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Comparative effect of single and dual bio inoculants on microbial properties and quality of sunflower

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Abstract
Liquid biofertilizer formulation is the promising and updated technology of the conventional carrier based production technology. Comparatively performance of liquid and carrier based bio-inoculants were studied for microbial count & quality parameters of sunflower. After harvest of sunflower and microbial properties also noted significantly better in bioinoculant treated plots as compared to uninoculated control and only RDF. Significantly higher values of actinomycetes population were noted in treatment received both the inoculants along with RDF in liquid form. However, in case of bacteria. and fungi dual inoculation with carrier based source (T5) and liquid source (T8) were noted statistically at par with each other. Through, liquid inoculants showed better performance but carrier based bioinoculants were also found equally at par with the respective N-fixer and P-solubilizer treatments. Quality in terms of oil yield, head diameter and number of seeds per head also improved with these bioinoculants.

Keywords: Bioinoculants, Microbial Properties of Sunflower, Liquid biofertilizers

Introduction
Biofertilizer like Azotobacter fix atmospheric nitrogen. Important characteristics of Azotobacter association with crop improvement is excretion of ammonia in the rhizosphere in the presence of root exudates in which help modification of nutrient uptake by the plants. Seed application of Azotobacter increase seed germination and also controls plant diseases due to synthesize and secretion of biologically active substances. The higher phosphorus uptake can increase seed sets and improve yields. Supply of adequate phosphorus increased oil content in seed (Zubillaga *et al*.2002) [20]. PSB plays a vital role for making unavailable phosphorus to available phosphorus by mineralization of organic phosphate or by solubilization of inorganic phosphate by production of acids (Rodriguez and Fraga, 1999) [7].

Liquid inoculants are special formulation containing desired microorganisms in viable form, their nutrients and certain chemicals primarily function as microbial cell protectant and amendments that promote cell survival during storage and after application to seed or soil. Various liquid media have been used to prepare the liquid biofertilizers of rhizobia. These media normally consists of carbon, nitrogen and vitamin sources, which promote the growth of bacteria.

Liquid biofertilizers formulation is the promising and updated technology of the conventional carrier based production technology which in spite of many advantages over the agrochemicals, left a considerable dispute among the farmer community in terms of several reasons major being the viability of the organism. Shelf life is the first and foremost problem of the carrier based biofertilizers which is up to 3 months 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 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. The range of possible contamination is very high as bulk sterilization does not provide the desirable results in the case of carrier-based biofertilizers, where as the contamination can be controlled constructively by means of proper sterilization techniques and maintenance of intensive hygiene conditions by appropriate quality control measures in the case of liquid biofertilizers.

Treatments
T1: Un-inoculated and unfertilized (control)
T2: Only RDF (60:40:30 kg N, P_2O_5 and K_2O ha^{-1})

Carrier based inoculants (with RDF)
T3: RDF + *Azotobacter*
T4: RDF + *Bacillus megaterium (PSB*)
T5: RDF + *Azotobacter* + *Bacillus megaterium* (PSB)

Liquid inoculants (with RDF)
T6: RDF + *Azotobacter*
T7: RDF + *Bacillus megaterium (PSB)*
T8: RDF + *Azotobacter*.+ *Bacillus megaterium* (PSB)

Material and Methods
The treatments comprising inoculation with carrier based and liquid inoculants of *Azotobacter* and *Bacillus megaterium* (PSB) for sunflower, used in alone and in combinations. Total eight treatments of bioinoculants were replicated three times in RBD. Seed treatment was done immediate before sowing with carrier based bioinoculants @ 250 g 10 kg^{-1} seed and liquid bioinoculants @ 100 ml 10 kg^{-1} seed. The crop was cultivated following recommended agronomic practices.

