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Evaluation of Biocontrol Agents and Chemical Fungicides Against *Sclerotium rolfsii* causing Stem Rot and Foliar Blight of Cowpea

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ABSTRACT

Sclerotium rolfsii is a serious pathogen which causes stem rot and foliar blight diseases in cowpea. Infected cowpea plant samples were collected from different locations of Kasargod district in Kerala. Six isolates of pathogen obtained could produce infection in cowpea during pathogenicity test and identified as *Sclerotium rolfsii* by morphological, cultural and molecular characterization. Among the biocontrol agents tested, highest inhibition of 84.44% was recorded with *Trichoderma harzianum* followed by *Trichoderma viride* (71.11). *Pseudomonas fluorescens* showed zero per cent mycelial inhibition whereas *Bacillus subtilis* inhibited the growth of pathogen by 38.89 per cent. Among the fungicides, mancozeb (75 WP) and propiconazole (25EC) were recorded with 100 per cent inhibition of pathogen at all the three concentrations (0.1%, 0.2% and 0.3%) tested followed by thiram (75WS) with 99.63 per cent inhibition at 0.3% concentration. Chlorothalonil (75 WP) and azoxistrobin 23SC were also found effective at all the tested concentrations (0.1%, 0.2% & 0.3%). Copper oxychloride (50WP) and carbendazim (50WP) were less effective against the pathogen with inhibition of 5.18 and 12.8 per cent at 0.1% and 0.05% concentrations, respectively.

Keywords: *Sclerotium rolfsii*, **chemical fungicides, biocontrol agents,** *Trichoderma harzianum*, *Pseudomonas fluorescens*, **stem rot, foliar blight.**

INTRODUCTION

Cowpea (*Vigna unguiculata* L.)) is one of the highly nutritious and indispensable vegetable crops which can be used for food, feed, fodder and green manure. It is being considered as the most demanded vegetable for people in Kerala and its cultivation is highly recommended. Stem rot and foliar blight of cowpea caused by *Sclerotium rolfsii* emerged as a major disease in farmer's fields and the severity was found more during rainy seasons. *S. rolfsii* is a soil born fungus reported by Rolfs in 1892 for the first time as a causal organism of tomato blight in Florida (Mahato *et al*, 2017). It is pathogenic to more than 500 species of plants of both monocots and dicots in the tropics and subtropics. Pathogen can survive in soil, crop residue and weeds for long period of time by forming sclerotial bodies (Kator *et al*, 2015). Since this pathogen became an important concern which result in yield reduction of cowpea and economic loss for farmers, efficiency of different biocontrol agents and chemical fungicides against this pathogen needs to be studied.

MATERIALS AND METHODS

Isolation of the pathogen

The pathogen *Sclerotium rolfsii* was isolated from plant parts of cowpea showing stem rot and foliar blight symptoms in the field (Pandi *et al*, 2017). Further, purification of the isolated fungus in PDA was done by hyphal tip method ensuring strict aseptic conditions. Pathogen was identified

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based on cultural, morphological and molecular characterization. Antagonist and fungicidal assay was conducted with the fungal isolate.

In vitro evaluation of biocontrol agents

KAU (Kerala Agricultural University) released fungal and bacterial biocontrol agents such as *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against the fungal isolate causing stem rot and foliar blight disease in cowpea by dual culture technique. The experimental design selected for statistical analysis was CRD with five treatments and four replications.

For *T. viride* and *T. harzianum,* 7 mm culture disc of pathogen and antagonist were cut out from the actively growing margin of four days old culture in the petridishes (Sekhar *et al*, 2020). These were placed on the PDA medium at opposite edges of the petridish (2 cm away from the edge) having 9 cm diameter. For *P. fluorescens* and *B. subtilis* antagonism was performed by inoculation of the pathogen at the centre of the sterile petri plate with nutrient agar (NA) medium. Bacterial cultures were streaked as parallel lines at both side of the pathogen keeping 2 cm distance from the periphery of the plate. Control plates were kept by inoculating only the pathogen at the centre of the petridish. Both dual culture and control plates were incubated at room temperature 28 ± 2^{0}C. Observations were taken at the interval of 24 h. Radial growth of the pathogen was recorded when complete growth was observed in control plates. Percent inhibition of the pathogen by antagonist fungi was calculated by using the following formula given by Girish and Sushma (2018).

$$\text{Percent Inhibition \% (I)} = \frac{C - T}{T} \times 100$$

Where

I: Percent inhibition

C: Mycelial growth in control (mm)

