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GENETICS AND PLANT BREEDING

Influence of Biofertilizers on Microbial Count and Nutrient uptake of *kharif* onion (*Allium cepa* L.)

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ABSTRACT

The experiment comprised of fifteen combinations of biofertilizers, organic manures and chemical fertilizers was conducted in RBD replicated thrice. The biofertilizers improved the microbial content and nutrient uptake of onion stover as compared to control and recommended chemical fertilizers. Highest Bulb dry mass (45.7 q/ha) and stover dry mass (27.9 q/ha) was observed in *Azospirillum* along with recommended fertilizer dose (T_3) followed by *Azotobacter* along with recommended fertilizer dose (T_1). Application of *Azospirillum* along with recommended fertilizer dose (T_3) resulted in significantly higher nitrogen uptake (210.3 Kg ha^{-1}) over all the treatments except *Azotobacter* along with recommended fertilizer dose (T_1). The highest phosphorus uptake (21.5 Kg ha^{-1}) was attained with application of *Azotobacter* along with *Vesicular-Arbuscular Mycorrhizae* and recommended fertilizer dose (T_9) over all the treatments. Organic manures improved the organic carbon status of soil and highest organic carbon of soil was observed in treatment where FYM @ 20 t/ha (T_{12}) and FYM @ 20 t/ha along with *Azotobacter* and *Vesicular-Arbuscular Mycorrhizae* (0.4%) was applied. While, highest bacteria (27.2×10^6) and actinomyctes (34.0×10^4) count was observed in FYM @ 20 t/ha treatment (T_{12}). *Azotobacter* along with recommended fertilizer dose (28.2×10^3) had highest fungal count at the time of harvesting. The present study highlights the need of use of biofertilizers along with organic and inorganic manures/fertilizer to enhance the nutrient availability and improve soil health.

Highlights

- Biofertilizers and organic manures not only supplies essential nutrients to onion but also improve crop yield potential, microbial count of soil and nutrient uptake of plant.
- Treatments of seedlings with the culture of *Azospirillum* and *Azotobacter* increase the dry mass of onion when utilized along with inorganic fertilizers and significantly improve the nutrient uptake in plants. These biofertilizers also increase the count of microbial organisms.

Keywords: Organic manures, microbial count, organic carbon, nutrient uptake

India is the second largest producer of onion (*Allium cepa* L.), in the world next to China and third largest exporter after Netherland and Spain. Onion a member of family Alliaceae is cultivated on an area of 1203 million hectare in the country (Anonymous, 2014). Onion is used in raw form as well as in dehydrated form to add flavour and taste to Indian foods as a culinary ingredient in wide range of food preparations. Raw onion has an antiseptic value. It promotes bile production and reduces blood sugar. In Punjab, it is cultivated over an area of 8.32 thousand hectare with a production of 185.40 thousand metric tonnes and average productivity of 22.28 tonnes per hectare (Anonymous, 2015). Onion, being a shallow rooted crop, it is quite responsive to heavy dose of inorganic fertilizers, but higher doses of these fertilizers deteriorates the soil health. Under inadequate and inappropriate fertilization considerable yield losses have been reported (Balemi *et al.*, 2007). For sustainable production

and productivity as well as quality, biofertilizers and organic farming may be the alternative means. Integrated nutrient management involving suitable combination of inorganic fertilizers, organic manures and biofertilizers helps in curtailing over dependence on inorganic fertilizers only. Hence, present investigation was conducted to study the effect of different biofertilizers and organic manures on microbial count and nutrient uptake of *kharif* onion so that dependence on inorganic fertilizers can be reduced.

