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REVIEW ARTICLE

TITLE:

**SEED TREATMENT WITH BIO-FERTILIZERS FOR IMPROVING
MAIZE PRODUCTIVITY UNDER VARIOUS FERTILITY
MANAGEMENT**

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SEED TREATMENT WITH BIO-FERTILIZERS FOR IMPROVING MAIZE PRODUCTIVITY UNDER VARIOUS FERTILITY MANAGEMENT

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Abstract: Background: Nitrogen (N) is required by plants for producing good yield, but its losses are the main reason for low availability. Biofertilizers have become an ecofriendly and cost-effective strategy in improving soil fertility and crop yield. For improving the N fertilizers efficiency in soil and reducing its losses, combination of biofertilizer and chemical N fertilizers are getting attention. This study was planned to study the effects of biofertilizer and chemical fertilizers on maize productivity. Experiment consisted of two factors 1- Fertilizer comprised of mineral nitrogen (N) and Farm Yard Manure (FYM) as 100% mineral N, 25% FYM+75% mineral N, 50%FYM+50% mineral N, while factor 2 comprised of bio-fertilizers (Control, *Azotobacter* spp. *Rhizobium* spp. *Pseudomonas* spp. and *Azospirillum* spp.) were applied as seed treatment before sowing. Early tasseling, silking and physiological maturity was recorded in plots where N was 25% from FYM and 75% from urea. The integration of FYM with urea either in 50:50 or 25:75 ratios had greater Leaf Area (LA), Leaf area index (LAI) , Grain Yield (GY), Biological Yield (BY), and 1000 grain weigh. Maximum leaf area (5269), LAI (3.16), Days to physiological maturity (103), grain/ear (411), 1000 grain weight (251.9), Grain yield kg/ha (3295) and biological yield (11031) was observed in 50% FYM+50%N. Tasseling, silking and physiological maturity was delayed but plant height, grains ear⁻¹, GY and BY were improved in plots where seed was treated with *Azotobacter* spp., *Azospirillum* and *Pseudomonas* spp. Greater LA and LAI was found for seed treated with *Azotobacter* as compared to *Azospirillum*,

Rhizobium and *Pseudomonas* spp. whereas least values were obtain in control. Application of either 50 % FYM + 50 urea or 25% FYM +75% urea and seed treatment with either *Azotobacter* spp., *Azospirillum* spp. or *pseudomonas* spp. had substantially increased yield attributing traits of maize.

Key words: Nitrogen Management, Bio-fertilizers, Farm Yard Manure, Seed Inoculation.

1. Introduction

Maize (*Zea mays* L.) is one of the major cereal crops in Pakistan. The cultivated area was about 1334 thousand hectares, and net production was 6130 thousand tons during 2016-17 (MNSFR, 2017). The Agro-climatic conditions of Pakistan especially of Khyber Pakhtunkhwa (KPK) are suitable to produce high quality maize. However, there are many aspects which are limiting maize production such as timely and appropriate use of fertilizer application. Regardless of the large amount of fertilizer application, yield is stagnant (Ali, S. 1999). Likewise, poor nutrient management and imbalance use of synthetic fertilizers are the other major constraints in maize production. Inorganic fertilizers are also expensive and less efficient (Twyford et al., 1995).

The World expanding population needs healthy food which can be produced by using essential nutrients in such a way that soil fertility remains healthy. Large amount of fertilizers are being used to provide essential nutrients for plants. The mechanism and features of the nutrient release from organic, bio-fertilizers and chemical fertilizers are not same (Henri et al., 2008). Plants need balanced and sufficient nutrients for optimum growth (Cakmak, I. 2002). The use of mineral fertilizers is fastest and most guaranteed way

to enhance crop production (Chianu et al., 2008) though its application is adversely affecting ecosystem through triggering pollution and ultimately deteriorating valuable soil flora and fauna (Shober, L.A. 2012). Crop yield can be maximized by the application of farmyard manure (FYM), which has been practiced for centuries to improve soil fertility and structure, increase microbiological activities and improve soil organic matter for better soil health (Zhang and Fang, 2007). Soil health cannot be improved due to continuous application of chemical fertilizers only; thus, it requires use of organic manure as well (Khanam et al., 2001). Bio fertilizer is the best alternative to synthetic fertilizer in developing countries to decrease the net cost of production which can be treated to roots, seeds or soil for improving soil health and nutrient availability (Nambiar, 1985). Living cells of different kinds of micro-organisms are said to be bio fertilizers (fungi, algae, bacteria), which can mobilize the non-available nutrients for plant use (Saini et al., 2004). These are more effective for sustainable environment (Amudha et al., 2014), soil quality and ultimately for better yield. Farmer get maximum advantages by applying less costly inputs (Kumudha and Gomathinayagam, 2007).

Microorganisms decompose organic matter and as a result nutrients are released for plants. Several authors stated that bio-fertilizer has important role in sustainable agriculture (Wani and Lee, 1999). Micro-organisms activities are improved with their method of application and application to more responsive crop species (Jilani et al., 2007). For instance, combined application of chemical and bio-fertilizer increased production of wheat (Das et al., 2001). Bacterial inoculation have prominent response to soil health when it is applied with FYM (Khalil and Sultan, 2001). Soil contains many different microbes which works in association (Grey and Smith, 2005) and so these microorganisms are responsible for energetic function in soil eco-system (Jalili et al., 2009). Decomposition of organic matter

by micro-organisms makes nutrients available to plant ultimately enhancing the soil fertility consequently improves soil health (Haas and Defago, 2005).

There are many management strategies for improving plant growth and yield of maize crop moreover, scientists have devised methods to reduce N losses from the soil. Research on separate application of chemical fertilizer, organic manure and biofertilizer is reported previously but a little work is previously reported on combination of chemical N fertilizers, organic fertilizer (FYM) and biofertilizers as seed inoculation and their combined effect on growth and yield attributes of maize plant. There is an urgent need to understand how to utilize the biofertilizers efficiently so that they may provide nutrients in full potential for plant growth and development and help in improving N availability to plants that ultimately improve the plant yield and growth. The current study was conducted to determine the influence of nitrogen management (NM) and seed treatment with bio fertilizer for enhancing yield and yield related traits of maize.

