JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2015. Vol. **9**(2), p. 1377-1381

Effect of Different Fortified Formulations on Growth of Maize and Soybean Grown under Green House Condition

M. Divya[1], B.L. Raghunandan[1], C.D. Srikanth[1] and K.R. Sreeramulu[2]

[1]Organic Farming Research Center, UAHS, Shimoga
[2]Department of Agricultural Microbiology, UAS, G. K. V. K. Bangalore - 65, India.

(Received: 16 December 2014; accepted: 26 January 2015)

Experiment was conducted to evaluate the effect of different fortified formulations on growth of maize and soybean grown under green house condition. microbial inoculants such as *Azotobacter chroococcum, Bradyrhizobium japonicum, Bacillus megaterium* and *Acinetobacter calcoaceticus* were fortified with the fortifying materials like Gibbrellic acid, PVP, PEG, glycerol, trehalose and $ZnSO_4$. Optimum concentration of fortifying materials for each organism were determined and fortifying combinations was developed. The two best fortified combination were found to be C_1 having GA_3+PVP+PEG+ Glycerol+Trehalose and C_2 having GA_3+PVP+PEG+Glycerol. These two combinations were selected for development of liquid, carrier, alginate beads and pellet formulations then these formulations were tested on growth of maize and soybean under green house condition, the growth of maize and soybean were significantly influenced by the inoculation of fortified microbial inoculant formulations. Among the four different fortified formulations tested liquid fortified formulations showed better growth compared to other formulations. The next best were carrier, alginate beads and pellet formulations. The lowest plant growth was recorded in the control plants.

Key words: *Azotobacter chroococcum, Bradyrhizobium japonicum, Bacillus megaterium, Acinetobacter calcoaceticus*, fortification, formulation, Maize, Soybean**.**

Agriculture in modern times is getting more and more dependent upon the steady supply of chemical fertilizers with the advent of green revolution technologies. To meet the food demand, one or other way farmers are depending on chemicals and fertilizers to increase their crop yields. However, use of fertilizers is becoming a threat to sustainability in agriculture. Many adverse effects are being noticed due to the continuous, excessive and imbalanced use of chemical fertilizers. Synthetic fertilizers have ruined the natural nutrient levels of the soil. Deaker *et al*., (2004)

* To whom all correspondence should be addressed.
Mob.: +91-8147281665;
E-mail: divyamicrobiology5@gmail.com

Now a days, biological means for production of agricultural commodities is gaining lot of importance, among biological means microorganisms being an integral component of soil ecosystem play a prestigious role by making the soil truly living. In this view biofertilizers stand first. Developing efficient biofertilizer formulation is a challenging step in commercialization of new microbial inoculants. Although research on developing new formulations is progressively slow, several available forms like powder, liquid and granular formulation have immensely contributed to the use of these beneficial microbial inoculants in crop production. fortification in a microbial product will add strength to the organism by reinforcement and invigoration and helps in increasing the metabolic activity and survivability of the microorganism, Kumaresan & Reetha (2011).

MATERIALS AND METHODS

In this experiment *Azotobacter chroococcum* and *Bacillus megaterium* was used as a nitrogen fixer and phosphorus solubilizers in maize crop, similarly *Bradyrhizobium japonicum,* and *Acinetobacter calcoaceticus* was used as a nitrogen fixer and phosphorus solubilizers in soybean crop.

Fortification of microbial inoculants

Fortification of *Azotobacter chroococcum*, *Bradyrhizobium japonicum, Bacillus megaterium* and *Acinetobacter calcoaceticus* was done by using fortifying combinations such as C_1 combination having GA_3 (Gibberellic acid) + PVP (Polyvinyl pyrrolidone) + PEG (Polyethylene glycol) + Glycerol + Trehalose and C_2 combination having GA_3 + PVP + Glycerol + Trahalose, The microbial inoculants were grown on their respective broth such as *A. chroococcum* on Waksmann No. 77 broth, *B. japonicum* on yeast extract mannitol broth, *B. megaterium* (PSB) on Pikovoskay's and *A. calcoaceticus* on Sperber's broth with above mentioned two fortifying combinations and incubated for 24 hrs at room temperature the broth culture was used for the preparation of carrier, liquid, granular and pellet based formulations.

