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Research article

PATHOGENICITY TESTS AND EVALUATION OF EFFICACY OF FUNGICIDES AGAINST *RHIZOCTONIA BATATICOLA*, THE CAUSAL AGENT OF DRY ROOT ROT OF CHICKPEA

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ABSTRACT: The pathogen was identified based on its mycelial and sclerotial characters and pathogenicity test was proved by soil inoculation method. Efficacy of two non systemic fungicides (copper oxychloride and captan), two systemic fungicides (hexaconazole and tebuconazole) and one antifungal antibiotic validamycin each at different concentrations were tested against *Rhizoctonia bataticola*, incitant of dry root rot of chickpea under *in vitro* conditions. The fungicides copper oxychloride, captan, hexaconazole and tebuconazole were found to be highly effective (100%) in inhibiting the mycelial growth of the highly virulent pathogen at all the concentrations tested.

Key words: Chickpea, *Rhizoctonia bataticola*, pathogenicity test, Fungicides, *In vitro* studies

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third important food legume crop. India is the leading producer of chickpea contributing to about 70 per cent of the world's chickpea production. Dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler is one of the most serious problem in chickpea growing belt of Andhra Pradesh. The pathogen is soil-borne, it infects the crop from seedling to maturity stages of the crop. Keeping in this view, an attempt was made to isolate and proving the pathogenicity of pathogen and to find out the suitable fungicides against causal agent of dry root rot under *in vitro* conditions.

MATERIALS AND METHODS

Isolation of Pathogen

A virulent isolate of *Rhizoctonia bataticola* was isolated from infected chickpea plants showing symptoms like withering and drying, by using tissue segment method (Rangaswamy and Mahadevan, 1999). Small pieces of tissue from the infected collar region along with some healthy tissue was cut with sterile scalpel. Then the pieces were surface sterilized with 1 per cent sodium hypochlorite for 2 minutes, followed by three washings in sterile distilled water to remove traces of sodium hypochlorite on the bits of tissue. The sterilized pieces were transferred to PDA plated Petri plates. Plates were incubated at 28 ± 2°C and observed periodically for growth of the fungus. The pathogen culture was purified by single hyphal tip method and maintained on PDA by periodical transfer throughout the present investigation.

Pathogenicity test

Pathogenicity test was conducted by soil infestation method. The pathogen was mass multiplied on sterilized sorghum grains in 250 ml conical flasks. The flasks containing sorghum seeds were autoclaved at 15 p.s.i for 20 min. Then the flasks were inoculated with 4 discs of 5.0 mm diameter mycelial growth of three days old culture of *Rhizoctonia bataticola* grown on PDA plate. The flasks were incubated at 28 ± 2°C for seven days. Then the inoculum was mixed with sterilized soil @ 100 g kg^{-1} soil and filled in the pots (22.5 cm diameter). The seeds of chickpea were sown simultaneously with pathogen inoculation @ 10 seeds per pot and an uninoculated control was maintained. The plants were observed for root rot symptoms. Each treatment replicated three times.

In Vitro Evaluation of Efficacy of Fungicides against the pathogen

In vitro efficacy of fungicides against the pathogen was evaluated by poisoned food technique (Nene and Thapliyal, 1993). The list of fungicides used in the present studies are given below table-1:

Table-1: The List of fungicides

S. No.	Common name	Trade name	Active ingredient	Concentration (%)	Source of supply
1.	Copper oxychloride	Blitox	50% WP	0.25	Rallis India Ltd., Mumbai
2.	Captan	Captaf	50% WP	0.25	Rallis India Ltd., Mumbai
3.	Hexaconazole	Contaf	5% EC	0.20	Hyderabad Chemical Supplies Ltd., Hyderabad
4.	Tebuconazole	Folicur	25.9% EC	0.10	Bayer Crop Science Ltd., Mumbai
5.	Validamycin	Sheathmar	3% L	0.10	Dhanuka Agritech Ltd., Mewat

Two non systemic (copper oxychloride,captan), two systemic(hexaconazole, tebuconazole) and one antifungal antibiotic validamycin each with different concentrations were used inorder to test the concentration of fungicide at which the pathogen growth inhibition is maximum.

