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EFFICACY OF BIOAGENTS AGAINST *SCLEROTIUM ROLFSII* OF CHILLI

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ABSTRACT:

Chilli (Capsicum annuum L.), is an important solanaceous vegetable-cum-spice crop.Chilli requires hot and humid conditions for growth and development and its cultivation is mainly confined to the tropical regions of the world. Chilli crop is suffered from many fungal, bacterial and viral diseases. Therefore, present in vitro study was conducted to assess Efficacy of bioagents against Sclerotium rolfsii of Chilli. Infected chilli plants showing typical root rot symptoms were collected from the fields and isolated on PDA. Pathogenicity of the test fungi were proved by sick soil (S. rolfsii) method in earthen pots, under screen house conditions. Nine bioagents were evaluated in vitro against the test fungi S. rolfsii, by applying dual culture technique and using potato dextrose agar (PDA) as basal culture medium. Observations on radial mycelial / growth colony diameter (mm) of the test fungi at an interval of 24 hrs of incubation were recorded and continued up to seven days or till the untreated PDA plates were covered fully with mycelial growth of the test fungi. All the nine bioagents evaluated in vitro, were found effective against S. rolfsii and significantly inhibited its mycelial growth over untreated control. However, highest mycelial growth inhibition recorded by T. asperellum (49.50 mm and 45.00 %), Aspergillus niger (51.50 mm and 42.77%), T. hamatum (55.00 mm and 38.88 %), T. koningii (55.00 mm and 38.88 %), Verticillium lacani (70.00 mm and 22.22 %), Metarhizium anisopliae (80.50 mm and 10.55 %), Pseudomonas fluorescens (88.50 mm and 1.66 %) and Bacillus subtilis (90.00 mm and 0.00 %).

Keywords:- Bioagents, *Sclerotium rolfsii*, Chilli

INTRODUCTION:

Chilli (*Capsicum annuum* L.), is an important solanaceous vegetable-cum-spice crop. Chilli is the native of new world tropics and sub-tropics which was introduced in India from Brazil in 16th century by Portuguese. It is a good source of vitamin A (292 I.U per 100 g), C (111 mg per 100g) and thiamine (0.19 mg per 100 gm). Pungency, one of the important quality attributes of Capsicum species is due to presence of alkaloid 'Capsaicin' in the fruit and also contain capsanthin and oleoresin. Chillies are widely used as a spices, condiments, culinary, supplements, medicines, and vegetables and for flavoring many vegetarian and non-vegetarian food products. Chilli requires hot and humid conditions for growth and development and its cultivation is mainly confined to the tropical regions of the world. Asian countries produces about 65.5 % of the world green chillies and pepper and stands at the top, European countries ranks second with production of 12.1 % chilli and African countries ranks third with production of 9.5 % of the total world chilli production.

Chilli crop is suffered from many fungal, bacterial and viral diseases and its major diseases are: damping off (*Pythium* spp, *Phyptopthora spp.*) anthracnose or fruit rot or dieback (*Colletotrichum capsici*), wilt (*Fusarium oxysporum* f.sp. *capsici),* bacterial leaf spot (*Xanthomonas campestris* p.v. *vesicatoria)*, fungal leaf spots

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(*Alternaria alternata, Cercospora capsici*), powdery mildew (*Leveillula tourica*), root rot (*Sclerotium rolfsii*) and leaf curl mosaic (virus). During recent years the root rot complex disease has been attaining serious proportion, causing severe yield losses in chilli crop. The pathogens commonly associated with chilli root rot complex *viz.*, *S. rolfsii, Fusarium* spp, *Phytopthora* spp. and *Rhizoctonia*. Were reported to cause yield losses to the tunes of 60.80 %, 34-65 %, 50 to 60% and 35 to 50%. (Kalmesh and Gurjar, 2001; Madhavi *et al.*, 2006; Muthukumar *et al.*, 2010).

METHODS :

Isolation, Identification and Pathogenicity

Those fungi associated with fungal root rot complex of chilli were isolated by applying tissue isolation technique (Tuite, 1969). Naturally infected chilli plants showing typical root rot symptoms were collected from the fields, thoroughly washed the root system with distilled water, blot dried and cut with sharp sterilized blade into small bits (5 mm). Plant root / stem pieces were taken from the lower hypocotyl and upper tap root were then surface sterilized with 1 per cent aqueous solution of Sodium hypochlorite (NaOCl) for two minutes. Subsequently, these root bits were washed thoroughly by giving three sequential changes with sterile distilled water to remove traces of Sodium hypochlorite if any, blot dried and aseptically transferred on to autoclaved and cooled Potato dextrose agar PDA medium in sterile glass Petri plates (90 mm), under Laminar air-flow cabinet and incubated in BOD incubator at 27 ± 2 ^{0}C temperature. These inoculated PDA plates were observed at regular interval for growth of the pathogenic fungi. After a weak of incubation, typical fungal growths developed on PDA plates were transferred into fresh PDA plates and incubated further. By applying hyphal tip isolation technique (Tutte, 1969), purified, and sub-cultured the cultures and their pure cultures on Agar slant tubes were maintained in refrigerator, for further studies.