2. Materials and Methods

The experiment was conducted at Palatoo Research Farm of Amir Muhammad Khan Campus Mardan, during spring 2016. The experiment was consisted of two factors i.e. NM (100 % urea, 25 % FYM+75 % urea, 50 % FYM + 50 % urea) and bio-fertilizers (Control, *Azotobacter* sp, *Rhizobium* sp, *Pseudomonas* sp. and *Azospirillum* sp.). The organic manure (FYM) was used for particular treatments on the basis of N content and mutual application of FYM and inorganic fertilizer, to obtain 150 kg N ha⁻¹. FYM was applied to the respective plots 10 days before sowing, basal dose of P, K and half dose of N from urea fertilizer were applied to the respective plots pre-sowing of maize and remains half dose of N from urea fertilizer was applied with 2nd irrigation. Bio-fertilizers were applied as seed treatment. The bio-fertilizers powder was obtained from

the National Agriculture council Islamabad Pakistan. Maize seeds were immersed in sugar solution (16 %) as a sticking agent and then mixed with the respective bio-fertilizer powders. The treated seeds were dried before sowing. Maize cultivar Azam was sown on plot size 4 x 3.5 m² with help of hand hoe at 70 cm row to row distance. The experiment was arranged in Randomized complete block design (RCBD) replicated three times and irrigation was applied according to requirement of the crop. Herbicides i.e. pre-emergence Premixtra was sprayed for effective and efficient management of weeds. All other agronomic practices were uniformly applied to each subplot. The experimental treatment's structure is below:

Factor A. Nitrogen management

F1 100 % urea (150 kg N ha⁻¹)

F2 FYM (25%) +75% Urea

F3 FYM (50%) + 50% Urea

Factor B. Bio-fertilizers

Control (no amendments/fertilizer)

Azotobacter spp.

Azospirillum spp.

Rhizobium spp.

Pseudomonas spp.

2.1. Procedure for physical data recording

2.1.1. Number of days to tasseling, silking and physiological maturity: The tasseling, physiological maturity and silking data were recorded by counting the number of days from the date of sowing to the date when 75% of plants formed tassels and silk in each plot.

2.1.2. Leaf area plant⁻¹ (cm²) (LA): Five plants from each experimental unit were selected to measure LA. Using a meter rod, the length and width of each leaf in its broadest position were measured. The formula below was used to record the amount of LA per plant.

Leaf Area per plant = lamina length x maximum width x 0.75 (correction factor) (Musa and Hassan, 2016).

2.1.3. Leaf area index (LAI): Data on the LAI were recorded by using the following formula.

$$LAI = \frac{\text{Leaf area plant}^{-1}}{100 \times 100} \times \text{number of plants m}^{-2}$$

2.1.4. Plants m⁻²: Number of plants m⁻² was recorded by counting number of plants in three central rows having one-meter length and number of plant m⁻² was recorded using following formula.

Plants m⁻² =

$$\frac{\text{Number of plants in three central rows}}{\text{Number of Rows} \times (\text{Row} - \text{Row distance}) \times (\text{Row Length})}$$

2.1.5. Plant height at maturity stage (cm): A meter rod was used to measure the average plant height in each plot from the base to the tip of each plant at the physiological maturity stage for five randomly chosen plants.

2.1.6. Number of grains ear⁻¹: Each plot had five ears removed at random and manually threshed. The grains that were collected were counted, and the average grain ear⁻¹ was calculated.

2.1.7. Biological yield (kg ha⁻¹) (BY): Data on BY were recorded by harvesting three central rows in each experimental unit, the plant was dried in sun for several days and was then weighed and converted to kg ha⁻¹ using the following formula.

Biological yield (kg ha⁻¹) =

$$\frac{\text{Biological weight (kg) of three rows}}{\text{Row-Row distance} \times \text{Row length} \times \text{number of rows}} \times 1000$$

2.1.8. 1000 grain weight (g) (TGW): Upon drying, the cobs were threshed for seeds and weight of 1000 grain was measured using electronic balance.

2.1.9. Grain yield (kg ha⁻¹) (GY): Three middle rows were harvested in each plot and were sun-dried and threshed. The grains collected were further dried and weighed and converted to kg ha⁻¹.

Grain yield (kg ha⁻¹) =

$$\text{HI (\%)} = \frac{\text{Grain weight of three rows}}{\text{R-R distance} \times \text{R length} \times \text{No. of Row}} \times 1000$$

2.1.10. Harvest index (%): Data on the harvest index were determined by using following formula.

$$\text{HI (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

2.2. Statistical Analysis: Data recorded on all studied parameters were statistically analyzed by using the appropriate procedure for RCB design using Statistix 8.1 software. Fisher Analysis of variance (ANOVA) was performed and means were generated. The means were separated on the basis of Least significance difference (LSD) at 5% probability level. The means values showing different distinguished groups were significantly different to each other (Jan et al., 2009). After that two way table were generated and means as well as interaction between the two studied parameters were compared.

3. Results

3.1. Days (D) to tasseling: Table 1 shows data regarding Silking and tasseling (ST) influenced by bio-fertilizers and NM. Bio-fertilizers and NM had a profound influence on ST. Significant effect was found in control vs rest of the treatments. Non-significant interaction was found in NM and bio-fertilizers for ST of maize. Delay in tasseling (51 D) were recorded in plots where N was applied from sole urea though it was

statistically similar (51 D) to plots where N was applied both from FYM+N (50: 50 ratio) (49). Plots where FYM+N was applied at 25:75 ratio, had early tasseling. In the case of bio-fertilizer treatment, tasseling was delayed in plots when seeds were treated with *Azospirillum* spp. which was at par with both *Azotobacter* spp and *Pseudomonas* spp. Tasseling was early both in control and seed treatment with *Rhizobium* spp. Tasseling was delayed in fertilizer plots (53 D) as compared to control plots where no N was applied (50 D).

3.2. Days to silking: For the days leading up to maize silking, significant differences in NM and bio-fertilizer use were seen. Control vs. rest of the treatments mean comparison was found to be significant. biofertilizer and N management interactions were found to be insignificant (Table 1). Delayed silking occurred in plots where N was applied solely from Urea followed by plots where FYM + Urea was applied at 50:50 ratios though it was statistically similar to the integration of FYM + Urea at 25:75 ratio. Comparing bio-fertilizer, silking was delayed in plots where the seed was treated with *Azospirillum* spp. (58 D) which was at par with *Azotobacter* spp. (57 D) and *Pseudomonas* spp. (57 D). Early silking occurred both in control (56D) and seed treatment with *Rhizobium* spp. (57 D). Planned mean comparison revealed that silking was delayed in fertilizer plots (59 D) compared to plots where no application of N was done (57D).

