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# MYCOFLORA OF STORED AND FRESHLY HARVESTED MUSTARD AND RAPESEED GROWN IN PAKISTAN

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A large number (1,273) of samples of mustard and rapeseed were collected from 116 locations belonging to 38 districts of Pakistan. Eighty nine known varieties were collected from different agricultural research institutes and studied for fungal infections. The dominant genus isolated from freshly harvested crop was *Alternaria* with 55% frequency of occurrence. In stored seeds, *Aspergillus* spp., *Alternaria* spp. and *Penicillium* spp. were present in that order. In case of meal and cake, the *Aspergillus* showed over 50% frequency of occurrence followed by *Penicillium* whereas. *Fusarium* spp. and *Stemphylium* spp. also showed significant dominance in freshly harvested seeds.

***Key words:*** Fungi, Mustard, Rapeseed.

## Introduction

The rapeseed cultivation started in the South Asia (India, Pakistan and Bangladesh) some 3,000 years ago. It occupies third place in the production after soybean and cottonseed in the world [1]. Rapeseed is the second most important oilseed crop after cottonseed, grown in Pakistan [2] and used as an edible oil and feed for livestock, its leaves and inflorescence are used as vegetable, condiments, and also for industrial purposes. Rapeseed cake is an important feed supplement for cattle and the meal is used in poultry ration which is on the rise. The presence of fungi on rapeseed cake and meal produces toxins, resulting in poor quality and thus cause economic loss. The presence of fungi on seed impairs seed viability and vigour [3]. Genus *Alternaria* is one of the most common groups of fungi that contaminate this crop in the field conditions. Members of this genus are ubiquitous and indigenous. Species of *Alternaria* are plant pathogens causing pre and post-harvest decay and wide spread contaminants of standing crop [4]. *Alternaria* blight causes major damage to rapeseed and mustard crops in India [5]. In a previous study, (1969-70), on the fungal flora of stored mustard and rapeseed, dominant fungi were *Aspergilli* spp. with more than 50% frequency of occurrence, followed by *Alternaria alternata*. Overall, eight fungal genera having sixteen species were isolated [6]. Seventeen fungal species belonging to ten genera were isolated from one hundred samples of mustard; in summer *Aspergillus* was the most dominant while in winter *Fusarium* had the highest incidence of occurrence [7]. Certain *Alternaria* species are capable of producing mycotoxins [8,9] which result in the contamination of agricultural commodities [10]. *Alternaria* species produce forty secondary metabolites of which ten are toxic to animals [11] and mammalian cells [12-15]. *Fusarium* species also produce various mycotoxins [16,17]. The ability of other fungi of genera *Aspergillus* and *Penicillium* to produce mycotoxins has also been recognized throughout the world as a major problem [18]. Thus there is enough circumstantial evidence to suggest the involvement of aflatoxins, produced by *Aspergillus flavus* and *A. parasiticus,* in acute toxicosis in human beings [19,20]. Moreover, there is an ever increasing demand for vegetable source of protein in poultry ration due to shortage of soybean and peanut meal, etc. In view of these reasons, a study was conducted to determine of the mycoflora of rape seed in field and storage conditions. (The presence of fungi deteriorates the quality of oilseed crops and has an ever increasing role in the world quality of oil, shoveled, germination and weight loss in vegetable oil industry).

## Materials and Methods

Eighty nine pre and post-harvest samples of known varieties and hybrids (Table 1) of *Brassica campestris*, *B. napus, B. juncea, B. carinata* and one variety of *B. chinensis* were collected from different institutes like Ayub Agricultural Research Institute (ARI), Faisalabad (Punjab); Agricultural Research Institute Tandojam, Hyderabad (Sindh), Agricultural University Research Station, Mingora, Swat (NWFP) and Agricultural Research Institute, Tarnab, Peshawar (NWFP). One thousand two hundred and seventy three samples of mustard and rapeseed and their products were collected from 116 locations belonging to 21 districts of Punjab, 6 districts of Sindh, 7 districts of NWFP and 5 districts of Balochistan during the crop years 1989-1992. The samples of unknown or mixed varieties were collected from the fields, wholesale markets and retail shops. Rapeseed crop was also grown in the experimental field of Mycotoxin Laboratory of Pakistan Council of Scientific and In-

dustrial Research, Karachi. The samples of rapeseed cake and meal were collected from Karachi, Lahore, Peshawar and Quetta only.

