



Evaluation of Seed Transmission of *Rhizoctonia solani* and Seed Mycoflora of Ajwain

Babu Lal Fagodia^{1*}, B. L. Mali¹, J. P. Tetarwal¹ and R. K. Fagodiya¹

¹Department of Plant Pathology, Rajasthan College of Agriculture, (MPUAT), Udaipur, Rajasthan, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author BLF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BLM and JPT managed the analyses of the study. Author RKF managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2017/35037

Editor(s):

(1) Yong In Kuk, Department of Development in Oriental Medicine Resources, Sunchon National University, South Korea.

Reviewers:

(1) Hamza Abdulmajeed, University of Ibadan, Ibadan, Nigeria.

(2) Rocky Thokchom, Uttar Banga Krishi Viswavidyalaya, India.

Complete Peer review History: <http://www.sciencedomain.org/review-history/19942>

Original Research Article

Received 25th June 2017

Accepted 5th July 2017

Published 8th July 2017

ABSTRACT

In vitro study was conducted Department of Plant Pathology, Rajasthan College of Agriculture, Udaipur (Rajasthan) and evaluated the seed transmission of *R. solani* from the seeds of eight popular cultivars of ajwain viz., Ajmer Ajwain-1, Ajmer Ajwain-2, Ajmer Ajwain-93, Pratap Ajwain, Lam selection-1, Gujarat Ajwain-1, Azad Ajwain and local cultivar. It was found that maximum recovery of the pathogens was from local cultivar and Ajmer Ajwain-93 exhibited lowest recovery of pathogens. It was also found in this study that there some differential results in recovery of *R. solani* from seeds of cv. Gujarat Ajwain-1 and cv. Azad Ajwain. Seed samples were collected and results revealed that in all seed samples were detected in both blotter and agar plate test methods from almost all the seed samples and these fungi were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceous*, *Alternaria alternata*, *Rhizopus oryzae*, *Rhizoctonia solani*, *Dreschlera australiensis* and *Fusarium sporotrichioides*.

Keywords: Ajwain seed; *Aspergillus*; *Rhizoctonia*.

*Corresponding author: E-mail: blfagodia25@gmail.com;

1. INTRODUCTION

Ajwain also (*Trachyspermum ammi* L.) known as Bishops weed and Carom seed, is one of the most important seed spices crop its, belongs to family *Apiaceae* is a native of Egypt. Ajwain is erect, glabrous or minutely pubescent branched annual herb which grows up to 75-80 cm in height. In India it is widely distributed and its production is concentrated mainly in Rajasthan followed by Gujarat, Madhya Pradesh, Bihar, Uttar Pradesh, Punjab, Tamil Nadu, Andhra Pradesh and West Bengal, respectively. Since ancient time the state of Rajasthan and Gujarat has emerged as seed spices bowl. Whose dried fruit of seeds are used as spices. In Rajasthan, it is cultivated in the districts of Chittorgarh, Udaipur, Jhalawar, Baran, Rajsamand, Bhilwara and Kota covering an area of 11658 hectares with the production and productivity is 4672 tonnes per annum and 401 kg/ha, respectively [1]. They are carrier of many important seed mycoflora inciting various diseases, which results in considerable losses in yield. Ajwain seeds carry a number of mycoflora. Although majority of them are saprophytes, a few are potentially pathogenic capable of ruining the crop. Several factors limiting the production of the crop in which poor health of the seed is one of the major factors which takes heavy toll of the crop at all the stages right from seedling to harvest and also during transport and storage. Seed germination and seedling growth greatly influenced by seed mycoflora [2].

One of the factors responsible for this is the use of contaminated seeds by farmers for the sowing purpose because several fungi are responsible for deterioration of ajwain grains/seeds in storage causing reduction in the germination potential and chemical constituents of seeds [3]. The aim of the present study to investigate the present status of seed mycoflora of Ajwain in Rajasthan and their incidence on seeds associated with fungi.

2. MATERIALS AND METHODS

An incubation study was carried out in Ph.D. laboratory, Department of Plant Pathology, Rajasthan College of Agriculture, (MPUAT), Udaipur, Rajasthan and experimental design was completely randomized design. The materials are provided by Maharana Pratap university of Agriculture and Technology, Udaipur.

