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The effects of the microbial amendments, Eco-T (*Trichoderma hazianum*) and Enrich (*Herbaspirillum seropedicae* and *Bacillus subtilis*) on growth and yield of cotton in Ghana

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ABSTRACT

Eco-T is a product containing the fungus *Trichoderma hazianum* strain KD. Enrich contains the bacteria *Herbaspirillum seropedicae* and *Bacillus subtilis.* Both were tested for their effects on growth and yield of cotton in Ghana in two trials. The first trial, in 2011, tested the effect of Eco-T on early growth of cotton. A second trial in 2012 tested the effect of Eco-T, Enrich and fertilizer rate on growth and yield. Results showed increases in growth rates and vigor with either product applied separately or in combination. Both products increased haulm and seed cotton yield in the 2012 trial. Average seed-cotton yield was 375, 835, 795 and 1709 kg ha^{-1} for the control, Eco-T, Enrich, and Eco-T plus Enrich treatments, respectively. Harvest Index (based on yields of seed-cotton and haulm) was 27.2%, 43.8%, 41.8% and 58.2% for the control, Eco-T, Enrich, and Eco-T plus Enrich treatments, respectively. The combination of double inoculation plus full fertilizer gave the highest yield.

1- Introduction

Eco-T is a formulation of spores of the fungus *Trichoderma hazianum* (strain KD), manufactured by Plant Health Products of South Africa (www.plant-health.co.za). This fungus is antagonistic to plant pathogens (Harman et al. 2004; Benitez et al. 2004) but is also known to enhance plant growth through rhizosphere associations (Bailey and Lumsden, 1998). *Trichoderma* increases resistance to stress and boosts plant vigor (Ozbay et al. 2004). These effects have been researched in several countries (e.g. Shanmugaiah et al. 2009; Windham et al. 1986). Eco-T is formulated as a dry powder that is applied to seed at planting.

Enrich is a freeze-dried formulation of selected strains of *Herbaspirillum seropedicae* and *Bacillus subtilis* produced by Thinkbio of Australia (www.thinkbio.com.au). These bacteria can become endophytic colonists of plants, mainly entering through stomata or through cracks in the epidermis or through soft tissue surface (Reis et al. 2000). They have been reported to improve the nitrogen nutrition of plants and to promote plant growth (Mclnroy and Kloepper, 1995; Muthukumarasamy et al. 2006). Reports suggest that

the mode of action for improving growth and yield is complex and not yet fully understood (Windham et al. 1986). Enrich is formulated as freeze-dried bacteria with long viability; this can be reformulated by mixing with water and spraying onto young plants in combination with an applicant that aids penetration into stomata and other points of entry.

There have been no previous trials of either Eco-T or Enrich on cotton in Ghana, but results from other places had led to the assumption that these products should improve the often poor establishment of cotton (Abudulai et al 2007), enhance efficiency of nutrient use and increase yield. The objective of these studies was to observe the effect of these products and their interactions on the growth and yield of cotton.

2- Materials and methods

The trials were conducted at the research farm of Ghana's Council for Scientific and Industrial Research, Savanna Agricultural Research Institute located in Nyankpala (9 º 42' N latitude, and 0 º 92' W longitude. and 184 m altitude). The area experiences a unimodal rainfall pattern with the main rains falling from May to October with a peak occurring in September and an average annual rainfall of 1022 mm (Kasei, 1990). The soil has a sandy loam texture, developed from voltaian sandstone and classified as Nyankpala series (SARI, 1994). Some weather data at the experimental site in 2011 and 2012 are shown in Figure 1.

- 2011 Trial:

The trial in 2011 monitored the early growth of cotton seedlings treated with Eco-T during the first 40 days of growth. There were two treatments: (1) seeds either treated with Eco-T or (2) untreated. No other seed dressing was used. Seeds were planted in pairs of plots with 10 replications. Eco-T was applied by mixing the dry powder with the seed at the rate of 2 grams per kilogram of seed prior to planting. The seeds and Eco-T were mixed by shaking them together in a large plastic bag. Plots were 4 rows wide and 5 m long with spacing of 0.75 m between rows and 0.30 m between plants in a row. A maximum of 100 seeds were planted per plot.

