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Influence of novel microbial formulations on enzymes and microbial indices of soil during wheat cultivation

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ABSTRACT

A field experiment was conducted to study the comparative influence of microbial preparations ,viz. phosphate solubilizing fungi (Jumpstart 2.0), phosphate solubilizing bacteria (PSB), Arbuscular mycorrhiza fungi and humic acid formulation (Bolt GR), in different combinations on soil microbial population and properties (Alkaline phosphatase, dehydrogenase, FDA hydrolytic activity, soil microbial biomass). Bacteria dominated the microbial population, with initial cfu level of 1.73×10^7 to a maximum of 6.2×10^7 gm^{-1} soil on 90 days after sowing (DAS) in plot, where PSB was applied @ 500 g/ ha, while plots with Jumpstart 2.0 application (@1.65 ml/ kg seed) recorded higher fungal population (7.4×10^6 cfu/g). Treatments and crop age had significant impact on microbial population and their interaction was also found to be significant. Soil microbial properties also increased significantly with crop aging and reached to a higher level on 90 DAS for all the treatments. Microbial activity was invariably found to be highest in T_7 treatment (application of PSF @ 0.83 ml/kg seed with humic acid @ 10 kg/ha), and lowest in control plots irrespective of sampling intervals. Results concluded that application of PSF, PSB, AM Fungi and organic formulations in different combination had favourably influenced the microbial properties of soil.

Key words: Biofertilizer, Enzymes, Microbial activity, Organic amendments, Soil quality

Productivity of any crop is mainly affected by prevailing environmental conditions and soil fertility. Although wheat production is increasing in the country, but further improvement in the quantity and quality of food crops are required to provide food security to the rising population. To bridge this gap between demand and supply more and more fertilizers are being used. However, rampant use of chemical fertilizers not only deteriorate soil quality, reduce soil organic matter, decrease soil water retention capacity but creates additional risk of environmental and food contamination. There is a need to adopt strategies for its substitution with natural and existing resources of macro and micronutrients. Organic amendments not only positively influence biological activities of soil but preserve structure of soil by building the organic matter content (De Forest *et al.* 2012). Presence of soil organic matter and its subsequent transformation by soil microbial biomass plays major role in determining the productivity of an ecosystem and its sustainability (Watts *et al*. 2010). Microbial activity is considered as important biological parameter of agricultural ecosystem and indicator of soil quality (Barros *et al*. 2007). Number and kind of microbial population in soil influence the overall nutrient cycling by regulating the decomposition of organic matter. Microbial population are in turn regulated by interactions between plant type, climate and management practices. soil microorganisms produce many extracellular enzymes such as dehydrogenase (DHA), acid and alkaline phosphatase (ACP/ALK), fluorescein diacetate (FDA) and cellulase, thereby regulate the release of plant nutrients, decomposition of organic matter and soil organic carbon (Fansler *et al*. 2005). Studies have shown that high microbial diversity in soils shows a healthy soil-plant relationship, whereas unhealthy soils often exhibit low microbial diversity (Tejada *et al*. 2011). Incorporation of organic manures influences soil enzymatic activity (De Forest *et al*. 2012). Application of farmyard manure (FYM) has been reported to improve the water holding capacity and overall structure of soil (Dejene *et al.* 2012; Tadesse *et al.* 2013) which in turn lead to a better root development. Among the many factors that influence the yield of wheat, phosphorus is considered as one of the most important factor in improving the yield. Phosphorus is present in large amount in soils but major proportion is considered as immobile. Phosphate solubilizing potential of fungus like *Aspergillus* and *Penicillium* has widely been evaluated in many plant production systems (Mendes *et al.* 2014; Gaind and Singh 2017). Field trials with *Penicillium billai* based phosphate solubilising fungi

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(PSF) biofertilizer (Jumpstart), developed by Novazyme India, have shown to improve the P availability, root growth and root hair formation in wheat crop (Patil *et al.* 2012). Jumpstart has been reported to improve the P uptake and yield of other crops as well (Patil *et al.* 2012; Singh and Gaind 2019). Besides phosphorus, humic acid content in soil also play important role in improving soil condition and plant growth (Baloach *et al.* 2004). Krishnakumar *et al.* (2005) reported that a combination of organic manures gave better results than single organic manure application. Meena *et al*. (2014) showed that microorganisms along with organic amendments not only increase organic matter status but also improve soil biological activities. Thus, the study was undertaken to evaluate the effect of different doses of PSF in combinations with organic amendments on native microbial population and enzymatic activities of soil under wheat crop.

