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STUDIES ON LEAF SPOT DISEASE OF MANGO AND ITS MANAGEMENT

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ABSTRACT

Mango (*Mangifera indica* L.) is susceptible to a number of diseases. One of the important diseases is leaf spot caused by *Alternaria alternata* affecting all the plant parts. The pathogen also attacks mango inflorescence resulting in a significant decrease in fruit set. The cultural characters of the pathogen were studied on Potato Dextrose Agar including the role of temperature and pH on the growth and sporulation. Certain fungicide and bioagents were evaluated for the control of the fungus. The temperature 25^0C and pH 7.0 was found optimum for the growth and sporulation of *Alternaria alternata.* The fungicide Indofil M-45 was highly effective in inhibiting the mycelial growth at all concentrations followed by Ridomil at 0.5%. The bio agents *Trichoderma harzianum* reduced the growth of *Alternaria alternata* by 85.4% followed by *T. viride, T. pseudokoningii, T. koningii , Aspergillus niger* and *A. flavus*

KEYWORDS:Mango leaf spot, pH, Temperature, Chemical control, Biological control, *Alternaria alternata*.

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INTRODUCTION

Mangoes (*Mangifera indica* L.) belonging to family *Anacardiaceae* are universally considered as one of the choicest fruits in tropical and subtropical areas of the world [1]. Mango is affected by a number of diseases at all stages of the development, including post harvest, storage and transit. Almost every part is affected by various pathogens [2], causing minor to major diseases of economic importance. Among these, leaf spot caused by *Alternaria alternata* is quite destructive hampering the increased yield of mango. They impoverish the leaves, diminish the phyto-synthetic efficiency and upset normal physiological activity of the host. An understanding of the role of environmental conditions and its effects on infection and survival of the pathogen is necessary to develop cultural disease management practices. In view of this, certain aspects of the disease and pathogen including abundance, cultural characters, temperature and pH requirements and control were studied.

MATERIALS AND METHODS

1. *Sampling and Isolation*

Mango leaves with leaf spot symptoms were collected from Horticulture experiment and training centre, Baruasagar, Jhansi (U.P.) in the months March and April. The common variety available at this centre was Amarapali, Neelam, Chuansa and Dashehari. The samples were collected from the variety Amarapali. Leaves with spots were cut into small pieces, surface sterilized with 1% NaOCl for 1 minute rinsed thoroughly in sterile distilled water and dried on sterile filter paper. The leaf pieces were placed onto potato dextrose agar (PDA) medium and incubated at 25±2^{0}C for 07 days to promote fungal growth and sporulation. Pure cultures were obtained from single spores and hyphal tips. The morphological and culture characteristic of the isolated organism was studied, conidia and conidiophores details were considered. The identification was further confirmed from NCFT by Dr. P. N. Chowdhry Principal Mycologist National Centre for Fungal Taxonomy Inderpuri, New Delhi.

2. *Environmental Studies*

Effect of different temperatures on the growth and sporulation of Alternaria alternata

The effect of temperature on growth and sporulation of the pathogen was studied on PDA by employing different temperatures as 15, 20, 25, 30 and 35°C. 1-2 mm mycelial disc of 7 days old culture was used to inoculate Petri plates. There were three replications for each treatment. Inoculated plates were kept in the incubator and temperature was adjusted to required level. The radial growth of the fungal colony was assessed by measuring the growth of the colony diameter in mm. The observations were recorded after every 24 hrs for seven days in each experiment.

3. *Physiological studies*

Effect of different pH of media on the growth and sporulation of Alternaria alternata.

Effect of different pH media on the growth and sporulation of *Alternaria alternata* was studied at pH levels, *viz.,* 5.0, 6.0, 7.0, 8.0 and 9.0. Observations were taken by Backman pH meter using N/10 sodium hydroxide and N/10 hydrochloric acid. The 1-2mm disc of pathogen was placed on the centre of the petri plate. The radial growth of the fungal colony was assessed by measuring the growth of the colony diameter in mm. The observations were recorded after every 24 hrs for seven days in each experiment. The plates were incubated at temperature 25±2^{0}C for seven days.

4. *Chemical and Bio-management*

Bio assay of fungicides against Alternaria alternata

For bio assay three fungicides viz., Bavistin, Ridomil and Indofil M-45 (Mancozeb) were assessed using poison-food technique [3]. Requisite quantities of fungicides were added in three different concentrations (0.5, 1.0 and 2.5) in 2 percent PDA medium, which was shaken well to make it homogenous. The medium was

then poured into petri plates. A circular disc of 1-2 mm diameter was taken from 7 days old culture, placed in the centre of each petriplates and were incubated at 25 ± 2^0C temperature for 7 days. The experiment was repeated thrice along with control. The radial growth of the fungal colony was measured in mm and interpreted in percent inhibition over control by following formula [4].

$$PI = \frac{RC - RT}{RC} \times 100$$

Where PI=Percent Inhibition, RC=Radial growth in control, RT= Radial growth in treatment.

