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Phenology, Growth and Biomass Yield Response of Maize (*Zea mays* L.) to Integrated Use of Animal Manures and Phosphorus Application With and Without Phosphate Solubilizing Bacteria

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Abstract

Phosphorus (P) unavailability and lack of organic matter under calcareous soils in semiarid climates are the major reasons for low crop productivity. Field experiment was conducted to investigate impact of P levels (40, 80, 120 and 160 kg P ha^{-1}) and animal manures (poultry, cattle and sheep manures) with (+) and without (-) phosphate solubilizing bacteria (PSB) on phenological development, growth, and biomass yield of hybrid maize "CS-200". The experiment was conducted at the Agronomy Research Farm of The University of Agriculture Peshawar, during summer 2014. The experiment was laid out in randomized complete block design with split plot arrangement, using three replications. Among the animal manures (AM), application of poultry manure was found to delayed phenological development (days to tasseling, silking and physiological maturity), improved growth (taller plants, higher mean single leaf area and leaf area index), and produced the highest biomass yield (poultry>sheep>cattle manures). Application of P at the rate of 120 kg ha^{-1} was found almost comparable to the highest P rate (120 kg ha^{-1}) but was more beneficial in terms of better growth and higher biomass yield than other P levels (120 ≥ 160>80>40 kg P ha^{-1}). The plots with (+) and without (-) PSB showed no differences in phenological development of maize. The plots with PSB (+) produced significantly taller plants with higher mean single leaf area and leaf area index and produced the highest biomass yield. We concluded from these results that combined application of 120 kg ha^{-1} and poultry manure along with seed treatment with PSB improve growth and total biomass of hybrid maize in the study area.

Keywords: *Zea mays* L; Hybrid; Phenology; Growth; Biomass; Phosphate solubilizing bacteria; Animal manures; Phosphorus; Semiarid climate

Introduction

Maize (*Zea mays* L.) ranks third most important cereal crop after wheat and rice in Pakistan [1]. Maize average yield in Pakistan is 3983 kg ha^{-1}, which is much lower than the other corn growing countries of the world [2]. Maize is a C_4 mode of carbon fixation plant efficiently utilizes inputs because of its rapid growth and high biomass [3], therefore require balanced nutrients application. Phosphorus (P) is very important for improving crop growth and yield [4-9]. Unfortunately under semiarid condition plants are not able to get the required P [10-12] due to high soil pH [13] and low organic matter [14-16]. Under semiarid condition, the applied P-fertilizer is converted into immobile form due to calcareous soils under semiarid climate [17]. About 30 to 40% crop yield is reduced due to the unavailability of P [18], and lower P use efficiency [19]. Phosphate solubilizing bacteria (PSB) has the ability of solubilizing the fixed soil P by releasing of organic acid, which convert the insoluble P to soluble form by increasing the P availability to plants [20,21]. Organic manures [22-25] and P application [19] under semiarid condition is very important for increasing P availability and yield [26,27] as well as profitability [14,28].

Unavailability of P and lack of organic matter in the soils are some of the major limiting factors for crop production under semiarid condition. Phosphorus is an essential macronutrient often limiting the plant growth due to its low solubility and fixation in the soils. Improving soil fertility by releasing bound P by microbial inoculants is an important aspect for improving crop growth and increasing crop productivity [29]. Hypothesis was tested that combined application of phosphate solubilizing bacteria (PSB) and animal manures could increase P availability which in turns will improve maize growth and total biomass. This research work was therefore designed with the objectives (1) to find out suitable/organic animal manure (AM) source, (2) to find out proper P level, (3) to find out proper combination of AM × P, (4) to find out proper combination of AM × PSB, (5) to find out proper combination of P × PSB, and (6) to find out proper combination of AM × PSB × P for improving phenological development, growth and biomass of maize hybrid (CS200) in the study area having semiarid climate.

Materials and Method

Site description

Field trial was conducted to investigate effects of animal manures (poultry, cattle and sheep manures each applied @ 5 t ha^{-1}) and phosphorus levels (40, 80, 120 and 160 kg P ha^{-1}) on growth and yield of maize with and without seed inoculation with phosphate solubilizing bacteria (Pseudomonas) at the Agronomy Research Farm of The University of Agriculture Peshawar, during summer 2014. The experimental farm is located at 34.01° N latitude, 71.35° E longitude at an altitude of 350 m above sea level. The farm Soil is silt clay loam, low

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in organic matter (0.87%), extractable P (5.6 mg P kg^{-1}), exchangeable, alkaline (pH 8.2) and is calcareous in nature [27].

