Maize Plant Growth, Soil Enzyme Activities and Bacterial Population as Affected by Soil Organic Amendments

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Abstract—*A pot experiment was carried out to determine the effect of corn cob, urea and Azotobacterchorococcum,alone or in combination on maize plant growth, soil enzymes and bacterial population. Corn cob alone or in combined treatment of corn cob and urea has indicated a significant increase in dehydrogenase activity in pot soil. Similarly, increase (25 to 72%) in nitrate reductase activity was also observed in Azotobacter alone and in combined amendment of Azotobacter and urea. However, increase in urease activity was found in all the amendments. Different soil amendments have stimulatory effect on bacterial population. Highest bacterial counts (210. 0 CFUs x 10^4 g^{-1}d. wt. soil) were found in Azotobacter treated pots followed by combined amendment of Azotobacter and urea. Plant height and yield in control pots were found 29 cm and 260 g/plant, respectively. Whereas, among all the amendments, maximum plant height (107cm) and yield (477g/plant) were observed in combined treatment of corn cob and urea, followed by Azotobacter and urea combined treatment, i. e. 77. 5 cm plant height with 455g/plant yield. Similarly, in all the single treatments, lowest plant height (44. 5 cm) and greater grain yield (380 g/plant) was observed in corn cob treated pots (44. 5 cm). However, no significant differences between Azotobacter and urea alone treatments were observed. Conclusively, the soil amendment with corn cob in combination with urea or Azotobacter, improves enzyme activity and microbial population in soil. Thus, corn cob alone or in combination with urea or Azotobacter may be applied in maize soil for better soil health and fertility*

1. INTRODUCTION

India is the fifth largest producer of maize, and its acreage and production are increasing continuously. Maize is a monocotyledon plant with shallow root zone system, hence needs adequate soil moisture and fertilizers. Along with maize production, a large amount of corn cob is produced as a byproduct, which is either burned or disposed uselessly after grain harvest [1]. Monaco et al. , [2] concluded that the application of maize straw in soilincreased organic matter and soil nutrientlevel. Availability of soil organic matter and various soil amendments directly affect the population and activities of micro-flora and fauna and ultimately soil fertility [3]. Therefore, present comparative study was aimed to determine the effect of different soil amendments on soil enzyme activities, bacterial population and growth of maize plants. Corn cob powder was appliedalone and in separate combinations with urea and*Azotobacter* in soil (microcosm).

2. MATERIALS AND METHODS

2. 1 Microcosm design

Pot experiments were carried outto determine the impact of urea, corn cob and *A. chorococcum*amendments on growth of maize (*Zea mays* L.) plants, soil enzymes such as, nitrate reductase, urease and dehydrogenase activities and bacterial population. Cultivated field soil samples were collected at the depth of 10-12 cm (from Karia village, Chamba, Himachal Pradesh, India). Two kg soil was used to fill each pot of 3 kg capacity and three replicates were made for each control and amendments (treatments). T_1R_1,T_1R_2, T_1R_3 for control, T_2R_1, T_2R_2, T_2R_3 for *Azotobacter,* T_3R_1, T_3R_2, $T_3R_3$for corn cob, T_4R_1, T_4R_2, T_4R_3 urea, T_5R_1, T_5R_2, T_5R_3 corn cob and urea and T_6R_1, T_6R_2, and T_6R_3 for*Azotobacter* and urea. Seeds of Baby Corn variety G-5406 were sown in each pot at 5 cm depth. The seeds were surface sterilized and rinsed with sterile water. Only single plant was maintained in each pot. Amendmentswere added as per handbook of agriculture (ICAR, India).

2. 2 Microcosm treatment

During entire crop period only three times i. e. 1, 30 and 60 days after the sowing, the urea treatmentsweregiven at a rate 0. 2 g urea/pot using a urea solution (2g/10ml). Similarly,for corn cob treatments, sixty grams of this powder was mixed with the soil before one week of the sowing. It was estimated that the one gram corn cob contains 0. 41% nitrogen and 28% carbon. Likewise the treatment of *A. chorococcum* was also given to pot soil. Pure and certified bacterial culture of *A. chorococcum* w5 strain was obtained from IARI, New Delhi, and aseptically multiplied in LauriaBertini (LB) medium at 32°C. Bacterial cells were harvested by centrifugation at 6000 rpm for 20 minutes and washed with sterile distilled water. A cell suspension of *A. chorococcum* was made in 12 ml sterile water (10^8cells ml^{-1}) and 3ml of this suspension was applied in all six pots during seed sowing and similar application of *A. chorococcum* was repeated 30 and 60 days after sowing.

