Integrative Biosciences 9: 37-40, 2005

Integrative **Biosciences**

http://www.ibiosci.or.kr

Pathogenicity of *Macrophomina phaseolina* and *Fusarium verticilloides* in Okra

Mashooda Begum, S. Lokesh* and T. Vasanth Kumar

Department of Studies in Applied Botany, Seed Pathology and Biotechnology, University of Mysore, Manasagangotri, Mysore-570 006, India

Abstract: In okra *Macrophomina phaseolina* and *Furasium verticilloides* cause collar-rot, seedling-rot and other severe diseases at fruit maturing stages. These stages were located in all the components of the seeds. The seeds collected from seeds infected with *Macrophomina phaseolina* and *Fusarium verticilloides* revealed 100% infection. Such seeds resulted in pre- and post-emergence mortalities. Inoculated seeds also showed pre- and post-emergence death of the seedlings. The fungi seed-transmitted showed disease symptoms at different growth of okra plant. *Fusarium verticilloides* causes the wilt and *Macrophomina phaseolina* causes the collar-rot. Until now seed transmission of these fungi have not been studied. Hence, in the present study an attempt has been made to fill this lacunae.

Key words: Collar-rot, seedling-rot, fruit-rot, seed transmission

Okra (*Abelmoschus esculentus* (L.) Moench) is an important vegetable crop grown mainly for its tender green fruits in India. The green fruits are rich in vitamins A and C and minerals like Ca, Mg and Fe. Okra seeds are also good sources of protein and vegetable oil (Yadav and Dhankhar, 2001). Okra crop is grown throughout the year and is susceptible to many fungal pathogens, among which few of them are seed-borne. A number of methods have been adopted for routine examination of seeds for the presence of seed-borne fungi. The location of the pathogen in the seeds also decides the disease severity. Firm establishment of the pathogen within the seed tissues is a prerequisite for its effective transmission. In routine seed health testing of okra samples *Macrophomina phaseolina* and *Fusarium verticilloides* infected samples were encountered to conduct investigations on their location and transmission aspects.

*To whom correspondence should be addressed.
E-mail: boramma@rediffmail.com

MATERIALS AND METHODS

Seed samples of okra including Arka Anamika, Arka Abhy, Pusa Sawani and a few local varieties were obtained from the Indian Institute of Horticulture Research and private seed companies at Bangalore. Following the procedures of ISTA the samples were assessed for the occurrence of *F. verticilloides* and *M. phaseolina*. The fungi were isolated from the seeds and cultured on potato dextrose agar medium (PDA) in the petri plates. Four hundred seeds of each sample were treated with 2% gum Arabic and rolled separately on 8-day-old sporulated colonies of *F. verticilloides* and *M. phaseolina*. The spore load was adjusted to 5×10^6 spores/g seed using a hemocytometer. Inoculated seeds were then sown in soil and maintained under greenhouse and field conditions, separately. In both cases, the uninoculated seeds maintained under similar conditions were served as corresponding control. In the other set, the seedlings raised from the uninoculated seeds were sprayed separately with the spore suspension of *M. phaseolina* and *F. verticilloides* at the concentration of 6×10^6 spores/ml. Furthermore, at regular intervals of 10 days the plants were sprayed with the spore suspension and the spray schedule was continued up to flowering. Such plants were maintained until fruit maturation stages. Seedlings raised from the seeds inoculated with the spore suspension of respective fungi were served as control.

During growing stages the parameters including the total number of fruits/plant, average fruit length, biomass were assessed. The fruits were harvested, extracted and their main nutritional ingredients such as proteins, carbohydrates and ascorbic acid content were determined. The densities of the seeds obtained from such treated plants were also determined. The seeds were also assessed for the occurrence of *M. phaseolina* and *F. verticilloides*.

On the other hand, the seeds obtained from *M. phaseolina* and *F. maniliforme* infected plants were separately soaked in distilled water for 16 h, then the seeds were carefully excised with the sterilized forceps and their components such as seed coats, cotyledons, radicals and plumules were separated and plated side by side on moistened blotter discs in the petri plates. The plates were incubated according to the procedures of ISTA for a period of one week. The components were then examined for the occurrence of fungi using stereoscopic microscope.

In the other set, 100 seeds each were plated separately on water agar and soil beds. These samples were maintained carefully for growing-on symptoms. Varied symptoms that developed on seedlings were recorded.

Carbohydrate content was determined using standard procedures as described by Dubois et al. (1956). Simultaneously a standard curve was developed for glucose for concentration range of 10–50 µg. Optical density of each sample was recorded at 490 nm to determine the carbohydrate content in each sample.

