Available Online at http://www.resealert.com

International Journal of Current Advanced Research
Vol. 1, Issue, 2, pp.22 - 25, October, 2012

International Journal of Current Advanced Research

RESEARCH ARTICLE

Studies on the Efficacy of Different Formulations of Bioinoculant Consortium on Sunflower (*Helianthus annuus* l.) Var. Modern

Sivasakthivelan.P* and D.Stella

Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608002, Tamilnadu - India.

*Corresponding author- K. Elumalai, Email- plantdoctorsiva@yahoo.co.in

ARTICLE INFO

Article History:

Received 10th August, 2012
Received in revised form 25th, August, 2012
Accepted 15th September, 2012
Published online 27th October, 2012

Key words:

Sunflower, Carrier, Alginate bead, Liquid Formulation, Bioinoculant consortium

ABSTRACT

The Bioinoculants are gaining importance in sustainable agriculture various complementing combinations of microbial inoculants for management of major nutrients such as nitrogen and phosphorus is necessary for sustainability. The present study was undertaken to assess the efficacy of carrier, alginate bead and liquid formulations and bioinoculant consortium of *Azospirillum lipoferum*, *Bacillus megaterium* and *Pseudomonas fluorescence* on the growth and yield of sunflower. The bioinoculant consortium improves the colonization potential, sustainability within the inoculants and enhances crop growth hence we hypothesize that microbial consortium enhances plant growth positively by a multitude of synergistic mechanisms when compared to single inoculant application. The results reveal that bioinoculant treatment of liquid formulation of *Azospirillum lipoferum* (AUAz-1) + *Bacillus megaterium* (AU Ba-1) + *Pseudomonas fluorescence* (AU Ps-1) produced the highest recorded values in all growth and yield parameters compared with carrier based and alginate bead formulations.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil seed crop grown throughout the year across India. The genus *Helianthus* includes about 70-80 species, some of them are perennials, *Helianthus annuus* L. species belongs to the family Asteraceae (Compositae).

Nitrogen and Phosphorus are two of the major essential elements for growth and development of crop plants, they are provided to plants in the form of chemical fertilizers which poses a health hazard and microbial population problem in soil besides the high cost of their application. Biofertilizers are the formulation of living microorganisms, which are able to fix atmospheric nitrogen and convert insoluble phosphorus to available one for the use of plants. Biofertilizers are inputs containing microorganisms which are capable of mobilizing nutritive elements from non-usable to usable form through biological processes (Subba Rao, 1993).The beneficial effects induced by the inoculated bacteria on plant growth and yield were Production of growth promoting substances such as gibberellins, cytokinins, IAA and other auxins that specially stimulate the root system and may also alter the endogenous plant phytohormone balance (Dobbelaere *et al*., 1999) Fixation of molecular nitrogen in rhizosphere that becomes available to inoculated plant (Skvortsova *et al*., 1998).

In the present study, the efficacy of bioinoculants with nitrogen fixers, phosphate solubilizing bacteria and chemical fertilizers, individually and in different combinations and formulations on the growth of sunflower *Helianthus annuus* under field conditions were studied aiming to reduce the chemical fertilizers usage, maximizing their use efficiency and to obtain highest growth and productive parameters.

MATERIALS AND METHODS

Culture Collection

Azospirillum lipoferum (AU Az-1), Phosphate solubilizing bacterial strain *Bacillus megaterium* (AU Ba-1) and *Pseudomonas fluorescens* (AU Ps-1) were obtained from the culture collections of the Department of Microbiology, Faculty of Agriculture, Annamalai University and used for this study.

Purification and maintenance of Cultures

Azospirillum lipoferum (AU Az-1), *Bacillus megaterium* (AU Ba-1) and *Pseudomonas fluorescens*, (AU Ps-1) were purified by streak plate method on N free malic acid, nutrient agar and king's B medium respectively. Individual colonies picked were streaked on respective slants and stored in a refrigerator at 4°C for further studies.