T: Mycelial growth in treatment (mm)

In vitro evaluation of chemical fungicides

Evaluation of seven fungicides including four systemic and 3 contact fungicides at three different concentrations was done by poisoned food technique (CRD with 22 treatments and 3 replications) against *S. rolfsii* (Subedi *et al*, 2019). Thiram (75WS), mancozeb (75 WP), chorothalonil (75 WP), copper oxychloride (50WP) were tested against the pathogen at concentrations of 0.1%, 0.2% & 0.3% and carbendazim (50WP), propiconazole (25EC) & azoxistrobin (23SC) were evaluated at 0.05%. 0.1% and 0.2%.

Required concentration of the fungicide was added to 50 ml sterilized water and shaken well for uniform mixing. Well mixed fungicidal suspension then transferred to 50 ml molten double strength potato dextrose agar medium (DPDA) ensuring that desired concentration was obtained. 15 ml poisoned media was poured into the petridishes, after solidification 5 mm mycelial disc of four days old culture of pathogen was inoculated at the centre of the petridish. Control plates were maintained by inoculating pathogen in the poison less PDA medium. All procedures mentioned above were strictly followed under aseptic conditions. Treatment plates were kept for incubation at room temperature 28 ± 2^{0}C. Growth of pathogen in the poisoned media were recorded and compared with control plate showing full growth of the fungus. Percentage inhibition was estimated by using the equation given by Girish and Sushma (2018).

RESULTS AND DISCUSSION

Identification of pathogen

Pathogen causing stem rot and foliar blight in cowpea was identified based on cultural, morphological and molecular characteristics. The pathogen showed rapid growth and covered petridish of 90 mm diameter within 4 days of inoculation. It produced cottony, fluffy and thick mycelium of pure white colony with slight zonations in PDA. White sclerotial initials having round shape were produced in the fungal colony after eight days of

Table 1. *In vitro* efficacy of biocontrol agents against *S. rolfsii*

Treatment	Biocontrol agents	Radial growth of pathogen in petridish (cm)*	Percentage of mycelial inhibition of *S. rolfsii* *
T1	*Trichoderma viride*	1.30	71.11 (57.47) [b]
T2	*Trichoderma harzianum*	0.70	84.44 (66.75) [a]
T3	*Pseudomonas fluorescens*	4.50	0.00 (0.00) [d]
T4	*Bacillus subtilis*	2.75	38.89 (38.56) [c]
T5	Control	4.50	0.00 (0.00) [d]
CD (0.05)			1.63
SE (m)			0.54

*mean of four replications, values in parenthesis are arcsine transformed Figures with same letter do not differ significantly from each other

inoculation. Total number of sclerotia formed in the fungal colony ranged from 25 to 30. Two types of hyphae were observed under microscope with one having coarse, straight and large cells (150-250 µm diameter) with clamp connection at septa. Some produced branching at the place of clamp connection. Another type of hypha was slender forming branches irregularly, but clamp connections were absent. Sclerotium was internally differentiated into four different layers having an outer thick skin followed by rind with thick cells, cortex with thin-walled cells and medulla region of loosely filamentous hyphae. Based on the D1/D2 Region- PCR analysis, the fungal culture showed 99.61 % similarity with *Athelia rolfsii* (Accession No:JN811675.1), which was the perfect stage of *Sclerotium rolfsii*

In vitro evaluation of biocontrol agents

Fungal biocontrol agents were found to be superior over bacterial agents against *S. rolfsii* for which highest inhibition percentage was recorded with *Trichoderma harzianum* (84.44 %) followed by *Trichoderma viride* (71.11%) (Table 1). The present findings were in agreement with Javeria *et al* (2014) who reported higher antifungal efficiency of *T. harzianum* with 70.82 per cent inhibition of *S. rolfsii.* Bacterial biocontrol agents showed less inhibition of pathogen under *in vitro* assay where *B. subtilis* checked radial growth of fungus to about 38.89 per cent. *P. fluorescens* showed no inhibition in the mycelial growth of the pathogen (Table 1). Low inhibition percentage of *P. fluorescens* against the pathogen were in accordance with observations made by Wankhade *et al* (2019). Kachhadia (2013) also revealed that *P. fluorescens* was not much effective in inhibiting *S. rolfsii*, but *B. subtilis* was tested to have more antifungal activity.

