JBiopest 6(2):112-116

Studies on host range and seed transmission nature of *Alternaria alternata* (Fr.) Keissler causing leaf blight of Isabgol

Rajesh Kumar Meena, S.S. Sharma and S.C. Meena

ABSTRACT

From the present investigation of host range, it can be concluded that *Alternaria* blight pathogen of Isabgol could produce visible symptoms on all the species except Ashwagandha. However, the appearance of symptoms was observed after 7-12- days of inoculation on these plants compared to 5-7- days in Isabgol. Hence, leaf blight of Isabgol (*Alternaria alternata*) has a wide host range. Seed is one of the important sources of external and internal mycoflora. With the help of two methods *viz.* SBT and PDA plate method, it was observed that six fungi i.e. *A. alternata, Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Curvularia lunata* and *Penicillium* spp. were frequent in all the tests. The Blotter and PDA proved to be an effective method to detect the mycoflora. However, the blotter test proved generally more sensitive, as the frequency of fungi recorded was higher in this test than PDA. Per cent frequencies of all species isolated from sterilized seeds were low as compared to unsterilized seed samples which happen quite naturally. All these mycoflora were examined for their pathogenic ability on Isabgol. Seeds and plants were inoculated with the mycoflora and closely observed for their disease reaction. It was observed that out of six fungi only *A. alternata* gave pathogenic reaction on the Isabgol and the pathogen is seed borne in nature.

MS History:25.5.2013 (Received)-28.8.2013 (Revised)-15.9.2013 (Accepted)

Key words: *Alternaria alternata*, host range**,** potato dextrose method, standard blotter technique, seed transmission and *Plantago ovata*.

INTRODUCTION

Isabgol (*Plantago ovata* Forsk.) is an annual herb that belongs to family Plantaginaceae. India is the largest producer and exporter of this crop in the world. Isabgol, *P. ovata,* belongs to a large genus of herbs or sub shrubs distributed mostly in the temperate regions and a few in the tropics. *P. ovata*, commonly known in English as Blond Psyllium or Indian Plantago in commercial is important for its seeds and husk which have been used as the indigenous medicine in many countries. The husk from the seeds is separated by physical process and it is exported largely to USA, West Germany, UK and France. The crop is challenged by a number of pathogens *viz., Fusarium wilt* (*F. oxysporum* Schlechtemendsnyd and Hnns.), damping off (*Pythium ultimum* Trow.), leaf blight (*Alternaria alternata* (Fr.) Keissler), downy mildews (*Peronospora plantaginis* Underwood, *Peronospora alta* Fuckel and *Pseudopernospora plantaginis*) and powdery mildew (*Erysiphecichora cearum* D.C.) *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 becomes very severe which results in total loss of yield.

Seed health largely determines the production. However, during seed selections many hidden pathogens may come along, either externally or internally which can not be detected by visual observations. Seed is one of the most important carriers of fungal propagules which help in dissemination of disease and survival of several plant pathogens. Seed borne inoculums have often been found to be a source of primary inoculum in case of some crops. In the present studies two methods *viz.* standard blotter paper method and potato dextrose agar method were used for detection of fungi from the seeds of Isabgol cultivar RI-89.

MATERIAL AND METHODS

Host range

The pathogen was artificially inoculated on different plant species for their sensitivity to *Alternaria alternata* to know the host range of various plant species including Wheat (*Triticum aestivum)*, Barley *(Hordeum vulgare)*, Mustard (*Brassica compestris)*, Ashwagandha *(Withania samnifera)*, Coriander *(Coriandrum sativum)*, Cumin *(Cuminum cyminum)*, Cauliflower *(B. oleracea var capitata)*, Cabbage *(B. oleracea var botrytis*), Chilli *(Capsicum annuum)* and Tomato (*Lycopersicon esculentum)* belonging to different families. The inoculated plants were kept under observation up to 30 days for any symptom development as per descriptions in materials and methods. All the plant species in test are *Rabi* season (winter season) plants.