The estimation of protein was performed employing the procedures of Lowry et al. (1951), using freshly prepared Lowry's reagent. Absorbance at 660 nm was measured in a spectrophotometer with a standard range of 10–100 µg of bovine serum albumin.

RESULTS AND DISCUSSION

Macrophomina phaseolina and *F. verticilloides* were found in all the four components of okra seeds, but the percent incidence was high in the seed coat (Table 1). Present study clearly showed that the transmission of *M. phaseolina* and *F. verticilloides* from seed to seedling. Pun et al. (1998) reported the seed-borne nature of *M. phaseolina*. Infection of *M. phaseolina* leads to pre- and post-emergence mortality and confirmed the seed to seedling transmission of the pathogen. Similar to present findings, systemic transmission of *F. verticilloides* has been clearly demonstrated by Foley (1962).

Young seedlings emerged out after seven days further showed circular, dark brown spots on the shoot tip. On 16th day these symptoms gradually spread to stem and leaves finally resulted in rotting. The infection of *M. phaseolina* and *F. verticilloides* was confirmed by incubating the diseased parts on moist blotters. Due to infection some of the seedlings showed abnormal features such as stunted growth of plumule and radicle.

Heavy colonization of the fungus was recorded in the seed coat components. Similar symptoms caused by *M. phaseolina* have been reported by many workers (Androus, 1938; Anonymous, 1940; Varada Rajan and Patil, 1946; Tarr, 1954; Meiri and Solel, 1963).

In the present findings, the fungus was found to spread both in stem and root. Both Tables 1 and 2 showed the percentage of infection in cotyledons and embryonal axis. The abnormal seedlings showed rotting of collar and root portions. Nath et al. (1970) reported the seed rot and seedling blight in mung bean due to fungi. Five percent of the naturally infected seeds and 80% of the artificially inoculated seeds did not germinate when sown in soil. Among the seedlings that emerged from the naturally infected seeds all of them showed collar-rot leading to the seedling death. This may be due to the colonization of

Table 1. Location of *M. phaseolina* and *F. verticilloides* in okra seeds

Seed components	% Incidence of fungi in different components of okra seeds	
	M. phaseolina	*F. verticilloides*
Seed coat	65	63
Cotyledon	36	39
Radicle	39	33
Plumule	33	37

Data based on 100 seeds.

Table 2. Pathogenicity of *M. phaseolina* and *F. verticilloides* in okra

Methods employed	Pathogenic effect of *M. phaseolina* and *F. verticilloides* in okra					
	% Ungerminated seeds		% Abnormal seedlings		% Disease symptoms	
	A	B	A	B	A	B
Water agar	39	35	8	7	21	17
Sand	36	31	9	7	27	18
Soil	37	31	9	9	26	16

Data based on 100 seeds. A = *F. verticilloides*, B = *M. phaseolina*, Symptoms : *Macrophomina phaseolina* showed the collor-rot, *F. verticilloides* showed the wilt.

Table 3. Percent transmission of *M. phaseolina* and *F. verticilloides* in okra under green-house and field condition

Treatment	% Occurrence of *M. phaseolina* and *F. verticilloides* in the seeds	
	M. phaseolina	*F. verticilloides*
Seeds inoculated with *F. verticilloides*	–	66.5 ± 0.5^{a} (67.5 ± 0.5^{a})
Plants sprayed with *F. verticilloides*	–	57.5 ± 0.5^{b} (58.5 ± 0.5^{b})
Seeds inoculated with *M. phaseolina*	61.5 ± 0.5^{a} (62.5 ± 0.5^{a})	–
Plants sprayed with *M. phaseolina*	52.5 ± 0.5^{b} (3.5 ± 0.5^{b})	–

Data based on 400 seeds. Data in paranthesis refer to field experiment. According to Duncan's multiple range test (DMRT), values followed by different superscript are significantly different at $P\leq0.05$.

Table 4. Varied response of okra due to *F. verticilloides* and *M. phaseolina* inoculation