2.1 *In vitro* Evaluation of Different Seed Mycoflora of Ajwain

2.1.1 Blotter method

Seed transmission of *R. solani* was studied on eight ajwain cultivars viz., Ajmer Ajwain-1, Ajmer Ajwain-2, Ajmer Ajwain-93, Pratap Ajwain, Lam selection-1, Gujarat Ajwain-1, Azad Ajwain and local cultivar. The sterilized plastic Petri plates of 90 cm layered with fresh and sterilized blotting paper on both the surfaces of Petri plate were used. Seeds of each cultivar were surface sterilized using 0.1 per cent mercuric chloride solution for one minutes followed by three washing with sterilized distilled water and then air dried. The blotter paper lined in the Petri plates were then moistened by using sterilized distilled water. Ten inoculated (*R. solani*) seeds per plate were used and maintained five replications for each cultivars, standard untreated check for each cultivar was also maintained with five replications for comparison. The plates were incubated at $28\pm 1^{\circ}\text{C}$ for 7 days and blotters were aseptically moistened on alternate days with sterilized distilled water to provide adequate moisture. Observation for seed germination, seedling mortality and healthy seedlings were recorded after seven days incubation.

2.1.2 PDA medium

Seed transmission of *R. solani* in all the eight cultivars was also studied using PDA medium. Twenty ml of sterilized melted PDA medium was aseptically poured in sterilized Petri plates and was allowed for 2 hrs to solidify. Seeds of all the cultivars were surface sterilized (using 0.1 per cent mercuric chloride solution for one minute followed by three washings with sterilized distilled water then dried). Ten seeds per plate were used keeping five replications for each cultivar. Standard untreated check for each cultivar was also maintained for comparison. The plates were incubated at $28\pm 1^{\circ}\text{C}$ for 7 days. Observation for seed germination, per cent seed germination, seedling mortality and healthy seedlings were recorded after seven days incubation.

2.2 *In vitro* Evaluation of Different Cultivars of Ajwain for Seed Transmission of Pathogens (*R. solani*)

Seed samples were collected from mandies of ajwain growing districts i.e., Udaipur, Pratapgarh,

Chittorgarh and Rajsamand of the Southern Rajasthan. Further, seed samples collected from seed producing agency, which produced seeds using improved cultural practices. All the collected seed samples were kept in cloth bags and brought to the laboratory following store at room temperature for further studies. Samplings were done by methods suggested by ISTA, [4]. Mycoflora associated with seeds samples were collected at post-storage stage and isolated by using two incubation methods *i.e.* Blotter Method and Agar Plate Method [5,6].

2.2.1 Blotter method

From each sample, four hundred seeds were selected and analyzed randomly. White blotting papers were cut into circles of 9 cm of diameter and sterilized in autoclave at 121°C, 15 psi for 15 minutes. Three circles of blotter papers were placed at the bottom of sterilized Petri-dishes aseptically and moistened by sterilized distilled water. Ten seeds were placed at an equal distance in each Petri dish. These dishes were incubated at 28± 1°C with 12 hours of light alternating with 12 hours of dark period. The seeds were examined on 7th day of incubation for emanating fungal colonies. Observed seed mycoflora developed on seeds in Petri dish and then examined the mycoflora under low and high objectives of compound light microscope.

2.2.2 Agar plate method

Two hundred seeds were taken from each sample for isolation of seed born mycoflora. Seeds were surface sterilized with 0.1 per cent mercuric chloride solution for one minutes followed by 3 washing with sterilized distilled water. Each sterilized Petri dishes contain 20 ml

of PDA medium was used for incubation of seeds. Aseptically ten seeds per Petri dish were incubated at 28±1°C with 12 hours with alternating light and dark period. The fungal colonies emanating from seeds were examined from 3rd and 8th day of incubation. Isolation of mycoflora from ajwain seeds were carried out and maintained on 2 per cent PDA medium. Further, identification of the isolates was made in the laboratory by using compound microscope.

3. RESULTS AND DISCUSSION

3.1 *In vitro* Evaluation of Different Cultivars of Ajwain for Seed Transmission of Root-rot Pathogens *R. solani*

3.1.1 Blotter method

The effect of root rot causing pathogens *R. solani* was studied on eight different cultivars of ajwain. The surface sterilized and un-sterilized ajwain seeds were aseptically kept on sterilized and wet blotter paper in Petri dishes and observed after 7 days of incubation at 28±1°C. The data revealed that the pathogens *R. solani* was recovered from almost all the test cultivars of ajwain.