Plant growth was assessed by measuring plant height at 10, 20, 30 and 40 days after emergence (DAE). Plant vigor was assessed by asking five groups of farmers who had no knowledge about the treatments to give their impression about the state of the plants by comparing the treated and untreated plots in each replication. At 40 DAE, all the plants were dug out, the roots were washed and root length measured. Plants were oven dried and dry weight recorded.

- 2012 Trial:

This trial was a 2 x 2 x 2 factorial experiment in a randomized complete block design with 4 replications. The three factors were: (1) Eco-T, (2) Enrich and (3) Fertilizer rate, each with two levels (Table 1). Cotton (cultivar FK 37) was planted on 3 July 2012. Plots were 4 rows 5 m long with spacing of 0.75 m between rows and 0.30 m within rows. The distance between plots was 1.5 m.

Seeds were treated with Eco-T with the same method as for the 2011 Trial. Enrich was prepared by adding the dried bacteria from a 1-acre pack of Enrich to 50 ml of non-chlorinated water. The mixture was left for five minutes for the bacteria to re-activate then added to water in a knapsack sprayer tank with the applicator liquid supplied with the pack. The Enrich mixture was applied when the cotton plants were knee-high on 14 August 2012. Hot dry conditions can reduce the chances of successful inoculation when spraying live bacteria, spraying was therefore done at 5:00 p.m., so following the manufacturer's recommendation to spray when cool and/or cloudy. A basal dressing of NPK fertilizer 23-10-5 was applied at the full rate of 250 kg ha^{-1} or at a rate of 162.5 kg ha^{-1} (65% of the full rate) on 6 August 2012. Plots were top-dressed with Sulphate of Ammonia at 125 kg ha^{-1} and 81.3 kg ha^{-1}, respectively on 20 August 2012. All other crop management practices such as weeding and pests control were carried out according to recommended practice for the area. Plots were harvested on 27 November 2012.

Data were collected on seedling height at 25, 40 and 55 days after seedling emergence (DAE). Plants were scored for greenness on 29 August 2012, three weeks after applying Enrich. The greenness scoring was a simple system where researchers judged plots as being green or not green in relation to the perceived average of the plots as a whole. Seed cotton yield and haulm weights were recorded at harvest.

- Statistical analysis:

All the data taken were analyzed using the general linear model procedure of SAS (SAS Institute, 1998). Data from each trial was analyzed separately. Where significant treatment effects were observed, means were separated using Fisher's Protected Least Significant Difference Test at $P < 0.05$. The sum of seed cotton yield and haulm yield were used as a reasonable approximation of total above ground crop biomass to calculate Harvest Index values, as seed cotton yield divided by sum of seed cotton yield and haulm yield multiplied by 100.

3- Results

3-1- 2011 Experiment

The plant population was not affected by Eco-T treatment (P > 0.05). The farmers' assessment of plant vigor used a score of 0 or 1, where a score of 1 is given to the most vigorous plot of each pair. The average for plots sown with Eco-T treated seed was about 0.60 compared to 0.40 for untreated plots. The respondent farmers also compared greenness between treatments and the average greenness score for plants from treated seeds was 0.60 compared to 0.40 for plants from untreated seeds. Plant height was significantly higher in treated than untreated seeds on all the assessment days except for the first assessment at 20 DAE (Table 2). Plants in treated plots also had significantly longer roots than those in untreated plots (Table 3). Dry biomass weight was higher for seedlings from the Eco-T treated plots but this difference was not significant (P > 0.05).