Seed transmission

To study the seed transmission of *A. alternata* in Isabgol, standard blotter paper and potato dextrose agar plate method were used (ISTA, 1976). The fungi isolated from seeds were identified in the laboratory with the available literature (Holliday, 1980; Sivanesan and Lawerence, 1988; Alexopoulos *et al*., 2004)

Standard Blotter Technique

Petri plates (90mm) containing blotter paper (internal diameter sized) were sterilized at 1.045 kg/cm^2 pressure for 20 minutes. These were moistened by flooding with sterile distilled water. The excess water was poured to maintain moisture level. Ten seeds were placed aseptically at equidistance in each Petri plate (9+1 seeds). Two hundred seeds were used. Half seeds were surface sterilized with 0.1% $HgCl_2$ solution for 3 minutes followed by 3 washings with sterile water to isolate internally seed borne fungi. The Petri plates were then incubated at 25 ± 1ºC under alternating cycle for 12 hrs light and 12 hrs darkness. The seeds were examined on 7^{th} day of incubation. The seed mycoflora were picked up and purified and further examined under microscope for their morphological studies leading to their identification.

Potato Dextrose Agar Plate Method

Potato dextrose agar medium was used as a basal medium for the isolation of seed mycoflora. Twenty ml of sterilized (at 1.045kg/cm^2 for 20 minutes) medium was poured into each sterilized Petri plate and was allowed to solidity. Twenty four hrs after the pouring, ten (9+1seeds) unsterilized seeds were plated at equal distance on the medium and were incubated at room temperature (25 ± 1ºC) while, surface sterilized with 0.1% $HgCl_2$ solution for 3 minutes followed by 3 washings with sterile water, seeds were plated for the detection of internally seed borne fungi. A total of two hundred seeds were used. The Petri plates containing sterilized and unsterilized seeds were incubated at 25±1ºC at room temperature giving alternate cycle of light and darkness for 7 days. The light was provided by fluorescent tube (40W). After incubation Petri plates were examined under a stereo-binocular microscope for per cent colonization of seed by different fungi. The initial growth pattern and number of fungal growths were recorded. Isolations were also made from these growths to detect the kind of fungi present in or on the seed.

RESULTS AND DISCUSSION

Host range of the pathogen

The result presented in Table 1 revealed that *Alternaria* blight of Isabgol pathogen could produce visible symptoms on all tested species except Ashwagandha. The symptom expression took longer time 10-12 days in Wheat and Barley, 9-11 days in Mustard, 7-9 days in Coriander, Cumin, Cauliflower, Chilli and Tomato compared to 5-6 days in Isabgol (*Platago ovata* Forsk) The pathogen was reisolated from tested plant leaves and the morphological characters of the reisolated pathogen were compared with the original culture and these were similar in all respects. Hence, *Alternaria* leaf blight of Isabgol (*A. alternata*) has wide host range. However, *A. alternata* has been reported from many other plants species too. This study indicates that sensitive plant species may be collateral hosts of this pathogen. The dispersal of pathogen may be through wind borne conidia on Isabgol. This needs further experimentation and confirmation. Similar

studies had been carried out by Agrawal (1985), Abbas *et al.*(1995), Green *et al.* (2001), Mangala *et al*. (2006); Surendra *et al.* (2009).

Table1. Host range of *Alternaria alternata* on different plants by using spray inoculation technique with reaction (RE) and incubation period (IP) (days)

Host	Scientific Name	RE	IP
Wheat	*Triticum aestivum*	+	10-12
Barley	*Hordeum vulgare*	+	10-12
Mustard	*Brassica compestries*	+	9-11
Ashwagandha	*Withania samnifera*	-	-
Coriander	*Coriandrum sativum*	+	7-9
Cumin	*Cuminum cyminum*	+	7-9
Cauliflower	*Brassica oleracea* var. *botrytis*	+	7-9
Cabbage	*Brassic aoleracea* var. *capitata*	+	7-9
Chilli	*Capsicum annuum*	+	7-9
Tomato	*Lycopersicon esculentum*	+	7-9

Note: + = Visible symptoms, - = Not visible symptoms

Seed transmission

The isolates seed mycoflora were observed under microscope and results thus obtained were presented in Table 2A. Six different fungi were recorded which occurred from unsterilized and sterilized seeds *viz., A. alternata, Fusarium oxysporum, Aspergillus niger, A. flavus, Curvularia lunata* and *Penicillium spp.* These were identified by using standard literature on Taxonomy of fungi. Maximum 16.00per cent *F. oxysporum* was recorded followed by *A. alternata* 14.00 per cent, *A. niger* 12.00 per cent, *Penicillium* spp 11.00 per cent and *C. lunata*10.00 per cent and least occurrence of 8.00 per cent of *Aspergillus flavus.* The mycoflora free seeds were 29.00 per cent in this category. In sterilized seeds, the *A. alternata* was higher by 10per cent than *F. oxysporum*, *A. flavus*, *Penicillium* spp and *C. lunata* and the seed which did not contain any mycoflora was 67.00 per cent. The study concludes that unsterilized seeds were carrying more amount and number of fungi as compared to sterilized ones as reported by Rawal (2002); Meena (2003); Samota (2003) (up to date the reference section). . Hence it suggests that seed treatment could be one of the best remedies to get rid of seed borne inoculum.

