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Field Screening of Chilli Leaf Spot caused by *Alternaria alternata* and the effect of Weather Parameters on the Development of the Disease

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ABSTRACT: Chilli is well known for its multipurpose functions and good nutritive value to the World. Several fungal, bacterial and viral diseases render chilli production at stake. In this present investigation, the screening of the most known disease of chilli that is its leaf spot caused by *Alternaria alternata* was done in and around Jabalpur district of Madhya Pradesh to calculate its per cent disease incidence in the given regions. Because the chilli crop is one of India's most stable spice crops and is plagued by pathogens, primarily fungi, adequate management is required. During the survey done in and around Jabalpur, the percent disease incidence ranged from 20% to 40%, according to the studies. Following the screening, the fungus pathogen was identified by isolating it and developing its pathogenicity test in both in vivo and in vitro conditions. The following were the findings: The pathogen generates white, cottony colonies with profusely expanding aerial mycelium that eventually turns grey, according to the cultural features. On the PDA medium, old civilizations have recognizable concentric rings. Conidiophores, which are olivaceous to brown in colour and simple to branching, are produced by the fungal pathogen. Conidia were produced in chains with transverse and longitudinal septa. The pathogenicity test shows that the symptoms start appearing from the older leaves and Alternaria readily produces symptoms on the inoculated leaves as small, circular necrotic spots having concentric rings in the centre of the spots. Analyzing illness development with meteorological factors revealed that disease development begins at temperatures ranging from 25°C to 35°C, with the most visible impacts at 28°C, and relative humidity also plays a vital role in disease development ranging from 70% to 90%. The observed disease incidence ranged from 2.50 percent to 12.67 percent.

Keywords: *Alternaria alternata* ,Chilli leaf spot, Disease incidence , Screening, , Survey, Weather parameter.

INTRODUCTION

Chilli (*Capsicum annum* L.) a perennial herbaceous plant that belongs to the family Solanaceae having 2n = 24 chromosome number is well known for its aroma, pungency and medicinal value. Both vegetarian and non-vegetarian dishes include chilli as the most important ingredient for its taste and flavour in every country. According to reports published on Indiastat.com by Professor Jayashankar Telangana State Agricultural University (Agricultural Market Intelligence Centre) showing the area, production, and productivity of chilli in India (2020-30th June 2021), the chilli covered 7.43 lakh ha area, produced 19.14 lakh tonnes, and had productivity of 2576 kg/ha, making India the world's largest producer, consumer, and exporter of chilli. According to the Spice Board India report (2019-2020 Estimate), the top chilli-producing states in India are Andhra Pradesh (6.60 lakh tonnes), Telangana (3.28 lakh tonnes), Madhya Pradesh (2.18 lakh tonnes), Karnataka (1.80 lakh tonnes), and West Bengal (1.80 lakh tonnes) (1.04 lakh tonnes).

Several fungal diseases such as damping-off (*Pythium aphnidermatum and Phytophthora* spp.), leaf spots (*Cercospora capsici*, *Alternaria alternata and Alternaria solani*), anthracnose and ripe fruit rot (*Colletotrichum capsici*), early blight (*Alternaria solani*), southern blight (*Sclerotium rolfsii*), fruit rot (*Alternaria alternata* and *Phoma destructive*), white mould (*Sclerotinia sclerotiorum*) and fruit rot (*Phomopsis* spp.), respectively (Sarkar *et al.*, 2017) are known to cause in chilli crop.

Alternaria leaf spot and fruit rot (*Alternaria alternata* (Fr.) Keissler) are considered to be the most important fungal disease as they pose a major threat in chilli growing areas (Dutt 1937, Sreekantiah *et al*., 1973). The pathogen (*A. alternata)* is known to cause seed, seedling, leaf and fruit diseases (Sreekantiah *et al*., 1973; Mehrotra, 1980). In severe cases, the fungus is also responsible for causing tender tip drying at different stages of crop growth (Courter *et al*., 1965). According to Bhale *et al.* (2001), the seed-borne nature of the pathogen *A. alternata* is known to cause a 30-60 per cent reduction in seed germination.