Pot culture studies

The soil samples used for pot experiment was collected from the forest soils of GKVK, Bangalore, which was red loamy in nature. Soil was sieved and potting mixture was prepared by mixing Soil: Sand: FYM in 2:1:1 proportion, 4 kg of soil was filled in to 5 kg capacity poly bags. The bags were punched with 2 or 3 holes at the bottom to drain out excess water. Before sowing, seeds were surface sterilized with 2 % sodium hypochlorite solution and washed with sterile distilled water for 5 times and three of maize / soybean seeds per pot were dibbled at a depth of 5 cm. The carrier (20g) and liquid formulations (4ml) were used as a seed treatment. Similarly five alginate beads and one pellet (having equal microbial load) were placed near the seed. The poly bags were kept on cement platforms in a randomized design under glass house conditions up to 60 days after sowing. Plant height was recorded on 15, 30, 45 and 60 days after sowing. In soybean after 60 days plants were uprooted, adhered soil particles were removed and washed with slow jet of water. The number of nodules in each treatment were counted and plant biomass was recorded.

RESULT AND DISCUSSION

The effect of different fortified formulation on growth of maize was studied under green house condition. The application of fortified formulation showed enhanced crop growth compared to control. All the fortified liquid, carrier, alginate and pellet formulations showed significant increase in plant growth

Table 1. Effect of different fortified formulations on plant height of maize grown under green house condition

Treatments	Plant height(cm)			
	15 DAS	30 DAS	45 DAS	60 DAS
T_1: C_1 + *Azotobacter chroococcum* + *Bacillus megaterium* (Liquid)	37.27	86.73	141.17	172.83
T_2 : C_1 + *A. chroococcum* + *B. megaterium* (Carrier)	33.60	83.56	137.20	159.40
T_3 : C_1 + *A. chroococcum* + *B. megaterium* (Alginate beads)	29.20	75.50	132.63	152.83
T_4 : C_1 + *A. chroococcum* + *B. megaterium* (Pellets)	25.37	70.63	126.40	146.63
T_5 : C_2 + *A. chroococcum* + *B. megaterium* (Liquid)	36.14	85.27	140.30	168.50
T_6 : C_2+ *A. chroococcum* + *B. megaterium* (Carrier)	31.50	80.20	135.83	157.67
T_7 : C_2 + *A. chroococcum* + *B. megaterium* (Alginate beads)	26.23	72.80	129.57	148.20
T_8 : C_2 + *A. chroococcum* + *B. megaterium* (Pellets)	24.40	67.40	121.57	142.03
T_9 : 100 % NPK	35.80	85.13	139.97	170.67
T_{10}: Control	20.30	53.37	60.67	72.00
S.Em+	0.54	0.58	0.61	0.86
C.D. at 5%	1.66	1.78	1.84	2.61

C_1 : GA_3 + Glycerol + PVP+ PEG + Trehalose **C_2 :** GA_3 + Glycerol + PVP+ PEG parameters of maize compare to control. Among the different formulations tested C_1 + *A. chroococcum* + *B. megaterium* (Liquid) showed maximum plant height at all the four stages of plant growth *viz.*, 15(37.27), 30(86.73), 45(141.17), and 60(172.83) DAS which was significantly differed from all other treatments but on par with the treatment of C_2 + *A. chroococcum* + *B. megaterium* (Liquid). The next best treatment which enhanced plant growth of maize was 100 % NPK. The lowest plant height was recorded in the control treatment (20.30 cm) The findings of this study are in accordance with the findings of Biari *et al*. (2008) who reported that bioinoculants have the ability to produce plant growth promoting substances like Indole Acetic Acid (IAA), Gibberellins (GA), B-vitamins, cytokinins and antifungal substances, which favour better growth of the crop plants. The results of this study were also in agreement with the findings of Rizwan *et al*. (2008) who reported such increased growth in maize due to application of plant beneficial microbial inoculants.

