Research Article

Effect of Biofertilizers along with Pesticides on Soil Biological Properties and Yield of Wheat

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Abstract

The present study was conducted for two successive *rabi* seasons (2016-18) in order to evaluate the combined effect of biofertilizers and pesticides on microbial activities and plant growth parameters of wheat crop. Sole application of biofertilizer or pesticides non-significantly affects soil rhizospheric microbial dynamics as well as plant growth parameters. In both years, maximum bacterial (9.05 $\log_{10}$ CFU g^{-1}soil in first year and 9.16 $\log_{10}$ CFU g^{-1}soil in second year), fungal (4.49 $\log_{10}$ CFU g^{-1}soil in first year and 4.54 $\log_{10}$ CFU g^{-1}soil in second year) as well as diazotrophic (5.79 $\log_{10}$ CFU g^{-1}soil in first year and 5.86 $\log_{10}$ CFU g^{-1}soil in second year) population was recorded in treatment having solitary application of biofertilizers at 90 DAS. Highest p-solubilizer population (4.30 $\log_{10}$ CFU g^{-1}soil in first year and 4.38 $\log_{10}$ CFU g^{-1}soil in second year) was recorded in the same treatment at 180 DAS. Highest soil enzymatic activities viz., dehydrogenase (10.05 µg TPF formed/g soil/h in first year and 12.6 µg TPF formed/g soil/h in second year), alkaline phosphatase (15.9 µg pNP formed/g soil/h in first year and 19 µg pNP formed/g soil/h in second year) and urease (407 µg urea hydrolyzed/g soil/h in first year and 401 µg urea hydrolyzed/g soil/h) were recorded in sole application of biofertilizers. Combined application of biofertilizers and pesticides yielded maximum number of effective tillers/m^2, plant height, number of grains per ear, 1000 grain weight, grain and straw yield as well. Therefore, application of pesticides at recommended rates have non-deleterious effects on biofertilizers functioning, rhizospheric microbial activities and plant growth parameters of wheat crop.

Keywords: Biofertilizers, Enzymatic activities, Microbial Population, Pesticides, Rabi, Wheat

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Introduction

The increasing trend of food demand prerequisite a need for an improvement in production system of the country. Wheat is a staple food crop for majority of world population and hence there is an undivided focus on improving the productivity of wheat all around the globe. It is the important food component of about two billion people (36% of the world population). On the other hand, an average of 35 % of potential global crop yield is lost to pre-harvest pests [1]. So, the reduction of total yield losses caused by pests, pathogens and weeds are major challenges to agricultural production [2]. To meet the demands for food grain, various pesticides and chemical fertilizers were extensively used in the fields since1960's. But these inputs deteriorated the soil health and harmed the natural microflora of soil ecosystem. Therefore, the aim of agriculture shifted from production to protection of soil ecosystem. Sustainable agriculture aims at improving crop yields without compromising with soil health. The conventional agricultural practices depended solely upon chemical inputs like fertilizers and pesticides. However, modern agriculture emphasises the benefits associated with the combined application of various organic and inorganic inputs. To minimize the negative implications caused by chemical inputs, the concept of biofertilization came into existence. Currently, the application of biofertilizers has been emerging because of their highly efficient plant growth promoting activities like indole acetic acid production, ammonia production, p-solubilization, zinc solubilisation etc [3]. Under field conditions, biofertilizers come in contact with various other agricultural inputs like pesticides and fertilizers which can affect the functioning and performance of biofertilizers. The combined application of these might have some effects on rhizospheric microbial activities and plant parameters of crop. Biofertilizers and pesticides, together, might stimulate or limit microbial activity in rhizosphere depending upon their concentration, environmental conditions etc. Biofertilizers enhance root exudation from crops and hence stimulate rhizospheric effect under field conditions [4]. Similarly, various microbial groups may degrade pesticides and later use them as carbon or nitrogen sources of nutrition [5]. These series of events in turn influence the yield parameters of crop under study. Therefore, current study was conducted to assess the impact of biofertilizers and pesticides (sole or combined application) on rhizospheric soil microbial activities and plant growth parameters of wheat crop.