Table 1. ST of maize as affected by application of bio- fertilizer and NM

BF	Days to tasseling				Days to silking			
	100 %N	25% FYM +75%N	50% FYM +50%N	Mean	100 %N	25%FYM +75%N	50%FYM +50%N	Mean
Control	49.4	48.5	49.2	49.0 ±0.4 b	56.4	55.2	56.4	56.0 ± 1.14 b
<i>Azotobacter</i> spp	51.8	49.5	50.9	50.7 ±0.3 a	58.4	56.5	57.3	57.4 ± 1.18 a
<i>Azospirillum</i> spp	51.7	50.0	51.4	51.0 ± 0.3 a	58.4	57.3	57.4	57.7 ± 1.14a

<i>Rhizobium</i> spp	49.9	48.9	49.2	49.3 ± 0.9 b	56.9	56.6	55.2	56.2 ± 1.11 b
<i>Pseudomonas</i> spp	51.7	49.5	50.8	50.7 ± 1.1 a	58.6	56.5	56.8	57.3 ± 1.23 a
Mean	50.9 ±1.19 a	49.3 ±0.53 b	50.3 ±1.31 a		57.7 ± 1.12 a	56.4 ± 1.21 b	56.6 ± 1.11 b	
Control vs Rest	Control Rest	50.2 52.9			Control Rest	56.9 59.4		
LSD for NM		0.9				0.9		
LSD for BF		1.1				1.2		
Interaction		ns				ns		

Means in column and row followed by different letters are significantly varied among each other at 0.05 P value, ns = non-significant

3.3. Leaf area plant⁻¹: Table 2 shows data regarding LA plant⁻¹. A perusal of data indicated application of bio-fertilizer and NM had a substantial influence on maize LA plant⁻¹. A significant difference was recorded between the control vs rest of the treatments for LA of maize. The interaction between BF and N was found non-significant. The N fertilized plots irrespectively of sources had higher LA plants (5806) than no fertilized plots (4274). The LA plant⁻¹ was higher (5269) when N was applied from 50% FYM and 50% Urea followed by 25% FYM + 75% urea (4952) while lesser LA plant (4872) was noted when the sole application of Urea was applied. In case of biofertilizer treatments, the higher LA plant⁻¹ was recorded from plots where seeds were treated with Azotobacter spp. (5806) followed by Azospirillum spp. (5078) which was statistically at par with pseudomonas and Rhizobium spp (5004 and 4995 respectively) while lesser LA (4274 cm²) was noted in control plots.

3.4. Leaf area index: Table 2 shows data on LAI. Analysis of data showed that NM and bio-fertilizers had significantly affected LAI of maize. No significant interaction was found between NM and bio-fertilizer for LAI of maize. The fertilized plots produced a higher LAI (3.0) as compared to no fertilized plots (2.2). The greater LAI (3.16) was recorded from plots where N was applied from 50% FYM + 50% Urea followed by plots where FYM and Urea were applied at 25:75 (2.95) while the lesser LAI (2.91) was recorded when sole urea was applied. In case of BF the seed primed with Azotobacter spp. had resulted in a higher LAI (3.50) followed by Pseudomonas spp (3.06) while a lesser LAI was obtained from control plots (2.56).

Table 2. LA plant⁻¹ (cm) and LAI of maize as affected by application of bio-fertilizer and NMs.

BF	LA plant ⁻¹				LAI			
	100 %N	25% FYM +75%N	50% FYM +50%N	Mean	100 %N	25%FYM +75%N	50%FYM +50%N	Mean
Control	4548	3928	4345	4274 ±199 b	2.73	2.36	2.61	2.56± 0.11 c
<i>Azotobacter</i> spp	5275	5876	6267	5806 ±212 a	3.22	3.53	3.76	3.50± 0.12 a

<i>Azospirillum</i> spp	4664	5096	5473	5078 ±233b	2.80	3.06	3.28	3.05± 0.16 b
<i>Rhizobium</i> spp	4937	4871	5176	4995 ± 198 b	2.63	2.87	3.05	2.85± 0.17b
<i>Pseudomonas</i> spp	4936	4989	5086	5004 ± 212 b	3.16	2.92	3.11	3.06± 0.10b
Mean	4872 ±282 b	4952 312 ± b	5269 322 ± a		2.91± 0.21 b	2.95± 0.16b	3.16± 0.11a	
Control vs Rest	Control	3728			Control	2.2		
	Rest	5031			Rest	3.0		
	Sign.	0.000			Sign.	0.000		
LSD for NM		281.5				0.2		
LSD for BF		363.4				0.3		
Interaction		ns				ns		

Means in column and row followed by different letters are significantly varied among each other at 0.05 P value, ns = non-significant

3.5. Days to physiological maturity: Data on the days until maize reached physiological maturity are presented in Table 3. Data analysis revealed that NM and bio-fertilizers (BF) had a significant impact on physiological maturity. A non-significant interaction between BF and NM was discovered. For physiological maturity, the control vs. rest of treatments was found to be significant. The planned mean of data showed that fertilized plots took fewer days to physiological maturity than no fertilized plots. Delayed physiological maturity occurred for treated seed with *Azotobacter* spp. (104 days) which was also at par with

Azospirillum spp. (103) and *Pseudomonas* spp. (103) whereas early maturity occurred in control plots (100). In case of NM, physiological maturity was delayed (103 days) by 50% Urea + 50% FYM application whereas the physiological maturity was early in both sole Urea fertilization and (102 days) in plots received N from 25% FYM and 75% Urea application.

3.6. Plants m²: Table 3 displayed the number of plants per square meter as a function of NM and biofertilizer application. Data analysis revealed that NM and bio-fertilizer had no discernible impact on plant area (m²). It was determined that there was no significant interaction between NM and bio-fertilizer. Additionally, control vs. rest of the treatments was found to be non-significant.

Table 3. Days to physiological maturity of maize as affected by application of bio-fertilizer and NM.

BF	Days to physiological maturity				Plant m ⁻²			
	100 %N	25% FYM	50% FYM	Mean	100 %N	25%FYM	50%FY	Mean
Control	99	100	101	100± c	6.7	7.1	7.6	7.1±b
<i>Azotobacter</i> spp	103	103	104	104± a	6.7	7.1	7.6	7.1±b
<i>Azospirillum</i> spp	102	103	105	103± ab	7.1	8.6	6.7	7.5±a
<i>Rhizobium</i> spp	99	101	103	101±	7.6	7.1	6.7	7.1±b
<i>Pseudomonas</i>	102	103	104	103	6.7	6.2	7.1	6.7±c
Mean	101± b	102± ab	103± a		7.0±b	7.2±a	7.1±ab	
Control vs Rest	Control	97.7			Control	7.1		
	Rest	102.2			Rest	7.1		

	Sign.	0.001			Sign.	0.953		
LSD for NM		1.6				ns		
LSD for BF		2.1				ns		
Interaction		ns				ns		

Means in column and row followed by different letters are significantly varied among each other at 0.05 P value, ns = non-significant

3.7. Grains ear⁻¹: Table 4 showed the effect of NM and BF on grains ear⁻¹. A perusal of data indicated that NM and bio-fertilizers had a significant effect on number of grains ear⁻¹. The interaction between biofertilizer and N management was not found to be significant. For grains per ear, a significant difference between control and rest was discovered. In fertilized plots, ear-1 grain counts were higher (420), while control plots had lower grain counts (373). A higher number of grains ear-1 was observed, for seeds treated with *Azotobacter* spp., (420) followed by seed treatment with *Azospirillum* spp. (411) and *Pseudomonas* spp. (399) while the lesser number of grains per ear was noted in no fertilizer plots (373). In NM, the maximum no. of grains per ear was noted in plots received N applied from FYM and urea at 50:50 ratios (411) followed by 25:75 ratio

(399) while sole use of mineral N had the lower number of grains ear⁻¹ (381).

