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Research Article

Compatibility of *Trichoderma* with Copper-Chitosan Graded Combinations

Erayya*, Nandani shukla, Rekha Balodi, Tewari, A.K. and J. Kumar
Department of Plant Pathology, GBPUA&T, Pantnagar-263145, Uttarakhand, India
*Corresponding Author E-mail: erayyapath@gmail.com
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ABSTRACT

A total of 22 Trichoderma isolates TCMS 2, 4, 5, 14b, 15, 16, 24, 32, 34, 36, 60, 62, 65 and 93, Th 1,3, 13, 14, 19 and 32, SBIT 32 and 76) were evaluated for their compatibility with dual combinations of copper hydroxide and chitosan (ChoF: liquid formulation) under in vitro conditions to check their maximum tolerance limit. The Trichoderma isolates were screened at graded (250+250, 500+500, 1000+1000 and 2000+2000 ppm) combinations of copper hydroxide and chitosan. At dual combination (250 ppm each), all the tested Trichoderma isolates showed cent per cent growth and thus found highly compatible. Only six isolates viz., TCMS-36, TCMS-60, TCMS-62, TCMS-65, SBIT-32 and SBIT-76 found compatible with exhibiting hundred per cent growth at 500 ppm (each) combination of copper hydroxide and chitosan. At 1000 ppm (each) combination only six isolates were found partially tolerant and none of the isolates was found completely tolerant. Maximum growth was observed in SBIT- 32 and TCMS-14b (3.6 cm), followed by TCMS-60 (3.5 cm), Th-1 with 2.9 cm colony diameter. Further, at 2000 ppm (each) combination, none of the isolates showed growth. Results indicated that optimum dose of copper and chitosan to use in combination with Trichoderma was 250 and 500 ppm (each). Further at level of 1000 ppm (each), the bio-control agent Trichoderma spp. itself affected by the fungicide (copper and chitosan). At 2000 ppm (each) combination inhibits completely the activities of Trichoderma spp. Hence, while using the triple combination of copper hydroxide (fungicide), chitosan (plant defense inducer) and Trichoderma spp. (biocontrol agent) for management of crop diseases, the optimum dose of the copper and chitosan might be selected as 250-500 ppm. The selected potential copper tolerant, Trichoderma isolates could be employed in sustainable, integrated disease management tactics along with the plant defence inducers and reduced level of fungicidal compounds.

Key words: *Trichoderma, Copper, Chitosan, Bio-control, Sustainability.*

INTRODUCTION

Trichoderma spp. present in nearly all type of soil habitates. In relation to other fungi *Trichoderma* spp. are easily culturable fungi. It belongs to the genus-Deuteromycotina, class-Hyphomycetes, order-Moniliales, and family-Moniliaceae. *Trichoderma* comprises large number of species *viz., T. asperellum, T. atroviride, T. harzianum, T. hamatum, T. koningii, T. virens* and *T. viride* which are widely used for bio-control of plant diseases incited by fungal pathogens[5].

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These species alone or in combination with other compatible chemical fungicides and /or plant defense inducers (chitosan) have been used for management of several crop diseases like root rots, wilts, damping off, white rot, collar rots etc[4]. *Trichoderma* spp. novel biological control properties against several plant pathogens, biotechnological applications and bio-remediation properties. Chemical compounds have been most commonly used to manage plant diseases. But, more specific the effect of a chemical on an organism, greater the probability of decreasing the effect through genetic shifts in the population. Whereas fungicides of broad spectrum produce undesirable consequences on non-target organisms (bio-agents) and on human beings. In contrast, use of biocontrol agents against plant diseases (biological control) is risk-free, eco-friendly and it results in enhancement of resident antagonists. Moreover, the combination of such biological control agents (*Trichoderma* spp.) with reduced levels of fungicide(s) and resistance inducer(s) such as chitosan reduces the diseases incidence/ severity similar to that achieved with higher doses of individual fungicide treatment. Application of bio-agents with lower doses of chemical pesticides is becoming an acceptable technological approach to biological equilibrium[2,6]. Identification of biological control agents tolerant to various fungicides would be more desirable than development of tolerance to single fungicide. So far, very few reports on the effect of graded concentrations of fungicides on growth of biocontrol agents and their cross tolerance to different fungicides are available. Prior to use of biocontrol agent in combination with fungicide to manage plant diseases, it should be evaluated/ screened for its compatibility / tolerance to fungicide/s. Keeping the above facts in view, a study was carried out on *In vitro* screening of *Trichoderma* spp. for their compatibility with copper-chitosan combinations.

