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Trichoderma-Enriched Biofertilizer: A Prospective Substitute of Inorganic Fertilizer for Mustard (*Brassica campestris*) Production

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Abstract

Effects of *Trichoderma*-enriched biofertilizer such as biofertilizer/compost [BioF/compost (household/kitchen wastes composted with *Trichoderma harzianum* T22)] and biofertilizer/liquid [BioF/liquid (*T. harzianum* T22 broth culture contains spores and mycelia)] alone or in combination with NPK fertilizer were evaluated for the growth, dry matter production, yield and yield attributes of mustard (*Brassica campestris*) grown under field condition. BioF/compost performed better than that of BioF/liquid. Recommended doses of NPK and 50% BioF/compost + 50% NPK showed similar effects on growth, dry matter accumulation and yield of mustard. Seed yield per plant was increased by 5.34% over the recommended dose of NPK, when the crop was fertilized with 50% BioF/compost along with 50% NPK. However, seed yield per plant was decreased only by 7.3 and 6.6% when BioF/compost, and 75% BioF/compost + 25% NPK were applied as compared to the recommended dose of NPK. Since 20% reduced yield is accepted in organic faming worldwide, the treatments namely BioF/compost, 50% BioF/compost + 50% NPK and 75% BioF/compost + 25% NPK might be recommended for mustard cultivation in Bangladesh, which may reduce cultivation cost and also reduce environmental pollution.

Keywords: Mustard, growth, dry matter production, seed yield, biofertilizer

1. Introduction

Inorganic fertilizer plays a significant role in environmental pollution. Among the inorganic fertilizers, nitrogen fertilizer increases denitrification, resulting in elevated emission of nitrous oxide (N_2O) to the atmosphere which contributes to global warming (Smith *et al*., 2008). It has also been reported that application of nitrogen fertilizers may deplete soil organic carbon in the long run (Khan *et al*., 2007). Instead, biofertilizers, the products containing living cells of different microorganisms, can prevent the depletion of the soil organic matter (Jeyabal and Kuppuswamy, 2001). It has also been reported that application of biofertilizers increases yield and reduce environmental pollution (Mia and Shamsuddin, 2010).

Fungi of the genus *Trichoderma* are biocontrol agents that are successfully used as biopesticides worldwide. Several species of *Trichoderma* are reported to produce secondary metabolites with antibiotic activity (Harman *et al.*, 2004; Reino *et al*., 2008; Vinale *et al*., 2008a). It is well documented that the interaction of *Trichoderma* strains with the plant may promote growth, improves crop yield, increase nutrient availability and enhance disease resistance (Harman *et al*., 2004; Benítez *et al*., 2004). In addition, some species of *Trichoderma* are able to colonize root surfaces, interact with the plant, and exchange compounds that can cause substantial changes in plant metabolism (Yedidia *et al*., 2001). It is also reported that *Trichoderma* metabolites or roots colonization by

Trichoderma, changes the proteome and transcriptome of plants (Marra *et al*., 2006; Alfano *et al*., 2007; Shoresh and Harman, 2008). Thus, the mechanisms of *Trichoderma*-plant interaction are too complex.

In Bangladesh, *Trichoderma* species are mainly used as biocontrol of soil and seed borne pathogens. In this study, performance of household wastes composted with *T. harzianum* T22 (here termed as BioF/compost) and broth of *T. harzianum* T22 containing spores and mycelia (termed as BioF/liquid) were evaluated alone or in combination with inorganic (NPK) fertilizer on plant growth, dry matter accumulation, yield components and seed yield of mustard (*Brassica campestris*) cv. BARI sorisa-16 grown under field condition.

2. Materials and Methods

2.1. Experimental area and whether data

A field experiment was conducted at agricultural research farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh during winter of 2009-2010 (Rabi season) using mustard as a test crop. The initial N, P, and K contents of the soils in the experimental plot were determined following standard procedures. Total N, available P and exchangeable K were 0.091, 16 μg/g soil and 0.32 g/kg soil, respectively. Data on maximum and minimum air temperature, soil temperature in different depth, relative humidity, rainfall, evaporation and ground water table were recorded in every 10 days from 1st November 2009 to 30th March 2010 (Table 1).

2.2. Seed sowing

Seeds of BARI sorisa-16 were sown on 15 November in line. The line spacing was 30 cm and plant to plant distance was 3-4 cm. The unit plot size was 2.5 m × 2.0 m. The crop husbandry operations such as thinning, weeding, irrigation etc. were done as and when necessary.