3.8. 1000 grains weight (g): Data regarding 1000 grains weight as affected by NM and bio-fertilizer are shown in Table 4. Analysis of the data indicated that NM had a significant effect on thousand-grain weight of maize while bio-fertilizers (BF) treatment and interaction between N management and biofertilizer had no significant effect on TGW. Control vs fertilized plots significantly affected thousand grain weight. The planned mean comparison showed that N fertilization had a higher 1000 grains weight (256) than control plots (239). In the case of NM, from 50% FYM and 50% Urea (251) followed by plots which received N from 75% urea and 25% from FYM (249) application sole urea fertilization had lower thousand grain weight (240).

Table 4. Grains ear⁻¹ and TGW (g) of maize as affected by application of bio-fertilizer and NM

BF	Grain ear ⁻¹				TGW (g)			
	100 %N	25% FYM +75%N	50% FYM +50%N	Mean	100 %N	25%FYM +75%N	50%FY M +50%N	Mean
Control	353	380	388	373 b	233.0	241.5	242.5	239.0
<i>Azotobacter</i> spp	417	418	426	420 a	243.0	257.7	268.2	256.3
<i>Azospirillum</i> spp	389	420	424	411 a	243.5	250.9	249.3	247.9
<i>Rhizobium</i> spp	373	373	397	381 ab	244.3	252.3	250.0	248.9
<i>Pseudomonas</i> spp	373	403	421	399 a	236.3	242.5	249.7	242.8
Mean	381± b	399± a	411± a		240.0±	249.0±	251.9±	
Control vs Rest	Control Rest	364.0 396.9			Control Rest	209.6 247.0		
LSD for NM		18.8				9.8		
LSD for BF		24.3				ns		
Interaction		ns				ns		

Means in column and row followed by different letters are significantly varied among each other at 0.05 P value, ns = non-significant

3.9. Grain yield (kg ha⁻¹): Table 5 displays the effect of NM and biofertilizer on maize GY. Data analysis revealed that NM and the use of bio-fertilizers had significantly increased maize GY. Significant interactions between NM and bio-fertilizer were discovered. Significant variations between the fertilized and control plots were noted. In the case of NM, the higher GY (3295) was obtained when N was applied from FYM and Urea at 50:50 ratios though it was not substantially different from plots received N from FYM and Urea at 25:75 ratios (3218 kg ha⁻¹) while sole urea fertilized showed lower GY (3086 kg ha⁻¹) of maize. The GY in fertilized plots was higher (3283 kg ha⁻¹) as compared to the control (3015 kg ha⁻¹). Higher GY (3283) was recorded for seed treatment with *Azotobacter* spp. which was statistically similar to plots where seeds were treated with *Azospirillum* spp. and *Pseudomonas* spp. while lower GYs were noted in control plots (3015) where no bio-fertilizers were applied. Interaction between NM and BF interaction indicated that GY decreased with the integration of Urea and FYM in no bio-fertilizer plots contrary to the

bio-fertilizer plots where GY was increased with the integration of FYM and Urea either in 25:75 or 50:50 ratios for all bio-fertilizer treatment except *Pseudomonas* spp.

3.10. Biological yield (kg ha⁻¹): Data on maize's BY are displayed in Table 5. The results of the data's statistical analysis showed that NM and bio-fertilizers had a significant impact on maize's BY. A non-significant effect was recorded in the interaction between bio-fertilizers and NM. In comparison to control plots, fertilized plots produced the highest BY (11189 kg ha⁻¹), according to the planned means comparison of the data (10010 kg ha⁻¹). Although it was comparable to *Azospirillum* spp. (10918 kg ha⁻¹) and *Pseudomonas* spp. (11189 kg ha⁻¹), seed treatment with *Azotobacter* spp. led to a higher BY. (10861 kg ha⁻¹) whereas lesser BY (10010) was obtained in control plots. In the case of NM, the integration of urea and FYM 50:50 ratios (11031 kg ha⁻¹) ratios had higher BY followed by plots where FYM + N in 25:75 was applied (10627 kg ha⁻¹) while minimum recorded from sole urea fertilization (10295 kg ha⁻¹).

Table 5. GY kg ha⁻¹ and BY (kg ha⁻¹) of maize as affected by the application of bio-fertilizer and NM.

BF	GY kg ha ⁻¹				BY (kg ha ⁻¹)			
	100 %N	25%FY M +75%N	50% FYM +50%N	Mean	100 %N	25%FYM +75%N	50%FYM +50%N	Mean
Control	3128	3096	2822	3015 b	10292	9990	9748	10010 b
<i>Azotobacter</i> spp	3176	3331	3481	3329 a	10057	11239	12271	11189 a
<i>Azospirillum</i> spp	3031	3203	3625	3287 a	10382	10732	11642	10918 a
<i>Rhizobium</i> spp	2988	3152	3115	3085 b	10459	10427	9941	10276 b
<i>Pseudomonas</i>	3110	3310	3431	3283 a	10282	10748	11553	10861 a
Mean	3086± b	3218± ab	3295± a		10295± b	10627± a	11031±a	
Control vs Rest	Control Rest	2272 3200			Control Rest	8847 10651		
LSD for NM		146.7				545		
LSD for BF		189.3				703		
Interaction						ns		

Means in column and row followed by different letters are significantly varied among each other at 0.05 P value, ns = non-significant

3.11. Harvest index (%): NM and bio-fertilizers application showed no significant influence on the maize crop harvest index (Table 6). It was determined that the BF and NM interaction was not significant. Control vs. rest of the treatments was found to be significant. Plot receiving N irrespective of the source had a higher harvest index (30.2 %) than the control plot where no N fertilizer was applied (25.9%).

3.12. Plant height (cm): Table 6 provides information on maize plant height. The use of bio-fertilizer and NM had a significant impact on plant height. The interaction between bio-fertilizer and N management was not found to be significant. The planned

mean comparison between fertilization and control was found to be significant. The fertilized plots produce taller plants (251) as compared to control plots (235). The taller plants were recorded in seed treatment with *Pseudomonas* spp (251.6) however it was at par with *Azospirillum* spp. (250.4) and *Azotobacter* spp. (248) followed by *Rhizobium* spp. (233.8) which was statistically similar to control plots (235). In case of NM, the application of FYM and urea at 50:50 ratios had attained taller plants (250.2) followed by 25% FYM and 75% Urea (243.4) though it was statistically similar to plots received N from sole urea application (238.2).

