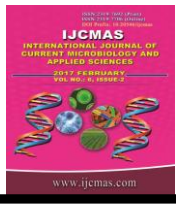
Int.J.Curr.Microbiol.App.Sci (2017) *6*(2): 140-146

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 2 (2017) pp. 140-146**
Journal homepage: http://www.ijcmas.com



Original Research Article http://dx.doi.org/10.20546/ijcmas.2017.602.020

Trichoderma pseudokoningii Showed Compatibility with Certain Commonly used Inorganic Pesticides, Fertilizers and Sticker Cum Spreaders

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Keywords

Inorganic fertilizers, Pesticides, Sticker, *Trichoderma pseudokoningii* .

Article Info

Accepted:
05 January 2017
Available Online:
10 February 2017

A B S T R A C T

Trichoderma pseudokoningii was tested for its compatibility with 11 insecticides, 7 fungicides, 2 herbicides, 1 sticker cum spreader, 4 different inorganic fertilizers in *in vitro* by poinson food technique. All the pesticides were tested for their compatibility with *T. pseudokoningii* at two different concentrations i.e., recommended dose (RD) and half of the RD and four different were tested for inorganic fertilizer and organic sticker cum spreader. All the pesticides significantly inhibited the mycelial growth of the fungus *T. pseudokoningii* except Thiamethonaus 25% WG at 0.125%. Ritha at all the tested doses was found compatible with the biocontrol gaent. Urea and muriate of potash were found compatible with *T. pseudokoningii* while single super phosphate and calcium ammonium nitrate inhibited its growth. The observed variations in the inhibitory potential could be due to inherent variability of chemical insecticide to biocontrol agents, *T. pseudokoningii* . CAN inhibited the colony growth of *T. pseudokoningii* at all the tested concentrations. Present investigations showed varying effects of pesticides, sticker, inorganic fertilizers on the fungi, their actual effects at cellular and field level need to be investigated to understand if the effects are permanent or temporary.

Introduction

Biological management refers the exploitation of beneficial fungal and bacterial microorganisms to attack and the disease caused by different pathogens. Biological management is gaining importance because of the deleterious effect of agrochemicals. It is an environmentally safe approach to curb plant diseases and pests. Fungal biocontrol agents like *Trichoderma* spp. has been identified as a potential agents for the management of the wide pathogenic range like *Fusarium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina* and root knot nematode, *Meloidogyne incognita* (Shylaja and Rao, 2012). It is known that biotic and abiotic factors influence the biocontrol efficacy of *Trichoderma* (Kredics *et al*., 2003).

Inorganic pesticides (Insecticides, fungicides, herbicides) and fertilizers (Urea, SSP, MOP and CAN) have played a vital role in supplementing the plant nutrients that get depleted during long term mono-cropping and land cultivation and to curb the biotic stresses.

Further for better efficacy in rainy days sticker and spreader are also used by the farmers.

The most promising possibility for the application of *Trichoderma* strains is within the framework of integrated plant protection management, based on the combined application of all the possible management tactics like physical, chemical and biological means.

The fungal biocontrol agents and selective fertilizer, pesticides may act synergistically increasing the efficiency of the control, allowing lower doses of pesticides, preserving natural enemies, minimizing environmental pollution and decreasing the likelihood of development of resistance to either agent (Moino and Alves, 1998; Ambettiger, 2009). Further, compatible combination can reduce the cost of cultivation by reducing the time of application of different component singly.

Materials and Methods

A starin of *T. psedokoningii* maintained at Indian Type Culture Collection (ITCC), Indian sgricultural Research Institute (IARI) New Delhi, India with accession number ITCC no. 8885.12 which was found to be effective against *Colletotrichum capsic*i, *Fusarium solani*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* was used for the experiment. In all, 11 insecticides, 7 fungicides, 2 herbicides, 1 sticker cum spreader, 4 different inorganic fertilizers were used in the study. The *In vitro* bio efficacy of the inorganic pesticides, fertilizers and organic sticker were determined using poisoned food technique (Nene and Thapliyal, 1997). All the pesticides were tested for their compatibility with *T. pseudokoningii* at two different concentrations ie., recommended dose (RD) and half of the RD. Sticker cum spreader was tested for their compatibility at three different does. On the basis of the contents of different elements different concentrations *viz.* 0.1%, 0.2%, 0.5%, 1%, 2% of the inorganic fertilizers were tested for their compatibility with the *T. pseudokoningii*. Each of the pesticides, sticker and inorganic fertilizers was added along with streptomycin sulphate at 0.3g/l to autoclaved but unset potato dextrose agar (PDA) separately in conical flasks and stirred until dissolved completely.

