

Appliance of Fungicides on the *Alternaria* Leaf Spot Disease of Red Delicious Apple (*Malus domestica* Borkh.) Caused by the Pathogen, *Aternariamali*

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ABSTRACT: Fungicides had drawn-out persistent and preventive effect on the growth and sporulation of the fungus, *Alternariamali* thereby preventing development of typical *Alternaria* leaf spot symptoms. Leaf lesions appear in the form of small, round, blackish spots, gradually enlarging to 2-5mm in diameter with a brownish-purple border. Most spots undergo a secondary enlargement phase and become irregularly shaped and much darker in colour, acquiring a “frog-eye” appearance. When fungicide was applied it reduced viable sporulation with deleterious effect on hyphae and mycelial growth. Carbendazim+Mancozeb completely (100%) had the maximum inhibitory effect on sporulation and colony growth while Ziram, Mancozeb, Dodine and Myclobutanil showed only a temporary inhibition of fungal growth and sporulation. The overall analysis of the results revealed that the combination of Carbendazim and Mancozeb at 2.0 g/l can be used for the control of foliar disease *Alternaria* leaf spot/blotch of *Malus domestica* Borkh.

KEYWORDS: Carbendazim, Mancozeb, *Alternariamali*, *Alternaria* leaf spot, frog-eye, *Malus domestica* Borkh.

I. INTRODUCTION

Red-delicious apple (*Malus domestica* Borkh.) is the most common variety grown in the Kashmir valley. It is identified as leading producer of apple in India which contributes a major portion of about 65% of total production in India, ranks 7th with an annual production of apple fruit (FAO, 2012). Like other horticultural crops apple is attacked by several pathogen which impair the quality and quantity of the fruit. Mostly, losses in huge of the crop are caused by fungal disease like collar rot, root rot, scoty blotch, powdery mildew, *Alternaria* leaf blotch, scab etc. Among these *Alternaria* leaf blotch caused by *Alternariamali* is prevalent in all the apple growing regions of the world and is one of the economically important apple diseases. Now, it has become the problem and challenge in Jammu and Kashmir to get rid of this disease in order to prevent from huge loss of crop. An outbreak of this disease in Kashmir was noted in year 1992 [1] and the occurrence of the disease (*Alternariamali*) was reported by Shahzad et al. [2] in Kashmir valley of Jammu and Kashmir.

Alternaria leaf spot disease caused by *Alternariamali* is a fungus disease recognized as important pathogen of apples resembled as frog-eye leaf spot appearance. Lesions appear as circular brown spots measuring 2-5 mm in diameter and sometimes can result in significant defoliation, decrease fruit quality and marketability due to severe infection [3,4,5]. The aim of this study was to estimate the disease incidence and evaluate different fungicides for effective control of leaf spot of Red-delicious apple (*Malus domestica* Borkh).

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Vol. 5, Issue 7, July 2016

II. MATERIALS AND METHODS

The fungicides were tested two times after the spray with an interval of 15 days and the final data was recorded 15 days after last (2nd spray).

A. SURVEY FOR THE LEAF INCIDENCE AND DISEASE SEVERITY:

A brief survey of important apple growing belts of Kashmir valley was conducted to assess the disease incidence and intensity of *Alternaria* leaf spot of apple. One hundred plants of orchards of different districts such as pulwama, Shopian, Kulgam and Budgam were randomly selected. The leaves of the plants bearing *Alternaria* leaf symptoms were picked up and counted to estimate the disease incidence percentage.

B. COLLECTION OF DISEASED PLANT MATERIAL:

Symptoms of *Alternaria* leaf spot on apple leaves were collected and brought to the laboratory in clean polythene bags and stored in refrigerator at 4± 1°C for further investigations.

C. ISOLATION AND MAINTENANCE OF PATHOGEN:

For isolation and maintenance, diseased leaves were collected from different locations. These were washed by running tap water and dried under turn papers. Small segments of the diseased tissues were made by a sharp sterilized blade along with some healthy portions. These segments were surface sterilized in 0.1% mercuric chloride (HgCl₂), for about 30 seconds followed by three washings in sterilized distilled water [5]. Then the segments were blotted on the filter papers and transferred into plates containing potato dextrose agar (PDA) and incubated at 25 ± 1°C for three days. The mycelium emerging from diseased bits was aseptically transferred to fresh PDA culture slants. These slants were incubated at 25 ± 1°C for 7 days. For the further characteristics of isolates the procedure of Khandewal and Prasad was adopted [6].