Experimentation

The experiment was laid out in randomized complete block design with split plot arrangement using three replications. Combinations of three animal manures (AM) and phosphate solubilizing bacteria (PSB) [inoculated seed with PSB (+) and seed not inoculated with PSB (-)] were applied to the main plots, whereas four phosphorus (P) levels were applied to subplots. All the animal manures sources (poultry manure, sheep manure and cattle manure) were applied at the rate of 5 t ha^{-1} two weeks before seed bed preparation. Poultry has 2.14% N, 1.73% P_2O_5, and 0.98% K_2O, sheep manure has 1.47% N, 0.29% P_2O_5, and 0.11% K_2O, and cattle manure has 1.13% N, 0.11% P_2O_5, and 0.07% K_2O. All P was applied at sowing time. The PSB obtained from NARC, Islamabad was mixed with the seed just before sowing time. A sub-plot size of 4 m × 3.5 m, having 5 rows, 4 m long and 70 cm apart was used. A uniform dose of 120 kg N ha^{-1} as urea in two equal splits, i.e., half at sowing, and half at knee height was applied. Maize hybrid “CS-200” was used as a test crop. All other agronomic practices were kept uniform and normal for all the treatments.

Data recording

Data on days to tasseling were calculated from the date of sowing to the date when 75% tasseling appear in each subplot. Days to silking, when 75% silking was emerged in each subplot, those dates were noted. Days to physiological maturity were recorded from the date of sowing till date when all the plants gets physiological maturity in each subplot. Data on plant height (cm) at physiological maturity was recorded with the help of meter rod by selecting ten plants randomly from each subplot and then average was worked out. Leaf area was calculated by measuring the lengths and widths of three middle leaves of five representative plants from each treatment at silking. The mean single leaf area was calculated by the following formula.

$$Leaf\ area = leaf\ length \times leaf\ width \times 0.75$$

Leaf area index was calculated as leaf area per plant divided by ground area $plant^{-1}$.

Data on biomass yield was recorded by harvesting four central rows in each subplot, the material was sun dried for several days and weighed, and then was converted into biomass yield kg ha^{-1} using following formulae:

$$Biomass\ yield\ \left(kg\ ha^{-1}\right) = \frac{Biomass\ yield\ (kg) \times 10{,}000\ m^2}{Number\ of\ rows \times row\ length \times row - row\ distance}$$

Statistical analysis

Data was statistically analyzed according to [30] for randomized complete block design with split plot arrangement and means among different treatments were compared using least significant differences (LSD) test ($p \le 0.05$).

Standard deviation was calculated for each parameter using the following formula:

$$s = \sqrt{\frac{\sum\left(x - \bar{x}\right)^2}{N - 1}}$$

Where s=standard deviation

X=each value of replicated data

$\bar{x}$ =mean of the replicated data

N=the number of values [24]

Results

Days to tasseling

Phosphorus (P) and animal manures source (AM) as well as the interaction between AM × PSB was found significant (Table 1). Interactions of AM × P, PSB × P and AM × PSB × P was found non-significant. Mean values of phosphorus shows that days to tasseling was enhanced with the increase the phosphorus level. Delayed tasseling was recorded with the application of 40 kg P ha^{-1} (63 days), followed by the 80 kg P ha^{-1} (61 days) and lower days to tasseling was recorded with 160 kg P ha^{-1} (60 days). Among the animal manures poultry manure delayed days to tasseling (62 days) was observed which is at par with sheep manure (61 days) and lower days to tasseling was recorded with cattle manure. In case of interaction between AM × PSB maximum days to tasseling was recorded with the poultry manure with PSB and lower (60 days) was recorded with cattle manure with PSB and without PSB (Figure 1).