MATERIALS AND METHODS

The present investigation was undertaken during the *kharif* season of 2014 at Vegetable Research Farm, Punjab Agricultural University, Ludhiana. The onion cultivar Agrifound dark red comprised the plant material. The soil of the experimental field was loamy sand in texture, having good water holding capacity, pH 7.8 and moderate soil fertility status. The treatments detail was given in Table 1. Bio-fertilizer was applied, on day of transplanting by seedling treatment except VAM which was mixed in soil. The nutrient uptake by bulb was computed by multiplying the percentage nitrogen, phosphorus and potassium content in bulb with bulb dry mass respectively. Stover nutrient uptake was computed by multiplying the nitrogen, phosphorus and potassium content with Stover dry mass respectively. Final total nutrient uptake was calculated by sum of nitrogen, phosphorus and potassium uptake by bulbs and by stover respectively.

In case of microbial count, organic carbon was determined by Walkley and Black method (1934). Bacterial count was determined by using serial dilution pour plate method (Gerhardt *et al.*, 1981). Actinomycete count was determined by using serial dilution pour plate method (Nonomura and Ohara 1969). Fungi count was determined by using serial dilution pour plate method (Kanwar *et al.*, 1997).

RESULTS AND DISCUSSION

Bulb Dry Mass

Application of *Azospirillum* and *Azotobacter* along with recommended dose of fertilizers (T_3 and T_1 respectively) resulted in maximum bulb dry mass of 45.7 qha^{-1} on an average (Table 2) which was significantly higher than that attained with control and rest of treatments except T_7, T_9 and T_{10}. Minimum bulb dry mass content was found in control. Among organic manures, application of FYM along with VAM and *Azotobacter* (T_{11})

Table 1: Treatment Detail

Treatments	Treatment detail
T_1	*Azotobacter* + Recommended dose of NPK
T_2	*Azotobacter* + 75% of recommended dose of N + Recommended dose of PK
T_3	*Azospirillium* + Recommended dose of NPK
T_4	*Azospirillium* + 75% of recommended dose of N + Recommended dose of PK
T_5	*Phosphate Solubilizing Bacteria* (PSB)+ Recommended dose of NPK
T_6	*Phosphate Solubilizing Bacteria* (PSB)+ 75% of recommended dose of P + Recommended dose of NK
T_7	*Vesicular-Arbuscular Mycorrhizae* (VAM) + Recommended dose of NPK
T_8	*Vesicular-Arbuscular Mycorrhizae* (VAM) + 75% of recommended dose of P + recommended dose of NK
T_9	*Azotobacter* + *Vesicular-Arbuscular Mycorrhizae* (VAM) + Recommended dose of NPK
T_{10}	*Azotobacter* + *Vesicular-Arbuscular Mycorrhizae* (VAM) + 75% of recommended dose of N + 75% recommended dose of P + recommended dose of K
T_{11}	FYM @ 20 t/ha + *Azotobacter* + *Vesicular-Arbuscular Mycorrhizae* (VAM)
T_{12}	FYM @ 20 t/ha
T_{13}	Poultry Manure @ 5t/ha
T_{14}	Recommended dose of NPK
T_{15}	Control

RD: Recommended dose of fertilizers (100:50:50, N:P:K kg per hectare)

resulted in significantly higher bulb dry mass than that attained with application of FYM @20 t ha^{-1} alone and control (T_{15}). Bulb dry mass attained with recommended dose of N, P and K was significantly higher than that of control.

Stover Dry Mass

The minimum stover dry mass was found in control plot where no fertilizer was applied which was 9.80 q ha^{-1} was significantly lower than that attained with T_1, T_2 and T_3, T_4, T_5 T_7, T_9 and T_{10}. Stover dry mass attained with recommended dose of fertilizers (T_{14}) was significantly higher from control (Table 2). The higher stover biomass might be attributed to the higher nutrient availability to stover that resulted from applying *Azosprillum* and *Azotobacter* along with recommended dose of fertilizers. The increased availability of nitrogen by nutrient application may also increase the weight of stover organs, through its role in increasing the food assimilation. Slow release of nutrients, when FYM alone or in combination with fertilizers and poultry manure alone was applied. Prajapati *et al.* (2016) also reported that onion dry leaf weight was improved with application of *Azospirillum* along with Salicylic acid. Hasanabadi *et al.* (2010) also found that *Azospirillum* increase the shoot dry weight and root dry mass, facilitating higher uptake of all nutrients in barley.