Materials and Methods
Experimental design

A field experiment on wheat crop (var. PBW 725) was conducted during *rabi* season for two successive years (2016 and 2017) at experimental area of School of Organic Farming, Punjab Agricultural University (PAU), Ludhiana, India. Different combinations of consortium biofertilizer (*Azotobacter*, PSB and PGPR) and pesticides (as recommended in Package of Practices of PAU) were made and enlisted as

T1: Control (without Biofertilizer+without Pesticide)
T2: Only Biofertilizers
T3: Only Pesticides
T4: Combined application of Pesticide + Biofertilizer

Collection of soil samples

Soil was collected 0-15 cm beneath the surface of soil from rhizospheric soil of wheat crop at regular time intervals (0 DAS, 90 DAS and 180 Days after Sowing). Randomly five samples were collected from each treatment and mixed to obtain one representative sample for analysis.

Enumeration of microbial population

The bacterial, fungal, actinomycetes, diazotrophic and p-solubilizer populations were enumerated using Nutrient agar medium [6], Glucose Yeast Extract agar medium [7], Kenknight's agar medium, Jensen's agar medium [8] and Pikovaskaya's agar medium [9], respectively using serial dilution spread plate method. The culture media were sterilized in an autoclave at 121° C for 15-20 minutes (15 psi pressure). Different dilutions were chosen for the bacteria (10^6 to 10^8), fungal (10^2 to 10^3), actinomycetes, PSMs and diazotrophic (10^2 to 10^4) enumeration in accordance to their relative abundance in soil.

Assay of microbial enzymatic activities

Dehydrogenase activity was assayed using the method given by Tabatabai [10] in which dehydrogenation of triphenyl formazon (TPF) from triphenyl tetrazolium chloride (TTC) was recorded calorimetrically at 480 nm. Soil alkaline phosphatise activity was assayed with the method given by Tabatabai and Bremner [11] in which the formation of p-nitrophenol was recorded calorimetrically at 420 nm. Soil urease activity was assayed by following the procedure given by Bremner and Douglas [12].

Yield attributes and yield

Various plant growth parameters like plant height (cm), yield attributes viz., number of effective tiller/m^2, ear length (cm), number of grains per ear, thousand grain weight (g) and yield (grain and straw in q/ha) were recorded for two consecutive years using standard methods of measurement.

Statistical analysis

The data was analysed with two-way analysis of variance (ANOVA) to determine the effect of different combinations of treatments, different day intervals and their interaction on various microbial population and enzymatic activities. The data related to yield attributes and crop yield were analysed with one way ANOVA to determine effect of different treatments using OP-stat software @5% level of significance.

Results and Discussions
Initial soil properties

Microbial activities were recorded at the time of sowing during the *rabi* seasons of both the years (2016-17 and 2017-18) listed in **Table 1**.

