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MYCOTOXIN PRODUCING POTENTIAL OF *ALTERNARIA ALTERNATA* ISOLATED FROM MUSTARD/RAPESEEDS.

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To evaluate the relative importance of fungal species, the known varieties of mustard/rapeseed collected from various research institutes located throughout Pakistan, were investigated. *Alternaria* species were the predominant fungi isolated from the samples of freshly harvested crops. The toxin producing potential of various strains of *Alternaria alternata* was investigated. The maximum amount produced by these isolates was 27.5mg/kg for tenuazonic acid and 22.9 mg/kg for alternatriol monomethyl ether.

Key words: *Alternaria alternata, Alternaria* toxins, Mustard/rapeseed.

Introduction

Occurrence of the genus *Alternaria* is common in agricultural commodities. It causes an extensive damage to grains, fruits and vegetables before and after harvest, during transportation and storage [1,2]. As pathogen, it causes black mold of tomatoes and peppers [3], mandarin fruit rot [4],and black point of cereal grains [5] and plant products. It is also known to produce secondary metabolites which are connected with illness in farm animals and have an adverse effect on mammalian systems [6-9]. The major toxic compounds produced by *Alternaria* spp. and reported in literature are alternariol (AOH), alternariol monomethyl ether (AME), altertoxin-I (ATX-I), altertoxin-II (ATX-II) and tenuazonic acid (TeA) [7-9]. AME and AOH were found in discoloured pecans [10], sorghum [11] along with Alteune and ATX-I [7]. Tenuazonic acid was detected in tomato paste [12] and in about 50% of tomato samples [13].

Oilseed rape is an important oilseed crop of Pakistan which is grown in Central, Northern and Southern parts of the country. This crop is vulnerable to aphids and fungal attack which results in huge economic losses, and hence the area under this crop is decreasing every year [14].

A study was conducted (1989-1992) to evaluate the relative importance of fungal species. It was found that the genus *Alternaria* was most prevalent with a frequency of occurrence of 55% in respect to other fungi [15]. In this study, isolates of *A. alternata* were tested for their toxin-producing potential.

Materials and Methods

Samples of known varieties and hybrids of rape and mustard seed were collected from Ayub Agricultural Research Institute (ARI) Faisalabad, Punjab; ARI Tandojam, Sindh; Agriculture University Research Station Mingora, Swat (NWFP) and ARI Tarnab, Peshawar.

Samples of unknown or mixed varieties of rapeseed were collected from fields and markets of major cities of the four provinces of Pakistan for a period of three years . The major species of genus *Alternaria* were isolated by inoculating the seed, cake and meal, on blotting paper and agar media. Single spore colonies of the *Alternaria* species were obtained on potato-dextrose-agar medium and identified in accordance with the classification of Ellis [1.2].

Fifty grams of broken rice grains with a moisture level of 50% were taken in 500 ml conical flasks and put in an autoclave for 20 min. at 15 1bs/sq inch presure and 12°C temperature. On cooling, these flasks were inoculated by the spore suspension of *A. alternata* and then kept in the dark for three weeks for the production of toxins. The strains of *A. alternata* used were isolated from the known varieties of mustard and rapeseed collected from various Institutes. Only few strains isolated from the unknown varieties of mustard and rapeseed, collected from the fields and the markets, were used for this purpose.

After blending the sample with 75 ml of methanol for two minutes, the homogenate was filtered. 40 ml of the filtrate was added to 80 ml of 20% aqueous ammonium sulphate and then 90 ml was twice extracted with 5 ml of methylene chloride. The extracts were evaporated to dryness, dissolved in 1 ml methanol, and analyzed for AME, AOH, and ATX-II by thin layer chromatography [16]. For extracting TeA, aqueous solution was acidified with HCl (pH_2) and extracted twice with 50 ml methylene chloride. The combined methylene chloride extracts were washed with 25 ml water and evaporated to dryness. The residue was dissolved in 2 ml methanol for spotting. The reference standards were purchased from Sigma and the Silica gel precoated HPTLC plates (10x10 cm) were purchased from E.Merck. The developing solvents were chloroform:acetone (88:12) and toluene:ethy1 acetate:90% formic acid (60::30:10) [17].

Results and Discussion

The most dominant species of genus *Alternaria* isolated from the mustard and rapeseed crops in Pakistan were *A. alternata* with 28% frequency of occurrence in respect to all fungi, while *A. tenuissima* had 17% and rest of *Alternaria* species like *A. brassicae, A. soloni* and *A. brassicola* had combined frequency of 12% [18]. Only *A. alternata* isolates were tested for their toxic potential and found to produce high quantities of toxins. The toxicity of AME, AOH, TeA and other *Alternaria* toxins is established (6,7,), whereas AME and AOH are foetotoxic [18], AOH is photosensitizing [19] while AME is mutagenic in Ames' test [20]. Hence, this study was undertaken to see the potential of *Alternaria* species, found in our environment, for toxin production. Fifty nine isolates of *A. alternata* were tested to determine the potential.