In vitro antagonistic potential of Bio-agents against Alternaria alternata

The antagonistic efficacy of different species of *Trichoderma* viz. *Trichoderma harzianum*, *T. viride*, *T. pseudokoningii, T. koningii* and *Aspergillus niger and A. flavus* were used by dual culture experiment [5]. *A. alternata* was inoculated at 4 cm apart with the bio agents. The plates were incubated at 25±2°C for 5 days.Three replicates were maintained for each treatment. In order to record inhibition of the test pathogen, radial growth was measured on an interval of 24 hrs for five days. The per cent inhibition over check/control was calculated after 5 days by the formula mentioned above.

RESULTS

1 Sampling and Isolation

Symptoms were observed as small, brownish circular spots on the surface of leaves and fruits and as black patches on the twigs. There were minute sunken spots concentrated on midrib and side veins and a dark brown band on the dorsal surface Fig 1. The tender leaves were more susceptible than the mature ones. The fungus on PDA produced moderately fast growing colony, dull to white in color, fluffy, circular and later turning into dark greenish olive with abundant sporulation Fig.2. Conidiophores originate solitary or in groups, simple or branched, straight or flexuous, smooth and pale to mid olivaceous or golden brown sometimes geniculate. Conidia were ovoid to obclavate, 20-36 × 9-9.5µm, 18-44×7.5-12.5µm, 3-5 septate, born in long chains Fig.3.

Figure 1
Symptoms on mango Leaf

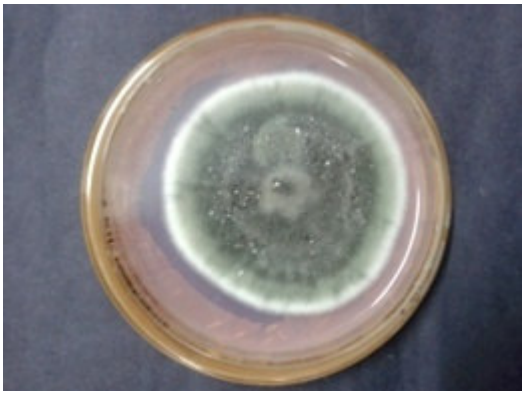

Figure 2
Culture of A. alternata on PDA

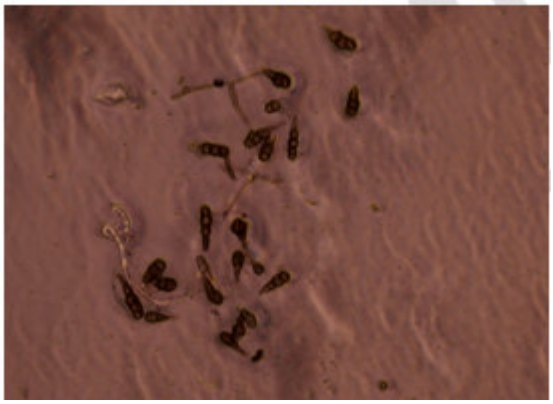

Figure 3
Micrograph of A. alternata

2 Environmental Studies

Effect of different temperatures on the growth and sporulation of Alternaria alternata

Effect of different temperature on the growth and sporulation of *Alternaria alternata* was studied and the results are presented in Table 1. It is seen from the table that after 168 hours, the colony growth and sporulation increased with the increase in temperature and attained maximum (33.3 mm) at 25^0C. Later, it decreased with the rise in temperature up to 35^0C. Mean radial growth and sporulation was low (13mm) at 35^0C.

3 Physiological Studies

Effect of different pH of media on the growth and sporulation of Alternaria alternata

Effect of different pH of media on the growth and sporulation of *Alternaria alternata* was studied. The results are presented in Table. 2. It is clear from the table that pH 7.0 was ideal in producing maximum mycelial mean growth (42.3mm) and sporulation. The mean mycelial growth and sporulation was low at pH 9.0 (16.3mm).

Table No 1

Effect of different temperatures on growth and sporulation of A. alternata

Different Temperature	Colony Growth & Sporulation*							
	Colony Growth in mm							Sporulation
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs	168hrs	
15^0C	1	2.6	7.6	10.6	12.6	13.6	15	-
20^0C	2.6	10.6	15	18.3	21.3	26.6	30	+++
25^0C	3.6	12.3	16	19.3	25	29.6	33.3	++++
30^0C	3	6.6	8.3	12	13	16	19.3	++
35^0C	2.6	4.3	6.3	7.6	9.3	10.6	13	+

Excellent ++++, Good +++, Average ++, Poor +, Ni l -. *Mean of three replications.

Table No 2

Effect of different pH of media on growth and sporulation of A. alternata

Different pH media	Colony Growth & Sporulation*							
	Colony Growth in mm							Sporulation
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs	168hrs	
pH 5	7.3	12	14.3	16	18	20	21	++
pH 6	6	8.6	10.6	14	16.6	20.3	23.3	+++
pH 7	13.3	18.3	24.6	29.6	35	39.3	42.3	++++
pH 8	5.6	12	16	18.6	22	25.3	28	+++
pH 9	5.6	8.3	10	12	13	14.3	16.3	+

Excellent ++++, Good +++, Average ++, Poor +, Ni l -. *Mean of three replications.