Potato Dextrose Agar Plate Method (PDA)

The isolated seed mycoflora were observed under microscope and results were recorded and presented in Table 2.

Table2. Per cent seed mycoflora isolated from Isabgol seed by Standard Blotter Technique (SBT)

Fungal mycoflora	Seed sample			
	Standard Blotter Paper Technique (SBT)		Potato Dextrose Agar Method (PDA)	
	Unsterilized	Sterilized	Unsterilized	Sterilized
*Alternaria alternata**	14.00	10.00	11.00	5.00
Fusarium oxysporum	16.00	8.00	8.00	6.00
Aspergillus niger	12.00	-	9.00	4.00
Aspergillus flavus	8.00	6.00	5.00	1.00
Curvularia lunata	10.00	4.00	2.00	-
Penicillium spp.	11.00	5.00	7.00	2.00
Seed without mycoflora	29.00	67.00	58.00	82.00
Total	100.00	100.00	100.00	100.00

No. of plated seeds 200 for each; *Pathogenic

In all six different fungi were recorded from unsterilized and sterilized seeds *viz., A. alternata, F. oxysporum, A. niger, A. flavus, C. lunata* and *Penicillium* spp. unsterilized seeds, the maximum occurrence of *A. alternata* was recorded as 11.00 per cent in seed followed by *Aspergillus niger* 9.00 per cent, *F. oxysporum* 8.00 per cent, *Penicillium spp.* 7.00 per cent, *Aspergillus flavus* 5.00 per cent and minimum 2.00 per cent occurrence was recorded for *C. lunata* and also the healthy seeds were 58.00 per cent. Further, in sterilized seeds the maximum 6.00 per cent occurrence was of *F. oxysporum* followed by *A. alternata* 5.00 per cent,

A. niger 4.00 per cent, *Penicillium spp.* 2.00 per cent and also minimum 1.00 per cent was observed for *A. flavus*. The fungus free seeds were 82.00 per cent. Seed carries inoculum externally and internally and with the help of these two methods, six fungi were detected in/on Isabgol seeds. Seeds and plants were inoculated with the mycoflora and closely observed for their disease reaction. Similar results had been reported by Kumar, 2005; Habib *et al.* 2007; Menaria 2011).

Seed transmission nature of *A. alternata* in Isabgol

The tests for presence of *A. alternata* carried out in earlier experiments indicated that this pathogen could be often seed transmitted. To know this the seeds having growth of *A. alternata* were transferred into pots directly and it was observed that after 45 days of sowing, the infection started in some of the plants. The typical symptoms were visible after 55 days of the sowing. This shows that infected seeds could be source of primary inoculum for this disease. It was observed that out of six fungi, only one fungus, i.e. *A. alternata* gave pathogenic reaction on the host Isabgol. The present investigations are in close conformity with the results of Chand *et al*. (1999).

ACKNOWLEDGMENT

The authors are highly grateful to the Head, Department of Plant Pathology and Dean, Rajasthan College of Agriculture, Udaipur (Raj.) for providing necessary facilities and Project Director, AINP – M & AP for financial support.

REFERENCES

Abbas, H.K., Grant, H. E. and Rex, N. P. 1995. Effect of conidia production, temperature on germination and infectivity of *Alternaria helianthi*. *Phytopathology,* **85**: 677- 685.

Agarwal, D.K. 1985. New host records of *Alternaria* spp. from India. *Indian Phytopathology,* **38** (2):392-393.

Alexopoulos, C.J., Mims, C.W. and Blackwell, M. 2004. Introductory Mycology. John Wiley and Sons, Inc., New York.

Chand, K., Jain, M.P. and Jain, S.C. 1999. Seed-borne nature of *Alternaria alternata* in cumin, its detection and location in seed. *Journal of Mycology and Plant Pathology,* **29**: 137-138.

Green, S., Mortensen, K. and Bailey K.L. 2001. Host range, temperature response, survival and overwintering of *Alternaria cirsinoxia*. *Biological Control,* **20**:57-64.