For control appropriate amount of streptomycin sulphate alone was added in the PDA media. About 15 ml of PDA with dissolved pesticides or stickers or inorganic fertilizers was poured into hot air oven sterilized petriplate (90 mm diameter) under sterile conditions and left to solidify. A 7 mm mycelia disc from 4 days old fresh culture of *T. pseudokoningii* was inoculated at the centre of each petriplate and incubated at 25-28˚C for 5-7 days. One set of petridish of PDA without the addition of any pesticides or stickers or inorganic fertilizer was maintained as a control. Each treatment was replicated for five times. The diameter of the fungal colony in each petriplate was recorded by taking the average of 10 measurements at different direction per petriplate.

The percent growth inhibition in the colony diameter was calculated by using the formula

$$\text{Percent inhibition} = \frac{C\text{-}T}{C} \times 100$$

Where, C = colony diameter in control
T = Colony diameter in treatments

The percent increase in the colony diameter was calculated using the following formula

$$\text{Percent inhibition} = \frac{C\text{-}T}{C} \times 100$$

Where, T = Colony diameter in treatments
C = Colony diameter in control

The data were statistically analysed and critical difference (CD) calculated at P = 0.05

Results and Discussion

Among the chemicals tested for compatibility all the pesticides significantly inhibited the mycelial growth of the fungus *T. pseudokoningii* at recommended and half of the recommended doses except Thiamethonaus 25% WG at 0.125% and Imidacloprid 17.8% SL at 0.2% and 0.1% (Table 1). Captan 50% WP at 0.25%, Carbendazim 12% + Mancozeb 63% at 0.25% and 0.125 %, Carbendazim 50% WP at 0.05% and 0.025% and Difenoconazole 25% EC at 0.125% and 0.25% were found highly detrimental to the fungus by retarding the growth totally to 100%. This was followed by Lambdacyhalothrin 5% EC at 0.25% and 0.50% and Paraquat dichloride 24% SL at 0.30% which inhibited the growth to an extent of 94.66% and 93.92% respectively.

There was no significant difference between Captan 50% WP at 0.25%, Carbendazim 12% + Mancozeb 63% at 0.25% and 0.125%, Carbendazim 50% WP at 0.05% and 0.025%, Difenoconazole 25% EC at 0.25% and 0.125%, Lambdacyhalothrin 5% EC at 0.25% and Paraquat dichloride 24% SL at 0.30% in inhibition of the radial growth of *T. pseudokoningii*. Bhatt and Srivastava (2003) found that Mancozeb exhibited fungistatic action against *T. viride* while copper oxychloride inhibited the growth of *Trichoderma* spp to a maximum extent. Maximum growth of the fungus was recorded in Thiamethoxam 25% WG at 0.125%, and Imidacloprid 17.8% SL at 0.2% and 0.1% with no inhibition on growth of the fungus. Methomyl 40%W/W at 0.02% and Mancozeb 75% WP at 0.125% was found compatible with the fungus with 7.29 cm and 7.24 cm radial growth of the fungus. As such the chemicals were found to be compatible compared to rest of the chemicals tested thiamethonaus 25% WG at 0.125% and imidacloprid 17.8% SL at 0.2% and 0.1% were found safe to the fungus, as there was no inhibition of growth. Methomyl 40%W/W at 0.02% and mancozeb 75% WP at 0.125% was found compatible with less detrimental effects (18.96% and 19.58% respectively) on the inhibition of growth of the fungus. The safety of imidachloprid to *Trichoderma* was also observed by Bhat and Sabalpara (2001), Vijayaraghavan and Abraham (2004). It was also found that the growth of *T. pseudokoningii* was more in recommended dose of Lambdacyhalothrin 5% EC and Metalaxyl-M 4% + Mancozeb 64% 1.42cm and 4.02cm respectively than half of the recommended dose 0.48cm and 3.84cm respectively.