MATERIALS AND METHODS

The field and laboratory studies were conducted during *rabi* season (2018) at ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India. The field experiment was laid out in a randomized block design (RBD) with three replications of each 12 combinations consisting of different combinations of Phosphate solubilizing fungi (PSF) namely Jumpstart and Bolt GR formulation along with PSB, AMF and recommended dose of fertilizers (Untreated control) and control without any fertilizer (P Control). Twelve treatments included, T_1: Untreated control (Recommended dose of P), T_2: Jumpstart @ 0.41 ml/ kg seed,T_3: Jumpstart @ 0.83 ml/ kg seed, T_4: Jumpstart @ 1.25 ml/ kg seed, T_5: Jumpstart @ 1.65 ml/kg seed, T_6: Soil application of Bolt GR @ 10 ml/kg (20-30 DAS at first irrigation), T_7: Jumpstart @ 0.83 ml/ kg seed + Soil Application of Bolt GR @ ml/kg, T_8: Soil application of Bolt GR @ ml/ kg (basal before sowing), T_9: 50% of Rec. P+ Jumpstart @ 0.83 ml/ kg seed, T_{10}: AM Fungi @ 10 kg/ha (basal before sowing), T_{11}: PSB @ 500 g/ha and T_{12}: P Control. Recommended doses of phosphorous and potassium were applied as basal before sowing of wheat through di-ammonium phosphate and muriate of potash. Nitrogen was applied through urea. Wheat variety HD-2967 developed at ICAR-IARI was used as test crop. Soil samples from a depth of 0–15 cm were collected from the experimental field at an interval of 30, 60 and 90 days after sowing (DAS) and microbial population and enzymatic activities of soil were recorded. Moist soil samples collected from the experimental field, were sieved through a 2-mm sieve, homogenized and stored in a cold room at 4°C in plastic bags for a few days to stabilize the microbiological activity. The population of different microbial groups, *viz*. bacteria (Nutrient glucose agar medium), fungi (Martin's rose Bengal agar medium) in soil samples were computed by using standard serial dilution plating techniques (Waksman *et al.* 1922). Dehydrogenase activity in soil samples was estimated by using 2, 3, 5 triphenyl tetrazolium using method suggested by Casida *et al*. (1964)**.** Alkaline phosphatase activity was assayed by following the method of Tabatabai *et al*. (1980). Fluorescein diacetate hydrolysis assay was performed according to the Swisher *et al*. (1980). Soil microbial biomass carbon was estimated by Chloroform Fumigation method (Anderson *et al.* 1993). All the observations were subjected to analyses of variance (ANOVA) and Critical Difference (CD) at 5% level of significance.

RESULTS AND DISCUSSION

Soil microbial population

Initial population of bacteria and fungi in soil were 1.73 × 10^7 and 3.1 × 10^6cfu g^{-1}, respectively. Bacterial and fungal population increased with growth of crop and reached to a maximum level on 90 DAS (flowering stage of the crop). With the advancement in crop growth, the growth in root biomass might have been responsible for increased microbial population. Results are in agreement with the earlier reports (Meena *et al.* 2013). Bacterial population reached to a maximum level of 6.2 × 10^7 cfu/g in T_{11}, where PSB was applied @500 g/ha at 90 DAS, followed by T_6 (4.3 × 10^7) and T_8 (4.2 × 10^7) where humic acid based Bio-stimulant was applied (Table 1). Maximum fungal population (7.4 × 10^6) cfu/g were recorded in T_5, where PSF was applied

Table 1 Influence of different treatments on microbial population in soil at different stages of growth

Treatment	Number of bacteria (cfu/g soil ×10^7)			Number of fungi (CFU/g soil × 10^6)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Untreated control	1.8	2.3	2.4	2.4	2.5	2.6
PSF @ 0.41 ml/kg seed	3.2	3.5	3.7	5.2	5.3	5.8
PSF @ 0.83 ml/kg seed	3.4	3.6	3.9	5.5	5.7	5.9
PSF @ 1.25 ml/kg seed	3.5	3.7	3.7	5.9	6.0	6.4
PSF @ 1.65 ml/kg seed	3.5	3.8	4.0	6.6	6.9	7.4
Bolt Gr @ 10 kg/ha	3.9	4.1	4.3	3.7	4.0	4.2
PSF @ 0.83 ml/kg seed + Bolt Gr	3.5	3.7	3.8	5.3	5.6	5.8
Bolt Gr @ 10 kg ha (BS)	4.0	4.1	4.2	4.0	4.2	4.3
PSF @ 0.83 ml kg seed + 50% P	3.6	3.8	3.9	4.4	4.5	4.6
AMF @ 10 kg/ha	3.7	3.8	3.9	5.2	5.4	5.6
PSB @ 500 g/ha	5.5	5.8	6.2	2.9	3.2	3.5
Control	2.0	2.3	2.3	2.5	2.9	3.1
CD	2.68	4.02	2.21	2.82	2.45	1.79
SE(m)	0.91	1.36	0.75	0.96	0.83	0.61
SE (d)	1.29	1.92	1.06	1.35	1.17	0.86