Table 6. Harvest index and plant height (cm) of maize as affected by application of bio-fertilizer and NM

BF	Harvest index				Plant height (cm)			
	100 %N	25% FYM +75%N	50% FYM +50%N	Mean	100 %N	25% FYM +75%N	50% FYM +50%N	Mean
Control	30.5	31.0	29.0	30.2	234.6	231.4	241.3	235.8 b
<i>Azotobacter</i> spp	31.6	29.7	28.4	29.9	238.3	245.5	260.0	248.0 a
<i>Azospirillum</i> spp	29.2	30.1	31.2	30.2	237.5	257.0	256.6	250.4 a
<i>Rhizobium</i> spp	28.8	30.3	31.6	30.2	228.5	233.8	239.2	233.8 b
<i>Pseudomonas</i>	30.3	30.9	29.9	30.3	251.9	249.1	254.0	251.6 a
Mean	30.1±	30.4±	30.0±		238.2±	243.4± ab	250.2± a	
Control vs Rest	Control	25.9			Control	216.9		
	Rest	30.2			Rest	243.9		
	Sign.	0.027						
LSD for NM		ns				8.2		
LSD for BF		ns				10.6		
Interaction		ns				ns		

Means in column and row followed by different letters are significantly varied among each other at 0.05 P value, ns = non-significant

3.13. Correlation analysis between maize parameters: Figure 1 shows the correlations between the different growth and yield parameters of maize. The strongest positive correlation is calculated between TGW and LA, LA and LAI, Days to maturity (DM) and ST, DM and DS. A moderately significant and positive correlation was recorded between TGW and GY, TGW and LAI, DM and GY, HI and GY. A negative and

moderately significant correlation was observed between TGW and DS, TGW and TS, DM and LA, DM and LAI, HI and DS, HI and ST, and Grain/ear and NL while a highly significant and negative correlation was recorded between TGW and DM indicating that if the value of one of this parameter increases, the other decreases. The results also show that BY, plant m⁻², and PH are independent of this study. This affects all correlations with values close to 0.

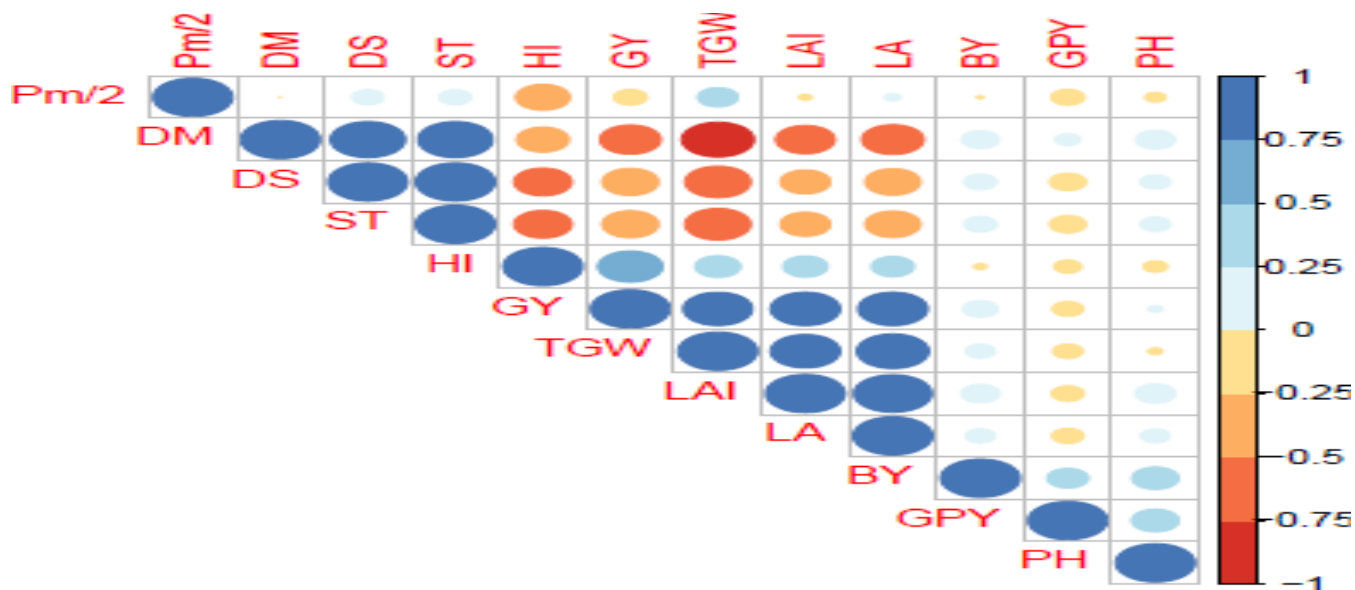


Figure 1. Correlation between different growth and Yield parameters of maize under the influence of biofertilizers and N. BY= Biological yield, GPY= Grain per year, PH= Plant height, P m⁻²= Plant per square meter, DS= Days to silking, DM= Days to maturity, ST= Days to tasseling, LA= Leaf area, LAI= Leaf are index, GY= Grain Yield, TGW= 1000 grain weight, HI= Harvest index