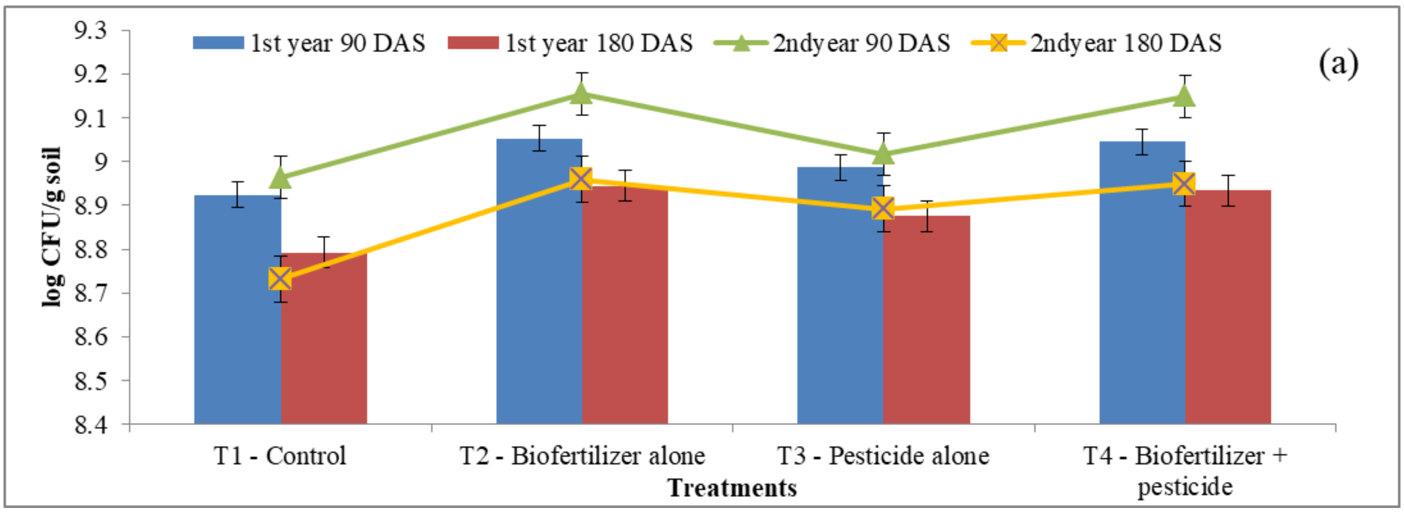
Table 1 Initial soil microbial flora and soil enzymatic activities

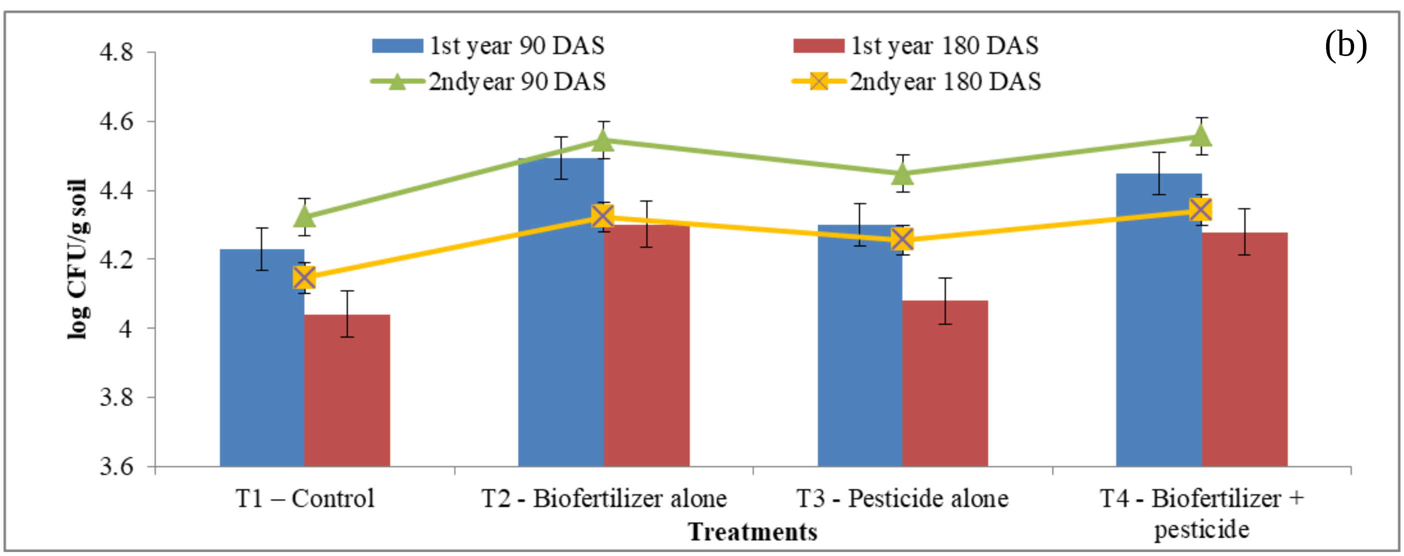
Parameter		Year 2016-17	Year 2017-18
Soil Microbial flora (log_{10} CFU g^{-1} soil)	Total Bacteria	7.50	7.53
	Total Fungi	2.95	3.04
	Total Actinomycetes	4.30	4.32
	Total Diazotrophs	4.08	4.13
	P-solubilizing Bacteria	2.78	2.81
Soil Enzymatic activity (µg activity/g soil/h)	Dehydrogenase activity	1.25	1.39
	Alkaline phosphatise activity	2.83	3.06
	Urease activity	112.38	114.01

