J Krishi Vigyan 2019, 7 (2) : 94-99 DOI : 10.5958/2349-4433.2019.00048.5

Effect of Liquid Bioinoculants on Biocontrol Activities and Growth Promotion of Amarathus (*Amaranthus cruentus*)

H Gurumurthy[1], M K Shivaprakash[2] and C C Maina[3]

Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore-560 065(Karnataka)

ABSTRACT

An experiment was conducted to evaluate the best consortia of liquid bioinoculantsfor biocontrol of pathogens and to enhance the plant growth and biomass of *Amaranthus cruentus* under greenhouse conditions. Liquid bioinoculants *viz*., *Azotobacter chroococcum*, *Bacillus megaterium*, *Frateuria aurantia*, *Psuedomonas fluorescens*, *Bacillus subtilis* and *Trichoderma viridae*were used either singly oras consortia in different treatment combinations. Treatment T_8 (Pathogens+ *Bacillus subtilis* + *Pseudomonas fluorescens* + *Trichoderma viride*) recorded maximum biocontrol efficiency (72.89 %) and vigour index (2423.56) followed by T_7 (62.25 % and 2298.11). Growth parameter like maximum plant height (8.82 cm at 15 DAS, 29.0 cm at 21 DAS and 40.87 cm at 30 DAS) was recorded in T_5. Maximum number of leaves (6.23 at 15 DAS, 9.13 at 21 DAS and 12.57 at 30 DAS) was recorded in T_5which received consortia of *Azotobacter chroococcum, Bacillus megaterium, Frateuria aurantia, Pseudomonas fluorescens, Bacillus subtilis* and *Trichoderma viride*. Maximum root length, shoot length, plant fresh weight and plant dry weight were 7.80 cm, 40.87 cm, 7.87 g and 3.07 g, respectively recorded in T_5 followed by other treatments.

Key Words: Amaranthus, Biocontrol, Efficiency, Liquid bioinoculants, Seedling, Vigour index.

INTRODUCTION

The health and nutrition of expanding world population is major upcoming challenges especially in developing countries. Green leafy vegetables were used since ancient periods as source of food as they contain many nutrients and minerals which are helpful in maintaining human health. Leafy vegetables being richest in their nutritional value hold an important place in well-balanced diet and are the cheapest of all the vegetables within the reach of poor man. They are also sources of energy, micronutrients and nutrients essential for health, in addition to phytochemicals with health benefits including glycemic control, immuno-stimulation or antioxidant activity (Belanger *et al*, 2004). *Amaranthus cruentus*(L) is a popular leafy vegetable originated from South America. Apart from its uses as a vegetable, it has also been used as an effective alternative to drug therapy in people with hypertension and cardiovascular disease (CVD) Martirosyan and Miroshnichenko (2007). The demand for this crop as vegetable has increased, especially in the urban centres where people are not involved in primary production and hence, these vegetables have become an important commodity in our market and its production an important economic activity for the rural women.

Liquid biofertilizer formulation is a promising and updated technology of the conventional carrier based production technology wherein its shelf life is up to 3m and it does not retain throughout the crop cycle. Liquid biofertilizers on the other hand facilitates the long survival of the organism by providing the suitable medium which is

Corresponding Author's Email: gurumurthyh.8031@gmail.com

[1] Senior Research Fellow, Division of Soil Science and Agricultural Chemistry, ICAR-IIHR, Hesaraghatta Lake Post, Bengaluru-560089.

[2] ICAR Emeritus Professor, Department of Agricultural Microbiology, UAS, GKVK, Bengaluru-560065, Karnataka.

[3] Research Fellow, Scheme on Popularisation of Biofertilizer, Department of Agricultural Microbiology, UAS, GKVK, Bengaluru-560065.

sufficient for the entire crop cycle. Carrier based bio fertilizers are not so tolerant to the temperature which is mostly unpredictable and uncertain in the crop fields while temperature tolerance is the other advantage of the liquid biofertilizers (Mahdi *et al*, 2010). Keeping all these aspects in mind, this study was conducted to evaluate the biocontrol efficiency, seedling vigour index, growth and biomass of Amarathus (*Amaranthuscruentus*) by using liquid bioinoculants.