In vitro evaluation of chemical fungicides

Among the fungicides, mancozeb (75 WP) and propiconazole (25EC) were found superior with 100 % inhibition at all the three concentration (0.1 %, 0.2 % and 0.3 %) followed by thiram (75WS) with 99.63 per cent inhibition at its highest concentration (0.3 %) (Table 2). Shirsole *et al* (2019) concluded that propiconazole, thiram and mancozeb showed more efficacy in inhibition of fungi under *in vitro* conditions. Chlorothalonil (75 WP) was also tested effective at all the tested concentrations (0.1 %, 0.2 % & 0.3 %) having inhibition percentage of 79.6 %, 72.22 % and 60.37

Table 2. *In vitro* evaluation of different fungicides against *S. rolfsii*

Treatment	Fungicide- concentration in per cent	Radial growth of pathogen in petridish (cm)*	Percentage mycelial inhibition of *S. rolfsii* (%)*
T1	Thiram (75WS) – 0.1 %	3.10	65.60 (54.00) [g]
T2	Thiram (75WS) – 0.2 %	2.63	70.70 (57.20) [e]
T3	Thiram (75WS) – 0.3 %	0.03	99.60 (88.00) [a]
T4	Mancozeb (75 WP) – 0.1 %	0.00	100.00 (90.00) [a]
T5	Mancozeb (75 WP) – 0.2 %	0.00	100.00 (90.00) [a]
T6	Mancozeb (75 WP) – 0.3 %	0.00	100.00 (90.00) [a]
T7	Chorothalonil (75 WP) – 0.1 %	3.56	60.40 (51.00) [h]
T8	Chorothalonil (75 WP) – 0.2 %	2.50	72.20 (58.20) [d]
T9	Chorothalonil (75 WP) – 0.3 %	1.83	79.60 (63.20) [b]
T10	Copper oxychloride (50WP) – 0.1%	8.53	5.20 (13.10) [n]
T11	Copper oxychloride (50WP) – 0.2%	7.60	15.60 (23.20) [m]
T12	Copper oxychloride (50WP) – 0.3%	6.26	30.70 (33.70) [j]
T13	Carbendazim (50WP) – 0.05 %	7.83	12.80 (21.00) [m]
T14	Carbendazim (50WP) – 0.1 %	7.06	21.50 (27.60) [k]
T15	Carbendazim (50WP) – 0.2 %	6.10	32.20 (34.57) [i]
T16	Propiconazole (25EC) – 0.05 %	0.00	100.00 (90.00) [a]
T17	Propiconazole (25EC) – 0.1 %	0.00	100.00 (90.00) [a]
T18	Propiconazole (25EC) – 0.2 %	0.00	100.00 (90.00) [a]
T19	Azoxistrobin (23SC) – 0.05 %	2.83	68.50 (55.90) [f]
T20	Azoxistrobin (23SC) – 0.1 %	2.51	72.18 (58.20) [d]
T21	Azoxistrobin (23SC) – 0.2 %	2.10	76.70 (61.10) [c]
T22	Control	9.00	0.00 (0.00) [op]
		CD (0.05)	1.03
		SE (m)	0.36

**Values in parenthesis are arcsine transformed, Figures with same letter do not differ significantly*

per cent, respectively. Chlorothalonil 75WP at its recommended concentration (0.2%) was at par with recommended concentration of azoxystrobin 23 SC (0.1 %) in suppressing mycelial growth of the pathogen. Among all the treatments, 16 treatments showed more than 60 per cent inhibition of fungus. Least inhibition percentage was recorded with copper oxychloride (50WP) followed by carbendazim (50WP) having inhibition of 5.18 and 12.8 per cent at 0.1 % and 0.05 %, respectively. These were not effective at inhibiting the fungi even at their highest concentration tested such as 0.3% for copper oxychloride (50WP) and 0.2 % for carbendazim (50WP) where inhibition percentage was below 35 % only(Table. 2). The results of current studies were in conformity with Rakholiya

(2015) who reported low effectiveness of copper oxychloride against *S.rolfsii* and 100% inhibition by mancozeb.

CONCLUSION

It can be concluded that, *S. rolfsii*, the causal organism of stem rot and foliar blight disease can be highly inhibited by fungal biocontrol agents like *Trichoderma harzianum*, *Trichoderma viride* and chemical fungicides like mancozeb (75WP) and propiconazole 25EC. Least fungicidal effect was exhibited by *Pseudomonas fluorescens*, *Bacillus subtilis*, carbendazim 50WP and copper oxychloride (50WP). .

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