D. IDENTIFICATION OF THE PATHOGEN:

The fungus pathogen *Alternaria mali* was identified by its colony characteristics and morphological characteristics [7].

E. APPLICATION OF FUNGICIDES:

Five fungicides at the concentration of 1.0, 2.0, 3.0, 2.5, 1.5 g/l were used to determine the efficacy against the *Alternaria mali* in laboratory and field experiments [8,3,9]. The chemicals were purchased locally. The combination of Carbendazim + Mancozeb was formulated and supplied by Indofil Industries limited India.

Table 1. List of fungicides used, active ingredient and their chemical name.

Trade name	Active ingredient	Chemical name
Dithane M-45	Mancozeb	Zinc-manganese ethylene bisdithiocarbamate
Cuman 27%	Ziram	Zinc dimethyl Dithiocarbamate
Companion	Carbendazim+ Mancozeb	Methyl <i>1H</i> -benzimidazol-2-ylcarbamate + Zinc-manganese ethylene bisdithiocarbamate
Syllit 65 WP	Dodine	n-dodecyl guanidine acetate
Systhane WSP	Myclobutanil	2-(4-Chlorophenyl)-2-(1,2,4-triazol-1-ylmethyl)hexanenitrile

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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F. INHIBITION OF SPORULATION :

Slide germination technique (SGT) described by wellman and McCallan [10] was employed to test the fungicity for inhibition of sporulation. Germination percentage was calculated using the formula.

$$\text{Per cent germination} = G/T \times 100$$

G. MYCELIAL GROWTH :

Poisoned food technique using potato dextrose broth (PDA) as basal medium was used to assess the effect of fungicides on mycelial growth .The mycelia dry weight was determined .Inhibition of sporulation and reduction in mycelia dry weight was calculated over control using the formula given by Vincent[9,30].

$$\text{Percentage inhibition} = C-T/C \times 10$$

Where, C=mycelia dry weight in control and T=mycelia dry weight in treatment

III.STASTICAL ANALYSIS

The collected data were stastically analyzed by using Random Block Designs. Analysis of data was formed accordingly (Panse and Sukhate, 1978).

IV.RESULTS AND DISCUSSION

A. SURVEY:

Being a foliar disease, the symptoms on apple “Red delicious” leaves were recorded periodically in the field. Initially the disease spots were minute, circular, light brown in color and 0.5mm in diameter. The spots later on turned circular dark brown blackish spots and gradually enlarged to 2-5 mm in diameter with a brownish-purple border and finally most resembled as frog-eye of appearance.

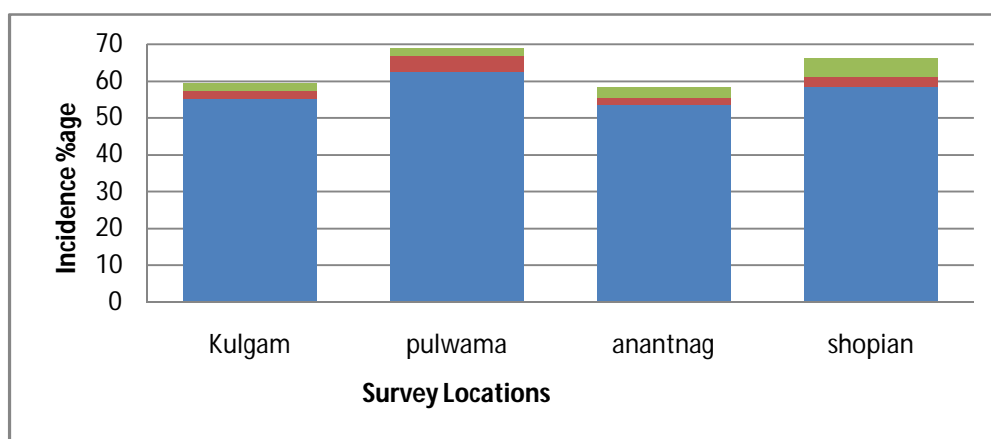


Fig.1. Survey for incidence of *Alternaria* leaf spot disease in *Malus domestica* Borkh. at different locations for collection of diseased samples.