Nutrient content in Bulb

The results have shown that all treatments showed similar trends for percentage nitrogen content in onion bulb (Table 2). However maximum nitrogen content of 3.05% was observed in T_3 and range of 2.0 to 3.05% nitrogen content in onion bulb was observed in different treatments. Similarly, Abd-Elrazzag (2002) reported that biofertilizers and organic manures improves the nitrogen concentration in onion.

Maximum P content in onion bulb (0.33%) was recorded with T_5 and T_9 and it was significantly higher than rest of treatments (Table 2) except T_6, T_7 and T_{13}. Among organic manure treatments, maximum P content was recorded in T_{13} but all the organic manure treatments show similar trends and show non-significant results. The minimum phosphorus content of 0.23% in the onion bulb was found in control (T_{15}), *Azotobacter* along with 75% of recommended dose of N and Recommended dose of PK (T_2) and VAM along with 75% of recommended

Table 2: Effect of different treatments on total nutrient uptake by onion

Treatments	Dry Mass		Nutrient content (%)						Nutrient Uptake (Kg ha^{-1})		
	Bulb Dry mass (q/ha)	Stover Dry mass (q/ha)	Bulb			Leaves					
			N (%)	P (%)	K (%)	N (%)	P (%)	K (%)	Total N	Total P	Total K
T_1	45.7	25.9	2.84	0.24	2.20	2.35	0.28	0.85	190.4	18.1	122.9
T_2	37.7	20.3	2.55	0.23	1.97	2.12	0.23	0.86	139.2	13.4	92.0
T_3	45.7	27.9	3.05	0.25	2.27	2.52	0.26	0.75	210.3	18.6	123.4
T_4	37.4	18.9	2.68	0.25	2.29	2.21	0.23	0.68	141.6	13.6	97.8
T_5	35.6	22.9	2.36	0.33	2.27	2.06	0.32	0.88	132.0	19.0	100.1
T_6	31.7	17.0	2.28	0.28	2.23	1.99	0.24	0.76	105.1	12.8	85.1
T_7	38.7	18.2	2.44	0.32	2.26	2.00	0.28	0.8	130.0	17.9	100.9
T_8	28.4	16.3	2.60	0.23	2.21	2.15	0.21	0.94	109.3	10.2	78.3
T_9	44.8	21.6	2.71	0.33	2.32	2.29	0.32	0.77	169.7	21.5	121.3
T_{10}	39.4	18.9	2.40	0.25	2.28	1.95	0.20	0.92	130.5	13.6	106.3
T_{11}	35.7	17.2	2.59	0.27	2.29	2.14	0.27	0.8	128.6	14.1	98.5
T_{12}	25.9	14.8	2.80	0.25	2.31	2.05	0.26	0.74	97.5	9.9	67.3
T_{13}	30.3	11.8	2.51	0.28	2.30	2.20	0.27	0.84	98.1	11.1	77.9
T_{14}	34.3	17.5	2.46	0.24	2.12	2.54	0.26	0.79	125.6	12.5	84.9
T_{15}	22.4	9.8	2.00	0.23	1.95	1.90	0.21	0.60	63.5	7.2	51.0
CD (P = 0.05)	7.9	7.6	NS	0.05	NS	NS	0.05	NS	38.5	3.8	40.5

dose of P and recommended dose of NK (T_8) respectively.

The increased phosphorus content in the onion bulb may be due to the higher availability of phosphorus in the PSB when it is applied at higher concentration. PSB and VAM improve the P content in the onion bulb which may be due to solubliztion of P with PSB and VAM. VAM mobilizes the available P and also improves the uptake of other micro nutrients like K, Ca, S, Mg and Fe through proper association with the roots of plants (Jaishankar *et al.*, 2005).