The maximum amount of TeA produced on rice grain in vitro was 27.5 mg/kg by an isolate of *A. alternata* from Torch variety of *Brassica campstris* collected from ARI, Peshawar. The maximum amount of AME produced was 22.9 mg/kg by an isolate of *A alternata* isolated from an unknown variety of brassica collected from the city of Sialkot, out of 59 strains of *A. Alternata,* 9 did not produce any of the *Alternaria* toxins, while the remaining 50 strains produced on one or all of the five toxins were studied (Table-1).

The species of *Alternaria* including *A. alternata, A. tenuissima* and others have great ability to produce high amounts of toxins which may cause great hazards to human and animal health. As reported by other workers [21] some strains of *A. alternata* produced as high as 9800 mg/kg of this toxin on artificial rice grain culture. Considering this potential of the isolates of *Alternaria,* it is likely that these toxins are found in high amounts in mustard/rapeseed and also in other agricultural commodities around us. More crops and products are necessary to be investigated for the natural occurrence of these toxins and also more strains of *Alternaria* be screened for their potential to produce these toxins.

TABLE 1. PRODUCTION OF *ALTERNARIA* TOXINS BY STRAINS OF *ALTERNATA* ISOLATED FROM DIFFERENT *BRASSICA* SPP. IN mg/kg.

Alternaria alternata isolated from.		TeA	AME	AOH*	Alteune*	Atx-11*
1. Shariee	*B. napus*	nd	nd	nd	nd	nd*
2. P-98	*B. juncea*	2.8	0.3	+ve	nd	+ve*
3. R-18	*B. Juncea*	4.2	0.6	+ve	nd	+ve
4. Poorbi-raya	*B. Carinata*	nd	nd	nd	nd	nd
5. 3-P-269	*B. juncea*	5.4	1.2	+ve	+ve	nd
6. Altax	*B. napus*	0.3	nd	+ve	nd	+ve
7. Koral	*B. napus*	nd	nd	nd	nd	nd
8. Tower	*B. napus*	nd	0.1	nd	nd	nd
9. PNS-tall	*B. napus*	0.6	0.036	+ve	+ve	+ve
10. E-20	*B.campestris*	6.6	nd	+ve	nd	nd
11. 1245	*B.campestris*	1.0	4.4	+ve	+ve	+ve
12. PYT-14	*B. napus*	0.2	nd	nd	nd	nd
13. DESI	*B.campestria*	8.5	3.2	+ve	nd	+ve
14. P-53-48-2	*B. juncea*	nd	nd	nd	nd	nd
15. P-129	*B. juncea*	4.3	3.4	+ve	nd	nd
16. P-159/4	*B. napus*	12.5	6.8	+ve	+ve	nd
17 P-56/72	Hybrid	3.1	nd	+ve	nd	+ve
18. RC-23	Hybrid	9.5	7.3	+ve	+ve	+nd
19. RH-78	Hybrid	1.7	nd	nd	nd	nd
20. S-9 X RH-30	Hybrid	nd	0.6	nd	nd	nd
21. S-9 x RH-30	Hybrid	0.8	0.5	+ve	nd	nd
22. P-33/72	*b juncea*	10.3	4.6	+ve	+ve	+ve
23. 3-P-269	*B. juncea*	3.5	1.9	+ve	+ve	nd
24. Pela raya	*B. carinata*	11.5	7.7	+ve	+ve	+ve
25. Early Raya	*B. juncea*	11.3	5.2	nd	nd	+ve
26. Toria selection	*B. campestris*	3.7	nd	nd	nd	nd
27. D.G.L.	*B. napus*	7.5	3.1	+ve	+ve	+ve
28. Raya anmol	*B. juncea*	3.3	2.6	+ve	nd	nd
29. Brown Rayanda	*B. carinata*	9.5	nd	nd	nd	nd
30. Peela Raya	*B. arinata*	1.7	0.5	+ve	+ve	nd
31. Varuna	*B.Juncea*	1.7	0.5	+ve	+ve	nd
32. B.S.A.	*B. ampestris*	23.6	16.0	+ve	+ve	nd
33. RL-18	*B. juncea*	nd	0.3	+ve	nd	nd
34. Shiralee	*B. napus*	0.5	nd	nd	nd	nd
35. S-9	*B.Juncea*	nd	9.2	+ve	+ve	nd
36. Toria A	*B. campestris*	15.2	16.7	nd	+ve	+ve
37. SM-83000	*B. juncea*	3.7	6.0	nd	nd	+ve
38. BM-I	*B. juncea*	6.8	2.5	+ve	+ve	nd
39. P-53	*B. juncea*	nd	nd	nd	nd	nd
40. