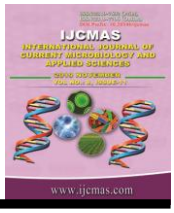
Int.J.Curr.Microbiol.App.Sci (2019) *8*(11): 2381-2389

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 11 (2019)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2019.811.276

Evaluation of Microbial and Enzyme Activity of Rhizospheric Soils under Integrated Nutrient Management of Apricot

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Keywords

Microbial and enzyme activity, Rhizospheric soils, Apricot

Article Info

Accepted:
22 October 2019
Available Online:
10 November 2019

A B S T R A C T

The present study was conducted at Horticultural Research and Training Station and Krishi Vigyan Kendra (HRTS & KVK), Kandaghat, Solan, Himachal Pradesh, India. This study was done to evaluate the change in microbial and enzyme activity of rhizospheric soil by application of biofertilizers, organic and chemical fertilizers during the year 2017-18. The results showed that maximum azotobater, PSB and actinomycetes population was found significantly higher with 80% RDF (25% CN + 75% Urea) + 20% N (VC) + (*Azotobacter* + PSB @ 25g/tree) during both the years of study. However, minimum was found in 100% RDF (100% N through CN). Soil enzyme activities were also found significantly higher with biofertilizes in combination with organic and chemical fertilizers. Soil enzyme activities (Urease, dehydroginase and alkaline phosphotase) were observed maximum when chemical fertilizers are applied in combination with vermicompost and biofertilizers as compared to control (chemical fertilizers without vermicompost and biofertilizers). It can be concluded that application of chemical fertilizers along with organic and biofertilizers can enhance the biological properties soil.

Introduction

Nutrition plays an important role in improving productivity and quality of apricot fruits. The key to mineral nutrition is the judicious application of manures and fertilizers to the plants.

Manures and fertilizers are regularly applied in the field to meet the requirement of nutrient elements by plants. Microbial communities are important for the functioning of the ecosystem both in relations to direct interactions with plants and with regard to nutrient and organic matter cycling (Debnath *et al.*, 2015).

Soil enzymes play key biochemical function in the overall process of organic matter decomposition in the soil system.

They are important in catalyzing several vital reactions necessary for the life process of microorganisms in soils and the stabilization of soil structure, the decomposition of organic

wastes, organic matter formation and nutrient cycling (Das and Varma, 2011). Soil enzymatic activities have been proposed as appropriate indicators because of their intimate relationship to soil biology and rapid response to changes in nutrient management.

Dehydrogenase (DH) exists as an integral part of intact cells, involved in oxidative phosphorylation, and reflects in the total oxidative potential of the soil microbial community (Kuma *et al.,* 2017).

Inorganic fertilizers are the chemical substances produced synthetically from inorganic products that are used to enrich soil with nutrients mostly nitrogen, phosphorus and potassium to help plants in growth and metabolic processes.

From the last two decades, inadequate and imbalanced use of inorganic fertilizers accompanied with restricted use of organic manures and bio-fertilizers has contributed considerably in raising agricultural productivity and reducing hunger worldwide.

On the other hand, they made the soils not only deficient in certain nutrients and also deteriorated the soil health (Lui and Tain, 2017).

Effect of Vermicompost promotes plant growth is well reported but mostly it is used as a main source of nitrogen and phosphorus which are nutrients as a part of some key plant structural components and act as a catalyst in the change of numerous biochemical reactions in plants and has very high porosity, aeration, drainage and water holding capacity.

Vermicompost application is one of the useful methods to renew the depleted soil fertility and augment the available pool of nutrients and conserve more water, maintain soil quality and conserve additional biological resources (Sinha, 2009). Biofertilizers contain primarily potent strain of microorganism in sufficient number. These microorganisms play a beneficial role in the soil fertility of rhizosphere and growth of plants. They are safe to handle with field application.

Their application rebalances the ratio between plant nutrients in the soil for improving crop yield and decrease the cost of some agricultural practices.

In rhizosphere soil, a large number of plant growth promoting rhizobacteria bacteria, particularly phosphate solubilizing bacteria, which have an ability to promote plant growth and increase plant production. These bacteria potentially convert insoluble P into an available form by release of organic acid like maleic, lactic, acetic, citric and succinic acid to dissolve insoluble P and other mechanism is the secretion of enzymes by PSB that are able to degrade insoluble phosphorus (Wang *et al.,* 2017).

The present study was done with an objective to find out the effect of integrated nutrient management on microbial population and enzyme activities of soil.

