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# Evaluation of Mycoflora and Mycotoxins Contamination of Mustard Seeds ( *Brassica juncea* L.)


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**ABSTRACT :** Oil seeds are of great economic importance and their significance in life of human next to cereals .Mustard (Brassica juncea L.) is an major oil seed crops of Northern India including Bihar state. Samples of mustard collected from pre-harvest stage from different farms. The seed Mycoflora of different seeds were screened by blotter paper ,agar plate method and seed washing methods ,The agar plate method was found to be suitable as there was higher percent incidence of seed mycoflora. Species of Aspergillus, Alternaria, Curvularia, Fusarium, Penicillum and Rhizopus were found associated with seed, percentage incidence of which varied from 2-15 %. Some of the isolates of Aspergillus flavus, Alternaria, Curvularia, Fusarium moniliforme and Penicillum citrinum were found toxigenic which produces aflatoxins ,Zearalenone and Citrinin respectively in the culture media. Sahay(1988) recorded the incidence of aflatoxins in mustard seeds .The samples were found naturally comtaminated with Aflatoxins ,Zearalenone and Citrinin.

**KEYWORDS :** Northen, Aspergillus flavus, Alternaria, Aflatoxins, Zearalenone and Citrinin.

# RESEARCH PAPER

## Introduction :

The production and supply of high quality grain remains of prime importance.oil seeds must thus be protected in the field against disease and in store after harvest, against fungal attack. These fungal strains not only reduce the quality of the crops but some species of moulds can produce highly toxic chemicals known as mycotoxins. According to McKee (1995) and Hasheem and Alumri (2010) many agricultural products may be exposed to a wide range of microbial contamination during pre and post harvest. In recent years several human diseases have been found to be associated with the consumption of mustard oil adulterated with the oil of Argemone Mexicana seeds, which causes renal failure leading to death of person. Mustard seeds facilitate the development of toxigenic fungi as well as mycotoxins contamination. Natural contamination of aflatoxins and other mycotoxins are found . In these attempt made investigation for mycoflora of mustard seeds and incidence of mycotoxins have been analysed in samples**Materials and Methods :**

Mustard seeds **were** collected from the crops at pre-harvest stage from different farms. Oil seeds are reported to carry many moulds both in field and during storage (Chauhan and Kaur,1975). The fungi associated with seeds at the stage of harvest and under storage bring about several undesireable changes making them unfit for consumption and sowing (Vaidehi and Lalitha, 1985**)** **.**The seeds carry number of fungi which include Aspergillus, Alternaria, Curvularia, Fusarium, Penicillum , Rhizopus **s**tolonifer. Attempts were made during present investigation to study mycoflora and mycotoxins of seeds. The seeds sample were collected such as MSS-18,MSS-19 ,MSS-20 and MSS-27 **. T**he mustard seed samples were collected and stored in plastic containers, Seeds were treated with 0.1 % mercuric chloride solution for surface sterilization of seeds. Isolation of the mycoflora associated with seed sample done by Agar plate method, Standard blotter paper method and seed washing method as recommended by International seed testing Association (ISTA,1966).

**1. Agar Plate Method(Muskett and Malone 1941):** Presterilized petriplates were taken pour with Glucose Nitrate Agar medium (GNA).After cooling 15 seeds per plate to be studied were equidistantly placed .The plates were incubated at 25+_2°c.After 7 days seeds were examined under microscope by preparing slides.

**2. Standard blotter paper method(Doyer 1938):** A blotter paper of 8.5cm diameter were soaked with sterile distilled water and placed in petri plates 10-15 seeds were placed equidistantly on moist blotter paper . The plates were incubated at 25+_2°c.After 7 days seeds were examined under microscope by preparing slides.

**3. Seed Washing method**: 50-100 seeds of mustard were taken in flask with sterile water. The flask were shaken for 5-10 minutes in electrical shaker .1 ml of seed washing obtained were plated on GNA medium. The plates were incubated at room temperature. The fungal colonies were immediately transferred to GNA slants for further study.

Natural occurrence of mycotoxins in mustard samples was analysed by the method of Jones (1972) , Roberts and Patterson (1975).Estimation of mycotoxins were performed by TLC plates .50 µl of chloroform extract was spotted on TLC plate along with the standard mycotoxins. The spotted chromatoplate was developed by solvent system TEF (Toulene: Ethyl acetate: Formic acid 6:3:1 v/v/v for aflatoxins components (Reddy etal.1970).After preparation of TLC plate ,chemical confirmation of mycotoxins was performed by spraying the spots with suitable reagents. Aflatoxins were confirmed by treating the extract with trifluroacetic acid (TFA).Aflatoxins $B_1$ appeared as a blue fluorescent spot at Rf about ¼ to that of aflatoxins $B_1$ on TLC plate. In some cases ,where the amount of aflatoxins $B_1$ was very low, the spot was sprayed with 25% $H_2SO_4$ which changed the color of fluorescence from blue to yellow. Confirmation of Citrinin was done by spraying the TLC plate with freshly prepared mixture of 0.5ml p-anisaldehyde in 85 ml of methanol containing 10 ml of acetic and 5ml of conc.$H_2SO_4$ and then by heating the TLC plate at 130°c for 10 minutes .This changed yellow streak of Citrinin to yellowish green under long wave uv light (Scott.etal 1970). Zearalenone was also confirmed by spraying TLC plate with acidic p-anisaldehyde solution by which greenish blue fluroescence turned faint brown (invisible light) and faint yellow in long wave UV light.

**Table: Fungi Isolated from seeds of Mustard**

| Fungi | % Incidence of Mycoflora | | | | | |
|---|---|---|---|---|---|---|
| | MSS-19 | | | MSS-27 | | |
| | AP | BP | SW | AP | BP | SW |
| Alternaria alternata | 35 | 30 | 31 | 36 | 32 | 33 |
| Alternaria brassicae | 38 | 35 | 35 | 38 | 35 | 20 |
| Aspergillus flavus | 35 | 30 | 32 | 36 | 32 | 19 |
| Aspergillus niger | 36 | 32 | 30 | 37 | 35 | 22 |
| Chaetomium sp | 32 | 28 | 28 | 30 | 28 | 29 |
| Cladosporium sp | 30 | 25 | 26 | 32 | 25 | 08 |
| Curvularia lunata | 10 | 09 | 10 | 11 | 09 | 09 |
| Fusarium moniliforme<br>Penicillium | 15<br>15 | 05<br>10 | 07<br>09 | 16<br>17 | 08<br>12 | 28<br>13 |
| Helminthosporium sp<br>Rhizopus (Ehremb) | 32<br>20 | 25<br>15 | 24<br>10 | 30<br>25 | 29<br>13 | 28<br>12 |

AP-Agar Plate BP-Blotter Paper SW-Seed Washing

**Result :** From table 1.fungi isolated from seeds of mustard were Alternaria alternata, Alternaria brassicae ,Aspergillus sp. Cladosporium ,chaetomium sp. Penicillium and Rhizopus. The percentage incidence varies from 2-12%. From the table it is clear that the incidence percentage is higher in Agar Plate as compared to Blotter Paper and seed washing .From the table it is clear that the species MSS-27 Show succeptible to fungal growth. Curvularia and Rhizopus are least. During the investigation three important mycotoxigenic fungi such as Penicillium citrinum the main producers of Aflatoxins, Zearalenone and Citrinin

respectively. Seed samples were analysed aflatoxins were found to be most common mycotoxins in those samples followed by Zearalenone and Citrinin.

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