2. 3 Dehydrogenase activity

Determination of dehydrogenase activity in soil was carried out by standard analytical method [10]. Concentration of TPF in μg^{-1}d. wt. soil was determined by calibration curve [12].

2. 4 Nitrate reductase analysis

Nitrate reductase activity was determined by using standard colorimetric technique [7]. The concentration of produced NO_2^-- N μgg^{-1}dm was determined by measuring the intensity of colour through a Labomed spectrophotometer (UVD-3500) at 520 nm.

2. 5 Urease activity

Urease activity was analyzed according to Kandeler [8] unbufferedmethod. Optical density was observed using spectrophotometer against the blank at 690nm. Enzyme activity was calculated using a standard curve.

2. 6 Soil microbial analysis

Thorton agar media was prepared for bacteria [9] and Jensen for*Azotobacter*. "Dilution plating" was used for bacterial count. Soil dilutions were prepared by adding 10g soil in 100ml sterilized double distilled water. Serial dilutions of $10^{-4}$for bacteria was made to determine the bacterial populationin g^{-1}d. wt. soil.

Statistical Analysis

Data obtained in triplicate from treated and control soilswere used as raw data for randomized block design and analysis ofvariance. Correlation analysis was used to explore relationshipsamong variables. Significance was defined as $p \leq 0.05$, using SASstatistical software (Statistical AnalysisSoftware Inc. , 1990).

3. RESULT AND DISCUSSION

The soil physico-chemical analysis showed that the soil was sandy loam with 7. 56±0. 8 pH; 2. 11±0. 17% organic matter, 92±0. 40% dry matter and 6. 75±0. 40% water content.

3. 1 Dehydrogenase enzyme activity

Dehydrogenase activity gives correlative information of microbial populations [10]. Results of present experiment pointed out a significant ($p \leq 0.05$) decrease of dehydrogenase activity in pots where *Azotobacter* was amended alonei. e. 13. 5% reduction in enzyme activity was observed in 30th day samples. However, it recovered within 60 days and in 90thday samples, 67. 4% increase in enzyme activity was observed and this trend was continued *i. e.* 38. 6% increase in dehydrogenase enzyme activity was observed on 60th day(fig. 1).

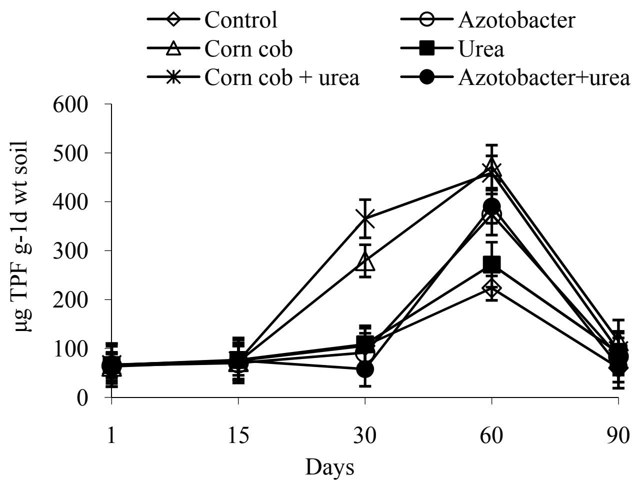

Fig. 1 Average of dehydrogenase activity.

Corn cob treatment also indicates a significant ($p \leq 0.05$) stimulatory effect on dehydrogenase activity. No significant changes were observed up to 15 days of the amendment. But in 30th day samples, 164. 7% increase in dehydrogenase activity was observed. Corn cob treatment indicates significantstimulation ofdehydrogenase activity up to 90 days *viz.* up to harvesting. In 90th day samples 90. 7% increase in enzyme activity was observed as compared to the control (fig. 1). In urea alone treated pots, no significant changes in dehydrogenase activity were found till 30 days. Whereas, in 60thday samples, 21. 5% increase in enzyme activity was observed, this was comparatively lower than the other treatments. This trend continued till harvesting and 50. 2% increase in enzyme activity was observedin 90thday samples (fig. 1).

