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LOCATION OF FUNGI IN GROUNDNUT SEED

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Abstract

Using ISTA techniques, component plating of groundnut seed showed that seed coat (testa) was greatly infected by fungi viz., *Alternaria citri* Ellis & Pierce apud Pierce, *Aspergillus flavus* Link ex Gray, *A. niger* van Tieghem, *Fusarium oxysporum* Schlecht, *F. semitectum* Berk & Rav., *F. solani* (Mart) Sacc., *Macrophomina phaseolina* (Tassi) Goid., and *Rhizoctonia solani* Kuhn., followed by cotyledon and axis (radicle and plumule). Reduced number of fungal species in surface sterilized seed indicates that most of the fungi were located on seed coat (testa). Blotter method showed greater incidence of fungi on seed parts followed by agar plate and deep freezing methods. In seedling symptom test, *Macrophomina phaseolina*, *Fusarium* spp., *Rhizoctonia solani* *Aspergillus flavus* and *A. niger* showed pre- emergence and post emergence rot resulting in root rot and damping off of seedling caused by root infecting fungi.

Introduction

Groundnut (*Arachis hypogea* L.) is considered as a valuable legume crop cultivated over an area of 99.4 hectares in Pakistan with a production of about 1017 Kg/hectare or 101 tones during 2001-02 (Anon., 2002). Groundnut seed contain 50% edible oil. Seeds are rich in fats, protein, vitamin B_1, B_2, B_6, nicotinic acid and other vitamins. It is also a good source of lecithin present to the extent of 0.5-0.7% in decorticated nuts. Peanut butter has become a common edible diet. Groundnut cake has high nutritive value. Peanut flour is suitable for supplementing wheat flour (Sastri, 1948). Of the various disease producing organism, *Fusarium solani*, *F. oxysporum* cause damping off of groundnut seedlings (Reddy & Rao, 1980). Root infection in groundnut is caused by *F. solani* and *R. solani* with *Glomus mosseae* (Parvathi *et al*., 1985). Infection of *Macrophomina phaseolina* reduced germination and vigour of sunflower seeds besides producing pre- and post-emergence seedling blight and charcoal rot disease (Sadashivaiah *et al*., 1986). *Aspergillus flavus* attack germinating groundnut seed (Clinton, 1960). *A. niger* caused crown rot disease of peanut (Gibson, 1953). A knowledge of the exact location of the pathogen in seed or the depth of seed infection by particular pathogen can be helpful in the control of seed borne infection. Studies were, therefore, carried out to isolate fungi from different component of groundnut seed which is presented herein.

Materials and Methods

Seven seed samples were collected from different localities of Pakistan viz., Karachi (3), Lahore (3) and Chakwal (1). The method used to detect the location of seed-borne fungi was as given by Mathur *et al.*, (1975) with slight modification. Seed soaked for 4 hrs in sterilized distilled water in test tubes were dissected aseptically into testa (Brown covering), and embryo. Embryo was further dissected into cotyledons and axis (redical and plumule). ISTA techniques (Anon., 1976) were used to detect fungal infection of different parts of seeds where 20 untreated and 20 seeds treated with 1% Ca $(OCl)_2$ were

used in Blotter, Agar plate and Deep freezing methods. Using blotter method, the treated and untreated seed components were plated in a Petri dish on three layers of sterilized moistened blotters. For Agar plate method, the treated and untreated seed components were plated on PDA, pH 5.5. The dishes were incubated at 24°C for 7 days. For deep freezing method, the treated and untreated seed parts were placed on blotters and incubated for 1 day each at 20°C and -2°C in deep freezer followed by 5 days incubation at 24±1°C under 12 h alternating cycle of ADL and darkness. Fungi growing on different parts of seeds were identified after reference to Barnett (1960), Booth (1971) Domsch *et al*., (1980), Ellis (1971), Nelson *et al*., (1983) and Raper *et al*., (1965). For seedling symptom test, one seed was placed in a test tube containing 10ml of 1% plain water agar. The tubes were closed by loose cotton plug and incubated for 14 days at 20°C under 12h alternating cycle of ADL and darkness. The cotton plug was removed when seedling reached the mouth of test tube. After 12 to 14 days the seedling showing fungal infection were counted (Khare *et al*., 1977). Data were subjected to Analysis of Variance (ANOVA) or Factorial Analysis of Variance (FANOVA) depending upon the experimental design following the procedure as given by Gomez & Gomez (1984).