Poisoned Food Technique

To 50 ml of sterilized distilled water, required quantity of fungicide was added and mixed thoroughly. This solution was added to 50 ml of sterilized cool molten double strength PDA medium, mixed thoroughly and poured into Petri plates. Six mm discs of four days old culture of pathogen were inoculated at the centre of Petri plates and then incubated at 28 ± 2°C. Three replications were maintained for each fungicide. Medium without fungicide was kept as control. Per cent inhibition of the growth of the fungus over the control was calculated using the formula:

$$I = \frac{C - T}{C} X100$$

where,
I = Per cent inhibition in growth of test pathogen
C = Radial growth (mm) in control
T = Radial growth (mm) in treatment

RESULTS AND DISCUSSION

Identification of pathogen

The fungus produced radial hyaline colonies, which later become carbonaceous brown to black. Mycelium was septate and dark brown in colour. Typical right angled branching of mycelium was observed. Sclerotia were black, varied from spherical to irregular in shape and measured 80 to 85µm in diameter. Pycnidial production was not observed in culture plates. The colony characters and morphological characters of mycelium and sclerotia were in agreement with the descriptions of Barnett and Barry (1972) and Sajeena *et al.* (2004). Thus, the fungus under present investigation was identified as *Rhizoctonia bataticola* (Taub.) Butler. (Figure-1).

Pathogenicity test

Pre-emergence rot of seedlings was observed and the survived plants showed stunted growth followed by wilting and drying of leaves and stems. When the infected plants were pulled out the tap root was blackened and devoid of lateral as well as finer roots. On re-isolation, the characters of the pathogen showed similarity with the original pathogen isolated from the field thus fulfilling Koch's postulates. Several workers have found soil inoculation method as most suitable in establishing the disease caused by *Rhizoctonia bataticola* (Katariya *et al*, 2007) in chickpea.

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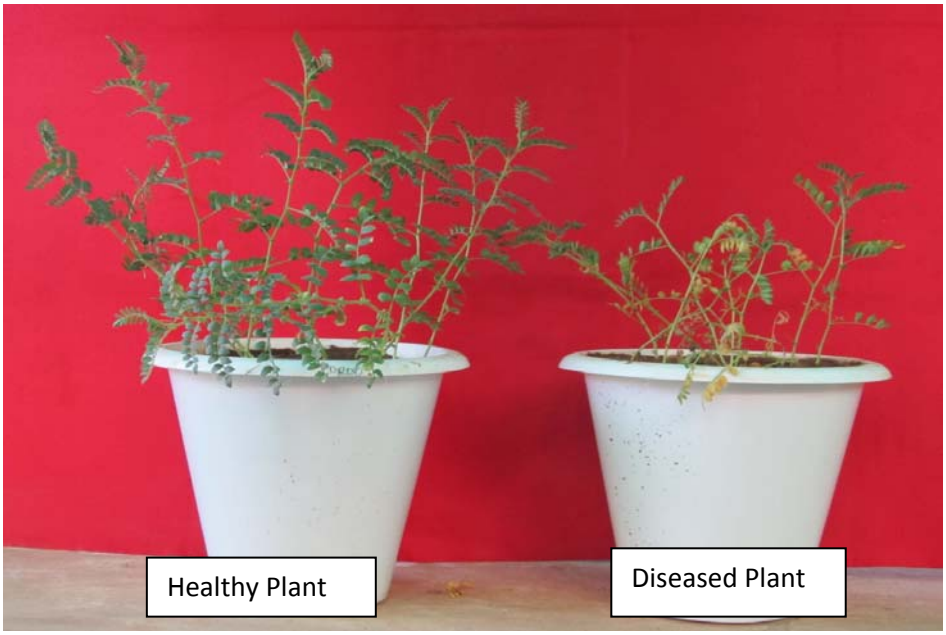
Figure-1: Pure culture of *Rhizoctonia bataticola* (Mycelial stage)