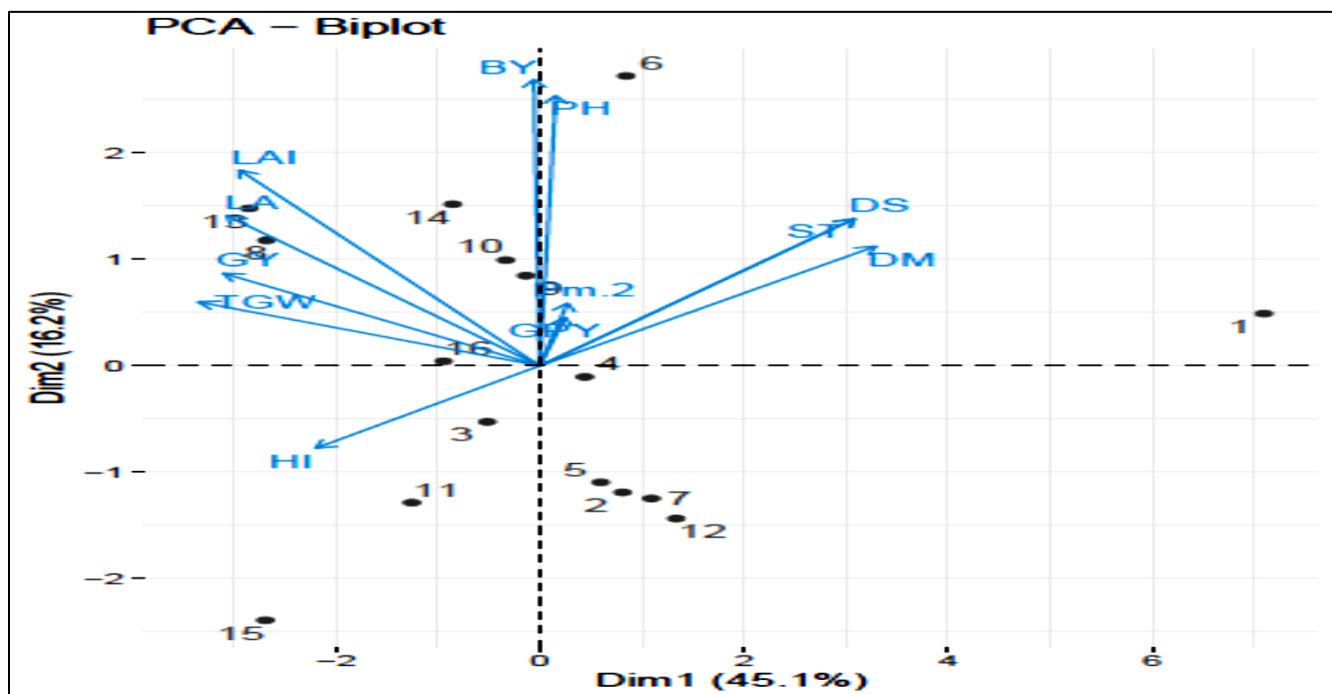


Figure 2. PCA biplot showing the effect of biofertilizers and N on the growth and yield of maize plants. BY= Biological yield, GPY= Grain per year, PH= Plant height, P m⁻²= Plant per square meter, DS= Days to silking, DM= Days to maturity, ST= Days to tasseling, LA= Leaf area, LAI= Leaf are index, GY= Grain Yield, TGW= 1000 grain weight, HI= Harvest index. 1-16 numbering shows the treatments.

3.14. PCA Biplot analysis between growth and yield parameters:

Figure 2 shows the

correlation between maize yield parameters and on the other hand the influence of the

effects of organic fertilizer and NM on these correlations. This principal component analysis (PCA) provides 45.1% of the information on dimension 1 and 16.2% on dimension 2, representing 61.3% of the information for the entire projection. For dimension 1, the most explanatory variables are the different treatments 2, 3, 4, 5, 7, 9, 10, 11, 12, and 14. However, the most explicative variables of dimension 2 are the Yield parameters of maize and other treatments including 1, 8, 13, and 15. PH, BY, GPY and $P\ m^{-2}$ are strongly correlated to each other and with dim 1, which implies that they are positively influenced by treatments 6, 10, 9, and 14 while being negatively influenced by 2, 3, 5, 7, 11, 12. LA, LAI, GY, and TGW are strongly correlated to each other and on dim 2. This shows they are positively influenced by 9, 10, and 14 while negatively influenced by 8, 13, and 16. ST, DS, and DM are strongly correlated to each other and axis 2 and are positively affected by 1 and 4 while negatively influenced by 3, 11, and 15. HI was negatively influenced by 15, 3, and 11 while correlated to both dimes.

4. Discussion

This study was carried out to check the combined effect of different NMs, manure and biofertilizer on growth and yield of maize. ST were greatly influenced by NM and bio-fertilizer. Tasseling was late by one day both in plots where sole Urea or integration of 50% Urea and 50% FYM was applied than in the plots where 75% Urea and 25% FYM were integrated. A two days delay in tasseling due to seed treatment with bio-fertilizer compared to control might be the more availability of plant nutrients for enhanced growth of the crop consequently tasseling was extended. These results are in a similarity to the results recorded by Farooqi (1999) and Amanat (1998) who also observed that more nutrients are available, especially in the maize delayed tasseling period. Maize crop took 47 days to flower when N was applied at $130\ kg\ ha^{-1}$ under semi-arid conditions. DS were substantially influenced

by NM and bio-fertilizer. Silking was delayed in plots where sole Urea was applied compared to the combined application of both Urea and FYM in either combination. This delay was probably because FYM provides nutrients gradually to crops due to their resilient nature for decomposition in their early stages compared to sole Urea application. Amanullah et al. (2009) also reported that silking of maize had delayed by application of urea at $180\ kg\ ha^{-1}$ compared to no fertilization. More DS were recorded when seeds were treated with *Azospirillum* spp, *Azotobactor* spp., and *Pseudomonas* spp as compared to no treatment and *Rhizobium* spp. The delay in silking due to bio-fertilizer might be due to increased nutrient availability from soil that extended silking of maize compared to no bio-fertilizer treatment.

LA $plant^{-1}$ was substantially influenced by N management and bio-fertilizer. Combine application of FYM and Urea either 50:50 or 25:75 ratios increased the LA of maize compared to the sole application of Urea. However, an increase in LA was observed in the fertilized plots compared with the unfertilized plots. The increase in LA in the integration of FYM and urea might have increased nutrient availability and enhanced soil physical and biochemical properties such as increased water holding capacity, aeration, and reduction of nutrient losses through leaching, enhancing nutrient uptake and consequently increasing LA $tiller^{-1}$ of maize. LA is a very important plant growth parameter because the efficiency of photosynthesis depends on a large and efficient assimilation surface area, provided with adequate solar and CO_2 energy and favorable conditions (Jain et al., 1966). The results are supported by Badr and Authman (Badr and Authman, 2006), Makinde (2007), and Laekemariam and Gidago (2012) who reported that LA per plant was higher under the integration of organic with inorganic fertilizer. Organic material slowly releases nutrients which decreases leaching losses. Many studies have shown that organic materials gradually release nutrients and

reduce the leaching losses, mainly N (Nevens and Reheul, 2003; Naveed et al., 2008). More LA per plant was recorded where seeds were treated with Azotobacter, following the rest of the bio-fertilizer compared to no bio-fertilizer. This increase might be due to the enhanced nutrient availability in the rhizosphere due to that bio-fertilizer. The results are similar to those of Sardrood et al. (2013) who found that LAI, plant dry weight, LA ratio, and leaf-specific weight of sorghum were also improved by using bio-fertilizers such as Pseudomonas, Azotobacter spp. The results are consistent with Yasari and Patwardhan (2007). They found that greater LA was recorded for seed treatment with biofertilizer i.e. Azotobacter and Azospirillum spp. than control plots. This might be due to the vigorous growth during vegetative periods under optimal nutrient availability that resulted in greater LA.

