoculants	Gibberellic Acid (GA)		Indole Acetic Acid (IAA)		Cytokinin	
	Diameter of Zone (mm)	GA (µg)	Root Length (mm)	IAA (µg)	Chl µg /ml	Cytokinin (µg)
Azotobacter chroococcum	16.32	1.91	1.15	130.96	0.97	1.19
Bacillus megaterium	15.10	2.29	1.38	173.41	1.62	3.56
Frateuria aurantia	14.47	1.47	1.13	127.37	0.86	0.94
Pseudomonas fluorescens	23.04	4.75	1.44	183.07	2.12	4.94
Bacillus subtilis	18.85	2.57	1.27	160.67	1.39	2.69
Trichoderma viridae	14.22	1.43	0.86	91.32	0.55	0.88
SEM±	0.19	0.05	0.03	0.73	0.02	0.05
CD @ 5%	0.59	0.14	0.09	2.24	0.06	0.17

25 ml^{-1} broth whereas, in our findings gibberellins production was in the range of 25-60 µg ml^{-1}. So the selected *Pseudomonas* isolates were quite efficient for the production of gibberellins like substances.

*Production of Indole Acetic Acid (*IAA): The IAA bioassay is based on the inhibition of root growth in cucumber by IAA. As the concentration of IAA increases the root elongation of germinating seedlings is inhibited. The results of the bioassay are presented in Table-I. The liquid bio-inoculants containing *Pseudomonas fluorescens* showed highest production of IAA (183.07 µg) which was followed by *Bacillus megaterium* (173.41 µg) and *Trichoderma viridae* (91.32 µg) recorded least IAA production. Karnwal (2009) isolated pseudomonad strains from rhizosphere soils and observed that the *Pseudomonas fluorescens* AK1 and *Pseudomonas aeruginosa* AK2 showed the best plant growth-promoting activity. These isolates were tested for their ability to produce IAA in pure culture for both strains, indole production increased with increases in tryptophan concentration . *P. aeruginosa* AK2 was less effective in production of indole acetic acid than *P. fluorescens* AK1.

*Production of Cytokinins:*The cucumber cotyledon greening bioassay is frequently used for detecting cytokinins and the results of the test are shown in Table I. Cytokinins accelerate chloroplast differentiation as well as regulate and stimulate chlorophyll (Chl) production in etiolated cucumber cotyledons. The increase in Chlorophyll production is proportionate to the concentration of cytokinins and this response provides a sensitive yet rapid bioassay for cytokinins. The significantly highest cytokinin production was observed in *Pseudomonas fluorescens* (4.94 µg) and the least was recorded in *Trichoderma viridae* (0.88 µg). Yildirim (2011) demonstrated that heads of cabbages inoculated with the three strains and tested strains have higher chlorophyll content than controls (14.7 %, 14.0 %, and 13.7 %, respectively, in plants inoculated with *B. cereus, R. rubi*, or *B. reuszeri*).

Effect of liquid bio-inoculants on growth parameters of *Amaranthus* crop under greenhouse condition.

Plant height: The plant height was recorded at different intervals and it is presented in Table II. At 15 DAS, treatment T_5 (8.82 cm) showed significantly highest plant height and it was statistically on par with treatments T_8 and T_6 (8.70cm and 8.63 cm), respectively. The least plant height was noticed in

TABLE II

Effect of liquid bio-inoculants on growth parameters of Amaranthus crop under greenhouse condition

Treatments	Plant height (cm)			Number of leaves		
	15 DAS	21 DAS	30 DAS	15 DAS	21 DAS	30 DAS
T_1- Control	5.45	14.60	23.37	4.93	7.53	10.13
T_2- A.c + B.m + F.a	6.85	18.33	27.53	5.27	8.10	10.80
T_3- A.c + B.m + F.a + B.s	7.50	22.47	28.97	5.00	8.03	11.50
T_4- A.c + B.m + F.a + T.v	8.40	23.23	38.23	5.90	8.53	11.43
T_5- A.c + B.m + F.a + T.v + P.f + B.s	8.82	29.00	40.87	6.23	9.13	12.57
T_6- A.c + B.m + F.a + P.f	8.63	25.77	40.53	6.13	8.50	11.83
T_7- A.c + B.m + F.a +T.v+ B.s	7.82	25.53	36.43	6.10	8.27	11.40
T_8- A.c + B.m+ P.f + T.v	8.70	28.70	39.07	5.83	8.13	11.77
SEM±	0.15	0.35	0.25	0.34	0.15	0.16
CD @ 5%	0.44	1.05	0.76	1.03	0.45	0.48

Note : *A.c- Azatobacter chroococcum, B.m - Bacillus megaterium, F.a – Frateuria aurantia, P. f- Pseudomonas fluorescens, B.s- Bacillus subtilis* and *T.v -Trichoderma viridae*

control (5.45 cm). At 21 DAS, treatment T_5 (29 cm) showed significantly highest plant height followed by treatment T_8 (28.70 cm) and T_6 (25.77 cm). The least plant height was noticed in the control (5.45 cm). At 30 DAS the highest plant height was recorded in the treatment T_5 (40.87) which was on par with T_6 (40.53 cm). The efficacy of three species microbial consortium of *Bacillus* sp, *Azotobacter* sp and *Frauteria* sp. for its plant growth promoting efficacy in black gram (*Vigna mungo* (L.) Hepper has been reported by Maiyappan *et al*. (2010).