The observed variations in the inhibitory potential could be due to inherent variability of chemical insecticide to biocontrol agents, *T. pseudokoningii*. Their inhibitory potential varies both between and within chemical classes (Inglis *et al*., 2001). A given insecticides may have different fungitoxic effects on various developmental stages of the fungus (Li and Holdom, 1994). The potential inhibitory effects of pesticides on germination and mycelia growth of biocontrol fungi vary from taxa and strains (Anderson *et al.*, 1989). However results may differ in field because fungi are exposed maximum to pesticides *In vitro* which doesn't occur under field conditions. Additionally, fungi may recover after some chemical pesticides are decomposed on plant leaves. Therefore, once a chemical insecticide is proved to be compatible in the laboratory, it must be selective in field conditions. On the other hand, high *In vitro* toxicity of the product will not always be same in the field (Butt and Brownbridge, 1997) but is likely to be occur (Alves *et al*., 1998).

Table.1 *In vitro* compatibility of *Trichoderma pseudokoningii* with chemicals

Pesticides	Treatment	Dose (%)	Colony diameter (mm) *	% Mycelial growth inhibition
	Control	-	0.90^{q}	100
Insecticides:	**Deltamethrin 2.8% EC**	0.014	16.30^{cd}	81.85
		0.028	12.70^{cd}	85.92
	Quinalphos 25% EC	0.0125	34.30^{hij}	61.81
		0.025	23.80^{ef}	73.55
	Dimethoate 30 % EC	0.030	25.80^{fg}	71.25
		0.060	11.30^{bc}	87.40
	Chlorpyriphos 20% EC	0.010	18.90^{de}	78.96
		0.020	15.40^{cd}	82.88
	Propargite 57%EC	0.100	31.30^{gh}	65.81
		0.200	18.80^{de}	79.03
	Malathion 50 % EC	0.075	49.20^{lm}	45.33
		0.150	22.90^{ef}	74.51
	Methomyl 40 % W/W	0.020	72.90^{p}	18.96
		0.040	57.50^{no}	36.07
	Thiamethonaus 25% WG	0.125	0.90^{q}	0.00
		0.050	51.80^{mn}	42.44
	Diafenthiuron 50% WP	0.040	41.80^{k}	53.48
		0.080	37.50^{ijk}	58.29
	Imidacloprid 17.8% SL	0.200	0.09^{q}	0.00
		0.100	0.09^{q}	0.00
	Lambdacyhalodrin5% EC	0.250	0.05^{a}	94.66
		0.500	14.20^{cd}	84.22
Fungicides:	**Captan 50% WP**	0.125	0.00^{a}	100.00
		0.250	0.00^{a}	100.00
	Carbendazim 12% + Mancozeb 63%	0.125	0.00^{a}	100.00
		0.250	0.00^{a}	100.00
	Carbendazim 50% WP	0.025	0.00^{a}	100.00
		0.050	0.00^{a}	100.00
	Metalaxyl-M 4% + Mancozeb 64%	0.125	38.40^{ijk}	57.33
		0.250	40.20^{jk}	55.33
	Mancozeb 75% WP	0.125	72.40^{p}	19.58
		0.250	43.10^{kl}	52.07
	Difenoconazole 25% EC	0.125	0.00^{a}	100.00
		0.250	0.00^{a}	100.00
Herbicides:	**Glyphosate 41 % SL**	0.150	61.70^{o}	31.40
		0.300	32.50^{hi}	63.81
	Paraquate Dichloride 24% SL)	0.150	15.70^{cd}	82.51
		0.300	0.054^{ab}	93.92
CD (P=0.05)			**0.60**	

*****Data are mean of four replications,
Mean followed same letter are at par with each other.

Table.2 Compatibility of *Trichoderma pseudokoningii* with different concentration of commonly used inorganic fertilizers