The samples of rapeseed from the fields were collected at different places randomly. After harvest, the rapeseed on the farms are kept in heaps on the ground before it is taken to market. Samples of about 2-3 kg of seeds from these heaps were collected at different points from surface to bottom, with the help of a probe. In market, the rapeseed is stored in jute bags and stocked in different types of godowns. The samples were collected from various jute bags randomly through sample probes and collected in plastic bags for chemical and microbiological analysis. The bags for sampling were selected

TABLE 1. LIST OF VARIETIES OF BRASSICA COLLECTED FROM VARIOUS AGRICULTURAL RESEARCH INSTITUTES IN PAKISTAN.

| S.No. | Varieties | Species |
|---|---|---|
| 1. | Early Raya | *B. juncea* |
| 2. | S - 9 | *B. juncea* |
| 3. | Toria selection | *B. campestris* |
| 4. | P-43 Yellow seeded | *B. juncea* |
| 5. | P-269 | hybrid |
| 6. | Toria-A | *B. campestris* |
| 7. | DGL | *B. juncea* |
| 8. | RL-18 | *B. juncea* |
| 9. | Raya Anmol | *B. juncea* |
| 10. | Toria | *B. campestris* |
| 11. | B. carinata 12 | *B. carinata* |
| 12. | P - 43 | *B. juncea* |
| 13. | P - 61 - 26 - 2 | *B. juncea* |
| 14. | P-53-72-21-S/6 | hybrid |
| 15. | China Sarsoon | *B. chinensis* |
| 16. | Poorbi Raya | *B. juncea* |
| 17. | Peela Raya | *B. carinata* |
| 18. | SM-83000 | *B. juncea* |
| 19. | S-9 x P-15 | *B. juncea* |
| 20. | P-98 | *B. juncea* |
| 21. | RD - 80 | *B. juncea* |
| 22. | BM - I | *B. juncea* |
| 23. | P-53-59-2/1 | *B. juncea* |
| 24. | P-53/72-2/-5 | *B. juncea* |
| 25. | P-53 | *B. juncea* |
| 26. | S-9 x RH-30 | hybrid |
| 27. | RH - 7859 | hybrid |
| 28. | P-269 | hybrid |
| 29. | Rc-280 x Early Raya | hybrid |
| 30. | P-33/72 | hybrid |
| 31. | P-53-48-2 | hybrid |
| 32. | P-56/72 | hybrid |
| 33. | Rc-23 | hybrid |
| 34. | RH - 78 | hybrid |
| 35. | Early Raya/22 | hybrid |
| 36. | Early Raya x S-9 | hybrid |
| 37. | Rc-247 | hybrid |
| 38. | ORI-50-6 | *B. juncea* |
| 39. | Toria composite | *B. campestris* |
| 40. | BSA | *B. campestris* |
| 41. | Shiralee | *B. napus* |
| 42. | Brown Raya (A) | *B. carinata* |
| 43. | Peela Raya | *B. carinata* |
| 44. | P-169/72 | *B. campestris* |
| 45. | RH-7859 | *B. juncea* |
| 46. | RH-30 | *B. juncea* |
| 47. | 4-53-48-2 | *B. juncea* |
| 48. | P-102/72 | *B. campestris* |
| 49. | P-33/72 | *B. juncea* |
| 50. | B. carinata 1 | *B.carinata.* |
| 51. | 3-P-269 | *B. juncea* |
| 52. | P-159 | *B. napus* |
| 53. | Varu | *B. juncea* |
| 54. | P-129 | *B. juncea* |
| 55. | P-159 | *B. napus* |
| 56. | P-53-48-2 | *B. juncea* |
| 57. | E-20 | *B. juncea* |
| 58. | ALTEX | *B. napus* |
| 59. | RL-18 | *B. juncea* |
| 60. | 1245 | *B. juncea* |
| 61. | S-191 | *B. napus* |
| 62. | Desi | *B. campestris* |
| 63. | S-26 | *B. campestris* |
| 64. | S-102 | *B. campestris* |
| 65. | PYT-14 | *B. napus* |
| 66. | Tower | *B. napus* |
| 67. | Pak China-89 | *B. napus* |
| 68. | 1245 | *B. campestris* |
| 69. | Torch | *B. campestris* |
| 70. | RD-71 | *B. juncea* |
| 71. | S.262 | *B. napus* |
| 72. | E.20 | *B. campestris* |
| 73. | RL-18 | *B. juncea* |
| 74. | PNS-tall | *B. juncea* |
| 75. | Tower | *B. napus* |
| 76. | Desi | *B. campestris* |
| 77. | Wester | *B. napus* |
| 78. | Alten | *B. napus* |
| 79. | KORAL | *B. napus* |
| 80. | Regent | *B. napus* |
| 81. | Global | *B. napus* |
| 82. | ALTAX | *B. napus* |
| 83. | DGL | *B. napus* |
| 84. | S-28 | *B. napus* |
| 85. | SWEEDEN:5 | *B. napus* |
| 86. | Tobin Tobin | *B. campestris* |
| 87. | P-6-1 | *B. juncea* |
| 88. | SWEEDEN:6 | *B. napus* |
| 89. | P-98-1 | *B. napus* |