MATERIAL AND METHODS

Biocontrol efficacy of selected biocontrol agents in seedling trays under green house conditions

A seedling tray experiment was conducted to evaluate the antagonistic and growth promoting effect of the consortia of biocontrol agents in substrate enriched with biocontrol agents against pathogen under greenhouse condition in the Department of Agril. Microbiology UAS. GKVK, Bangalore with the following treatments. T_1:Control, T_2: *Bacillus subtilis* (Bs), T_3:*Pseudomonas fluorescens* (Pf), T_4:*Trichoderma viride* (Tv), T_5:*Bacillus subtilis* (Bs) + *Pseudomonas fluorescens* (Pf),T_6:*Bacillus subtilis* (Bs) + *Trichodermaviride* (Tv), T_7:*Pseudomonas fluorescens* (Pf) + *Trichodermaviride* (Tv), T_8:*Bacillus subtilis* (Bs) + *Pseudomonas fluorescens* (Pf) + *Trichodermaviride* (Tv)

Preparation of substrates and seedling trays

The substrate was prepared by mixing 10 kg of coir pith with 2.5 kg each of red earth, perlite, vermicompost and pongamia cake. The substrates were autoclaved in autoclavable polybags and filled into large polythene bags. The mass multiplied pathogen inoculum *viz., Fusarium*sp., *Phytophthora*sp., *Pythium*sp. and *Rhizoctonia*sp. and biocontrol agents were added to substrate mixture @ 100g /kg to each polybag and mixed properly one week prior to sowing. The mixed substrate was added at the rate of 100g/tray at the time of sowing. The trays with 18 cups each with 100g capacity were used for the experiment. The trays were labelled and substrate infested with pathogen was filled in the trays and the sowing was taken up in replication. The trays were watered daily and the following observations related to germination *viz.*, germination percentage, percent pre-emergence and post emergence disease incidence, Seedling vigour index (SVI), shoot and root length , bio control efficiency were recorded.

Percent pre-emergence disease incidence was calculated using formula100 (GA-GT)/ GA where GA-Germination percentage in absolute control and GT- Germination percentage in treatment. Percent post- emergence disease incidence was calculated using formula 100 (GP-ND)/ GP where GP-Number of healthy plants left in control and ND- Number of healthy plants left in treatment. The seedling vigour index was calculated by adopting the method suggested by (Abdul- Baki and Anderson,1973)and expressed in number by using the formula SVI = Germination (%) x [shoot length (cm) + root length (cm)]

The shoot length of ten randomly selected plants was measured from collar region to the tip of the plant with the help of a scale and the mean shoot length was expressed in centimetres. The root length ten randomly selected plants was measured from collar region to the tip of primary root with the help of a scale and the mean root length was expressed in centimetres .Biological control efficacy was calculated using the formula BCE = (DIPC-DIT/ DIPC) x 100 given by (Guo *et al*, 2004)where DIPC- Disease incidence in pathogen control and DIT- disease incidence in treatment group

Growth promotion and biocontrol activities of liquid formulations on Amaranthus in raised beds

For this experiment, land was dug and soil was brought to fine tilth. FYM and vermicompost were applied at the rate of 12.5 t/ha 15d prior to sowing. Small plots having a dimension of 1m × 1m were prepared. Microbial inoculants were applied to each plot as per required treatment as soil application seven days before sowing. Sowing was taken up and the beds were regularly watered and maintained and observations were recorded

at regular intervals. The plants were sprayed with consortia of biocontrol agents*viz*., *Pseudomonas fluorescens. Bacillus subtilis, Lactic acid bacteria, Azotobacter chroococcum, Saccaharomyces cerevisiae* at 14 and 21d after sowing. The treatment details were as follows. T_1**:**Control,T_2**:** *A. chroococcum*(A.c) + *B. megaterium*(B.m) + *F. aurantia*(F.a)**,** T_3**:** *A. chroococcum*A.c) + *B. megaterium*(B.m) + *F. aurantia*(F.a) + *B.subtilis*(B.s), T_4**:** *A. chroococcum*(A.c) + *B. megaterium*(B.m) + *F. aurantia*(F.a) + *T.viride*(T.v), T_5**:** *A. chroococcum*(A.c) + *B. megaterium*(B.m) + *F. aurantia*(F.a) + *T.viride*(T.v) + *P. fluorescens* (P.f) + *B.subtilis*(B.s)**,** T_6**:** *A. chroococcum*(A.c) + *B. megaterium*(B.m) + *F. aurantia*(F.a) + *P. fluorescens* (P.f), T_7**:** *A. chroococcum*(A.c) + *B. megaterium*(B.m) + *T.viride*(T.v) + *B.subtilis* (B.s) T_8**:***A. chroococcum*(A.c) + *B. megaterium*(B.m) + *P. fluorescens* (P.f) + *T.viride*(T.v)