Number of leaves: Application of bio-inoculants have positive effect on number of leaves and the results pertaining to number of leaves at different days after sowing were interpreted in Table II. At 15 DAS, Treatment T_5 recorded higher number of leaves per plant (6.23) compared to other treatments. The treatments T_6, T_7 and T_4 recorded 6.13, 6.10 and 5.90 respectively. Control (T_1) recorded least number of leaves (4.93). At 21 DAS, Treatment (T_5) recorded highest number of leaves of 9.13 compared to treatments T_6 (8.53), T_4 (8.50), T_7 (8.27), T_8 (8.13). At 30 DAS, T_5 recorded maximum number of leaves (12.57) followed by T_6 (11.83) which was on par with T_8 (11.77), T_3 (11.50), T_4 (11.43) and T_5 (11.40). Control (T_1) recorded least number of leaves (10.13). Uma Maheswari and Elakkiya (2014) studied the application of liquid biofertilizers on *Vigna mungo* and observed for number of leaves which were increased in combined inoculation of liquid biofertilizer treatments such as *Rhizobium* + *Azospirillum* + *Azotobacter* on 60th day showed maximum response in leaves (27.6) followed by other treatments and control.

Root and Shoot Length: The results pertaining to root and shoot length of Amaranthus plants are presented in Table III. Maximum root length (7.80 cm) was recorded in T_3. Least root length is noticed in Control (T_1). Similarly the shoot length of treatment T_5 recorded maximum shoot length (40.87 cm) compared to other treatments. Treatment T_6 (40.53cm) was on par with T_5. Least shoot length was recorded in control (23.37cm). Uma Maheswari and Elakkiya, (2014) their study was clearly highlighted that combined inoculation of liquid biofertilizers such as *Rhizobium Azospirillum, Azotobacter* (Treatment T_7) could enhance the morphological parameters such as height of the plant (31.62cm), number of leaves (27.6), Shoot length (20.5 cm), Root length (15.6 cm), number of roots (21.8), root nodules(15.4) at 60th day compared to individual inoculation and control.

TABLE III

Effect of liquid bio-inoculants on plant biomass of Amaranthus under greenhouse condition

Treatments	Root length (cm)	Shoot length (cm)	Root & Shoot Fresh weight (cm)	Root & Shoot Dry weight (cm)
T_1- Control	3.40	23.37	2.48	0.94
T^2- A.c + B.m + F.a	5.30	27.53	4.60	1.47
T_3- A.c + B.m + F.a + B.s	6.30	28.97	5.71	1.73
T_4- A.c + B.m + F.a + T.v	6.50	38.23	7.59	1.93
T_5- A.c + B.m + F.a + T.v + P.f + B.s	7.80	40.87	7.87	3.07
T_6- A.c + B.m + F.a + P.f	7.00	40.53	7.77	2.83
T_7- A.c + B.m + F.a +T.v+ B.s	6.70	36.43	7.71	2.68
T_8- A.c + B.m+ P.f + T.v	6.17	39.07	7.19	2.93
SEM±	0.07	0.25	0.06	0.08
CD @ 5%	0.21	0.76	0.17	0.24

Note : *A.c- Azatobacter chroococcum, B.m - Bacillus megaterium, F.a – Frateuria aurantia, P. f- Pseudomonas fluorescens, B.s- Bacillus subtilis* and *T.v -Trichoderma viridae*.

Root and Shoot Fresh Weight: There was significant difference among the treatments in Root and shoot fresh weight and the highest root and shoot fresh weight was recorded in T_5 (7.87g) which was on par with T_6 (7.77g) and T_7 (7.71g). Control (T_1) recorded least root and fresh weight (2.48g). Singaravel *et al*., 2008 reported the application of liquid bio-fertilizer both Symbion N and Symbion P significantly increased the growth character, yield character and yield of okra. Among various treatments, Symbion N and Symbion P both applied in soil was significantly superior in increasing the growth and yield of okra. This treatment recorded the highest okra yield of 6280 Kg ha^{-1}.

Root and Shoot dry weight: The Root and shoot dry weight of Amaranthus crop was recorded at harvest stage and the highest was recorded in treatment T_5 (3.07g). The treatments T_8 (2.93g) and T_6 (2.83g) were on par with each other. Uninoculated control (T_1) recorded least root and shoot dry weight (1.47g). The efficacy of three species microbial consortium of *Bacillus* sp., *Azotobacter* sp. and *Frauteria* sp. for its plant growth promoting efficacy in black gram (*Vigna mungo* L.) has been reported by Maiyappan *et al*. (2010).

The study indicated that application of consortia of liquid bio-inoculants enhance the growth and biomass of Amaranthus which can be attributed to the plant growth promoting ans biocontrol activity of the organisms.

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