Enzyme Activity of Bt Cotton Grown Soils as Influenced by Bio-Fertilizers and Foliar Application of Nutrition

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Abstract
A filed experiment was conducted during rainy season of 2014 to study the effect of bio-fertilizer and foliar application of macro nutrients (N, P and K) on enzyme activity at 60, 90 and 120 days after sowing. Enzyme activity was higher during the flowering period (60 days after sowing) and thereafter the activity decreased with the age of the crop, the lowest activity was recorded at final harvest. Consortia of microbes applied to soil + foliar application of 18:18:18 at 1.5 per cent recorded significantly higher enzyme activity *viz.,* dehydrogenase (11.3 µg TPF g^{-1} day^{-1}), acid phosphatase (142.2 µg PNP released g^{-1} h^{-1}), alkaline phosphatase (101.3 µg PNP released g^{-1} h^{-1}) and urease (95.3 µg NH_4^+ g^{-1} 2 h^{-1}) over other treatments at 60 DAS Significantly higher seed cotton yield also recorded with microbial consortia + foliar application of 18:18:18 at 1.5 per cent (1670 kg ha^{-1}) over other treatments and the control (1004 kg ha^{-1}). This study concludes that, soil application of the bio-fertilizers increases tested enzyme activity during the flowering in rain-fed situations and decreases with the age of the crop.

Keywords: Bio fertilizers, Consortia, Dehydrogenase, Foliar nutrition, Phosphatase, Urease
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Introduction
Cotton (*Gossypium hirsutum* L.) is the most important commercial crop of India cultivated in an area of 12.65 million ha with a production of 40 million bales of lint. Cotton contributes to 80 per cent of the raw material to the textile industry and provides employment to nearly 60 million people. India ranks first in area and second in global cotton production.

Although there are diverse benefits of Bt cotton, public concern also exist because both *in vitro* and *in vivo* studies on Bt cotton have shown that Bt toxin is produced in leaves, stems and roots of Bt cotton plants when introduced to the soil. Bt-toxin from Bt cotton plants are released into the soil through two mechanisms, i.e., biomass incorporation and root exudates. Bt toxin released into soil is adsorbed or bound on clay particles, humic components, or organic mineral complexes and thus is protected against degradation by soil microorganisms. Although Bt toxin is found naturally in many soils, continuous growing of Bt crops on same location can increase the toxin levels to a concentration that might affect the composition and activity of soil biochemical properties.

Soil enzymes are important bio-chemical constituents of the soils, influencing the nutrient transformations, determining the nutrient availability to plant and, ultimately the soil quality. Soil enzyme activity is known to be significantly influenced by the soil/climatic conditions as well as plant nutrition through bio-fertilizers and foliar applied fertilizers (Mastero *et al.*, 2006 and Sarkar *et al.,* 2009).

Studies on impact of soil applied microbial consortia alone or in combination with foliar nutrition of N, P and K on important soils enzyme activity in Bt cotton growing soils, under rain fed conditions, is scanty. With this view, an experiment was conducted to study the influence of microbial consortia of Phosphate solubulizing Bacteria (PSB) +Potassium solubulizing Bacteria (KSB) + *Azotobacter* + Vesicular Arbusular Mycorrhizha (VAM) fungi and foliar application of nutrition on the soil enzyme activity.

Material and Methods
A field experiment was conducted at College Farm, Rajendranagar during rainy season (kharif) 2014 on a sandy clay loam soil with neutral pH (7.4) and low organic carbon (0.34 %). The soil was low, medium and high in the available N (174.8 kg ha^{-1}), P_2O_5 (49.3kg ha^{-1}) and K_2O (422.4 kg ha^{-1}), respectively. The experiment was laid out in a randomized block design (RBD) with 10 treatments replicated thrice with a net plot area of 5.4 m X 3.6 m. An intra hirsutum cotton hybrid Jadhu (Boll-Gaurd II) having semi determinate plant type was used as a test cultivar. Treatments in the experiment included,

T_1 Control (RDF-150:60:60 N, P_2O_5 and K_2O g ha^{-1})
T_2 Consortia of microbes (PSB+KSB+VAM+*Azotobactor*) to soil at 1

