DISTRIBUTION, ISOLATION, PURIFICATION, PATHOGENCITY AND IDENTIFICATION OF *ALTERNARIA ALTERNATA* (KESSLER) CAUSING LEAF BLIGHT OF ISABGOL (*PLANTAGO OVATA)*

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Abstract: In the year 2008, the maximum per cent mortality 34.78% was recorded at Sayala of district Jalore followed by Keshwana and Bhundwa at 60 days after sowing. Whereas, during the year 2009, the maximum per cent plant mortality 37.85% was observed at Sayala of district Jalore followed by Keshwana and Bhundwa at 60 DAS. The overall disease incidence as per cent plant mortality was more in 2009 as compared to 2008. The fungal isolates were collected from five different Agro climate zone of Rajasthan i.e. R.C.A. farm (Udaipur), Kapasan (Chittorgarh), Mandore (Jodhpur), Sumerpur (Pali) and Keshwana (Jalore). On the basis of morphological, cultural and pathogenic characteristics, the isolates were identified as *Alteraria alternata* (Fr.) Keissler. Pathogenicity test was done according Koch's postulates for all the five isolates. The identity of R.C.A. farm (Udaipur) isolate was confirmed by Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi-110012 (The ITCC Code no.6317, 2008).

Keywords: *Alternaria alternata*, Distribution, Isolation, Purification and *Plantago ovata*

INTRODUCTION

Isabgol (*Plantago ovata* Forsk.) or blond psyllium is one of the important ancient herbal medicines which are being used over centuries due to its several medicinal uses. It is indigenous to the Mediterranean region and West Asia. In India, it is mainly cultivated in Mehsana and Banaskantha district of Gujarat and adjoining districts of Rajasthan and to a limited extent in Haryana. Presently Rajasthan is a dominating state in Isabgol production. The husk from the seeds is separated by physical process and it is exported largely to USA, West Germany, UK and France. This is one of the largest contributors of foreign exchange among medicinal crops. In recent years, its annual export has increased to more than 600 Crores. It has been introduced in India and cultivated specially in Gujarat and some parts of Rajasthan. Now India is the largest producer of Isabgol and exports seed and husk worth Rs 25 million annually. The husk is the rosey- white membranous covering of the seed which constitutes the drug and is given as a safe laxative, particularly beneficial in habitual constipation, chronic diarrhoea and dysentery. *Alternaria* blight has become a serious problem in recent years. It has been found that downy mildew affected crop is more prone to be attacked by *Alternaria alternata*. It causes considerable damage every year and sometimes become very severe which results in total loss of yield.
Hence, present investigations were carried out to Distribution, Isolation, Purification, Pathogencity and Identification of *Alternaria alternata* (Kessler) causing Leaf blight of Isabgol (*Plantago ovata*)

MATERIAL AND METHOD

Distribution and prevalence of leaf blight disease in Isabgol growing area in Rajasthan

Forays were conducted to know the distribution and prevalence of *Alternaria* leaf blight of Isabgol during the year 2008-09 and 2009-10 in Isabgol growing areas, which includes districts *viz.*, Udaipur, Chittorgarh, Pali and Jalore on Farmer's fields. Such visits were aimed to record the incidence of the disease, exploring possibility of existence of different species and variability of leaf blight of Isabgol pathogen isolates from different locations i.e. cultural, spore morphology, aggressiveness, toxin and molecular variability. To assess the incidence of disease three fields randomly selected and 10 Square meter area in field in each village and per cent disease incidence / per cent plant mortality was observed at 60 days after sowing. To know the, diseased and healthy plants were counted and the average plant mortality due to disease was calculated using following formula:

Per cent plant mortality =

$$\frac{\text{Number of diseased/dead plants}}{\text{Total number of plant observed}} \times 100$$

Isolation of the Pathogen

Diseased samples were brought to laboratory for isolation and purification of the pathogen. A herbarium was also prepared to carry out the symptomatology and histopathological studies in future. For isolation of the pathogen, small pieces of diseased leaves with blighted spots were taken and surface sterilized by dipping the bits in 0.1% mercuric chloride solution for 2 min. followed by

three washings in sterilized water and plated on 2% PDA contained Petri plates aseptically. These were incubated at 25±1ºC in incubator for growth. Sub-cultures were prepared from the periphery of the mycelial growth of 4-5 days old colonies developed from the infected bits on (PDA) slants. The cultures were incubated at 25±1ºC for growth and sporulation. The microscopic examination of cultures indicated that the fungus belongs to the genus *Alternaria.*

