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In vitro Efficacy of Fungicides and Bioagents Against Dry Root Rot of Pigeonpea Caused by *Rhizoctonia bataticola* (Taub.) Butler

Maruti[1*], Savitha, A. S.[1], Gururaj Sunkad[1] and Amaresh Y. S.[1]

[1]Department of Plant Pathology, College of Agriculture, UAS, Raichur-584104, Karnataka, India

*Corresponding Author E-mail: msbaganal143@gmail.com

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ABSTRACT

Pigeonpea [Cajanus cajan (L.) Millsp.] Is one of the protein rich legume of the semi-arid tropics grown predominantly under rainfed condition. It is affected by several fungal diseases among them the dry root rot caused by Rhizoctonia bataticola which cause severe economic yield loss. An attempt was made to manage the disease with fungicides and bio control agents. In vitro efficacy of non systemic, systemic and combi fungicides are evaluated against R. bataticola. Among contact fungicides tested, ziram recorded 100 per cent inhibition at all the concentrations (i.e., 0.1, 0.2 and 0.3 %) with the mean inhibition, which was significantly superior over all the treatments whereas, mancozeb and thiram showed 100 per cent inhibition at 0.3 per cent concentration. Among systemic fungicides tested, tebuconazole showed complete inhibition at all the concentration (i.e., 0.05, 0.10 and 0.15), whereas, propiconazole showed 100 per cent inhibition of R. bataticola at 0.10 and 0.15 per cent concentration. Among combi products tested, carbendazim 12% + mancozeb 63% WP, trifloxystrobin 25% + tebuconazole 50% EC and carboxin 37.5% + thiram 37.5% WP showed cent per cent (100%) inhibition at all the concentrations (i.e.,0.10%, 0.20% and 0.30 %). Where as the bio-agents tested, Trichoderma viride (Tv-B) was found more effective and statistically significant over other bio-control agents in inhibiting the mycelial growth (77.20 %) of R. bataticola followed by Trichoderma virense (Tvn-B) (75.76 %) and rest of other treatments.

Key words: *Pigeonpea, Dry root rot, Rhizoctonia bataticola, Fungicides*

INTRODUCTION

Pigeonpea (2n=22) [*Cajanus cajan* (L.) Millsp.] is one of the important grain legume crop of tropical and sub-tropical regions of the world and globally, it is grown on area of 4.75 mha with 3.68 mt of total production[1]. It is the second most important pulse crop of India after chickpea. In India, this crop is grown in an area of 3.86 m ha with an annual production of 2.90 m tonnes and productivity is 751 kg ha^{-1} [2], which accounts for 90 per cent of the pigeonpea area and production of the world. In India, it is mainly grown in Maharashtra, Uttar Pradesh, Madhya Pradesh, Gujarat, Andhra Pradesh, Karnataka and Tamil Nadu, constitutes 90 per cent of the area and production of pigeonpea.

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In Karnataka, this crop is considered as most important pulse crop with an area of 6.80 lakh ha with the production of 4.80 lakh tonnes and productivity of 712 kg ha^{-1} [2].

Even though, the crop is accounting about 90 per cent of world area and production, there is constraint in the productivity over the years. Pigeonpea is known to be attacked by more than 50 pathogens reported from 23 different countries. Among them few are economically important and wide spread causing heavy losses *viz.,* wilt caused by *Fusarium udum*, *Phytopthora* blight by *Phytophthora drechsleri* f. sp. *cajani*, stem canker *by Macrophomina phaseolina*, pigeonpea sterility mosaic disease caused by Tenui virus and more recently dry root rot caused by *Rhizoctonia bataticola* which is primarily a soil inhabitant generally affects the fibro vascular system of the roots affect the transport of nutrients and water to the upper parts of the plant. Hence, in the present study an attempt was made to manage this soil borne disease with some fungicides and bioagents.

MATERIALS AND METHODS

Collection and isolation of the pathogenic isolates:

The field was survey carried out during month of October-November 2015, a large number of infected pigeonpea roots were collected from different place *viz*., Raichur, Kalaburgi, Bidar and Yadgir districts. These samples were subjected to standard tissue isolation. The pigeonpea roots showing typical bark feeling and disintegrated roots were cut into small bits measuring about 2 mm and surface sterilized in ($HgCl_2$) (0.1%) for one minute such bits were transferred to Petri dishes containing sterile water successively for three times and then into the Petri dishes containing 20 ml of potato dextrose agar (PDA) medium and incubated at ± 28 ^oC for 10 days and observed for fungal growth. The culture of *R. bataticola* was maintained at 5 ^oC in the refrigerator and sub cultured periodically at an interval of 20 to 25 days during the course of the investigation.

