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Efficacy of Seedborne *Alternaria helianthi* on Germination, Seedling Vigour and Blight Incidence in Sunflower

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ABSTRACT

Sunflower seeds collected from Alternaria leaf blight affected plants were tested to detect the pathogen and categorize seeds into apparently healthy, naturally infected (partially infected) and artificially infected (completely infected). All these three categories of infected seeds, naturally infected with A. helianthin and artificially inoculated showed 32.8 and 19.0 per cent reduction in germination respectively. The shoot and root length of seedlings were also reduced in both cases. The vigour index was very low (400) in naturally infected seeds, artificially inoculated seeds recorded a vigour index of 533.5 whereas in control it was 1799.8. With the increase in spore load of *A. helianthi* on seeds, there was an increase in the number of seedlings showing blight incidence. Seeds were tested to detect the pathogen from the seed coat, endosperm and embryo by a component plating method. In all these three categories of infected seeds, the infection of *A. helianthi* was more on seed coat than cotyledons and embryos. In cotyledons and embryos of the apparently healthy seeds, the fungal infection was not detected. In partially infected seeds, only cotyledons showed infection while the embryo was found to be free from the pathogen. Completely infected seeds showed the lowest rate of germination (60-64%) in blotter towel and pot culture experiments. Disease incidence was high in completely infected seeds (40%) in the blotter towel test. The fungus is both externally as well as internally seed-borne. The result indicated that the level of seed infection influences germination, seedling vigour and disease incidence in sunflowers.

Key words: Alternaria leaf blight; sunflower; seedling blight; seedborne; seedling vigour

1) INTRODUCTION

Alternaria spp. plant pathogens are found across the world, affecting a variety of species, including sunflowers. It is a major defoliating pathogen in warm humid climates like India. Farmers and businesses produce sunflowers (*Helianthus* spp.) for manufacturing oil, edible seeds, and ornamental flowers displays. Sunflower (*Helianthus annuus* L.) is an important oilseed crop in India with high quality edible oil and wider adaptability. Several diseases are known to cause yield loss in sunflower and some of these diseases are seed borne viz., Alternaria leaf blight, downy mildew, charcoal rot and head rot. Among them Alternaria blight incited by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara is an important fungal disease of sunflower in India and estimates of yield losses due to this disease range upward to 80% [1, 3]. Transportation of infected sunflowers and agricultural practices spread the pathogen worldwide and cause diseases in places, like India, where sunflowers are an important source of oil production. In India, sunflower cultivated over an area of 4.006 lakh hectare with a production of 2.840 lakh tones and productivity of 709 kg/ha. The pathogen that causes this disease is part of the *Alternaria* genus, it is ubiquitous and abundant and can cause a high mycotoxicological risk during harvest, which causes devastation to entire crop production [11]. *Alternaria helianthi* is an Ascomycetes (Div-Ascomycota) from the Pleosporaceae family. This pathogen produces simple, meaning rarely branched conidiophores that bear solitary conidia. These conidia are light brown coloured, ellipsoid or broadly ovoid, and rarely form longitudinal septa. The pathogen (conidia) spreads through plant debris, seed and air [2, 3, 5, 10, 12] and incites severe leaf spot and stem spot resulting in premature defoliation and stem breakage. In addition, a seedling blight due to *A. helianthi* has also been reported [9, 13]. However, there is little information on germination of such seeds carrying infection and the intensity of leaf blight infection on seedlings. The proportion of infection by *A. helianthi* in sunflower seed components was quantitatively determined. The influence of seed infection on germination was also investigated using in vivo and in vitro screening techniques. The objective of the present study was to determine the effect of seedborne inoculum of *A. helianthi* on sunflower seedling vigour and seedling

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blight incidence.

2) MATERIALS AND METHODS

Detection of Pathogen*:* To detect the seed mycoflora associated with severely infected sunflower heads (cv. Morden), two standard detection methods, viz., standard blotter method and agar plate method (ISTA, 1985) [7] were employed. The experiment was conducted with four replications and under each replication hundred seeds were tested. The seeds were placed in 90 mm Petri plates @ 10 seeds per plate and 10 such plates were maintained under each replication. Fungal colonies or sporulating structures obtained from seeds after incubation through both the methods were isolated separately onto fresh PDA medium in Petri plates. Pure cultures of the *Alternaria helianthi* was isolated by adopting hyphal tip method or single spore isolation technique and maintained on potato dextrose agar slants at 40 C in a refrigerator [14].