Days to silking

Animal manures, P levels and interactions PSB × P and AM × PSB × P had significantly affected days to silking, while PSB and interactions AM × PSB and AM × P was found non-significant (Table 2). Delayed silking (68 days) were observed with 40 kg P ha^{-1}, followed by 80 kg P ha^{-1} (67 days) which was statistical at par with 120 kg P ha^{-1} (67 days) and early silking was recorded with 160 kg P ha^{-1} (66 days). In case of AM, poultry manure delayed silking (68 days), followed by sheep

Phosphorus (kg ha^{-1})	**Days to tasseling**	**Days to silking**	**Days to physiological**	**Plant height (cm)**	
40	63 a	68 a	104 a		174 d
80	61 b	67 b	103 b		177 c
120	61 c	67 b	102 c		185 a
160	60 d	66 c	102 d		181 b
LSD	**0.25**	**0.24**	**0.33**		**1.33**
Animal manure (5 t ha^{-1})					
Sheep	61 a	67 b	103 b		180 a
Poultry	62 a	68 a	104 a		181 a
Cattle	60 b	66 c	102 c		176 b
LSD	**0.52**	**0.4**	**0.31**		**1.72**
PSB					
without PSB	61	67	103		177 b
with PSB	61	67	103		181 a
LSD	**ns**	**ns**	**ns**		*
Interactions					
AM × PSB	*(Figure 1)	ns	*(Figure 4)	ns	
AM × P	ns	ns	ns		ns
PSB × P	ns	*(Figure 2)	ns		ns
AM × PSB × P	ns	*(Figure 3)	ns		*(Figure 5)
Standard Deviation	± 1.15	± 1.11	± 1.28		± 5.14

Means followed by different letter (s) with in each category are significantly different using LSD test ($p \le 0.05$)
ns=non-significant at 5% level of probability * =significant at 5% level of probability

Table 1: Days to tasseling, days to silking, days to physiological and Plant height (cm) of maize hybrid as affected by affected by phosphorus, animal manures and phosphatesolubilizing bacteria.

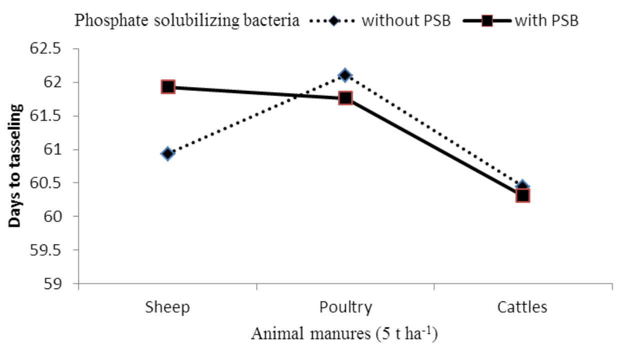

Figure 1: Response of days to tasseling of maize hybrid to phosphate solubilizing bacteria and phosphorus interaction (PSB × P).

Phosphorus (kg ha^{-1})	Mean single leaf area (cm^2)	Leaf area index	Biomass (kg ha^{-1})
40	412 d	3.75 d	11143 d
80	424 c	4.01 c	11799 c
120	435 a	4.20 a	12753 a
160	428 b	4.19 b	12437 b
LSD	**2.44**	**0.02**	**307.47**
Animal manure (5 t ha^{-1})			
Sheep	422 b	4.02 b	12010 b
Poultry	431 a	4.10 a	12368 a
Cattle	421 b	4.00 b	11722 b
LSD	**4.84**	**0.045**	**357.77**
PSB			
	422 b	4.01	11819
with PSB	427 a	4.06	12247
LSD	*	ns	ns
Interactions			
AM × PSB	ns	ns	
	ns	ns	ns
PSB × P	ns	ns	ns
AM × PSB × P	*(Figure 6)	*(Figure 7)	ns
Standard Deviation	± 10.60	± 0.20	± 768

Means followed by different letter (s) with in each category are significantly different using LSD test (p ≤ 0.05)
ns=non-significant at 5% level of probability * =significant at 5% level of probability

Table 2: Mean single leaf area (cm^2), Leaf area index and biomass (kg ha^{-1}) of maize hybrid as affected by affected by phosphorus, animal manures and phosphatesolubilizing bacteria.

manure (67 days) and early silking was recorded with cattle manure. Interaction between PSB × P indicated that delayed silking (68 days) was recorded with 40 kg P ha^{-1} with and without PSB and early silking (66 days) were recorded with 160 kg P ha^{-1} without PSB (Figure 2). Interaction among AM × PSB × P showed that delayed silking (66 days) was recorded with cattle manures and with the increase in P levels with and without PSB (Figure 3).

