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# STUDIES ON SEED-BORNE MYCOFLORA OF CHILLIES AND CONTROL OF FRUIT ROT DISEASE

Nasreen Sultana, S.A. Jamil Khan and A.K. Khanzada

*Crop Diseases Research Institute, Pakistan Agricultural Research Council, Karachi University Campus*

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Fifteen chillies seed samples from different places in Sind, were analysed by ISTA techniques. 24 fungi were isolated, including 12 species in Pakistan. The seedling symptoms test showed that species *Fusarium moniliforme* and *Alternaria alternata* are seed transmitted. Both methods of infection (wounded and unwounded) differed significantly, enhancing fruit rot by different fungi. Fungicides viz., Benomyl and Captan were found effective in controlling fruit rot of chillies.

*Key words:* Chillies, Seed-borne mycoflora, Incubation, Techniques, Transmission, Fruit rot, Fungicides, Pakistan.

## INTRODUCTION

Chillies or red peppers (*Capsicum annum* L.) is one of the important vegetable crops, grown throughout Pakistan. It is attacked by diseases of fruit rot and die back (*Colletotrichum capsici* (Syd.) Butl. & Bisby. Wilts (*Fusarium* and *Verticillium*), bacterial blight, bacterial leaf spot and virus diseases [6]. The crops in Pakistan suffers from two serious diseases, the fruit rot (*Alternaria tenuissima*) (Nees ex Fr) Wilt, and die back caused by anthracnose fungus *Colletotrichum capsici* [7,9]. Despite the widespread use as green and ripe pods as spice, no systemic work on seed mycoflora of chillies seed has been done in Pakistan. The present studies were initiated, to know the occurrence of different fungi of chillies seed, to select procedures for reliable application for the isolation of potentially pathogenic fungi from seed samples, and to study the efficacy of fungicides against fruit rot of chillies.

## MATERIALS AND METHODS

Fifteen seed samples collected from different places in Sind during 1985-86 season. The samples were analysed in the same year for seed mycoflora. Different seed-borne fungi were detected by the method suggested by International Seed Testing Association [1] for isolation of seed-borne fungi.

*Standards blotter method.* The seeds were sown in three layers of moistened blotters placed in petridishes at the rate of 25 seeds per dish. 400 seeds of each samples were used. The dishes were incubated at $25^{o}$ ($\pm$ $1^{o}$) under 12 hours alternating cycle of A.D.L. (Artificial Day Light supplied by cool white fluorescent tube) and darkness. After seven days seeds were examined for the fungal infection under stereoscopic binocular microscope. The fungi appeared were transferred to potato dextrose agar and grown for identification and study.

*Deep freezing blotter method.* Two hundred seeds of each samples were soaked for 5 minutes in 1% sodium hypochlorite and plated as described in the blotter method. The petridishes were incubated for one day each at $20^{o}$ and $-20^{o}$ respectively followed by five day incubation at $24^{o}$ ($\pm$ $1^{o}$) under 12 hours alternating cycles of ADL and darkness.

*Agar plate method.* Ten seeds per plates were sown on potato dextrose agar (PDA) after treatment with 1% sodium hypochlorite solution for 10 minutes. 200 seeds of each samples were used. Incubated at $24^{o}$ under 12 hours of alternating cycle of ADL and darkness. After seven days colonies were examined for mycelial growth, colour, pigmentation and sporulation. The fungi growing on seeds were identified after reference to Barnett & Hunter [2] Barron [3], Booth [4] and Ellis [5].

*Seedling symptoms test.* Seed transmission studies were performed using water agar seedling symptoms test recently described by Khare, Mathur and Neergard [10] for the detection of *Septoria nodorum* berk, in wheat grains. The test was conducted in test tubes, one seed per tube on 2% water agar, (20 ml per tube.), 50 seeds for each of the two samples were used. Symptoms were recorded after 12-15 days. To confirm transmission of pathogens, isolations were made from roots, attached seeds, stem and leaves of the diseased seedling on PDA.

*Field studies.* The pathogenicity test of *Alternaria alternata* and *Fusarium moniliforme* was carried out in vivo 360 healthy seedlings were sown in six plots which were randomized and replicated three times and the plot size was 6 x 3 mm. Out of 6 rows of plants per plot, 300

fruits were selected from 3 row for inoculation of wounded pods, An equal number of unwounded pods were inoculated in the remaining three rows. Two wounds of 5 mm length were made on pericarp of the fruit.

Pure culture of each fungus was obtained by the single spore culture technique. Conidial suspension of seven days' old culture of test fungi were prepared in sterilized distilled water by flooding these culture with 1:2000 Tween-20 solution and dislodging conidia. The spore concentration of *Alternaria alternata* ($6 \times 10^5$ spores/ml) and *Fusarium moniliforme* ($8 \times 10^8$ spores/ml) were determined with haemocytometer.