The maximum recovery of *R. solani* on surface sterilized seeds was observed in Local cultivar (18.0%) followed by Gujarat ajwain-1 (15.8%), Lam selection-1 (13.0%), Azad ajwain (11.6%), Pratap ajwain (9.50%), Ajmer ajwain-1 (8.50%), Ajmer ajwain-2 (7.00%) and lowest on Ajmer ajwain-93 (6.50%). Similarly maximum recovery of *R. solani* on unsterilized seeds was observed on cv. Local cultivar (50.0%) followed by Gujarat ajwain-1 (40.5%), Lam selection-1 (30.0%),

Table 1. Seed transmission of *R. solani* in different cultivars of ajwain after 7 days of incubation at 28 ± 1°C

S. no.	Ajwain cultivars	Blotter method (Per cent recovery on seeds)		PDA (Potato Dextrose Agar) (Per cent recovery on seeds)	
		Surface sterilized seeds (%)	Unsterilized seeds (%)	Surface sterilized seeds (%)	Unsterilized seeds (%)
1	Ajmer Ajwain-1	8.50 (16.9)	18.0 (25.0)	11.0 (19.3)	22.0 (27.9)
2	Ajmer Ajwain-2	7.00 (15.3)	14.0 (21.9)	8.90 (17.3)	17.0 (24.3)
3	Ajmer Ajwain-93	6.50 (14.7)	12.5 (20.7)	8.00 (16.4)	13.8 (21.8)
4	Pratap Ajwain	9.50 (17.9)	24.0 (29.3)	13.5 (21.5)	27.0 (31.3)
5	Lam selection-1	13.0 (21.1)	30.0 (33.2)	16.5 (23.9)	39.0 (38.6)
6	Gujarat Ajwain-1	15.8 (23.4)	40.5 (39.5)	17.0 (24.3)	44.0 (41.5)
7	Azad Ajwain	11.6 (19.9)	25.0 (30.0)	14.8 (22.6)	32.0 (34.4)
8	Local cultivar	18.0 (25.1)	50.0 (45.0)	21.0 (27.2)	54.0 (47.3)
	S.Em.±	0.249	0.428	0.263	0.484
	CD at 5%	0.755	1.298	0.798	1.467

*Average of three replications. Figures in parentheses are arcsine $\sqrt{\text{per cent angular transformed values}}$

Azad ajwain (25.0%), Pratap ajwain (24.0%), Ajmer ajwain (18.0%), Ajmer ajwain-2 (14.0%) and lowest on Ajmer ajwain-93 (12.5%), respectively (Table 1). In general the maximum recovered of pathogen *R. solani* was on cv. Local cultivar from surface sterilized and unsterilized seeds. Lowest recovered of *R. solani* was from cv. Ajmer ajwain-93.

3.1.2 PDA (Potato Dextrose Agar) medium

The seed and soil borne nature of the root rot causing pathogen *R. solani* was studied on eight different cultivars of ajwain. The surface sterilized and un-sterilized ajwain seeds were aseptically kept on PDA (Potato Dextrose Agar) medium in sterilized Petri dishes and observed after 7 days of incubation at $28 \pm 1^\circ\text{C}$. The data revealed that the pathogens *R. solani* was recovered from almost all the test cultivars of ajwain. The maximum recovery of *R. solani* on surface sterilized seeds was observed in Local cultivar (21.0%) followed by Gujarat ajwain-1 (17.0%), Lam selection-1 (16.5%), Azad ajwain (14.8%), Pratap ajwain (13.5%), Ajmer ajwain-1 (11.0%), Ajmer ajwain-2 (8.90%) and lowest from Ajmer ajwain-93 (8.00%). Similarly maximum recovery of *R. solani* on unsterilized seeds was observed on cv. Local cultivar (54.0%) followed by Gujarat ajwain-1 (44.0%), Lam selection-1 (39.0%), Azad ajwain (32.0%), Pratap ajwain (27.0%), Ajmer ajwain-1 (22.0%), Ajmer ajwain-2 (17.0%) and lowest from Ajmer ajwain-93 (13.8%), respectively (Table 2). In general the maximum recovered of pathogens *R. solani* was on Local cultivar in surface sterilized and unsterilized seeds. Lowest recovered of *R. solani* was on cv. Ajmer ajwain-93. The experiment was conducted both on surface sterilized and un-sterilized

seeds, suggesting external as well as internal seed borne nature of both the pathogen. The similar results earlier had been reported from other crops like soybean [7].

3.2 In vitro Evaluation of Seed Mycoflora of Ajwain

3.2.1 Blotter method

The effect of different seed mycoflora on seed of ajwain was studied. The surface un-sterilized and sterilized ajwain seeds were aseptically kept on sterilized and wet blotter paper in Petri dishes and observed after 7 days of incubation at $28 \pm 1^\circ\text{C}$. The data revealed that the fungal mycoflora was recovered from almost all the seeds samples of ajwain. The maximum recovery of mycoflora on unsterilized seeds was observed in *Aspergillus niger* (12.5%) followed by *Aspergillus flavus* (11.0%), *Alternaria alternata* (10.0%), *Aspergillus ochraceous* (9.50%), *Fusarium sporotrichioides* (8.50%), *Dreschlera australiensis* (8.00%), *Rhizopus oryzae* (7.00%) and lowest on *R. solani* (6.50%). Similarly maximum recovered of mycoflora on sterilized seeds was observed in *Aspergillus niger* (9.00%), *Aspergillus flavus* (7.50%), *Aspergillus ochraceous* (6.50%), *Fusarium sporotrichioides* (6.00%), *Alternaria alternata* (5.00%), *Dreschlera australiensis* (4.81%), *Rhizopus oryzae* (4.00%) and lowest on *R. solani* (0.00%), respectively.