Fungal inoculated seeds	Seed germination (%)	MRL±SE (cm)	MSL±SE (cm)	Vigour index	Green-house condition		Field condition	
					% Of seedling emergence	% Of seedling with disease symptoms	No. of seedling emergence	No. of seedling with disease symptoms
Control	40.5±0.5^{b}	2.4±0.0^{b}	8.2±0.0^{a}	428.0± 4.0^{a}	40.5±0.5^{c}	15.5±0.5^{a}	40.5±0.5^{d}	16.5±0.5^{b}
Seeds obtained from the plants spread with spore suspension	50.5±0.5^{a}	2.6±0.0^{a}	5.4±0.2^{c}	405.5±15.5^{a}	44.5±0.5^{b}	7.5±0.5^{b}	42.5±0.5^{c}	7.5±0.5^{c}
Control	39.5±0.5^{b}	2.1±0.0^{a}	6.3±0.1^{b}	332.8± 9.1^{b}	38.5±0.5^{c}	17.5±0.5^{a}	39.5±0.5^{d}	20.5±0.5^{a}
Seeds obtained from the plants spread with spore suspension	50.5±0.5^{a}	2.7±0.0^{a}	5.2±0.0^{c}	392.5± 2.5^{a}	44.5±0.5^{b}	6.5±0.5^{b}	45.5±0.5^{b}	7.5±0.5^{c}
Control	50.0±0.0^{a}	2.5±0.0^{a}	5.2±0.0^{c}	394.0± 4.0^{a}	49.5±0.5^{a}	6.5±0.5^{b}	50.5±0.5^{a}	6.5±0.5^{c}

Data based on 400 seeds. Data refers to the quality parameters of okra plants raised from *M. phaseolina* and *F. verticilloides* inoculated seed/plants. According to Duncan's multiple range test (DMRT), values followed by different superscript are significantly different at $P \leq 0.05$.

different tissues which resulted in the inactivation of the living tissues. Among 50% of the seedlings emerged from the artificially inoculated seeds, 45% showed disease leading to seedling mortality. The remaining 5% seedlings exhibited the disease symptoms only at fruit maturation stages. The uninoculated seeds yielded healthy seedlings. The present observation clearly revealed the seed infection due to fungi lead to seedling disease, confirmed the seed to seedling transmission. The seeds procured from the fruits of infected plants were found heavily colonized by the fungi.

Four hundred seeds were tested for the transmission of the pathogen on 1% water agar in glass petri dishes by the method described by Khare et al. (1977). Healthy seeds plated and maintained similarly were served as control. Seedlings with brown symptoms lead to the rotting after 10 to 12 days at the tip of the leaves and were progressed backward and ultimately resulted in the collapse of seedlings within 20 days.

The previous literature suggested the transmission of *F. verticilloides* through air and soil. Baker (1938) trapped the spores from air over grape orchids. All these reports support the dispersal, survival and infective capability of the fungus. The pathogen may be introduced to an uncontaminated area through seeds. When once introduced to uninfected soil, it may later spread through air, rain and irrigation water to new fields and crops. As the pathogen invariably located in the embryo, probably no seed treatment can efficiently eliminate the infection. Hence, some of the fungicidal or biocontrol treatment should be aimed to preventing the invasion of pathogen into embryos of the seeds.

Table 5. Pathogenic effect of *F. verticilloides* and *M. phaseolina* on the quality of okra

Seeds / plants treatment	No. of leaves on 30th day	No. of fruits per plant	Mean Length of the fruit (cm)	Mean Girth of the fruit (cm)	Mean biomass of the fruit (g)	No. of seeds per plant	1000 seed weight (g)	Total Protein (mg) / 100 g of fruit	Total Carbo hydrate (mg) / 100 g of fruit	Ascorbic acid (mg) / 100 g of fruit
Seeds inoculated with *Fusarium verticilloides*	2.0±0.0^{b}	2.0±0.0^{b}	5.5±0.5^{a}	4.5±0.5^{b}	4.0±0.0^{b}	4.0±0.0^{b}	20.5±0.5^{c}	27.0±1.0^{c}	15.5±0.5^{b}	13.0±1.0^{c}
Plants sprayed with *Fusarium verticilloides* spore suspension	2.0±0.0^{b}	2.0±0.0^{b}	5.0±0.0^{b}	4.5±0.5^{b}	4.5±0.5^{b}	4.5±0.5^{b}	24.0±0.0^{b}	34.0±0.0^{b}	14.0±0.0^{b}	14.0±0.0^{c}
Seeds inoculated with *Macrophomina phaseolina*	2.0±0.0^{b}	2.0±0.0^{b}	4.0±0.0^{c}	4.0±0.0^{c}	4.0±0.5^{b}	5.0±0.0^{b}	21.5±0.5^{c}	21.5±0.5^{d}	10.5±0.5^{c}	16.5±0.5^{b}
Plant sprayed with *Macrophomina phaseolina* spore suspension	2.0±0.0^{b}	2.0±0.0^{b}	3.0±0.0^{c}	4.0±0.0^{c}	5.0±0.0^{a}	3.5±0.5^{b}	20.5±0.5^{c}	24.0±1.0^{d}	14.0±0.0^{b}	16.0±0.0^{b}
Control	3.0±0.0^{a}	3.0±0.0^{a}	6.0±0.0^{a}	5.0±0.0^{a}	6.5±0.5^{a}	7.0±0.0^{a}	35.0±0.0^{a}	40.0±0.0^{a}	34.5±0.5^{a}	20.5±0.5^{a}

Data based on 400 plants. According to Duncan's multiple range test (DMRT), values followed by different superscript are significantly different at $P \leq 0.05$.