The results have shown that all treatments showed similar trends for percentage potassium content in onion bulb (Table 2). However minimum K content (1.95 %) was recorded in T_{15} where no fertilizer was applied and maximum K content (2.32%) was observed in T_9. Potassium content among different treatments did not show any significant effect. Similar results were found by Chatterjee and Bandyopadhyay (2014) biofertilizers did not put any significant effect on potassium uptake in tomato.

Nutrient content in Onion stover

The results have shown that all treatments showed similar trends for percentage nitrogen content in onion stover (Table 2). However maximum nitrogen content of 2.52% was observed in T_3 and range of per cent nitrogen content was 1.90 to 2.52 in onion leaves among different treatments was observed. *Azotobacter* and *Azospirillum* recorded maximum N content in onion leaves it may be due to bacterium fix the non available form of nitrogen (nitrate) to available form (nitrite) of nitrogen and improves the content of N utilized by the plants. Nitrogen content in plant was higher when nitrogen was applied along with *Azospirillum* and *Azotobacter*, but the difference was not significant.

Maximum P content in onion leaves (0.32%) was recorded with T_5 and T_9 and it was significantly higher than rest of treatments except T_1, T_7, T_{11} and T_{13}. Among organic manure treatments, maximum P content was recorded in T_{13} and T_{11} but the difference among the organic manure treatments was non-significant and treatments show similar treands. PSB improve the P content in the onion bulb it may be due to solubliztion of P with PSB. The results are in line with the findings of Kavvadias *et al.* (2012) who reported increased plant concentration of phosphorous with phosphorus application.

All treatments show similar trends in terms of potassium content in the onion plant (Table 2). But the maximum potassium content (0.94%) in plant

Table 3: Effect of different treatments on microbial count in soil

Treatments	Microbial Count			
	O.C. (%)	Bacterial (10^6)	Actinomyctes (10^4)	Fungi (10^3)
At Transplanting time	**0.32**	**17.3**	**24.6**	**14.8**
At harvesting time				
T_1	0.32	24.8	25.8	28.2
T_2	0.30	23.2	25.4	18.0
T_3	0.34	22.6	27.8	18.4
T_4	0.28	21.0	27.6	18.0
T_5	0.32	22.6	28.4	18.6
T_6	0.30	21.8	28.0	18.4
T_7	0.32	22.6	27.2	18.2
T_8	0.28	22.4	27.6	18.6
T_9	0.34	22.2	28.8	18.4
T_{10}	0.28	22.0	28.4	18.6
T_{11}	0.40	26.8	33.0	27.6
T_{12}	0.40	27.2	34.0	25.3
T_{13}	0.38	25.8	32.6	27.4
T_{14}	0.32	18.4	25.6	16.2
T_{15}	0.26	18.0	25.0	15.6

was observed in T_8. The potassium content varied from 0.60 to 0.94% in different treatments of onion stover.

Nutrient uptake

Different treatment put a significant effect on total N uptake of onion (Table 2). Minimum total N uptake was observed in T_{15}. Maximum N uptake was observed in T_3 which was significantly higher than rest of treatments except T_1. Application of recommended dose of fertilizers resulted in significantly higher N uptake as compare to control.

Minimum total P uptake was observed in T_{15} (Table 2). Maximum total phosphorus uptake was observed in T_9 which was statistically at par with T_1, T_3, T_5 and T_7 and was significantly higher from rest of the treatments. Among organic manure, maximum P uptake was recorded by T_{11} which was significantly higher from T_{12} and statistically at par with T_{13}.

The maximum K uptake attained with T_3 which was significantly higher than T_8, T_{12}, T_{13} and T_{15} and was statistically at par with rest of treatments. Among organic manure, maximum K uptake was attained with T_{11} and it was statistically at par with other organic manure treatments i.e. T_{12} and T_{13}. Total K uptake with organic manures alone or with biofertilizers was significantly higher from control.

