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# Seed transmission nature of pathogenic seed borne myco flora of wheat (*Triticum aestivum* L. Em. The Ll.) Seed Samples

**Hansraj Dhakar, RS Ratnoo and Anita Jat**

**Abstract**
Seed (Chittorgarh and Udaipur) were surveyed. A total of 80 seed samples, 40 of each district, were collected during 2016. Eight wheat seed borne myco flora *viz.*, *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Curvularia lunata*, *Fusarium moniliforme*, *Rhizopus stolonifer*, *Mucor* spp. and *Trichoderma viride* were detected and isolated from eighty seed samples. Among the seed borne fungi, *A. alternate and F. moniliforme* produced distinct seed rot and seedling infection symptoms.

**Keywords:** Wheat, Seed Borne Myco Flora, pathogenic

## 1. Introduction
Seeds are regarded as highly effective means for transporting of plant pathogens over long distances. Numerous examples exist in agriculture literature for the international spread of plant diseases as a result of the importation of seeds that were infected or contaminated with pathogens. Seed borne diseases have been found to affect the growth and productivity of crop plants. Any infections agent (bacteria, fungi, nematode, etc.) which is associated with seeds having potential of causing a disease in a seedling or plant, is teemed as seed borne pathogen (Agarwal and Srivastava 1981) [1].

## 2. Material and Method
### Experimental location
The experiment was conducted at Department of Plant Pathology, Rajasthan College of Agriculture (RCA) MPUAT, Udaipur during 2016.

### Sources of experimental materials
Seed to plant transmission studies were carried out *in vitro* and pot culture conditions. Under *in vitro* condition, seed transmission test was carried out by employing plain agar test tube-seedling symptoms test.

To confirm the seed transmission nature of pathogenic seed borne myco flora, randomly selected one hundred discoloured and infected seeds were used. The seedlings were raised in 160 x 16 mm test tubes each containing 10 ml of 1 per cent water agar. The discoloured and diseased seeds were placed over plain agar media @ one seed per test tube. The control was also maintained by inoculating seeds with pure culture of pathogenic test myco flora and with surface sterilized healthy seeds. Test tubes were plugged with a cotton plug and were incubated at 25±2 $^0$C under alternating 12 h of light with 12 h of dark period. After 10 days, plugs were removed and kept for incubation. Observations on seedling symptoms appearance were recorded 15 days after germination.

Similarly, to confirm the seed transmission nature of pathogenic seed borne myco flora in pot culture condition, randomly selected discoloured infected seeds were used and were grown in pots filled with sterilized soil. The separate control with surface sterilized healthy seeds and inoculated seeds with pure culture of pathogenic test myco flora were also maintained. The test myco flora was multiplied in sterilized rice medium (20 gm rice + 10 ml distilled water) in five hundred ml conical flasks by inoculating with 7 days old culture. The inoculated flasks were incubated at 25±2 $^0$C for 10 days and were shaken every day to avoid clumping. The pots were filled with sterilized soil and inoculated upper 4 cm layer of the soil with culture grown in rice medium. The inoculated pots were kept in pot house for 24 h for proper soil infestation before sowing. The surface sterilized wheat seeds were sown at the rate of 10 seeds per pot. A set of control was also kept with surface sterilized seeds sown in sterilized un inoculated soil.

Subsequently, the pots were incubated in pot house and watered regularly. Observations on seedling symptoms were examine and recorded 21 days of after germination. The diseased seedling tissues from test tubes and pot condition grown seedlings were collected, surface sterilized and plated on PDA medium for isolations.

## 3. Results

All the seed samples (forty from each district) collected were mixed well and used for observing myco flora effect on germination, seedling vigour and seedling symptoms.

### (A) Agar Test: seedling symptoms in plain agar Test Tubes

The wheat seed borne myco flora *viz., Alternaria alternata, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium moniliforme, Rhizopus stolonifer, Mucor* spp. and *Trichoderma viride* were detection by standard incubation techniques and seedling symptoms test. Among the myco flora, *Alternaria alternata*, and *Fusarium moniliforme* were recorded pathogenic in seedling symptom in plain agar test and pot experiment.