Microbial dynamics in wheat rhizosphere

Bacterial population

Bacteria are known to multiply very rapidly in comparison to other microbial groups in soil. In our study, maximum bacterial population (9.053 log_{10} CFU g^{-1} soil in first year and 9.155 log_{10} CFU g^{-1} soil second year was recorded in treatment having the application of biofertilizer in *rabi* seasons (**Figure 1a**) However, the bacterial population (9.045 log_{10} CFU g^{-1}soil in first year and 9.149 log_{10} CFU g^{-1} soil in second year) of treatment having the combined application of biofertilizer and pesticides (T4) was at a non-significant difference with the population of treatment T2 at 90 DAS. Application of biofertilizer might have stimulated the root exudation and thus promoted the proliferation of bacteria in wheat rhizosphere. Similar results were reported by Yuvraj [13].





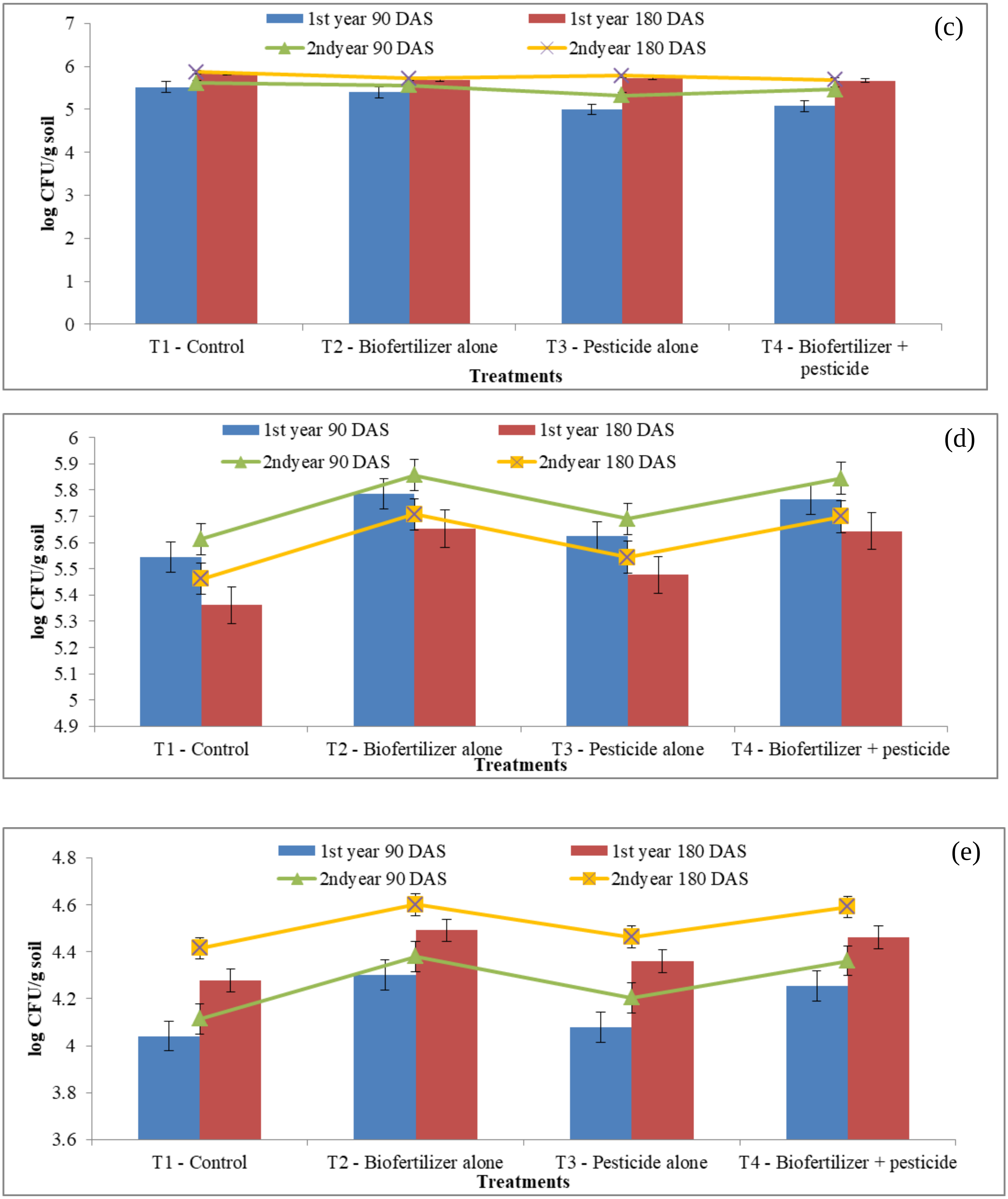


Figure 1 Microbial dynamics of wheat rhizosphere as affected with application of different combination of pesticides and biofertilizers a) Total bacteria, b) Total fungi, c) actinomycetes, d) Diazotrophs and e) P-solubilizers

Fungal population

Maximum fungal population (4.491 log_{10} CFU g^{-1} soil) was recorded in treatment having solitary application of biofertilizer (T2) during first year. Whereas, during second year, maximum fungal population (4.556 log_{10} CFU g^{-1}

