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TRICHODERMA AUREOVIRIDAE: A POTENTIAL ORGANISM FOR BIO-PROSPECTING AGAINST *MACROPHOMINA PHASEOLINA* (TASSI) GOID. IN JUTE

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Abstract

Trichoderma aureoviridae was isolated from jute (*Corchorus olitorius* L.) rhizosphere soil in West Bengal, India and the isolate was confirmed by 18S rDNA gene (AB916337). *In vitro* potentials of *Trichoderma aureoviridae* were evaluated against *Macrophomina phaseolina* (Tassi) Goid. infecting jute by dual culture technique, siderophore and cell wall degrading enzyme (CWDE) production. Besidpaires disease control, *T. aureoviridae* was found good growth promoting character when applied alone or in combinations of plant growth promoting bacteria and synthetic chemicals. *T. aureoviridae* enhanced the biomass production of jute, showed 72% *in vitro* and 62% *in vivo* growth inhibition of pathogen in this crop and has the great potential to induce plant defence system under stress condition and for further application in jute production.

Microbial communities are abundant in rhizosphere or areas under the influence of the root and its close vicinity. The rhizosphere gives support to many active microbial populations capable of exerting beneficial, neutral or detrimental effects on plant growth (Whipps 2000). Numerous species of soil fungi and bacteria flourish in the rhizosphere of plants and activate or stimulate plant growth by a plethora of mechanisms. The difficulty of controlling the pathogen lies in the long surviving ability of sclerotia, its broad host range and lack of resistant jute (*Corchorus olitorius* L.).

Trichoderma spp. is endophytic plant opportunistic symbionts widely used as biocontrol agents for plant diseases, relatively easy to isolate and ranks first in order of importance (Lorito *et al*. 2010). The main mode of action of the biocontrol agent include competition for space cum nutrients, because it is one of the predominant members of the rhizosphere, grow quickly on many substrates and provides mycoparasitism being a proteinaceous non-enzymatic elicitor (Djonovic *et al*. 2007). *Trichoderma* produces antifungal diffusible and volatile metabolites with antibiotic activity besides its positive influence on growth promotion and increased tolerance to various abiotic stresses (Gravel *et al*. 2007).

Different *Trichoderma* spp. along with other microorganisms with plant growth promoting properties were isolated from the rhizosphere soils using potato dextrose agar (PDA) and nutrient agar (King *et al*. 1954). The DNA of the selected species was isolated from mycelium grown in the broth culture and the specific 18S rDNA gene was electrophoresed using ITS_1 (5'-TCCGT AGGTGAACCTGCGG-3') and ITS_4 (5'-TCCTCCGCTTATTGATATG-3') primers. Amplicon was electrophoresed in a 1% agarose gel, purified using nucleospin purification column (Macherey-Nagel) and visualized under UV. Concentration of the amplicon was checked in a nanodrop - ND 2000. The isolate confirmed as *T. aureoviridae* (AB916337) was tested *in vitro* against *M. phaseolina* using dual culture technique (Bell *et al.* 1982, Bandopadhyay *et al.* 2006) for its antagonistic potential.

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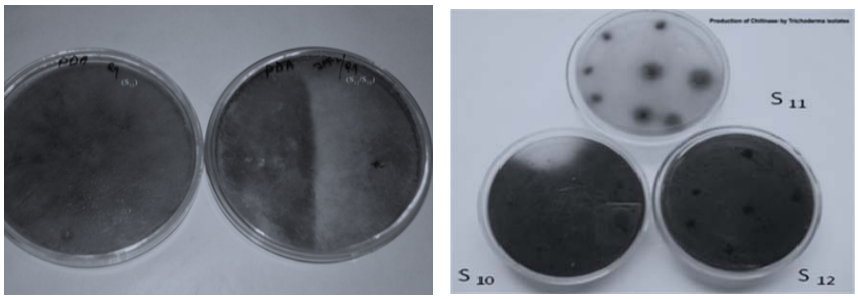
Observations of mycoparasitism was compared at different intervals and per cent reduction over control was calculated after Vincent (1947). The isolates of *Trichoderma* were tested for chitinase production using commercial chitin powder (HiMedia) as source of chitin biopolymer following the method of Agarwal and Kotasthane (2012). The growth promoting activity of *T. aureoviridae* was evaluated against the production of IAA (Patten and Glick 1996), volatile growth-stimulating compounds as HCN following the method of Alstrom and Burns (1989) and siderophore formation was done by the methods described by Schwyn and Neilands (1987). Compatibility among selected strains of bacteria and fungi including *Trichoderma* strains were tested to see the mutual/synergistic relations following the standard method (Fukui *et al*. 1994).