Habib, A., Sahi, S.T., Ghazanfer, M.U. and Ali, S. 2007. Evaluation of some fungicides against seed borne mycoflora of eggplant and their comparative efficacy regarding seed germination. *International Journal of Agricultural and Biology,* **9**(3): 519-520.

Holliday, P. 1980. Fungus disease of tropical crops. Cambridge University Press, Cambridge, UK. pp: 289-295.

ISTA, 1976. International rules for seed testing annexure. *Seed Science Technology,* **4**: 41-177.

Kumar, S. 2005. Seed mycoflora of cumin (*Cuminum cyminum* L.) and their management. M. Sc. (Ag.) Thesis, Maharana Pratap University of Agriculture and Technology, Udaipur.

Meena, K.R. 2003.Seed mycoflora of sorghum [*Sorghum bicolour* (L.) Moench] and their management. M. Sc. (Ag.) Thesis, Maharana Pratap University of Agriculture and Technology, Udaipur.

Mangala, U.N. Subbarao, M. Ravindrababu, R. 2006. Host range and resistance to *Alternaria alternata* leaf blight on chilli. *Journal of Mycology and Plant Pathology,* **36**(1): 84-85.

Menaria, D. 2011. Patho-physiological studies on *Curvularia* fruit rot of bell pepper. M. Sc. (Ag.) Thesis, Maharana Pratap University of Agriculture and Technology, Udaipur.

Rawal, P. 2002. Investigation on pathogenic potential and management of micro-organisms associated with fennel (*Foeniculum vulgare* Mill.) seed in Rajasthan. Ph. D. (Ag.) Thesis, Maharana Pratap University of Agriculture and Technology, Udaipur.

Samota, R.K. 2003. Seed mycoflora of coriander (*Coriandrum sativum* L.) and its management. M. Sc. (Ag.) Thesis, Maharana Pratap University of Agriculture and Technology, Udaipur.

Sivanesan, A. and Lawerence, Z. 1988. International Course on the Identification of Fungi and Bacteria of Agricultural Importance.CAB, IMI, Kew, UK.

Surendra, V., Mahapatra, S.S. and Swain, N.C. 2009. Detection of *Alternaria* isolates from different host plants and testing their degree of virulence by cross inoculation method. *Journal of Plant Protection and Environment,***6**:103:106.

Rajesh Kumar Meena[1], Sharma,S.S.[2] and Meena, S.C.[3]
Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan
[1,3]Department of Plant Pathology, RCA, MPUAT, Udaipur-313001, Rajasthan, India.
[2]Department of Plant Pathology, RCA, MPUAT, Udaipur-313001, Rajasthan, India.
Email address: rajeshpatho@gmail.com

ACKNOWLEDGMENTS

Along with **Ilkka Havukkala, New Zealand**, The Editor-In-Chief; The Managing Editor, Editorial Advisory Board Members, Associate Editors and Editorial Board Members gratefully acknowledge the valuable time spared by the following external reviewers in reviewing the manuscripts of the issue **6 (2), 2013**.

Dr. Amar N. Sharma, Directorate of Soybean Research-ICAR, Indore 452 001, MP, **India**

Dr. Ashwell R. Ndhlala, University of KwaZulu-Natal, Pietermaritzburg, **South Africa**

Dr. Carmelo Peter Bonsignore, Mediterranean University of Reggio Calabria, **Italy**

Prof. A P Garg, Opp. Agriculture University, Modipuram, Meerut, **India**

Dr. S. Manisegaran, TNAU, Madurai**, India**

Dr. Mohamed E. I. Badawy, Alexandria University, Alexandria, **Egypt**

Dr. S. Malarvannan, MS Swaminathan Research Foundation, Chennai, **India**

Dr. B.Parameswari, Sugarcane Breeding Institute Regional Centre, Karnal, **India**

Dr C.Rettinassababady, PAJANCOA & RI, KARAIKAL, Pondicherry, **India**

Dr. K. Selvaraj, Central Research Institute for Jute and Allied Fibres, Kolkata, **India**

Dr. Sabu K Thomas, St.Joseph's College, Calicut, Kerala, **India**

Dr. M. P. Srivastava, Tulip Garden, Sector 57, Sushant Lok Gurgaon, New Delhi, **India**

Dr. El-Sayed A. El-Sheikh, Plant Protection Department, Zagazig University, **Egypt**