Efficacy of different bioagents and fungicides against *Rhizoctonia bataticola*:

Six bioagents were evaluated for their efficacy through dual culture technique. The fungal bio-agent and the test pathogen were inoculated side by side on a single Petri plate containing solidified PDA medium. Whereas, the bacterial bioagents were streaked one day earlier to the test pathogen. Three replications were maintained for each isolate with one control by maintaining only pathogen and bioagent. They were incubated for seven days. The diameter of the colony of both bioagent and the pathogen was measured in both directions and average was recorded and the per cent inhibition on growth of the test pathogen was calculated by using the formula given below by Naik et al[3].

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

Where,

I = Per cent inhibition

C= Radial growth of the pathogen in control

T = Radial growth of pathogen in treatment

Poison food technique[4] was followed to test the efficacy of the mentioned different fungicides. The pathogen *R. bataticola* was grown on PDA medium in Petri-plates for ten days prior to setting up the experiment. Fungicide suspension was prepared in PDA by adding required quantity of fungicide to obtain the desired concentration on the basis of active ingredient present in the chemical. Twenty ml of poisoned medium was poured in each of the sterilized Petri plates. Mycelial disc of 0.5 cm was taken from the periphery of nine day old culture and placed in the centre and incubated at 28 ± 2 ^oC till growth of the fungus touched the periphery in control plate. Suitable checks were also maintained without addition of any fungicide, three replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was worked out. The per cent mycelial inhibition was calculated by using the formula given by Vincent[5] and data were analyzed statistically by using Completely Randomized Design (CRD).

RESULTS

Efficacy of fungal and bacterial bio agents against *R. bataticola* under dual culture

Seven bio-agents were tested against the inhibition of mycelia growth of *R. bataticola* through dual culture (Table.1). Among the bio-agents tested, *T. viride* (Tv-B) was found more effective and statistically significant over other bio-control agents in inhibiting the mycelial growth (77.20 %) of *R. bataticola* followed by *T. virense* (Tvn-B) (75.76 %) and rest of other treatments. However, the least mycelial inhibition was recorded in *B. subtilis* (BS-1) (27.87 %) and (RBS-1) (31.19 %).

Efficacy of non-systemic fungicides against *R. bataticola* under *in vitro*

Efficacy of seven contact fungicides was tested against mycelial growth of *R. bataticola* by poisoned food technique (Table. 2). Among contact fungicides tested, ziram recorded 100 per cent inhibition at all the concentrations (*i.e.,* 0.1, 0.2 and 0.3 %) with the mean inhibition, which was significantly superior over all the treatments where as mancozeb and thiram showed 100 per cent inhibition at 0.3 per cent concentration. In case of copper oxychloride no inhibition was recorded at all the concentrations tested against the target pathogen *R. bataticola.*

Efficacy of systemic fungicides against *R. bataticola* under *in vitro*

Efficacy of six systemic fungicides was tested against *R. bataticola* by poisoned food technique (Table.3). Among the systemic fungicides tested, tebuconazole showed complete inhibition at all the concentration, propiconazole showed 100 per cent inhibition of *R. bataticola* at 0.10 and 0.15 per cent concentration. Whereas difenconazole, carbendazim and hexaconazole showed 100 per cent inhibition at 0.15 per cent concentration. Least mycelial per cent inhibition of the pathogen was recorded in thiophanate methyl with 95.95, 96.49 and 97.40 at 0.05, 0.10 and 0.15 per cent concentrations respectively with significant differences.

Efficacy of combi-fungicides against *R. bataticola* under *in vitro*

Efficacy of six combi fungicides was tested against *R. bataticola* by poisoned food technique (Table.4). Among these combi products tested, carbendazim 12% + mancozeb 63% WP, trifloxystrobin 25% + tebuconazole 50% EC and carboxin 37.5% + thiram 37.5% WP showed cent per cent (100%) inhibition at all the concentrations (0.10%, 0.20% and 0.30 %). However zineb 68% + hexaconozole 4% WP, captan 70% + hexaconazole 5% WP and tricyclazole 18% + mancozeb 62% WP recorded 100 per cent inhibition at the concentrations of 0.2 and 0.3 per cent.