The results showed the disease incidence percent ranged between 55.5-62.5 % at different locations. Maximum (62.5 %) in Budgam, followed by Pulwama(58.4 %), Kulgam (55.1 %) and Anantnag (53.5 %). Plant leaves showing leaf spot disease symptoms were collected from different locations in Kashmir valley during survey and brought to the laboratory for isolation and identification of causal agent fungus.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 7, July 2016

B. ISOLATION OF THE PATHOGEN:

The fungus *Alternariamali* causative agent of *Alternaria* leaf spot disease was isolated from infected leaves of several Red-delicious plants, collected from different locations.

C. PURE CULTURE MULTIPLICATION:

Pure culture PDA medium with standard method for further studies was multiplied for the isolated leaf spot causing fungus, *Alternariamali*[24].

D. IDENTIFICATION OF THE PATHOGEN:

The cultural and morphological characteristics of the isolated fungus closely resembled to those described for *Alternaria alternate* (Fr.) Keissler by many workers in available literature. The fungus revealed that mycelium was septate, hyaline and irregularly branched. The conidiophores were dark brown in color, septate, straight and branched[13].

E. PATHOGENCITY TEST

Artificial inoculations of isolated fungus resulted in production of light brown circular spots on leaves at 7 days of inoculation. The lesions later enlarged deepened to brownish-purple. These symptoms almost resembled the diseased leaves from which the isolations were made[14,20,23,32].

F. FUNGUS RE-ISOLATION

Re-isolations from artificially produced lesions yielded the same fungus after the pathogenicity test[30].

G. EFFECT OF FUNGICIDES ON *ALTERNARIA* LEAF SPOT DISEASE IN RED DELICIOUS APPLE

Different fungicides were screened for their efficacy in inhibiting and employing mycelia inhibition techniques. The data on the efficacy of various fungicides at different concentrations in inhibiting the mycelia growth of *Alternariamali* in vitro is summarized in Table 2. It is evident from the table that all the fungicides tested inhibited the growth of fungus significantly at all concentrations tested. However, carbendazim+ Mancozeb at 2.0 g/l had a maximum disease reduction 55.7 Percent, followed by mancozeb 41.4 percent, dodine 37.1 percent, myclobutanil 21.4 percent and ziram 10.9 percent[15,17,18,22,33,34,35,36,37].

Table 2. Effect of different fungicides on *Alternaria* Leaf spot disease of *Malus domestica* Borkh.

Fungicides	Dose (g/lit.)	Leaf spot score	% disease reduction
Mancozeb	2.5	4.1	41.4
Ziram	3.0	6.8	10.9
Carbendazim+ Mancozeb	2.0	2.1	75.7
Dodine	1.0	4.4	37.1
Myclobutanil	1.5	5.5	21.4

Note: effect of fungicides in rows and columns followed by different case letters are significantly ($P < 0.05$) different according to Duncan's Multiple Range Test.

V. CONCLUSIONS

In general, *Alternaria* leaf spot on Red delicious apple (*Malus domestica* Borkh.) is an important disease and a limiting factor in the successful production because it causes premature defoliation resulting in reduced photosynthetic activity.

From the above studies, it is observed that *Alternariamali* is a very destructive pathogen causing a widespread destruction in the Kashmir valley. But with the utilization of modern methods and techniques, it becomes easier to control this fungus. As far as the control of *Alternaria* is concerned, application of fungicides is the common method for the same. But keeping in view, the survey proved that the disease was prevalent in all the apple growing areas of valley, with varied incidence and intensity. The results showed the disease incidence percent ranged between 55.5-62.5 % at different locations. Maximum (62.5 %) in Budgam, followed by Pulwama (58.4 %), Kulgam (55.1 %) and Anantnag (53.5 %). A temperature of 25+₋ 1^oc was found optimum for the germination of *Alternariamali* spores. However best growth and sporulation was observed in malt extract and potato dextrose media. Some contact and systematic fungicides were tested in vitro at different concentrations for their efficacy in inhibiting the germination of

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(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 7, July 2016

spores and mycelial growth. However, carbendazim+ Mancozeb at 2.0 g/l had a maximum disease reduction 75.7 Percent, followed by mancozeb 41.4 percent, dodine 37.1 percent, myclobutanil 21.4 percent and ziram 10.9 percent. On the basis of above inferences it can be concluded that *Alternaria* leaf spot disease of *Malus domestica* Borkh. Is economically an important disease with great potential to threaten existing healthy plantation of apple Red delicious and to lower the productivity, if not cured properly and timely. Further, the efficacy of fungicides used in vitro need to be evaluated in vivo prior to their recommendation for the control of the disease.

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International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 7, July 2016

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