The most popular and moderately susceptible variety of jute (JRO 524) was collected for further *in vivo* study from ICAR-CSRSJAF; Budbud, Burdwan, India. The inoculum suspension were mixed, air-dried under sterile conditions and jute seeds were treated (Nandakumar *et al.* 2001). Control seeds were soaked in distilled water. Treated seeds were also subjected to the blotter plate method and the seeds were evaluated following the procedures of International Seed Testing Association (ISTA 1996). After incubation, the germination percentage and root-shoot lengths of the seedlings were assessed and the vigour index was calculated (Abdul-Baki and Anderson 1973). Charcoal-based seed treatment (10 g/kg of seed) of respective bio-agent with 2% CMC (as sticker) based soil bio-formulation mixture contains spore suspension (2×10^8 spores/ml) was applied and compared with carbendazim 50 WP (bavistin) @ 2 g/kg seed and 25 ppm IAA soaked seed for compatibility. The experiment was set up in randomized block design under the temparature controlled ploy-green house condition. In each treatment, 1g of leaf sample was used at different time intervalas for assaying isoperoxidase (Putter 1974).

All the bio-agents tested alone were found to be effective in inhibiting the mycelial growth of *M. phaseolina* in dual culture technique (Fig. 1a). Among two fungal antagonists of *Trichoderma* species, *T. aureoviridae* (S_{12}) was found much superior than other species (S_{10}) by inhibiting the growth of *M. phaseolina* 72% next to *A. faecalis* (S_3) and *B. amyloliquefaciens* (S_7) by 76% under *in-vitro* condition (Table 1). The *in vivo* percentage of disease control (62%) by the same fungi was also significantly higher and appreciable over the fungicides used (50 WP carbendazim) as T_4 (32%) (Table 2).



a b

Fig. 1. *T. aureoviridae* (S_{12}) showing development of: (a) antagonism, (b) chitinase.

However, the biocontrol agent like *Trichoderma* spp. may exert direct antagonism and parasitsm against many fungal pathogens and growing towards them due to the sequential, synergistic expression of CWDEs *viz.*, chitinase (Fig.1b), which serve the hyphae of *Trichoderma* spp. to firmly attach to the surface of its host mycelium and penetrate the host (Dennis and Webster 1971). Thus, understanding the induction process it is necessary to select the most

efficient *Trichoderma* spp. for biocontrol (Haran *et al*. 1996). In the present investigation, growth promotion by *T. aureoviridae* isolate, significant increase in biomass in terms of dry weight of aerial parts and roots (T_3) with other isolates (T_8 + T_9) was evident (Table 2). These results are in accord with those observed in other agricultural crops inoculated with specific strains of *Trichoderma* spp. (Harman *et al*. 2004).

Table 1. *In vitro* evaluation of antagonism by dual culture.

Sample	Colony diameter/radial growth (mm)		Inhibition zone (mm)	% inhibition
	Pathogen	Antagonist		
Bradyrhizobium sp.[b]	25.0	61.0	4.0	72.22
Alcaligenes faecalis[b]*(S3)	22.0	64.0	4.0	75.55
Bacillus sp.[b]	24.0	64.0	2.0	73.33
A. faecalis[b]	23.0	62.0	5.0	74.44
Pseudomonas sp.[b]	24.0	63.0	3.0	73.33
Bacillus amyloliquefaciens[b]*(S7)	22.0	63.0	5.0	75.55
Trichoderma spp.[c] (S10)	28.0	60.0	2.0	68.88
Trichoderma aureoviridae[c]*(AB916337)	25.0	64.9	0.1**	72.22
Macrophomina phaseolina[a]	90.0	-	-	-
CD at 5%	0.708	0.626	0.353	1.652

****=** Almost over growth, - = Not applicable, a = Pathogen, b = Bacterial isolates, c = Fungal isolates, b* and c* = Selected isolates.

The results on bio-priming of seeds in green house suggested that, the fungal isolate *T. aureoviridae* was found to have intermediate effect with respect to the potential in enhancing the root length, shoot length and seedling biomass on dry weight basis over the control, which are almost at par with other treatments when it was applied singly and compatible combinations either in terms of seed treatment or soil application (Table 2). The development of biocontrol strategies involving a mixture of microbials is an emerging area in crop protection to reduce the damage caused by plant pests and diseases on economically important crops. Our isolate *T. aureoviridae* as a component of a new combination of microbial consortia exhibited unique role as a biocontrol agent in comabating the *Macrophomina* disease complex in jute. Similar results on the enhanced bio-efficacy of bio-formulations contains fungal and bacterial biocontrol agents have been reported for the management of root rot disease in greengram (Thilgavathi *et al*. 2007). In addition to control of the stem rot of jute, *T. aureoviridae* enhanced the germination, biomass as well as to induce the plant defence system.