2.3. Experimental design and treatments

The experiment was conducted in a randomized complete block design (RCBD) using eight treatments with three replications. The treatments were: (T_1) control (without biofertilizer and inorganic fertilizer), (T_2) recommended dose of NPK, (T_3) BioF/compost (household/kitchen wastes composted with *T. harzianum* T22), (T_4) 50% BioF/compost + 50% NPK, (T_5) 75% BioF/compost + 25% NPK, (T_6) BioF/liquid (the broth contains spores and mycelia of *T. harzianum* T22 grown in liquid media), (T_7) 50% BioF/liquid + 50% NPK, and (T_8) 75% BioF/liquid + 25% NPK.

2.4. Collection of biofertilizers and application of fertilizers

Trichoderma harzianum T32 enriched biofertilizers (BioF/compost and BioF/liquid) were collected from Natore Development Society (NDS) and M/S RASH Agro Enterprise, Natore-6400, Bangladesh**.**

For the high yield goal of mustard, the required amount of N, P and K were determined using the following equation (Anonymous, 2005)

$$F_r = U_f - C_i/C_s \times (S_t - L_s)$$

Where, F_r = Fertilizer nutrient required for given soil test value, U_f = Upper limit of the recommended fertilizer nutrient for the respective STVI class, C_i = Units of class intervals used for fertilizer nutrient recommendation, C_s = Units of class intervals used for STVI class, S_t = Soil test value and L_s = Lower limit of the soil test value within STVI class.

Recommended dose of urea for N, triple super phosphate (TSP) for P and muriate of potash (MP) for K were 79, 33.5 and 17 g for 5 m^2 plot. The whole triple super phosphate and muriate of potash and half of urea were applied during final plot preparation and the remaining urea was applied after 25 days of seeding.

N, P, K contents of the BioF/compost was determined. N, P and K were 1.63, 0.142 and 1.35%, respectively. BioF/compost was applied at the rate of 618 g/plot during final land preparation. However, BioF/liquid was applied at the rate of 25 ml/line after seed germination.

3.5. Data collection

Data were collected on an individual plant basis from 10 randomly selected plants of each plot in such a way that the border effect was avoided for high precision. Plant height and number of branches per plant were recorded during harvest. Root and shoot dry (oven dried at 70°C for 3 days) weight (g) of plants were determined at 50% flowering stage. Number of siliqua per plant, length of siliqua (cm) and number of seeds per siliquae were recorded after harvesting of the crop. However, seed yield per plant (g) was estimated after cleaning and proper drying.

3.6. Statistical analysis

Analysis of variance and comparison of means were calculated separately with statistical package MSTAT-C. The means were compared using the least significance difference (LSD) test. The significance of difference between the pairs of treatment means was evaluated by Duncan's Multiple Range Test (DMRT) at 95% confidence levels ($P \leq 0.05$).

3. Results and Discussion

3.1. Effect on vegetative growth

Plant height and number of branches per plant were significantly influenced by the applied biofertilizer alone or in combination with NPK fertilizer (Table 2). The plant height of mustard (cv. BARI sorisa-16) was significantly ($P \leq 0.05$) higher in recommended dose of NPK, BioF/compost, 50% BioF/compost + 50% NPK and 50% BioF/liquid + 50% NPK. However, the shortest plant height was recorded in control, BioF/liquid and 75% BioF/liquid + 25% NPK. The maximum number of branches (8.33) per plant was achieved by the 50% NPK + 50% BioF/liquid, which was statistically similar to recommended dose of NPK (7.85) and 50% BioF/compost + 50% NPK (8.0). BioF/compost and 75% BioF/compost + 25% NPK were identical with recommended dose of NPK, 50% BioF/compost + 50% NPK and 50% BioF/liquid + 50% NPK. The control treatment produced the lowest number of branches per plant, followed by BioF/liquid and 75% BioF/compost + 25% NPK. Shoot length of *B. campestris* was significantly enhanced by combined exposure of chemical fertilizer, biofertilizer and compost (Datta *et al*., 2009). Harzianic acid, a secondary metabolite produced by *T. harzianum*, was reported to have increased the growth of canola (Vinale *et al*., 2009a). The enhanced plant growth by *Trichoderma* (*T. harzianum* strain T22, T39 and A6) might be due to production of secondary metabolites which may act as an auxin like compound (Vinale *et al*., 2008a, b). It has been shown that *Trichoderma* spp. increased nutrient uptake through enhanced root growth or promoted availability of necessary nutrients leading to growth of the plants (Harman *et al*., 2004). Moreover, *Trichoderma* reduced the concentrations of substances in soil that are inhibitory to plant growth (Windham *et al*., 1986; Kleifeld and Chet, 1992; Wang *et al*., 2000). It has also been reported that *T. harzianum* 1295-22 could improve nitrogen use efficiency and could solubilize a number of poorly soluble nutrients, such as Mn^{4+}, Fe^{3+} and Cu^{2+} etc. leading to plant growth and development (Altomare *et al*., 1999). Thus, one or several mechanisms might be involved in the regulation of growth of mustard by *Trichoderma*-enriched biofertilizer alone or in combination with NPK fertilizer.