Fresh and dry weight of shoot and root

Table 2. Effect of different fortified formulations on plant biomass of maize grown under green house condition

Treatments	Plant biomass (g Plant^{-1})			
	Fresh weight of shoot	Dry weight of shoot	Fresh weight of root	Dry weight of root
T_1: C_1 + *Azotobacter chroococcum* + *Bacillus megaterium* (Liquid)	65.03	6.50	4.45	0.86
T_2 : C_1 + *A. chroococcum* + *B. megaterium* (Carrier)	57.40	5.20	3.23	0.76
T_3 : C_1 + *A. chroococcum* + *B. megaterium* (Alginate beads)	50.73	4.32	2.43	0.69
T_4 : C_1 + *A. chroococcum* + *B. megaterium* (Pellets)	48.63	3.89	1.57	0.59
T_5 : C_2 + *A. chroococcum* + *B. megaterium* (Liquid)	64.17	6.12	3.70	0.84
T_6 : C_2+ *A. chroococcum* + *B. megaterium* (Carrier)	53.77	4.86	2.88	0.72
T_7 : C_2 + *A. chroococcum* + *B. megaterium* (Alginate beads)	45.13	4.07	2.29	0.65
T_8 : C_2 + *A. chroococcum* + *B. megaterium* (Pellets)	41.27	3.75	1.40	0.55
T_9 : 100 % NPK	63.46	6.06	3.45	0.83
T_{10}: Control	38.60	1.73	0.93	0.28
S.Em+	0.40	0.15	0.34	0.02
C.D. at 5%	1.21	0.47	1.02	0.06

C_1: GA_3 + Glycerol + PVP+ PEG + Trehalose C_2: GA_3 + Glycerol + PVP+ PEG

Table 3. Influence of different fortified formulations on plant height of soybean grown under green house condition

Treatments	Plant height (cm)		
	15 DAS	30DAS	45 DAS
T_1: C_1 + *Bradyrhizobium japonicum* + *Acinetobactor calcoaceticus* (Liquid)	26.30	46.10	62.40
T_2 : C_1 + *B. japonicum* + *A. calcoaceticus* (Carrier)	21.38	42.47	58.67
T_3 : C_1 + *B. japonicum* + *A. calcoaceticus* (Alginate beads)	19.27	35.13	51.24
T_4 : C_1 + *B. japonicum* + *A. calcoaceticus* (Pellets)	16.63	28.37	44.13
T_5 : C_2 + *B. japonicum* + *A. calcoaceticus* (Liquid)	25.70	45.26	61.20
T_6 : C_2+ *B. japonicum* + *A. calcoaceticus* (Carrier)	19.35	41.33	57.10
T_7 : C_2 + *B. japonicum* + *A. calcoaceticus* (Alginate beads)	17.83	32.58	48.17
T_8 : C_2 + *B. japonicum* + *A. calcoaceticus* (Pellets)	15.77	26.43	39.13
T_9 : 100 % NPK	25.20	44.15	60.72
T_{10}: Control	15.07	23.62	35.58
S.Em+	0.45	0.67	0.60
C.D. at 5%	1.38	2.13	1.82

C_1: GA_3 + Glycerol + PVP+ PEG + Trehalose C_2: GA_3 + Glycerol + PVP+ PEG was maximum in C_1 + *A. chroococcum* + *B. megaterium* (Liquid) which was significantly differed from all other treatments but was on par with the treatment of C_2 + *A. chroococcum* + *B. megaterium* (Liquid). The next best treatment which highest biomass in maize was 100 % NPK. This may be due to better plant growth and more accumulation of photosynthates in plant system due to efficient mobilization of plant nutrients by beneficial microorganisms present in different formulations and in crop rhizosphere. The results of this study are in agreement with the findings of Biari *et al*. (2008) who reported such increased fresh and dry weight of plant due to application of bifertilizers.

