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Full Length Research Paper

Increasing the efficacy of *Trichoderma harzianum* for nutrient uptake and control of red rot in sugarcane

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The application of *Trichoderma* multiplied culture (TMC) of *T. harzianum* strain Th 37 @ 20 kg/ha on the stubbles at the ratoon initiation stage increased the availability of nitrogen (N), phosphorus (P) and potassium (K) by 27, 65 and 44%, respectively. Availability of some of the micronutrients viz., Cu, Fe, Mn and Zn were also enhanced, respectively by 6, 100, 79 and 66%. There was a considerable increase in organic carbon (55%) with concomitant decrease in soil pH (6%).There was significant difference in nutrient uptake between highly red rot susceptible variety (CoLk 7710) and moderately resistant variety (CoS 96268). The increase of N uptake was more in susceptible CoLk 7701 (69%) where as the uptake was only 45% in moderately resistant CoS 96268. P uptake increased by 96% in CoLk 7701 and 69% in CoS 96268. K uptake increased 128% over control of CoLk 7701 and 59% in CoS 96268. The level of protection against red rot increased up to 78% in combination with TMC + salicylic acid (SA) and 86% with metabolites + SA where as the protection was 60 and 71%, respectively with TMC and metabolites. Application of TMC + SA resulted in significant growth promotion through improvement in ratoon initiation (15%), number of tillers (8%), cane height (5%), girth (4%), number of internode (8%), length of internode (2%), single cane weight (5%) and number of millable cane (4%) which was reflected in the improvement in average yield by 23%.

Key words: Sugarcane, *Trichoderma harzianum*, red rot, *Colletotrichum falcatum*, biocontrol, nutrient uptake, salicylic acid, agrochemicals, growth promotion, management.

INTRODUCTION

Biocontrol of plant pathogens with *Trichoderma*, *Pseudomonas*, *Bacillus* and *Acetobacter* has been established by several workers (Harman, 2006). *Trichoderma spp.* is among the most commonly isolated soil fungi with high bicontrol potential. Due to their ability to protect plants and contain pathogen populations under different soil conditions, these fungi have been widely studied and commercially marketed as biopesticides, biofertilizers and soil amendments. *Trichoderma* spp. also produce numerous biologically active compounds, like cell wall degrading enzymes, secondary metabolites etc. (Vinale et al., 2008). In the last two decades, agricultural soils of the subtropical India have been intensively used for conventional crop production. To boost crop productivity farmers relied heavily on inorganic fertilizers. Excessive and continuous use of inorganic fertilizers is deteriorating soil quality and crop productivity (Dawe et al., 2003). Of late, many producers have adopted organic amendments in sugarcane based production system for sustainable crop growth. Bioagents like *Trichoderma* spp. are now being used to improve the efficacy of organic amendments. Among different species *T. harzianum* is one of the po-tent bioagents against red rot of sugarcane and several other diseases (Singh et al., 2008a, b; Harman, 2006). Improvements in uptake of nutrients and

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growth due to application of *Trichoderma* were also noticed (Srivastava et al., 2006; Yadav et al., 2008; Shukla et al., 2008). Nearly 50 - 55% canes were found free after red rot challenge due to the application of TMC and /or metabolites of *T. harzianum* strain Th 37 (Singh et al., 2008a; Singh et al., 2009). Efforts were, therefore, made to improve upon the level of protection against *C. falcatum* infection by testing the combinations of salicylic acid (SA), carbendazim (Bavistin), $CuSO_4$, $FeSO_4$ and $ZnSO_4$ with *Trichoderma* multiplied culture(TMC) and metabolites (culture filtrate).