Compatibility test of the Bioinoculants

Azospirillum lipoferum (AU Az-1), *Bacillus megaterium* (AU Ba-1) and *Pseudomonas fluorescens* (AU Ps-1) were investigated for their compatibility with each other by Cross streak assay method. Nutrient agar medium was prepared, autoclaved and poured to a Petri plate. *Azospirillum lipoferum* was streaked at one end as a single streak and the plates incubated at 32°C for 48 h. After robust growth of the *Azospirillum lipoferum* (AU Az-1), *Bacillus megaterium* (AU Ba-1) and *Pseudomonas fluorescens* (AU Ps-1) were streaked vertically and the plates were incubated for 96 hours.

Preparation of carrier based inoculant

The selected isolates were multiplied in large quantities in appropriate culture broths by incubating at 28±2ºC in an incubator shaker till they attained log phase with a cell load of $1x10^9$ cfu ml^{-1} and were used for inoculant preparation. Press mud collected from East India Distilleries (EID Parry), Nellikuppam were used as carriers. The carrier materials were powdered and the pH was brought to neutral by adding $CaCO_3$ and sterilized at 15 *psi* for 1 hour and allowed to cool over night and then mixed with the log phase culture ($1x10^9$ cfu ml^{-1}) of the selected agriculturally beneficial microbial isolates *viz.*, *Azospirillum lipoferum* (AU Az-1) *m*, *Bacillus megaterium* (AU Ba-1) and *Pseudomonas fluorescens* (AU Ps-1) individually in appropriate quantities of sterile carrier in shallow trays.

The moisture content was adjusted to 30-35 per cent. Curing in shallow trays for 24 hr in aseptic rooms and packed in high density opaque polythene bag (300 gauge) at the rate of 200 g bag^{-1} and sealed. Individual inoculant was prepared by mixing equal volumes of each culture broth with sterile carrier and combined inoculant was also prepared by mixing equal volumes of broth with the carrier materials.

Preparation of alginate beaded inoculant

The *A. lipoferum* (AU Az-1), *B. megaterium* (AU Ba-1) and *P. fluorescens* (AU Ps-1) were grown in respective medium to get a population of $1x10^9$ cfu ml^{-1}. Sodium alginate beaded inoculant was prepared as per the methods described by Hegde and Brahmaprakash (1992). Two gram of sodium alginate was added to 100 ml of culture broth of PGPB and mixed for 30 min in a magnetic stirrer. The mixture was added drop wise through a 10 ml syringe into 100 ml sterile 0.1N $CaCl_2$ to obtain uniform alginate beads. One gram of material contained 16 to 17 beads, each bead approximately weighing 60 mg. The beads were washed twice in sterile distilled water and incubated in respective broth containing PGPB isolates for seven days in a psychrotherm (model environ shaker, Orbitec Ltd.,India) incubator at 28 ± 2ºC to allow PGPB to multiply inside the beads. The beads were again washed in sterile distilled water and air dried in laminar air flow chamber under aseptic condition.

Preparation of liquid inoculant

For developing liquid formulation of *Azospirillum*, N free malate (NFb) broth was prepared in combination with different chemicals to increase the survival of *Azospirillum* cells. The chemical amendments *viz.*, trehalose at 10 mM, polyvinylpyrollidone (PVP) at 2 % and glycerol at 10 mM were added to one litre of NFb broth separately.

One ml of log phase culture of *Azospirillum lipoferum* was inoculated individually in each broth. An uninoculated control was maintained for each broth and the flasks were incubated at room temperature.

Determination of seedling vigour index under in vitro condition

The selected plant growth promoting bacteria isolates *viz., A. lipoferum* (AU Az-1), *B. megaterium* (AU Ba-1) *and P. fluorescens* (AU Ps-1) were used to study their effect as consortium on seed germination and seedling growth of sunflower by sterile growth pouch method. The seeds were treated with the individual and combined PGPB inoculants (5 ml 10^{-1} seeds) and shade dried for 30 min. ($1x10^8$ cells $seed^{-1}$). The treated seeds were placed in plastic cups filled with sterile sand and incubated at 28±1ºC at 95% relative humidity. The seeds were periodically moistened with Jensen's nutrient solution.