Table 1. Location of fungi in groundnut seed.

Name of fungi	Agar plate		Blotter method		Deep freezing	
	A	B	A	B	A	B
	1%±SD	1%±SD	1%±SD	1%±SD	1%±SD	1%±SD
	Seed coat (Testa)					
Alternaria citri	2±5.6	2 ± 56	2±5.6	2±5.6	1±3.7	2±5.6
Aspergillus flavus	20±6.5	30±10.6	25±11.8	37±7.9	10 ±7.6	17±6.4
A. niger	22±9.2	26±11.3	21±4.8	29±10	10±4.4	16±7
Fusarium oxysporum	3±11.3	4±13.2	3±12.2	5±17	1±5.6	3±11.3
F. semitectum	3±10.3	5±18.8	2±7.5	4±15.1	1±3.7	3±11.3
F. solani	9±3.4	9±12.1	10±13.8	12±15.6	5±10.2	6±8.5
Macrophomia phaseolina	11±16.3	10±14.3	13±18.8	18±17.6	9±12.9	9±14.3
Rhizoctonia solani	2±7.5	5±17.0	3±11.3	5±17	2±9.4	4±13.2
	Cotyledon					
Alternaria citri	3±9.6	2±5.6	2 ±6.6	2+5.6	2±7.5	2±7.5
Aspergillus flavus	16±6.5	28+10.8	19±7.4	25±12.6	13±8.3	20±9.2
A. niger	17±4.4	20±6.4	19±7.8	25±12.6	9±7.5	11±8.3
Fusarium oxysporum	3±11.3	4±15.1	4±13.2	4±15.1	1±5.6	2±7.5
F. semitectum	2±6.6	4±13.2	2±8.5	4±16	1±5.6	2±7.5
F. solani	9±12.7	11±14.4	10±16.3	12±16.6	9±13.1	8±11.3
Macrophomia phaseolina	12±17	13±17.3	13±18.8	15±21	10±15.1	9±13.9
Rhizoctonia solani	4±13.2	4±13.2	3±11.3	5±17	2±9.4	4±15.1
	Axis					
Alternaria citri	2+5.6	2±7.5	2±5.6	3±9.4	2±7.5	3±11.3
Aspergillus flavus	13±11.8	19±13.7	10±8.4	18±10.9	7±2.8	15±11.4
A. niger	8±5.5	12±10.3	12±9	16±6.3	5+6.9	7±6.7
Fusarium oxysporum	1±7.5	3±11.3	2±9.4	4±15.1	1±5.6	2±9.4
F. semitectum	1±3.7	1±3.7	0±0	1±5.6	0±0	0±0
F. solani	10±16.9	11±15.3	10±13.7	13±18.4	10±15.9	11±15.9
Macrophomia phaseolina	12±19.5	12±16.7	13±17.7	12±17.7	10±16.1	13±18.4
Rhizoctonia solani	1±2.8	5±17	2±9.4	4±15.1	2±7.5	4±15.1

Results

Component plating of groundnut seed showed that most of the fungi were located on seed coat (testa) followed by cotyledons and axis of groundnut seed (Table 1). Of the 7 samples tested, Chakwal sample showed highest colonization of pathogenic fungi like *R. solani, M. phaseolina* on all parts of seed whereas other samples collected from Karachi and Lahore showed higher incidence of storage fungi like *A. flavus* and *A. niger* on all parts of seed. Infection of *A. flavus* significantly reduced due to surface sterilization with 1% Ca $(OCl)_2$ ($p<0.001$). Of the three methods used, blotter method showed significant infection of *A. flavus* and *A. niger* ($p<0.001$) as compared to agar plate and deep freezing method. Seed coat (testa) was significantly infected by *A. flavus* as compared to other parts ($p<0.001$). Limonard (1968) also reported that microbial contamination was eliminated by chlorine disinfection. Infection of *M. phaseolina* was recorded in all parts of surface sterilized and non sterilized seeds of seed coat (testa), cotyledons and axis. Infection of *R. solani* and *Fusarium* spp., were observed in all parts viz, seed coat (testa), cotyledon and axis. Seedling symptom test of groundnut seed on 1% plain water agar showed 7% pre-emergence death by infection of *M. phaseolina* which caused root discoloration and death of emerging radicle where as 3% seedling died and showed charcoal rot symptoms on root (Table 2). *R. solani* caused 3% pre-emergence death in sterilized seeds and 5% pre-emergence death in non-sterilized seeds (Table 2) and seedling showed root rot symptoms. *F. solani* showed 6% pre-emergence death of seedlings in surface sterilized seeds and 3% in non sterilized seeds. Infection by *A. flavus* showed 10% pre-emergence death of groundnut seed in surface sterilized seeds. *Alternaria citri* also caused pre and post-emergence death groundnut seedling (Table 2).

