

LOCATION OF *MACROPHOMINA PHASEOLINA* IN SEEDS OF PUMPKIN AND DEVELOPMENT OF CHARCOAL ROT DISEASE

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Abstract

Using component plating technique *Macrophomina phaseolina* was isolated from seed coat, tegment, cotyledons and embryo of pumpkin seeds. Infected seeds give rise to infected seedlings that transmit the pathogen into the fruits.

Introduction

Macrophomina phaseolina (Tassi) Goid., a soil inhabiting fungus is known to produce seed rot, seedling blight, wilt and root rot on over 500 species of plants in different parts of the world (Sinclair, 1982). At least 67 hosts of *M. phaseolina* have been reported from Pakistan (Mirza & Qureshi, 1988; Shahazad & Ghaffar, 1986; Shahzad *et al.*, 1988). *M. phaseolina* has been reported from seeds of bottle-gourd and musk melon (Maholay, 1988, 1989). The fungus has been detected in association with seed coat and cotyledons of melon and was found to penetrate the fruit via the peduncle to infect the seed (Reuveni *et al.*, 1983). Experiments were carried out to study the location of *M. phaseolina* in the seeds of pumpkin (*Cucurbita pepo*) and its effect on the germination of seed and development of the disease.

Materials and Methods

Seed samples of pumpkin were collected from Hyderabad, Sindh, Pakistan. Based on macroscopic observation, the seeds were separated into three categories viz., i) seeds with black dotted spots; ii) healthy looking seeds with few dotted spots, iii) healthy looking seeds with no dotted spots. For determining the location of fungi in seed, the seeds were washed and soaked in distilled water for 2 hours and then aseptically dissected to separate different component viz., seed coat, tegment, cotyledons and embryo. Dissected seed parts were surface disinfected with 0.5% NaOCl solution and placed on blotters in Petri dishes (Du-Hyunglee *et al.*, 1984).

For transmission studies, ten seeds were placed on 3 layers of well soaked blotter in 9 cm diam Petri dishes. After five days, the lids were removed and dishes put in polyethylene bags to study seedling symptoms for 15 days. Single seed was placed in 200x20 mm test tube containing 15 ml of 1% plain water agar. The cotton plug was removed as the seedling reached to the top. A set of 15 Petri dishes and 150 test tubes for each category of seeds was incubated at 25°C under 12 hours of alternating

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Table 1. Percent recovery of *Macrophomina phaseolina* from seeds of pump kin.

Parts of seed	Categories		
	A	B	C
Seed Coat	100a ²	62a	22a
Tegment	87a	67a	7b
Cotyledon	12b	7b	0b
Embryo	7b	9a	0b

A: Seeds with black dotted spots, B: Healthy looking seeds with few black dotted spot, C: Healthy looking seeds. ²Number followed by the same letter within a column are not significantly different ($P=0.05$) according to Duncan's multiple range test.

cycle of ADL and darkness (Khare *et al.*, 1977). In another experiment, five seeds were sown in earthen pots containing 3 kg of autoclaved soil. A set of 20 pots was used for each category. Seeds were also sown in 3.5x5 m plot in two rows, 3.5 m long and 3 m apart. The pots and field experiments were carried out during winter (20-28°C) and then in summer (28-40°C) and emergence of seeds and *M. phaseolina* infection on seedling were recorded. The data were analysed using Duncan's Multiple Range test.

Table 2. Seed to seedling transmission of *Macrophomina phaseolina* under laboratory conditions in pumpkin seeds.

Categories	Premergence infection	Petriplates			Test Tube	
		At hypo-cotyl & radicle axis	Aerial part	Premergence Infection	At hypo-cotyl & radicle axis	Aerial part
Seeds with black dotted spots	9.3a	11.0a	3.0a	15.0a	8.0a	1.7a
Healthy looking seeds with few black dotted spots	4.7a	7.0a	4.7a	11.7a	6.0a	2.0a
Healthy looking seeds	0b	3.0b	0b	0.7b	2.0b	0b

Means followed by the same letter within a column are not significantly different ($P=0.05$) according to Duncan's multiple range test.

Results and Discussion

Where seeds with dotted spots were used, *M. phaseolina* infection in seed coat and in tegment was significantly higher than in cotyledons and embryo. *M. phaseolina* infection in healthy looking seeds was low in seed coat and tegment and was not detected on cotyledons and embryo (Table 1).

There was a direct correlation between cotyledons and embryo infection and loss in germination (Table 2,3). Most of the ungerminated seeds were covered with gray to black loose mycelium with minute black sclerotia. The seedling in which the seed coat was either attached to the peg or cotyledons showed browning of hypocotyl and root axis resulting in death of the seedling. In seedling where seed coat remained attached to the cotyledons during germination, there was yellowing and browning of cotyledonary leaves resulting in death of the aerial parts. Seedling mortality was significantly greater ($p < 0.05$) during summer as compared to winter sowing in both the pot and field experiments. Such similar results have been reported by Reuveni *et al.*, (1983) who found that infected seed gave rise to infected plant with more severe symptoms under warmer conditions.

Adult plant grown from seed having dotted spot showed upto 14% *M. phaseolina* infection during summer with no infection in winter sowing. Reuveni *et al.*, (1982) also reported that infection was more prevalent in summer than in winter grown *Cucumis melo*. Similarly, high temperature has been found to favour *M. phaseolina* infection on soybean (Agarwal *et al.*, 1973; Meyer *et al.*, 1974), pine (Hodges, 1962), corn and sorghum (Livingston, 1943) and bean and cowpea (Thompkins & Gardner, 1935). Disease severity in castor bean was highest where warm and moist spring were followed by hot and dry weather (Cook, 1955). In the present study infected plants showed stunted growth, vine decline, root and basal stem rot, reduction in number and size of fruit, fruit shedding just after formation and rotting of the developed fruit. Minute black sclerotia were also seen on the surface of seeds and inner mesocarpic tissues of

Table 3. Effect of sowing seed infected with *Macrophomina phaseolina* on plant emergence and seedling mortality.

Categories		POTS		FIELD	
		Emer- gence	Seedling Mortality	Emer- gence	Seedling Mortality
Seeds with black dotted spots	Summer	17.0b	10.8a	17.6b	9.3a
	Winter	19.3b	3.0b	19.0ab	4.0b
Healthy looking seeds with with few black dotted spots	Summer	20.0ab	7.8a	21.0ab	5.8ab
	Winter	20.5ab	2.0bc	21.8ab	1.5c
Healthy looking seeds	Summer	24.0a	4.3b	23.5a	5.0b
	Winter	25.0a	0.5c	25.0a	1.0c

Means followed by the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

infected fruit. *M. phaseolina* was isolated from the disease plant part when placed on PDA medium. The characteristic charcoal rot symptoms appeared in the basal portion of few plants. Reuveni *et al.*, (1983), reported that the fungus can penetrate melon fruit via peduncle and infect the seeds as well.

The results of the present study showed that *M. phaseolina* infection is extra as well as intra-embryonal which affects the germination of the seeds and produces disease symptoms on seedling and the adult plant. There is therefore need to use healthy seeds to prevent spread of inoculum in non-infested field.

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