5. *Chemical and Bio-management*

Bio assay of fungicides against A. alternate

The results presented in the Table No.3 indicated that all the fungicides were superior over control. The fungicide Indofil M-45 proved effective as it inhibited the growth completely at all concentrations followed Ridomil where as Bavistin was the least effective. The superior and most fungi toxic quality of these fungicides was in descending order, Indofil M-45 > Ridomil > Bavistin. The affectivity of fungicides increased with higher concentration of dose. In Bavistin change in the concentration percentage increased inhibition percentage of the pathogen. Ridomil was effective at high concentration, the growth of the test pathogen was 4mm in 0.5% concentration, after an increase in concentration (1.0% and 2.5%) the pathogen showed total inhibition.

In vitro antagonistic potential of Bio-agents against A. alternata

In vitro high antagonistic potential of Bio-agents against *A. alternata* was found in species of *Trichoderma,* followed by *Aspergillus* species. The inhibition percentage ranged from 85.4% to 75.9% and 68.9% to 65.4% with species of *Trichoderma* and *Aspergillus* respectively. The *Trichoderma harzianum* was superior to all bio agents tested Table. 4. The inhibitory potential of these bio agents have been in desending order as; *Trichoderma harzianum* > *T. viride* > *T. pseudokoningii* > *T. koningii* >*Aspergillus niger* > *A. flavus.*

Table No 3
Bio assay of fungicides against A. alternata.

S. No.	Fungicides	Doses (%)	Colony growth (mm)	Inhibition (%)
1.	Indofil M-45	0.5	0.0	100
		1.0	0.0	100
		2.5	0.0	100
2.	Ridomil	0.5	4	95.12
		1.0	0.0	100
		2.5	0.0	100
3.	Bavistin	0.5	60	26.83
		1.0	50	39.02
		2.5	40	51.21
4.	Control	--	82	--

Table No 4
In vitro antagonistic potential of Bio-agents against A. alternata

Bio-agent	Radial growth of *A. alternata* in mm					Inhibition Percentage				
	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Trichoderma harzianum	06.0	07.9	10.3	12.0	12.2	40.0	65.6	71.4	80.3	85.4
T. viride	06.1	08.0	11.2	12.5	17.1	39.0	65.2	73.3	79.6	79.6
T. pseudokoningii	06.2	09.5	12.9	16.0	19.9	38.0	58.6	69.2	73.7	76.3
T. koningii	06.4	08.1	13.2	18.3	20.2	36.0	64.7	68.5	70.0	75.9
Aspergillus niger	06.9	11.1	16.5	22.2	26.1	31.0	51.7	60.7	63.6	68.9
A. flavus	06.9	12.7	18.3	22.8	29.0	31.0	44.7	56.4	62.6	65.4
Control	10	23	42	61	84.0					

DISCUSSION

The fungus *A. alternata* was grown on artificial media. The morphological characters like fast growing colony, dull to white color mycelium, abundant sporulation, conidiophores and shape of conidia closely resemble with the description given by Prakash[6].Temperature is an important environment factor affecting the growth and sporulation of the fungi. Fungi are capable of surviving under varied temperatures. It is usually reported between 10 to 35^0C with few exceptions[7]. The optimum temperature for the growth of *A. alternata* ranged from 20-30^0C [8, 9]. In the present studies, the fungus grew well at 25^0C, fully agreeing to earlier reports[10, 11] . Thus, it can be concluded that temperature 25^0C was most suitable for growth and sporulation of the pathogen The pH 7.0 was most suitable for the maximum growth and sporulation of the pathogen. Cakarevic and Boškovic [9] reported that the optimal pH value of fungus growth is between 6 and 8 depending upon the isolate The results of the present study are in agreement with the conclusions made by Curran[8] who observed pH 7.0 as the optimum for vegetative growth of *Alternaria* sp. However, Hubballi *et al*., [12] reported pH range 6.00- 6.50 as optimum for growth of *A. alternata.* The fungicide Indofil M-45 proved superior to other fungicides. In earlier studies, Indofil M-45 has been found superior against culture of *A.*

alternata [13]. Further, Kamble *et al.,* [14] also reported that Indofil M-45 was highly effective in inhibiting the mycelial growth of the fungus. *In vitro* antagonistic potential against *A. alternata* was found in all the species of *Trichoderma* and *Aspergillus* evaluated. *Trichoderma harzianum* was most superior followed by *T. viride.* Pandey [15] also recorded the maximum control of *Alternaria alternata* by *Trichoderma harzianum* followed by *T. viride.* However, Ambuse *et al.,* [16] reported *T. viride* followed by *T. harzianum* against leaf spot diseased caused *A. tenuissima.*

CONCLUSION

From the above findings it can be concluded that the temperature 25^0C and pH 7.0 was most suitable for optimum growth and sporulation of the pathogen. The fungicide Indofil M-45 was highly effective in inhibiting the mycelial growth at all concentrations followed by Ridomil at 0.5%. *Trichoderma harzianum* was most superior followed by *T. viride*. The maximum inhibition of 84.5% was achieved by *Trichoderma harzianum.* However, further studies are needed to evaluate their potential under field conditions.

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