Biological properties
For isolation of bacteria, fungi and actinomycetes from soil. Three different media were used for specific group of flora. The composition of these media is given below

Nutrient Agar medium (For Bacteria)

1.	Beef extract	:	3 g
2.	Peptone	:	5 g
3.	NaCl	:	1 g
4.	Agar Agar	:	15 g
5.	Distilled water	:	1000 ml
6.	pH to be adjusted	:	7

Ken knight medium (For actinomycetes)

1.	Dextrose	:	1 g
2.	KH_2SO_4	:	0.1 g
3.	$NaNO_3$	:	0.1 g
4.	KCl	:	0.1 g
5.	$MgSO_4$	:	0.1 ml
6.	Agar- Agar	:	15 g
7.	Distilled water	:	1000 ml
8.	pH to be adjusted	:	7

Rose Bengal Agar medium (for fungi)

1.	Dextrose	:	10 g
2.	Lactose	:	5 g
3.	KH_2SO_4	:	1 g
4.	$MgSO_4$	:	1 g
5.	Rose Bengal	:	1 ml
6.	Streptomycin	:	3 ml
7.	Agar- Agar	:	15 g
8.	Distilled water	:	1000 ml
9.	pH to be adjusted	:	7

Preparation of medium
Agar-Agar was boiled in 500 ml of distilled water in a pan. In another pan about 500 ml distilled water and all chemical ingredients were added and mixed properly. Both these ingredients were mixed together properly, filtered and made up the volume to 1000 ml with distilled water. The respective media were distributed in 500 ml conical flasks, plugged with non-absorbent cotton, tied these plugs with paper by thread and sterilized at 6.82 kg (15 lb) pressure for 15 min in an autoclave.

Method
Procedure (Dhingra and Sinclair, 1993)
Transfered of 1 g of soil sample in 10 ml of sterile distilled water in test tube (1:10), shake properly. Then Transfered 1 ml suspension from this test tube to another tube containing 9 ml of sterile distilled water (1:100) again transfered 1 ml of suspension from this test tube to tube containing 9 ml sterile distilled water (1:1000). Similarly, the dilution process was continued as per requirement for fungal isolation $1:10^4$. For bacteria $1:10^7$ and for actinomycetes $1:10^5$dilution were preferred. The concerned diluted samples were poured at rate of 1 ml/plate. The respective melted medium (cool to 45^0) was poured at rate of 20 ml/plate. Spread the medium by an inclined rotary motion of the plate. After solidification of medium, these plates were incubated at $30\_+ 2^0$ in an inverted position in incubator.

Head diameter
Head diameter was determined at physiological maturity stage by harvesting 5 plants of sunflower per experimental unit.

Number of seeds per head
The number of seeds per head were recorded from selected five plants from plots. Counted the number of seeds from head.

Test weight
The seeds from selected samples from each plot were removed and weight of 100 seeds was taken on digital top pan electronic balance from each of the treatments and designated as test weight.

Oil content
The oil content of sunflower was determined by Soxhlet extraction method using petroleum ether as extractant (Plummer, 1998).

Oil yield
Oil yield (kg ha^{-1}) was calculated by multiplying the respective oil content with respective seed yield using following formula.
Oil yield (kg ha^{-1}) = oil content in seed (per cent) x seed yield (kg ha^{-1})/ 100

Result and Discussion
The scrutiny of the data given in Table 1.shows significant increase in microbial population in soil after harvest of sunflower crop was also noted with single as well as dual inoculation of *Azotobacter*and PSB along with recommended dose of fertilizers over control. Significantly highest values of actinomycetes population (29.22 CFU × 10^{-5}) were noted in treatment received both the inoculants along with RDF in liquid form. However, in case of bacteria and fungi dual inoculation with carrier based source (T5) and liquid source (T8) were noted statistically at par with each other.
Noted that the application of 75% RDF + 25% N through FYM + PSB + *Rhizobium* recorded significantly more total microbial count at harvest than that of 100% RDF. However, concluded that the rhizobial population was increased from 30 days onwards and highest was recorded at 60 days in combined application of *Rhizobium japonicum* + *Azotobacter brasilence* in soybean. Found that seed inoculation with

Rhizobium significantly increased nodulation and root colonization over uninoculated control treatment in chickpea. Further, reported that VAM fungi and *Rhizobium* both showed similar root colonization and spore count, spore number and root colonization increased upto 50% RDF but decreased slightly at 100% RDF in groundnut. Earlier, also noted that in green gram crop. Combination of *Rhizobium* inoculation with 50% recommended dose of nitrogen and phosphorus fertilizers increased VAM fungi colonization. Similar findings were reported by.