DISCUSSION

Chemicals are spectacular, impressive and quick and convincing even to an uneducated farmer, but there is also an intensified worldwide concern about environmental pollution due to escalated use of hazardous pesticides and fungicides. A multitude of microbes has been implicated to be biocontrol agent of plant pathogen, sometimes with excellent documentation. Hence studies were carried out to find effective biocontrol agent against *R. bataticola* and to develop biocontrol technique as a feasible component in the present day integrated disease management (IDM) strategy. In the present investigations, bacterial antagonists, *P. fluorescens* (+DAPG)-RP46, *P. fluorescens* (+DAPG)-RPF-20 and *B. subtilis* (BS-1 and RBS-1) were screened against *R. bataticola* under *in vitro*. Among them *P. fluorescens* (+DAPG)-RP46 recorded maximum (34.74 %) inhibition of mycelia growth of *R. bataticola* followed by *P. fluorescens* (+ DAPG)-RPF-20 (34.74%) and the least was in *B. subtilis* (BS-1) (27.87 %). The present findings are supported by Anand *et al*.[6] and Vinod Kumar *et al.*[7], they observed and identified a superior performance of *P. fluorescens* isolate against a broad spectrum of pathogens. The mechanism involved in the inhibition of growth might be due to the agglutination potential of *P. fluorescens* and colonization of sclerotia of *M. phaseolina* as observed by Jana *et al.*[8]. Further, Karunanithi

et al.[9] found production of antibiotics *viz*., siderophore, HCN, pyrrolnitrin, phenazine and 2, 4-diacetyl phloroglucinol and lytic enzymes by *P. fluorescens* which inhibited the mycelial growth of *M. phaseolina* in dual culture by producing inhibition zone of 12 mm as reported by Vanithal and Ramjegathesh,[10], Manjunatha *et al.*[11], Saravanakumar, *et al*. [12], Sendhilvel, *et al*[13].

Among the fungal bio-agents tested, *T. viride* (Tv-B) (77.20 %) was found more effective as compared to other biocontrol agents in mycelial inhibition of *R. bataticola* followed by *T. virense* (Tvn-B) (75.76 %). These findings are similar to that of other researchers (Elad *et al*.[14]; Ghaffar *et al*.[15]; Indra *et al*[16]; Suriachandraselvan *et al*.[17]; Manjunatha,[18]; Ammajamma *et al*.[19]; Shekhar and Kumar[20]; Nagamma *et al*.[21]; Kumari *et al*.[22] and Manjunatha *et al*.[23]. Mechanisms for bio-control of plant pathogens by *Trichoderma* are antibiosis, competition and mycoparasitism. *T. harzianum* and *T. viride* both suppressed the growth of *R. bataticola* and that might be due to mutual intermingling of antagonistic isolate with the test pathogen[3,24,25,26]. The present findings showed that effectiveness of antagonistic activity of *T. harzianum* and *T. virense* against a major soil borne pathogen *R. bataticola.*

In vitro evaluation of fungicides provides useful preliminary information regarding its efficacy against a pathogen with in a shortest period of time and therefore serves as guide for further field testing. In the present study among contact fungicides, ziram showed compete inhibition (100 %) at all the concentrations (0.1, 0.2 and 0.3 %), where as mancozeb and thiram recorded 100 per cent inhibition at 0.2 and 0.3 per cent concentration but copper oxychloride showed no inhibition at all the concentration. Among the systemic fungicides, tebuconazole (0.05 %) showed 100 per cent mycelial inhibition followed by propiconazole, which has showed 100 per cent inhibition at 0.10 and 0.15 per cent concentration but difenconazole, carbendazim and hexaconazole showed 100 per cent inhibition at 0.15 per cent concentration. The least inhibition of pathogen was recorded in thiophanate methyl with 95.95, 96.49, and 97.80 per cent at 0.05, 0.10 and 0.15 concentration respectively. Among the combi fungicides, carbendazim 12% + mancozeb 63 % WP, trifloxystrobin 25% EC+ tebuconozole 50 % EC, carboxin 37.5 % + thiram 37.5 % WP showed 100 per cent inhibition at all the concentration of 0.10, 0.20 and 0.30 per cent, whereas zineb 68 % + hexaconazole 4 % WP, captan 70 % + hexaconazole 5% WP and tricyclazole 18 % + mancozeb 62 % WP showed 100 per cent inhibition at the concentration of 0.2 and 0.3 per cent tested. The effectiveness of the triazoles fungicides in a combi form such as hexaconazole and tricyclazole may be attributed to their interference with the biosynthesis of fungal sterols and inhibition of ergosterol biosynthesis. In many fungi, ergosterol is essential to the structure of cell wall and its absence cause irreparable damage to cell wall leading to death of fungal cell. A similar study was reported for the effectiveness of triazoles, which inhibit the biosynthesis pathway in fungi[21,22,27,28,29,30,312].