Poisoned Food Technique

The data revealed that all the fungicides at all concentrations were found to be significantly superior in reducing mycelial growth of *R. bataticola* when compared to control. The non systemic fungicides *viz*., copper oxychloride (Plate1) @ 1000, 1500, 2000 and 2500 ppm, captan (Plate 2) @ 1000, 1500, 2000 and 2500 ppm showed 100 per cent inhibition of mycelial growth of *R. bataticola* whereas the antibiotic validamycin (Plate 5) has shown increased inhibition of 86.66 to 95.55% with increasing concentration from 250 to 1000 ppm with a mean inhibition of 91.66 per cent. The results were in agreement with Ebenezar and Wesely (2000) who reported that the fungicides copper oxychloride (0.25%), captan (0.2%) inhibited the growth of M*acrophomina phaseolina* causing root rot disease in greengram by 95.89 and 78.56 per cent respectively. Both the systemic fungicides *viz*., hexaconazole (Plate 3) @ 500, 1000, 1500 and 2000 ppm and tebuconazole (Plate 4) @ 250, 500, 750 and 1000 ppm suppressed 100% the mycelial growth of the pathogen. Similar findings were reported by Konde *et al*. (2008) who evaluated the fungicides against *R. bataticola* causing root rot of soybean and revealed that combination of carbendazim + thiram (0.1 + 0.2 %), penconozole (0.1%) and thiophanate-Methyl (0.1%) were significantly effective in completely inhibiting the radial growth of *R. bataticola*. It is concluded that the copper oxychloride, captan, hexaconazole and tebuconazole were proved to be best in arresting the mycelial growth of the pathogen under *in vitro*. The fungicides has to be tested under field conditions along with potential biocontrol agents for further research work (Table-2).

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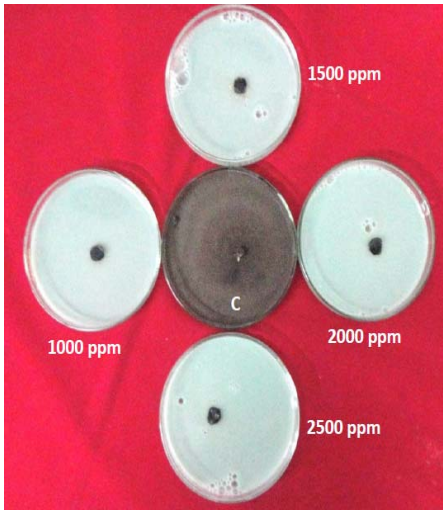
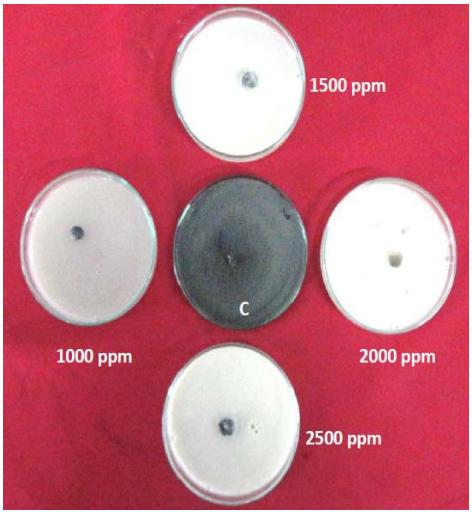
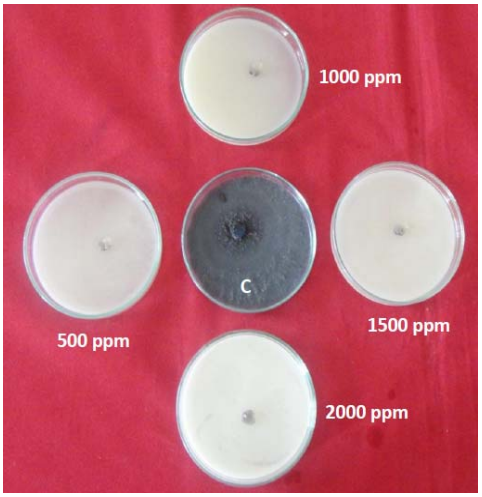