A combine treatment of corn cob and urea has indicated a highest dehydrogenase enzyme activity among all the treatments after 30 days, which was 246. 4% more than the control and this trend was continued till harvestingand found to be 57. 6% more than the control (fig. 1). On the other hand combine treatment of *Azotobacter* and urea has also indicated a significant ($p \leq 0.05$) decrease in dehydrogenase activity after 15 days, which was again found to be decreased in 30th day samples i. e. 47. 7% less enzyme activity, was observed in 30thday samples. Comparatively, it was also lower than the *Azotobacter* alonetreatment. Whereas, enzyme activity recovered between 30 and 60 days and in 60thday samplesa significant increase (74. 6%) in enzyme activity was observed. No further significantchanges were found during harvesting; while some increased enzyme activity was observed in all other treatments including *Azotobacter* alone treatment (fig. 1). Similar observations have been reported by Peinadoet al. ,

[11]. Among the three samplings, highest dehydrogenase activity was found at the 30 to 60 days. This might be associated with optimal plant growth and relatively increase in root exudates, which might help the better bacterial growth in the rhizosphere.

3. 2 Nitrate reductase enzyme activity

Nitrate reductase activity in soil indicates anaerobic nitrate reduction. In the present study, no significant effect on nitrate reductase enzyme activity was found up to 15 days, except *Azotobacter* alone treatment. In *Azotobacter* alone treated pots 25. 7% more enzyme activity was observed in 15[th]day samples and again 71. 6% increase in nitrate reductase activity was foundin 30[th]day samples. Whereas, after 60 days a significant ($p \leq 0.05$) decrease in nitrate reductase activity was observed, which was43. 7% lower than the control pots*viz.* 0. 64 and 0. 36 µg NO_2-N g^{-1}d. wt. soil was found in control and *Azotobacter* treated pots, respectively.

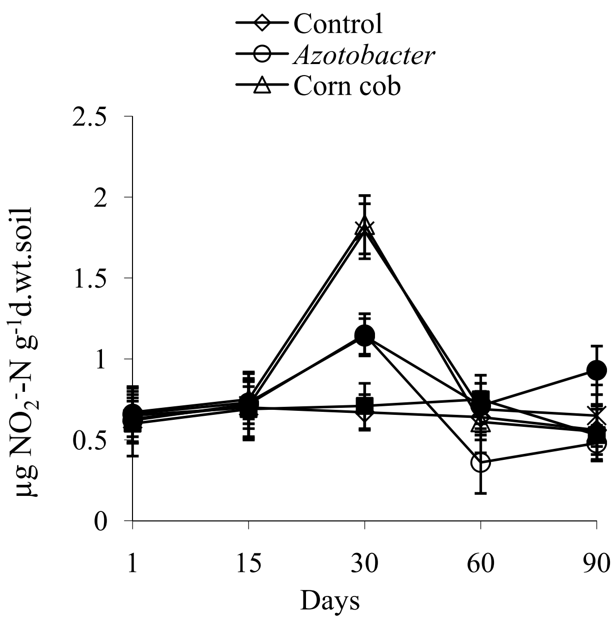

Fig. 2: Average of nitrate reductase enzyme

Corn cob treatment has also indicated a significant ($p \leq 0.05$) and highest 173. 1% increased nitrate reductase enzyme activity in 30[th]day samples. But in 60[th] day samples enzyme activities deceased significantly, which remained almost same till harvesting(fig. 2). Nitrate reductase activity was not significantlyaffected in urea treatment alone i. e. only 17. 2% increase in nitrate reductase activity and further there wasno significant change. But when pots were treated with corn cob and urea in combination, a second highest increase (167. 2%) inenzyme activity was observed (fig. 2). Combine treatment of *Azotobacter* and urea has also indicated a continuously increased nitrate reductase activity from 30 days of treatment to 90[th]day i. e. up to harvesting. In 30[th]-day samples 70. 1% increase in enzyme activity was observed, which wassignificantly more than the control,throughout the experiment*viz.* in 90[th]day samples 66. 1% enhanced enzyme activity was found (fig. 2). Increased soil nitrate reductase activity was also reported in sugarcane field after application of *Azotobacter* as bio-fertilizer [22]. Similarly, corn cob treatment has indicated a significantly increased nitrate reductase enzyme activity.