MATERIAL AND METHODS

Compatibility of *Trichoderma* with different concentrations of copper and chitosan

Twenty two *Trichoderma* isolates viz., TCMS 2, 4, 5, 14b, 15, 16, 24, 32, 34, 36, 60, 62, 65 and 93, Th 1,3, 13, 14, 19 and 32, SBIT 32 and 76) were screened against different combinations (0, 250, 500, 1000, 2000 ppm each) of copper and chitosan compounds to check compatibility of these compounds with the *Trichoderma* isolates. Three replications were maintained for each treatment.

Preparation of fungicides stock solution

Stock solution of fungicide was prepared by dissolving weighted quantity of copper hydroxide in a measured volume of sterilized distilled water. The amount of stock solution to be added to potato dextrose agar medium was calculated by the following formula:

$$C_1V_1 = C_2V_2$$

Where,

C_1 = Concentration of stock solution (µg/ml)

C_2 = Desired concentration (µg/ml)

V_1 = Volume (ml) of the stock solution to be added

V_2 = Measured volume (ml) of the PDA medium

Poisoned food technique

Potato dextrose agar medium was poisoned with various concentrations of copper hydroxide and chitosan stock solution was poured @ 20 ml per Petri plates, aseptically. For each concentration (250+250, 500+500, 1000+1000 and 2000+2000 ppm) of copper and chitosan combination, three replications were maintained. After solidification of medium, each plate was centrally inoculated with 5 mm mycelia disc of three days old culture of *Trichoderma* using sterilized sharp cork borer. Petri plates without copper hydroxide-chitosan inoculated with *Trichoderma* were served as check. All inoculated Petri plate was incubated at 28 ± 1^0C. Radial growth was measured 72 hr after inoculation with the help of measuring scale. Per cent radial growth inhibition over control was calculated by applying the following formula**.**

$$I = \frac{C-T}{C} x100$$

Where,

I = Per cent Inhibition

C = Colony diameter in control

T = Colony diameter in treatment

RESULTS AND DISCUSSION

A total 22 *Trichoderma* isolates (14 isolates of TCMS series, six isolates of Th-series and two SBIT series) were screened against different combination of copper and chitosan at different grades *viz.,* 250 + 250, 500 + 500, 1000 + 1000 and 2000 + 2000 ppm to check the compatibility of *Trichoderma* spp. with the copper hydroxide-chitosan combination/s.

Among all the 22 *Trichoderma* isolates screened for their compatibility to copper-chitosan combination (250 ppm each), all the tested *Trichoderma* isolates showed cent percent growth, which indicate the compatibility of all the tested *Trichoderma* isolates with copper hydroxide and chitosan at this combination. The maximum colony diameter of 8.0cm was observed in all the treatments, which was similar to control. Thus, the combination was having no inhibitory effect on any of the tested *Trichoderma* isolates.

Six isolates *viz.*, TCMS-36, TCMS-60, TCMS-62, TCMS-65, SBIT-32 and SBIT-76 recorded maximum colony diameter of 8.0cm and thus found highly compatible with copper hydroxide and chitosan combination of 500 ppm each. However, all the tested isolates produced the colony diameter of >7.0 cm which was at par with the control. The maximum inhibition (12.5 %) at this combination was observed on Th-1 followed by TCMS-14b (7.5 % inhibition), TCMS-2 and th-3 with 6.25 % inhibition over control.

At copper hydroxide and chitosan combination (1000 ppm each), only six *Trichoderma* isolates were able to tolerate and grow for some extent. SBIT-32 and TCMS-15 were recorded maximum colony diameter of 3.6 cm followed by TCMS-4 (3.5 cm), Th-14 (3.0 cm), TCMS-32 (2.9 cm) and SBIT-76 with 2.6 cm of colony diameter. The growth of TCMS-5, TCMS-14b, TCMS-16, TCMS-24, TCMS-34, TCMS-36, TCMS-60, TCMS-62, TCMS-65, TCMS-93, Th-1, Th-3, Th19, Th-32, Th-13 was completely inhibited and thus these isolates were not shown compatibility at this combination.

Further, at 2000 ppm combination of each copper hydroxide and chitosan, none of the isolates showed any growth i.e., hundred per cent inhibition of the colony growth of all the tested isolates was observed at this combination (Table 1). Results indicated that optimum dose of copper hydroxide and chitosan to be used in combination with *Trichoderma* is 250-500 ppm for any triple combination to manage the plant diseases effectively and economically.