NM and bio-fertilizer considerably influenced LAI of maize. The integration of FYM and Urea either in a 25:75 or 50:50 ratio gives rise to the LAI of maize compared to the sole application of Urea. N fertilization irrespective of sources of application had higher LAI than no fertilizer application. The higher LAI in fertilized plots might be due to the optimal availability of nutrients to the crop which enhances growth and consequently increases LAI. Athar and Ashraf (2005) found that maize yield is highly dependent on LAI, LAD, and leaf efficiency on solar radiation absorption for photosynthesis. For most plant species, N deficiency leads to a decrease in LA development, changes in leaf tissue composition, leaf cell structure, and plant water content (Casa, 2003), ultimately reducing GY. An increase in LAI was found in plots where seeds were treated with Azotobacter spp as compared to no treatment. This might be due to the fact that those bio-fertilizers provide better growing conditions for vigorous growth of the crop that had reached to higher LAI. Biological fertilizers such as Azotobacter spp. in addition to N₂ fixation, also synthesize and secrete a

significant amount of biologically active substances such as vitamin B, nicotinic acid, pantothenic acid, biotin, heterotoxin, gibberellin, help improve the growth of plant roots (Rao, 1986). Another important property of Azotobacter spp. related to crop improvement is the secretion of ammonia into the rhizome in the presence of root exudates, which alter nutrient uptake by plants (Narula and Gupta, 1986). Our results are like Sardrood et al. (2013) who reported that LAI grain of sorghum was enhanced with bio-fertilizers (Azotobacter and Pseudomonas spp.)

Duration of physiological maturity was considerably extended by the integration of FYM and Urea in 50: 50 ratios followed by the same combination but in 25:75 ratio though it was similar to sole use of Urea. The early physiological maturity in sole urea might be due to deficient N requirement probably due to potential losses that occurred due to leaching or volatilization. This may also be explained that the combined application of FYM and Urea reduced N losses and provide more N to the crop that extended maturity. The early maturity in no fertilized plots and over-fertilized plots might be due to the lesser N availability in soil. Amanullah et al. (2009) also reported that with increased N levels, maize plants would accumulate more heat units until flowering, flowering, and physiological ripening. Oikeh et al. (1997) reported that more days to physiological maturity were observed in plots where seeds were treated with Azotobacter spp., Azospirillum spp., and Pseudomonas spp. compared with other treatments. Physiological maturation delay may be due to better growing conditions and prolonged availability of phytonutrients.

Plant height was affected significantly by N management and bio-fertilizer. Combined application of FYM and Urea in 50:50 ratio had taller plants which were similar to the same combination in 25:75 while sole urea had short plants. The combined application of urea with organic fertilizer enhances nutrient use efficiency which increases the growth of

maize. Many researchers declared that leaching losses of nutrients especially N can be reduced by the application of organic material which gradually releases nutrients. Similar results were also reported by Dadarwal et al. (2009). They evaluated the effect of integrated nutrient management in maize and found that maximum plant height and dry matter accumulation in the treatment consisted of 75% NPK + 2.25 tons vermicompost ha⁻¹ compared with other treatments. Taller plants were recorded in N-fertilized plots compared to no fertilization. Similar results were reported by Ghafoor and Akhtar [40] who stated that the plant height of maize is significantly increased by the application of N. The taller plants were recorded in Azotobacter spp which was at par with Azospirillum spp. and Pseudomonas spp. as compared to rest of bio-fertilizer and control. Bio-fertilizer may enhance crop growth and yield due to supplementation of fixed N and plant growth enhancing substances for better growth and development. Our results are similar to finding of Baral and Adhikari (2013) who reported that maize treated with bio-fertilizer (Azotobacter spp.) has significantly resulted in the increasing of plant height and LA. Seed treatment with Azospirillum spp. Might give significant increase in the crop growth (Hossain et al. 2015).

Thousand grains weight was significantly influenced by NM and biofertilizer. Thousand grain weight increased with the integration of FYM and Urea either in 50:50 or 25:75 ratios over sole application of Urea. The results are in similarity to Sial et al. (2007) who found that combined application of inorganic fertilizers with organic manure enhanced the assimilated production and their storage in economical parts of plants. The energy source that compost provides to the bacteria, which enhances biological activities and N availability, may be responsible for the improvement in grain weight of one thousand grains. Abdullahi et al. (2014) also reported similar findings and conclusions. The influence of bio-fertilizer

was found non-significant on TGW of maize. However, there was non-significant increase with seed treatment with Azotobacter spp. compared to rest of the bio-fertilizer and control. The same or least variation among those treatments might be due to the fact that enough nutrients and growing conditions were met for portioning of assimilate to the reproductive units. However, Naseri et al. (2013) found significant effect of bio-fertilizer inoculation on TGW of maize. In a study by Iqbal et al (2016), TGW of mung bean crop increased significantly when seed were inoculated with biofertilizers.

Grains per ear were considerably influenced by NM and bio-fertilizer. Combine application of FYM and Urea in 50:50 ratio had higher number of grains per though it was similar with same combination at 25:75 while sole Urea fertilization resulted in a lesser number of grains ear⁻¹. This might be due to uptake of more nutrients which enhances better root development is due to integration of urea with organic fertilizers. Leaching losses can be controlled by use of organic manure which slowly releases nutrients. Kumar et al. (2002) also found that both N fertilizer and FYM had significantly affected the number of grains per cob and higher number of grains was recorded when N was applied from both FYM and Urea in 50:50 ratio. More grains per ear in plots where seeds were treated with bio-fertilizer compared to control. This might be due to the positive effect of bio-fertilizers on enhancing nutrient uptake and provide better soil conditions. As those micro-organisms also produced vitamins and plant growth hormone which promote growth of plant (Anup Das et al., 2010). The combine use of Azospirillum spp and Azotobacter spp. was the most effective method for enhancing yield components of common buckwheat (Singh et al., 2015). Similarly, Obaid et al. (2016) reported that bio-fertilizer (Azotobacter spp.) increases N availability in the soil which might have enhanced the numbers of grain per ear.

BY was substantially influenced by NM and bio-fertilizer. Combine application of FYM

and Urea in 50:50 ratio resulted higher BY though it was non-significant with same combination but different ratio (25:75) while lower BY was recorded in sole urea. This could be because of the integration of FYM and Urea have positive effect on increasing LA, plant height, more number of grains consequently increased the BY of maize. The same was also reported by Makinde et al. (2010) found that by increasing the N rate, a significant increase in BY has been observed and when N was applied both half from FYM and Urea. Likewise, El-Lattief (2013) found that integrated use of urea with bio-fertilizer have synergistic effect on biomass of wheat as compared to either sole application of urea or bio-fertilizer. According to Akbari et al. [53], GY, plant height, harvest index and BY of sunflowers were increased by a combination of synthetic and organic fertilizers. Moreover BY in plots where seeds were treated with *Azotobacter* spp. which was similar to *Azospirillum* and *Pseudomonas* spp. as compared to no treatment. An increase in BY and growth could be described with N₂-fixing bacteria. This might be also due to the ability of bio-fertilizer to transport major nutrients like N and phosphorous as well releasing plant growth regulators for example gibberellins, IAA and ABA. Organic manure released organic acid which increased soil acidity and thus converted insoluble forms of phosphorus into soluble ones (Abou-El-Seoud, and Abdel-Megeed, 2012). Similar conclusions reported by Dobbelaere et al. (2002) by *Azospirillum* spp. inoculation growth of spring wheat. Jat et al. (2013) found a substantial increase in stover yield from 11.9 to 13.0% for *Azotobacter* over no seed treatment. A positive influence of Bio-fertilizers (*Azotobacter* and *Azospirillum*) on sorghum yield was also reported by Van Oosterom et al. (2010). Likewise, seed treatment with *Azotobacter* and *Azospirillum* enhanced the dry matter yield of sorghum Dobbelaere et al. (2002) also found that seed treatment with *Azotobacter* spp. enhanced cereal crop yields up to 30 %.