The effect of different fortified

Table 4. Influence of different fortified formulations on number of branches, nodules and nodule dry weight of soybean grown under green house condition

Treatments	Number of branches $plant^{-1}$ at harvest	Number of nodules $plant^{-1}$	Nodule dry weight (mg $plant^{-1}$)
T_1: C_1 + *Bradyrhizobium japonicum* + *Acinetobacter calcoaceticus* (Liquid)	11.33	19.66	0.96
T_2 : C_1 + *B. japonicum* + *A. calcoaceticus* (Carrier)	8.67	16.00	0.65
T_3 : C_1 + *B. japonicum* + *A. calcoaceticus* (Alginate beads)	7.33	12.67	0.42
T_4 : C_1 + *B. japonicum* + *A. calcoaceticus* (Pellets)	6.67	10.33	0.34
T_5 : C_2 + *B. japonicum* + *A. calcoaceticus* (Liquid)	10.67	18.33	0.89
T_6 : C_2 + *B. japonicum* + *A. calcoaceticus* (Carrier)	7.67	15.00	0.58
T_7 : C_2 + *B. japonicum* + *A. calcoaceticus* (Alginate beads)	6.67	12.33	0.40
T_8 : C_2 + *B. japonicum* + *A. calcoaceticus* (Pellets)	6.00	9.00	0.28
T_9 : 100 % NPK	10.33	3.00	0.13
T_{10}: Control	5.33	2.30	0.11
S.Em+	0.40	0.59	0.03
C.D. at 5%	1.21	1.78	0.09

C_1: GA_3 + Glycerol + PVP+ PEG + Trehalose C_2: GA_3 + Glycerol + PVP+ PEG

Table 5. Influence of different fortified formulations on plant biomass of soybean grown under green house condition

Treatments	Plant Biomass (g $Plant^{-1}$)			
	Fresh weight of shoot	Dry weight of shoot	Fresh weight of root	Dry weight of root
T_1: C_1 + *Bradyrhizobium japonicum* + *Acinetobacter calcoaceticus* (Liquid)	22.56	4.85	4.24	0.57
T_2 : C_1 + *B. japonicum* + *A. calcoaceticus* (Carrier)	19.28	3.75	3.13	0.42
T_3 : C_1 + *B. japonicum* + *A. calcoaceticus* (Alginate beads)	16.12	2.92	2.90	0.34
T_4 : C_1 + *B. japonicum* + *A. calcoaceticus* (Pellets)	15.18	1.80	2.63	0.27
T_5 : C_2 + *B. japonicum* + *A. calcoaceticus* (Liquid)	22.12	4.42	3.96	0.49
T_6 : C_2 + *B. japonicum* + *A. calcoaceticus* (Carrier)	18.12	3.39	3.08	0.37
T_7 : C_2 + *B. japonicum* + *A. calcoaceticus* (Alginate beads)	15.76	2.44	2.86	0.32
T_8 : C_2 + *B. japonicum* + *A. calcoaceticus* (Pellets)	14.64	1.25	2.49	0.22
T_9 : 100 % NPK	21.82	4.35	3.89	0.46
T_{10}: Control 11.80	0.80	1.89	0.12	
S.Em+ 0.22	0.16	0.14	0.04	
C.D. at 5% 0.76	0.53	0.46	0.13	

C_1: GA_3 + Glycerol + PVP+ PEG + Trehalose   **C_2**: GA_3 + Glycerol + PVP+ PEG formulation on growth of soybean was studied under green house condition. The application of fortified formulation showed enhanced crop growth compared to control. All the fortified liquid, carrier, alginate and pellet formulations showed significant increase in plant growth parameters of soybean compared to control. Among the different formulations tested C_1 + *B. japonicum* + *A. calcoaceticus* (Liquid) showed maximum plant height at all the four stages of plant growth *viz.*, 15(26.30 cm), 30(46.10 cm) and 45 (62.40 cm) DAS which was significantly differed from all other treatments but was on par with the treatment of C_2 + *B. japonicum* + *A. calcoaceticus* (Liquid) followed by the treatment100 % NPK.