Observations related to plant growth *viz.,* plant height (cm), number of leaves, shoot length (cm), root length (cm), fresh weight and dry weight of plants (g) were recorded at regular intervals.

RESULTS AND DISCUSSION

Biocontrol efficiency of Amaranthus

The results pertaining to the effect of liquid bioionculants on biocontrol efficiency in amaranthus grown in seedling trays under greenhouse condition is presented in Table 1. Per cent germination (89.07) was maximum in Treatment T_8 (Pathogens + *Bacillus subtilis* + *Pseudomonas fluorescens* + *Trichodermaviride*) followed by T_7 (Pathogens + *Pseudomonas fluorescens*+ *Trichodermaviride* (88.70) which was on par with T_8. Lowest per cent germination (70.20) was recorded in control T_1 (pathogens alone).There were no significant differences observed between the treatments regarding days taken for 50 per cent germination. The treatments T_5 and T_6 recorded less number of days taken for 50 per cent germination (3.00) followed by T_4, T_7 and T_8 which took 3.33 days taken for 50 per cent germination. Lowest pre-emergence disease incidence (3.0%) was observed with T_8 followed by T_6 (3.26 %) T_7 (4.05 %) and T_5 (4.07 %). Treatment T_8 which received *Trichoderma viride* as liquid bioinoculant recorded lowest post emergence disease incidence (4.01%) which was significantly lesser than other treatments. Maximum biocontrol efficiency (72.89 %) was observed in the treatment T_8 which was significantly higher than T_7 (62.25 %). The highest biocontrol efficiency of T_8 can be attributed to the use of biocontrol agents to suppress the disease incidence with the production of antibiotics, siderophores, and cell wall degrading enzymes (chitinase and glucanse) as well as induction of systemic resistance, root colonization efficacy, and rhizosphere competence (Beneduzi *et al*, 2012).

Biocontrol efficiency was lowest in control which may be attributed to lack of any biocontrol agent in the treatment.Compared to individual inoculation and control, collective effect of liquid consortia can reduce the use of chemical fertilizers. Inoculation with plant growth promoting rhizobacteria (PGPR) may enhance crop productivity either by making the nutrients available to plants or by protecting plants from pathogenic microorganisms. Our results were similar to the results obtained by (Karthikeyan*et al*, 2001)who reported the use of consortia of *Trichodermaviride*and *T. harzianum*and *Paecilomyces lilacinus* against damping off of brinjal and Mohan (2006)who reported a decrease in the disease incidence of brinjal seedlings when treated with a consortia of biocontrol agents and PGPRs.

Seedling vigour index of Amaranthus

The data pertaining to the efficiency of liquid bioinoculants in enhancing seedling vigour of Amaranthus are presented in Table 2. Highest root length (6.50 cm) was recorded in T_8(Pathogens +*Bacillus subtilis*+*Pseudomonas fluorescens* +*Trichoderma viride*). Lowest root length (2.30 cm) was recorded with control which was treated with only pathogens. Treatment T_8 (Pathogens + *Bacillus subtilis, Pseudomonas fluorescence* and

Table 1. Biocontrol efficiency of liquid bio inoculants in Amaranthus grown in seedling trays under green house condition.

