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Seed-borne Fungi of Some Vegetables in Saudi Arabia and their Chemical Control

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ABSTRACT. The present studies were undertake to find out the fungi associated with the seeds of locally cultivated okra, pepper, radish and soybean and their control by fungicidal seed treatment. Seeds were obtained from the local market and farmers in September, 1991. Using the methods suggested by the ISTA, seed-borne fungi were isolated by moist blotter and agar plate methods. A total of 15 species of fungi belonging to the genera, *Alternaria, Aspergillus, Botrytis, Cladosporium, Colletotrichum, Curvularia, Epicoccum, Fusarium, Penicillium,* and *Stemphylium* were isolated from the tested seeds. These fungi are reported for the first time from the tested seeds in Saudi Arabia. Five fungicides viz., Bavistin, Benlate, Cozib, Dithane M-45 and Ridomil MZ-58 were used as seed-treatment. The number of fungal species were greatly reduced when the seeds were treated with 0.2 percent of the tested fungicides before placing them on agar plates. Benlate was found to be very efficient as seed fungicide followed by Cozib and Dithane M-45.

Okra (*Hibiscus esculentus* L.), Pepper (*Capsicum annum* L.), Radish (*Raphanus sativus* L.) and soybean (*Glycine max* L.) are some of the important vegetables grown in Saudi Arabia. Seeds play a vital role for the healthy production of the crop but can be carriers of important diseases which cause considerable reduction in the yield of plants (Noble and Richardson 1968, Neergaard 1977, Nene and Agarwal 1978, Suryanarayana 1978, Siddiqui *et al.* 1979, Elarsoi 1993, Michail 1993).

Schneider *et al.* (1971), Singh *et al.* (1973) and Mathur (1983) have reported a number of fungi from soybean seeds which could cause seed rot and other diseases. *Colletotrichum capscisi, C. piperatum* (causing die-back, anthracnose and fruit rot) and other fungal pathogens of chillies are known to be seed borne (Grover and Bansal 1970, Narain and Pangrahi 1971, Rout and Rath 1973). Nobel and Richardson (1968) have reported that *Fusarium solani* transmitted by okra seeds can cause rot diseases while *Macrophomina phaseoli* was also found on okra fruit and proved to be transported by seeds (Childambaram and Mathur 1975). Mathur (1983) has listed *Alternaria brassicae, A. brassicola, Sclerotinia sclerotiorum* and *Phoma lingam* as seed pathogens of *Brassica* spp causing different symptoms. Many workers have also reported various fungi from vegetable seeds (Grovers and Bensal, 1970, Khare *et al.* 1972, Esuruoso *et al.* 1975, Karwasra and Singh 1982). By reviewing the literature, it is revealed that seed fungi are generally very destructive and cause seed rot, reduction in germination, pre and post emergence death and many other diseases in the field (Ellis *et al.* 1975, Bolkan *et al.* 1976, Ashokan *et al.* 1979, Subeiah *et al.* 1982).

Many workers have successfully controlled the seed borne fungi by using various fungicides both on vegetables and other crops (Narain and Paningrahi 1971, Singh *et al.* 1973, Ellis and Paschal 1979, Agarwal and Singh 1974, Sharma and Bosnchaudhry 1975, Karwasra and Singh 1982, Abou-Heilah 1984, Kassim 1985, 1987, 1989).

The control of seed borne fungi is not possible unless we know the type of fungus associated with a particular type of seed and their potential to cause disease. In the present study an attempt has been made to investigate the fungi associated with seeds of some of the important vegetable crops (Okra, pepper, radish and soybean) grown in Saudi Arabia. Some fungicides were also used, as an effort, to protect the seeds from the seed borne fungi and to minimize the chances of losses after they are grown.

Materials and Methods

Seeds of okra, pepper, radish and soybean, obtained from the local market and farmers in September , 1991, were used in these experiments.

The method suggested by the International Seed Testing Association (Anonymous 1973) for the isolation of seed-borne fungi was used. Isolation of fungi was made by agar plate method (Muskett and Malone 1941) and blotter technique (de Tempe 1951).

Seeds were surface sterilized conventionally, with 0.1% $HgCl_2$ for 2 min; washed three times in sterile distilled water and then air dried. This may have eradicated the fungi carried on the surface of the seeds. Seeds were placed on moist papers in petri dishes or on PDA plates for the growth of seed-borne fungi. A total of 400 seeds of each vegetable were used. The plates were incubated under alterate cycles of 12 h light and 12 h darkness at 20°C for one week. The isolated fungi were identified after reference to Barnett and Hunter 1972, Booth 1971, Ellis 1971, and Raper and Fennell 1965.