MATERIALS AND METHODS

Location and soil characteristics

Field experiments were conducted during the crop seasons of 2006 - 2007 and 2007 - 2008 at the Indian Institute of Sugarcane Research, Lucknow located at 26° 56' N, 80° 52' E and 111 m above mean sea level. Lucknow has semi arid sub-tropical climate with dry hot summer and cold winter. The soil of the experimental field was sandy loam (13.3% clay, 24.5% silt and 62.2% sand) of Indo - Gangetic alluvial origin, very deep (> 2 m), well drained, flat and classified as non calcareous *udic ustochrept*. Area per plot was 27 m^2 with three replications in randomized block design. The crop was planted during 2006 - 2007 using 38000 three-bud cane setts ha^{-1} on 15 March, 2006 at 75 cm row to row spacing.

Soil and plant samples were analyzed to assess the availability of macro and micro nutrients before (0 day) and after 60 days of *Trichoderma* application. Soil samples from 0 - 15 cm depth were collected by soil auger from five spots in the field, thoroughly mixed and homogeneous sample was prepared. Homogenous soil was used for estimation of organic carbon, by the Walkley and Black rapid titration method (Walkley and Black, 1934). Available nitrogen was determined by alkaline $KMnO_4$ method (Subbiah and Asija, 1956), available phosphorus was extracted with 0.5 M sodium bicarbonate solution (pH 8.5) and was determined colorimetrically (Jackson, 1973). Exchangeable potassium was extracted by 1 N ammonium acetate solution and was determined using flame photometer (Jackson, 1973). Soil samples were also analyzed for the DTPA-extractable iron, manganese, copper and zinc following the method of Lindsay and Norvell, 1978 using atomic absorption spectrophotometer.

Development of basic culture of potent strain of *T. harzianum* (Th 37) under laboratory

T. harzianum strain Th 37 was found most potent in the biocontrol of red rot. It was isolated from sugarcane rhizosphere of Kushinagar, Uttar Pradesh, India (Singh et al., 2008 a). For the development of basic culture it was grown on potato dextrose agar (PDA) medium for one week at 28 ± 2°C. Conical flasks (500 ml capacity) containing 200 g of autoclaved (121° C for 1 h) wheat bran: saw dust: tap water mixture(3:1:4; w/w/v) were inoculated with 5 - 6 mm dia agar disks obtained from one week old PDA culture. These flasks were incubated in a BOD incubator under continuous illumination for 15 days. The culture thus developed was taken as nucleus culture for preparation of *Trichoderma* multiplied culture (TMC).

Development of TMC under laboratory

Sulphitation press mud (20 kg) was autoclaved at 121° C for 1 h in two consecutive days. The nucleus culture developed above was mixed thoroughly with press mud and covered with transparent polythene sheet and watered daily to maintain 30% moisture for luxuriant growth of *Trichoderma* in two weeks (Singh and Joshi, 2007). This *Trichoderma* growth on press mud (TMC) was taken for field experimentation.

Development of metabolites under laboratory

The *Trichoderma* culture was grown in potato dextrose broth for 15-20 days at 28 ± 2°C and the culture filtrate was collected through Whatman filter paper No. 1 and subsequently passed through sterilized millipore filter (0.45 µm) to remove the mycelia and spores (Kao and Hsieh,1986). The culture filtrate thus obtained was referred to as metabolites and it was stored in freezer (4°C).

Crop culture

Normal dozes of N, P and K were applied @ 150, 60 and 60 kg ha^{-1} through urea (46.4% N), single super phosphate (6.98% P) and potassium chloride (49.8% K). The N was applied half at planting and half at tiller stage. In addition to the fertilizers TMC was applied @ 20 kg ha^{-1} in furrows before the planting of cane setts. Before covering the setts with soil, chlorpyriphos (20% EC) was sprayed over them to guard against the infestation of termite and early shoot borer. The crop was raised with three pre-monsoon irrigations (23 April, 24 May and 21 June during 2006 in plant crop). The remaining half dose of N (75 kg ha^{-1}) was top-dressed on 22 June.