The present results support work of many previous researchers. The integration of bio-agents with lower or sub-lethal doses of agrochemicals is becoming an acceptable technological-approach in sustainable agriculture[2,6]. There are certain microorganisms, which can survive in high concentrations of metals and have the potential to accumulate different metals. It is necessary to collect data about the effect of metal-containing compounds on *Trichoderma* isolate from the point of the view of sustainable agricultural system and integrated pest management due to the application of *Trichoderma* spp. in combination with metal-containing fungicides and chemical fertilizers[3,1]. Yap *et al.*[7], investigated the tolerance of Cu by *Trichoderma atroviride*, a tolerant fungus isolated from the drainage surface sediment of the Serdang industrial area under *in vitro* conditions, and observed that *T. atroviride* can tolerate up to 600 mg/l of Cu on solid medium tolerance upto 300 mg/l of Cu concentration when grown in liquid medium). Yazdania[8]. also reported the copper tolerance in *Trichoderma* spp. was upto 600 mg/l on potato dextrose agar.

Table 1: Compatibility of *Trichoderma* isolates with graded combinations of copper hydroxide and chitosan

Sl. No.	*Trichoderma* Isolate	Control		Copper hydroxide + chitosan (250ppm each)		Copper hydroxide + chitosan (500ppm each)		Copper hydroxide + chitosan (1000ppm each)		Copper hydroxide + chitosan (2000 ppm each)	
		G (cm)	I (%)	G (cm)	I (%)	G (cm)	I (%)	G (cm)	I (%)	G (cm)	I (%)
1	**TCMS2**	8.00	0.00	8.00	0.00	7.50	6.25	0.00	100.00	0.00	100
2	**TCMS4**	8.00	0.00	8.00	0.00	7.60	5.00	3.50	56.25	0.00	100
3	**TCMS5**	8.00	0.00	8.00	0.00	7.70	3.75	0.00	100.00	0.00	100
4	**TCMS14b**	8.00	0.00	8.00	0.00	7.40	7.50	0.00	100.00	0.00	100
5	**TCMS15**	8.00	0.00	8.00	0.00	7.90	1.25	3.60	55.00	0.00	100
6	**TCMS16**	8.00	0.00	8.00	0.00	7.80	2.50	0.00	100.00	0.00	100
7	**TCMS24**	8.00	0.00	8.00	0.00	7.80	2.50	0.00	100.00	0.00	100
8	**TCMS32**	8.00	0.00	8.00	0.00	7.90	1.25	2.90	63.75	0.00	100
9	**TCMS34**	8.00	0.00	8.00	0.00	7.90	1.25	0.00	100.00	0.00	100
10	**TCMS36**	8.00	0.00	8.00	0.00	8.00	0.00	0.00	100.00	0.00	100
11	**TCMS60**	8.00	0.00	8.00	0.00	8.00	0.00	0.00	100.00	0.00	100
12	**TCMS62**	8.00	0.00	8.00	0.00	8.00	0.00	0.00	100.00	0.00	100
13	**TCMS65**	8.00	0.00	8.00	0.00	8.00	0.00	0.00	100.00	0.00	100
14	**TCMS93**	8.00	0.00	8.00	0.00	7.80	2.50	0.00	100.00	0.00	100
15	**Th-1**	8.00	0.00	8.00	0.00	7.00	12.50	0.00	100.00	0.00	100
16	**Th-3**	8.00	0.00	8.00	0.00	7.50	6.25	0.00	100.00	0.00	100
17	**Th-14**	8.00	0.00	8.00	0.00	7.70	3.75	3.00	62.50	0.00	100
18	**Th-19**	8.00	0.00	8.00	0.00	7.90	1.25	0.00	100.00	0.00	100
19	**Th-32**	8.00	0.00	8.00	0.00	7.90	1.25	0.00	100.00	0.00	100
20	**Th-13**	8.00	0.00	8.00	0.00	7.80	2.50	0.00	100.00	0.00	100
21	**SBIT- 32**	8.00	0.00	8.00	0.00	8.00	0.00	3.60	55.00	0.00	100
22	**SBIT 76**	8.00	0.00	8.00	0.00	8.00	0.00	2.60	67.50	0.00	100
SEm ±		-		**NS**		**0.23**		**0.23**		**NS**	
CD (0.01)						**0.85**		**0.87**			
CV (%)						**0.52**		**5.2**			

Index: G: Radial growth of *Trichoderma* isolate (diameter) and I: Inhibition of *Trichoderma*.by copper hydroxide-chitosan combination.

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