Days to physiological maturity

Physiological maturity was significantly affected by AM, P levels and interaction between AM × PSB. All other interactions, AM × P, PSB × P and AM × PSB × P had no significant effect on days to physiological maturity. Mean values for P levels indicated that delayed (104 days) day to physiological maturity was observed with the application of 40 kg P ha^{-1}, followed by 80 kg P ha^{-1} (103 days). Early physiological maturity (102 days) was recorded in plots where P was applied at the highest rate of 160 kg ha^{-1}. Delayed days to physiological maturity (104 days) were observed with poultry manure, followed by sheep manure (103 days) and early physiological maturity (102 days) was observed with cattle manure. Interaction between AM × PSB indicated that delayed physiological maturity (104 days) was recorded with poultry manure with PSB and early days to physiological maturity (101 days) was observed with cattle manure and with PSB (Figure 4).

Plant height

Plant height was significantly affected by AM, P levels and

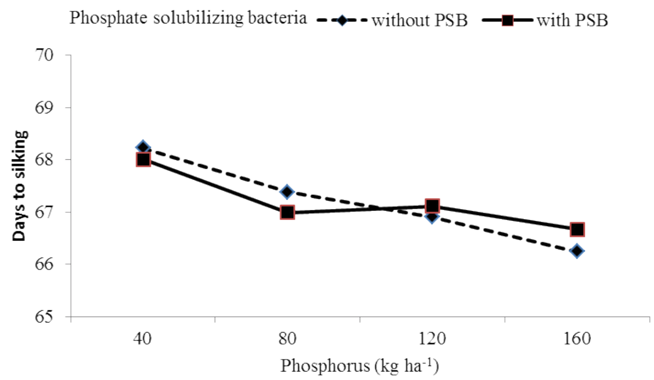

Figure 2: Response of days to silking of maize hybrid to interaction of Phosphate solubilizing bacteria and phosphorus levels (P × PSB).

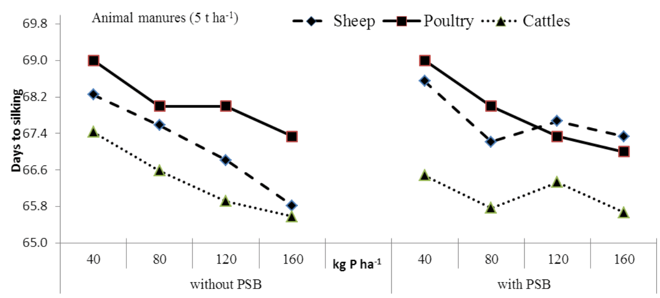

Figure 3: Response of days to silking of maize hybrid to phosphate solubilizing bacteria, phosphorus and animal manure (PSB × P × AM).

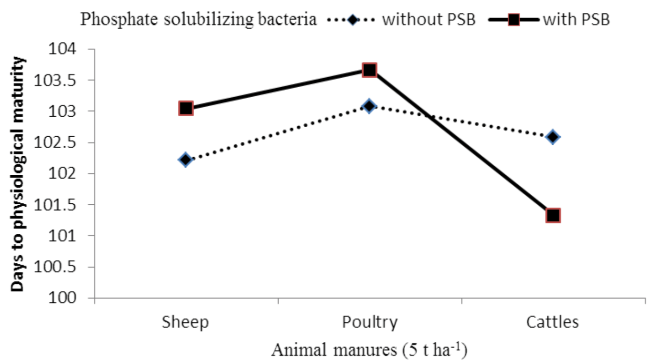

Figure 4: Response of days to physiological maturity of maize hybrid to animal manures sources and phosphate solubilizing bacteria (AM × PSB).

interaction among AM × PSB × P, while PSB and all remaining interactions (AM × P, AM × PSB and PSB × P) had not significant effect on plant height. Taller plants (185 cm) were recorded with 120 kg P ha^{-1}, followed by 160 kg P ha^{-1} (181 cm) whereas short stature plants (174 cm) were produced when 40 kg P ha^{1} was applied. In case of AM taller plants (181 cm) were recorded with poultry manure being at par with sheep manure (180 cm), and shorter plants were recorded with cattle manure (176 cm). In case of interaction AM × PSB × P, taller plants (189 cm) were recorded with application of 120 kg P ha^{-1} and poultry manure along with PSB (Figure 5), and dwarf plants (171 cm) were recorded with 40 kg P ha^{-1} and cattle manure along with PSB.