Bacterial infections such as bacterial leaf spot (*Xanthomonas campestris pv. vesticatoria*), bacterial wilt (*Ralstonia solanacearum*), and crown gall are also

known to reduce chilli output (*Agrobacterium tumefaciens*). Some parasitic nematodes, such as *Meloidogyne* sp., which causes root-knot disease, and sting nematode (*Belonolaimus longicaudatus*), have an effect on chilli. There are several viral diseases that afflict chilli plants, including alfalfa mosaic (*Alfalfa mosaic virus),* tobacco leaf curl virus, chilli venial mottle virus, and beet curly top virus (*Beet curly top virus*). Some post harvest rots occur in chilli caused by *Alternaria terreus*, *Alternaria candidus*, *Alternaria niger*, *Fusarium moniliforme* and *Penicillium corylophilum* (Sarkar *et al*., 2017).

MATERIAL AND METHODS

Disease survey. An intensive roving survey was conducted in the major chilli growing areas in and around Jabalpur, Madhya Pradesh. The villages covered were Sukha village, Padwar Kalan (Katangi Road), Onriya (Bhedaghat), Pipariya village, Imaliya village and J.N.K.V.V. farms as well - Maharajpur Horticulture Farm and Experimental field of Department of Plant Pathology.

Calculation of Per cent disease incidence:

$$\text{Per cent Disease Incidence} = \frac{\text{Number of infected leaves}}{\text{Number of total plants}} \times 100$$

The per cent disease incidence was worked out by using the formulae devised by McKinney (1923).

The disease incidence was categorized into six categories following a 0-5 scale, based on the per cent leaf area infected by leaf spots of the pathogen.

Grade	Area of infection (Per cent)
0	No disease
1	1-10% leaf area covered
2	11-20% leaf area covered
3	21-50% leaf area covered
4	51-80% leaf area covered
5	More than 80% of the leaf area covered

Isolation. From different areas, diseased samples were collected during the season and then taken to isolate in the laboratory. Initially collected diseased samples were washed thoroughly under the tap water and then cut into small pieces 2-4 mm in size by using a sterilized blade such that the sample contained a 50 per cent healthy portion as well as a 50 per cent diseased portion. The surface sterilization of the pieces was done by using 1 per cent Sodium hypochlorite solution for 30 seconds to 1 minute, and then finally washed well with the three changes of sterilized distilled water afterwards to remove excess water from the pieces; they were placed on blotter paper. By using a sterilized inoculating needle place the sample pieces on Petri-plates containing potato dextrose agar medium under aseptic conditions. Three replications were made by placing three pieces of PDA media on each plate. Inoculated Petri plates were kept in an incubator at 25°±2°C and examined at frequent intervals to check the growth of the target fungal pathogen.

Purification and maintenance of the target pathogen (*A. alternata*). The purification of the culture was done with the help of the single spore method. At regular intervals, sub-culturing was done. The pure culture was maintained in slants and stored at 4°C temperature in the refrigerator.

Identification of the isolated pathogen (*A. alternata*). It starts by observing the characteristics of the colony developed on the PDA medium on which the target isolated pathogen was grown and incubated at 25°± 2°C. The examination of the pathogen was done after 7 days. While the morphology of the conidia was studied properly when observing 20-30 day old cultures of the fungal pathogen.

Pathogenicity test for the isolated pathogen (*A. alternata*). The spore suspension method was used on the detached leaves of chilli *in vitro* as well as under *in vivo* conditions on the chilli plant. For making inoculums spore suspension, ten day old culture was used. An atomizer is used for spraying the inoculum suspension under both conditions. Humidity levels were maintained around the chilli plant by using loosely polythene bag wrapping. Un- inoculated pots served as control. At regular intervals, the examination was continued till the expression of the typical symptoms appeared. To confirm its pathogenicity, mother cultures were used.

To study the effect of weather parameters on the development of the disease. Randomly, five chilli plants were selected and tagged irrespective of the appearance of the disease at the pre-flowering stage. The tagged plants were observed for disease appearance and progressive development that had been recorded at a 7-day interval based on the standard meteorological weeks. By analyzing, the per cent disease incidence, the relationship between temperature and relative humidity with the disease appearance has been observed.