TABLE 2. PERCENT PREVALENCE OF VARIOUS FUNGI ISOLATED FROM MUSTARD/RAPESEED AND THEIR PRODUCTS.

| Province | Punjab | | | | Sindh | | | | NWFP | | | | Balochistan | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Samples | Seed | Seed | Cake | Meal | Seed | Seed | Cake | Meal | Seed | Seed | Cake | Meal | Seed | Seed | Cake | Meal |
| | fresh | stored* | | | fresh | stored | | | fresh | stored | | | fresh | stored | | |
| No. of Samples | 204 | 135 | 53 | 117 | 162 | 98 | 86 | 92 | 99 | 30 | 31 | 25 | 52 | 40 | 31 | 18 |
| *Alternaria brassicae* | 4.0 | 0.4 | 2.2 | 0.0 | 3.4 | 0.8 | 0.0 | 0.0 | 1.2 | 2.2 | 0.4 | 0.0 | 2.6 | 0.2 | 0.0 | 0.0 |
| *Alternaria tenuissima* | 16.8 | 9.0 | 1.0 | 0.6 | 15.6 | 5.8 | 2.0 | 1.0 | 14.6 | 12.2 | 4.2 | 0.2 | 16.8 | 6.4 | 0.8 | 0.2 |
| *Alternaria alternata* | 27.6 | 14.2 | 1.6 | 2.0 | 28.4 | 13.4 | 0.4 | 1.0 | 25.4 | 17.2 | 3.6 | 1.6 | 29.2 | 17.6 | 2.0 | 1.2 |
| *Alternaria solani* | 4.5 | 1.0 | 0.0 | 0.0 | 6.0 | 3.2 | 0.0 | 0.0 | 1.6 | 1.8 | 0.6 | 0.0 | 7.8 | 3.0 | 0.0 | 0.0 |
| *Alternaria brassicola* | 2.6 | 0.0 | 0.0 | 0.0 | 3.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 0.0 | 0.0 | 2.6 | 0.0 | 0.0 | 0.0 |
| *Alternaria* spp. | 1.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.8 | 2.0 | 0.0 | 0.2 | 0.4 | 2.8 | 2.6 | 1.0 | 0.0 |
| Sum ----> | 56.5 | 24.6 | 5.8 | 2.6 | 57.2 | 23.2 | 2.4 | 2.8 | 44.8 | 33.8 | 9.0 | 2.2 | 61.8 | 29.8 | 3.8 | 1.4 |
| *Stemphylium* spp. | 15.0 | 8.2 | 4.2 | 2.8 | 8.6 | 8.0 | 2.6 | 1.4 | 2.0 | 0.0 | 0.0 | 0.0 | 4.2 | 1.4 | 0.0 | 0.0 |
| *Fusarium semitectum* | 1.0 | 2.4 | 0.8 | 3.6 | 3.4 | 4.6 | 2.8 | 2.0 | 0.0 | 3.2 | 2.8 | 3.2 | 3.2 | 4.6 | 4.8 | 0.2 |
| *Fusarium solani* | 1.6 | 0.6 | 0.0 | 0.0 | 2.8 | 0.2 | 0.0 | 0.0 | 2.4 | 1.8 | 0.0 | 1.6 | 2.0 | 1.8 | 0.4 | 0.0 |