Concentration of inorganic fertilizer (%)	Urea		Single super phosphate (SSP)		Muriate of potash (MOP)		Calcium ammonium nitrate (CAN)	
	Colony diameter (mm)	% increase over control	Colony diameter (mm)	% increase over control	Colony diameter (mm)	% increase over control	Colony diameter (mm)	% increase over control
Control	70.00	-	70.00	-	70.00	-	70.00	-
0.1	72.00	+2.86	64.00	-8.57	73.20	+4.57	67.00	-4.29
0.2	74.00	+5.41	60.00	-14.29	76.40	+8.38	64.00	-7.14
0.5	79.00	+12.86	58.00	-27.71	80.30	+12.83	61.66	-11.91
1.0	84.00	+20.00	50.00	-28.57	90.00	+28.57	59.40	-15.14
2.0	90.00	+28.57	47.40	-32.29	90.00	+28.57	33.00	-52.86
CD (P = 0.05)	3.26	-	3.47	-	3.33	-	2.48	-

*****Data are mean of five replications

Table.3 Compatibility of *Trichoderma pseudokoningii* with organic sticker

Treatment	Dose (%)	Colony diameter (mm)*	% Mycelial growth inhibition
Ritha	0.0025	90.00	0.00
	0.005	90.00	0.00
	0.010	88.00	2.23
	0.020	85.00	5.56
Control (*T. pseudokoningii*)	-	90.00	-
CD (P=0.05)		**6.45**	

*****Data are mean of five replications

When data presented in table 2 showed urea and muriate of potash were compatible with *T. pseudokoningii* while single super phosphate and calcium ammonium nitrate inhibited its growth. At 0.1% and 0.2% urea did not affect the growth of *T. pseudokoningii*.

The colony diameter of *T. pseudokoningii* increases slowly with the increase in concentration of urea from 0.1%, 0.2%, 0.5% and 1%. At 2% concentration of urea significantly highest colony diameter of 90 mm diameter was observed with 28.57% increase of growth over control. In this study none of the concentrations of urea tested inhibited fungal growth (Table 2).

With SSP, the fungal growth was inhibited at all the tested concentrations. At 0.1% the colony was restricted to 64.00 mm as against the 70 mm in the control with a significant inhibition of 8.57%. At 0.2%, 0.5%, 1% and 2% the colony diameters were 60 mm, 50.6mm, 50mm and 47.4 mm respectively. The values were at par with each other, but the inhibition was significantly greater than at 0.1%. the corresponding percent inhibition were 14.29%, 27.71%, 28.57% and 32.29% respectively for 0.2%, 0.5%, 1% and 2% concentration.

CAN inhibited the colony growth of *T. pseudokoningii* at all the tested

concentrations. The largest inhibition was recorded at 0.1% with a colony diameter of 67 mm and significantly smaller than the control (70 mm). At 0.2% the colony diameter was 64 mm and significantly different from 0.1% concentration. The colony diameter of the fungus was 61.66 mm and 59.4 mm at 0.5% and 1% respectively, where were statistically at par with each other but significantly less than those in control and 0.2% concentration.

Earlier worker Sharma *et al.,* (1999) reported the incompatibility of *T. harzianum* with calcium nitrate at higher concentration. Similarly they also reported the compatibility of *T. harzianum* and *Pochonia chlamydosporia* with urea, superphosphate and muriate of potash (Table 3).

Present investigations showed varying effects of pesticides, sticker, inorganic fertilizers on the fungi, their actual effects at cellular and field level need to be investigated to understand if the effects are permanent or temporary. In case of temporary arrest of fungus activity, it may recover after degradation of toxicant and such chemicals can be employed in combination with the fungus under field conditions. The results are of permanent importance for both the development of protocols for fermentation technology and field applications of the application and efficacy of biocontrol agents.

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How to cite this article:

Pranab Dutta, N. Kakati, A. Das, H. Kaushik, S. Boruah, P. Bhowmick, P. Kaman, K.C. Puzari, R.P. Bhuyan and Hazarika, G.N. 2017. *Trichoderma pseudokoningii* Showed Compatibility with Certain Commonly used Inorganic Pesticides, Fertilizers and Sticker Cum Spreaders. *Int.J.Curr.Microbiol.App.Sci.* 6(2): 140-146. doi: http://dx.doi.org/10.20546/ijcmas.2017.602.020