Treatment	Per cent Germination	Days taken for 50 per cent germination	Pre-emergence disease incidence (%)	Post –emergence disease incidence (%)	Biocontrol efficiency (%)
T_1	70.20^{d}	4.00^{a}	28.33^{a}	28.56^{a}	0.00^{h}
T_2	75.37^{cd}	4.00^{a}	15.25^{b}	19.03^{b}	12.00^{g}
T_3	80.37^{bc}	4.33^{a}	8.23^{c}	14.27^{c}	29.11^{e}
T_4	78.17^{c}	3.33^{a}	5.07^{cd}	9.53^{d}	20.23^{f}
T_5	79.30^{bc}	3.00^{a}	4.07^{d}	9.28^{d}	32.25^{d}
T_6	84.23^{ab}	3.00^{a}	3.26^{d}	7.14d^{e}	54.12^{c}
T_7	88.70^{a}	3.33^{a}	4.05^{d}	8.00^{d}	62.25^{b}
T_8	89.07^{a}	3.33^{a}	3.00^{d}	4.01^{e}	72.89^{a}
SEM±	1.29	1.03	0.79	0.77	0.75

Table 2. Efficiency of liquid bio inoculants in enhancing seedling vigour of Amaranthus grown in seedling trays under green house condition.

Treatment	Root length (cm)	Shoot length (cm)	Root dry weight (g)	Shoot dry weight (g)	Vigour index
T_1	2.30^{f}	10.11^{d}	0.410^{a}	0.087^{d}	871.03^{d}
T_2	4.30^{bc}	17.13^{bc}	0.053^{c}	0.090^{d}	1615.19^{c}
T_3	2.70^{ef}	16.31^{c}	0.092^{b}	0.073^{e}	1527.81^{c}
T_4	3.33^{de}	17.00^{bc}	0.061^{c}	0.084^{d}	1591.43^{c}
T_5	4.00^{cd}	15.13^{c}	0.088^{b}	0.124^{c}	1517.38^{c}
T_6	6.00^{ab}	19.00^{ab}	0.091^{b}	0.221^{a}	2109.25^{b}
T_7	5.60^{a}	20.31^{a}	0.089^{b}	0.163^{b}	2298.11^{ab}
T_8	6.50^{bc}	20.71^{a}	0.093^{b}	0.223^{a}	2423.56^{a}
SEM±	1.01	0.58	0.002	0.002	75.17

Trichoderma viride) recorded maximum shoot length (20.71 cm) followed by T_7 (20.31), T_8 and T_7 which were on par with each other. Lowest shoot length (10.11 cm) was recorded in Control T_1. Maximum root dry weight (0.093 g) was observed in T_8 followed by T_6 (0.091 g) and was on par with each other. Highest shoot dry weight (0.223 g) was also noticed in T_8 (Pathogens + *Bacillus subtilis, Pseudomonas fluorescens* and *Trichoderma viride*) followed by T_6 (0.221 g). Least shoot dry weight (0.087 g) was recorded in control (T_1).

There was a significant increase in the vigour index of Amaranthus plants of T_8 receiving pathogens, *Bacillus subtilis, Pseudomonas fluorescence* and *Trichoderma viride* (2423.56) when compared to T_7 receiving pathogens, *Pseudomonas fluorescens* and *Trichoderma viride* (2298.11). Plants treated with only pathogens recorded the lowest T_1 vigour index (871.03). Higher growth parameters were observed in T_8 attributed to biocontrol activity of *Trichoderma viride, Pseudomonas fluorescens* and plant growth promoting activity of *Bacillus subtilis* and *Pseudomonas fluorescens.* This finding was similar to the findings of Gamalero *et al* (2004).

Growth and biomass of Amaranthus as influenced by liquid bio inoculants on raised beds under green house condition

The results pertaining to growth and biomass of Amaranthus as influenced by liquid bio inoculants on raised beds under green house condition at different days after sowing (DAS) is interpreted in Table 3. At 15 DAS, plants of T_5 receiving consortia of *Azotobacter chroococcum, Bacillus megaterium, Frateuria aurantia, Pseudomonas fluorescens, Bacillus subtilis* and *Trichoderma viride* recorded maximum height (8.82 cm) followed by T_8 (*Azotobacter chroococcum, Bacillus megaterium, Pseudomonas fluorescens* and *Trichoderma viride)* which recorded 8.70 cm and T_6 recorded 8.63 cm. The lowest plant height was recorded in the control (5.45 cm). At 21 DAS, T_5 recorded maximum plant height of 29.00 cm followed by T_8 which recorded 28.70 cm. After 30 DAS, the maximum plant height (40.87 cm) was observed in T_5 treated with *Azotobacter chroococcum, Bacillus megaterium, Frateuria aurantia, Pseudomonas fluorescens, Bacillus subtilis* and *Trichoderma viride* followed by T_6 (40.53 cm) which was treated with microbial consortia of *Azotobacter chroococcum, Bacillus megaterium, Frateuria aurantia* and *Pseudomonas fluorescens* and T_8 (39.07 cm). The treatment control recorded least plant height of 23.37 cm

