Location and Transmission of Seed-borne Fungi Associated with Some Cucurbits Seeds and its Effect on Infection Incidence

Baraka M. A.*; I. N. Ali*; M. I. Ghoneem and M. I. Mohamed****

* Agricultural Botany Dept., Suez Canal University, Faculty of Agriculture, 41522 Ismailia, Egypt

** Plant Pathology Research Institute, Agriculture Research Center, Dokki, Egypt

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Abstract: Blotter method was more accurate than PDA method for detect seed-borne fungi on watermelon, melon and squash seeds respectively. Seed-borne fungi were dominant in seed testa followed by cotyledons. Seed transmission by water agar method, clearly indicated transmission of *Fusarium solani*, *Macrophomina phaseolin*, *Rhizoctonia solani* from watermelon, melon and squash seeds to seedlings. Typical symptoms of the diseases were appeared on plants like vascular wilt, charcoal rot, stunted growth, brown spots and root rot on hypocotyls and cotyledons. *F. solani* was transmitted through roots, stems and shoots in watermelon and melon but through stems only in squash, while *M. phaseolina* and *R. solani* were transmitted through roots, stems and shoots in watermelon, melon and squash.

Keywords: Location, Transmission, Seed-borne Fungi, Cucurbits Seeds.

INTRODUCTION

Sowing of infected seeds reduced germination, vigor and potential yield by transmitting pathogen from seed to plants. Seed infection usually occurs during three distinct physiological phases in the seed production, development and maturation, the pathogen can be involved in all these stages of growth and transmit from planted to the new crop thus developing a systemic that can colonize the seed (McGee, 1995).

Nasreen and Ghaffar (2009) reported that, ISTA techniques were studied to detect seed-borne mycoflora of cucumber (*Cucumis sativus*). The blotter method was the most suitable technique for detection of fungi in cucumber seeds. A deep freeze blotter method was preferable for the detection of *Fusarium* spp. A total of 18 genera and 33 species were isolated, of which 25 have not been recorded from seeds of cucumber. Seed-borne fungi of bottle gourd (*Lagenaria siceraria*) were studied. Both blotter and deep freeze blotter methods yielded quantitatively as well as qualitatively more fungi than agar plate method.

Seed borne fungi are known to be located in different components of seed. The pathogen is located on and in the seed coat and transmits from seed to seedling in cucumber and pumpkin Lee *et al.*, (1984). In this regard, Sudisha *et. al.* (2006) mentioned that, seedling symptom test showed varied incidence of black spot on hypocotyls, lesions on cotyledon and pycnidia on ungerminated seed.

The following investigation was designed to detect location of the seed-borne fungi on three cucurbit seed and their transmission to mature plants

MATERIALS AND METHODS

Seed-health testing methods:

Moist blotter method:

Ten seeds from each of watermelon (cv.Giza-1), melon (cv.Ismaillawy) and squash (cv.Iskandrany) were plated in a 9-cm diameter Petri-dish containing three layers of blotter paper moistened with sterilized tap water. Five replicates were incubated at 20±2°C for 7 days under cool white fluorescent light with alternating cycles of 12 hours light and 12 hours dark.

Agar plate method:

The infected seed samples were carefully washed in running tap water and soaked in 2% solution of sodium hypochlorite for 3 minutes. The seeds were washed four times in sterilized water and dried between sterilized filter papers. Seed samples were then placed in sterilized Petri-dishes containing PDA medium. The plates were incubated at 20±2°C for 7 days under cool white fluorescent light with alternating cycles of 12 hours light and 12 hours dark. Incidence of growing fungi from different seed parts was recorded.

Isolation of associated fungi:

Sample of 60 seeds from each of three cucurbit crops were washed several times in sterilized water and soaked in sterilized water for one hour using the component plating method as described by (Maden *et al.* 1975). The seeds were dissected into two parts *i.e.* seed coat and cotyledon. These parts were plated separately in sterilized Petri-dishes contained three layers of moist blotter at the rate of 20 seed coat, 20 cotyledons per Petri dish respectively. The dishes were incubated at 20 °C in alternative regime of light and darkness (12/12 hours) for one week. Fungal growth was examined using stereomicroscope and light microscope. Incidence of growing fungi from seed parts was recorded.

