IN VITRO COMPATIBILITY STUDIES OF *TRICHODERMA HARZIANUM* WITH INORGANIC FERTILIZERS

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Summary. A strain of the biocontrol agent *Trichoderma harziaum* was tested *in vitro* for its compatibility with different concentrations of commonly used inorganic fertilizers. Four different inorganic fertilizers *viz*., urea, single super phosphate (SSP), muriate of potash (MoP) and calcium ammonium nitrate (CAN) were used, each at concentrations of 100, 200, 500, 1000 and 2000 ppm. Urea at 1000 ppm and above increased the colony diameter of *T. harziaum* by 11.1%. MoP increased the growth of the biological control agent at all concentrations tested while SSP and CAN both inhibited it. The inhibition ranged from 8.8% to 13% for SPP and from 11.1% to 71.9% for CAN and increased with the increase in concentration.

Key words: Biological agents, fungal growth.

Biological control of plant diseases is gaining importance because of the deleterious effects of agro-chemicals. *Trichoderma harzianum* Rifai has been identified as a very promising biocontrol agent. It is antagonistic to a wide range of plant pathogenic fungi, such as *Fusarium* sp. (Muhammad and Amusa, 2003; Sivan *et al.,* 1987), *Pythium* spp. (Sivan *et al*., 1984; Howell 1982), *Rhizoctonia solani* Kühn (Shalini *et al*., 2006; Elad *et al*., 1980), *Sclerotium rolfsii* Sacc. (Upadhyay and Mukhopadhyay, 1986; Elad *et al*., 1980, 1982) and *Macrophomina phaseolina* (Tassi) Goid. (Adekunle *et al.,* 2006). *Trichoderma* isolates have also been reported to control the root knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw. in sunflower (Kaushal, 1990) and to suppress nematode reproduction, reduce root galling and increase yield of tomato (Affokpon *et al*., 2011). The control of *Meloidogyne javanica* by *T. harzianum* has also been demonstrated (Sahebani and Hadavi, 2008). *Trichoderma* formulations are commercially available under different brand names throughout the world. It is known that biotic and abiotic factors influence the biocontrol efficacy of *Trichoderma* (Kredics *et al*., 2003). Fertilizers have played a vital role in the success of India's green revolution and consequent self-reliance in food grain production. The use of inorganic fertilizers is a common practice for supplementing plant nutrients that get depleted during long term mono-cropping and land cultivation. Urea is one of the most common nitrogenous fertilizers applied to crops. In India, other common fertilizers are single super phosphate (SSP), muriate of potash (MoP) and calcium ammonium nitrate (CAN). 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 physical, chemical and biological means of control. Many investigations have been undertaken to assess the effect of herbicides (Kredics *et al.,* 2003), fungicides (McLean *et al*., 2001; Gowdar *et al*., 2006; Kredics *et al.,* 2003; Malathi *et al*., 2002) and phytohormones (Sharma *et al*., 1999) on growth and bio-efficacy of *Trichoderma* spp., both *in vitro* and *in vivo*. Studies on the compatibility of bio-control agents with chemical pesticides and inorganic fertilizers were conducted by Sharma *et al*. (1999), but not with *T. harzianum*. Therefore, an investigation was undertaken to assess the effects of the most common fertilizers used in India on a local strain of *T. harzianum* having potential as a biological control agent.

MATERIALS AND METHODS

The strain IIHR TH-2 of *Trichoderma harzianum*, maintained at Indian Type Culture Collection (ITCC), Indian Agriculture Research Institute (IARI), New Delhi, India with accession number ITCC NO 6888), which was found to be effective against root knot nematodes (Rao, 2007), was used for the experiment. Different concentrations of four different inorganic fertilizers *viz.,* urea (46% nitrogen), single super phosphate (SSP, 16% phosphorus), muriate of potash (MoP, 60% potassium) and calcium ammonium nitrate (CAN, 20% nitrogen, half in nitrate form and half in ammonium form) were used in the study. On the basis of their contents of the various elements, different concentrations, *viz*. 100, 200, 500, 1000 and 2000 ppm, of these inorganic fertilizers were prepared. Each inorganic fertilizer, was added to autoclaved but unset potato dextrose agar (PDA) separately, in conical flasks, and stirred until dissolved.

About 15 ml of PDA, with dissolved inorganic fertilizer, was poured into Petri plates (90 mm diameter) under sterile conditions and left to solidify. A 10 mm disc of a 4-day-old bio-agent culture was inoculated in each

Petri plate and incubated at 28-30 °C for 5 days. One set of dishes of PDA without the addition of any inorganic fertilizer was maintained as a control. Each treatment had six replicates. The diameter of the fungal colony in each Petri plate was recorded by taking the average of two measurements at right angles per plate.