3. 3 Urease enzyme activity

Urease enzyme activity increased significantly ($p \leq 0.05$) in those cases where urea treatment was given. In *Azotobacter* amendment alone, no significant changes were observed up to 30 days. But at 60[th]day samples, 45. 6% increase in urease activity was observed. Further changes in enzyme activities were not significant. Similarly, amendment of corn cob alonedid not indicateany significant change in urease activity up to 30[th]day. Whereas, 81. 7% increase in urease activity was observed in 90[th]day samples (fig. 3). In urea treated pots, significant increases ($p \leq 0.05$) in urease activities were found from the day of application upto the 60 days. Similar trend was observed in all the amendments where urea was used for the treatment. Alone urea treatment indicates 17. 6% increase in urease activity in first day samples and this trend was continued up to the 90[th]day. A highest increase in urease activity in urea alone treated pots was found on 60[th]day samples. Whereas in 90 days samples, 45. 2% decreased in urease activity was observed.

Combine treatment of corn cob and urea has indicated more significant increase($p \leq 0.05$) in urease than urea alone treatment. In first day sample, 34. 8% increase in urease activity was observed. Similar to the urea alone treatment, the trend of increase in enzyme activity was found up to 60 days. In 60[th]-day samples 93. 9% increase in urease activity was observed (fig. 3).

Increase in urease activity was found from 1[st] to 60 days of amendment. In first day samples, 28. 9% increase in urease activity was observed, whichcontinued tillthe 60 days. During this period highest increase in urease activity was found in 30[th]day samples i. e. 58. 5% more enzyme activity was observed. After this increase,enzyme activity was reduced and in 60[th]day sample only 10. 7% increase in enzyme activity was found. Whereas, in 90[th]day samples 79. 4% decreased urease activity was observed.

Highest urease activities among the different treatments i. e. corn cob+urea, *Azotobacter* +urea and urea were found. Kanchikerimath and Singh [18]have pointed out higher urease activity due to addition of manure in maize-wheat-cowpea cropping system. Similarly, high urease activity was also reported by Roscoe et al. , [19]. They achieved similar results in nontillage soil under maize crop. As in our case of corn cob amendment, higher enzymatic activities have been observed, which might be due to improved organic matter of the soil.

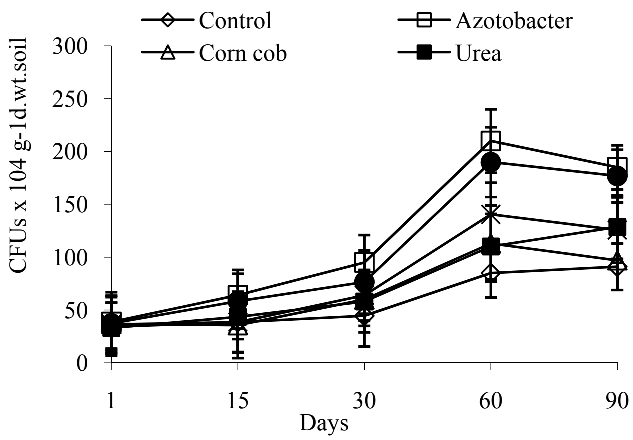

Fig. 3: Average of urease enzyme activity

3. 4 Bacterial population

In the present experiment, a significant increase($p \leq 0.05$)in bacterial population was found in *Azotobacter* treated pots from 15 to 90 days of sowing. After 15 days, 68. 4% more bacterial CFUs were observed as compared to control. A highest increase (147. 0%) in bacterial population was found in 60thday samples *viz.* 85. 0 CFUs x $10^4 g^{-1}$d. wt. soil in control and 210. 0 CFUs x $10^4 g^{-1}$d. wt. soil was observed in *Azotobacter* amended pots. But in 90thday samples, some reduction in bacterial population was found i. e. only 88. 8% more bacterial population was found as compared to control (fig. 4).