Effect of *A. helianthi* on seed germination and seedling vigour*:* To study the efficacy of the fungus on seed germination and seedling vigour, paper towel method prescribed by ISTA was adopted [7]. Germination towels of 44×33 cm size was dipped off. Conidial suspension of the pathogen was obtained from 15 days old cultures of *A. helianthi* grown on oatmeal agar. The concentration of conidia (105 conidia / ml) in suspension was adjusted using haemocytometer. 10 ml of this suspension was used to treat 400 dry surface sterilized seeds of cv. Morden and air dried. Fifty inoculated seeds were distributed over wet paper towel at equidistance and another wet towel was laid over the seeds and both were rolled together. The three such rolls were rolled in a wax paper sheet and kept in a germinator at 25± 10 C temperature and 90-95% relative humidity for 10 days. In one treatment naturally infected seeds were kept for germination. Seeds of cv. Morden collected from disease free fields served as control. All treatments were replicated three times. Observations on seed germination, root length and shoot length were recorded after ten days. The vigour index was determined by multiplying the percentage of germination with the sum of root length and shoot length in centimetres [6].

Effect of spore load of *A. helianthi* on the incidence of seedling blight: Various concentration of conidial suspensions (230, 3500 and 41000 conidia / ml of suspension) were obtained. 4 ml suspension of each concentration was used separately to treat 100 seeds. The treated seeds were air dried and sown in 20 cm diameter plastic pots (25 seeds / pot) with sterile soil. Uninoculated seed served as control. Four replications were maintained for each treat. This experiment was repeated twice.

Effect of *A. helianthi* infection on seedling rot incidence in blotter towel test and in pot culture: The seeds were grouped into three categories as apparently healthy, partially infected and completely infected based on the level of infection on flower head and colour of seeds by visual observation. Seed borne infection of *A. helianthi* in sunflower seeds were studied by component plating, blotter towel and pot culture tests.

Blotter towel method: In this method, 100 seeds of each category were surface sterilized and plated on layers of blotter towel and incubated at 25±2C. The germination percentage was recorded after 7 days of incubation and also percentage of disease incidence was recorded. One week after incubation, the total numbers of emerged seedlings as well as the number of seedlings showing lesions on the hypocotyls or cotyledons were recorded.

Pot culture experiment: Thirty seeds of each category were used. Six replicates for each category of seeds and five seeds were sown per pot (30 cm) containing sterilized mixture of field soil: farmyard manure: sand. Twelve days after sowing, the total numbers of emerged seedlings as well as the number of seedlings showing lesions on the hypocotyls or cotyledons were counted.

3) RESULTS AND DISCUSSION

Effect of *A. helianthi* on seed germination and seedling vigour: Seed germination was reduced significantly in all treatments when compared to control (Table 1). There as 32.8 % reduction in germination with naturally infected seeds whereas in treatment with artificially inoculated seeds 19.0 % reduction in germination was observed. 29-35 % loss in germination was recorded in seed samples of sunflower collected from heavily infected fields [4, 6].

Shoot and root length reduced significantly in all treatments when compared to control (Table 1). In control the shoot length was 8.9 cm whereas in naturally infected seeds and artificially inoculated seeds it was 3.3 and 3.5 respectively. Likewise, root length was also reduced significantly. The rot length was 3.9 and 4.4 cm in naturally infected seeds and artificially inoculated seeds, respectively, and in control it was 12.6 cm. The vigour index also decreased significantly as a result of reduction in germination and shoot and root length. Vigour index was only 400.4 in the treatment with naturally infected seeds it was 533.5. In control the vigour index was 1799.8.

Effect of spore load of *A. helianthi* on the incidence of seedling blight: Inoculation of sunflower seeds with various concentrations of conidial suspensions of *A. helianthi* resulted in slight reduction in emergence of seedlings. The emergence of seedlings in treatments with 230 conidia/ml was 84% which was at par with control whereas at 3500 and 41000 conidia/ml treatments the emergence was decline 82.6 and 80.0% respectively (Table 2). There was significant increase in seedlings showing stem and leaf lesions with increase in spore load. At higher conidial concentration (41000 conidia/ml) 44.4% of seedlings showed leaf lesions. In each of the three concentration, leaf lesions developed on greater number of seedlings than did stem lesions.

Effect of *A. helianthi* infection on seedling rot incidence in blotter towel test and in pot culture: Seed-borne inoculum was found to be the primary source of inoculum of leaf blight during the growth of seedlings. This study demonstrated that the infection levels on outer and inner portions of the sunflower seeds to vary.

