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Differential sensitivity of *Trichoderma* to Selected Fertilizers

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ABSTRACT

Based on sensitivity of *Trichoderma harzianum* and *Trichoderma virens* i*n vitro,* fertilizers were categorized as dangerous, cautious and safe to *Trichoderma.* Observations on radial growth and spore germination indicated that zinc sulphate was dangerous with 100 per cent inhibition of either radial growth or spore germination or both of *Trichoderma*. While urea and DAP were found place in cautious group, muriate of potash, ammonium sulphate, single super phosphate and potassium nitrate were found safe for *Trichoderma* spp.

Key words : Categorization, Fertilizers, Sensitivity, *Trichoderma*

Soil borne plant pathogenic fungi such as *Fusarium, Phytophthora, Pythium, Rhizoctonia, Sclerotium etc.* cause diseases in most of the economically important crop plants. Chemical means of managing the diseases caused by these pathogens are not practicable owing to high cost of chemicals and environmental pollution. Biological control offers a novel approach when applied either alone or in combination with other management practices without the demerits of chemical control (Papavizas, 1985 and Mukhopadhyay, 1987). *Trichoderma* is one of the most common soil inhabitants and extensively studied biocontrol agent in the management of soil borne plant pathogens (Elad *et al.,* 1980).

Species of *Trichoderma* are being used either as seed treatment or soil application. In both the cases, the antagonist has been continuously exposed to different agrochemicals applied to the field either in soil or as foliar sprays and is likely to influence the efficacy of native or applied biocontrol agents like *Trichoderma*. Hence it is necessary to assess influence of fertilizers on the sensitivity of *Trichoderma* in order to use in the Integrated Disease Management systems (Singh *et al*., 1995). The present investigation was conducted to evaluate the sensitivity of two isolates of *Trichoderma* spp. *viz., Trichoderma harzianum* and *Trichoderma virens* to selected fertilizers.

MATERIAL AND METHODS

Trichoderma harzianum (from cotton fields) and *Trichoderma virens* (from citrus orchard) available in the Department of Plant pathology, Agricultural College, Bapatla were utilized in the present study. Seven fertilizers *viz.,* urea, muriate of potash, ammonium sulphate, single super phosphate (SSP), diammonium phosphate (DAP), potassium nitrate and zinc sulphate were used to assess the *in vitro* sensitivity of *Trichoderma* isolates by using the poisoned food technique for the radial growth (Nene and Thapliyal, 1993). Ten ml stock solution of 1, 00, 000 ppm concentration was prepared in sterilized distilled water. To obtain the desired concentration of fertilizer in the medium, amount of stock solution to be added was calculated by using the formula $\mathbf{C_1V_1 = C_2V_2}$, Where, C_1 = concentration of the stock solution (ppm); V_1 = volume of the stock solution to be added (ml); C_2 = desired concentration (ppm) and V_2 = volume of PDA in which fungicide is to be amended (ml). Required amount of stock solution was added in 60 ml sterilized distilled water (to get the double strength) and then mixed with 60 ml molten double strength PDA to get desired concentration. There after 20 ml of the poisoned medium was poured into sterilized petriplate (9.0 cm diameter) under aseptic conditions and allowed to solidify. Each plate was inoculated in the centre with 3mm diameter disc cut from the periphery of actively growing 3rd day old test *Trichoderma* culture individually under aseptic conditions and incubated at 28±1^{0}C in a BOD incubator. Unamended PDA plates inoculated with individual test isolates of *Trichoderma* served as checks. Three replications were maintained for each treatment. Radial growth of the test isolates was recorded after 24hours (day 1) and 48 hours (day 2) of incubation. Per cent inhibition of growth over control was calculated using the formula

$$I = \frac{C - T}{C} \times 100$$

Table 1. Effect of fertilizers on *Trichoderma* growth (dia. in cm).

S. No.	Fertilizers	*T.harzianum*		*T.virens*	
		Day1	Day 2	Day1	Day 2
1.	Urea	1.6 (1.6)^{c}	3.2 (2.0)^{b}	1.6 (1.6)^{c}	3.6 (2.2)^{d}
2.	Muriate of Potash	4.4 (2.3)^{a}	9.0 (3.2)^{a}	3.7 (2.2)^{a}	9.0 (3.2)^{a}
3.	Ammonium sulphate	3.5 (2.1)^{b}	8.4 (3.1)^{a}	3.0 (2.0)^{b}	6.4 (2.7)^{c}
4.	Single super phosphate	4.3 (2.3)^{a}	9.0 (3.2)^{a}	3.9 (2.2)^{a}	8.5 (3.1)^{ab}
5.	Diammonium phosphate	0.6 (1.3)^{d}	1.3 (1.5)^{c}	0.6 (1.3)^{d}	1.5 (1.6)^{e}
6.	Potassium nitrate	3.6 (2.1)^{b}	9.0 (3.2)^{a}	2.9 (2.0)^{b}	8.1 (3.0)^{b}
7.	Zinc sulphate	0.4 (1.2)^{d}	1.1 (1.4)^{c}	0.0 (1.0)^{e}	0.0 (1.0)^{f}
8.	Check	4.3 (2.3)^{a}	9.0 (3.2)^{a}	3.8 (2.2)^{a}	9.0 (3.2)^{a}
	CV (%)	3.7	2.3	2.8	2.4
	CD (P=0.01)	0.16	0.15	0.11	0.14

Figures in parentheses are square root transformed values. Figures with similar alphabets do not differ significantly. All the fertilizers were evaluated at 2 per cent concentration.

