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Effect of *Alternaria alternata* on seed germination and seedling vigour

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Abstract

Isabgol seed samples were collected from different mandis, shops, farms, farmers, and seed division of Jaipur, Jodhpur, Jalore, Nagour. Experiment were conducted to evaluate the effect of *Alternaria alternata* fungi of Isabgol on seed germination and seedling vigour. Seed germination and seedling vigour tests were conducted using seed inoculation (in Petri plates), seed inoculation (in pots) and foliar application. *Alternaria alternata* caused blight symptoms and observed highly pathogenic causing pre- and post- emergence mortality and reducing vigor index.

Keywords: *Plantago ovata*, germination, *Alternaria alternata*, seed inoculation, seedling growth

Introduction

Blond psyllium (*Plantago ovata* Forsk.) commonly known as *isabgol*, is an annual herb with narrow linear rosette like leaves belonging to the family Plantaginaceae. *Isabgol* is an important cash crop cultivated for its export and being of important medicinal value is reported to have larger demands and is traded in major medicinal markets of the world. *Isabgol* has pharmaceutical importance to treat dysentery, chronic constipation and chronic diarrhoea and as laxative demulcents, emollients and diuretics. India commands nearly monopoly in the production and export of the seed and husk to the world market. India is earning about Rs. 1600 million as foreign exchange from the export of blond psyllium products to countries like USA, Germany, France, England, Spain and Belgium^[1].

In India, the *isabgol* crop is mainly grown as commercial crop in Gujarat, Rajasthan and Madhya Pradesh. However, the crop is spreading to other non-traditional parts of the country such as Haryana, Uttar Pradesh and Karnataka. In Rajasthan, it is being cultivated in 190081 hectares area with a total production of 99950 tonnes of seeds with an average productivity of 525 kg/ha^[2]. In Rajasthan, *isabgol* mainly cultivated in Barmer, Jalore, Nagaur, Jodhpur and Jaisalmer districts. Presently, Rajasthan is on the top in productivity in India.

^[3] reported a number of pathogens viz., Fusarium wilt (*Fusarium oxysporum*), damping off (*Pythium ultimum* Grow), leaf blight (*Alternaria alternata* (Fr.) Keissler), downy mildews (*Peronospora plantaginis*) and powdery mildew (*Erysiphe cichoracearum* D.C.) affecting this crop. *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 *A. alternata*. It causes considerable damage every year and sometimes become very severe which results in total loss of yield^[4]. Hence, present investigations were carried out to test the efficacy of plant extracts and fungicides against leaf blight of *isabgol* incited by *A. alternata*.

Material and Methods

Effect of *Alternaria alternata* on seed germination and seedling vigour (Pathogenicity Test)

For this, two tests were performed as follows:

Seed Inoculation Technique

One hundred apparently healthy surface sterilized seeds were taken. The seeds were then rolled on 10 days old sporulating culture of *Alternaria alternata*, thriving on PDA contained in Petri dishes.

Inoculated seeds were plated on sterilized moist blotter in Petri dishes and same number of inoculated seeds were also sown in 30 cm earthen pots (Pre-sterilized and having autoclaved soil) at the rate of 5 seeds per pot x 20. The uninoculated surface sterilized apparently healthy seeds served as control. These Petri dishes and pots were kept in incubator (24 ± 1 °C) and cage house (22-25 °C), respectively. The pots were watered as and when required. Observations on seed germination and pre emergence mortality were recorded on 10th day of sowing whereas, post emergence mortality and root and shoot length were recorded at 30 DAS. Seedlings showing symptoms were recorded on 60th day of sowing. Seedling vigour was also calculated by following formula of [5].

Seedling Vigour Index = Germination % x (Root length + Shoot length)

Spray Inoculation Technique

The method suggested by [6] was followed to observe the symptoms on seedlings and growing on plants. Thirty days old seedlings, raised from disinfected seeds in 30 cm earthen pots having autoclaved soil (soil : FYM = 3 : 1) were inoculated by spraying with spore suspension of 7 days old culture of *Alternaria alternata*, having concentration @ 10^6 spores/ml. The seedlings were sprayed to run off. Earthen pots having inoculated seedlings were kept in a humid chamber for 48 hours. Check plants were sprayed with distilled water only. Each treatment was comprised of 100 seedlings (5 seedlings/ pots x 20). The sprayed seedlings were accommodated in a cage house where temperature ranged from 20 to 25 °C. Plants were inspected on 15th day of incubation. Isolations were carried out from inoculated plants parts showing typical disease symptoms.