soil) was observed in treatment T4 having integrated application of biofertilizer and pesticides (Figure 1b). This might be due to the fact that the microbes degraded the pesticides applied in field and thus the end products of chemicals were consumed as source of nutrition by fungal population. The results are supported by study conducted by He *et al* [14]. The two treatments (T2 and T4) were at a non-significant difference in both seasons at 90 DAS.

Actinomycetes population

Harvesting stage was observed to be most suitable for actinomycetes growth as maximum actinomycetes population (5.690 log_{10} CFU g^{-1} soil for first year and 5.716 log_{10} CFU g^{-1} soil for second year) was recorded at 180 DAS in treatment having neither the application of biofertilizer nor pesticides (T1) in both years (Figure 1c). Actinomycetes dwell under those conditions where the competition of other microbial groups is limited. Also the application of pesticides might have inhibited the growth of actinomycetes due to their toxic effects. The results are in accordance with study conducted by Bruggen and Semenov [15].

Diazotrophic population

Application of biofertilizer (T2) positively stimulated the diazotrophic population (5.785 log_{10} CFU g^{-1} soil for first year and 5.857 log_{10} CFU g^{-1}soil for second year) at flowering stage. Nevertheless, the population in treatment having combined application of biofertilizer and pesticides (T4) was at a non-significant difference with the population of treatment T2 (Figure 1d). This clearly indicates that pesticides had no negative impact on the efficiency of biofertilizer. Similar results were recorded in study conducted by Gopal *et al* [16].