Where, I = per cent inhibition; C = growth of *Trichoderma* spp. in unamended medium and T = growth of *Trichoderma* spp. in amended medium.

Slide germination technique (Montgomery and Moore, 1938) was used to assess sensitivity of Trichoderma spores to selected fertilizers. Spore suspension of *Trichoderma* spp. was prepared by washing seven day old culture in the petriplate with sterilized water. Required concentration of test fertilizer was prepared by using sterile distilled water and 0.1ml of the solution was placed at the centre of a clean and sterilized glass slide and allowed it to dry at room temperature (30-35^{0}C). Spore suspension prepared in PDB (0.1ml) was placed on the same spot where fungicidal suspension was placed. Later slides were placed in moist chambers prepared using wet blotting papers placed in petriplate. Entire experimental procedure was followed under aseptic conditions in a laminar air flow chamber. The moist chambers with slides in it were incubated at 28±1^{0}C in a BOD incubator. Observations on number of spores germinated were recorded 12hours after incubation under high power (40X objective) of the microscope. Four replications were maintained for each fertilizer. From each replication three microscopic fields were observed for averaging number of spores germinated per microscopic field.

Per cent spore germination inhibition was calculated by using the following formula.

$$I = \frac{C - T}{C} X\ 100$$

Where, I = per cent inhibition; C = number of spores germinated in control; T = number of spores germinated in treatment.

RESULTS AND DISCUSSION

Both the isolates of *Trichoderma*, *viz*., *T harzianum* and *T virens* grew equally well with a diameter of 3.7 cm and 9.0 cm after 24 and 48 h of incubation at 28±1^{O}C respectively on control PDA

Table 2. Effect of fertilizers on *Trichoderma* growth - per cent inhibition

S. No.	Fertilizers	*T.harzianum*	*T.virens*	Mean
1.	Urea	65.0 (53.6)	59.6 (50.5)	62.3 (52.1)[c]
2.	Muriate of Potash	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)[g]
3.	Ammonium sulphate	7.0 (15.2)	29.2 (32.7)	18.1 (23.9)[d]
4.	Single super phosphate	0.0 (0.0)	5.1 (13.1)	2.5 (6.6)[f]
5.	Diammonium phosphate	85.1 (67.4)	83.3 (65.9)	84.2 (66.6)[b]
6.	Potassium nitrate	0.0 (0.0)	10.0 (18.8)	5.0 (9.4)[e]
7.	Zinc sulphate	88.1 (69.9)	100.0 (90.0)	94.0 (79.9)[a]
	Mean	35.0 (29.4)[b]	41.0 (38.7)[a]	
	CV (%)		4.82	
	CD (P=0.01)	Fertilizers	Isolate	Interaction
		2.6	1.4	3.7

Figures in parentheses are square root transformed values. Figures with similar alphabets do not differ significantly. All the fertilizers were evaluated at 2 per cent concentration.

plates (Table 1). Similarly, spore germination was on par in both the isolates with 100 per cent germination by 48th h of incubation in fertilizer un-amended potato dextrose broth.

In fertilizer amended medium, all the fertilizers showed inhibitory effect either on radial growth or spore germination or both. Variation existed between *Trichoderma* isolates in their sensitivity to different fertilizers between the growth stages of the same test fungus, *i. e.,* assimilative phase (radial growth) and spore phase (spore germination) and among different fertilizers in their toxicity to *Trichoderma* isolates.

Variation between *Trichoderma* isolates:

Observations made on the radial growth of *Trichoderma* indicated significant variation in the sensitivity of *Trichoderma* isolates to fertilizers or toxicity of fertilizers towards *Trichoderma* isolates. When observations were recorded on radial growth for two consecutive days both the *Trichoderma* isolates were found insensitive to MOP and SSP on both days of incubation (Table 2). While difference in sensitivity was observed on second day of incubation where in *Trichoderma harzianum* was found insensitive to ammonium sulphate and potassium nitrate while *T. virens* was found sensitive. When mean inhibitory per cent in the radial growth of *Trichoderma* isolates was analyzed, cotton isolate *Trichoderma harzianum* was found less sensitive (35% inhibition) compared to citrus isolate *Trichoderma virens* (41%) (Table 2). Similar result was obtained with spore germination where in *Trichoderma harzianum* was less sensitive (50%) compared to *Trichoderma virens* (58.5%) (Table 3). This difference in per cent inhibition of radial growth was due to more sensitvity of *Trichoderma virens* to four out of seven fertilizers. Compared to *Trichoderma virens*, sensitivity of *Trichoderma harzianum* was higher only with respect to urea and DAP. Though both the *Trichoderma* isolates were sensitive to seven test fertilizers in spore phase, urea, MOP, ammonium sulphate and DAP had more effect on *Trichoderma virens* than on *Trichoderma harzianum*, while SSP had more effect on *Trichoderma harzianum* than on *Trichoderma virens*. Zinc sulphate exhibited complete spore inhibition in both the isolates. Higher sensitivity of *Trichoderma virens* in comparison to *Trichoderma harzianum* to the selected fertilizers may be attributed to application of relatively lower amount of fertilizers in citrus compared to that in cotton fields and hence less adopted to fertilizer toxicity.

