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***J. Mycopathol. Res.* 61(1) : 115-118, 2023;
ISSN 0971-3719**

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Bio-efficacy of Antagonists and Phytoextracts against *Alternaria alternata* causing Leaf spot of chilli

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Received : 13.12.2022

Accepted : 01.02.2023

Published : 27.03.2023

Chilli (*Capsicum annuum* L.) is one of the important vegetable crops in India. Chilli leaf/fruit is affected by many fungal pathogens, but Leaf spot of chilli caused by *Alternaria alternata* is the most destructive disease leading losses in field as well as market. It adversely affects the fruit quality, quantity and ultimately reduces the market value. An investigation was carried out to test the efficacy of bioagents *in vitro* by dual culture technique against *Alternaria* leaf spot of chilli. Four bioagents i.e. *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated and among them highest per cent growth inhibition (75.67%) was observed in *T. harzianum* Rifai. NAU followed by *T. viride* (71.33%), *B. subtilis* (71.00%) and *P. fluorescens* (67.33%), respectively. Five phytoextracts were tested at 10% *in vitro* against the mycelial growth and among them garlic phytoextract was found most effective (75.98%) for inhibiting the pathogen followed by neem (55.26%), tulsi (47.69%), lemon grass (46.71%) and nilgiri leaf extract (44.40%), respectively.

Key words: *Alternaria alternata*, antagonist, botanicals, leaf spot

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the important cash crop. It is mainly cultivated for the vegetable purpose which has potential vegetable market and medicinal values also. Chilli is cultivated over an area of 309 thousand hectares in India with annual production of around 3592 MT followed by China and in Gujarat it was 22.07 MT in 2017-2018 (Anonymous, 2019).

Alternaria leaf spot of chilli affects quality and quantity of fruit with economic loss to the chilli growers. Fruit rot is a major constraint during chilli cultivation causing several losses in terms of quality and quantity and it is reported that 5-85 per cent yield losses occur due to *Alternaria* leaf spot. The injudicious application of chemicals

results in health hazard problems to common people. The search for alternative approaches which have minimal deleterious effects, more ecofriendly and will contribute to the goal of sustainability in agriculture is needed (Jain and Sharma, 2006; Waghunde *et al.* 2016). The present investigation was done to reduce *Alternaria* leaf spot of chilli by the utilization of an antagonist and phytoextract under south Gujarat condition.

MATERIALS AND METHODS

Collection, isolation and purification

The naturally disease infected leaves of chilli plant showing the small, dull to dark brown spot with yellow dry lesions, circular or irregularly shaped spots symptoms of *Alternaria* leaf spot were collected from Regional Horticulture Research Station (R.H.R.S.), Navsari Agricultural University,

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Navsari farm during crop season. Small pieces of diseased tissues along with adjoining healthy tissues were cut and surface sterilized by dipping in (0.1%) NaOCl₂ solution for one minute followed by three successive washings with distilled sterile water. The sterilized pieces were then transferred aseptically under laminar air flow system in sterilized Petri plates containing 20 ml Potato Dextrose Agar (PDA) medium. The Petri plates were incubated at 27 ± 1°C in BOD incubator for seven days. The culture was purified by hyphal tip method. As soon as the mycelia growth were observed in the Petri plates, advancing hyphal tips growing out of the tissue segments were cut off with sterilize inoculation needle and transferred to PDA slants for further growth.

***In vitro* evaluation of bioagent**

Antagonistic effect of different bioagents i.e. *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were tested by dual culture technique for their antagonism against the *A. alternata* causing leaf spot in chilli.

Seven day- old culture of the bio agents and the pathogen were employed by following dual culture method. Mycelial disc of 5mm diameter were kept at periphery of Petri plate by keeping 80mm distance between antagonist and *A. alternata*. In case of bacterial bio agents, half portion of the plates were streaked and 5 mm diameter mycelial discs was placed at the corner of Petri plates. In control, only pathogen was kept in the center of Petri plate. The Petri plates were incubated at 27 ± 1°C in BOD incubator for 7 days. The observations on mycelia growth (mm) and per cent growth inhibition were recorded after 7 days of incubation.

***In vitro* evaluation of phytoextract**

Bioefficacy of five phytoextracts of plant species having medicinal value i.e. garlic, tulsi, nilgiri, lemon grass and neem were tested *in vitro* by poisoned food technique against *Alternaria* leaf spot of chilli. All the phytoextracts were tested at 10 per cent concentration. Fresh and healthy 100gm plant parts of each species were thoroughly washed with tap water and then with distilled sterilized water. They were macerated separately in grinder by adding 100ml. ethanol.

The mixture was filtered through two fold sterilized muslin cloth and the filtrate were centrifuged at

5000 rpm for 10 min. and the clear supernatant extract were collected in sterilized conical flasks. After evaporating the ethanol from extract, the clear extract were collected and diluted with 100 ml distilled sterile water to make volume 1:1 (W/V). This was considered as 100 per cent concentration for the study to test the efficacy of plant extracts. Each phytoextracts (10%) were mixed thoroughly in sterilized 100ml. PDA medium filled in 250 ml flask under aseptic condition. The medium were supplemented with streptomycin sulphate @ 50 ppm to prevent bacterial contamination. The 5 mm disc of 7 days old cultures of isolates of *A. alternata* were placed in Petri plate containing PDA medium along with phytoextracts. Observations on mycelial growth (mm) and per cent growth inhibition were recorded 7 days after inoculation.

