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Studies on leaf spot of chilli

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ABSTRACT :

The leaf spot of chilli is important and destructive disease in chilli growing areas of Maharashtra, which is caused by *Alternaria alternata*. Therefore studies were undertaken at PGI, MPKV, Rahuri during the year 2016-17. For this leaves of chilli leaf spot were collected from the PGI farm MPKV Rahuri, which yielded the pathogen *Alternaria alternata*. The pathogenicity of *Alternaria alternata* was tested by Koch's postulates, which proved that the test pathogen was pathogenic to chilli. *In vitro* evaluation of effect of temperature and humidity revealed that test pathogen grew well with maximum sporulation at optimum temperature of 27°C with 80 per cent relative humidity. Six bioagents such as *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma hamatum*, *Pseudomonas fluorescens*, *Bacillus subtilis* and yeast (*S. cerevisiae)* were evaluated *in vitro* against isolated pathogen applying Dual Culture Technique. Among them *T. harzianum* was found most effective in inhibiting the growth of *Alternaria alternata* of about (76.23 %) this was followed by *T. hamatum* (70.46 %), yeast (*S. cerevisiae)* (57.98 %), *Bacillus subtilis* (43.81%), *Pseudomonas fluorescens* (36.01%) and *T. viride* (34.81%), respectively. Among the six botanicals leaf extracts (2%) tested *in vitro* against the pathogen, *Neem* leaf extract was found most effective inhibiting (55%) of the pathogen *Alternaria alternata* followed by garlic cloves (41.25%), *Tulsi* leaf extract (36.25%), nilgiri leaf extract (32.5%), mixture of onion and garlic leaf extract (30%) and parthenium (28.75%) respectively. A total number of six fungicides *viz.*, Carbendazim (0.05%), Chlorothalonil (0.1%), Hexaconazole (0.1%), Mancozeb (0.1%), Propiconazole (0.1%) and Captan (0.1%) were evaluated *in vitro* against the isolated pathogen applying poison food technique. Among them Mancozeb @ 0.1 %, Carbendazim @ 0.05 % and Captan @ 0.1% recorded maximum growth inhibition of 78.99, 78.66 and 74.78 per cent, respectively of the test pathogen with minimum colony diameter of 17.33 , 17.66 and 20.83 mm, respectively.

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INTRODUCTION

Chilli (*Capsicum annuum* L.) is an imperative spices, vegetable as well as commercial crop. Chilies are having good nutritious value and can be used as

multipurpose throughout the world for its aroma, pungency and medicinal value. The most important chilli growing states in India are Andhra Pradesh (49%), Karnataka (15%) Maharashtra (6%) and Tamil Nadu (3 %) which constitute nearly 75 per cent of the total area under chilli (Yadav *et al.*, 2015). This crop is attacked by large number of bacterial, fungal and viral diseases. Among them leaf spot of chilli caused by *Alternaria alternata* is one of the most destructive disease in India. which are seed borne and reduce the seed germination and yield loss upto 30-60 per cent. The present investigations were undertaken with objectives *viz.*, Isolation, identification, pathogenicity, *in vitro* evaluation of fungicides, biological control, botanicals and effect of temperature and humidity on growth and sporulation of the pathogen.

MATERIAL AND METHODS

Isolation:

Diseased leaf sample of chilli were collected from various fields were brought to the laboratory and washed thoroughly in running tap water. These diseased specimens (leaves) were blot dried and cut with sharp sterilized blade into small bits (5mm) keeping half healthy and half diseased portion intact. These pieces were surface sterilized with 0.1 per cent aqueous solution of mercuric chloride ($HgCl_2$) for two minutes and then washed by giving three changes with sterile distilled water to remove traces of mercuric chloride and blot dried. The surface sterilized diseased pieces were then inoculated on the solidified and cooled PDA (Potato dextrose Agar) medium in petriplates under aseptic conditions of Laminar-air-flow cabinet. Inoculated plates were then incubated in BOD incubator at 27±1°C temperature. Three to four days of incubation, the well-developed mycelial growth free from any contaminant was obtained.

Identification :

Based on microscopic observations of spores and mycelium of the fungus, the fungus was identified upto species level and confirmed.

