Research Paper :

Location and transmission of *Macrophomina phaseolina* and *Alternaria alternata* in okra

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SUMMARY

A total of 115 okra seed samples collected from different sources were subjected to Standard Blotter Method (SBM) for isolation. Five samples showing higher infestation of seed borne fungi in SBM were selected for studying the location and transmission of the pathogen. *Macrophomina phaseolina* recorded 2-5% in seed coat, 0-5% in fringe layer, 0-4% in cotyledons, 0-4% in endosperm and 0-3% in embryonic axis. *Alternaria alternata* was 0-4% in seed coat, 0-3% in fringe layer, 0-2% in the cotyledons, 0-2% in endosperm and 0-2% in embryonic axis in *Kharif* (monsoon). In *Rabi* (post-monsoon), *M. phaseolina* was 0-4% in seed coat, 0-5% in fringe layer, 0-10% in cotyledons, 0-2% in endosperm and 0-1% in embryonic axis while *A. alternata* 2-3% in the seed coat, 0-3% in fringe layer, 1-3% in cotyledons, 0-2% in endosperm and 0-1% in embryonic axis. The seeds harvested during *Kharif* and *Rabi* season favoured the more number of pathogens in the seed coat than in the other components. The transmission of *M. phaseolina* and *A. alternata* was 12.2% in *Kharif*. In *Rabi*, the transmission was 18.2% in all the five seed samples. The present study revealed that the disease transmission was more during *Rabi* than *Kharif* season.

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Okra [*Abelmoschus esculentus* (L.) Moench.] is one of the important summer, rainy season vegetable crop. It is used in soups, stews and creole dishes together with many other vegetables. In Karnataka, the okra is cultivated in 7181 hectares with an annual production of 58057 tones (Anonymous, 2007). This crop is affected by number of fungal diseases. Important pathogenic fungi are *M. phaseolina, A. alternata, Fusarium oxysporum* and *F. solani*. These pathogen cause brown lesion spots, leaf spots, blight, wilt, rot, die-back and discoloration of seeds. Present study was undertaken on the location and transmission of *M. phaseolina* and *A. alternata* in okra seeds during *Kharif* and *Rabi* seasons in Karnataka.

MATERIALS AND METHODS

A field survey was carried out during 2007-2008 and a total 34 seed samples of okra in *Kharif* and 28 in *Rabi* were collected during 2007. During 2008, 29 seed samples in *Kharif* and 24 in *Rabi* season were collected. The samples were collected from farmers, agro-agencies and the seeds were extracted from the fruits grown at different agro-climatic regions of Karnataka. The collected seed samples were dried in sunlight to bring down the safe seed moisture and were subjected to Standard blotter method (SBM) for isolation of fungi.

Standard blotter method (ISTA, 1976):

Three blotter discs were dipped in sterilized water and placed in 9 cm diameter Petri plates. Twenty five seeds were plated at equidistantly on moist blotter discs and were incubated at 23 ± 2^0C under alternating cycles of NUV light and darkness. The seeds were examined on 7th day using stereobinocular microscope and the seed mycoflora were recorded.

Five samples showing higher incidence of *M. phaseolina* and *A. alternata* in standard blotter method were selected for studying the location and transmission studies.

Location of the pathogen by component plating method :

This method is adapted to know the location of the pathogen in different components of the seed (Basak, 1998). Two hundred seeds from each sample were selected and washed four to five times with distilled water and soaked separately in sterilized distilled water for 24 hours. The seeds were dissected for different components as seed coat, fringe layer, cotyledons, endosperm and embryo using sterilized scissors and forceps under aseptic condition. Each part was surface sterilized by 1% mercuric chloride ($HgCl_2$) solution and washed with sterile water and placed directly on moistured blotters. All the components plated individually and kept for incubation.

Transmission studies :

Among the total seed samples, five samples showing higher incidence of *M. phaseolina* and *A. alternata* were selected for disease transmission in the experimental plot. The seed samples were sterilized with 1% mercuric chloride solution for 2-3 minutes and in the distilled water and sown. The experimental plot were prepared by 10 x 10 m. One hundred seeds were selected from each with four replications. The proper agronomical practices were followed for raising the plants. The severity of the disease was assessed by using 0-9 scale (Mayee and Datar, 1986) and per cent disease index calculated using the formula:

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of individual ratings}}{\text{No. of leaves examined} \times \text{Maximum disease grade (g)}} \times 100$$

Seed to seed transmission of *M. phaseolina* and *A. alternata* were studied.