RESULTS

Survey. In and around Jabalpur, a survey was undertaken are Sukha village, Padwar Kalan, Onriya village (Bhedaghat), Imaliya, Pipariya village, and Maharajpur Horticulture Farm was among the villages assessed. In and around Jabalpur, the incidence of Alternaria leaf spot on chilli is as follows:

Table 1: A survey on disease incidence of Alternaria leaf spot of chilli.

Name of villages/areas	Per cent disease incidence (%)	Rating is given according to a Scale (0-5)
Sukha village	23	3
Padwar Kalan	20	2
Maharajpur Horticulture farm	35	3
Imaliya	33	3
Pipariya	22	3
Onriya, Bhedaghat	40	3

Calculation of Per cent disease incidence is done based on the below formula devised by McKinney (1923):

$$\text{Per cent Disease Incidence} = \frac{\text{Number of infected leaves}}{\text{Number of total plants}} \times 100$$

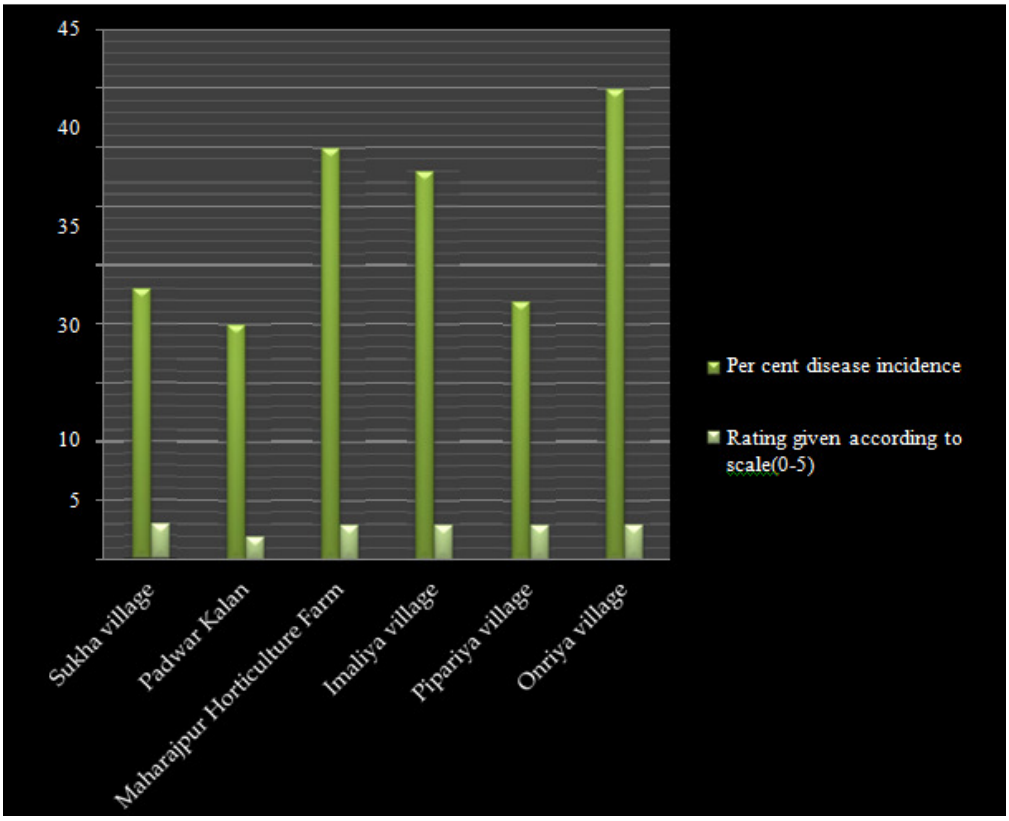

Fig. 1. Per cent disease incidence of Alternaria leaf spot of chilli in and around Jabalpur.

Collection of diseased samples. Disease samples having concentric rings and reddish-purple spots on leaves were the most common symptoms that appear on older leaves. The biggest circular to angular dark patch with concentric rings giving a target-like appearance was chosen from the field and brought to the laboratory for isolation.