Seed transmission of some pathogenic fungi:

In vitro.:

Ten seeds from each of three cucurbit crops were artificially inoculated with *Fusarium solani* (Mart) Sacc., *Macrophomina phaseolina* (Tassi) Goid and *Rhizoctonia solani* Kuhn. Ten percent of water agar was prepared and 10ml of the medium was poured into each sterilized test tube, one seed was placed in each test tube and incubated for 21 days under 12/12 h alternate cycles of NUV light and darkness. Ten seeds each three cucurbits crops were left without inoculation and placed in water agar test tube to serve as control. Seedlings were regularly examined for the occurrence of disease symptoms associated with *F. solani*, *M. phaseolina* and

R. solani. Data was scored up to three weeks of incubation and tabulated as percentage of infection for each fungus (Sudisha *et. al.* 2006).

In vivo. **:**

Artificially inoculated for seeds from each of three cucurbit crops with *F. solani, M. phaseolina* and *R. solani* was carried out by sterilizeing pots 25 cm in diameter by immersing in 5% formalin solution for 15 minutes and left for two weeks to get rid of any toxicity. The tested fungi were inoculated on barely medium to prepare the inoculums. Flasks (500 ml) contained 100g; clean barley grains and 100 ml water was mixed and autoclaved at 121°C for 20 minutes. Sterilized barley medium was inoculated with an equal disks (5 mm diameter) taken from 7 days old pure culture and incubated at 20-25 °C for two weeks. Soil (50% sand and 50% clay w/w) was sterilized by autoclaving at 121 °C for 1 hr. then left for 7 days before useing. Sterilized pots were filled with sterilized soil at the rate of 4 kg soil/pot and mixed with the previously isolated fungi growing in barley media at a rate of 5 g /kg soil (w/w). Pots infested with uninoculuted media were used as a control. Three replicates of pots were sown by five infested seeds for each treatment. Another set of pots were sown by noninfested seeds and used as a control treatment. Emerged seedlings were left to growth. The transmission rates of the fungi from different plant parts at 90 days were determined. Five plants were removed from the pots, washed, disinfected and dissected under sterile condition. The various plant parts (roots, stems, and shoots) were plated on sterile moist blotters in the rat of 15portion / replicate and incubated for 7-10 days at 24 °C. Recovered fungi from each treatment were identified, and the transmission percentage was recorded.

RESULTS AND DISCUSSION

Seed-health testing methods:

Data in Table (1) demonstrate that, *Penicillium* sp. showed the highest percentage of isolated fungi of watermelon seeds for blotter method followed by *Aspergillus niger, Rhizoctonia solani, Macrophomina phaseolina, Alternaria solani* and *Fusarium solani* (52.6, 51.2, 32.6, 28.0, 26.0 and 21.2 respectively).Whereas, *Botrytis* sp., *F. oxysporum, Cladosporium* sp., *A. flavus, A. alternate, Phoma* sp. and *Verticillium* sp. were the lowest percentage. At the same time, PDA method, *Penicillium* sp. recorded the highest percentage followed by, *A. niger, M. phaseolina* (36.0, 22.0 and 12.6, respectively). While, *R. solani, A. solani, A. flavus, F. solani* and *Trichoderma* sp. were the lowest percentage. Similar results were recorded for seed-borne fungi on melon seeds and squash seeds.

It is clear from our data that, in the case of watermelon seeds the blotter method was more accurate than PDA method for detecting most of seed-borne fungi, similar results was observed in case of melon seeds and squash seeds.

Several methods have been developed to detect seed-borne mycoflora which have been reviewed by (De Tempe 1961 and Neergaard 1973). The standard blotter method yielded maximum number of fungi such similar results have been observed from the detection of seed borne fungi in rice (Khan *et al.* 1988), cotton (Bhutta, 1988) and sunflower (Dawar 1994, Begum and Momin 2000). In addition, Khan *et al.* (1988) preferred the use of agar plate method over the blotter method for isolation of *Curvularia* spp., and *Drechslera* spp., from disinfected seeds of rice. Presence of *Aspergillus* spp., especially *A. niger* and *A. flavus* on seeds of bottle gourd in higher frequencies and its association with ungerminated seeds of bottle gourd confirmed the findings that species of *Aspergillus* though occur as saprophytes may cause low germination in seeds (Shakir and, Mirza 1992 and Dawar 1994).