L ha^{-1}

T_3 Foliar application of urea at 2 per cent

T_4 Foliar application of KNO_3 at 2 per cent

T_5 Consortia of microbes + Foliar application of urea at 2 per cent

T_6 Consortia of microbes + foliar application of KNO_3 at 2 per cent

T_7 Foliar application of 18:18:18 at 1.5 per cent

T_8 Foliar application of 17:44:0 at 2 per cent

T_9 Consortia of microbes + foliar application of 18:18:18 at 1.5 per cent

T_{10} Consortia of microbes + foliar application of 17:44:0 at 2 per cent

Consortia (PSB and *Azotobactor* are in the form of liquid at 250 ml L-1 and KSB and VAM in the form of powder at 250 g) were mixed well and the mixture was spread uniformly on well decomposed FYM (100 kg/ha) one day before application. FYM was incubated overnight by mainlining optimum moisture and applied to the soil at the time of sowing along with the seed. Foliar sprays were applied at 60, 90 and 120 DAS.

Recommended dose of fertilizers and other package of practices were uniformly adopted in all the treatments for growing healthy crop. Enzymatic activity *viz.,* dehydrogenase, phosphatases and urease in rhizosphere soils was studied by employing standard procedures at the time of flowering (60, 120 DAS) and harvest (153 DAS).

Results and Discussion

Dehydrogenase

Dehydrogenase activity is thought to reflect the total scope of activity of soil microflora and is consequently a good indicator of microbial activity (Nannipieri *et al.*, 2002). Dehydrogenase activity ranged from 3.2 to 11.3 µg TPF produced g^{-1} day^{-1} and highest dehydrogenase activity recorded during flowering stage. As the crop aged, the activity reduced and thus at the final harvest, the least value was recorded. At 60 DAS, significantly higher dehydrogenase activity of 11.3 µg TPF produced g^{-1} day^{-1} (Table 1) recorded with consortia of microbes applied to soil combined with foliar application of 18:18:18 at 1.5 percent than other treatments where consortia of microbes was not applied to soil and was on par with consortia of microbes combined with foliar application of KNO_3 (10.8 µg TPF produced g^{-1} day^{-1}) at 2 per cent and Consortia of microbes applied to soil (10.4 µg TPF produced g^{-1} day^{-1}). Significantly lower activity of 7.4 µg TPF produced g^{-1} day^{-1} recorded with control than the remaining treatments. At final harvest, significantly higher dehydrogenase activity of 5.4 µg TPF produced g^{-1} day^{-1} recorded with consortia of microbes with foliar application of18:18:18 at 1.5 per cent than all the treatments. Control recorded significantly lower activity of 3.2 µg TPF produced g^{-1} day^{-1} and was on par with all foliar nutrition treatments. Similar results were reported by Sarkar *et al.,* 2009.

Phosphatases (Acid and Alkaline)

Activity of acid and alkaline phosphatase was significantly higher 142.2 (µg TPF g-1 day^{-1}) and 101.3 µg PNP released g^{-1} h^{-1} in soils at 60 DAS recorded with microbial consortia applied to soil and foliar application of 18:18:18 at 1.5per cent than all other treatments. Significantly lower acid and alkaline activity (99.5 µg PNP released $g^{-1}h^{-1}$ and 54.9 µg PNP released $g^{-1}h^{-1}$) in soils recorded with control. At final harvest, significantly higher acid phosphatase activity of 116 µg PNP released g^{-1} h^{-1} in soils was recorded with application of microbial consortia with foliar application of 18:18:18 at 1.5 per cent which was at par with combination of microbial consortia and foliar application of KNO_3 (106.8 µg PNP released g^{-1} h^{-1} in soils) at 2 per cent, alkaline phosphatase activity of 82.6 µg PNP released g^{-1} h^{-1} in soils was recorded with (T_9).