The plant crop was harvested on 10, March, 2007 and recorded 68 t ha^{-1} cane yields. After harvest of plant crop, dry cane leaves left in the field were spread uniformly and the field was immediately irrigated to facilitate stubble sprouting / ratoon initiation. TMC @ 20 kg ha^{-1} was applied on stubbles at the time of ratoon initiation. N was applied @ 200kg ha^{-1} in ratoon crop. Half dose (100 kg N ha^{-1}) was applied at the time of ratoon initiation in interrow spaces and remaining half (100 kg N ha^{-1}) was applied in second week of April along the rows. Ratoon crop was harvested leaving the border rows and net plot yield was recorded. Two formulations of *T. harzianum* that is, TMC and metabolites were tested in combinations with each of salicylic acid (SA), carbendazim, $CuSO_4$, $FeSO_4$, $MnSO_4$ and $ZnSO_4$.The uniform concentrations (500 ppm) of each of the chemicals, fungicide and SA were taken.

Plant sampling and chemical analysis

Five plants having intact leaves (both dry and green) were selected randomly from sample row (2^{nd} row of plot) of each plot. The selected plants were chopped (2 mm size), homogenized and dried at 70°C for 72 h. The dried samples were ground in a stainless steel Wiley mill. 1 g of the grinded samples was taken and wet digested in concentrated H_2SO_4 for determination of total N and in di-acid-mixture (HNO_3 and $HClO_4$ mixed in 4:1 ratio) for determination of total P and K. The N content was determined by Kjeldahl method using Kjeltec auto - analyzer (Blakemore et al., 1972). P content was determined by vanadomolybdate yellow color method (Piper, 1966) using an UV-spectrophotometer. Total K content was determined using flame photometer. At harvest, five plants were randomly selected from each plot for estimation of growth attributes.

Assessing bicontrol of red rot

During July - August at appropriate temperature (28 - 30°C and the relative humidity 90 - 95%), 30 canes in each treatment were inoculated with *Colletotrichum falcatum* (pathotype Cf 09, 10^6 conidia ml^{-1}) using parafilm technique of inoculation (Duttamajumder and Mishra, 2004). Development of the disease was recorded in 0 - 9 scale (Srinivasan and Bhat, 1961). Complete and partial cane protection from red rot

Table 1. Availability of macro and micro nutrients in soil as influenced by the application of *Trichoderma harzianum.*

SN	Nutrient- unit	TMC applied soil	Control (check)	Increase in nutrient	% increase	SE	CD at 5%
1	N -kg /ha	279.52	219.52	60.00	27.33	3.7882	16.3008
2	P -kg/ha	75.88	45.96	29.92	65.10	1.0307	4.4355
3	K -kg/ha	274.00	190.00	84.00	44.21	5.5075	23.6990
4	Cu- ppm	1.41	1.33	0.08	6.01	NS	NS
5	Fe-ppm	14.68	7.32	7.36	100.54	1.1820	5.0862
6	Mn- ppm	9.98	5.56	4.42	79.49	0.4801	2.0660
7	Zn- ppm	0.88	0.53	0.35	66.00	0.0300	0.1290
8	Organic- C (%)	0.56	0.36	0.20	55.55	0.0115	0.0496
9	Soil pH	7.32	7.82	-0.5	-6.39	-	-

Table 2. Uptake of NPK in CoLk 7701 by application of *Trichoderma harzianum.*

Treatment	N %	P %	K %
TMC	0.71	0.11	1.14
TMC + Bavistin	0.67	0.09	1.06
TMC + $CuSO_4$	0.59	0.08	1.00
TMC + $FeSO_4$	0.64	0.09	0.05
TMC + $ZnSO_4$	0.70	0.11	1.13
TMC + SA	0.64	0.09	1.02
Metabolite	0.70	0.11	1.14
Meta. + Bavistin	0.64	0.09	1.05
Meta. + $CuSO_4$	0.67	0.09	1.10
Meta + $FeSO_4$	0.64	0.08	1.06
Meta. + $ZnSO_4$	0.67	0.09	1.09
Meta. + SA	0.64	0.08	1.07
Control	0.42	0.056	0.50
SE	0.0078	0.0061	0.0136
CD at 5%	0.0161	0.0126	0.0280

infection were recorded as per the methodology described by Singh et al. (2008 a, b).

RESULTS AND DISCUSSION

Increase in availability of macro and micro nutrients in *Trichoderma harzianum* applied soil

The analysis of soil was done after 60 days of treatment. Data of ratoon soil (Table 1) clearly indicate that increase in availability of P was highest (65%) followed by K (44%) and N (27%) in case of TMC applied on stubbles at ratoon initiation stage. Among the micro nutrients, Fe increased maximum (100%). Other nutrients like Mn (79%), Zn (66%) and Cu (6%) also increased. By addition of TMC in soil, organic carbon content increased by 55% whereas the soil pH reduced by 6%. Increased nutrients availability has helped the plants in absorbing higher amount of nutrients to improve the growth and vigour.

Challenge inoculation of red rot was carried out on a highly susceptible variety CoLk 7701 to assess the protection of the disease by the use of *Trichoderma.* N - uptake increased from 0.42 - 0.71% by either of TMC or metabolite. There was little less absorption due to combinations of agrochemicals, carbendazim and SA with TMC and metabolites (Table 2). In these treatments N - uptake was higher by 54%. N-uptake by the CoS 96268, a moderately red rot resistant genotype, was similar to that of CoLk 7701. It was increased from 0.42 - 0.61%. TMC or metabolites singly was better than their combinations with other treatments (Table 2). The combinations increased N uptake by 35%. P-uptake enhanced from 0.056 - 0.110 by the TMC or metabolites alone. Other treatments (combinations) were effective to increase uptake of P by 60% (Table 2). The treatment of TMC + $ZnSO_4$ was also similar to TMC alone in CoLk 7701 for uptake of P. In CoS 96268 the P- uptake was similar to CoLk 7701 (96% higher than check). TMC combined with

Table 3. Uptake of NPK in CoS 96268 by application of *Trichoderma harzianum.*

Treatment	N %	P %	K %
TMC	0.61	0.091	0.82
TMC + Bavistin	0.57	0.089	0.74
TMC + $CuSO_4$	0.57	0.086	0.76
TMC + $FeSO_4$	0.55	0.079	0.70
TMC + $ZnSO_4$	0.57	0.084	0.75
TMC + SA	0.57	0.086	0.76
Metabolite	0.61	0.091	0.81
Meta. + Bavistin	0.57	0.086	0.75
Meta. + $CuSO_4$	0.58	0.089	0.78
Meta + $FeSO_4$	0.55	0.079	0.72
Meta. + $ZnSO_4$	0.59	0.089	0.78
Meta. + SA	0.57	0.084	0.75
Control	0.42	0.053	0.51
SE	0.0125	0.0007	0.0083
CD	0.0259	0.0015	0.0171

Table 4. Increased growth and yield of sugarcane by application of *Trichoderma harzianum.*

Growth character	TMC applied	Control (check)	Total increase	% increase	SE	CD at 5%
Clumps- 000/ha	14.81	12.77	2.04	15.97	0.0057	0.0248
Tiller-000/ha	140.28	128.73	11.55	8.93	0.9468	4.0743
Cane height-m	1.82	1.73	0.09	5.20	0.0057	0.0248
Cane girth-cm	2.39	2.28	0.11	4.82	NS	NS
Internode no./cane	20.56	18.90	1.66	8.78	0.0923	o.3974
Internode length-cm	9.93	9.70	0.23	2.37	0.0115	0.0496
Single cane wt-kg	0.546	0.516	0.030	5.81	0.0034	0.0149
NMC000/ha	108.2	103.2	5.0	4.84	0.0333	0.1434
Yield -t/ha	68.48	55.34	13.14	23.76	0.0288	0.1242