REFERENCES

Androus CF (1938) Seed transmission of *Macrophomina phaseolina. Phytopath* 28: 620-634.

Anonymous (1940) Indian central jute committee. *Ann Rep Agric Res Sch* 1939-1940, pp 50.

Anonymous (1996) Proceedings of the international rules for seed testing. *Seed Sci Technol* 21: 25-30.

Arinze AK, Naqui SHZ, and Exundayo (1975) Storage rot of sweet potato (*Ipomea baticola*) and the effect of fungicides on extracellular cellulolytic and pectolytic enzymes of causal organisms. *J A Int Bioterior Bull* 11: 41-45.

Baker RED (1938) Studies in the pathogenicity of tropical fungi. The occurrence of latent infections in tropical plants. *Ann Bot* 8: 919-931.

Bhargava SN, Ghosh AK, Srivastava MP, Singh RH, and Tandon RN (1960) Studies on fungal diseases of some tropical fruits. VII, Effect of temperature on the decay of mango banana and guava caused by some important pathogens. *Proc Nat Acad Sci India Sect B* 35: 393-398.

Brown GE (1971) Pycnidial release and survival of *Diplodia natalensis* spores. *Phytopath* 61: 559-561.

Chahal DS and Suryanarayana D (1970) Root rot of okra (*Abelmoschus esculentus* (L.) Moench) caused by *Rhizoctonia batalicola.* (Taub) Butler. *Punjab J Res* 7: 651-653.

Del Rosario MSE (1954) The fungus causing black kernel rot of corn. *Philpp Agric* 37: 623-631.

Dubey SC and Jha AK (1999) Influence of dates of sowing and environmental factors on collar rot of okra. *Ind Phytopathol* 52: 291-293.

Dubois M, Gultes KA, Hamilton JK, Rebers PA, and Smith F (1956) Corbohydrate estimation by phenol sulphuric acid method. *Ann Chem* 26: 350.

Foley DC (1962) Systemic infection of corn by *Fusarium verticilloides. Phytopath* 52: 870-872.

Grover RK and Singh G (1970) Pathology of wilt of okra (*Abelmoschus esculentus* (L.) Moench) caused by *Fusarium oxysporum.* Synder and Hansen its host range and histopathology. *Ind J Agric Sci* 42: 989-995.

Jackson GMH and Gollifer DE (1975) Storage rot of taro *(Celopasia esculenta)* in British solomn islands. *Ann Appl Bot* 80: 217-230.

Khare MN, Mathur SB, and Neergaard P (1977) A seedling symptom test for detection of septoria nodorum in wheat seed. *Seed Sci Technol* 5: 615-617.

Lowry OH, Rosen Brough NJ, Farr AL, and Randall RJ (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* 193: 265-275.

Madan S, Singh D, Mathur SB, and Neergaard P (1975) Detection and location of seed borne inoculum of ascochyte rebiei and its transmission in chick pea. *Seed Sci Technol* 3: 667-681.

Meiri A and Solel Z (1963) Transmission of charcoal rot by sesame seeds. *Phytopath Medit* 2: 90-92.

Naseema A, Balakrishnan S, and Nair MC (1983) Pathology and control of seed mycoflora of some vegetables in Kerala. *Agri Rev J Kerala* 21: 32-27.

Nath S, Mathur SB, and Neergaard P (1970) Seed-borne fungi of mung bean *(Phaseolus ameus)* from India and their significance. *Proc Int Seed Test Assoc* 35: 225-241.

Neergaardp (1977) *Seed Pathology.* Vol. 1. Macmillan Press Ltd., London, pp 1-839.

Tarr SAJ (1954) Macrophomina phaseolina recorded on phaseolus mungo var. radiatus, plant pathology. *Rep Res Div Minist Sudan,* pp 71-80.

Vardana Rajan BS and Patel JS (1946) Seed transmission of stem rot of jute and its control. *Ind J Agri Sci* 16: 193-206.

Yadav SK and Dhankhar BS (2001) Correlation studies between various field parameters and seed quality traits in okra cv. Varsha uphar. *Seed Res* 29: 84-88.

[Received October 19, 2004; accepted February 21, 2005]