3.2. Effect on dry matter accumulation

Dry matter production was significantly ($P \leq 0.05$) influenced by biofertilizer and chemical fertilizer (Table 2). Recommended dose of NPK, BioF/compost, 50% BioF/compost + 50% NPK and 50% BioF/liquid + 50% NPK produced the highest root and shoot dry matter weight per plant. The lowest root and shoot dry matter weight was obtained in control. The increased root dry matter weight might have increased the nutrient uptake through maximum exploitation of soils. Datta *et al*., (2009) showed that root length of *B. campestris* was increased by combined application of NPK fertilizer, Azophos (biofertilizer) and organic manure. It has been reported that sole application of *Trichoderma* spp. increased both root and shoot growth of corn (Björkman *et al*., 1994).

Table 1. Weather data 10 days intervals during the study period

Month	Days	Air tem. (^oC)		Soil temp. (^oC) in depth (cm)			Humi-dity	Rainfall (mm)	Ground water table
		Max.	Min.	10	20	30	(%)		(m)
November 2009	1	28.0	26.0	27.5	28	28.5	84.0	00.0	16.58
	10	27.0	25.0	27.0	27.5	28.0	76.0	00.0	16.61
	20	26.0	22.0	26.0	26.5	27.0	83.0	00.0	16.67
	30	24.0	16.0	23.0	23.5	23.5	76.0	00.0	16.02
December 2009	40	21.0	16.0	21.5	22.0	22.5	91.0	00.0	15.62
	50	19.0	16.0	20.0	20.5	20.5	91.0	00.0	16.48
	60	16.0	13.0	18.0	18.5	18.5	92.0	00.0	16.59
January 2010	70	14.0	11.0	16.0	16.5	17.0	90	00.0	16.72
	80	16.0	13.0	17.0	17.5	18.0	92.0	00.0	16.80
	90	18.5	14.5	18.0	18.5	19.0	92.0	00.0	16.50
February 2010	100	24.0	20.0	22.0	22.5	22.5	78.0	00.0	15.60
	110	24.0	18.0	20.5	21.0	21.0	91.0	00.0	18.36
	118	27.0	22.0	22.0	22.5	23.0	98.0	00.0	18.41
March 2010	130	29.0	24.0	22.0	22.5	23.0	68.0	00.0	18.56
	140	34.0	27.0	23.5	23.5	24.0	83.5	00.0	17.70
	150	30.0	24.0	24.5	25.0	25.5	83.5	2.92	17.60

Table 2. Effect of chemical fertilizer alone or in combination with biofertilizer on plant height, number of branches and dry matter production of mustard

Treatments	Plant height (cm)	Number of branches	Dry matter weight (g)	
			Root	Shoot
Control (T_1)	71.20 b	4.06 d	0.27 b	1.46 c
Recommended dose of NPK (T_2)	134.94 a	7.87 a	1.84 a	8.85 a
BioF/compost (T_3)	129.47 a	7.00 ab	1.38 a	7.10 a
50% BioF/compost + 50% NPK (T_4)	127.60 a	8.00 a	1.61 a	8.81 a
75% BioF/compost + 25% NPK (T_5)	129.47 a	6.43 abc	1.43 a	8.95 a
BioF/liquid (T_6)	77.30 b	4.60 cd	0.40 b	1.86 bc
50% BioF/liquid + 50% NPK (T_7)	113.80 a	8.33 a	0.62 b	3.63 b
75% BioF/liquid + 25% NPK (T_8)	72.10 b	5.40 bcd	0.43 b	2.74 bc
CV (%)	11.76	17.45	28.73	18.86
LSD at 0.5%	21.78	1.95	0.49	1.77

Figures in a column having common letters did not differ significantly at 5% level of significance