Seed germination

The number of days taken for 50 per cent of the seeds to show radicle emergence was taken as the day for first count of germination. Uninoculated seeds with distilled water served as control. Seedling height and germination percentage were recorded and vigour index was calculated as per the procedure suggested by Abdul-Baki and Anderson (1973).

Vigour index = Germination per cent x Total length of seedling (Cm)

Seedling height

Seedling from each treatment were taken at random and the length between the collar and tip of primary root and the length of plumule was measured between collar and tip of primary leaves and mean value was expressed in centimeters.

Studies on the growth promoting efficacy of Bioinoculants on sunflower in pot culture experiment

The pot culture trials were conducted at the pot culture yard of the Department of Microbiology which is situated at 11° 24' North latitude and 79° 44' East longitude at an altitude of + 5.79 m above mean sea level. The cement pots of size 1' x 2' x2' filled with land soil and sand in the ratio of 1:1.The seeds of sunflower Var. Morden were surface sterilized with 80 per cent ethanol and 0.1 per cent mercuric chloride and washed the seeds with sterile distilled water for 3 to 4 times. The seeds were mixed with carrier based bioinoculant as individual organisms and consortium of organisms separately having a cell load of $1x10^9$ cfu ml^{-1} and shade dried for 30 min. After shade drying, the seeds were sown at 25 of seeds per pot and finally five seeds were maintained.

Biometric observations

Flower Head Diameter (Cm)

Capitullum diameters of three representative plants from each treatment were measured at harvest and their mean values were recorded.

Percentage of filled seeds head^{-1}

Percentage of filled seeds head^{-1} was worked out by dividing the number of filled seeds head^{-1} by the total number of seeds head^{-1}.

Seed Yield (kg ha^{-1})

Seed yields of the crop kg ha^{-1} were determined at the time of the harvest. Mean value of three plants was recorded.

Treatments Details

The experiments were conducted using Completely Randomized design (CRD) with the following eight treatments and with three replications of each treatment.

T_0 – Control
T_1 -75% N & P
T_2 - 75% N & P + *Azospirillum lipoferum* (AU Az-1)
T_3 - 75% N & P + *Bacillus megaterium* (AU Ba-1)
T_4 - 75% N & P + *Pseudomonas fluorescens* (AU Ps-1)
T_5 - 75% N & P + *Azospirillum lipoferum* (AU Az-1) + *Bacillus megaterium* (AU Ba-1)
T_6 - 75% N & P + *Azospirillum lipoferum* (AU Az-1) + *Pseudomonas fluorescens* (AU Ps-1)
T_7 - 75% N & P + *Bacillus megaterium* (AU Ba-1) + *Pseudomonas fluorescens* (AU Ps-1)
T_8 - 75% N & P + *Azospirillum lipoferum* (AU Az-1) + *Bacillus megaterium* (AU Ba-1) + *Pseudomonas fluorescens* (AU Ps-1)

RESULTS AND DISCUSSION

Seed treatment with bioinoculant consortium significantly increased the germination percentage and vigour index of sunflower, when compared with dual, single inoculation and control. In the present study, increase in germination percentage and vigour index of sunflower might be due to the increased survivability exhibited by the microbial consortium on the spermosphere and spermoplane this increased survivability may in turn enhance the production of growth hormones such as Auxins, Gibberellins and cytokinins. Inoculation of sunflower with T_8 - 75% N & P + *Azospirillum lipoferum* (AU Az-1) + *Bacillus megaterium* (AU Ba-1) + *Pseudomonas fluorescens* (AU Ps-1) increased the germination percentage and vigour index over control.

Effect of Different formulation of bioinoculant consortium on the Germination Percentage, Plant height and Vigour Index of Sunflower were presented in Table.1and the highest germination percentage (93.60%) was observed in the liquid formulation of microbial consortium which was higher than beaded inoculums (92.22%) and carrier based inoculum (91.32%) of microbial consortium.