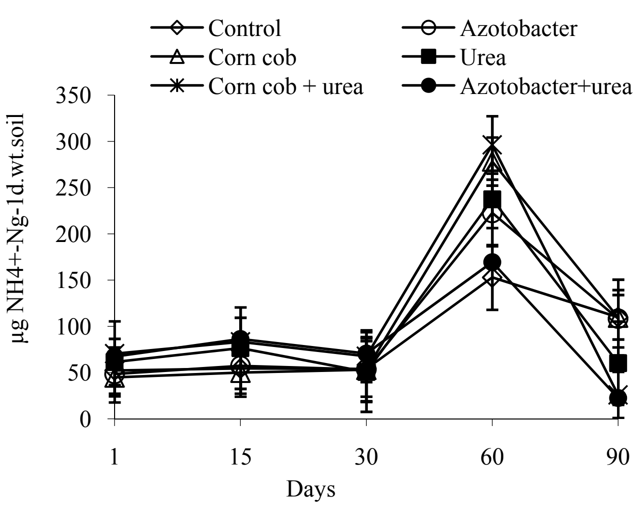

Fig. 4: Total bacterial population after different amendments in soil

As compared to control some significant increase in bacterial population was found in all the amendments. Corn cob alone treatment indicates the significant increase($p \leq 0.05$) in bacterial population from 15 to 60 days after sowing. In 15thday samples 44. 8% increase in bacterial population was observed and which remained more than the control till 60thday. Whereas, in urea amended pots increase in bacterial population was observed from 30 to 60thday (fig. 4). Similarly, combine treatment of corn cob and urea also indicated more stimulatory effect on bacterial population. Increase in bacterial population was found from 15 to 90th day. Almost similar results were observed in *Azotobacter* and urea amended pots. In these pots also an increase in bacterial population was found from 15 to 90th day (fig. 4). This agrees with the findings of Albiachet al. , [20] according to them long-term application of organic fertilizers positively influences the soil available nutrients and results in increased microbial proliferation.

3. 6 Plant growth

Plant growth and health arevaluable parameters to determine the soil fertility and health. Plant height wassignificantly more in all the treated pots as compared to the control pots. In control pots average plant height was29 cm and it wassignificantly ($p \leq 0.05$) lower than all the treatments. Whereas, among all the amendments, maximumplant height (107cm) were observed in combine treatment ofcorn cob and urea, followed by *Azotobacter* and urea combine treatmenti. e. 77. 5cmplant height. Similarly, in all the single treatments, lowest plant height (44. 5 cm) was observed in corn cob treated pots (44. 5cm). No significant differencesbetween *Azotobacter* and urea single treatments were observed. Khorsandi and Nourbakhsh [1] found increased soil nitrogen after application of corn residue and reported significantly increased plant height and yield as compared to untreated plots.

4. CONCLUSION

In this study, we report the use of corn cobs in combination with urea and *Azotobacterchorococcum*as effective manure for enhancing soil fertility and maize plant growth. In pot experiments, we have used corn cobs, Urea or *A. chorococcum*either individually or in various combinations to assess their effect on soil biochemical properties and plant height. Biochemical properties were measured in terms of dehydrogenase, urease and nitrate reductase activity and soil bacterial population. With growing concerns related to the excessive use of chemical fertilizers in crop cultivation and in the era where people are looking for ecofriendly alternatives, this is an interesting and new line of study. We have observed that soil amendment with corn cob in combination with urea or *Azotobacter* resulted in the buildup of high bacterial population and enzyme activity in soil. The measurement of soil enzymes can be used as indicative of the biological activities and natural biochemical processes in soil. Since corn cob is the byproduct of maize, hence it may contain more useful plant available nutrients for the maize plants. Therefore, based on the present findings, corn cob alone or incombination with urea or *Azotobacter* may be applied for maize soil for better soil health and fertility. However more molecular studies are required to evaluate the effect of different amendments on actual bacterial community.

5. ACKNOWLEDGEMENT

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