To confirm the seed transmission nature of *Alternaria alternata* and *Fusarium moniliforme* the discoloured and infected seeds were selected from collected wheat seed samples and were placed in plain agar test tubes and control was also maintained by healthy seeds. Test tubes were incubated at $25\pm2^0$C under alternating 12 h of light with 12 h of dark period. Observations on seedling symptoms were recorded after 10 days of germination.

Results presented in table 1 revealed that, yellowing of leaf followed by blight symptoms were observed seedlings after 10 days of inoculation. The re-isolation of the pathogens was made from infected leaves tissues taken from seedlings of discoloured/diseased seeds and seedlings raised from inoculated seeds, which yielded the fungus identical with the original *Alternaria alternata* which was isolated from seed samples. Wilting and stunted growth symptoms were observed on seedlings after 15 days of inoculation. Similarly, re-isolation of the pathogens was made from infected leaf and root tissues which yielded the fungus identical with the original *Fusarium moniliforme* which was isolated from seed samples. The similar symptoms were observed in seedlings raised from discoloured/diseased seeds and seedlings grown by seed inoculations with test pathogens.

### (B) Pot experiment

To confirm the seed transmission nature of *Alternaria alternata* and *Fusarium moniliforme* the discoloured and infected seeds were selected from collected wheat seed samples and were grown in pots filled with sterilized soil and separately control was also maintained by healthy seeds. Pots were incubated in pot house and watered regularly. Observations on seedling symptoms were recorded after 15 days of germination.

Results presented in table 2 showed that yellowing of leaf followed by blight symptoms were observed after 15 days germination. The re-isolation of the pathogens was made from infected leaves tissues which yielded the fungus identical with the original *Alternaria alternata*.

However, stunting growth and wilting symptoms were observed after 15 days of incubation. The re-isolation of the pathogens was made from infected root tissues which yielded the fungus identical with the original *Fusarium moniliforme* which was isolated from seed samples pathogen was identical with the original one that was inoculated.

It was also observed that the symptoms produced in plain agar test tube method were similar to the symptoms produced in pot culture condition. Hence, it was proved that the pathogenic seed borne myco flora *viz., A. alternata* and *Fusarium moniliforme* were observed seed transmissible in nature.

**Table 1:** Studies on seed transmission nature of isolated seed myco flora in agar seedling symptom test

| S. No. | Seed myco flora | Seed germination (%) | Seedling Mortality (%) | Seedling symptoms |
|---|---|---|---|---|
| 1 | *Alternaria alternata* | 77.21 (61.47) | 16.69 (24.09) | Yellowing of leaves, blight |
| 2 | *Aspergillus flavus* | 48.33 (44.02) | 17.64 (24.81) | Seed rot |
| 3 | *Aspergillus niger* | 76.79 (61.18) | 14.86 (22.64) | Seed rots, root rot |
| 4 | *Curvularia lunata* | 78.39 (60.78) | 15.73 (23.35) | Seedling rot |
| 5 | *Fusarium moniliforme* | 53.51 (46.99) | 18.99 (25.76) | Yellowing of leaves, Stunted growth, wilting |
| 6 | *Rhizopus stolonifer* | 81.69 (64.63) | 7.45 (15.81) | Seed rot |
| 7 | *Mucor* Spp. | 81.17 (64.26) | 7.34 (15.68) | Seed rot |
| 8 | *Trichoderma viride* | 83.27 (65.83) | 5.42 (13.45) | Seed rot |
| 9 | Control | 84.37 (66.71) | 4.19 (11.80) | Healthy seedlings |
| SEm± | | 0.622 | 0.363 | |
| CD at 5% | | 1.849 | 1.079 | |
| CV% | | 1.81 | 3.19 | |