Phosphate solubilizer population

Maximum p-solubilizer population (4.491 log_{10} CFU g^{-1}soil for first year and 4.602 log_{10} CFU g^{-1}soil for second year) was recorted in treatment with the application of biofertilizer alone (T2). However, the population of p-solubilizer in this treatment was at a non-significant difference with the population (4.462 log_{10} CFU g^{-1}soil for first year and 4.591 log_{10} CFU g^{-1}soil for second year) of treatment T4 at 180 DAS (Figure 1e). This might be due to reduced competition at this stage of crop growth. Brar [4] also observed an enhancement in p-solubilizer population with the application of biofertilizers.

Soil Enzyme Activities

Soil enzymatic activities indicate the effect of various farming practices on soil health status. It was inevitably indicative in the present study that the application of various agricultural amendments yielded improved soil enzymatic activities in comparison to the treatment without fertilizers.

Dehydrogenase activity

Soil dehydrogenase activity is a direct indicator of living intact cells present in the soil. The application of biofertilizer alone (T2) or in combination with pesticides (T4), reported maximum dehydrogenase activity in both years (**Table 2**). This might be due to fact that the application of biofertilizers stimulated microbial growth in rhizosphere and therefore higher dehydrogenase activity was also observed in treatments having application of biofertilizer. The results were in agreement with study conducted by Yuvraj [13].

Alkaline phosphatase activity

Alkaline phosphatase is of immense value in agricultural sector since it converts unavailable form of phosphorus to available form which is assailable by the plants. In both years, maximum alkaline phosphatise activity (15.9 µg pNP formed/g soil/h in first year and 19 µg pNP formed/g soil/h in second year) was reported at 180 DAS which might be due to higher population of p-solubilizers at this stage. This enzymatic activity was at a non-significant difference between treatments having solitary application of biofertilizer (T2) and integrated application of biofertilizer and pesticides (T4) as mentioned in Table 2. The results are supported in study conducted by Demanou *et al* [17] where they observed no negative effect of pesticides on soil alkaline phosphatase activity.

Urease activity

The hydrolysis of urea fertilizer in fields is carried out by urease enzyme and therefore this enzyme holds a huge significance in agriculture and soil microbiology. In first year, maximum urease activity (407µg urea hydrolyzed/g soil/h) was recorded in treatment T2 whereas in second year the same was reported to be maximum (401µg urea hydrolyzed/g soil/h in treatment T4, with combined application of biofertilizer and pesticides at 90 DAS (Table 2). There was a non-significant difference in urease activities of both treatments. There was a decline in urease activity at harvesting stage which might be due to limited N source at this stage of plant growth. Frankenberger and Dick [18] and Kaur et al [19] also observed maximum urease activity at flowering stage and a decline at harvesting stage.

Table 2 Effect of pesticides and biofertilizers on rhizospheric enzymatic activities

Treatment	Dehydrogenase activity (µg TPF formed/ g soil/h)				Alkaline phosphatase activity (µg PNP formed/g soil/h)				Urease activity (µg urea hydrolysed/ g soil/h)			
	1st year		2nd year		1st year		2nd year		1st year		2nd year	
	90 DAS	180 DAS	90 DAS	180 DAS	90 DAS	180 DAS	90 DAS	180 DAS	90 DAS	180 DAS	90 DAS	180 DAS
T1	4.2	2.1	6.3	2.05	3.5	9.4	5.4	11.6	213	123	216	132
T2	10.05	4.1	12.6	6.1	5.2	15.9	10.9	19	407	254	400.6	263.7
T3	5.9	2.24	8.2	2.3	4.1	11.7	7.3	13.7	298	156	268	152
T4	9.99	3.5	12.58	6.052	4.9	15	10.5	18.6	389	249	401	264
CD@5%												
Treatment	0.08		0.03		0.44		0.35		4.87		0.22	
Environment	0.05		0.02		0.31		0.25		3.44		0.17	
Interaction	0.11		0.05		0.63		0.50		6.89		0.34	

Yield attributes and crop yield

Plant height

Plant height indicates the growth index of crop. The plant height between treatments having combined application of biofertilizers along with pesticides (88.6 cm in first year and 90.2 cm in second year) and solitary application of biofertilizers (88.6 cm in first year and 90 cm in second year), was non-significantly different in both years (**Table 3**). The results are supported by study conducted by Mubeen *et al* [20] where they reported an increase in plant height with application of biofertilizers alone or in conjunction with pesticides.