The application of microbial consortia also had significant positive effect on number of leaves of Amaranthus raised in beds under green house conditions. At 15 DAS and 21 DAS, significant number of leaves (6.23 and 9.13, respectively) was observed in T_5 which received microbial consortia of *Azotobacter chroococcum, Bacillus megaterium, Frateuria aurantia, Pseudomonas fluorescens, Bacillus subtilis*and*Trichoderma viride*) followed by and on par with the treatments T_6 (6.13 and 8.50, respectively) and T_7 treatments containing microbial consortia of *Azotobacter chroococcum, Bacillus megaterium, Frateuria aurantia*and *Pseudomonas fluorescens* (T_6) and microbial consortia of *Azotobacter chroococcum, Bacillus megaterium, Frateuria aurantia, Bacillus subtilis* and *Trichoderma viride* (T_7) respectively. The lowest number of leaves (4.93) was observed in the control.

After 30 days of sowing, the highest root length(7.80 cm), shoot length (40.87), plant fresh weight (7.87 g), plant dry weight (3.07 g) was recorded in the treatment (T_5) treated with *Azotobacter chroococcum, Bacillus megaterium, Frateuria aurantia, Pseudomonas fluorescens, Bacillus subtilis* and*Trichoderma viride.*

There was a significant difference among growth parameters between the plants which received different combination of liquid bioinoculants. Treatment T_5 recorded maximum growth parameters compared to other treatments. The findings coincides with (Murugesan, 2008, Ramakrishnan and Selvakumar, 2012), overall utilization of liquid biofertilizers with single and combined treatments in addition to increased yield could be a strategy to achieve sustainable agriculture. The results are attributed to the cumulative beneficial effect of PGPRs and biocontrol agents. These results were in conformity with the findings of (Chrispaul*et al*, 2010) who reported that the treatment receiving effective microorganisms recorded highest values in all the parameters measured except the root dry matter accumulation. There were significant differences ($p \leq 0.05$) in shoot length, growth, stem diameter, leaf numbers per plant, leaf area, leaf fresh and dry weight and root fresh and dry weights among treatments. The results demonstrated that growth and yield of Amaranthus may be improved by inoculating the plants with effective microorganisms, and as a result reducing the use of chemical fertilizers in production of this vegetable hence promoting sustainable agriculture.

REFERENCES

Abdul- Baki A and Anderson JD(1973). Vigour determination in Soybean seed by multiple criteria. *Crop Sci***13**: 630-633.

Belanger J, Balakrishna M, Latha P, Katumalla S and Johns T (2004). Contribution of selected wild and cultivated leafy vegetables from South India to lutein and β-carotene intake. *Asia Pacific J Clinical Nutr* **46** (4): 221-227.

Table 3. Growth and yield of Amaranthus as influenced by liquid bio inoculants in raised beds under green house condition.