Table 1: Comparative effect of single and dual bio inoculants on soil microbial population After Harvest of Sunflower

Sr. No.	Treatment	Bacteria (CFU X10^{-7})	Actinomycetes (CFU X10^{-5})	Fungi (CFU X 10^{-4})
T1	Uninoculated and unfertilized (Control)	30.66	16.98	4.83
T2	Only RDF (60:40:30 kg NPK / ha^{-1})	35.04	20.28	6.12
Carrier based inoculants				
T3	RDF + *Azotobacter*	36.64	21.61	6.83
T4	RDF + *Bacillus megaterium* (PSB)	35.77	22.09	6.50
T5	RDF + *Azotobacter* + *Bacillus megaterium* (PSB)	39.17	24.54	7.39
Liquid inoculants				
T6	RDF + *Azotobacter*	37.54	22.71	7.10
T7	RDF + *Bacillus megaterium*(PSB)	36.73	24.54	6.73
T8	RDF + *Azotobacter* +*Bacillus megaterium* (PSB)	42.75	29.22	7.75
	S.E.±	1.21	0.92	0.32
	C.D. at 5 %	3.66	2.77	0.98
	C.V. %	5.70	7.00	8.47

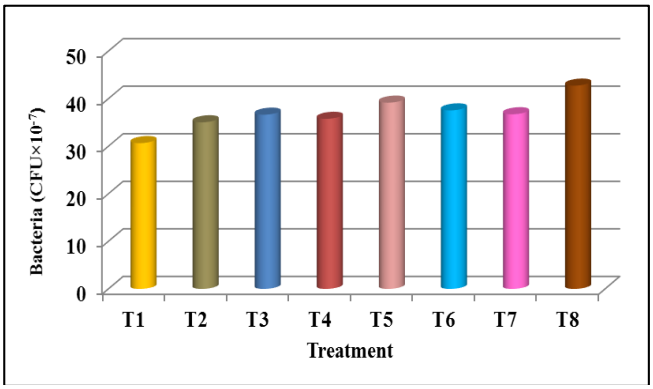

Fig 1: Bacteria population

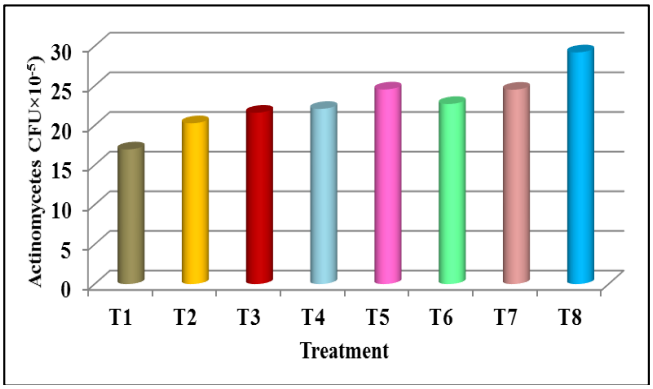

Fig 2: Actinomycetes population

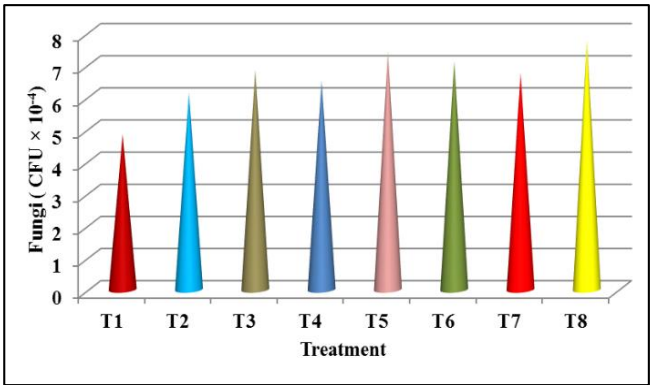

Fig 3: fungal population

Comparative effect of single and dual bioinoculants on seed quality attributes of sunflower

Scrutiny of the results presented in Table 2. reveals that the seed quality attributes after harvest of sunflower crop showed non-significant results for test weight. However, oil content, oil yield, number of seeds per head and head diameter were found increased in dual inoculation with carrier based sources (T5) and liquid sources (T8) which were noted statistically at par with each other.