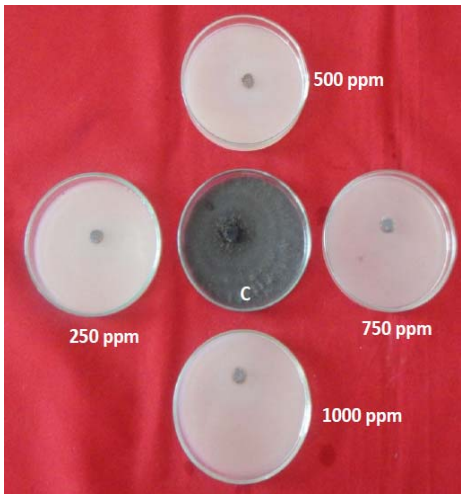
Copper oxychloride (Plate 1)



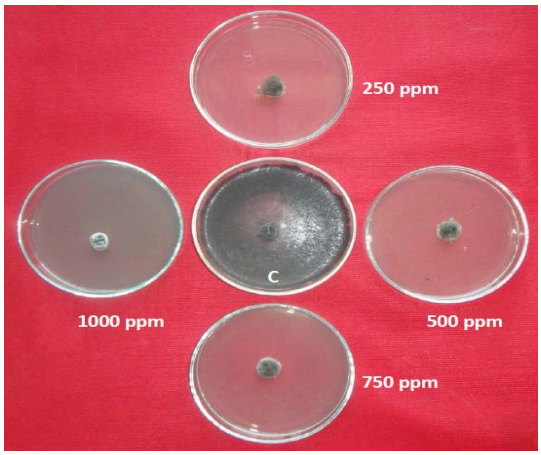
Captan (Plate 2)



Hexaconazole (Plate 3)



Tebuconazole (Plate 4)



Validamycin (Plate 5)

Table 2. *In vitro* evaluation of efficacy of different fungicides on mycelial growth of *Rhizoctonia bataticola* in poisoned food technique

S.No.	Fungicides	Concentration (ppm)	Mycelial growth of pathogen (cm)*	Growth inhibition over control (%)	Mean
1.	Copper oxychloride	1000	0.0	100.00 (90.00)	100 (90.00)
		1500	0.0	100.00 (90.00)	
		2000	0.0	100.00 (90.00)	
		2500	0.0	100.00 (90.00)	
2.	Captan	1000	0.0	100.00 (90.00)	100 (90.00)
		1500	0.0	100.00 (90.00)	
		2000	0.0	100.00 (90.00)	
		2500	0.0	100.00 (90.00)	
3.	Hexaconazole	500	0.0	100.00 (90.00)	100 (90.00)
		1000	0.0	100.00 (90.00)	
		1500	0.0	100.00 (90.00)	
		2000	0.0	100.00 (90.00)	
4.	Tebuconazole	250	0.0	100.00 (90.00)	100 (90.00)
		500	0.0	100.00 (90.00)	
		750	0.0	100.00 (90.00)	
		1000	0.0	100.00 (90.00)	
5.	Validamycin	250	1.2	86.66 (68.71)	91.66 (73.15)
		500	0.8	91.11 (72.68)	
		750	0.6	93.33 (75.07)	
		1000	0.4	95.55 (78.00)	
	Control	-	9.0	-	-
	S. Em ±	-	-	0.57	-
	C.D (0.05)	-	-	1.63	-

*Mean of three replications
Figures in parentheses are angular transformed values

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