Phosphotase activity in these treatments was significantly superior to other treatments. Significantly lower acid and alkaline Phosphotase activity than the remaining treatments (73.2 µg PNP released g^{-1} h^{-1} and 50.0 µg PNP released g^{-1} h^{-1}) in soils recorded with control. Acid phosphatase at this stage was at par with foliar application of urea (84 µg PNP released g^{-1} h^{-1} in soils) at 2 per cent. Alkaline phosphatase activity at final harvest on par with Foliar application of urea (50.7 µg PNP released g^{-1} h^{-1} in soils), KNO_3 (50.7 µg PNP released g^{-1} h^{-1} in soils), 17:44:0 (53.3 µg PNP released g^{-1} h^{-1} in soils) at final harvest than the remaining treatments. This might be due to large quantity of phosphorus fertilizer addition to soil as well as foliar application to meet the crop demand and low initial soil available P. Insect-resistant Bt crops have the potential to change the

Table 1: Soil enzyme activity as influenced by soil application of Microbial consortia and foliar nutrition in rain fed Bt cotton

Treatments	Urease (µg NH_4^+ g^{-1} 2 h^{-1})		Dehydrogenase (µg TPF g-1 day^{-1})		Phosphatase (µg PNP released g^{-1} h^{-1})			
					Acid phosphatase		Alkaline phosphatase	
	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest
Control (150:60:60)	68.8	53.3	7.4	3.2	99.5	73.2	54.9	50.0
Consortia of microbes* to soil @ 1 L ha^{-1}	85.4	67.7	10.4	4.4	122.3	83.5	85.3	62.6
Foliar application (FA*) of 2 per cent Urea	78.8	67.8	9.8	3.7	111.5	84.0	57.1	49.3
Foliar application (FA) of 2 per cent KNO_3	77.0	64.2	9.1	3.5	109.7	95.7	60.1	52.6
Consortia of microbes + FA of 2 per cent Urea	91.3	73.1	10.8	4.1	109.0	100.1	78.6	68.2
Consortia of microbes + FA of 2 per cent KNO_3	84.8	70.9	9.3	4.0	119.9	106.8	82.8	62.1
Foliar application of 1.5 per cent 18:18:18 WSF	79.8	63.3	9.9	3.6	110.9	95.6	67.5	53.3
Foliar application of 2 per cent 17:44:0 WSF	77.8	64.8	9.9	3.8	110.2	94.9	63.2	51.0
Consortia of microbes + FA of 1.5 per cent 18:18:18 WSF	95.3	82.2	11.3	5.4	142.2	116.0	101.3	82.6
Consortia of microbes + FA of 2 per cent 17:44:0 WSF	84.0	66.7	9.3	4.1	116.5	102.3	81.9	61.2
SEM	3.8	3.2	0.4	0.3	3.7	5.6	2.6	3.0

microbial dynamics, biodiversity and essential ecosystem functions in soil, because they usually produce *cry* protein through all parts of the plant, similar reports given by Gregory (2009).

Urease Activity

Activity of urease in soils at 60 DAS recorded with consortia + foliar application of 18:18:18 at 1.5 per cent (95.3 µg NH_4^+ g^{-1} 2 h^{-1}) was on par with all other treatments with consortia of microbes and was significantly higher than the treatments without consortia of microbes. Significantly lower urease activity (68.8 µg NH_4^+ g^{-1} 2 h^{-1}) in soils was recorded with control than foliar application treatments and was on par with remaining treatments without consortia of microbes.

At final harvest, urease activity of 82.2 µg NH_4^+ g^{-1} 2 h^{-1} recorded with consortia of microbes applied to soil with foliar application of 18:18:18 at 1.5 per cent was on par with consortia with foliar application of urea (73.1 µg NH_4^+ g^{-1} 2 h^{-1}) at 2 per cent and was significantly higher than other treatments. Significantly lower activity of urease (53.3 µg NH_4^+ g^{-1} 2 h^{-1}) was recorded with control than remaining treatments.

This might be due to inhibition of urease activity under levated levels of N availability. Studies of Yanyu *et al.* (2011) also indicated negative correlation between urease activity and soil N concentration. The results of Ajwa *et al.* (1999) also support these findings where an inhibitory effect of mineral fertilizers and 15 percent decreased urease activity with application of excess doses of mineral fertilizer were reported.

Conclusions

This study concludes that, application of microbial consortia and foliar application of macro nutrients to *Bt* cotton fields influences the activity of Dehydrogenase, phosphatases and urease enzymes in soil at flowering and harvesting stages of the crop. These enzyme activities are higher during the flowering period (60 DAS) and thereafter steadily decreased with the age of the crop up to the harvesting stage. Soil application of the biofertilizers increased the enzyme activity during the flowering in rain fed situation, however the activity decreased with the age of the crop.

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