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Short Communication

Location of Seed-borne Inoculum of *Lasiodiplodia theobromae* and its Transmission in Seedlings of Pumpkin (*Cucurbita pepo*)

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Abstract. Using naturally infected seeds of pumpkin, the fungus pathogen *Lasiodiplodia theobromae* (syn. *Botryodiplodia theobromae*) was located on and in the seed coat, including tegmen, and in the tissues of the cotyledons and embryo. Apparently healthy seeds showed incidence of the fungus in seed coat and tegmen. The fungus was transmitted to seedlings and caused pre- and post-emergence damping-off desease, resulting in increase in the inoculum potential of *L. theobromae* in the agricultural fields.

Keywords: pumpkin, seed-borne fungus, *Lasiodiplodia theobromae*, seedling mortality, *Cucurbita pepo*, *Botryodiplodia theobromae*, seedling damping-off

Lasiodiplodia theobromae (syn. *Botryodiplodia theobromae)* has been reported to attack various cucurbitaceous fruits, such as cantaloupes, bottle gourd, squash, pumpkin and fluted pumpkin (Sultana, 2003; Neufo and Emebiri, 1990; Maholay, 1988; Beraha *et al*., 1976; Laxminarayan and Reddy, 1976). The fungus was detected from the mature fruits of pumpkin that were left either in the field or kept stored for seed collection. The infected fruits were observed to be ash grey with dotted spots externally, but were dark black inside. The fungus is also reported to be seed-borne in various other cucurbitaceous crops, such as water melon, bottle gourd, squash and pumpkin (Sultana, 2003; Maholay, 1989; Maholay and Sohi, 1982; 1976; Sohi and Maholay, 1974). *L. theobromae* is further reported to be seed-transmitted, and a cause of pre- and post-emergence death of maize seedlings (Kumar and Agarwal, 1997). Studies were carried out to determine the location of *L. theobromae* in seeds of pumpkin (*Cucurbita pepo*), and its effects on seed germination. The development of disease in pumpkin is also reported in the present communication.

Pumpkin seeds were collected from the growers' fields in Hyderabad, Sindh, Pakistan. Based on visual observations, the seeds were separated into three categories, namely, dark black, slightly black, and apparently healthy seeds. For determining the location of the fungus in the seeds, the seeds were washed and soaked in distilled water for 2 h, and then aseptically dissected to separate different components, viz., seed coat, tegmen, cotyledons and embryo. Dissected seed parts were surface disinfected with 0.5% sodium hypochlorite (NaOCl) solution for 5 min, washed with distilled water for several times, and placed on blotters in petri dishes (Du-Hyunglee *et al*., 1984). For the disease transmission studies, 10 seeds were placed on three layers of well-soaked blotters in 9 cm dia petri dishes. After five days, the lids were removed and dishes put in polyethylene bags to study the development of seedling disease symptoms for 15 days. Single seeds were separately placed in 200 x 20 mm test tubes containing 15 ml of 1% plain water agar. Sets of 15 petri dishes and 150 test tubes, for each category of seeds were incubated at 25 °C under 12 h alternating cycle of artificial daylight and darkness (Khare *et al*., 1977). In another experiment, five seeds were sown in earthen pots containing autoclaved soil. Sets of 20 pots were used for each category of seeds.

By the separated seed components plating of severely and moderately infected seeds of pumpkin, *L. theobromae* was found in the seed coat, tegmen, cotyledons and embryo. *L. theobromae* infection in the seed coat and tegmen was significantly higher than in cotyledons and embryo. Whereas infection in the healthy looking seeds was low in the seed coat and tegmen, it was not detected in cotyledons and embryo (Table 1). Similar results of incidence of *L. theobromae* in apparently healthy seeds have been reported (Maholay and Sohi, 1982; 1976; Srivastava, 1964).

Seed germination was noted to be directly correlated with cotyledons and tegmen infection. Most of the ungerminated seeds were covered with cottony greyish mycelium with superficially or partially embedded pycnidia. The seedlings, in which the seed coat was either attached to the peg or cotyledons, showed browning of root axis, root tip and hypocotyls, ultimately resulting in the death of seedlings. In the seedlings where the seed coat remained attached to

Table 1. Detection of *Lasiodiplodia theobromae* from different parts of the seeds of pumpkin

Parts of seed	Infection rate (%) categories of seeds		
	A	B	C
Seed coat	100	58	14
Tegmen	58	28	8
Cotyledons	14	8	0
Embryo	8	4	0

A = seeds dark brown; B = seeds slightly black; C = apparently healthy seeds

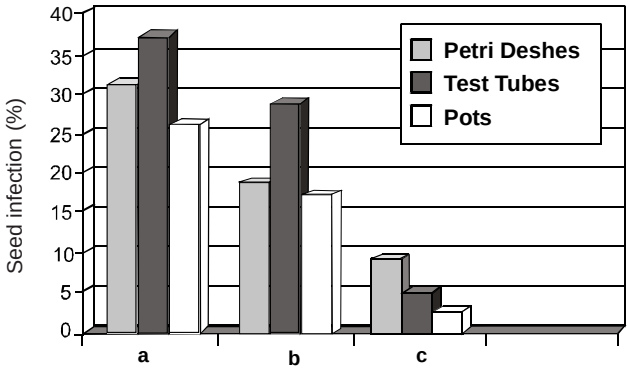

Fig. 1. Percentage of seed infection caused by *Lesiodiplodia theobromae* by categories, on pumpkin seeds of dark black category when germinated under different conditions; a = pre-emergence mortality, b = hypocotyl and radical axis infection, c = aerial parts infection.

Table 2. Seed to seedling transmission of *Lasiodiplodia theobromae* in pumpkin seeds

Categories of seed	Petri-dishes (with moist blotters)			Test tubes (with agar)			Pots (with sterilized soil)		
	pre-emergence infection (%)	post-emergence infection (%)		pre-emergence infection (%)	post-emergence infection (%)		pre-emergence infection (%)	post-emergence infection (%)	
		hypocotyl and radical axis	aerial parts		hypocotyl and radical axis	aerial parts		hypocotyl and radical axis	aerial parts
Seed dark black	32	19	9	37	29	5	26	18	3
Seeds slightly black	19	15	6	16	13	8	12	12	4
Apparently healthy seeds	10	5	3	7	4	4	9	0	0

the cotyledons during germination, there was blackening of cotyledonary leaves and the first formed leaves, followed by the collapse of aerial parts. The pathogen was commonly found in the lesions on root axis, hypocotyls and cotyledonary leaves, where pycnidia were easily observed under stereobinocular. Symptoms caused by the fungus on the cotyledons also extended to the hypocotyls and killed the seedlings. Many of the first formed leaves were infected by contact with infected cotyledons resulting in the development of secondary infections. Seedling mortality was significantly greater in the heavily and moderately infected seeds as compared to the apparently healthy-looking seeds (Table 2). Maximum infection of *L. theobromae* was recorded after 6-10 days and 6-14 days of incubation on blotter and test tubes, respectively. Disease development on seedlings in the pots was observed 7-16 days after planting. The three methods used for transmission studies, namely, blotter, agar in test tubes, and sterilized soil pots, were all found to yield comparable results. The observations from the present study reveal that seed infection caused pre-emergence and post-emergence damping-off and its transmission from seed to seedlings and soil was an important factor of spreading inoculum in non-infested agricultural field.

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