In another experiment, seeds of the tested vegetables were separately treated by dipping the seeds into 2% suspension of Bavistin [(carbendazim 50% a.i) (2-methoxyl-carbamoyl) benzimidazol]. Benlate [(benomyl 80% a.i) methyl-1-(butyl-carbamoyl)-2 benzimidazolcarbamate]; Cozib 62 [(copperoxychloride 60% a.i) miconized copperoxychloride and (Zineb 20% a.i.) Zinc ethylen-bis-dithiocarbamate]; Dithane M45 [(mancozeb 80% a.i.) manganes-ethylene bis dithiocarbamate complex with zinc salt]; Ridomil M2-58 [(metalaxyl 10% and mancozeb 48% a.i) 2-(methoxyl-carbamoyl)-benzimidazole], as recommended by the manufacturer of these fungicides. A total of 100 gm of each vegetable seed were used in each treatment. This experiment was carried out on agar plates.

Results and Discussion

Fungi isolated by the blotter technique are shown in Table 1. Eight fungal species *viz; Alternaria alternata, Aspergillus niger, Botrytis cinerea, Cladosporium cladosporioides, Colletotrichum capsici, Curvularia lunata, Fusarium solani* and *Penicillium* sp. were isolated from the four kinds of tested seeds.

Table 1. Percentage incidence of seed-borne fungi based on the number of fungal colonies arising out of the vegetable seeds by blotter technique

Fungi	Okra	Pepper	Radish	Soybean
Alternaria alternata	5	0	10	5
Aspergillus fumigatus	0	0	0	0
A. niger	20	20	30	10
Botrytis cinerea	0	0	0	5
Cladosporium cladosporioides	0	5	0	0
C. herbarum	0	0	0	0
Colletotrichum capsici	0	15	0	0
C. piperatum	0	0	0	0
Curvularia intermedia	0	0	0	0
C. lunata	10	0	5	0
Epicoccum nigrum	0	0	0	0
Fusarium moniliforme	0	0	0	0
F. solani	5	0	0	0
Penicillium sp.	15	10	15	10
Stemphylium botryosum	0	0	0	0

Fungi isolated by the agar method are shown in Table 2. A total of 15 species *viz., Alternaria alternata, Aspergillus fumigatus, A. niger, Botrytis cinerea, Cladosporium cladosporioides, C. herbarum, Colletotrichum capsici, C. piperatum, Curvularia intermedia, C. lunata, Epicoccum nigrum, Fusarium moniliforme, F. solani, Penicillium* sp. and *Stemphylium botryosum* were isolated from the four tested seeds.

Table 2. Percentage incidence of seed-borne fungi based on the number of fungal colonies arising out of the vegetable seeds by PDA plates

Fungi	Okra	Pepper	Radish	Soybean
Alternaria alternata	5	0	15	10
Aspergillus fumigatus	0	10	0	0
A. niger	25	15	20	15
Botrytis cinerea	0	0	10	15
Cladosporium cladosporioides	0	10	0	10
C. herbarum	10	15	0	0
Colletotrichum capsici	0	20	0	0
C. piperatum	0	15	0	0
Curvularia intermedia	15	0	0	5
C. lunata	20	0	10	0
Epicoccum nigrum	5	0	0	10
Fusarium moniliforme	10	0	0	0
F. solani	15	0	10	0
Penicillium sp.	15	5	15	10
Stemphylium botryosum	5	10	0	0

Table 3. Effect of various fungicides on the seed-borne fungi of the vegetable seeds (+ – indicate the presence of fungi after treatment)

Fungi	Okra						Pepper						Radish						Soybean					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Alternaria alternata	+	+	+	+	+	+	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus fumigatus	–	–	–	–	–	–	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
A. niger	+	–	–	+	+	+	+	–	–	–	+	+	+	–	–	+	+	+	+	–	–	–	–	+
Botrytis cinerea	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	+	–	–	–	+	+
Cladosporium cladosporioides	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	+
C. herbarum	+	–	–	–	–	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Colletotrichum capsici	–	–	–	–	–	–	+	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
C. piperatum	–	–	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Curvularia intermedia	+	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
C. lunata	+	–	–	+	+	+	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–
Epicoccum nigrum	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+
Fusarium moniliforme	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
F. solani	+	–	–	+	+	+	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–
Penicillium sp.	+	–	–	–	+	+	+	–	–	–	–	+	+	–	–	+	+	+	+	–	–	–	+	–
Stemphylium botryosum	+	–	–	–	+	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–

1 = Untreated 3 = Cozib 5 = Bavistin

2 = Benlate 4 = Dithane M-45 6 = Ridomil MZ-48

Many of these isolated fungi are reported to be pathogenic to the seeds of the tested vegetables. *Colletotrichum capsici* and *C. piperatum* cause fruit rot of chillies (Narain and Pangrahi 1971, Rout and Rath 1972, Rajendra *et al.* 1970). Beside *Colletotrichum* spp. Rout and Rath (1972) have reported the presence of