other treatments (Table 3) the P-uptake enhanced by 76% while metabolites combinations it was higher by 69%. K-uptake was higher than the N and P. K increased from 0.5 - 1.14% by TMC or metabolites alone. In combined treatments the K uptake was increased from 0.55 - 1.05%. K uptake was increased from 0.50 - 1.07% by the combined treatments of agrochemical, bavistin and SA with the *Trichoderma* formulations. K uptake was increased by 60.78% due to TMC and 58.82% due to metabolites. In CoS 96268, K uptake was lesser than CoLk 7701. In TMC combinations the K uptake was increased from 0.51 - 0.74%. In metabolites combinations the K uptake was increased from 0.57 - 0.75%.

Plant growth promotion and increase in yield

Results (Table 4) clearly indicate that 23% higher yield of ratoon was achieved with the combined treatment of *T. harzianum* and SA. The increase in yield may be due to higher clumps (15%), tillers (8%) cane height (5%), girth (4%), internode (8%), length of internode (2%), single cane weight (5%) and number of millable cane (4%).

Biocontrol of red rot

Highest level of protection was achieved with the treatment of metabolites + SA (86%) followed by the treatment of TMC + SA (78%). TMC treatment alone could protect red rot in 71% plants and metabolites alone protected 60% plants (Table 5). However, in treatments of TMC combined with agrochemicals and carbendazim the protection was 61 - 70% and with metabolites 48 - 56%. This clearly indicates that addition of SA has boosted the protection level significantly and helped *T. harzianum* for inducing systemic resistance in larger number of plants (Singh et al., 2008 a). Among the other combinations, carbendazim + TMC was better (70% protection) than carbendazim + metabolites (56% protection).

Trichoderma does not adversely affect the beneficial

Table 5. Bio-control of red rot with *Trichoderma harzianum* combined with salicylic acid and agrochemicals in CoLk 7701.

Treatments	Cane basis control of red rot			Total	% control
	R-1	R-2	R-3		
TMC	21	22	21	64	71.12
TMC + Bavistin	24	17	22	63	70.00
TMC + $CuSO_4$	15	20	18	53	58.89
TMC + $FeSO_4$	20	20	15	55	61.11
TMC + $ZnSO_4$	19	23	16	58	64.45
TMC + SA	24	27	20	71	78.89
Metabolite	14	19	21	54	60.00
Meta. + Bavistin	21	12	18	51	56.67
Meta. + $CuSO_4$	16	15	19	50	55.56
Meta + $FeSO_4$	15	18	20	53	58.89
Meta. + $ZnSO_4$	17	18	19	54	60.00
Meta. + SA	27	24	27	78	86.67
Control	8	6	5	19	21.12
SE					8.75
CD					18.06

Canes challenged with red rot / treatment = 30.

microorganisms in the rhizosphere and facilitates the biocontrol of plant diseases (Munnecke, 1972; Papavizas and Lewis, 1981; Singh et al., 2008a). It also possesses the required tolerance to several broad spectrum pesticides. *T. harzianum* has been reported to compete with *F. oxysporum* f. sp. *melonis* (Sivan and Chet, 1989). *T. harzianum* has strong capacity to mobilizes and take up of soil nutrients (Benitez et al., 2004). Results reported here in are in agreement with the above findings.

Yedidia et al. (2001) observed the colonization of *T. harzianum* in cucumber roots to enhance the availability of nutrients and over all antagonism to the pathogen. Similar effects were also reported in tomato, chilli and lettuce plants under field conditions. Up take of K was found to be higher than N and P in sugarcane as also reported by Shukla et al., 2008.The higher uptake of nutrients might be due to increased biomass in ratoon crop (Muchow et al., 1996; Singh et al., 2008b). Increased nutrition may be directly linked with enhanced yield of ratoon.

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