3.3. Effect on seed yield components

Seed yield components such as number of siliqua per plant, length of siliquae, number of seeds per siliquae and seed yield per plant were significantly influenced by chemical and biofertilizer (Table 3). Number of siliqua per plant was significantly higher in recommended dose of NPK that was statistically similar to BioF/compost, 50% BioF/compost + 50% NPK and 50% BioF/liquid + 50% NPK. The lowest number of siliqua per plant was found in control and BioF/liquid. The longest siliquae was also found in recommended dose of NPK that was not statistically different from the treatments 50% BioF/compost + 50% NPK and 50% BioF/liquid + 50% NPK. Recommended dose of NPK and 50% BioF/compost + 50% NPK produced significantly higher number of seeds per siliquae, followed by the treatments of BioF/compost, 75% BioF/compost + 25% NPK, BioF/liquid and 50% BioF/compost + 50% NPK. However, the least number of seeds per siliquae was recorded in control, which was statistically similar to 75% BioF/liquid + 25% NPK. Length of siliquae and number of seeds per siliquae were also significantly increased in *B. campestris* by application of chemical, biofertilizer and organic manure in India as reported by Datta *et al*.(2009)

3.4. Effect on seed yield

Seed yield per plant was significantly influenced by chemical and biofertilizer (Table 4). Recommended dose of NPK produced significantly the higher seed yield per plant (4.93 g), which was followed by the 50% BioF/compost + 50% NPK, BioF/compost and 75% BioF/compost + 25% NPK. 2009).

Table 3. Effect of chemical fertilizer alone or in combination with biofertilizer on seed yield contributing characters of mustard

Treatments	Number of siliq	Length of siliquae (cm)	Number of seeds/siliquae
Control (T_1)	41.27 c	4.13 b	9.77 b
Recommended dose of NPK (T_2)	191.77 a	5.02 a	14.10 a
BioF/compost (T_3)	174.30 a	4.63 ab	12.16 ab
50% BioF/compost + 50% NPK (T_4)	187.90 a	5.00 a	13.43 a
75% BioF/compost + 25% NPK (T_5)	90.40 b	4.88 a	11.67 ab
BioF/liquid (T_6)	49.23 c	4.34 ab	11.03 ab
50% BioF/liquid + 50% NPK (T_7)	166.53 a	4.75 ab	11.16 ab
75% BioF/liquid + 25% NPK (T_8)	67.80 bc	4.82 ab	9.67 b
CV (%)	15.78	8.27	14.58
LSD at 0.5%	33.09	0.67	2.93

Figures in a column having common letters did not differ significantly at 5% level of significance

Table 4: Effect of chemical fertilizer alone or in combination with biofertilizer on seed yield of mustard

Treatments	Seed yield per plant (g)	% Yield increased over control treatment	% Yield increased over recommended dose of NPK
Control (T_1)	1.10 d		76.50 (-)
Recommended dose of NPK (T_2)	4.68 ab	325.5	
BioF/compost (T_3)	4.34 ab	294.5	7.3 (-)
50% BioF/compost + 50% NPK (T_4)	4.93 a	348.2	5.34 (+)
75% BioF/compost + 25% NPK (T_5)	4.37 ab	297.3	6.62 (-)
BioF/liquid (T_6)	1.27 d	15.5	72.86 (-)
50% BioF/liquid + 50% NPK (T_7)	2.71 c	146.4	42.09 (-)
75% BioF/liquid + 25% NPK (T_8)	1.82 cd	65.5	61.11 (-)
CV (%)	18.05		
LSD at 0.5%	0.95		

Figures in a column having common letters did not differ significantly at 5% level of significance. (-) = decrease and (+) = increase