Mean single leaf area

Mean single leaf area was significantly affected by AM, PSB and P levels and interaction among AM × PSB × P. However, the interactions AM × P, PSB × P and AM × PSB was found non-significant. Phosphorus applied at the rate of 120 kg ha^{-1} produced larger mean single leaf area (435 cm^2), followed by 160 kg P ha^{-1} (428 cm^2) while reduced mean single leaf area (412 cm^2) was obtained from plots applied 40 kg P ha^{-1}. Application of PSB produced significantly larger mean single leaf area (427 cm^2) as compared to without PSB, (422 cm^2) mean single leaf area. Among the animal manures poultry manure resulted in higher mean single leaf area (431 cm^2), followed by cattle manure (421 cm^2) however it was not significantly different from sheep manure (422 cm^2). Interaction among AM × PSB × P, indicated that maximum mean single leaf area (443 cm^2) was obtained with 120 kg P ha^{-1} along with PSB and poultry manure, and lower mean single leaf area (401 cm^2) was recorded with 40 kg P ha^{-1} along with sheep manure and without PSB (Figure 6).

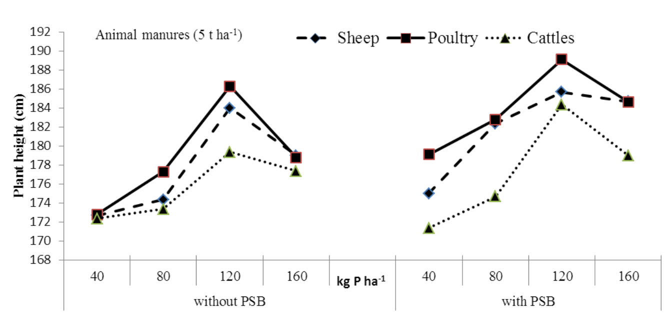

Figure 5: Response of plant height of maize hybrid to animal manure, phosphate solubilizing bacteria and phosphorus (AM × PSB × P).

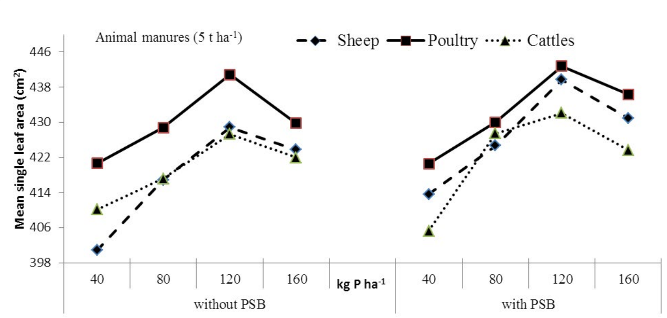

Figure 6: Response mean single leaf area of maize hybrid to animal manure, phosphate solubilizing bacteria and phosphorus levels (AM × PSB × P).

Leaf area index

Leaf area index (LAI) was significantly affected by animal manure and phosphorus and interaction among AM × PSB × P, while PSB and all remaining interactions (AM × P, AM × PSB and PSB × P) had no significant effect on LAI. Phosphorus application at the rate of 120 kg ha^{-1}gaves higher LAI (4.20); followed by 160 kg P ha^{-1} (4.19) and lower LAI (3.75) was recorded with 40 kg P ha^{-1}. Maximum LAI (4.10) was recorded with poultry manure and minimum LAI (4.00) was observed with cattle manure being statistical at par with sheep manure (4.02). In case of interaction among AM × PSB × P, higher LAI (4.28) was recorded with application of 120 and 160 kg P ha^{-1},poultry manure with PSB, while lower LAI (3.65) was recorded with sheep manure+40 kg P ha^{-1} without PSB (Figure 7).