Materials and Methods

A field experiment was conducted during 2017-18. The location of the experiment was in the orchard of apricot at Horticultural Research and Training Station and Krishi Vigyan Kendra (HR & TS and KVK), Kandaghat, Solan (H.P.).

Where the field studies were conducted is located at an altitude of 1425 meters above the mean sea level, having latitude of 30.59^{o}N and longitude of 77.07^{o}E.

The experiment was laid in Randomized Blocked Design using "New Castle" variety. A total of eleven different fertilizer combinations were made as follows:

Treatments code	Fertilizers
T_1 **(Control)**	100% RDF (100% N through CN)
T_2	100% RDF (100% N through Urea) + (*Azotobacter* + PSB @ 25g/tree)
T_3	100% RDF (25% CN + 75% Urea) + (*Azotobacter* + PSB @ 25g/tree)
T_4	100% RDF (50% CN + 50% Urea) + (*Azotobacter* +PSB @ 25g/tree)
T_5	100% RDF (75% CN + 25% Urea) + (*Azotobacter* +PSB @ 25g/tree)
T_6	90% RDF (25% CN + 75% Urea) + 10% N (VC) + (*Azotobacter* + PSB @ 25g/tree)
T_7	90% RDF (50% CN + 50% Urea) + 10% N (VC) + (*Azotobacter* + PSB @ 25g/tree)
T_8	90% RDF (75% CN + 25% Urea) + 10% N (VC) + (*Azotobacter* + PSB @ 25g/tree)
T_9	80% RDF (25% CN + 75% Urea) + 20% N (VC) + (*Azotobacter* + PSB @ 25g/tree)
T_{10}	80% RDF (50% CN + 50% Urea) + 20% N (VC) + (*Azotobacter* +PSB @ 25g/tree)
T_{11}	80% RDF (75% CN + 25% Urea)+ 20% N (VC) + (*Azotobacter* + PSB @ 25g/tree)

*RDF (Recommended Dose of Fertilizers); (500 N: 250 P_2O_5: 500 K_2O g/tree) + FYM- 40 kg/tree). CN (Calcium Nitrate); VC (Vermicompost); PSB (Phosphorus Solubilizing Bacteria)

Biofertilizer consisting of Azotobacter and PSB were used in combination with organic and inorganic fertilizers having different doses. The biofertilizer was purchased from Department of Microbiology, IARI, New Delhi, India. Plants were inoculated with charcoal based biofertilizer having 10^{-8} cells/ g applied @ 25g/ plant.

The recommended dose of fertilizers (RDF) of apricot is (500 N: 250 P_2O_5: 500 K_2O g/tree) + FYM- 40 kg/tree). Calcium ammonium nitrate is a recommended fertilizer of apricot but this is not available in the market due to highly hygroscopic and explosive. The government of India banned to open sale, purchase and manufacturer of calcium ammonium nitrate (CAN) in the market under the explosive Act, 1884. The substitute of Calcium ammonium nitrate is calcium nitrate. The cost of calcium nitrate is Rs 48 kg^{-1} and the farmers cannot afford to purchase this fertilizer. So the treatments combinations were made in this experiment in such a way to get profitable yield.

Application of Single super phosphate (SSP) and muriate of potash (MOP) along with farm yard manure was applied during mid of January. Nitrogen was applied through urea and calcium nitrate in two split doses according to treatments made. First half dose was applied about two weeks before flowering and remaining half dose was applied one month after first application. *Azotobacter* and PSB was mixed along with 30 kg of vermicompost and left as such for one week for multiplication of microbes. The manures and fertilizers were broadcasting in the basin under the spread of trees, 30 cm away from the tree trunk and thoroughly mixed with soil.

Soil microbial population assay

Soil samples for microbial analysis were taken at a depth of 7.5 cm of the top soil in the rhizospheric region of plants (Ogunmwonyi *et al.,* 2008). The enumeration of bacteria in fresh soil samples was carried out by following serial dilution plate count technique as given by Dhingra and Sinclair (2000) and Martin (1950), respectively.

Soil enzyme activity assay

Soil enzymatic activities were analysed from soil samples collected at a depth of 10 cm from the three different location of rhizosphere of each treatment in the month of July. We determined the activity of four enzymes, dehydrogenase, urease, acid phosphatase and alkaline phosphotase. The

reference method for the determination of urease enzyme activities is described by (Tabatabai and Bremner, 1972). Dehydrogenase activity was assayed by quantifying the ug of TPF (2, 3, 5- triphenyl formazan) produced and expresses as g^{-1} sample h^{-1} as described by (Casida *et al.,* 1964). The alkaline phosphotase activity was quantifying the amount of P- nitrophenol released and expressed as ug of P-nitrophenol released g^{-1} sample h^{-1} as described by (Tabatabai and Bremner (1969).