Pathogenicity of the test fungi were proved by sick soil (*S. rolfsii*) method in earthen pots, under screen house conditions. Earthen pots (30 cm dia.) disinfected with 5 per cent Copper sulphate solution were filled with autoclaved potting mixture of soil: sand: FYM (2:1:1) and inoculated (@ 50 g / kg soil) separately with mass multiplied (sand: maize) culture of the test fungi *viz., S. rolfsii* mixed thoroughly, watered adequately and incubated in screen house for two weeks. Earthen pots filled with sterilized potting mixture without cultures of the test fungi were maintained as uninoculated control. Surface sterilized (1% Sodium hypochlorite) healthy seeds of chilli. Parbhani Local seeds were sown (@ 20 seeds / pot) in these pots, kept in screen house and watered regularly. Observations on seed germination, pre-emergence seed rot and post-emergence seedling mortality were recorded, respectively at 7 and 30 days after sowing.

In vitro evaluation of the bioagents

Most potential fungal and bacterial biocontrol agents were evaluated *in vitro* against the test fungi *S. rolfsii*, by applying Dual culture technique (Dennis and Webter, 1971) and using PDA as basal culture medium. Seven days old cultures of the test bioagents and test fungi grown on respective culture media were used for the study. One each 5 mm culture disc of the test fungus and the test fungal bio agents cut out with sterilized cork-borer was placed at equidistance and exactly opposite to each other on autoclaved and solidified PDA medium in Petri plates. For bacterial biocontrol agents, a culture disc (5 mm) for the test fungus was placed along periphery of the PDA plate and exactly opposite to it pure culture suspension of the test bacterial biocontrol agent was streaked with wire / inoculation needle loop. The PDA plates separately inoculated (in the centre) alone with pure culture disc of the test fungus were maintained as untreated control.

Experimental details:

Design : CRD

Replications: Three

Treatments: Ten

Treatment details:

Tr. No.	Treatments	Tr. No.	Treatments
T_1	*Trichoderma asperellum*	T_6	*Metarhizium anisopliae*
T_2	*T. harzianum*	T_7	*Verticillium lacani*
T_3	*T. hamatum*	T_8	*Bacillus subtilis*
T_4	*T. koningii*	T_9	*Pseudomonas fluorescens*
T_5	*Aspergillus niger*	T_{10}	Control (untreated)

Observations on linear colony growth (mm) of the test fungus and the test bioagent was recorded at an interval of 24 hrs of incubation and continued up to seven days or till the untreated control plates were fully covered with mycelial growth of the test fungi. Based on cumulative data, per cent mycelial growth inhibition of the test fungus with the test bioagents, over untreated control was calculated by applying the following formula (Arora and Upadhay, 1978).

Colony growth in - Colony growth in

Control plate intersecting plate

Per cent Growth = -------------------------- x 100

Inhibition Colony growth in control plate

RESULT:

In vitro efficacy of bioagents

In vitro efficacy of bioagents against S. rolfsii

The results revealed that all the bioagents evaluated in vitro exhibited antifungal activity against S. rolfsii and significantly inhibited its growth, over untreated control.

In vitro efficacy of bioagents against S. rolfsii

Tr. No.	Treatments	Colony Dia. (mm) of Pathogen*	% Inhibition
T_1	*Trichoderma asperellum*	49.50	45.00 **(42.13)**
T_2	*T. harzianum*	19.00	78.88 **(62.64)**
T_3	*T. hamatum*	55.00	38.88 **(38.58)**
T_4	*T. koningii*	55.00	38.88 **(38.58)**
T_5	*Aspergillus niger*	51.50	42.77 **(40.84)**
T_6	*Metarhizium anisopliae*	80.50	10.55 **(18.95)**
T_7	*Verticillium lacani*	70.00	22.22 **(28.12)**
T_8	*Bacillus subtilis*	90.00	0.00 **(0.00)**
T_9	*Pseudomonas fluorescens*	88.50	1.66 **(7.41)**
T_{10}	Control (untreated)	90.00	0.00 **(0.00)**