Similar to plant height more number of branches were produced in the treatments supplemented with C_1 + *B. japonicum* + *A. calcoaceticus* (Liquid) (11.33) followed by the treatment C_2 + *B. japonicum* + *A. calcoaceticus* (Liquid) (10.67) and 100 % NPK (10.33) supplimented treatments. These results are in conformity with the findings of many research workers who reported such increased plant height due to liquid biofertilizers application in crops like soybean (Kalhapure *et al.*, 2003) and pea (Asghar *et al.* 2003).

More number of nodules (19.66) and nodule dry (0.96 g) weight were recorded in the treatment of C_1 + *B. japonicum* + *A. calcoaceticus* (Liquid) which was on par with the treatments of C_2 + *B. japonicum* + *A. calcoaceticus* (Liquid). In liquid formulations due to presence of more number of viable cells of *B. japonicum* and there interaction with dicot crop like soybean plant produces more number of nodules. The results of this study are in accordance with the research findings of Deshmukh *et al.* (2004) who reported the more number of nodules in soybean plants. Similarly, Kalita *et al.* (2006) also reported increased number of nodules in blackgram and green gram due to inoculation of rhizobium.

The fresh and dry weights of shoot and root were more in C_1 + *B. japonicum* + *A. calcoaceticus* (Liquid) which was significantly differed with all other treatments but on par with the treatment of C_2 + *B. japonicum* + *A. calcoaceticus* (Liquid). The next best treatment which showed more biomass was 100 % NPK. Similarly Kalhapure *et al.* (2003) reported that inoculation of *Bradyrhizobium japonicum* to soybean has increased the seed germination, nodulation and other growth parameters like number of branches per plant, dry weight per plant and grain yield compared to uninoculated control.

The growth of maize and soybean were significantly influenced by the inoculation of fortified microbial inoculant formulations. Among the four different fortified formulations tested liquid fortified formulations showed better growth compared to other formulations. The next best were carrier, alginate beads and pellet formulations. The lowest plant growth was recorded in the control plants.

REFERENCES

1. Asghar, A., Muhammad, I. And Noor, E.J, Effect of *Rhizobium leguminosarum* inoculation on growth and yield of different pea cultivars. *Sarhad J. Agric.*, 2003; **19** : 56-59.
2. Biari, A., Gholami, A., And Rahmani, H.A., Growth promotion and enhanced nutrient uptake of maize (*Zea mays* L.) by application of plant growth promoting rhizobacteria in arid region of Iran. *J. Biol. sci*., 2008; **8**(6): 1015-1020.
3. Deaker, R., Roughley, R.J. And Kennedy, I.R., Legume seed inoculation technology – a review. *Soil Biol. Biochem*., 2004: **36**: 1275-88.
4. Deshmukh, P.J., Potdukhe, S.R., Pardey, V.P. And Ingle, Y.V, Effect of *Rhizobium japonicum* and chemical herbicides on nodulation, *Rhizobium* survival and grain yield of Soybean (*Glycine max* (L.) Merrill.). *Biova.*, 2004; **15** : 89-93.
5. Kalhapure, D.J., Memane, S.A., Rasal, P.H. And Pawar, K.B., Varietal response of Soybean to different strains of *Bradyrhizobium japonicum*. *J. Maharashtra Agric. Univ.*, 2003; **28** : 161-163.
6. Kalita, R., Deka, A.K. And Azad, P., Evaluation of native *Rhizobium* from acid soils of Assam on Pigeonpea. *Legume Res*., 2006; **29** :157-162.
7. Kumaresan, G. And Reetha, D., Survival of *Azospirillum brasilense* in liquid formulation amended with different chemical additives. *J. Phytol.*, 2011; **3**: 48-51
8. Rizwan, A., Muhammad, A., Azeem, K., And ZAHIR, A., Effectiveness of organic/biofertilizer supplemented with chemical fertilizers for improving soil water retention, aggregate stability, growth and nutrient uptake of maize (*Zea mays* L.). *J. Sustainable Agri.*, 2008; **3**(4):57-77.