Pathogenicity Test

Among various fungi isolated from seeds. Results on the effect of seed borne fungi on germination and seedling vigour obtained by seed and foliar inoculation methods are presented in the (Table 1, 2 and 3) *Alternaria alternata* was most predominantly associated with them. Hence, for further studies pathogenicity was tested for *Alternaria alternata* only.

Seed inoculation (in Petri plates)

Inoculation of healthy seeds with *Alternaria alternata* (Table 1) caused both pre- and post-emergence mortality (11.42% and 3.07%, respectively), reduction in vigour index (292.5) in comparison to control (1003.3). Amongst the seedlings raised in the test, 74.60 percent seedlings showed symptoms and they were yellowing and brown or black necrotic spots on the leaves.

Seed inoculation (in pots)

In seed inoculation, *Alternaria alternata* caused higher pre- and post-emergence mortality i.e. 9.37% and 4.41%, respectively and reduction in vigour index (639.2) in comparison to control (850.5). Amongst the seedlings raised in the test, 83.07 percent seedlings showed symptoms like seedling blight, leaf blight and yellowing of leaves (Table 2).

Foliar inoculation

Out of 100 seedlings sprayed with conidial suspension of *Alternaria alternata*, 85.0 percent of seedlings showed symptoms (Table 3). Symptoms observed at seedling stage were yellowing, tip burning, marginal blight and brown to black small spots on the leaves. Re-isolation from affected tissues revealed the presence of the pathogen.

Table 1: Pathogenicity of seed borne *Alternaria alternata* of isabgol by Seed Inoculation Technique (in Petri plates)

Fungus	Percent Germination	Percent seedlings showing symptoms*	Percent seedling mortality		Elongation (cm)		Vigour index	Type of symptoms
			Pre-emergence	Post- emergence	Root	Shoot		
<i>Alternaria alternata</i>	65.00	74.60	11.42	3.07	1.75	2.75	292.50	Yellowing, small isolated brown / black necrotic spots on leaves
Control (uninoculated seed)	79.00	3.89	4.76	2.53	5.70	7.00	1003.30	Yellowing and tip burning of leaves

No. of seed tested =100

* Based on emerged seedlings

Table 2: Pathogenicity of seed borne *Alternaria alternata* of isabgol by Seed Inoculation Technique (in pots)

Fungus	Percent Germination	Percent seedlings showing symptoms*	Percent seedling mortality		Elongation (cm)		Vigour index	Type of symptoms
			Pre-emergence	Post-emergence	Root	Shoot		
<i>Alternaria alternata</i>	68.00	83.07	9.37	4.41	4.10	5.30	639.20	Seedling blight, leaf blight and yellowing of leaves.
Control (uninoculated)	81.00	1.25	5.26	1.23	5.10	5.40	850.50	Yellowing, burning of leaves.

No. of seed tested =100

* Based on emerged seedlings

Table 3: Pathogenicity of *Alternaria alternata* by foliar inoculation technique (in pots)

Fungus	No. of seedlings sprayed	Percent seedlings showing symptom	Type of symptoms
<i>Alternaria alternata</i>	100	85.00	Yellowing, tip burning, marginal blight and brown to black small spots on the leaves.-
Control (uninoculated)	100	0.00	

Average of 5 replications (5 seedlings/pot x 4/replication)

Seed germination and seedling growth are generally influenced by seed borne fungi [7-10]. In the present investigation, the effect of *A. alternata* as estimated by seed and foliar inoculation methods showed significant reduction in the seed germination and seedling vigour and proved to be pathogenic. It is likely that fungi which reduced seed germination are pathogenic to host seedlings at the pre-emergence stage [11]. Our findings of seed and foliar inoculation techniques revealed that *Alternaria alternata* reduced seed germination, root and shoot elongation by causing higher percentage of pre- and post- emergence mortality and subsequently it also reduced vigor index, whereas in control of both the test showed good germination, root and shoot elongation and higher vigor index.

Symptoms like chlorotic/ necrotic leaf spot and leaf blight were observed on young seedlings by inoculating them with conidial suspension of *Alternaria alternata* through Spray Inoculation Technique. Association of seed borne *Alternaria alternata* with infected plant parts has also been reported by [12-16].

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