| *Fusarium oxysporum* | 3.0 | 2.8 | 3.0 | 2.4 | 0.0 | 3.4 | 4.6 | 0.0 | 5.6 | 2.6 | 3.2 | 2.4 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Fusarium equisiti* | 0.0 | 0.8 | 1.4 | 3.0 | 0.8 | 0.0 | 0.8 | 2.0 | 0.0 | 1.2 | 2.6 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Fusarium moniliforme* | 0.0 | 0.0 | 1.2 | 0.0 | 0.0 | 4.2 | 1.2 | 0.0 | 2.6 | 0.0 | 1.8 | 0.2 | 1.2 | 5.2 | 1.2 | 0.6 |
| *Fusarium* spp. | 1.2 | 1.4 | 1.0 | 0.0 | 0.6 | 0.0 | 0.0 | 0.0 | 1.8 | 0.0 | 0.8 | 0.6 | 1.6 | 1.4 | 0.0 | 0.0 |
| Sum ----> | 6.8 | 8.0 | 7.4 | 9.0 | 7.6 | 12.4 | 9.4 | 4.0 | 12.4 | 8.8 | 11.2 | 9.0 | 8.0 | 13.0 | 6.4 | 0.8 |
| *Cladosporium spheaerospermum* | 2.5 | 7.8 | 8.6 | 7.6 | 6.6 | 9.6 | 8.2 | 4.2 | 9.6 | 4.4 | 10.0 | 4.0 | 2.2 | 6.2 | 1.2 | 0.0 |
| *Cladosporium* spp. | 3.2 | 1.2 | 1.2 | 2.4 | 1.2 | 2.6 | 1.2 | 3.4 | 1.4 | 0.0 | 0.0 | 0.0 | 0.0 | 1.8 | 0.0 | 0.0 |
| Sum ----> | 5.7 | 9.0 | 9.8 | 10.0 | 7.8 | 12.2 | 9.4 | 7.6 | 11.0 | 4.4 | 10.0 | 4.0 | 2.2 | 8.0 | 1.2 | 0.0 |
| *Curvularia lunata* | 1.0 | 2.4 | 1.0 | 1.2 | 0.0 | 0.6 | 1.4 | 1.6 | 2.8 | 3.0 | 1.2 | 0.8 | 3.2 | 3.0 | 1.0 | 0.0 |
| *Drechslera hawaiiensis* | 2.4 | 1.8 | 1.0 | 1.6 | 3.6 | 2.6 | 2.0 | 2.4 | 3.6 | 2.8 | 0.6 | 1.0 | 1.2 | 1.6 | 0.2 | 0.0 |
| *Aspergillus nidulans* | 0.8 | 1.2 | 0.0 | 8.2 | 0.6 | 1.4 | 3.6 | 4.2 | 0.0 | 0.0 | 4.6 | 3.2 | 2.6 | 2.0 | 4.2 | 5.4 |
| *Aspergillus flavus* | 3.6 | 9.6 | 18.6 | 23.0 | 3.6 | 9.6 | 20.6 | 21.4 | 3.0 | 8.6 | 18.0 | 22.2 | 2.8 | 9.8 | 17.2 | 23.4 |
| *Aspergillus niger* | 1.0 | 8.6 | 19.0 | 13.0 | 2.4 | 6.4 | 12.6 | 13.0 | 2.2 | 7.4 | 13.4 | 11.4 | 0.6 | 6.8 | 21.2 | 18.0 |
| *Aspergillus sydowi* | 0.8 | 0.0 | 2.8 | 5.2 | 0.0 | 0.0 | 1.6 | 3.6 | 0.0 | 0.4 | 1.0 | 2.2 | 0.2 | 2.2 | 6.0 | 3.6 |
| *Aspergillus versicolor* | 0.0 | 1.4 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 4.4 | 1.0 | 2.4 | 0.8 | 0.0 | 0.0 | 0.0 | 2.4 | 4.8 |
| *Aspergillus terreus* | 0.0 | 0.4 | 1.2 | 0.0 | 0.6 | 2.8 | 1.2 | 0.0 | 0.0 | 0.0 | 3.5 | 1.6 | 0.0 | 0.0 | 1.8 | 4.2 |
| *Aspergillus sulfurus* | 0.0 | 2.0 | 3.0 | 2.4 | 1.2 | 0.8 | 0.0 | 1.6 | 1.4 | 1.8 | 2.8 | 3.4 | 1.8 | 2.6 | 0.2 | 3.2 |