Pathogenicity test :

The surface sterilized (0.1% $HgCl_2$) seeds of Phule Jyoti were sown (@ 2 seeds /pot) in the earthen pots (20 cm dia) filled with steam sterilized potting mixture of soil : sand : FYM (2:1:1). The test pathogen (isolated) was mass multiplied on the basal culture medium PDA in petridishes. Spore suspension of the test pathogen was prepared by harvesting freshly sporulating 7-8 days old culture. The resultant spore-cum-mycelial suspension was filtered through double-layered muslin cloth and filtrate obtained was suitably diluted with sterile distilled water to get inoculums concentration of 3-5 x 10^6 spores/ml. Thirty days old seedlings of Phule Jyoti were artificially inoculated by spraying with atomizer. Seedlings sprayed with sterile water (without inoculums) were also maintained as suitable control. Inoculated plants were incubated in the glass house where high humidity (>80 %) and optimum temperature (27 ±1°C) were maintained for further development of leaf spot in chilli.

***In vitro* evaluation of effect of temperature and humidity on growth and sporulation of pathogen:**

Experiment on effect of temperature and humidity on growth and sporulation of test pathogen were conducted *in vitro* on PDA medium. The Petri plates were inoculated with fresh culture of effective test isolates and incubated at (5,10,15,27,35,45 and 50) °C temperature with humidity (95,90, 85, 80, 65, 55 and 40) per cent, respectively. Each treatment replicated for three times. Growth of each isolates in each Petri plate was recorded on 7th days after inoculation.

Sporulation :

The cavity slides containing 0.1 ml of conidial suspension (10^6 conidia/ml) were placed in Petri plates containing moist filter papers at the bottom were incubated for 24 hrs. Each treatment was replicated for three times and in each replication 100 conidia were counted for the germination.

All Observations on redial mycelial growth and sporulation of test organism was recorded in each treatment and replication and mean colony diameter and per cent inhibition is calculated.

$$\text{Per cent growth inhibition (I)} = \frac{(C - T)}{C} \times 100$$

where,

C = Growth of test fungus (mm) in control plate

T = Growth of test fungus (mm) in treatment plate.

***In vitro* evaluation of biological agent :**

Six bioagents such as *Trichoderma viride*,

Trichoderma harzianum, Trichoderma hamatum, Pseudomonas fluorescens, Bacillus subtilis and yeast (*S. cerevisiae)* were evaluated *in vitro* against isolated pathogen applying Dual culture technique and using potato dextrose agar (PDA) as basal culture-method.

Bioagents were evaluated for their efficacy by dual culture technique. Disc (5 mm) of isolated pathogen was placed at the center of one corner of different Petri plates containing solidified PDA medium and disc of *Trichoderma viride, Trichoderma harzianum, Trichoderma hamatum* was placed at other corner of different Petri plates. The disc of each bioagents was kept with isolated pathogen disc in three Petri plates. A loopful of 24 hour old culture of *Pseudomonas fluorescens, Bacillus subtilis* and yeast (*S. cerevisiae)* was inoculated at 2 cm just opposite to the pathogen on each plate.

***In vitro* evaluation of botanical extract :**

Leaves of *Tulsi, Neem,* parthenium, onion, nilgiri and garlic cloves were brought to the laboratory and washed with running tap water. Then surface sterilize with 40 per cent ethyl alcohol solution then separately chopped. Each leaf sample was then separately grind and homogenized in mechanical grinder with equal quantity of sterile distilled water (1:1, w:v). The homogenate obtained was then strained through double layered muslin cloth and filtrate collected was then filtered through Whatman No. 1 filter paper using volumetric flasks (50 ml capacity). An appropriate quantity of leaf extracts was incorporated separately in the molten and cooled PDA medium in conical flask (250 ml capacity) to get desired concentration (2%) of extract of different samples. Plant extracts amended PDA was then poured (15-20 ml/plates) in sterilized Petri plates (90 mm dia.) under aseptic conditions. Treatment of different leaf extracts with respective concentration was replicated for three times by placing at the center with 5.0 mm uniform mycelial disc, obtained from 6-7 days old culture of isolated pathogen and multiplied on agar plates.