Statistical analysis

One-way analysis of variance (ANOVA) was performed to determine the effect of different fertilizer treatments on microbial load and enzyme activity of rhizospheric soil. The level of significance referred in the results is $p< 0.05$.

Results and Discussion

Application of 80% RDF (25% CN + 75% Urea) + 20% N (VC) + (*Azotobacter* + PSB) resulted in maximum population (20.43×10^{-5} CFU/g soil) of *Azotobacter* depicted in Table 1. This was statistically at par with T_{10} and T_{11}. The minimum azotobacter population (17.23×10^{-5} CFU g^{-1} soil) was found in T_1: 100% RDF (100% N through CN) during the year 2017. In the year of 2018, maximum azotobacter population (23.33×10^{-5} CFU g^{-1} soil) of azotobacter was observed in T_9: 80% RDF (25% CN + 75% Urea) + 20% N (VC) + (Azotobacter + PSB) whereas minimum azotobacter population (12.43×10^{-5} CFU g^{-1} soil) was obtained in T_1: 100% RDF (100% N through CN) which was statistically at par with T_2.

Phosphorus solubilising bacteria (PSB)

In the year 2017, application of different source of fertilizer treatment had not shown significant difference on PSB population. During the year 2018, it is evident from the data presented in Table 1 indicated that the maximum PSB population (29.43 CFU g $soil^{-1}$) was noticed in treatment T_9: 80% RDF (25% CN + 75% Urea) + 20% N (VC) + (*Azotobacter* + PSB) which was highly significant over the other treatments, whereas minimum PSB population (12.43 CFU g^{-1} soil) was observed in treatment T_1: 100% RDF (100% N through CN) which was statistically at par with T_2.

Similar reports have also been given by Laishram *et al.,* (2013) maximum azotobacter and PSB viable count were found with chemical fertilizers NPK+ Vermicompost + bio-fertilizers. The increased microbial population due to application of different types of organic manures in turn provide adequate biomass as feed to microbes and helps in increasing microbial population in soil Mir *et al.,* (2013).

The results are in conformity with those of Singh *et al.,* (2010) who have observed that inoculation of *Azotobacter, Azosprllium* and PSB in strawberry seedlings increased viable count of Azotobacter and PSB over the rest of the control. Khare *et al.,* (2018) who have found that microbial population of *Azotobacter* and PSB were higher in the rhizosphere of seedlings raised in microbial enriched vermicompost at the time of uprooting of plants.

Vermicomposts are rich in microbial populations and diversity, particularly fungi, bacteria and actinomycetes Edwards (1998). The increased microbial population level of biomass of the soil might be application of organic fertilizers enriched with different types of bio-fertilizers in turns provide adequate supply of nutrients for microbes and helps in increased level of microbial population in soil.

Actinomycetes

Perusal of data presented in Table 1 showed that different treatment of INM had significant effect on *actinomycetes* population during both the years of study. Maximum actinomycetes population (18.27 CFU g soil^{-1}) was perceived in the treatment T_{10}: 80% RDF (50% CN + 50% Urea) + 20% N (VC) + (*Azotobacter* +PSB) which was statistically at par with T_9 and minimum actinomycetes population (11.87 CFU g^{-1} soil) was seen in treatment T_1: 100% RDF (100% N through CN) which was statically at par with T_2 and T_3 during the year 2017.

In 2018, the maximum actinomycetes population (23.33 CFU g^{-1} soil) was counted in T_9 80% RDF (25% CN + 75% Urea) + 20% N (VC) + (Azotobacter + PSB) which was statistically at par with T_5, T_6, T_8, T_{10} and T_{11}. Whereas the minimum actinomycetes population (14.10 CFU g^{-1} soil) was reported in T_1: 100% RDF (100% N through CN) which was statistically at par withT_4.

Similar results were reported by Panchal *et al.,* (2018) in wheat. Actinomycetes population were significantly higher in those treatments receiving bio-fertilizers, FYM and vermicompost. This may be attributed to the vermicompost containing higher amount of growth promoting substances, vitamins and enzymes, which in turn increased microbial population and in addition to this azotobacter + phospho solubilising bacteria increased the root biomass production, which resulted in higher production of root exudates increasing the beneficial bacteria, fungi and actinomycetes population in rhizosphere Panchal *et al.,* (2018).

Urease enzyme activity

It was observed that inorganic fertilizers in combination with organic and bio-fertilizers had significant effect on urease activity of soil. The maximum urease activity (221.98 µg g^{-1} soil hr^{-1}) was estimated in the treatment T_{10}: 80% RDF (50% CN + 50% Urea) + 20% N (VC) + (*Azotobacter* +PSB) which was statistically at par with all treatments except T_1, T_2 and T_3 during the year 2017. Whereas minimum urease activity (219.16 µg g^{-1} soil hr^{-1}) was recorded in treatment T_1: 100% RDF (100% N through CN) which was statistically at par with T_2 and T_3.

In case of second year, the maximum urease activity (222.11 µg g^{-1} soil hr^{-1}) was estimated in the treatment T_{10}: 80% RDF (50% CN + 50% Urea) + 20% N (VC) + (*Azotobacter* +PSB) which was statistically at par with T_3, T_6, T_7, T_8, T_9 and T_{10} whereas minimum urease activity (221.12 µg g^{-1} soil hr^{-1}) was recorded in treatment T_1: 100% RDF (100% N through CN) which was statistically at par with T_4 and T_5.

The present results are in harmony with the findings of Uz and Tavali, (2014) have detected a slight but statistically significant differences in urease activity of soil by using organic amendments like FYM and vermicompost as compared to control. Incorporation of organic manures influenced soil enzyme activity either because of the composition added materials themselves or because they increased microbial activity of soil Verma *et al.,* (2018). Higher urease activity probably resulted from an increase in soil organic matter content and microbial population resulting in the secretion of urease, although no urea was applied Chang *et al.,* (2007).

Application of different sources of nutrients had significant effect on dehydrogenase activity of soil. The maximum (2.79 µg g^{-1} soil hr^{-1} in the year 2017) was observed in T_9: 80% RDF (25% CN + 75% Urea) + 20% N (VC) + (*Azotobacter* + PSB) which was statistically at

par with T_{10} and T_{11}. The minimum dehydroginase activity (1.27 µg g^{-1} soil hr^{-1} in the year 2017) was obtained in T_4: 100% RDF (50% CN + 50% Urea) + (*Azotobacter* + PSB) which was statistically at par with T_1, T_3, T_2, T_5, T_6 and T_8.

Data collected in the year, 2018 had also significant value on dehydrogenase activity of soil. The highest dehydrogenase activity (2.77 µg g^{-1} soil hr^{-1}) was observed in T_9: 80% RDF (25% CN + 75% Urea) + 20% N (VC) + (*Azotobacter* + PSB) which was statistically at par with T_8. Whereas minimum (1.32 µg g^{-1} soil hr^{-1}) was observed in T_1: 100% RDF (100% N through CN).

According to Adak *et al.*, (2014) dehydrogenase enzyme activity was augmented with soil application of 10 Kg vermicompost + N:P:K (100:50:100 g/tree/year of age)+ Azotobacter +PSM+ *Tricoderma harzianum* + Organic mulching. Dehydrogenase activity is influenced more by the quality than quantity of organic matter incorporated into soil. Vandana *et al.*, (2012) reported that the effect of nutrient management on enzyme activity on acid phosphatase was increased with a range from (15.5- 130.7 µg of 4-nitrophenol g^{-1} soil h^{-1}) and alkaline phosphatase activity from (17.2 - 50.3 µg of 4-nitrophenol g^{-1} soil h^{-1}). The results on dehydrogenase activity of soil of the present was fit with the findings of Uz and Tavali (2014) reported that in plants receiving organic material started increase in dehydrogenase activity after one month of the experiment and then decreased to control level at around three months. Elevated dehydrogenase activity is possibly due to utilization of nutrients provided by the organic materials by microorganisms resulting in an increase in microbial activity.

Alkaline phosphotase

The data related to alkaline phosphatase activity of soil observed after harvest were presented in Table 2 results revealed that alkaline phosphatase activity indicated that there was significant influence by different treatments over control. The highest alkaline phosphotase activity (9.67 µg g^{-1} soil hr^{-1}) was found in T_{10}: 80% RDF (50% CN + 50% Urea) + 20% N (VC) + (*Azotobacter* +PSB) which was at par with T_6, T_7, T_8, T_9 and T_{11} whereas the lowest alkaline phosphotase activity (6.97 µg g^{-1} soil hr^{-1}) was recorded in T_3: 100% RDF (25% CN + 75% Urea) + (*Azotobacter* + PSB). However, it was at par with T_1, T_2, T_4 and T_5 during the year 2017. In the second year of study the highest alkaline phosphotase activity (19.40 µg g^{-1} soil hr^{-1}) was found in T_{11}: 80% RDF (75% CN + 25% Urea)+ 20% N (VC) + (*Azotobacter* + PSB) which was at par with T_9 and T_{10}, whereas the lowest alkaline phosphotase activity (10.06 µg g^{-1} soil hr^{-1}) was recorded in T_2: 100% RDF (100% N through Urea) + (*Azotobacter* + PSB). However, it was at par with T_1, T_3 T_4, T_5, T_6, T_7 and T_8 during the year 2017. The results on alkaline phosphotase activity of soil presented in Table 2 showed that its activity was much higher than acid phosphotase.