RESULTS AND DISCUSSION

Bioagents

Total four antagonists viz., *Trichoderma viride* Pers. NAU isolate, *T. harzianum* Rifai. NAU isolate, *Pseudomonas fluorescens* NAU isolate and *Bacillus subtilis* NAU were evaluated for their antagonism against *A. alternata* causing *Alternaria* leaf spot of chilli by dual culture method under *in vitro* condition. The observations on mycelial growth (mm) and per cent growth inhibition (PGI) were recorded after seven days of incubation and results presented in Table 1.

All the antagonists were significantly inhibiting the mycelial growth of *A. alternata* over the control. The significantly lowest mycelial growth (18.25 mm) with highest per cent growth inhibition (75.67%) was observed in *T. harzianum* Rifai. NAU isolate seven day after incubation. The next best bioagent was *T. viride* Pers. NAU isolate recorded 21.50 mm mycelial growth with 71.33 per cent growth inhibition followed by *B. subtilis* NAU isolate recorded 21.75 mm mycelial growth with 71.00 per cent growth inhibition. The highest mycelial growth (24.50 mm) and lowest inhibition (67.33PGI) was recorded in *P. fluorescens* NAU isolate against *A. alternata* at seven days after incubation (Table 1).

Similar results to present investigation was reported by Sarkar *et al.* (2017) who recorded maximum mycelial growth inhibition in *T. harzianum* (76.23 %) followed *T. hamatum* (70.46%), yeast (*S. cerevisiae*) (57.98%), *B. subtilis* (43.81%), *P.*

Table 1: *In vitro* bio-efficacy test of antagonists on the mycelial growth inhibition of *A. alternata*

Treatment	Bioagent	Mycelial growth 7 DAI (mm)	PGI (%)
T ₁	<i>Trichoderma viride</i> Pers. NAU isolate	21.50	71.33
T ₂	<i>Trichoderma harzianum</i> Rifai. NAU isolate	18.25	75.67
T ₃	<i>Pseudomonas fluorescens</i> NAU isolate	24.50	67.33
T ₄	<i>Bacillus subtilis</i> NAU isolate	21.75	71.00
T ₅	Control	75.00	-
S.Em.±		0.89	
CD at 5%		2.68	
CV %		5.52	

Table 2: *In vitro* bio-efficacy of phytoextracts on the mycelial growth inhibition of *A. alternata*

Treatment	Phytoextract	Mycelial growth 7 DAI(mm)	PGI (%)
T ₁	Garlic (<i>Allium sativum</i> L.)	18.25	75.98
T ₂	Tulsi (<i>Ocimum sanctum</i> L.)	39.75	47.69
T ₃	Nilgiri (<i>Eucalyptus</i> sp.)	42.25	44.40
T ₄	Lemon grass (<i>Cymbopogon flexuosus</i> Steud.)	40.50	46.71
T ₅	Neem (<i>Azadirachta indica</i> A. Juss)	34.00	55.26
T ₆	Control	76.00	-
S.Em.±		0.99	
CD at 5%		2.96	
CV %		4.76	

fluorescens (36.01%) and *T. viride* (34.81%) against *A. alternata* infecting chilli. Jakatimath *et al.* (2017) found that mycelial inhibition was highest in *T. harzianum*-72 (87.00%) followed by *T. harzianum*-21 (83.00%), *T. harzianum*-29 (81.32%), *T. harzianum*-28 (80.00%), *P. fluorescens* (12.00%) and *B. subtilis* (7.66%) against *A. alternata* infecting brinjal. *T. viride* was found most effective under *in vitro* condition against *A. alternata* infecting chilli was reported by Pandey *et al.* (2019).

Phytoextracts

Total five phytoextracts at 10 per cent concentrations were tested against mycelial growth of *A. alternata* causing leaf spot of chilli under *in vitro* condition by poison food technique. The observations on the mycelial growth (mm) and PGI were recorded after seven days of incubation and the results are mentioned in Table 2.

All the phytoextracts were found significantly superior in inhibiting the mycelial growth of *A. alternata* over the control. The significantly lowest mycelial growth (18.25mm) with highest per cent

growth inhibition (75.98%) was observed in garlic over the rest of phytoextracts. The next best treatments in order of merit were neem and tulsi (34.00 and 39.75mm respectively) with 55.26 and 47.69 PGI at seven days after incubation. The lemon grass (40.50mm) and nilgiri (42.25mm) recorded 46.71 and 44.40 PGI which were statistically at par to each other (Table 2).

The highest growth (42.25mm) and lowest inhibition (44.40 PGI) was recorded in nilgiri against *A. alternata* at seven days after incubation (Table 2). Among the evaluated five phytoextracts highest growth inhibition was recorded in garlic and found most effective in growth inhibition of *A. alternata* followed by neem. The nilgiri was found least effective as compared to others.

The results of present investigation are in conformity with the results obtained by Balai and Ahir (2011) who recorded maximum mycelial growth inhibition (73.7%) in garlic clove extract and minimum mycelial growth inhibition (33.4%) in bel against *A. alternata* infecting brinjal at 5, 10 and 15 per cent concentrations. More or less similar results have been reported by Sarkar *et al.* (2017) who noted that neem leaf extract was found the most effective (55%) in inhibiting the mycelial

growth of *A. alternata* causing leaf spot in chilli followed by garlic clove extract (41.21%).

ACKNOWLEDGEMENTS

The authors are highly grateful to Principal, N. M. College of Agriculture, Navsari and all staff of the Department of Plant Pathology, N.M.C.A., Navsari for providing necessary facilities during the present investigation.

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