Cladosporium cladosporioides, C. herbarum, Stemphylium botryosum, Aspergillus spp, *Penicillium* spp, *Alternaria* spp and *Pythium* spp. These fungi are reported to cause fruit rot, damping off, wilt and foliar diseases. Most of these fungi have also been isolated from the pepper seeds used in the present study which could be potentially pathogenic to the tested seeds. Similarly, *Aspergillus* spp. *C. cladosporioides, Curvularia intermedia, Botrytis cinerea* and *Penicillium* spp. were frequently isolated from soybean seeds (Singh *et al.* 1973 and Scheinder *et al.* 1971), which can be pathogenic to soybean seeds causing seed rot and poor emergence. In the present study, many fungi from the list above have been isolated which could prove pathogenic and cause losses. *Macrophomina phaseolina* was found on okra fruit (Chidambaram and Mathur 1975) while *Fusarium solani* caused a rot disease of okra (Nobel and Richardson 1968). Beside *F. solani* many other fungi were isolated from the okra seeds tested in the present study. *Aspergillus niger, A. flavus, Cladosporium* spp may cause seed rot during germination while others may invade the seedling (Gupta and Chohan 1970). Mathur (1983) has reported *Alternaria brassicae, A. brassicola, Sclerotinia sclerotiorum* and other fungal spp. from *Brassica.* In the present study, beside *F. solani* which is root rot pathogen, *A. alternata, Curvularia lunata* have been isolated. They are reported to cause leaf spots of many crop plant (Elarosi 1993). Most of the fungi isolated from the tsted seeds are reported for the first time in Saudi Arabia.

The number of the isolated fungi was considerably reduced when the seeds were treated with 0.2% fungicides before placing them on the agar plates. Growth of most isolated fungi were completely inhibited by the tested fungicides. Benlate was found to be the most effective fungicide with the rate of 90% followed by Cozib with 87%, Dithane M-45 with 61% and Bavistin with 42%. Ridomil MZ-48 was found to be least effective against the isolated fungi with the rate of 10%. Benlate, bavistin and dithane M-45 are also reported to be effective in the control of seed-borne fungi by other workers (Gupta and Chohan 1970, Sharma and Basuchaudhry 1975, Ellis and Paschal 1979, Abou-Heilah 1984, Kassim 1985, 1989). Grover and Bansal (1970) and Narain and Panigrahi (1971) have reported that *Colletotrichum capsici* was controlled both *in vitro* and *in vivo* when the infected seeds of pepper were treated with different fungicides. Fungi occurring on soybean seeds were successfully reduced and seed emergence was increased through use of captan and thiram (Singh *et al.* 1973). However, none of the tested fungicides were effective against *Alternaria alternata,* thus confirming the results of Abuo-Heilah (1984) and Kassim (1985, 1987, 1989).

As it is evident from the results of the present study most of the fungi isolated from the seeds of the vegetables are recorded for the first time in Saudi Arabia. Little is known in Saudi Arabia about the role of seed borne fungi of vegetable seeds

which may cause seed rot alone or in association of other organism. They could cause seedling mortality and post emergence diseases of the plant. So their control, before going to the field is necessary. The control of the seed borne disease (pathogen) is not possible unless we know the type of the fungi associated with the seeds. So further research is required to investigate thoroughly, the ecology of the fungi on seeds, germination, pre and post emergence effects and effects of the seed fungicides. In future, further studies may be taken up to study all the above mentioned factors and to see the efficiency of the different fungicides on seed borne fungi.

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الفطريات المنقولة ببذور بعض أنواع الخضار في المملكة العربية السعودية ومقاومتها الكيميائية

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تمت هذه الدراسة لمعرفة الفطريات المرافقة لبذور البامية ، الفلفل ، الفجل وفولَ الصويا المزروعة محلياً ومقاومتها الكيميائية وقد تم الحصول على البذور من المزارعين والأسواق المحلية وقد استعملت طريقة ورق الترشيح المبلل وأطباق الأجار وتم عزل ١٥ نوعاً من الفطريات تتبع للأجناس التالية : *Alternaria, Aspergillus, Botrytis, Cladosporium, Colletotrichum, Curvurlaria, Epicoccum, Fusarium, Penicillium,* and *Stemphylium* وهي تسجل لأول مرة في المملكة العربية السعودية على هذه البذور وقد اختبر فعالية كل من المبيدات التالية : الفيز ، البافستين ، البنليت ، الكوزيت ، الدياثين م٤٥ والريدوميل م ز ٥٨ وقد اختزلت هذه المبيدات نمو جميع الأنواع الفطرية المعزولة ولكن وجد أن مبيد البنليت كان أكثر تلك المبيدات فعالية ثم يتبعه مبيد الكوزيت ومبيد الدياثين م٤٥ الذي جاء في المرتبة الثالثة .