Among the treatments, alkaline phosphotase activity was found to increase with application of T_9, this may be due to the fact that in general, the enzyme activity in the soil is closely related to organic matter build up Bayer *et al.*, (1993). These results were further confirmed by the findings of Robles *at al.* (2016) who reported increase in acid and alkaline phosphotase activity with mineral fertilizers + organic fertilizers in *vitis venifera* Cv. Thompson seedless. Soil phosphatase activity was strongly inhibited by inorganic phosphate. Manure can stimulate phosphatase activity by providing soil microorganisms with sources of C, N, and P Heidi *et al.*, (2011). Phosphatases can also affect environmental quality following mismanagement of manure, as P in surface runoff is related to organic P content and phosphatase activity Yu *et al.*, (2006).

Table.1 Effect of integrated nutrient management on Azotobacter, PSB and Actinomycetes population of soil

Treatments	Azotobacter (CFU × 10^5 g^{-1} soil)		PSB (CFU × 10^5 g^{-1} soil)		Actinomycetes (CFU × 10^4 g^{-1} soil)	
	2017	2018	2017	2018	2017	2018
T_1	17.23	14.10	16.60	12.43	11.87	15.83
T_2	18.03	17.66	18.40	13.23	11.90	17.33
T_3	17.80	19.00	18.40	15.17	12.70	19.00
T_4	17.56	13.90	19.63	18.63	13.93	17.10
T_5	18.43	21.16	20.37	18.03	13.73	21.17
T_6	18.83	21.40	21.50	25.37	13.77	21.40
T_7	18.96	20.50	20.50	23.63	15.77	20.50
T_8	19.00	22.60	21.67	20.63	15.97	22.60
T_9	20.43	23.33	22.60	29.43	17.77	22.67
T_{10}	19.70	21.23	22.00	27.63	18.27	21.23
T_{11}	19.23	22.33	21.53	25.63	16.03	21.67
Mean	18.65	19.74	20.29	20.89	14.70	20.05
CD $_{(0.05\%)}$	0.31	0.26	NS	1.43	1.40	3.40

Table.2 Effect of integrated nutrient management on enzyme activities of soil

Treatments	Urease activity (µg g^{-1} soil hr^{-1})		Dehydrogenase activity (µg g^{-1} soil hr^{-1})		Alkaline phosphotase (µg g^{-1} soil hr^{-1})	
	2017	2018	2017	2018	2017	2018
T_1	219.16	221.12	1.29	1.32	7.23	10.34
T_2	220.06	221.65	1.34	1.77	7.09	10.06
T_3	219.94	221.71	1.32	1.54	6.97	11.81
T_4	221.70	221.48	1.27	1.33	8.00	13.71
T_5	221.75	221.44	1.31	1.71	8.07	12.98
T_6	221.59	221.95	1.89	1.47	8.89	13.88
T_7	221.70	221.96	1.87	1.59	9.08	13.30
T_8	221.32	221.91	1.45	2.25	9.41	13.73
T_9	221.72	221.92	2.79	2.77	9.23	15.51
T_{10}	221.98	222.11	2.36	1.58	9.67	16.08
T_{11}	221.58	222.01	1.97	1.95	9.58	19.40
Mean	221.14	221.75	1.71	1.75	8.47	13.71
CD $_{(0.05\%)}$	1.04	0.42	0.88	0.66	1.57	4.49

Acknowledgement

This research was supported by Department of Fruit Science, Dr YS parmar University of Horticulture and Forestry, nauni, Solan (H.P). I would like to express my great appreciation to Dr. D D Sharma for assistance in this research programme.

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How to cite this article:

Kuchay, M.A., Sharma, D.D. and Vijay Kumar. 2019. Evaluation of Microbial and Enzyme Activity of Rhizospheric Soils under Integrated Nutrient Management of Apricot. *Int.J.Curr.Microbiol.App.Sci.* 8(11): 2381-2389. doi: https://doi.org/10.20546/ijcmas.2019.811.276