Recovery of the pathogens from seeds :

Seed samples collected from the experimental plot in *Kharif* season, subjected for seed health testing methods. Again the seeds sown in *Rabi* season in experimental plot for recovery of the pathogens were studied. These seeds also yielded the *M. phaseolina* and *A. alternata*. The study showed that, *M. phaseolina* and *A. alternata* were transmitted from seed to seedlings and to the seeds.

RESULTS AND DISCUSSION

During the field survey, the collar rot or brown lesion spots and leaf spots of okra was noticed in all the visited fields during *Kharif* and *Rabi* season, 2007-2008. The severity of the collar rot or brown lesion spots and leaf spots were more in *Kharif* than *Rabi* seasons in both the years.

Location of the pathogen in different seed components :

Five different seed samples were used for studying the location and transmission. The present study revealed that in okra, *M. phaseolina* was recorded 5-15% and *A. alternata* was 6-14% in Standard blotter method (SBM). *M. phaseolina* ranged from 2-5% in seed coat, 0-5% in fringe layer, 0-4% in cotyledons, 0-4% in endosperm and 0-3% in embryonic axis. *A. alternata* ranged from 0-4% in seed coat, 0-3% in fringe layer, 0-2% in cotyledons, 0-2% in endosperm and 0-2%, in the embryonic axis in *Kharif* (Table 1). Agarwal and Singh (2000) have recorded 20-60%, 70-100% has extra-embryonal and 20-30%, 50-70% as intra-embryonal in okra.

In *Rabi*, *M. phaseolina* was recorded 10-20% and *A. alternata* infected 5-18% in all the five seed samples. *M. phaseolina* showed 0-4% in seed coat, 0-5% in fringe layer, 0-10% in cotyledons, 0-2% in endosperm and 0-1% in embryonic axis. While *A. alternata* 2-3% in seed coat, 0-3% in fringe layer, 1-3% in cotyledons, 0-2% endosperm and only 0-1% in the embryonic axis (Table 1).

Location of the pathogen in the seed is important to control seed borne pathogens. Based on the location of the pathogen in the seeds, the chemicals are selected to prevent the seed borne inoculation of the pathogens. Majority of the seed borne pathogens are lodged on the seed coat, some pathogens are in the cotyledons and some are in embryonic axis. Many researchers have reported (Agarwal and Singh, 2000; Shrestha *et al*., 2000; Basak, 1998 and Vishunavat and Sanjay Kumar, 1994) the location of the pathogen in seed coat, cotyledons, endosperm and embryonic axis of various crops.

The expression of *M. phaseolina* and *A. alternata* was more in seed coat than other components. The seeds harvested during *Kharif* and *Rabi* seasons favoured more number of pathogens in the seed coat than other components. The seeds harvested during *Rabi* season showed less incidence of mycoflora in the seed components when compared to the *Kharif* season. This was because of the pervailing environmental factors during the growth stages of crop.

Transmission studies :

The present study revealed that the seeds having

Table 1 : Location of the *Macrophomina phaseolina* and *Alternaria alternata* in different seed components of okra during *Kharif* and *Rabi* season, 2008