***In vitro* evaluation of fungicides :**

A total of six fungicides *viz.*, Carbendazim (0.05%), Chlorothalonil (0.1%), Hexaconazole (0.1%), Mancozeb (0.1%), Propiconazole (0.1%) and Captan (0.1%) were evaluated *in vitro* against the isolated pathogen applying poison food technique and using potato dextrose agar (PDA) as basal culture media. The requisite quantity of each fungicide was calculated and thoroughly mixed with autoclaved and cooled (40-45°C) PDA in conical flasks to obtain desired concentrations. Petri plates containing plain PDA without any fungicide, where inoculated with 5.0 mm disc of the test pathogen and maintained as suitable untreated control. All the treatment (inoculated) and control Petri plates where then incubated at 27 ± 1°C in BOD incubator till the control plates were fully covered with mycelial growth of the test pathogen.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Isolation :

The culture of the fungal colony was initially white, cottony with profuse aerial mycelium which gradually turned grey colour. Aged culture appeared completely greyish with aerial mycelium and distinct concentric rings

Table 1: *In vitro* evaluation on effect of temperature and humidity on radial growth and sporulation of *Alternaria alternata*

Sr.No.	Temperature (°C)	Humidity (%)	Mean colony dia 7th DAI (mm)*	Sporulation
1.	5	95	00.00	-
2.	10	90	9.83	-
3.	15	85	13.50	+
4.	27	80	80.50	+++
5.	35	65	51.16	++
6.	45	55	00.00	-
7.	50	40	00.00	-
	S.E.±	0.75		
	C.D. (P=0.05)	1.64		

was formed on medium. Conidiophores were short to long, simple or branched arising singly. Conidiophores were golden to brown coloured with 2-9 transverse and 0-2 longitudinal septa. Conidia were borne in long chains (6-11) on conidiophores, they were thick walled, beaked and brown in colour. Based on the characters of the colony and morphological characters of conidiophores and conidia the fungus has been identified as *Alternaria alternata*. These results are mostly in agreement with Devappa and Thejakumar (2016) who isolated fungus *A. altemata* from diseased leaves of chilli on PDA medium.

Identification :

The fungus was identical as *Alternaria alternata* at the Department of plant pathology and Agril.

Table 2 : *In vitro* evaluation of effect of bio-agents on radial growth of *Alternaria alternata*

Sr. No.	Biological agents	Mean colony dia 7th DAI (mm)*	Growth inhibition (%)
T_1	*Trichoderma harzianum*	19.83	76.23
T_2	*Trichoderma viride*	54.33	34.81
T_3	*Trichoderma hamatum*	24.66	70.46
T_4	*Pseudomonas fluorescens*	53.33	36.01
T_5	*Bacillus subtilis*	46.83	43.81
T_6	Yeast (*S. cerevisiae)*	35.00	57.98
T_7	Control	83.33	00.00
	S.E.±	3.79	
	C.D. (P=0.05)	8.22	

Table 3 : *In vitro* evaluation on effect of botanicals on radial growth of *Alternaria alternata*

Sr. No.	Botanicals	Mean colony dia 7th DAI (mm)*	Growth Inhibition (%)
T_1	*Neem* leaf extract	36.00	55
T_2	*Tulsi* leaf extract	51.66	36.25
T_3	Garlic	47.33	41.25
T_4	Nilgiri	54.33	32.5
T_5	Parthenium	57.00	28.75
T_6	Onion+garlic	56.00	30
T_7	Control	80.00	00.00
	S.E.±	1.38	
	C.D. (P=0.05)	2.98	

Table 4: *In vitro* evaluation on effect of fungicides on radial growth of *Alternaria alternata*

Treat. No.	Treatments	Concentration (%) used	Mean colony dia 7th DAI (mm)*	Growth inhibition (%)
T_1	Mancozeb	0.1%	17.33	78.99
T_2	Carbendazim	0.05%	17.66	78.66
T_3	Chlorothalonil	0.1%	39.50	52.12
T_4	Hexaconazole	0.1%	32.66	60.48
T_5	Propiconazole	0.1%	21.16	74.42
T_6	Captan	0.1%	20.83	74.78
T_7	Control		82.50	0.00
	S.E.±	1.60		
	C.D. (P=0.05)	3.46		

Microbiology, PGI, Mahatma Phule Krishi Vidyapeeth, Rahuri. Morphological characters of conidiophores and conidia of the fungus has been identified by adapting slide culture technique under microscope. Identification of *A. alternata* causing leaf spot of chilli was discovered by several workers. Similar results were reported by Rao (1965) and examined that conidiophores were short to long, septate, branched, straight to slightly wave and Conidia were obclavate to typically muriform dark.