3-2- 2012 Experiment

There were significant differences between the heights of plants in the different treatments (Table 4). T4 (Eco-T with Enrich and full rate of fertilizer) had the highest average plant height at 25, 40 and 55 DAE. However, the difference was only significant (P<0.05) against mean values in treatments where no microbial products were used (T1 at 25, 40 and 55 DAE and T5 at 55 DAE). At 55 DAE the average height of plants in T4 plots were about 30% higher than in T1 plots.

There were significant differences between treatments with regard to greenness scores (Table 5). At either fertilizer rates, plants were perceived by scorers to be greener in plots that received either Eco-T or Enrich or both treatments, compared to untreated control. The highest greenness score was recorded in plots that received Enrich with or without Eco-T. The average score for the high fertilizer rate treatments was 0.719, while the average score for the lower rate treatments was 0.5625.

The mean seed cotton yield for all plots receiving either full or reduced fertilizer was 973.7 and 883.3 kg ha^{-1} respectively, a significant increase in yield due to additional fertilizer. However, the effect of Enrich and Eco-T on yield was larger (Table 6). Haulm weight was lower at the lower rate of fertilizer and lower without either Eco-T or Enrich, but the difference was only significantly lower in untreated seeds when compared to treated seeds at the higher rate of fertilizer. Harvest index was significantly different (P<0.05) for plants treated with Eco-T and/or Enrich and the untreated control (Table 6). For treated plants, harvest index was significantly higher (P<0.05) for plants treated with both Eco-T and Enrich compared with those treated with either Eco-T or Enrich. Mean harvest Index was 27.2%, 43.8%, 41.8% and 58.2% for the control, Eco-T, Enrich, and Eco-T plus Enrich treatments, respectively.

4- Discussion and conclusions

An assumption made when planning the trials was that these products should increase plant growth rates; therefore plant height, root length and greenness scoring were used as indirect indicators of growth and vigor. Results showed that treatment with Eco-T positively affected growth and vigour of cotton plants, as evidenced by increased plant height, root length and farmer assessment in 2011. In the 2012 trial plant height and greenness scoring also indicated a positive effect of both products on plant growth. The fact that *T. hazianum* induces a growth response has been reported for many crops including cotton (Windham et al. 1986; Bailey and Lumsden, 1998; Ozbay et al. 2004). Endophytic bacteria such as *H. seropedicae* and *B. subtilis* have been reported to improve nitrogen use efficiency and growth in plants in other research (Mclnroy and Kloepper, 1995; Rudresh et al. 2005; Muthukumarasamy et al. 2006). The mode of action appears complex and some researchers credit increased crop performance to better efficiency of uptake of phosphate and other nutrients including nitrogen (Muthuumarasamy et al. 2006). These plant microorganisms are also known to be antagonistic to minor pathogens once they have colonized host plants, and this would also contribute to stronger growth and better yield (Ousley et al. 1993; Inbar et al. 1994). *T. hazianum* treatment has been reported as shortening the emergence time of tomato, tobacco and corn (Windham et al. 1986), however, this effect was not observed in these trials. Seed cotton yield measured in 2012 ranged from 350 kg ha^{-1} in untreated plots up to 1770 kg ha^{-1} in plots that were treated with the full dose of fertilizer (250 kg ha^{-1}) and with both inoculants. This large range showed that these products can substantially increase yields when conditions are favourable. This is consistent with trials in California which also reported that *T. hazianum* treatment resulted in increased seed cotton and lint yield (Windham et al. 1986). In this 2012 study, square initiation and onset of boll formation coincided with a dry spell in August (see Figure 1) and it may be that the inoculants had increased above and below ground plant vigor allowing treated plants to yield well in conditions where otherwise they would have failed to thrive. However, the differences in haulm weights between treatments in 2012, although large, were smaller than the differences in yield. This perhaps suggested that during the reproductive growth, from flowering to boll maturity,

the treated plants produced more flowers and retained more through to boll maturity. For untreated plots, more flowers and immature bolls perhaps abscised due to stress factors. The increased seedling height, greenness and harvest weights confirm that there should be more assimilates available to be partitioned to reproductive organs. Muthukumarasamy et al. (2006) also reported that co-inoculation enhanced biomass production in sugarcane under nitrogen limited condition; other researchers have noted the same effect and also linking this to improved nutrient uptake and utilization (Rudresh et al. 2005).