Initial population of bacteria: 1.73 ×10^7 and initial population of fungi: 3.1 × 10^6

@1.65 ml/kg seed. The increase in the fungal population could be attributed to the presence of the organic matter. Application of humic acid based Bio-stimulant also resulted into an increase in fungal population as compared to control and the plots where no fungal inoculation were carried out. This indicated that humic acid based Bio-stimulant has positively influenced the bacterial as well as fungal population in the soil. The results are in agreement with the earlier reports (Deepa 2001; Sellamuthu and Govindaswasmy 2003). Sellamuthu and Govindaswamy (2003) reported an increase in population of bacteria, fungi and actinomycetes in sugarcane rhizosphere on application of humic acid @ 20 and 30 kg/ha. Soil microbial activity of an ecosystem plays important role in determining its fertility as majority of soil processes are mediated by microorganisms (Nannipieri *et al*. 2003). Soil is home for a mixed diverse microbial community which live together in harmony and carry out functions like biodegradation, nitrogen fixation, improving soil fertility, phosphate solubilization etc. (Umi and Sariah 2003). They also play role in protecting host from disease causing microorganisms. Therefore, any organic or inorganic supplementation in soil should not have adverse affect on native microflora.

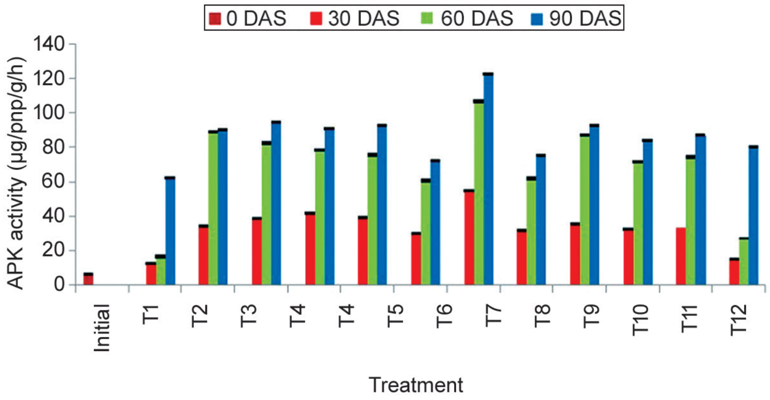

Fig 1 Influence of different treatments on alkaline phosphatase activity (µg PNP/g soil/hr) in soil at different stages of crop growth.

Soil enzymatic activities

Soil enzymes are group of enzymes originated and released from living and dead microorganisms, roots and residues of plants and animals. They are important in catalyzing several vital reactions necessary for the life processes of soil microorganisms, soil structure stabilization, organic wastes decomposition, organic matter formation and nutrient cycling hence, playing an important role in crop production (Dick *et al.* 1994). Effect of PSB, AM, Jumpstart 2.0 (phosphate solublizing fungi) and Bolt GR in different combinations (10 different combinations) were studied on soil microbial properties (alkaline phosphatase, dehydrogenase, FDA activities and soil microbial biomass).

Alkaline phosphatase enzyme

Organic P compounds undergo mineralization and the resulting P is taken up as nutrient by the plants. This mineralization process is mediated by the enzyme phosphatase (Tarafdar and Claassen 1988) released by the soil microbes. Numerous soil microbes or rhizosphere microflora possesses the ability to transform organic P into soluble forms of P (Rodriguez *et al.* 2006). The mechanism involves production of organic acids, release of protons and ammonium ion assimilation and secretion of phosphatase enzyme. Phosphatase enzyme activity in soil before sowing of wheat crop was 6.5 µg PNP/g soil/hr, which increased significantly with crop aging, *i.e.* at 30, 60 and 90 DAS in all the treatments studied (Fig 1). The activity was found to be maximum in T_7 treatment (integrated application of PSF @0.83 ml/kg seed with Bolt Gr @10 kg/ha), where the enzyme activity reached to a level of 122.61 µg PNP/g soil/hr. Application of humic acid has also been reported to lead the enhancement in catalase and alkaline phosphatase activity in sugarcane crop (Sellamuthu and Govindaswamy 2003). Alkaline phosphatase activity was significantly affected by different treatment as well as crop age. The sole inoculation of PSB and Bolt GR and AMF also showed significant increase in enzyme activity over control.