Location of some pathogenic fungi associated with different seeds parts of three cucurbit crops:

Location of fungal species associated with different seeds parts of the three cucurbit crops was detected. Data presented in Table (2) reveal that, *F solani, M. phaseolina, R. solani, A. solani, Phoma* sp. and *Verticillium* sp. were the highest appearance in seed coat of watermelon seeds (20.0, 16.6, 10.0 and 3.3%)respectively, while, *F. solani* and *M. phaseolina* isolated from cotyledon (3.3 and 3.3%) respectively. Data also show that, *R. solani, F. solani, M. phaseolin* and *Verticillium* sp were the highest appearance in seed coat on melon seeds (20.0, 16.6, 10.0 and 3.3%) respectively, while, *F. solani* and *M. phaseolin* were found on cotyledon by (6.6 and 3.3%) respectively. Similar results were recorded on squash seeds, *A. flavus, F. solani* and *M. phaseolina* were the highest appearance in seed coat (6.6, 3.3 and 3.3%)respectively.While, *F. solani* was isolated only from cotyledon (3.3%).

The present investigation clearly revealed that seed-borne fungi were dominant in seed coat than cotyledons. Many studies are in agreement with our results such as Lee *et al.* 1984, when reported that *Didymella bryoniae* was found lying dominant in seed coat of cucumber and pumpkin. A study from elsewhere indicated that seed-borne fungi in water melon invaded epidermis, cotyledons and embryos (Rankin, 1954). Agarwal and Sinclair, (1997) reported that seed borne pathogen can be transmitted either by infection of embryo, endosperm or by contamination of seed coat.

Transmission of the pathogenic fungi from seeds to plants of some cucurbit crops:

Among the seed-borne fungi associated with watermelon, melon and squash seeds, the three genera, *F. solani, M. phaseolina* and *R. solani* were selected to study their transmission in plant parts during the growth of three hosrs.

In vitro.:

Transmission of some seed-borne fungi by water agar method was observed in this trial. Data in Table (3) reveal that, seedling symptom varied incidence of deferent symptoms on hypocotyls and cotyledon of watermelon, melon and squash seedlings. Vascular wilt appeared on hypocotyls and cotyledon of watermelon, melon and squash seeds when infected with *F. solani* (13.3, 6.7 and 13.3%) respectively and initiated from 8th day of incubation compared with the control. While, watermelon, melon and squash seeds infected with *M.*

phaseolina resulting hypocotyls and cotyledon infected with charcoal rot and stunted growth (13.3%) and initiated from 8th day of incubation compared with the control. Whereas, watermelon, melon and squash seeds were infected with *R. solani* resulting hypocotyls and cotyledon infected with brown spots and root rot (13.3, 6.6 and 6.7) respectively initiated from 8th day of incubation compared with the control.

In water agar method, *F. solani*, *M. phaseolina* and *R. solani* were transmission from watermelon, melon and squash seeds to seedling and cause typical symptoms, vascular wilt, charcoal rot, stunted growth, brown spots and root rot on hypocotyl and cotyledon. Our results are agreement with Narseen *et al.* (2009) who mention that *M. phaseolina* can transmitted to seedling and caused pre and post emergence infection which was significantly high under water stress conditions.

In vivo.:

Seed-plant transmission of pathogenic fungi under greenhouse condition was recorded. Data in Table (4) show that, watermelon plant parts (root, stem and shoot) recorded 13.3, 20 and 6.7% incidence of *F. solani* respectively, 22.2, 20 and 0.0% incidence of *M. phaseolina* respectively and 2.2, 24.4 and 0.0 incidence of *R. solani*. On the other hand, melon plant parts (roots, stems ant shoots) recorded 8.88, 24.4 and 4.4 % incidence of *F. solani* respectively,11.1, 31.1and 0.0% incidence of *M. phaseolina* respectively and 8.9, 26.7 and 0.0 incidence of *R. solani*. Whereas squash plant parts (roots, stems ant shoots) recorded 0.0, 2.2 and 0.0 % incidence of *F. solani* respectively,8.9, 15.6 and 0.0% incidence of *M. phaseolina* respectively and 8.9, 15.6 and 0.0 incidence of *R. solani*.

It is clear from data that, in the field, *F. solani* was transmitted through roots, stems and shoots in watermelon and melon but through stems in squash, while *M. phaseolina* and *R. solani* were transmitted through roots, stems and shoots in watermelon, melon and squash. In this respect, Reuvani *et al.* (1983) detection *M. phaseolina* in seed coat and cotyledons of melon and found the fungus to penetrate the fruit *via* peduncle to infect the seed. *Macrophomina phaseolina* has been reported to be transmitted from seed to seedling of soybean (Anwar *et al.*, 1995) and sunflower (Dawar, 1994 and Bhutta *et al.*, 1996).