Isolation and Identification of the target pathogen. Initially white, cottony, and abundant in aerial mycelium fungal colonies were seen, but they progressively turned grey with the period and thus old cultures appeared entirely grey, and clear concentric rings formed on the PDA medium. Conidiophores' appearance ranged from simple to branched, and singly. Conidia were observed from golden to brown in appearance and found in lengthy chains on conidiophores, with beaked edges and strong walls, and had transverse and longitudinal septation. The identifying fungus was found to be *Alternaria alternata* based on colony characteristics and morphological characteristics of conidiophores and conidia.

Purification of the Pathogen. Once the pathogen was identified, pure cultures were obtained and periodic sub-culturing was done on PDA slants to keep them in pure culture form. This pure culture was employed, throughout the investigation process.

Pathogenicity. The pathogenicity test was confirmed by using the inoculum spore suspension method on healthy chilli plants under *in vivo* conditions and on detached chilli leaves in the lab by inoculating them with the same inoculum spore suspension.

Symptomatology. Under natural conditions, the most noticeable characteristic of the fungus is the development of concentric rings in the centre of the spots on leaves.

Symptoms appear on different parts of the plants such as on leaves, twigs and fruits. The presence of brown to black necrotic lesions on older leaves, ranging one to five mm wide and producing a bull's eye appearance of concentric rings. On getting favourable conditions, several lesions coalesce and thus result in leaf drop. Usually, the spots formed by the *Alternaria* are surrounded by a chlorotic halo.

Effect of weather parameters on the development of the disease. On analyzing the disease development with the weather parameters like temperature, relative humidity and rainfall, the per cent disease incidence was observed. The results show that the maximum disease incidence (12.67 per cent) was found in the first week of January when the temperature was 26.5°C and relative humidity was 87 per cent with 0.5 mm rainfall. The least disease incidence was observed in March when the temperatures go high at 33.9°C and relative humidity decreased to 67 per cent with 0.0 mm rainfall. Thus with the help of meteorological data (2020-2021), the effect of temperature and relative humidity on disease development was established in Table 2.

Plate 1: Pure culture of *Alternaria alternate.*

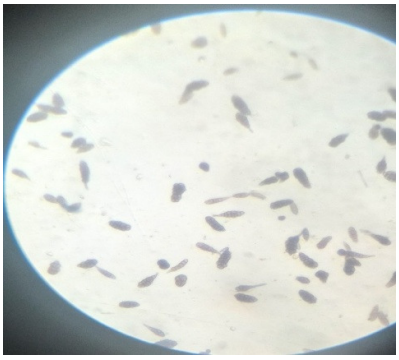

Plate 2: Spores of *A. alternate.*

Healthy Chilli Plant Inoculated Chilli Plant

Plate 3: Pathogenicity test under natural condition.

Plate 4: Pathogenicity test under lab condition (detached chilli leaf).

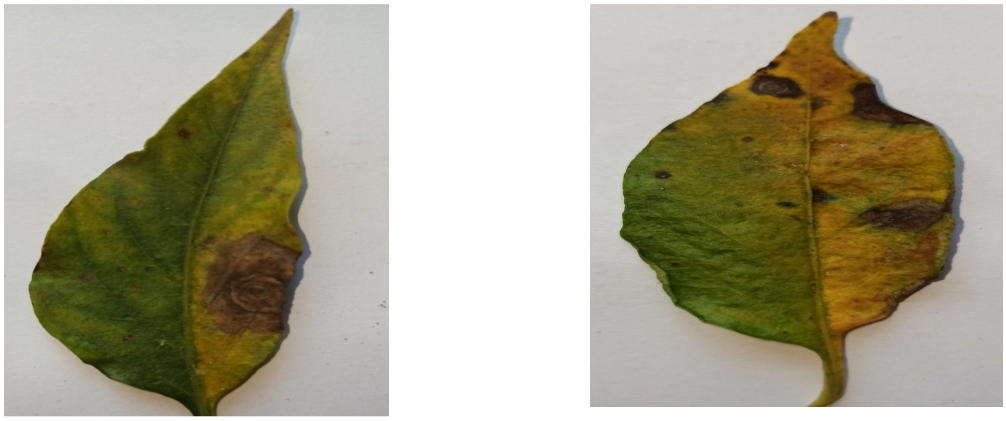

Plate 5: Disease symptoms of leaf spot of chilli caused by *A. alternate.*

Table 2: Effect of weather parameters on disease incidence.