Dehydrogenase enzyme

Dehydrogenase (DHA) is an oxidoreductase enzyme which is present only in viable cells and does not accumulated extracellularly in soil. This enzyme oxidizes soil organic matter by transferring protons and electrons from substrate to acceptors and thus become a part of respiratory pathway. The enzyme activity invariably increased in all the treatments with crop duration with maximum at 90 DAS of wheat crop (Fig 2). This might be due to the fact that wheat crop used in the current study flowers in 85-90 DAS, earlier studies have also reported increase in enzyme activity at flowering stage (Marinara *et al.* 2000). Among the various treatments applied the application of PSF @0.83 ml/kg seed with humic acid @10 kg/ha showed the maximum dehydrogenase activity (Fig. 2). The humic acid consists of moderate organic carbon which might have increased the soil dehydrogenase activity. This was in accordance with Meena *et al.* (2013) who reported higher DHA values in soils amended with compost and humic acid as compared to soil fertilized with chemical fertilizers. Chemical fertilizers may also promote the growth of microbial communities as compared to control by countering the limitation of mineral nutrients (Gaind and Singh 2016).

FDA activity

Fluorescein diacetate hydrolysis (FDA) is an indicator of overall microbial activity, involved in hydrolysis and decomposition of organic matter and indicates broad spectrum of soil enzyme activities on substrates including carbohydrates, lipids and proteins (Dick *et al.* 1996). FDA

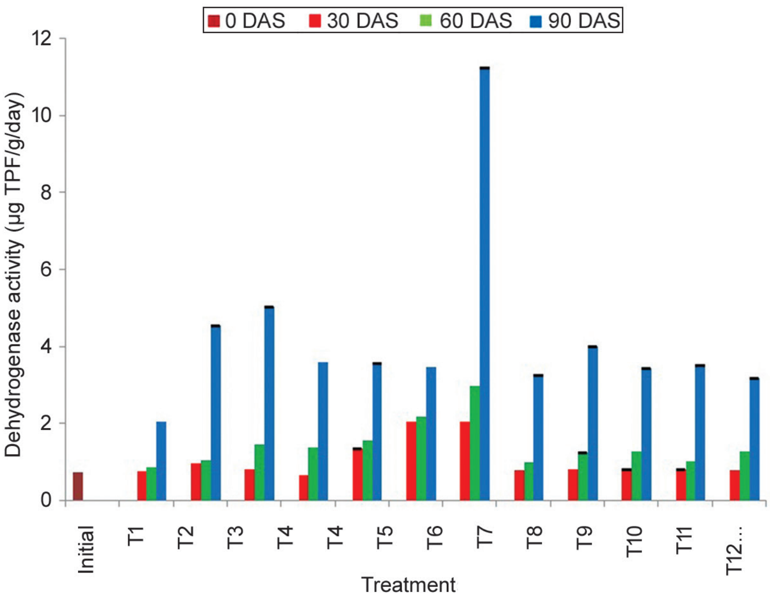

Fig 2 Influence of different treatments on dehydogenase activity in soil at different stages of crop growth.

is hydrolyzed by a number of different enzymes, such as proteases, lipases and esterases. The product of this enzymatic conversion is fluorescein, which can be estimated spectrometrically. FDA hydrolytic activity in soil was significantly increased with crop aging, i.e. minimum activity was recorded in 0 day sample (Fig 3), which increased with increase in days of sowing and maximum activity was recorded in soil samples at 90 DAS of wheat crop. As in case of dehydrogenase activity, FDA hydrolytic activity was also found to be highest in T_7 treatment (integrated application of PSF @0.83 ml/kg seed with Bolt Gr @10 kg/ha) in all the sampling intervals, with maximum in samples after 90 DAS (1.36 µg fluorescein/g/hour), while lowest was recorded in Control (0.76 µg fluorescein/g/hour). The higher value of FDA hydrolysis is a sign of positive microbial activity and soil health. The maximum FDA was observed in treatment of PSF@ 0.83 ml/kg seed along with humic acid @10 kg/ha. Organic manure and fertilizer are known to increase the microbial biomass of soil which might have led to increase in FDA hydrolysis (Biswas *et al.* 2018). Analysis of data indicated that alkaline Fluorescein diacetate hydrolysis was significantly affected by different treatment as well as crop age.