Purification of the pathogen through single spore technique

To purify *Alternaria* culture, a spore suspension was prepared in sterilized distilled water from 10 days old culture on PDA and flooded on 2% plain agar in Petri plates. The excess suspension was drained out and the Petri plates were then incubated in inverted position at 25 ±1ºC. After eight hours, a single germinating spore was marked with the help of dummy objective and then transferred individually with a piece of plane agar medium to PDA slants by inoculating needle under aseptic conditions. These mono-conidial isolates were maintained on PDA slants and were used for further experimentation

Pathogencity test of the Pathogen

The pathogencity test was conducted by growing Isabgol plants in pots. When plants were 45-50 days old, they were inoculated by using spray inoculation technique. Spore suspension prepared from sporulating culture in sterile water with minimum of 50 spores per ml. After inoculation, the pots were watered and kept covered with polyethylene bags containing moist sterilized cotton swab within it for 48 hours to maintain high humidity. Suitable controls by spraying sterilized distilled water were also maintained. After 4 days of inoculation the typical symptoms started appearing on the leaves initially as yellow islands. The polythene bags were removed and it was observed that typical blighted areas appeared on the leaves after 7-10 days of inoculation. In order to confirm the pathogencity, reisolations were made from these artificially produced diseased symptoms which yielded the same species of fungus *Alternaria*, identical with the type inoculated. Similar results were obtained repeatedly and in this way Koch's postulates were proved.

Identification of the pathogen

The pathogen was identified at generic level by studying their cultural, morphological and pathogenic characteristics. For confirmation of identity, the pure culture was sent to Indian type culture collection (ITCC), Division of plant pathology, IARI, New Delhi-110012. The letter was received from ITCC and the results are mentioned in chapter of experimental results and discussion.

RESULT AND DISCUSSION

Distribution and prevalence of leaf blight disease in Isabgol growing area in Rajasthan

The disease *Alternaria* leaf blight is distributed in almost all the Isabgol growing areas of Rajasthan. However, there are no reports which reveal about its exact time of occurrence and other epidemiological factors. Fields surveys were carried out from 25 December to 31 December 2008 and 2009 as given in Table 1. In the year 2008, the maximum per cent mortality 34.78% was recorded at Sayala village of district Jalore followed by Keshwana (32.24%), Bhundwa (29.60%) and Nosar (26.90%) and minimum mortality was found at Jaitaran (16.30%) at 60 days after sowing.

In the year 2009, the maximum per cent plant mortality 37.85% was observed at Sayala village of district Jalore followed by Keshwana (36.40%), Bhundwa (30.17%) and Nosar (28.40%) and minimum per cent mortality was found at Jaitaran (18.30%) at 60 days after sowing.

The per cent plant mortality varied from 16.30 – 34.78 at 60 DAS and 18.30 – 37.85 at 60 DAS in the both years i.e. 2008 and 2009 respectively. The overall disease incidence as per cent plant mortality was more in 2009 as compared to 2008. This shows that mortality may increase at maturity because under favorable weather conditions the disease progress is fast, which ultimately affects the yield. Similar surveys for *Alternaria alternata* were conducted for Senna by Tetarwal and Rai (2007) and Osiru *et al*. (2007).

Symptoms of the disease

At 45 days after sowing in 2008, leaf blight of Isabgol (*Plantago ovata* Forsk.) was observed in an Isabgol field of Agronomy at Rajasthan College of Agriculture, MPUAT, Udaipur because at this time disease initiates as per observations during day to day visit of experimental fields. Symptoms on affected plants started with yellowing and browning of the lower leaves, progressing upwards under high humidity conditions. Yellowing often develops from the leaf tips and along the margins of the leaf petiole. Under severe infection, lesion enlarged and coalesced causing blighting of leaf portions. Concentric circles which are characteristics symptom of *Alternaria* with dark layers of conidia were observed under moist conditions on infected leaves. Infection under favorable conditions was found to cause severe defoliation, with considerable yield losses when it occurred prior to flowering. In *Alternaria* leaf blight, it has been found that downy mildew affected crop is more prone to be attacked by *Alternaria alternata*. The already weakened plants under favourable weather conditions become a good reservoir for *Alternaria alternata* and they cause mortality of plants. Present results are well supported by workers like Patel *et al.* (1982) and Mondal (2010).

Collection of diseased samples

The samples collected from survey were isolated and the pathogen was given code according to numbering in the Table 2. The fungal isolates were prepared from five different locations i.e. R.C.A. farm (Udaipur), Kapasan (Chittorgarh), Mandore (Jodhpur), Sumerpur (Pali) and Keshwana (Jalore) Districts of Rajasthan and collected samples were brought to laboratory for isolation and further studies. For convenience, the isolates of *Alternaria alternata* were named as Aa-1, Aa-2, Aa-3, Aa-4 and Aa-5 for R.C.A. farm (Udaipur), Kapasan (Chittorgarh), Mandore (Jodhpur), Sumerpur (Pali), and Keshwana (Jalore) Isolates, respectively.