This increase in root colonization as influenced by these cells would increase the density and length of root hairs, as well as the appearance and elongation rate of lateral roots, thus increasing surface area (Fallik *et al*., 1994).

Table 1 Effect of Different formulation of bioinoculant consortium on the Germination Percentage, Plant height and Vigour Index of Sunflower

Treatments	Germination (%)			Plant height (cm)			Vigour Index		
	Different formulations of Bioinoculants								
	Carrier	**Liquid**	**Bead**	**Carrier**	**Liquid**	**Bead**	**Carrier**	**Liquid**	**Bead**
T_0 - Control	70.60	74.20	72.40	16.80	17.40	17.00	1186.08	1291.08	1230.80
T_1 - 75% N & P	75.00	79.90	76.20	18.90	19.80	18.00	1417.50	1582.00	1371.60
T_2 - 75% N & P + AU Az-1	80.30	82.03	81.20	19.80	20.22	19.25	1589.94	1656.40	1559.04
T_3 - 75% N & P + AU Ba-1	76.40	76.84	75.02	17.23	18.76	17.97	1314.08	1436.16	1342.50
T_4 - 75% N & P + AU Ps-1	79.30	81.22	84.40	18.45	19.53	18.04	1459.12	1583.40	1519.20
T_5 - 75% N & P + AU Az-1 + AU Ba-1	86.56	89.20	88.62	20.85	21.47	20.69	1972.20	2005.12	1796.32
T_6 - 75% N & P + AU Az-1 + AU Ps-1	89.60	91.86	90.94	21.63	22.49	21.84	2056.89	2318.32	2186.90
T_7 - 75% N & P + AU Ba-1 + AU Ps-1	84.36	86.42	85.05	20.47	21.37	20.09	1719.72	1840.32	1700.00
T_8 - 75% N & P + AU Az-1 + AU Ba-1 + AU Ps-1	91.32	93.60	92.22	22.44	24.00	23.05	2059.82	2321.28	2188.48
SEd	0.47	0.85	0.62	0.40	0.73	0.59	1.42	1.48	1.46
CD(p=0.05)	0.99	1.72	1.26	0.82	1.52	1.21	2.90	2.96	2.94

Table 2 Effect of Different formulation of Bioinoculant consortium on the Flower Head Diameter, Percentage of filled seeds and Seed Yield of Sunflower

Treatments	**Flower Head Diameter (Cm)**			**Seed Filling Percentage (%)**			**Seed Yield (kg ha^{-1})**		
	Different formulations of Bioinoculants								
	Carrier	**Liquid**	**Bead**	**Carrier**	**Liquid**	**Bead**	**Carrier**	**Liquid**	**Bead**
T_0 - Control	16.60	16.82	16.70	49.38	68.09	63.42	830	825	828
T_1 - 75% N & P	17.10	16.94	17.24	50.17	71.80	69.52	842	860	850
T_2 - 75% N & P + AU Az-1	17.80	17.62	17.54	52.69	84.98	76.86	914	923	918
T_3 - 75% N & P + AU Ba-1	17.70	17.92	17.80	53.69	87.56	79.58	857	896	882
T_4 - 75% N & P + AU Ps-1	17.12	17.29	17.32	56.92	84.43	72.18	895	902	898
T_5 - 75% N & P + AU Az-1 + AU Ba-1	18.35	18.92	18.46	86.50	90.93	83.97	965	974	971
T_6 - 75% N & P + AU Az-1 + AU Ps-1	18.56	19.92	18.84	87.46	90.13	88.98	972	980	975
T_7 - 75% N & P + AU Ba-1 + AU Ps-1	18.20	18.64	18.52	83.10	89.46	78.01	951	972	958
T_8 - 75% N & P + AU Az-1 + AU Ba-1+ AU Ps-1	19.96	21.88	20.62	88.49	91.26	89.83	975	983	978
SEd	0.76	1.00	0.86	0.50	0.58	0.49	1.53	1.62	1.58
CD(p=0.05)	1.42	1.96	1.78	1.03	1.13	1.10	3.26	3.48	3.36