GY of maize was greatly influenced by NM and bio-fertilizer. The integration of FYM and Urea either in 25:75 and 50:50 ratios had higher and similar GY compared to sole Urea fertilization. The GY in fertilizer treated plots was higher as compared to the control plots. The same was also reported by Nagassa et al. (2005) who found that FYM and N in combination had significantly affected GY. The GY decreased with the integration of Urea and FYM in no bio-fertilizer plots contrary to the bio-fertilizer plots where GY was increased with integration of FYM and Urea either in 25:75 or 50:50 ratios for all bio-fertilizer treatment except *Pseudomonas* spp. These results are in similarity to Bocchi and Tano (1994) and Kader et al. (2002) who reported that the addition of manures and seed treatment with *Azotobacter* spp. increased the availability of nutrients and optimized the plant growth which resulted in higher GY. The health of the soil was correlated with an increase in the biomass of the microbial community because this affected the balance and availability of nutrients in the rhizosphere that result in a greater harvest (El-Kholiyetal., 2005; Biari et al, 2008). The synergistic effect of Bio-fertilizers (*Azotobacter* and *Azospirillum*) on yield was recounted by Van Oosterom et al. (2010). The seed treatment with *Azotobacter* enhanced cereal crop yield up to 30 %. Akbari et al. (2009) also found that a combination of bio and chemical fertilizers increased the GY of sunflower. The higher GY with seed treatment of *Azotobacter* spp., *Azospirillum* spp. and *Pseudomonas* spp. compared to control plot may be due to the production of vitamins, amino acids and plant growth promoting substances like (GA) gibberellic acid and IAA by beneficial microbes which resulted in a rise in nutrient uptake, translocation, and photosynthate assimilate synthesis, which improved plant growth characteristics and generated a profitable yield (Singh and Singh, 2006). Seed treatment with *Azotobacter* spp enhanced maize GY from 15 to 35% over control plots. Likewise, number of

researchers i.e. Gholami et al. (2009), and Hajnal-Jafari et al. (2012) also found that *Azotobacter* spp. enhanced maize yield. Increased crop production due to seed treatment with *Azotobacter* spp. has been investigated for several crops. *Azotobacter* spp. has improved GY of several crops in the range of 10-12 % (Jaga and Singh, 2010). An increase in seed yield of wheat with seed treatment with *Azotobacter* spp. was also reported by Ahmed et al. (2011); Kader et al. (2002) and Abd El-Lattief (2014). Yousefi and Barzegar (2014) indicated that the GY, harvest index, BY, and protein content of wheat were all increased by 34.3, 7.7, 12.5 and 13.6%, respectively, when *Azotobacter* spp. and *Pseudomonas* spp. were applied together. Additionally, seed treatment plus fertilization with *Azotobacter* and *Pseudomonas* spp. reduced the use of fertilizers from 25 to 50%. Likewise, Seed treatment with *Azospirillum* spp. had a considerable increase in GY (Rodrigues et al., 2008). NM and bio-fertilizer had no substantial influence on the harvest index of maize. Though N fertilization irrespective of the source had a higher harvest index than no fertilized plots.

In this study, a significantly positive correlation was found between TGW and GY, TGW and LAI, DM and GY, HI and GY while the strongest positive correlations are obtained between DM and ST, DM and DS, TGW and LA, LA and LAI. This suggests that as one of these parameters increases in value, the parameter to which it is significantly correlated also increases (Malik et al., 2011). The relationship between this parameter and its positivity is a key indicator of how the crop will behave. GY was positively related to LA, HI, LAI, and TGW while negatively correlated to DM, ST, and DS. Plant indicating that delay or irregularity in plant growth at the reproductive stage would lead to poor GY. Kasu-Bandi et al. (2019) reported that NP cannot be attributed directly to the collar diameter in soybean crops.

The biplot analysis showed a visual analysis and the effect of N and biofertilizers on the parameters of maize. About 62% of the variability is presented by biplot analysis of different parameters on dim 1 and dim 2. This variability proportion can be considered low compared to previous studies by different researchers where Kasu-Bandi et al. (2019) reported a 75 % variability projection in different growth and yield parameters of soybean plants. Bhatta et al. (2019) found that the first two major elements connected to West Siberian synthetic's agronomic and qualitative traits and bread wheat accessions had an overall variation of 54%. Mohammadi et al. (2012) also reported a low variability of about 68% in a study of wheat yield and growth parameters. Positive correlation between maize growth parameters, plant m⁻², PH, DM, ST, DS with BY and GY as suggested by the biplot's acute angles that these growth parameters contribute a lot to the yield of maize. BY had a longer vector that showed more responsiveness of this parameter to biofertilizers and NM treatment. Similar findings were reported by Msundi et al. (2021) on wheat genotypes growth and yield parameters, Where GY showed more responsiveness indicated by the longest vector. Kasu-Bandi et al. (2019) and soybean growth and yield parameters where biofertilizer application showed a more influence on yield parameters of the soybean crop.

5. Conclusion

It is concluded that the application of either 50 % FYM + 50 Urea or 25% FYM +75% Urea had increased yield-attributing traits of maize as compared to sole urea fertilization in all plots Among microbial inoculates seed treatment with either *Azotobacter* spp., *Azospirillum* spp. or *pseudomonas* spp. had substantially increased yield and yield-attributing traits of maize compared to rhizobium and control. Combination of all three treatments delayed silking, tasseling, physiological maturity and increased plant height, leaf area and LAI by decreasing or

reducing N losses. Correlation analysis showed a strong correlation in most of the growth and yield parameters indicating that yield attributes are not independent. It is pertinent to mention the popularization of using bio-fertilizers, such as Azotobactor, Pseudomonas, and Azospirillum as the low-cost technology for promoting plant growth and reducing huge rely on mineral fertilizers, especially Urea. A combination of chemical fertilizer, organic fertilizer and biofertilizer may also reduce the contribution to environmental pollution which is a requirement for sustainable agriculture. Application of either 25 % FYM and 75 % Urea or 50% FYM and 50% Urea is recommended for higher GY and improved yield-attributing parameters of maize along with seed treatment with Azotobactor spp., Azospirillum spp., or pseudomonas spp. under the agro-ecological conditions of Mardan.

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