Treatment	Plant height (cm)			Number of leaves			Root length (cm)	Shoot length (cm)	Plant Fresh weight (g)	Plant Dry weight (g)
	15 DAS	21 DAS	30 DAS	15 DAS	21 DAS	30 DAS				
T_1	5.45^{e}	14.60^{e}	23.37^{f}	4.93^{a}	7.53^{c}	10.13^{d}	3.40^{g}	23.37^{f}	2.48^{f}	0.94^{e}
T_2	6.85^{d}	18.33^{d}	27.53^{e}	5.27^{a}	8.10^{bc}	10.80^{cd}	5.30^{f}	27.53^{e}	4.60^{e}	1.47^{d}
T_3	7.50^{c}	22.47^{c}	28.97^{d}	5.00^{a}	8.03^{bc}	11.50^{bc}	6.30^{de}	28.97^{d}	5.71^{d}	1.73^{cd}
T_4	8.40^{ab}	23.23^{c}	38.23^{b}	5.90^{a}	8.53^{ab}	11.43^{bc}	6.50^{cd}	38.23^{b}	7.59^{b}	1.93^{c}
T_5	8.82^{a}	29.00^{a}	40.87^{a}	6.23^{a}	9.13^{a}	12.57^{a}	7.80^{a}	40.87^{a}	7.87^{a}	3.07^{a}
T_6	8.63^{a}	25.77^{b}	40.53^{a}	6.13^{a}	8.50^{ab}	11.83^{b}	7.00^{b}	40.53^{a}	7.77^{ab}	2.83^{ab}
T_7	7.82^{bc}	25.53^{b}	36.43^{c}	6.10^{a}	8.27^{b}	11.40^{bc}	6.70^{c}	36.43^{c}	7.71^{ab}	2.68^{b}
T_8	8.70^{a}	28.70^{a}	39.07^{b}	5.83^{a}	8.13^{c}	11.77^{b}	6.17^{e}	39.07^{b}	7.19^{c}	2.93^{ab}
SEM±	0.15	0.35	0.25	0.34	0.15	0.16	0.07	0.25	0.06	0.08

Note: Means with the same superscript donot differ significantly @ P=<0.05 as per DMRT

T_1: Control (Pathogens alone) T_5: Pathogens + *Bacillus subtilis* + *Pseudomonas fluorescens*

T_2: Pathogens + *Bacillus subtilis* T_6: Pathogens + *Bacillus subtilis* + *Trichodermaviride*

T_3: Pathogens + *Pseudomonas fluorescens* T_7: Pathogens + *Pseudomonas fluorescens* + *Trichodermaviride*

T_4: Pathogens + *Trichodermaviride* T_8: Pathogens + *Bacillus subtilis* + *Pseudomonas fluorescens* + *Trichodermaviride*

Beneduzi A, Ambrosini A andPassaglia LM(2012). Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetics and Molecular Biol* ***35*****(4)**: 1044-1051.

Chrispaul Mathaura, David M, Musyimi, Joseph A, Ogur Samuel V andOkello (2010). Effective microorganisms and their influence on growth and yield of pig weed. *ARPN J Agri and Biolo Sci* **5**(1): 17-22.

Gamalero E, Trotta A, Massa N, Copetta A, Martinotti MG and Berta G (2004). Impact of two fluorescent Pseudomonas and an Arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition. *Mycorrhiza* **14**:185–192.

Guo J H, Q I H Y, Guo Y H, Ge H L, Gong L Y, Zhang L X and Sun PH (2004). Biocontrol of tomato wilt by plant growth promoting rhizobacteria. *Biol Control***29:** 66-72.

Karthikeyan Doraisamy G and Sivakumar C V (2001). Biological control of *Pythiumaphanidermatum-Meloidogyne incognita* disease complex in chilli with organic amendments. *Madras Agric J* **86**: 320-323.

Mahdi S, Hassan GI, Samoon SA, Rather H A and Showkat A D (2010). Bio-fertilizers in organic agriculture. *J Phytology* **2**: 42-54.

Martirosyan D and Miroshnichenko L A (2007). Amaranth oil application for coronary heart disease and hypertension. *Lipids in Health and Disease* **476-591.**

Mohan S M (2006). *Biological control of Damping off disease of Solanaceous crops in Commercial Nurseries. M Sc (Agri.) Thesis*UnivAgriSci Bangalore.

Murugesan NV (2008). Effect of liquid biofertilizer on growth and yield of tomato *J Ecobiology* **23** (2): 153-158.

Ramakrishnan K and Selvakumar G (2012). Effect of biofertilizers on enhancement of growth and yield on tomato, *Int J Res in Botany* **2**(4): 20-23.

Saunder R A and Beciker R (1984). Amaranthus.A potential food and feed resource. In: "*Advanced Science Technology*" cereal chemist. St Paul MN. P. 352.

Received on 27/02/2019 *Accepted on 30/03/2019*