The per cent increase in the colony diameter was calculated using the following formula

$$\% \text{ increase} = \frac{\text{Colony diameter in the treated-Colony diameter in control}}{\text{Colony diameter in control}} \times 100$$

The per cent inhibition in the colony diameter was calculated using the formula

$$\% \text{ inhibition} = \frac{\text{Colony diameter in control-Colony diameter in treated}}{\text{Colony diameter in control}} \times 100$$

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

RESULTS

Urea and muriate of potash were compatible with *T. harzianum* while single super phosphate and calcium ammonium nitrate inhibited its growth (Table I, Fig. 1).

At 100 and 200 ppm, urea did not affect the growth of *T. harzianum*. The colony diameter of *T. harzianum* remained the same up to a concentration of 200 ppm (90 mm) and was at par with the growth in the control plates. As the concentration of urea increased above 200 and up to 1000 ppm, the diameter of the colony also increased. At 500 ppm, the colony diameter was 91.7 mm and at par with that in the control and at 100 and 200 ppm. At 1000 ppm, the colony diameter was 95 mm, significantly larger than those in control, 100 ppm, 200 ppm and 500 ppm. At 2000 ppm, the colony diameter increased to 100 mm (11.1% increase in growth). None of the concentrations of urea tested inhibited fungal growth (Fig. 1A).

With SSP, the fungal growth was inhibited at all concentrations tested (Fig. 1B). At 100 ppm the colony was restricted to 82.5 mm as against the 90 mm in the con-