The application of full dose of nitrogen along *Azospirillum* and *Azotobacter* resulted in significantly higher total nitrogen uptake as compared to reduced nitrogen application. This could be due to the more availability of nitrogen to plants because of N fixation by biofertilizers is helpful for their vegetative and reproductive cycle. The increased vegetative growth may also be the reason for higher nitrogen uptake. The *Azotobacter* and *Azospirillum* excretes plant growth promoting substances such as vitamins, kinetins and gibberellins which improved the vigour of the crop and subsequently resulted in enhanced productivity. It emerges that those strains of *Azotobacter* are effective in N fixation and possessing genetic information for curbing specific pathogens of crop plants, synthesis of plant growth promoting hormones and for proteins, enzymes and other factors that improve uptake of essential nutrients by plants utilized in farming. This also may be due to production of plant growth promoting hormones and nitrogen fixation or assimilation by *Azospirillum* (Kumar and Rao, 2012).

These results are in line with Abd-Elrazzag (2002) in which biofertilizers and organic manures increases the nutrient uptake in onion. Potash application enhanced the nitrogen and phosphorus content in tomato (Chatterjee and Bandyopadhyay, 2014).

Microbial count in soil at transplanting and harvesting time

At the time of transplanting organic carbon in the soil was 0.32%, bacteria was 17.3×10^6, Actinomyctes was 24.6×10^4 and fungi was 14.8×10^3 (Table 3). After harvesting, biofertilizers improved the organic carbon of soil and highest organic carbon of soil was found in T_{12} and T_{11} that was 0.4% followed by T_{13}. FYM and poultry manure improved the organic carbon in the soil. Highest number of bacteria was found in T_{12} followed by T_{11} followed by T_{13}. *Azotobacter* fix the N in the soil and made available to the plants. Biofertilizers also improves the Actinomyctes at the time of harvesting. Highest number of Actinomyctes was found in treatment T_{12} and T_{11}. Biofertilizers also improved the Fungi at the time of harvesting. T_1 had highest number of fungi microorganism at the time of harvesting followed by T_{11}. So, biofertilizers improved the micro flora in the soil.

FYM treatments improved the microbial count at harvesting time as compared to other biofertilizer treatments. It might be due to slow releasing of nutrients from FYM. Farm yard manure is a carrier of organic carbon and organic dry matter (Singh and Singh, 2005). Due to this reason, organic carbon and microbial count improved in onion with the application of FYM alone or in combination with biofertilizers. The treatments without inoculation of any bacteria and application of chemical fertilizer did not differ so much than inoculated ones initially at time of transplanting but during the remaining stages of growth especially at harvesting they gave minimum microbial count for bacterial group compared to inoculated ones. The distinguished increased in count indicated that the microbial counts were positively influenced by plant root exudates and surplus of nutrients (Verma *et al.*, 2011).

CONCLUSION

Biofertilizers are eco-friendly, non-toxic and relatively cheaper natural products than the inorganic/chemical fertilizers. The integrated use of biofertilizers along with organic and inorganic manures/fertilizers will help in reducing the soil and environmental degradation and protect nutrients against losses (such as leaching and volatilization losses). The results revealed that highest Bulb dry mass and stover dry mass was observed in T_3 followed by T_1 treatments. Application of T_3 treatment recorded significantly higher nitrogen uptake over all the treatments except T_1. The higher phosphorus uptake was attained with application of T_9 over all the treatments except T_1, T_3, T_5 and T_7. Treatment T_3 resulted in higher total potassium uptake. Organic manures improved the organic carbon status of soil and highest organic carbon of soil was observed in T_{12} and T_{11} treatments. While, highest bacteria and actinomyctes count was observed in T_{12} treatment. Treatment T_1 had highest fungal count at the time of harvesting. The application of biofertilizers along with recommended dose of fertilizers not only improves nutrient availability but also improves the yield and protects the soil against degradation.

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