In blotter towel method: The germination was 64% in completely infected seeds and 74% in partially infected seeds. Similarly, the infection of seedlings was more (40%) in completely infected seeds, while it was 28% in partially infected seeds. In case of apparently healthy seeds there was 91% germination with only 3.5% infection on

seedlings. There were significant differences in germination and seedling rot of the seed samples of all categories. In fungicide treated seeds, there was a cent percent germination with no disease incidence on the seedlings (Table 3). However, on seedlings raised from the completely infected seeds, necrotic lesions were observed on roots and cotyledons. Diseased seedlings showed symptoms of either dark necrotic lesion on cotyledons or necrotic areas on the roots.

In pot culture experiment: In fungicide treated seeds there was 98% germination, while in apparently healthy seeds, 87% germination was observed. However, in partially infected seeds, the germination was 77%. The germination was reduced to 60% in completely infected seeds (Table 3). Infection of seedlings was 18% in seedlings grown from completely infected seeds, but infection was not observed in seedlings grown from partially infected and apparently healthy seeds. There was an apparent inverse relation between the level of seed infection and germination percentage in blotter towel and pot culture tests.

The results of studies reported in this paper indicated that seedborne inoculum of *A. helianthi* has a role in reduction of seed germination and seedling vigour. Kim and Mathur [8, 15] detected the inhibitory effect of *Alternaria* spp. in carrot seeds on seeds germination and seedling growth. He also reported that the seed sample most severely infected with *Alertnaria radicina* showed reduced germination and emergence of normal seedlings but increased seedlings rot. However, seed infection of the fungus was not found to affect normal seedling growth of carrot in the soil test. The reduction in germination was more in naturally infected seeds. High inoculum levels on seeds will affect seedling emergence and the incidence of seedling blight will also be high. So, seeds from infected sunflower fields should be avoided for raising the crop.

4) CONCLUSION

Mycologists and pathologists are continuing to measure the potentiality of *Alternaria* spp on other several crops. However, the present study revealed that the pathogen is mostly externally seed-borne and is associated on seed

Table 1: Effect of *A. helianthi* on seed germination and seedling vigour of sunflower seeds

Treatment	Percent germination	Percent reduction in germination	Shoot length* (cm)	Root length* (cm)	Seedling vigour
Naturally infected seed	55.3 (48.0)	32.8	3.3	3.9	400.4
Healthy seeds inoculated with the pathogen	66.6 (55.4)	19.0	3.5	4.4	533.5
Control	82.3 (65.1)	0.0	8.9	12.6	1799.8
CD at 5%	2.2	-	0.5	0.7	-
* Average of 40 seedlings in each treatment; Figures in the parentheses are angular transformation					

Table 2: Effect of spore load of *A. helianthi* on the incidence of seedling blight of sunflower seeds

Conidia / ml	Percentage Emergence*	% Seedlings showing lesions**	
		Stem lesions	Leaf lesions
230	84.0 (66.5)	0.0 (0.0)	16.1 (4.0)
3500	82.6 (65.4)	31.5 (5.6)	51.5 (7.1)
41000	80.0 (63.5)	44.4 (6.6)	58.8 (7.6)
Control	84.0 (66.5)	0.0 (0.0)	0.0 (0.0)
CD at 5%	4.2	0.3	0.5
* Figures in the parentheses are angular transformations. ** Figures in the parentheses are square root transformations			

Table 3: Efficacy of *A. helianthi* infection on seed germination and seedling rot incidence of sunflower seeds in blotter towel test and on germination in pot culture

Seed type	Blotter towel test		Pot culture	
	Germination (%)	Seedling rot incidence (%)	Germination (%)	Seedling rot incidence (%)
Fungicide treated (Vitavax)	99.5	0.1	97.8	1.5
Apparently healthy	91.0	3.5	87.0	4.2
Partially infected	73.5	28.0	76.5	30.0
Completely infected	63.8	40.0	60.2	46.7
CD (*P=0.05*)	9.7	4.8	12.7	5.1
CV (%)	7	8	10	9
Figures were arc sin transformed before analysis				

coat and internally seed-borne associated on cotyledons and embryo. The seed samples, which were severely infected with *A. helianthi* showed low germination and high rate of seedling rot. However, in pot cultures, seed infection of fungus had shown less effect on seedling infection. Seeds treated with fungicide reduced the seedling rot and increased the germination. Diseased seedlings were observed for its symptoms of either dark necrotic lesion on hypocotyls or necrotic areas on the cotyledons. More research and innovations are needed to reduce the incidence of *Alternaria* blight caused by *Alternaria helianthi* on sunflower for supporting the agricultural needs of our economy.

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