The manufacturers and distributors of these products cannot guarantee the cost of either product to farmers, because this depends on a range of factors related to the local distribution network. However, they have indicated under normal trading conditions, the cost for both products combined should be as low as $30-$40 per ha. At this cost estimate and with 2012 farm-gate seed cotton values of $0.4 - $0.5 per kg, these products would increase farm profits if used in conditions similar to this trial.

In conclusion, the growth and yield of cotton plants were improved with the application of both Eco-T and Enrich. The inoculants increased seed cotton yield irrespective of whether or not plants received the full recommended or reduced dose of nitrogenous fertilizer. The highest yield increases were in treatments with both inoculants. An additional trial that measures the components of yield is needed to understand with more accuracy how these products increased yield to such high levels.

5- Acknowledgment

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Table 1- Trial design and treatments.

Treatments	Fertilizer Proportion of full rate	Eco-T 1=inoculation 0=no inoculation	Enrich 1= inoculation 0 =no inoculation
T1	100%	0	0
T2	100%	1	0
T3	100%	0	1
T4	100%	1	1
T5	65%	0	0
T6	65%	1	0
T7	65%	0	1
T8	65%	1	1

Table 2- Effect of Eco-T seed treatments on mean plant height in 2011.

Treatment*	Days after emergence			
	10	20	30	40
Treated seed	6.4 a	13.4 a	22.1 a	35.4 a
Untreated seed	5.9 b	13.3 a	20.0 b	31. 5 b
Prob.	0.0250	0.9254	0.0049	0.0043
CV (%)	10.6	8.3	10.4	12.0

*Treated seeds were coated with the dry powder of Eco-T at the rate of 2 grams per kilogram of seed prior to planting.

Table 3- Effect of Eco-T treatment on mean root length and mean plant dry weight in 2011.

Treatment*	Root length (cm)	Dry weight (g)
Treated seed	13.1 a	8.2 a
Untreated seed	12.0 b	6.7 a
Prob.	0.0042	0.0630
CV (%)	8.8	32.1

*Treated seeds were coated with the dry powder of Eco-T at the rate of 2 grams per kilogram of seed prior to planting.

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Table 4- Effect of treatments on cotton plant height (cm) in 2012.

Treatment*	Fertilizer rates	Eco-T	Enrich	Seedling height at 25 DAE	Seedling height at 40 DAE	Seedling height At 55 DAE
T1	100%	0	0	12.55	25.6	51.4
T2	100%	1	0	15.66	36.5	64.6
T3	100%	0	1	16.11	33.3	61.0
T4	100%	1	1	17.45	36.7	71.5
T5	65%	0	0	14.65	28.3	54.0
T6	65%	1	0	16.97	34.4	63.8
T7	65%	0	1	16.52	32.1	61.3
T8	65%	1	1	16.55	31.2	61.3
LSD (P<0.05)				3.28	9.50	14.66

*100% fertilizer rate applied was equivalent to the full dose of 250 kg while 65% rate was equivalent to 162.5 kg of NPK or 125 kg and 81.3 kg Sulphate of Ammonia, respectively. Eco-T was applied by coating seeds with the dry powder at the rate of 2 grams per kilogram of seed prior to planting. A 1-acre pack of Enrich bacteria was reactivated in 50 ml non-chlorinated water to prepare a foliar spray and applied to cotton plants at knee-high at 5.00 pm.

Table 5- Effect of treatments on cotton greenness score in 2012.