The yield attributing characters like test weight could be improved through better nutrition of crop only. This might be due to the direct role of nitrogen to seed growth and indirectly help in accommodating osmatic imbalances present during final stage of seed filling. Oil per cent was higher due to the fact that higher nitrogen absorption had enhanced more Acetyl Co-A formation which was directly related with oil formation and phosphorus plays an important role in carbohydrate metabolisms and helps in conversion of carbohydrates in to oil. This biochemical reaction requires ATP and inorganic phosphate (Lanje *et al.* 2005) [4]. Moreover, Shehata and El-Khawas (2003) [13] reported a significant increasing oil content in sunflower using biofertilizer. Further, the wider variation in oil yield was largely due to the differences in seed yield because oil yield is the result of seed yield and oil content. Jain and Trivedi (2005) found that different treatment combinations of *Bradyrhizobium* + PSB + 26.20 kg P ha^{-1} turn out to be the best in respect to oil yield in soybean.

Biofertilizer inoculation increases the more number of seeds per head than non inoculated plants than no treated plants with inoculums. Vassey (2003) [17] reported that *Azotobacter* and *Azospirillium* increase the available nitrogen in the soil which enhanced the grain number in sunflower. However, found Indole acetic acid followed by cytokinin generated by *Azotobacter* caused to increase preserved material through growing side and root weight improving vegetative, growth and increased portion of generating organs such as seeds per head. Reported that *Azotobacter*increased the available nitrogen in the soil which could enhance seed number per head in sunflower. Found seed inoculation increased head diameter of sunflower than un-inoculated plants.

Table 2: Comparative effect of single and dual bioinoculants on seed quality attributes of sunflower

Sr. No.	Treatment	Test wt. (g/100 Seed)	Oil Content (%)	Oil yield	Number of Seeds Per Head	Head Diameter (cm)
T1	Uninoculated and unfertilized (Control)	3.93	30.34	319.28	402	16.22
T2	Only RDF (60:40:30 kg NPK / ha^{-1})	4.06	32.23	516.28	481	18.90
	Carrier based inoculants					
T3	RDF + *Azotobacter*	4.15	33.26	549.12	548	18.98
T4	RDF + *Bacillus megaterium* (PSB)	4.13	33.77	545.36	505	19.20
T5	RDF + *Azotobacter* + *Bacillus megaterium* (PSB)	4.27	34.12	570.39	662	20.96
	Liquid inoculants					
T6	RDF + *Azotobacter*	4.10	33.57	560.39	595	19.20
T7	RDF + *Bacillus megaterium*(PSB)	4.19	33.93	560.65	537	20.04
T8	RDF +*Azotobacter* + *Bacillus megaterium*(PSB)	4.40	34.98	611.84	697	22.00
	S.E.±	0.152	0.52	22.74	21.66	0.48
	C.D. at 5 %	NS	1.57	68.67	65.41	1.44
	C.V. %	6.37	2.71	7.44	6.78	4.36

Conclusion

The significant increase in microbial population in soil after harvest of sunflower crop was also noted with single as well as dual inoculation of sunflower and PSB along with recommended dose of fertilizers over control. Significantly higher values of actinomycetes population were noted in treatment received both the inoculants along with RDF in liquid form. However, in case of bacteria and fungi dual inoculation with carrier based source (T5) and liquid source (T8) were noted statistically at par with each other.

The seed quality attributes after harvest of sunflower crop showed non-significant results for test weight. However, oil content, oil yield, head diameter and number of seed per head were found increased in dual inoculation with carrier based sources (T5) and liquid sources (T8) which were noted statistically at par with each other.

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