Among the different formulations tested T_8 - 75% N & P + *Azospirillum lipoferum* (AU Az-1) + *Bacillus megaterium* (AU Ba-1) + *Pseudomonas fluorescens* (AU Ps-1) liquid inoculums of microbial consortium (24.00 cm) significantly increased the plant height over the beaded (23.05 cm) and carrier based inoculums (22.44 cm). The highest vigour index of (2321.28) was recorded with liquid inoculums of microbial consortium T_8 - 75% N & P + *Azospirillum lipoferum* (AU Az-1) + *Bacillus megaterium* (AU Ba-1) + *Pseudomonas fluorescens* (AU Ps-1) which was followed by the beaded (2188.48) and carrier (2059.82) based inoculants respectively.

The different yield parameters such as flower head diameter, seed filling percentage and seed yield were recorded and the results are presented in Table.2. Inoculation of sunflower with T_8 - 75% N & P + *Azospirillum lipoferum* (AU Az-1) + *Bacillus megaterium* (AU Ba-1) + *Pseudomonas fluorescens* (AU Ps-1) increased the yield parameters and recorded flower head diameter of 21.88 cm in liquid, 20.62 cm in beaded and 19.96 cm in carrier based inoculants respectively. Along with this maximum seed yield (983 Kg ha^{-1}) was recorded in T_8 which was as followed by (978 Kg ha^{-1}) in beaded and (975 Kg ha^{-1}) in the carrier based inoculums. In addition Goel *et al.*,(1999) reported that the inoculation with certain PGPR may enhance crop productivity either by making the other nutrients available or protecting plants from pathogenic microorganisms.Chandrasekhar *et al.*,(2005) reported that both morphological and yield parameters showed a better results through the combination of Biofertilizers and chemical fertilizers than using either method alone.

CONCLUSION

The results of the present study clearly indicate that inoculation of sunflower with liquid formulation of microbial consortiums were highly beneficial for enhancing the yield besides effecting a reduction in the cost of inorganic fertilizers.

References

1. Subba Rao, N.S., 1993. Biofertilizers in Agriculture and Forestry.3 rd Edn.,Oxford and IBM Publishing Co., Oxford.
2. Chandrasekar, B.R., G. Ambrose and N.Jayabalan, 2005.Inflence of biofertilizer and nitrogen source level on the growth and yield of *Echinochloa frumentacea* (Roxb.) Link.J.Agric.Technol, 1:223-234.
3. Pereyra, M.A., F.M. Ballesteros, M.C. Cecilia, M. Creus, R.J. Sueldo and A.B Carlos. 2009. Seedlings growth promotion by *Azospirillum brasilense* under normal and drought conditions remains unaltered in Tebuconazole-treated wheat seeds. Eur. J. Soil. Biol., 45; 20-27
4. Falik, E., S. Sorig and Y. Okon. 1994. Morphology and Physiology of plant roots associated with *Azospirillum*: In *Azospirillum* plant root associations, Okon, V., ED., CRC press, Buca Raton, FL, 77.
5. Dobbelaere, S., A. Croonenborghs, A. Thys, A. Vande Broek and J. Vanderleyden, 1999. Phytostimulatory effect of *Azospirillum brasilense* wild type and mutant strains altered in IAA production wheat. Plant Soil, 212: 155-164.
6. Goel, A.K., R.D. Laura, D.V. Pathak, G. Anuradha and A. Goel, 1999. Use of biofertilizers: Potential, constrains and future strategies review. Int. J. Top. Agric., 17: 1-18.
7. Skvortsova, N.G., M.M. Umarov and N.V. Kostina, 1998. The effect of non leguminous plant inoculation with *Bacillus polymyxa*, *Pseudomonas* mixed cultures on nitrogen transformations in the rhizosphere. Microbiology, 67: 201-204.

****