Table 1: Efficacy of bioagents against *R. bataticola* under dual culture

Sl. No.	Bioagents	Per cent inhibition*
1.	*T. viride* (Tv-B)	77.20 (61.48)
2.	*T. harzianum* (Th-R)	73.91 (59.28)
3.	*T. virense* (Tvn-B)	75.76 (60.51)
4.	*P. fluorescens* (+DAPG)- RPF-20	34.74 (36.11)
5.	*P. fluorescens* (+DAPG)- RP-46	38.12 (38.13)
6.	*B. subtilis* (BS -1)	27.87 (31.87)
7.	*B. subtilis* (RBS-1)	31.19 (33.95)
	S.Em±	0.23
	C.D. at 1%	0.97

*Mean of three replication, Figure in parenthesis are arcsine value

Table 2: Efficacy of non-systemic fungicides on the growth of *R. bataticola* of pigeonpea under *in vitro*

SI. No.	Fungicides	Per cent inhibition*			
		0.1 %	0.2 %	0.3 %	Mean
1	Zineb 78 % WP	0.00(0.00)	25.00(30.00)	30.47(33.50)	18.49(21.17)
2	Copper oxychloride 50 % WP	0.00(0.00)	0.00(0.00)	0.00(0.00)	00.00(0.00)
3	Thiram 80 % WP	98.11(82.09)	98.62(83.33)	100.00(90.00)	98.91(85.14)
4	Captan 70 % WP	20.63(27.01)	96.08(78.59)	96.11(78.63)	70.94(61.41)
5	Chlorothalonil 75 % WP	85.09(67.28)	89.78(71.36)	92.98(74.64)	89.28(71.09)
6	Ziram 27 % SC	100.00(90.00)	100.00(90.00)	100.00(90.00	100.00(90.00)
7	Mancozeb 75 % WP	98.04(81.95)	100.00(90.00)	100.00(90.00)	99.34(87.32)

	S. Em±	C.D. at 1%
Fungicides (F)	0.10	0.39
Concentration (C)	0.06	0.24
F×C	0.18	0.67
CV	0.59	

*Mean of three replications, Figure in parenthesis are arcsine value

Table 3: Efficacy of systemic fungicides on the growth of *R. bataticola* of pigeonpea under *in vitro*

SI. No.	Fungicides	Per cent inhibition*			
		0.05 %	0.10 %	0.15 %	Mean
1	Difenoconazole 25 % EC	96.81(79.11)	98.89(83.97)	100.00(90.00)	98.56(84.56)
2	Carbandezim 50% WP	98.75(83.69)	99.44(85.92)	100.00(90.00)	99.36(86.51)
3	Thiophanate methyl 70 % WP	95.95(78.40)	96.49(79.21)	97.80(81.40)	96.74(79.70)
4	Hexaconazole 5 % SC	97.87(81.60)	99.23(85.02)	100.00(90.00)	99.03(85.54)
5	Propiconazole 25 % EC	98.80(83.73)	100.00(90.00)	100.00(90.00)	99.60(87.91)
6	Tebuconazole 29.9 % EC	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)

	S. Em±	C.D. at 1%
Fungicides (F)	0.16	0.62
Concentration (C)	0.11	0.40
F×C	0.28	1.07
CV	0.76	

*Mean of three replications, Figure in parenthesis are arcsine value

Table 4: Efficacy of combi fungicides on the growth of *R. bataticola* of pigeonpea under *in vitro*

SI. No.	Fungicides	Per cent inhibition*			
		0.1 %	0.2 %	0.3 %	Mean
1	Carbendazim 12% + Mancozeb 63% WP	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
2	Zineb 68% + Hexaconazole 4% WP	97.89 (81.65)	100.00(90.00)	100.00(90.00)	99.29(87.22)
3	Trifloxystrobin 25% + Tebuconazole 50% EC	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
4	Carboxin 37.5% + Thiram 37.5% WS	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
5	Captan 70% + Hexaconazole 5% WP	98.02 (81.91)	100.00(90.00)	100.00(90.00)	99.34(87.30)
6	Tricyclazole 18% + Mancozeb 62% WP	91.85 (73.42)	100.00(90.00)	100.00(90.00)	97.28(84.47)

	S. Em±	C.D. at 1%
Fungicides (F)	0.05	0.18
Concentration (C)	0.03	0.12
F×C	0.08	0.31
CV	0.21	

*Mean of three replications, Figures in parenthesis are arc sine value

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