Biomass yield

Data in association with regarding biomass yield (kg ha^{-1}) revealed that biomass yield was significantly affected by AM, PSB (Table 2). However, Interactions AM × PSB, AM × P, PSB × P and AM × PSB × P were found non-significant. Application of phosphorus at the of 120 kg ha^{-1} results in higher biomass yield (12753 kg ha^{-1}), followed by 160 kg P ha^{-1} (12437 kg ha^{-1}), and lower with (11143 kg ha^{-1}) was recorded with 40 kg P ha^{-1}. Among the AM, poultry manure produced higher biomass yield (12368 kg ha^{-1}), followed by sheep manure (12010 kg ha^{-1}) however it was statistical at par with cattle manure (11722 kg ha^{-1}). Whereas higher biomass yield (12247 kg ha^{-1}) was obtained from plot receiving PSB and lower biomass yield (11819 kg ha^{-1}) was recorded with plots had without PSB.

Discussion

Maize phenology (tasseling, silking and physiological maturity) was delayed with lower P levels (40 and 80 kg ha^{-1}) levels. Phenological development enhanced (early) with the application of higher P levels (120 and 160 kg ha^{-1}. The reason for early phenology with application of higher P levels might be due to better root development and thus facilitated the plants obtained more P and other nutrient from poultry manure for rapid plant growth and development. These findings are in line with those of [31] who reported that early in phenological parameters with high level of P. Phenological development in our study was also delayed with application of poultry manure as compared with other two manures (sheep and cattle manures) [32] reported delayed in phenology with application of poultry manure. However, in this study plots with (+) and without (-) PSB had showed no significant differences in the phenological development.

Growth parameters (plant height, mean single leaf area and LAI) were significantly improved with application of two higher P levels.

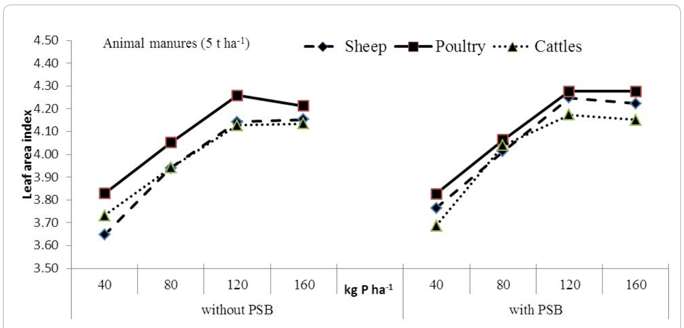

Figure 7: Response of Leaf is index of maize hybrid to animal manure, phosphate solubilizing bacteria and phosphorus.

These results are in accordance with [6] who reported that higher level of P helped the plant to attain maximum height [33] reported that application of P significantly increased the leaf area in maize. According to [34], LAI improved with application of P doses over control. The growth parameters were also significantly improved with application of poultry manure as compared to sheep and cattle manures [35] suggested that poultry manure enhanced the LAI in maize. According to [23], application rate of poultry manure enhanced lead area, total chlorophyll content, carbon content, water holding capacity, and decrease bulk density of soil. The growth parameters were also improved under plots with (+) PSB than without (-) PSB. According to [29], that application of phosphate solubilizing microorganism improving soil fertility by releasing bound P and therefore improve crop growth and increase crop productivity.

The biomass yield was significantly increased with the application of 120 or kg P ha^{-1} and poultry manure. Reduction in biomass yield was observed with application of 40 kg P ha^{-1} and cattle manure. The increase in biomass yield reflects the better growth and development of the plants due to balanced and more availability of nutrients which was associated with increased root growth due to which the plants explore more soil nutrients and moisture throughout the growing period. The increase in biomass yield with 120 or kg P ha^{-1} and poultry manure in our experiment was attributed to the improvement in growth parameters and vice versa. These results are in line agreement with [36] who stated that application of P-fertilizer significantly increased the biomass yield of maize. According to [24], application of poultry manure increased biomass yield in maize. In our study application of PSB had no significant effects on the biomass yield of maize. In contrast to our results, [37,38] reported that inoculation of maize (*Zea mays*) with PSB under greenhouse and field conditions increased biomass yield of maize. According to [36], biofertilizer (Pseudomonas) significantly increased the biomass yield of maize over control. Improvement in phenological development and growth parameters increased maize biomass [26,27]). The increase in maize biomass is responsible for higher grain yield per unit area [26,27]) and higher net returns under semiarid climate [14,28].

Conclusion

We concluded from this study that combined application of 120 kg P ha^{-1} along with poultry manure and inoculation of seed with phosphate solubilizing bacteria (PSB) improve growth and biomass yield of maize under semiarid climates.

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