* The value in parentheses is angular transformed

**Table 2:** Studies on seed transmission nature of isolated myco flora in pot experiment

| S. No. | Seed myco flora | Seed germination (%) | Seedling Mortality (%) | Seedling symptoms |
|---|---|---|---|---|
| 1 | *Alternaria alternata* | 77.52 (61.68) | 17.34 (24.59) | Yellowing of leaves, blight |
| 2 | *Aspergillus flavus* | 48.64 (44.20) | 17.81 (24.94) | Seed rot |
| 3 | *Aspergillus niger* | 78.72 (62.50) | 14.78 (22.59) | Seed rots, root rot |
| 4 | *Curvularia lunata* | 78.27 (62.19) | 16.30 (23.79) | Seedling rot |
| 5 | *Fusarium moniliforme* | 53.28 (46.86) | 18.17 (24.59) | Stunted growth, yellowing of leaves, wilting |
| 6 | *Rhizopus stolonifer* | 81.68 (64.63) | 6.67 (14.69) | Seed rot |
| 7 | *Mucor* Spp. | 81.36 (64.40) | 7.34 (15.71) | Seed rot |
| 8 | *Trichoderma Viride* | 83.20 (65.77) | 5.78 (13.90) | Seed rot |
| 9 | Control | 84.35 (66.69) | 4.63 (12.42) | Healthy seedlings |
| SEm± | | 0.493 | 0.371 | |
| CD at 5% | | 1.466 | 1.102 | |
| CV% | | 1.43 | 3.25 | |

* The value in parentheses is angular transformed

**Table 3:** Effect of different seed borne myco flora on germination and per cent mortality of wheat tested by seedling symptom test

| S. No. | Seed myco flora | Seed germination (%) | Mortality (%) |
|---|---|---|---|
| 1 | *Alternaria alternata* | 76.85 (61.24) | 17.85 (24.98) |
| 2 | *Aspergillus flavus* | 48.33 (44.02) | 18.55 (25.50) |
| 3 | *Aspergillus niger* | 58.81 (62.58) | 14.78 (22.60) |
| 4 | *Curvularia lunata* | 78.14 (62.11) | 16.52 (23.97) |
| 5 | *Fusarium moniliforme* | 55.27 (48.0) | 19.67 (26.31) |
| 6 | *Rhizopus stolonifer* | 81.36 (64.47) | 7.61 (16.00) |
| 7 | *Mucor* spp. | 81.68 (64.65) | 8.44 (16.86) |
| 8 | *Trichoderma viride* | 83.11 (65.73) | 6.23 (14.44) |
| 9 | Control | 84.35 (66.73) | 4.89 (12.77) |
| SEm± | | 0.875 | 0.198 |
| CD at 5% | | 2.604 | 0.590 |
| CV% | | 2.53 | 1.69 |