Month		Maximum Temperature(°C)	Minimum Temperature(°C)	Relative humidity Morning (%)	Relative humidity Evening (%)	Rainfall (mm)	Disease Incidence (%)
DEC	49	29.3	9.1	81	29	0.0	7.50
	50	26.4	14.7	88	57	2.3	5.50
	51	23.2	5.5	74	31	0.0	5.33
	52	23.8	7.2	83	43	0.0	5.40
JAN	1	26.5	12.4	87	50	0.5	12.67
	2	25.0	11.8	86	49	0.4	10.50
	3	25.4	8.0	75	31	0.0	11.67
	4	24.6	8.6	86	49	0.0	8.00
FEB	5	21.4	4.8	73	31	0.0	8.50
	6	26.4	8.9	72	34	0.0	9.50
	7	27.7	11.8	83	42	12.6	11.50
	8	28.6	10.6	79	28	0.0	12.50
MAR	9	32.7	12.4	74	25	0.0	5.00
	10	34.8	13.0	74	20	0.0	4.90
	11	32.7	15.3	78	29	6.2	3.00
	12	33.9	16.7	67	27	0.0	2.50

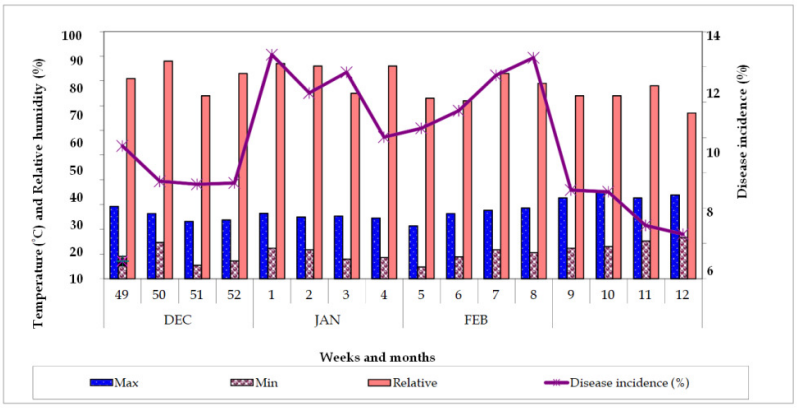

Fig. 2. Effect of weather parameters on disease incidence.

DISCUSSION

Survey. In and around Jabalpur, a random survey was conducted in the proximity of promising chilli farming areas. The highest per cent disease incidence was observed is 40 per cent in Onriya village, Bhedaghat, which received the third-highest rating on the rating scale, whereas the lowest per cent disease incidence was 20 per cent in Padwar Kalan village, which received the second-highest rating. Earlier, surveys were not as common, but by understanding the losses caused by the disease, 5–85 per cent as reported, attention has been given to this disease of chilli.

Isolation and Identification of the pathogen. The pathogen *A. alternata* was isolated on PDA media using infected chilli leaves. The fungal colonies showed white, cottony, and abundant aerial mycelium growth, but after some days of incubation, it turned into an olivaceous green colour showing concentric rings. Conidiophores arose singly ranging from simple to branched in appearance, bearing muriform brown colour conidia with transverse and longitudinal septation. The test fungus was then purified, maintained on agar slants, and kept at 4°C in the refrigerator. The identified fungus was *Alternaria alternata.* This

suggests that *A. alternata* is linked to the chilli leaf spot. On PDA media, the fungus showed abundant mycelial development. It evolved into a grey-brownish, multicelled, septate, and irregularly branching organism. Conidia were found to be muriform, born in chains, and varying in colour from light olivaceous to dark brown.