Place of collection	Per cent infection of seed in blotter method		Incidence in different seed components									
			Seed coat		Fringe layer		Cotyledons		Endosperm		Embryonic axis	
	M. p	A. a	M. p	A. a	M. p	A. a	M. p	A. a	M. p	A. a	M. p	A. a
***Kharif* season**												
Sindhanur	15.0	11.0	3.0	2.0	2.0	1.0	1.0	-	-	1.0	-	-
Shankaraghatta	13.0	14.0	3.0	3.0	-	1.0	2.0	2.0	3.0	-	1.0	-
Chitradurga	12.0	10.0	3.0	-	4.0	-	-	-	-	2.0	-	-
Harihara	7.0	8.0	2.0	2.0	3.0	3.0	4.0	1.0	2.0	1.0	1.0	-
Gadag	5.0	6.0	5.0	4.0	5.0	3.0	4.0	2.0	4.0	1.0	3.0	2.0
***Rabi* season**												
Pavagada	20.0	18.0	3.0	2.0	2.0	-	2.0	3.0	2.0	-	1.0	1.0
Mustur	16.0	12.0	-	2.0	-	-	-	2.0	-	-	-	-
Tarikere	15.0	13.0	4.0	3.0	5.0	3.0	10.0	2.0	1.0	2.0	-	-
Neralagunte	15.0	10.0	-	3.0	1.0	1.0	2.0	3.0	2.0	1.0	-	-
Honnali	10.0	5.0	3.0	3.0	3.0	3.0	2.0	1.0	-	2.0	-	-

M. p – *Macrophomina phaseolina*, A. a – *Alternaria alternata*

Table 2 : Seed transmission of *M. phaseolina* and *A. alternata* naturally infected in okra during *Kharif* and *Rabi* season, 2008

Place of collection	Per cent incidence in SBM		Germ. per cent	Mortality per cent		Diseased plants (per cent)	Healthy plants (per cent)	Recovery of pathogen	
	M. p	A. a		Pre-emergence	Post-emergence			M. p	A. a
***Kharif* season**									
Shankaraghatta	15	10	81	19	06	15	60	11	08
Gadag	21	13	76	24	03	13	60	12	10
Sindhanur	15	18	78	22	02	10	66	13	12
Chitradurga	07	15	85	15	05	08	72	10	12
Harihara	17	20	86	14	06	15	65	14	08
Mean	15.00	15.20	81.20	18.80	4.40	12.20	64.60	12.00	10.00
Sd	5.099	3.962	4.324	4.324	1.817	3.114	4.980	1.581	2.000
S.E.±	2.276	1.769	1.931	1.931	0.811	1.390	2.223	0.706	0.893
***Rabi* season**									
Shankaraghatta	18	10	66	34	11	19	36	14	12
Gadag	25	15	78	22	15	20	43	11	10
Sindhanur	30	20	83	17	10	17	56	17	12
Chitradurga	23	12	70	30	05	11	54	16	08
Harihara	14	08	67	33	08	24	35	04	13
Mean	22.00	13.00	72.80	27.20	9.80	18.20	44.80	12.40	11.00
Sd	6.205	4.690	7.396	7.396	3.701	4.764	9.834	5.225	2.000
S.E.±	2.770	2.094	3.302	3.302	1.652	2.127	4.390	2.333	0.893

Data based on 100 seeds (each sample in 4 replicates)

15% infection of *M. phaseolina* and 15.2% infection of *A. alternata*, showed the transmission of 12.2% in okra (Table 2).

Recovery of the pathogens from seeds :

Seed samples collected from the experimental plot subjected for seed health testing methods for recovery of disease transmission. The seeds collected from disease transmitted plants, sown again during *Rabi* season, infection having 22% of *M. phaseolina* and 13% infection of *A. alternata* showed 18.2% transmission (Table 2).

Reduction of the yield is based on the environmental conditions and the severity of the disease symptoms. The

mode of seed to seedling transmission of the pathogen depends on the aggressiveness of the pathogen and environmental conditions. Current study revealed that the transmission of the pathogens was more during *Kharif* than *Rabi* harvested seeds. But disease transmission was more in *Rabi* than *Kharif* season. Singh and Shukla (1986) have reported the disease appeared in the first fortnight of July and gradually increased upto November, declined in disease severity with the lowering of temperature and relative humidity upto December.

Many workers have recorded the transmission of diseases on different crops. Agarwal and Singh (2000) have studied *M. phaseolina* transmission in okra, Bhale *et al*. (2000) have studied *Colletotrichum dematium* and *A. alternata* transmission in chilli, Vidyasekaran and Thiagarajan (1981) have reported transmission nature of *Fusarium oxysporum* in chilli. Kulbe and Sati (1987) have reported *A. raphani* seed transmission nature in tomato.

The study reveals that the disease transmission was more during *Rabi* than *Kharif* season. Because, the environmental factors influence for the transmission of the pathogen.

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