Among two forms of formulation studied, the charcoal based seed treatment with respective bio agents singly (T_1 - T_3) or in compatible combination (T_9 - T_{10}) were close to each other in case of shoot length along with seed treating or soaking chemicals (T_4 and T_8) gave better responses with respect to enhanced germination of seed. Whereas Carboxy methyl cellulose (CMC) based Soil formulation gave better response in increasing seed vigour index (T_9) either in terms of calculated vigour index or estimated biomass on dry weight basis (T_8). Among three *Trichoderma* strains, *T. aureoviridae* (S_{12}) advocated the superior efficiency towards the production of chitinase and plant growth promoting properties such as IAA, HCN and siderophore in all and was automatic choice. The genomic DNA of S_{12} (567 bp) indicated 97.88 - 98.59% similarity at nucleotide level which revealed that *T. aureoviridae* (S_{12}) 98.59% closely related with FJ610285

and 97.88% distantly related with HQ596942 of China isolates. To evaluate the efficacy of bio formulations under challenge inoculation using *T. aureoviridae* in single (T_3) and in combinations (T_9) revealed very good effect in seedling vigour, controlling the stem rot disease and induced peroxidase activity compared to others (Table 2). Standard error of mean (SEm±) was computed in all cases. The difference in the treatment mean was tested by using critical difference (CD) or LSD at 5% level of probability.

Table 2. *In vivo* effects of the formulated isolates under green house study.

Treatments	Germi-nation (%)	Root length (cm)	Shoot length (cm)	Fresh weight (gm)	Dry weight (gm)	Vigour index (VI)	Disease control (%)
T_1= ST with S_3 + SA with S_3 (15 DAS)	92.01	3.76	11.91	3.46	0.50	1441.79	63.61
T_2= ST with S_7 + SA with S_7 (15 DAS)	95.33	4.95	13.20	4.14	0.60	1730.10	62.88
T_3= ST with S_{12} + SA with S_{12} (15 DAS)	88.67	3.82	12.20	3.64	0.40	1420.14	62.43
T_4= ST with carbendazim 50 WP @ 2 gm/kg	88.50	3.76	12.16	3.27	0.33	1407.98	31.91
T_5= ST with carbendazim 0.2% +S_3+ SA with S_7 & S_{12} (1:1) 15 DAS	84.52	3.75	11.62	3.01	0.30	1298.50	31.73
T_6= ST with carbendazim 0.2% +S_7 + SA with S_3 & S_{12} (1:1)15 DAS	83.26	3.76	12.02	2.88	0.30	1313.94	31.54
T_7= ST with carbendazim 0.2% +S_{12}+ SA with S_3 & S_7 (1:1) 15 DAS	83.99	3.75	11.45	3.24	0.30	1276.38	20.23
T_8= SS with IAA @ 25 ppm + SA with S_3+S_7+S_{12} (1:1:1) 21 DAS	85.08	4.21	12.00	3.76	0.53	1378.56	38.23
T_9= T_4+T_6+T_8 (1:1:1) + quizalofop ethyl 5% EC sprays @ 1.5 ml/lit. (30 DAS)	90.00	4.25	12.68	4.36	0.40	1523.77	48.34
T_{10}= ST with S_3+S_7+S_{12}+SA with S_3+S_7+S_{12} (1:1:1) 21 DAS	83.33	3.77	12.35	3.45	0.34	1341.80	25.6
T_{11}= Inoculated control	73.16	3.46	10.39	2.97	0.31	1013.46	_
T_{12}= Healthy control	83.23	3.80	11.24	3.00	0.32	1251.78	_
SEm (±)	2.69	0.05	0.31	0.20	0.05	41.30	_
CD at 5%	5.55	0.11	0.65	0.41	0.10	85.25	_

ST = Seed treatment, SS = Seed soaked, DAS = Days after sowing, S_3 = *Alcaligenes faecalis*, S_7 = *Bacillus amyloliquefaciens* and S_{12} = *Trichoderma aureoviridae* (AB916337).

The results depicted that *T. aureoviridae* (AB916337) has been found to be a unique isolate which has immense activity to control the stem rot of jute and enhanced the plant health comapred to the traditional biocontrol agents used in jute. It is also noticed that the new isoltae of *T. aureoviridae* could be used as efficiant bio-prospecting agent for controiling the diseases of other crops also.

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