	SE± (M)	**0.69**	**0.77**
	C.D (P=0.01)	**2.10**	**2.30**

*Mean of three replications, Dia.: Diameter, **Figures in parentheses are arcsine Transformed values

The results revealed *T. harzianum* as most effective bioagent with significantly least mycelial growth (19.00 mm) and significantly highest mycelial growth inhibition (78.88%), followed by *T. asperellum* (49.50 mm and 45.00 %), *Aspergillus niger* (51.50 mm and 42.77%), *T. hamatum* (55.00 mm and 38.88 %), *T. koningii* (55.00 mm and 38.88 %), *Verticillium lacani* (70.00 mm and 22.22 %), *Metarhizium anisopliae* (80.50 mm and 10.55 %), *Pseudomonas fluorescens* (88.50 mm and 1.66 %) and *Bacillus subtilis (*90.00 mm and 0.00 %*).*

These test bioagents found effective in present study against rot of chilli causing *S. rolfsii*, were also reported as potential antagonists against *S. rolfsii*, by several earlier workers (Bankar *et al.* 2017; Tabing and Tiwari, 2018; Vineela *et al.,* 2020; Sekhar *et al.*, 2020;)

Plate: *In vitro* efficacy of bio agents against *S. rolfsii* causing chilli root rot complex.

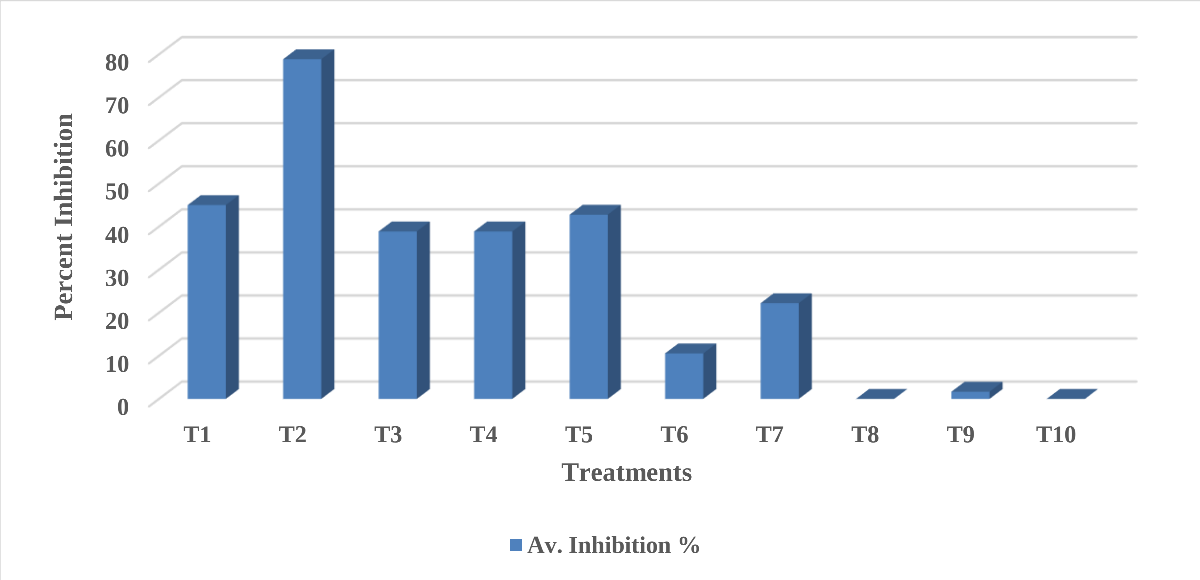

Fig. : *In vitro* efficacy of bio agents against *S. rolfsii* causing chilli root rot complex.

CONCLUSIONS

All nine bioagents evaluated in vitro significantly inhibited mycelial growth over untreated control, of the test pathogenic fungi S. rolfsii and F. solani, causing chilli root rot complex. However, in S. rolfsii, significantly highest mycelial growth inhibition resulted with T. harzianum (78.88 %) %), followed by T. asperellum (45.00 %), Aspergillus niger (42.77%), T. hamatum (38.88 %), T. koningii (38.88 %), Verticillium lacani (22.22 %), Metarhizium anisopliae (10.55 %), Pseudomonas fluorescens (1.66 %) and Bacillus subtilis (0.00 %). Similarly, in F. solani, significantly highest mycelial growth inhibition was resulted with T. harzianum (90.00 %), followed by T. koningii (87.77 %) Aspergillus niger (80.00 %), T. asperellum (77.77 %), T. hamatum (77.77 %), Metarhizium anisopliae (68.33 %), Verticillium lacani (47.22 %), Bacillus subtilis (36.66 %) and Pseudomonas fluorescens (32.77 %).

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