Microbial biomass carbon

Microbial biomass comprises a small proportion of soil organic matter but it reflects the total organic matter content within the microorganisms. Microbial biomass comprises 2-3% of soil carbon and 3-5% of soil N (Jenkinson and Ladd 1981). It acts as a catalyst for the conversion of plant nutrients into their available form (Coleman *et al.* 1983). The microbial biomass carbon increased with the crop ageing *i.e.* at 30, 60, 90 days after sowing in all the treatments studied (Fig. 4). Increase in the microbial biomass over time indicates the fertility of the soil. The maximum microbial biomass carbon was observed when PSF @0.83 mL/kg seed was applied with the humic acid (287.46 µg/g soil) and the minimum was observed when the chemical fertilizer was applied (175.2 µg/g soil). This was in accordance with the findings of Starling (1985) that soil system with high organic input have high microbial biomass and activity. Increased microbial biomass could be due to availability of suitable conditions for microbial growth. Thus microbial biomass is a useful indicator of soil quality and soil development. The interaction between crop stage and treatments also significantly influenced microbial biomass carbon of the soil. Soil microbial properties was found to be significantly increased with crop aging, i.e. minimum activity was recorded at 0 day sample, which increased with increase in days of sowing and maximum activity was recorded in soil samples on 90 DAS of wheat crop. In general these activities were found to be highest in T_7 treatment (application of

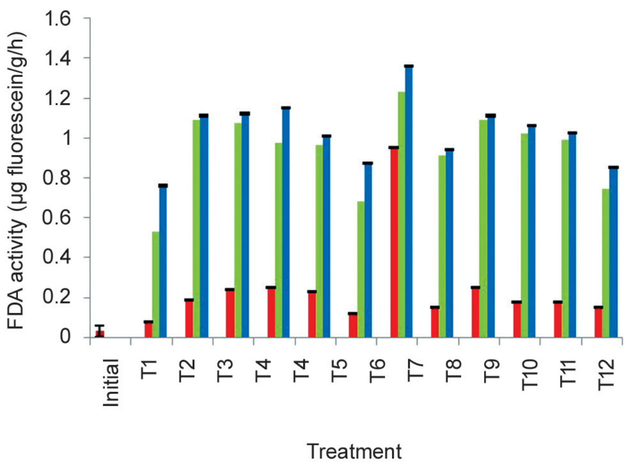

Fig 3 Influence of different treatments on Fluorescein diacetate hydrolysis (FDA) in soil at different stages of crop growth

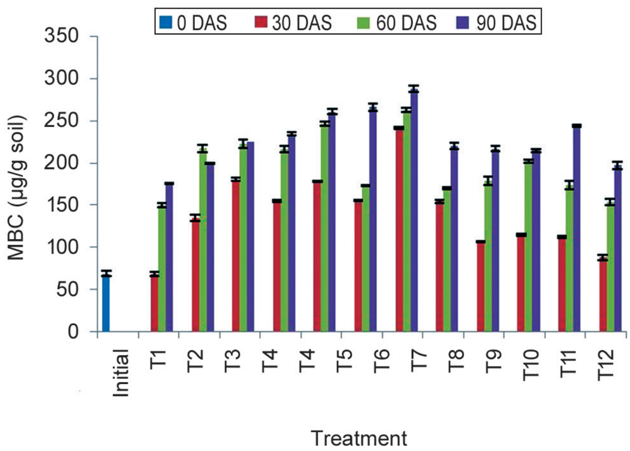

Fig 4 Influence of different treatments on MBC in soil at different stages of crop growth.

PSF @0.83 ml/kg seed with humic acid @10 kg/ha) in all the sampling intervals, while lowest was recorded in Control. Variability of soil microbial activity in the plots where treatments were applied might be due to micro-climate created by the various treatments, availability of substrate and microorganism density as reported by Balota *et al.* (1998).

It may be concluded from the results of the present investigation that organic input in the form of Phosphate solubilising fungi (PSF, Jumpstart 2.0), phosphate solublizing bacteria (PSB), AM Fungi and humic acid (Bolt GR), either alone or in different combinations may led to a higher microbial biomass and activity in the soil. These microbes/inputs not only supported in building up of population of native microflora but also resulted into improved enzyme activity in the soil, indicating their significant role in crop production by mediating the life process of soil microorganism, soil structure stabilization, organic wastes decomposition, organic matter formation and overall nutrient cycling.

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