Pathogenicity :

The pathogenicity of *Alternaria alternata* was confirmed, in which initial symptoms of Alternaria leaf spot was recorded seven to nine days after inoculation on the inoculated leaves with a small, circular necrotic spot. leaves usually spots with concentric rings develop. Alternaria causes small reddish – purple spots , most characteristic symptom is brown or dark spots on older leaves. These spots started to increase with irregular margin and it remained brown in colour surrounded by yellow hallow and the spotted leaves soon wither and the plant died. These results are mostly in agreement with Devappa and Thejakumar, 2016.

***In vitro* evaluation on effect of temperature and humidity on *Alternaria alternata* :**

The results presented in Table 1 reveals that the growth of pathogen was maximum at 27ºC temperature with humidity of 80 per cent. Temperature below 5ºC and above 45ºC were inhibit the growth of the test pathogen. There was no growth observed at 5ºC, 45ºC and 50ºC temperature. The formation of conidia was observed after 7th day at optimum temperature 27ºC.As regards to sporulation, the good sporulation was observed at 27ºC temperature with humidity 80 per cent and moderate sporulation at 35ºC with humidity 65 per cent. Scanty sporulation was recorded at 15ºC temperature with humidity 85 per cent. There was no sporulation at 5ºC, 10ºC, 45ºC and 50ºC temperature with relative humidity 95 per cent, 90 per cent, 55 per cent and 40 per cent, respectively. More or less similar results have been reported, earlier research workers as Shinde (1995) noticed that *A. alternata* was able to grow at temperature from 5°C to 35°C.

***In vitro* evaluation of bioagents :**

The data presented in Table 2 revealed that among the six bioagents *T. harzianum* was found most effective in inhibiting the growth of *Alternaria alternata* of about (76.23 %) this was followed by *T. hamatum* (70.46 %), Yeast (*S. cerevisiae)* (57.98 %), *Bacillus subtilis* (43.81%), *Pseudomonas fluorescens* (36.01%) and *T. viride* (34.81%) on growth of test pathogen. From the data presented it was observed that out of the six bioagents used *T. harzianum* was most effective for inhibiting the growth of *Alternaria alternata*. These results are more or less similar with the findings reported by Subash *et al*. (2013) and Gohel and Solanky (2011).

***In vitro* evaluation of botanicals :**

The data presented in Table 3 revealed that among the six botanicals, *Neem* leaf extract was found most effective inhibiting (55%) of the pathogen *Alternaria alternata* followed by garlic cloves (41.25%), *Tulsi* leaf extract (36.25%), nilgiri leaf extract (32.5%), mixture of onion and garlic leaf extract (30%) and parthenium (28.75%). More or less similar results have been reported by Meena and Mariappan (1993) and noted that *Neem* leaf extract were effective in controlling *Alternaria alternata*.

***In vitro* evaluation of fungicides :**

The data presented in Table 4 revealed that all the six fungicides evaluated *in vitro* against *Alternaria alternata* were found fungi toxic and recorded significant reduction in radial growth of the test fungus over untreated control. The fungicide Mancozeb inhibited (78.99%) radial mycelial growth of pathogen *Alternaria alternata* followed by Carbendazim (78.66%), Captan (74.78%), Propiconazole (74.42%), Hexaconazole (60.48%) and Chlorothalonil (52.12%). From the above data it was observed that out of six fungicides Mancozeb is most effective for inhibiting the growth of *Alternaria alternata*, These results are in agreement with those reported by Shinde (1995) tested relative efficacy of different fungicides against *Alternaria alternata in vitro.* Among fungicides tested Manocozeb (Indofil M-45) 0.25 per cent was found most effective showing 100 per cent inhibition of growth and sporulation of *A. alternata.*

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