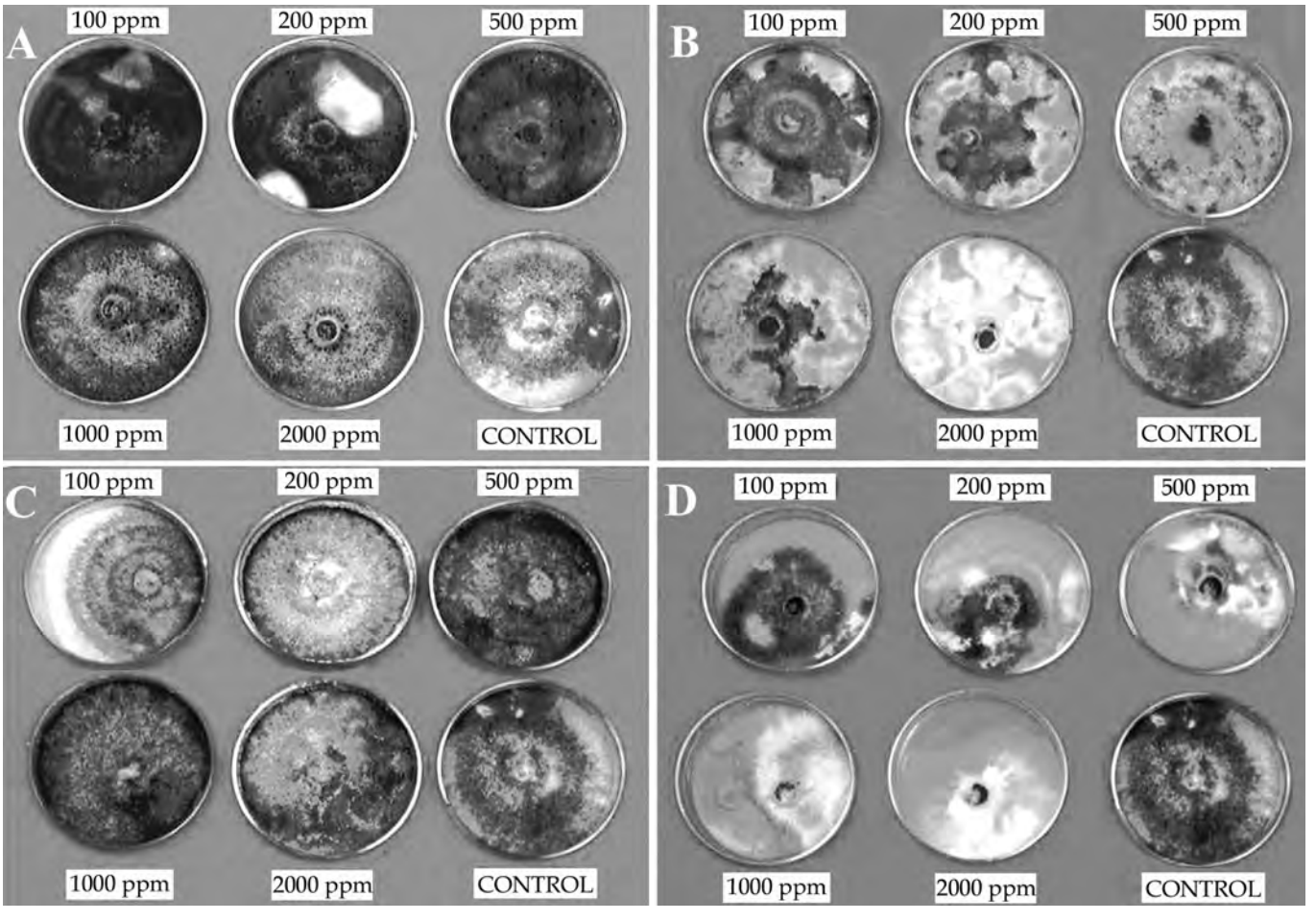

Fig. 1. Growth of *Trichoderma harzianum* as affected by different concentrations of inorganic fertilizers. A, urea; B, single super phosphate; C, muriate of potash; D, calcium ammonium nitrate. Note that the growth of the fungus is similar to that in the control at all concentrations of urea (A) and muriate of potash (C) while it is increasingly reduced by increasing concentrations of single super phosphate (B) and calcium ammonium nitrate (D).

Table I. *In vitro* compatibility of *Trichoderma harzianum* with different concentrations of some commonly used inorganic fertilizers.

Concentration of inorganic fertilizer (ppm)	Urea		Single super phosphate (SSP)		Muriate of Potash (MoP)		Calcium ammonium nitrate (CAN)	
	Colony diameter (mm)	Increase (%)	Colony diameter (mm)	Increase (%)	Colony diameter (mm)	Increase (%)	Colony diameter (mm)	Increase (%)
Control	90.00	0	90.00	0	90.00	0	90.00	0
100	90.00	0	82.50	- 8.83	95.66	+ 6.28	80.00	- 11.11
200	90.00	0	81.60	- 9.33	100.00	+ 11.11	72.60	- 19.33
500	91.66	+ 1.84	78.30	- 13.00	100.00	+ 11.11	66.00	- 26.66
1000	95.00	+ 5.55	78.30	- 13.00	100.00	+ 11.11	64.10	- 28.77
2000	100.00	+ 11.11	78.30	- 13.00	100.00	+ 11.11	25.30	- 71.88
CD (P=0.05)	4.95	-	4.89	-	4.33	-	8.17	-

trol, with a significant inhibition of 8.8%. At 200 ppm, 500 ppm, 1000 ppm and 2000 ppm the colony diameters were 81.6, 78.3, 78.3 and 78.3 mm, respectively. These values were at par with each other, but the inhibition was significantly greater than that at 100 ppm. The corresponding per cent inhibitions were 9.33, 13, 13 and 13, respectively.

All tested concentrations of MoP increased growth of *T. harzianum* (Fig. 1C). The least increase in colony diameter was recorded at 100 ppm (95.7 mm) but this diameter was, however, significantly larger than that in the control (90 mm) but significantly smaller than that of 100 mm observed at all larger MoP concentrations. As a consequence, the per cent increase in mycelial growth was the least (6.3%) at 100 ppm and significantly greater (11.1%) at all greater concentrations.

CAN inhibited the colony growth of *T. harzianum* at all tested concentrations (Fig. 1D). The smallest inhibition was recorded at 100 ppm, with a colony diameter of 80 mm and significantly smaller than in the control (90 mm). At 200 ppm, the colony diameter was 72.6 mm and significantly smaller than those in both the control and at 100 ppm. The colony diameter of the fungus was 66 mm and 64.1 mm at 500 ppm and 1000 ppm, respectively, which were at par but significantly less than those in the control and at 100 ppm. The per cent inhibition was 11.1% at 100 ppm, 19.3% at 200 ppm, 26.7% at 500 ppm and 28.8% at 1000 ppm. The least mycelial growth was recorded at 2000 ppm (colony diameter 25.3 mm).

DISCUSSION

Trichoderma harzianum reacted differently to different inorganic fertilizers. Its growth was compatible with urea, while single super phosphate inhibited it, with per cent inhibition increasing with the increase of its concentration. Interestingly, muriate of potash was compatible with both (this study) and *T. pseudokoningii* Rifai (Sharma *et al.*, 1999). The compatibility test of *T. harzianum* with calcium ammonium nitrate showed that, at larger concentrations, it was incompatible. Similar studies on the compatibility of bio-agents (*T. harzianum*, *T. pseudokoningii* and *Pochonia clamydosporia* Goddard) with the fertilizers urea, superphosphate and muriate of potash were reported by Sharma *et al.* (1999). These authors reported that the chemical fertilizers were compatible with all the biological control agents tested.

Our investigation has shown that not all inorganic fertilizers are compatible with the bio-agents. The results are of paramount importance for both the development of fermentation protocols and field applications of the bio-agents. They can be used to standardize a suitable medium for the bio-control agents, thereby increasing the yield of the fungus during mass production. Also, they can be used to determine the application schedule of fertilizer and bio-pesticides in farmers' field. However, to draw final conclusions, we suggest that the effects of inorganic fertilizers on *T. harzianum* also be investigated under field conditions.

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