| *Aspergillus fumigatus* | 0.8 | 3.6 | 4.2 | 1.8 | 0.0 | 2.6 | 4.2 | 4.6 | 1.2 | 2.8 | 3.2 | 6.2 | 0.0 | 0.0 | 0.0 | 1.4 |
| *Aspergillus tamarii* | 0.0 | 0.4 | 0.0 | 0.0 | 0.0 | 0.8 | 1.0 | 1.8 | 0.0 | 1.6 | 0.8 | 1.2 | 0.0 | 0.0 | 1.4 | 3.8 |
| *Aspergillus violaceus* | 0.0 | 0.0 | 0.0 | 0.4 | 0.2 | 0.0 | 1.2 | 0.0 | 0.0 | 0.0 | 1.6 | 0.0 | 0.0 | 0.0 | 0.0 | 1.4 |
| *Aspergillus* spp. | 0.0 | 0.2 | 0.0 | 1.2 | 0.0 | 1.0 | 0.0 | 0.8 | 1.6 | 2.0 | 2.5 | 2.4 | 1.0 | 1.6 | 4.2 | 4.8 |
| Sum ----> | 7.0 | 27.4 | 49.8 | 55.2 | 8.6 | 25.4 | 46.0 | 55.4 | 10.4 | 27.0 | 52.2 | 53.8 | 9.0 | 25.0 | 58.6 | 74.0 |
| *Penicillium jenethelium* | 2.6 | 6.8 | 3.2 | 6.6 | 2.4 | 5.0 | 4.2 | 3.6 | 1.6 | 3.0 | 1.8 | 3.6 | 0.0 | 0.0 | 0.2 | 0.8 |
| *Penicillium chrysogenum* | 0.0 | 4.2 | 2.6 | 0.0 | 0.0 | 5.4 | 6.4 | 3.2 | 3.8 | 4.2 | 1.8 | 1.2 | 0.0 | 6.8 | 10.2 | 4.6 |
| *Penicillium notatum* | 0.0 | 3.4 | 2.0 | 1.8 | 0.0 | 0.0 | 2.0 | 1.8 | 0.0 | 0.8 | 2.8 | 0.0 | 0.0 | 1.6 | 6.8 | 4.2 |
| *Penicillium cyclopium* | 0.0 | 0.0 | 1.8 | 2.2 | 0.6 | 2.4 | 1.8 | 0.0 | 0.0 | 3.4 | 2.8 | 4.6 | 5.6 | 4.2 | 5.8 | 3.8 |
| *Penicillium islandicum* | 0.0 | 1.0 | 2.2 | 3.2 | 0.8 | 0.0 | 2.4 | 2.6 | 2.6 | 0.0 | 0.0 | 4.8 | 0.0 | 0.0 | 1.2 | 0.8 |
| *Penicillium citrinum* | 0.8 | 0.0 | 2.2 | 1.2 | 0.0 | 0.4 | 0.0 | 3.2 | 0.0 | 3.2 | 1.8 | 4.2 | 0.0 | 0.0 | 0.4 | 0.6 |
| *Penicillium* spp. | 0.6 | 0.0 | 0.8 | 1.0 | 1.2 | 0.0 | 1.2 | 1.6 | 2.2 | 1.8 | 2.2 | 4.6 | 0.8 | 1.2 | 1.0 | 1.2 |
| Sum ----> | 4.0 | 15.4 | 14.8 | 16.0 | 5.0 | 13.2 | 18.0 | 16.0 | 10.2 | 16.4 | 13.2 | 23.0 | 6.4 | 13.8 | 25.6 | 16.0 |
| *Rhizopus arrhizus* | 0.0 | 0.0 | 4.2 | 0.0 | 0.0 | 0.0 | 3.0 | 3.2 | 0.2 | 0.0 | 1.0 | 3.2 | 0.0 | 0.0 | 1.2 | 2.2 |
| *Mucorales* spp. | 0.0 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 3.6 | 1.8 | 0.0 | 0.0 | 0.2 | 0.6 | 0.0 | 0.0 | 1.0 | 1.8 |
| Other Fungi | 1.6 | 2.6 | 2.0 | 2.4 | 1.6 | 2.4 | 2.2 | 3.8 | 2.6 | 3.8 | 1.8 | 3.2 | 4.0 | 4.4 | 2.0 | 3.8 |