Isolation and Purification of the pathogen

Infected leaves were taken for isolation under aseptic condition. The fungus emerging from leaf bits placed on PDA were observed to have olivaceous to dark brown mycelial growth showing concentric zones. The pure culture of the fungus was prepared by single spore technique and maintained on PDA media. On third day after incubation on the potato dextrose agar, fungal colonies were seen as olivaceous in colour surrounded by whitish fluffy growth of the fungus. It changed gradually on fourth day from light olivaceous to dark olivaceous in colourd, after 15-20 days of incubation, whole fungal growth converted into brown to black in colour. The fungus growth completely covered the Petri-dish within 7 days of incubation at 25±1ºC. Present results are well supported by Gaddanakeri and Kulkarni (1998) isolated *Alternaria alternata* using tissue isolation from infected leaves of turmeric (*Curcuma longa*).

Pathogencity test

To prove Koch's postulates, pathogencity test was conducted for all the five isolates collected from different places as per methods and control pots were sprayed with distilled water only. Disease symptoms were initiated within 3-4 days of inoculation. The first symptoms on affected plant of Isabgol started with yellowing and browning of the lower leaves, progressing upwards because of high humidity and temperature prevailing. Few leaves exhibited blighting from the leaf tip and along the margins of the leaf petiole. Under serve infection, lesions enlarged and coalesced causing blighting of entire leaf. The pathogens were re-isolated from such leaves and the morphological characters of the re-isolated *Alternaria alternata* was compared with the original culture of the pathogen which were similar in all respects (morphological and microscopic characters). Hence, the causal agent of the disease was confirmed as *Alternaria alternata*. Present results are well supported by Patel *et al.* (1982).

Identification of the pathogen

On the basis of morphological, cultural and pathogenic characteristics, the isolates were identified as *Alteraria alternata* (Fr.) Keissler and all cultures were nearly similar. The identity of Udaipur isolate was confirmed by Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi-110012 (The ITCC Code no.6317, 2008). The similar work done by Ramjegathesh and Ebenezar (2012) collected ten isolates of *A. alternata* causing leaf blight of onion from conventional onion growing areas of Tamil Nadu, India and their pathogencity was established. The cultures/ isolates were identified by ITCC with accession no. 5470.

Table 1: Distribution and prevalence of leaf blight in Isabgol growing areas of Rajasthan during 2008 and 2009 with average plant mortality in respective years

S.No	District	Name of village	Average plant mortality (%) 60 days after sowing (DAS)	
			2008	2009
1.	Udaipur	1. R.C.A Campus	22.10 (22.09)	24.15 (24.08)
		2. Fatehnagar	19.23 (19.22)	21.23 (21.22)
		3.Sutharo ka kheda	20.36 (20.35)	21.90 (21.89)
2.	Chittorgargh	1. Sathkhanda	23.10 (23.02)	25.23 (25.21)
		2.Sambhupura	22.55 (22.54)	24.50 (24.49)
		3.Kapasan	24.38 (24.37)	26.90 (26.83)
3.	Jodhpur	1.Mandore	25.76 (25.70)	27.85 (27.84)
		2.Charai	24.92 (24.91)	26.80 (26.79)
		3.Nosar	26.90 (26.89)	28.40 (28.39)
4.	Pali	1.Jaitaran	16.30 (16.29)	18.30 (18.29)
		2.Marwar Junction	18.36 (18.35)	19.40 (19.39)
		3.Sumerpur	17.20 (17.19)	18.75 (18.74)

5.	Jalore	1.Keshwana	32.24 (32.23)	36.40 (36.39)
		2.Bhundwa	29.60 (29.59)	30.17 (30.16)
		3.Sayala	34.78 (34.77)	37.85 (37.84)
	SEm±		0.712	0.753
	CD at 5%		2.063	2.183
	CV%		4.24	4.29

Figures in parentheses are angular transformed values and observations were recorded for mortality only which indicates the severity.

Table 2: Collection of disease samples and designation of isolates from different major Isabgol growing areas of Agro-climatic zones of Rajasthan

S.No	Location	Designation	Agro - climatic zone
1.	R.C.A. Farm (Udaipur)	Aa-1	IVb- Humid southern.
2.	Kapasan (Chittorgarh)	Aa-2	IVa- Sub humid Southern Plain.
3.	Mandore (Jodhpur)	Aa-3	Ia- Arid Western.
4.	Sumerpur (Pali)	Aa-4	IIb- Transitional Plain of Luni Basin.
5.	Keshwana (Jalore)	Aa-5	IIb- Transitional Plain of Luni Basin.

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