Table (1): Incidence of seed-borne fungi isolated from some cucurbit seeds by two health testing methods.

%Infection / Fungi	Watermelon (cv. Giza-1)		Melon (cv. Ismaillawy)		Squash (cv. Iskandrany)	
	Blotter	PDA	Blotter	PDA	Blotter	PDA
Alternaria alternate	4.6	0.0	10.0	0.6	5.2	0.0
Alternaria solani	26.0	6.6	26.6	5.2	10.0	4.6
Aspergillus flavus	5.4	3.2	5.3	1.2	20.6	0.6
Aspergillus niger	51.2	22.0	35.6	21.2	68.6	28.0
***Botrytis* sp.**	17.2	0.0	6.6	0.0	26.0	0.0
***Cladosporium* sp.**	6.0	0.0	2.0	0.0	5.2	0.0
Fusarium oxysporum	6.6	0.0	16.6	0.6	3.2	0.6
Fusarium solani	21.2	2.0	28.6	3.2	11.2	2.0
Macrophomina phaseolina	28.0	12.6	30.0	9.2	23.2	5.2
***Penicillium* sp.**	52.6	36.0	42.6	28.6	61.2	24.0
***Phoma* sp.**	0.6	0.0	2.6	0.0	0.0	0.0
Rhizoctonia solani	32.6	8.0	39.2	7.2	8.0	5.2
***Trichoderma* sp.**	0.0	1.2	2.0	2.6	4.0	3.2
***Verticillium* sp.**	0.6	0.0	5.2	0.0	0.0	0.0
L.S.D. at 5%	6.62	3.43	6.45	2.89	6.20	2.01

Table (2): Percentage of seed-borne fungi isolated from different seed parts of some cucurbit seeds using standard blotter method.

%Infection / Fungi	Watermelon (cv. Giza-1)		Melon (cv. Ismaillawy)		Squash (cv. Iskandrany)	
	Seed testa	Cotyledon.	Seed testa	Cotyledon.	Seed testa	Cotyledon.
Alternaria solani	3.3	0.0	0.0	0.0	0.0	0.0
Aspergillus flavus	0.0	0.0	0.0	0.0	6.6	0.0
Fusarium solani	20.0	3.3	16.6	6.6	3.3	3.3
Macrophomina phaseolina	16.6	3.3	10.0	3.3	3.3	0.0
***Phoma* sp.**	3.3	0.0	0.0	0.0	0.0	0.0
Rhizoctonia solani	10.0	0.0	20.0	0.0	0.0	0.0
***Verticillium* sp.**	3.3	0.0	3.3	0.0	0.0	0.0
L.S.D. at 5%	0.72	ns	0.58	0.32	0.44	ns

Table (3): Transmission of seed-borne fungi associated with some cucurbits seeds *in vitro*.

Fungi \ %Infection	Watermelon (cv.Giza-1)	Melon (cv. Ismaillawy)	Squash (cv. Iskandrany)
Fusarium solani	13.3	06.7	13.3
Macrophomina Phaseolina	13.3	13.3	13.3
Rhizoctoni solani	13.3	06.7	06.7
Control	0.0	0.0	0.0

L.S.D. at 1% for : Crops (C): 8.30, Fungi (F): ns, C x F: ns

Table (4): Transmission of seed-borne fungi associated with some cucurbits seeds *in vitro*.

Fungi \ % Infection	Watermelon (cv. Giza-1)			Melon (cv. Ismaillawy)			Squash (cv. Iskandrany)		
	Root	Stem	Shoot	Root	Stem	Shoot	Root	Stem	Shoot
Fusarium solani	13.3	20.0	6.7	8.9	24.4	4.4	0.0	2.2	0.0
Macrophomina Phaseolina	22.2	20.0	0.0	11.1	31.1	0.0	8.9	15.6	0.0
Rhizoctonia solani	2.2	24.4	0.0	8.9	26.7	0.0	8.9	15.6	0.0
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L.S.D. at 5%									
A (Fungi)		0.68			1.12			0.42	
B (Plant parts)		0.79			0.83			0.83	
A x B		2.40			2.86			ns	

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