* The value in parentheses is angular transformed

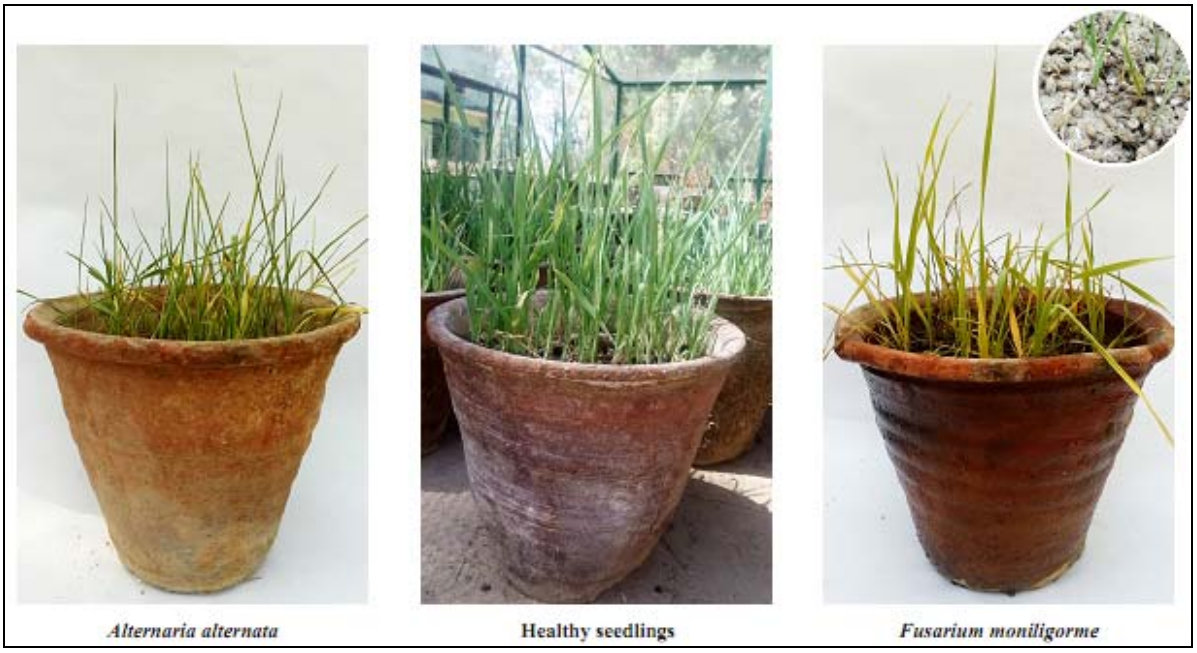


**Fig 1:** Pathogenic seed myoc floro symptons on wheat seedling by soil inoculation teachnique in pot experiment


**Fig 2:** Symptoms in agar test tubes

## 4. Discussion

In dates back to 1729, Michelli first demonstrated seed transmission of a pathogen while, Tillet (1755) [7] confirmed and established that *Tilletia caries* Tull, the fungus responsible for bunt disease of wheat is seed borne. Since then the knowledge of seed borne disease of crop has been greatly increased and now there is hardly any cultivated crop where at least one seed borne fungal parasite is not known. Consequently, it has become clear in Plant Pathology that seed borne myco flora is largely associated with the occurrence of number of diseases.

Many plant pathogenic fungi are known to produce phytotoxic metabolites Sharma (2001) [6]. The importance of the production of such toxic metabolites is more obvious when the pathogen is seed-borne because it may either inhibit seed germination or adversely affects the initial growth of seedling.

In present investigation to confirm the seed transmission nature of *Alternaria alternata*, and *Fusarium moniliforme* the discoloured and infected seeds were selected from collected wheat seed samples and were placed in plain agar test tubes, pot culture conditions and control was also maintained by inoculating seeds with pure culture of *A. alternata*, and *F. moniliforme.* Subsequently, test tubes were incubated at 25$\pm$ $2^0$C under alternating 12 h of light with 12 h of dark period and the pots were placed in pot house. Results revealed in both conditions that, yellowing of leaf followed by blight symptoms were observed on seedlings after 15 days after germination. The re-isolation of the pathogens was made from infected leaves tissues taken from seedlings of discoloured/diseased seeds and seedlings raised from inoculated seeds, which yielded the fungus identical with the original *A. alternata* which was isolated from seed samples. However, root rot and wilting symptoms were observed on seedlings after 25 days of inoculation. Similarly, re-isolation of the pathogens was made from infected root tissues which yielded the fungus identical with the original and *F. moniliforme* which was isolated from seed samples. The similar symptoms were observed in seedlings raised from discoloured/diseased seeds and seedlings grown by seed inoculations with test pathogens. Observations on seedling symptoms were recorded starting from 15 to 25 days of incubations. Arseniuk *et al*., (1993) [2] were observed transmission and the effect of seed borne inoculum on *Stagonospora nodorum* (blotch.) and *Fussarium* spp. in the field. Infected seed was shown to as a source of primary inoculum in epidemics of the disease in cereals. Seed borne *Stagonospora nodurum* (Berk.) was detected efficiently on oxgall agar medium as well as on yeast malt extract and potato dextrose agar media Similarly, Duthie and Hall (1987) [4] were found that infection of stem of winter wheat by *Fusarium graminearum* was directly related to incidence of infection of seed at planting. The efficiency of transmission of the pathogen from seed to stem ranged from 55 to 94% over from sampling dates in two trials. Results indicating that all transmission occurred during the autumn. Basak and lee, 2002 [3] were found that transmissions of all seed borne pathogens from seeds to seedlings were also detected by test tube seedling symptom test. Among the seed borne fungi, *A. alternate and F. moniliform*e produced distinct seed rot and seedling infection symptoms.

## 5. References


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