After the pod formation, the spore suspensions of aforesaid fungi were sprayed separately over the pericarp of wounded and unwounded pods by an ordinary syringe. The inoculated fruits were enclosed in internally moistened polyethylene bags for 36 hours to maintain high humidity. 100 inoculated wounded fruits were selected from one row were sprayed with Benomyl at the rate of 0.1 percent. Same number of fruits from another row were sprayed with Captan at the rate of 0.2 percent. Similar fungicidal application was followed for unwounded fruits. Untreated but inoculated fruits were used as check. The second application of fungicides was made after 15 days of the first application. The percent incidence of fruit rot on sprayed and non-sprayed in wounded and unwounded fruits were calculated (Table 3).

## RESULTS AND DISCUSSION

*Testing of 10 samples by blotter method.* The fungi associated with seeds, their average percent incidence and the range of occurrence is given in (Table 1). All of the fungi were isolated in range of 0.5-50%. *Alternaria alternata* was the most predominent pathogen and isolated from all the seed samples with an infection range of 2-44%. *Alternaria alternata* and *Fusarium moniliforme* are reported to be the main cause of fruit rot in wounded red pepper (Uma, N.U. [11]. *Alternaria tenuissima* and *Fusarium moniliforme* were encountered in 70% and 80% of seed samples respectively. The maximum infection due to *A. tenuissima* was 18.2 % whereas in *F. moniliforme* was 20.5 %. *A. tenuissima* has been reported causing chilli fruit rot in Pakistan [Hafiz, [7] and Kamal and Moghal [9]. Among other fungi *Curvularia* spp. and *Drechslera* spp., were isolated from chilli seeds from 1-18 %. *Aspergillus* spp. and *Chaetomium globosum* were also isolated from the seed samples.

*New records.* Twenty-four seed-borne fungi were isolated from ten seed samples tested by standard blotter method. Out of 24 fungi, 12 of them are new records of *Capsicum annum* L. in Pakistan, and have been indicated by asterisks in (Table 1).

*Evaluation of methods.* Data presented in (Table 2) indicates that infection counts of *Alternaria alternata* and *Fusarium moniliforme* were generally higher in the deep freezing method. The blotter method gave comparable results while the infection counts were substantially lower on the agar plate method.

*Transmission studies.* Samples number 3 and 9 with high infection of *Fusarium moniliforme* and *Alternaria alternata* respectively (Table 2) were used for transmission study. Observations were primarily recorded on symptoms produced by the two pathogens separately. In severe infection of *Fusarium* sp. either seed did not germinate or the seedling was killed soon after emergence. In both cases the fungus sporulated profusely on non-germinated seed and dead seedlings. In less severe infection brown rot developed at the base of the coleoptile and main root. Most of the seedling died within three week. No seed rot or death of seedlings was caused by *Alternaria alternata.* Seedling emergence from an infected seed should mild symptoms of brown discoloration in the coleoptile and *Fusarium moniliforme* and Alternaria alternata were isolated from diseased parts of the infected seedlings. *Fusarium moniliforme* frequently isolated from diseased root, coleoptile and leaves.

The water agar seedling symptoms test showed that *Fusarium moniliforme* and *Alternaria alternata* are seed transmitted. Almost all of the seedlings attacked by the *Fusarium moniliforme* died during experimental period of five weeks.

*Field studies.* The pathogenicity test revealed that species of *Fusarium moniliforme* and *Alternaria alternata* produced 100 and 80 percent fruit rot on wounded pods while 70 and 15 percent on unwounded pods respectively (Table 3). Unwounded fruit suffered less than the injured ones. These observation closely conform with the findings of Uma, N.U. [11], and Illyas *et al.* [8]. *Fusarium moniliforme* was found pathogenic as it was more or less equally effective in rotting chillies (wounded and unwounded) pods, whereas *Alternaria alternata* proved a week parasite that attacked wounded or weakened plant tissues.

Field spray with fungicides. Benomyl and Captan after inoculation of test fungi almost completely controlled the fruit rot in both wounded and unwounded chilli pods. The serious nature of the disease has been mentioned by Illyas *et al.* (1985). According to them damage due to fruit rot under favourable condition may be as high as 10-45%.

Table 1. Percentage incidence fungi associated with chillies seeds, according to relative abundance of each fungal species and proportion of host seed samples infected.