3.2.2 Agar-plate method

The effect of different seed mycoflora on seed of ajwain was studied. The un-sterilized and surface sterilized ajwain seeds were aseptically kept on

Table 2. Fungal mycoflora isolated from ajwain seeds by standard blotter test and plain agar medium method under sterilized and unsterilized condition

S. no.	Fungal mycoflora	Seed sample*			
		Blotter test		On media	
		Unsterilized	Sterilized	Unsterilized	Sterilized
1.	<i>Aspergillus niger</i>	12.5 (20.6)	9.00 (17.4)	11.0 (19.3)	8.00 (16.4)
2.	<i>Aspergillus flavus</i>	11.0 (19.3)	7.50 (15.8)	9.00 (17.4)	7.20 (15.5)
3.	<i>Aspergillus ochraceous</i>	9.50 (17.9)	6.50 (14.7)	8.00 (16.4)	6.00 (14.1)
4.	<i>Alternaria alternata</i>	10.0 (18.4)	5.00 (12.9)	6.50 (14.7)	4.50 (12.2)
5.	<i>Rhizopus oryzae</i>	7.00 (15.3)	4.00 (11.5)	5.80 (13.9)	3.00 (9.97)
6.	<i>Rhizoctonia solani</i>	6.50 (14.7)	0.00 (0.00)	4.50 (12.2)	0.00 (0.00)
7.	<i>Dreschlera australiensis</i>	8.00 (16.4)	4.80 (12.6)	6.20 (14.4)	4.00 (11.54)
8.	<i>Fusarium sporotrichioides</i>	8.50 (16.9)	6.00 (14.18)	7.50 (15.8)	5.00 (12.92)
9.	Without seed mycoflora	27.0 (31.3)	57.2 (49.14)	41.5 (40.1)	62.3 (52.1)
	Total	100	100	100	100
	S.Em. \pm	0.272	0.292	0.274	0.301
	CD at 5%	0.815	0.874	0.821	0.903

*Mean of three replications

PDA (Potato Dextrose Agar) medium in sterilized Petri dishes and observed after 7 days of incubation at $28\pm 1^{\circ}\text{C}$. The data revealed that the fungal mycoflora was recovered from almost all the seeds samples of ajwain. The maximum recovery of mycoflora on unsterilized seeds was observed in *Aspergillus niger* (11.0%) followed by *Aspergillus flavus* (9.00%), *Aspergillus ochraceous* (8.00%), *Fusarium sporotrichioides* (7.50%), *Alternaria alternata* (6.50%), *Dreschlera australiensis* (6.20%), *Rhizopus oryzae* (5.80%) and lowest on *R. solani* (4.50%). Similarly maximum recovered of mycoflora on sterilized seeds was observed in *Aspergillus niger* (8.00%), *Aspergillus flavus* (7.20%), *Aspergillus ochraceous* (6.00%), *Fusarium sporotrichioides* (5.00%), *Alternaria alternata* (4.50%), *Dreschlera australiensis* (4.00%), *Rhizopus oryzae* (3.00%) and lowest on *R. solani* (0.00%), respectively (Table 2). Amongst these, some of the fungal species have been already reported on seeds of fennel and ajwain including *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus oryzae* [3,8,9]. The association of *Rhizopus oryzae*, *Alternaria alternata*, *Fusarium sporotrichioides* and *Rhizoctonia solani* were found to be as the new fungus on ajwain seed in the present study.

4. CONCLUSION

The present study shows that the seed transmission of *R. solani* from the seeds of eight popular cultivars of ajwain. It was found that maximum recovery of the pathogen was from local cultivar and lowest in Ajmer Ajwain-93. Seeds samples of ajwain were detected in both blotter method and agar plate test from almost all the seed samples and there were maximum fungi was found *Aspergillus* spp.

ACKNOWLEDGEMENTS

We thank Dr. B.L. Mali, former Professor, Plant Pathology for comments and suggestions from time to time. Authors are thankful to Dr. S.S.

Sharma, Professor and Head, Dept. of Plant Pathology, RCA (MPUAT), Udaipur, Rajasthan for providing laboratory facility. First author is also thankful to Indian Council of Agricultural Research, New Delhi for financial support in the form of a Senior Research Fellowship.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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