Ear length

Ear length was observed to be maximum (11.8 cm in first year and 12 cm in second year) in treatment having solitary application of biofertilizer in both years (Table 3). Again, the application of pesticides in combination with biofertilizers had no significant negative effect on ear length. Biofertilizers increase nutrient availability of the crop and thus promote elongation of ear as supported in study conducted by Lavakusha *et al* [21].

Table 3 Effect of biofertilizers and pesticides on yield attributes and yield of wheat crop

Treat ment	Plant height(cm)		Ear length(cm)		No. of effective tillers/m^2		Number of grains/ear		1000 grain weight(g)		Grain yield (Q/ha)		Straw yield (Q/ha)	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
T1	86.00	88.40	10.80	11.20	386	392	38.10	39.20	40.80	40.92	45.75	46.25	67.90	69.10
T2	88.60	90.00	11.80	12.00	416	426	39.80	40.60	41.32	41.54	46.70	47.32	69.05	70.45
T3	86.40	88.60	11.00	11.20	402	408	38.40	39.40	40.86	40.94	45.88	46.40	68.10	69.35
T4	88.60	90.20	11.60	11.80	420	422	40.20	40.80	41.38	41.56	46.82	47.38	69.25	70.65
CD@5%	0.28	0.21	0.20	0.20	6.25	15.84	0.64	0.22	0.16	0.10	0.23	0.21	0.67	0.29

Number of effective tillers/ m^2

Grain production relies extensively on tillering of a crop. In the first *rabi* season, maximum number of effective tillers per m^2 (420) was observed in treatment having integrated application of biofertilizers and pesticides (T4) whereas the same parameter (426) was observed to be maximum in treatment T2 in the successive *rabi* season (Table 3). In both years, there was a non-significant difference in the number of effective tillers per mt^2 of treatments T2 and T4. A progressive increase in number of effective tillers per mt^2 was reported by Lavakusha *et al* [21] in rice crop.

Number of grains per ear

Maximum number of grains per ear (40.2 in first year and 40.8 in second year) were noted in treatment having combined application of pesticides and biofertilizers (T4) in both years (Table 3). The results were non-significantly higher than the number of grains per ear (39.8 in first year and 40.6 in second year) obtained in treatment having solitary application of biofertilizers (T2). Kumari *et al* [22] reported a positive effect of biofertilization on number of grains per ear.

Thousand grain weight

The treatment having combined application of biofertilizers and pesticides also gave highest 1000 grain weight (41.38 g in first year and 41.56 g in second year) as mentioned in Table 3. There was a non-significant difference in 1000 grain weight (41.32 g in first year and 41.54 g in second year) of treatment having solitary application of biofertilizers. The results are supported by study conducted by Kumari *et* al [22].

Grain and straw yield of wheat crop

The treatment having combined application of pesticides and biofertilizers gave non-signicantly higher grain (46.82 q/ha in first year and 47.38 q/ha in second year) and straw yield (69.25 q/ha in first year and 70.65 q/ha in second year) than treatment having solitary application of biofertilizers (Table 3). The results were superior to yield obtained in treatment having neither the application of biofertilizers nor pesticides i.e. farmer's practice. Devasenamma *et al* [23] also observed similar results in case of yield attributes of crop.

Conclusion

The study concluded that the combined application of biofertilizer and pesticide had no deleterious effect on soil microbial activities and plant growth parameters of wheat crop. Improved soil enzymatic activity is direct indicative of healthy soil status hence microbial activities.

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Publication History

Received	15.06.2020
Revised	20.07.2020
Accepted	04.08.2020
Online	30.08.2020