Variation between growth stages:

Sensitivity of *Trichoderma* isolates to selected fertilizer was found more in spore phase compared to assimilative phase (radial growth) as all the seven fertilizers could inhibit spore germination with least minimum inhibition in SSP (30.5%) and maximum in Zinc sulphate (100%). Variation in the effect of fertilizer on different growth

Table 3. Effect of fertilizers on *Trichoderma* spore germination.

S. No.	Fertilizers	Per cent inhibition		Mean
		T. harzianum	*T. virens*	
1.	Urea	50.0 (45.0)	61.7 (51.7)	55.8 (48.3)[b]
2.	Muriate of Potash	38.6 (38.4)	50.9 (45.5)	44.7 (41.9)[c]
3.	Ammonium sulphate	33.8 (35.5)	45.9 (42.6)	39.8 (33.4)[d]
4.	Single super phosphate	33.7 (35.4)	27.3 (31.4)	30.5 (33.4)[e]
5.	Diammonium phosphate	37.8 (37.9)	51.6 (45.9)	44.7 (41.9)[c]
6.	Potassium nitrate	29.7 (32.9)	45.4 (42.3)	37.5 (37.6)[de]
7.	Zinc sulphate	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)[a]
	Mean	50.0 (45.0)[b]	58.5 (49.9)[a]	
	CV (%)		4.60	
	CD (P=0.01)	Fertilizers 4.6	Isolate 2.4	Interaction 6.5

Figures in parentheses are square root transformed values. Figures with similar alphabets do not differ significantly. All the fertilizers were evaluated at 2 per cent concentration.

Table 4. Grouping of fertilizers based on per cent inhibition in the growth and spore germination of *Trichoderma*.

Parameter	Group	Fertilizers
100 % inhibition either in growth or in spore germination	Dangerous	Zinc sulphate
50 to 99% inhibition either in growth or in spore germination	Cautious	Urea, DAP
< 50 % inhibition either in growth or in spore germination	Safe	MOP, ammonium sulphate, SSP, potassium nitrate

stages of *Trichoderma* was maximum with MOP, ammonium sulphate, potassium nitrate which were found to be less toxic to assimilative phase (9, 6.4 and 8.1% inhibition respectively) but highly toxic to spore phase (44.7. 39.8 and 37.5% inhibition respectively) .

Variation in fertilizer toxicity:

Among the fertilizers MOP, ammonium sulphate SSP and potassium nitrate were found least inhibitory (0.0, 18.1, 2.5 and 5% in growth respectively) to *Trichoderma* isolates followed by 62.3 per cent with urea, 84.2 per cent with DAP and zinc sulphate 94 per cent. Urea (62.3 and 55.8%), MOP (0.0 and 44.7%), ammonium sulphate (18.1 and 39.8%) , SSP (2.5 and 30.5%) DAP (84.2 and 44.7%), potassium nitrate (5 and 37.5%) and zinc sulphate (94 and 100%) showed variation in their toxicity to assimilative and spore phases.

Among the nitrogenous fertilizers amide form of fertilizer *viz*., urea was found more toxic to both the isolates on both the phases of growth (spore and radial), compared to ammoniacal forms. Zinc sulphate containing heavy metal zinc was found more toxic on both phases of growth compared to all the fertilizers. Jayaraj and Ramabadran (1998) reported inhibition in the growth and sporulation of *T. harzianum* when the medium was amended with urea or calcium nitrate. Vijayaraghavan and Abraham (2004) reported that urea concentrations exceeding 1 per cent were found toxic to *Trichoderma*. Toxic effect of zinc sulphate on *Trichoderma* growth was reported by Sharma and Mishra (1995),

Based on the results obtained, all the test fertilizers were grouped in to 'dangerous', 'cautious' and 'safe' and presented in Table 4. Zinc sulphate was dangerous to *Trichoderma* spp with nearly 100 per cent inhibition of either radial growth and spore germination. Urea and DAP were found in cautious category with more than 50 per cent inhibition of either radial growth or spore germination of *Trichoderma* spp. MOP, ammonium sulphate, SSP and potassium nitrate were found safe to *Trichoderma*.

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