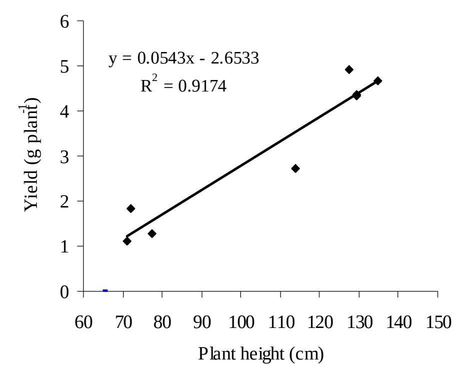

Fig. 1a. Relationship between plant height (cm) and seed yield

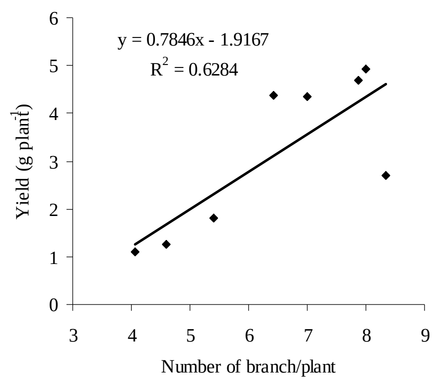

Fig. 1b. Relationship between number of branch per plant and seed and seed yield

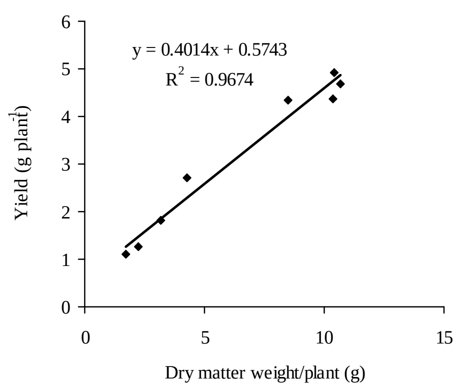

Fig. 1c. Relationship between dry matter weight/plant and seed yield

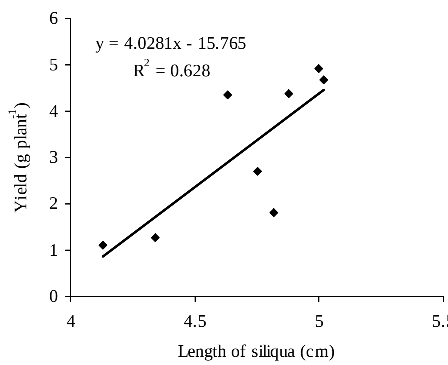

Fig. 1d. Relationship between length of siliqua and seed yield

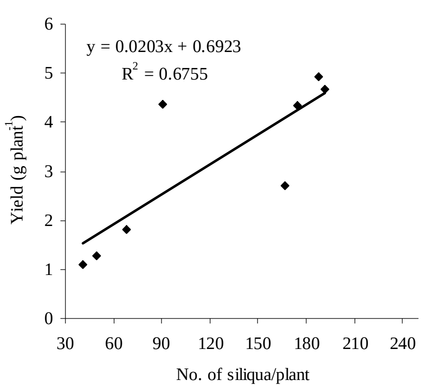

Fig. 1e. Relationship between no. of siliqua/plant and seed yield

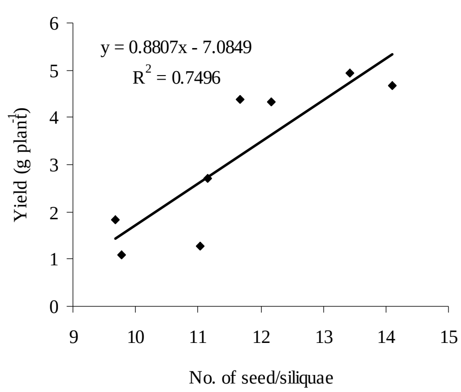

Fig. 1f. Relationship between no. of seed/siliquae and seed yield

However, the lowest seed yield per plant was found in control and BioF/liquid. In contrast to the control, seed yield per plant was dramatically increased in all the treatments such as recommended dose of NPK (T_2), BioF/compost (T_3), 50% BioF/compost + 50% NPK (T_4), 75% BioF/compost + 25% NPK (T_5), BioF/liquid (T_6), 50% BioF/liquid + 50% NPK (T_7) and 75% BioF/liquid + 25% NPK (T_8) by 325.5, 294.5, 348.2, 297.3, 15.5, 146.4, and 65.5%, respectively (Table 4). However, only 50% BioF/compost + 50% NPK (5.34%) gave superior yield over standard dose of NPK (Table 4). Seed yield per plant was decreased by 7.3 and 6.62% in the BioF/compost (T_3) and 75% BioF/compost + 25% NPK (T_5), respectively. Generally, 20% decreased yield is expected in plants cultivated in organic systems than conventionally produced crops (Rembiałkowska, 2007). Thus, BioF/compost (T_3), 50% BioF/compost + 50% NPK (T_4) and 75% BioF/compost + 50% NPK (T_5) may be recommended for mustard cultivation in Bangladesh.

3.5. Relationship between different attributes and seed yield of mustard

A positive linear relationships were found among seed yield/plant and plant height (R^2 =0.9174), number of branch/plant (R^2=0.6284), dry matter weight/plant (R^2= 0.9674), length of siliqua (R^2=0.628), number of siliqua/plant (R^2= 0.6755) and number of seed/siliquae (R^2=0.7496) (Fig. 1a-f).

4. Conclusions

Seed yield per plant was increased by 5.34% when mustard plants were fertilized with 50% NPK along with 50% BioF/compost over the recommended dose of NPK fertilizer application. In contrast, only 7.3 and 6.62% decreased seed yields per plant were observed when mustard plants fertilized with BioF/compost and 75% BioF/compost along with 25% NPK fertilizer over the recommended dose of NPK fertilizer, respectively. Thus, there is a scope of using *Trichoderma*-enrich biofertilizer for higher yield, reduced cultivation cost and environmental pollution.

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