Pathogenicity test of target pathogen (*A. alternata*). Using the spore suspension method the pathogenicity test was demonstrated both under a natural environment as well as *in vitro* conditions on detached chilli. Initial signs with a tiny and circular necrotic patch appeared on leaves after seven to nine days of inoculation. In severe infections, concentric rings appeared on the leaves. The most noticeable symptom observed on older leaves was brown or dark spots.

Thippeswamy *et al*. (2007), identified the cause of the leaf spot of chilli as *A. alternata* and demonstrated its pathogenicity on chilli seedlings.

Pathogenicity was demonstrated by inoculating the chilli plants with *Alternaria* spore suspension and also maintaining control without inoculation. Symptoms appeared as a tiny, circular necrotic patch on the inoculated leaves. The brown spots began to grow in size with a yellow halo. This discovery is consistent with that of Mangala *et al*. (2006); Li *et al*. (2011), all of whom found pathogenicity in *A. alternata.*

Symptomatology. The symptomatology produced by *A. alternata* was studied in detail on chilli leaves. The reddish-purple dots on leaves, with brown or dark spots on older leaves, are the most common symptom of *Alternaria* spp. These patches began to grow with an irregular edge and stayed brown with a yellow halo, eventually withering and killing the plant.

Sreekantiah *et al*. (1973) observed that within 7 days of treatment the chilli plants were showing early-stage circular-shaped spots up to 1 cm on leaves, which enlarge and turn brown into an irregular sunken patch with a dark brown edge. The most prevalent position of spots was found to be near the leaf's margins and resembled those found on fruits as well.

Effect of weather parameters on disease development. Kumar *et al*. (2013) studied the influence of temperature and relative humidity on fruit rots (*Alternaria alternata* and *Aspergillus niger*) of ber (*Zizyphus mauritiana* Lamk.) and found that maximum damage was observed at 25°C by *Alternaria alternata* while *Aspergillus niger* showed at 30°C. They also observed a positive relationship of relative humidity with the fruit rot by both the pathogens that showed the highest severity at 100 per cent RH.

Ghewande (1986) discovered that temperatures between 25°C and 29°C, with a relative humidity of 87 per cent were more favourable for the development of Alternaria leaf spot of groundnut induced by *Alternaria alternata*.

Pandey *et al*. (2019) worked on the effect of atmospheric temperature, relative humidity and rainfall on the disease development of Alternaria leaf spot of chilli and concluded that the maximum and minimum temperature and relative humidity have an impact on disease development, where the high temperature together with high humidity keeps the disease intensity low, whereas low temperature together with low humidity favour high disease development. During 2002-03, the average maximum and minimum temperatures and maximum and minimum relative humidities were 29.7°C and 13.6°C and 87.5 per cent and 39.2 per cent respectively, whereas, during 2003-04, the average maximum and minimum temperatures and maximum and minimum relative humidities were 34°C and 15.4°C and 73.3 per cent and 45.2 per cent respectively.

CONCLUSION

According to the cultural traits, the pathogen forms white, cottony colonies with profusely developing aerial mycelium that eventually turns grey. Old civilizations had distinct concentric rings on the PDA medium. Conidiophores, which are olivaceous to brown in color and simple to branching, are produced by the fungal pathogen. Conidia were generated in chains with transverse and longitudinal septa. The pathogenicity test reveals that symptoms occur on older leaves first, and Alternaria rapidly produces symptoms on infected leaves as small, circular necrotic spots with concentric rings in the centre of the spots. Analyzing illness development with climatic conditions indicated that disease development begins at temperatures ranging from 25°C to 35°C, with the most evident consequences at 28°C, and relative humidity also plays a role. Using meteorological elements to analyze illness development indicated that disease development begins at temperatures ranging from 25°C to 35°C, with the most evident consequences at 28°C, and relative humidity also plays a crucial part in disease development ranging from 70% to 90%. The observed illness incidence ranged between 2.50 and 12.67 percent.

FUTURE SCOPE

We can focus on molecular characterization of the mode of infection caused by fungus in addition to using chemical and botanical management tactics. We can also use any forecasting model based on climatic factors to forecast the disease and alert farmers about the loss caused by sickness.

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