Treatment*	Fertilizer	Eco-T	Enrich	Greenness score
T1	100%	0	0	0.375
T2	100%	1	0	0.625
T3	100%	0	1	1.000
T4	100%	1	1	0.875
T5	65%	0	0	0.125
T6	65%	1	0	0.375
T7	65%	0	1	1.000
T8	65%	1	1	0.750
LSD (P<0.05)				0.4061

*100% fertilizer rate applied was equivalent to the full dose of 250 kg while 65% rate was equivalent to 162.5 kg of NPK or 125 kg and 81.3 kg Sulphate of Ammonia, respectively. Eco-T was applied by coating seeds with the dry powder at the rate of 2 grams per kilogram of seed prior to planting. A 1-acre pack of Enrich bacteria was reactivated in 50 ml non-chlorinated water to prepare a foliar spray and applied to cotton plants at knee-high at 5.00 pm.

Table 6- Effect of treatments on seed cotton and haulm yield and harvest index in 2012.

Treatment*	Fertilizer rates	Eco-T	Enrich	Seed cotton Kg ha^{-1}	Cotton haulm Kg ha^{-1}	Harvest index %
T1	100%	0	0	400	1220	24.6
T2	100%	1	0	887	1203	42.7
T3	100%	0	1	837	1183	41.7
T4	100%	1	1	1770	1317	57.6
T5	65%	0	0	350	847	29.8
T6	65%	1	0	783	1003	44.9
T7	65%	0	1	753	1070	41.9
T8	65%	1	1	1647	1167	58.8
LSD (P<0.05)				109	325.6	8.0

*100% fertilizer rate applied was equivalent to the full dose of 250 kg while 65% rate was equivalent to 162.5 kg of NPK or 125 kg and 81.3 kg Sulphate of Ammonia, respectively. Eco-T was applied by coating seeds with the dry powder at the rate of 2 grams per kilogram of seed prior to planting. A 1-acre pack of Enrich bacteria was reactivated in 50 ml non-chlorinated water to prepare a foliar spray and applied to cotton plants at knee-high at 5.00 pm.

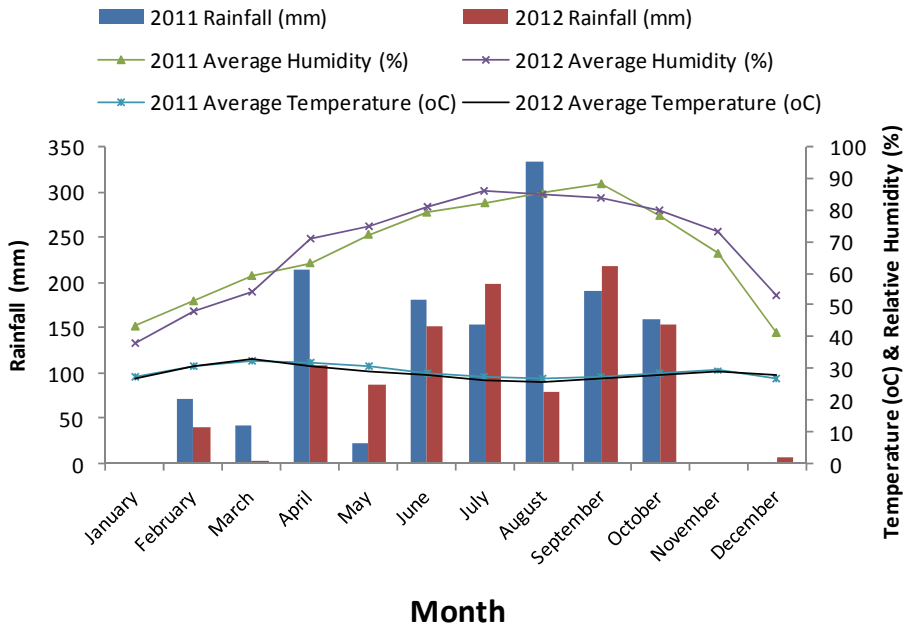

Fig. 1- Mean monthly rainfall, relative humidity and temperature at the experimental site during 2011 and 2012.

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