* Stored for over six months.

randomly and numbers determined according to the size of the lot. Seeds were placed on blotter as well as on agar plates and incubated at 28° C ± 2, accordingly. Pure cultures were obtained from the fungi emerging from seeds and kept on PDA slants. Single spore cultures were made from the mixed cultures for identification. Dilution technique was used for isolating fungi from rapeseed cake and meal. Classification was done by the method of Ellis [21,22].

## Results and Discussion

During the period of study, dominant species of fungi (Table 2), isolated from fresh rapeseed crop, was the genus *Alternaria*, with 56.5% dominance in Punjab, 57.2% in Sindh, 44.8% in NWFP and 61.8% in the province of Balochistan. The genus *Stemphylium* was the second most dominant after *Alternaria* from fresh crop, followed by 5 species of *Fusarium*. However, from the stored mustard/rapeseed, the dominant genus was *Aspergillus* with 27.4% occurrence in Punjab, 25.4% in Sindh, 27% in NWFP and 25% in Balochistan. The dominance of *Aspergillus* and *Penicillium* increased to 61.6% in cake and 69.2% in meal in Punjab, 51% in cake and 70.4% in meal in Sindh, 60.4% in cake and 75.8% in meal in NWFP, and 84.2% in cake and 90% in meal in Balochistan. Five species of *Alternaria*, 11 of *Aspergillus*, 7 of *Penicillium*, 5 of *Fusarium*, 2 of *Cladosporium* and 1 each of *Curvularia, Drechslera, Rhizopus* and *Stemphylium* were isolated. Some members of *Mucorales* group and few other fungi or mycelia sterila were also isolated. The fungal flora of 8 *Brassica* varieties belonging to 4 species, cultivated in the experimental field of Mycotoxin Laboratory, was slightly different from the fungal flora isolated from the fresh crop collected from different locations in Pakistan, mainly due to the time factor between harvest and isolation of fungi. Here fungi were isolated on the same day after harvest. Fungal spores were found on 36% rapeseed. *Alternaria alternata* and *A. brassicae* were the most dominant fungi followed by *A. tenuissima*, *Stemphylium* spp. and *Cladosporium* spp. Overall combined prevalence of *Alternaria* species was 93.4%. After surface sterilization, about 6.1% seeds showed the presence of a fungal infection.

The species of *Aspergillus, Penicillium, Fusarium, Alternaria* and others have great potential to produce large number of mycotoxins in large amounts. The toxins are reported to be hazardous to human and animal health [18]. A lot of work has been done on the toxin producing potential of different fungi using various substrates [23]. Keeping in view the potential of these fungi for toxin production, there are chances that these toxins may be found in large amount in mustard/rapeseed and also in other agricultural commodities around us. The presence of these fungi does not necessarily confirm the presence of their toxins but may indicate the chances for the production of mycotoxins, provided conditions are optimum and the strains of fungi are mycotoxigenic.

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