| Fungi | Samples | | Incidence (%) | |
|---|---|---|---|---|
| | Number infected | Percentage infected | Range | Average |
| *Alternaria alternata* | 10 | 100 | 2.0-44 | 28.0 |
| **A. tenuissima* | 7 | 70 | 1.0-22 | 18.2 |
| **Aspergillus candidus* | 6 | 60 | 0.5-2 | 1.5 |
| *A. flavus* | 7 | 70 | 1.0-21 | 9.5 |
| *A. niger* | 7 | 70 | 2.0-12 | 6.5 |
| *A. sulphureus* | 2 | 20 | 0.5-4 | 2.2 |
| **A. terreus* | 3 | 30 | 1.0-5 | 3.0 |
| *Chaetomium globosum* | 8 | 80 | 1.0-13 | 7.5 |
| **Cladosporium cladosporoides* | 2 | 20 | 1.0-5 | 3.0 |
| **Chalariopsis thielavioides* | 4 | 40 | 0.5-3 | 1.5 |
| **Curvularia clavata* | 7 | 70 | 1.0-18 | 10.5 |
| *C. lunata* | 5 | 50 | 1.0-10 | 6.0 |
| *C. pallescens* | 3 | 30 | 1.0-4 | 2.5 |
| **C. robusta* | 2 | 20 | 0.5-3 | 2.2 |
| *D. hawaiiensis* | 4 | 40 | 1.0-5 | 2.0 |
| **D. spicifera* | 5 | 50 | 0.5-6 | 3.5 |
| **Epicocum purpurascens* | 3 | 30 | 1.0-3 | 2.0 |
| **Fusarium moniliforme* | 8 | 80 | 1.0-32 | 20.5 |
| *F. semitectum* | 1 | 10 | 0.0-0.5 | 1.5 |
| **Memnoniella echinata* | 5 | 42 | 1.0-4 | 2.5 |
| **Myrothecium roridum* | 3 | 22 | 1.0-2 | 1.2 |
| **Penicillium purpurogenum* | 2 | 16 | 0.5-4 | 2.2 |
| *Rhizopus nigricans* | 4 | 33 | 1.0-50 | 25.5 |
| *Stachybotrys atra* | 3 | 22 | 0.5-3 | 2.0 |

* New records of the fungi associated with chilli seeds.

Table 2. Percentage infection by *Alternaria alternata* and *Fusarium moniliforme* in six samples of *Capsicum annum* L. tested by different methods observation based on 200 seeds per method.

| Sample No. | Blotter method | | Deep freezing method | | Agar plate method | |
|---|---|---|---|---|---|---|
| | *Alternaria alternata* | *Fusarium moniliforme* | *Alternaria alternata* | *Fusarium moniliforme* | *Alternaria alternata* | *Fusarium moniliforme* |
| 2 | 25.5 | 25.5 | 32.0 | 27.5 | 21.5 | 18.0 |
| 3 | 31.0 | 32.5 | 38.5 | 38.0 | 26.0 | 29.5 |
| 4 | 26.5 | 29.0 | 28.0 | 32.5 | 20.5 | 20.5 |
| 6 | 38.5 | 6.5 | 40.5 | 10.0 | 31.5 | 8.0 |
| 8 | 28.0 | 16.0 | 30.5 | 18.0 | 24.5 | 12.5 |
| 9 | 44.0 | 19.5 | 42.5 | 21.0 | 34.0 | 17.5 |
| Mean | 32.6 | 21.5 | 35.3 | 24.5 | 26.3 | 17.6 |

Table 3. Effect of fungicides on the incidence of pod rot of chillies in field

| Treatments | Percentage of infection by | | | |
|---|---|---|---|---|
| | *Fusarium moniliforme* | | *Alternaria alternata* | |
| | Wounded pods | Unwounded pods | Wounded pods | Unwounded pods |
| Benlate | 1 | 0 | 2 | 0 |
| Captan | 4 | 2 | 1 | 0 |
| Non-treated | 100 | 70 | 80 | 15 |

Therefore the application of effective fungicides should be carried out in order to minimize loss of chillies due to fruit rot disease.

## REFERENCES


1. Anonymous, Proc. Int. Seed Test. Assoc., **4**, 3 ((1976).
2. H. Barnett, B.B. Land Hunter, *Illustrated Genera of Imperfecti Fungi* (Bugess Publishing Co., Minneapolis M.N., 1972), pp. 241.
3. G.L. Barron, *The General of Hyphomycetes from Soil* (Robert and Krieger Publishing Co., New York 1968), pp. 364.
4. C. Booth, *The Genus Fusarium* (Commonwealth Mycological Instt., Kew, Surrey, England, 1971), p. 1-237.
5. M.B. Ellis, *Dematicious Hyphomyces* (C.M.I., Kew, Surrey, England, 1971), p. 608.
6. G. Frohlich and W. Rodewald, *Pest and Diseases of Tropical Crops and their Control* (Pergamen Press Oxford, 1970).
7. A. Hafiz, *Plant Diseases* (Pub. P.A.R.C., 1986), pp. 552
8. M. Ilyas, M. Sultan Khan and Inamullah Khan, Pakistan Agri. Sci., 188-180 (1985).
9. M. Kamal and S.M. Moghal, *Studies on Plant Diseases of South West Pakistan* (Agri. College Tandojam, Pakistan, 1968), pp. 207.
10. M.N. Khare, S.B. Mathur, P. Neergaard, Seed Sci. Technol., **5**, 613 (1977).
11. N.U. Uma, Plant Diseases, **65**, 915 (1981).