Discussion

The component plating of groundnut seed, showed *M. phaseolina* infection in all parts of seed viz., seed coat (testa), cotyledon and axis (radicle and plumule). Such similar observation has been made on sunflower seed (Dawar & Ghaffar, 1990) and pumpkin seed (Sultana *et al*., 1994). Sadashivaiah *et al*., (1986) reported *M. phaseolina* infection only in pericarp and seed coat. In the present study, *R. solani* was found in all parts viz., seed coat (testa), cotyledon and axis. Dawar & Ghaffar (1990) also found *R. solani* infection in all parts of sunflower seed viz., pericarp, seed coat, cotyledons and axis. Vasiljevic (1951) also found that *R. solani* produces intracellular mycelium in endosperm, embryo and seed coat of *Capsicum*. In the present study, seedling symptoms test of naturally infected seeds on water agar slant showed 7% pre emergence death of seeds due to *M. phaseolina* infection where seed germinated but the pathogen caused root discoloration and showed charcoal rot symptoms. Such similar observation have been made by Sadashivaiah *et al*., (1986) who found that *M. phaseolina* caused pre emergence and post emergence seedling rot where it penetrated in the root and stem tissues including the wood vessel and thus interrupting the water supply. Dawar (1994) also reported infection of *M. phaseolina* during pre and post emergence of sunflower seedling. Present observation showed that *R. solani* caused 4% pre emergence and 3% post emergence infection on groundnut. Deighton (1931) reported that *R. solani* affected plant lose turgidity, fall over and finally decay. *R. solani* has also been reported to cause dieback of leaves, stem and root rot disease in *Capsicum* (Szimai 1941). In the present study

Table 2. Seedling symptom test of groundnut seed.

Name of fungi	Pre-emergence seed rot %		Post-emergence rot %			
			Dead seedling		Healthy looking seedling	
	A	B	A	B	A	B
Alternaria citri	0	4	2	3	1	4
Aspergillus flavus	4	16	0	2	1	3
A. niger	3	28	0	2	0	0
Fusarium oxysporum	3	8	0	3	2	4
F. semitectum	2	3	2	3	1	3
F. solani	6	3	1	3	0	2
Macrophomina phaseolina	5	9	3	2	0	0
Rhizoctonia solani	3	5	0	3	0	3

A = Sterilized

B = Non-Sterilized

Fusarium solani, F. semitectum and *F. oxysporum* showed 13% pre emergence and 12% post emergence infection. Dawar (1994) reported that *F. solani* caused pre and post emergence rotting of sunflower seed. The present observation showed that *A. niger* caused 15% pre emergence and 2% post emergence rot of groundnut. Gibson (1953), found. *A. niger* causing most serious disease of crown rot of peanut. Similarly Jain & Neema (1952) reported that *A. niger* produced circular brownish spot on the cotyledon and this discoloured area rapidly rotted and spread to the stem and hypocotyls. The fungus attacks the plant soon after germination and apparently exerts its effect in substantial part by production of oxalic acid. *A. flavus* showed 10% pre emergence and 3% post emergence infection. Clinton (1960) has also reported *A. flavus* as one of the fungus attacking germinating groundnut seed. As the groundnut seeds is an important source of oil and its seeds are also used as roasted and salted, there is therefore need to control the seed borne fungi for obtaining good quality of seeds.

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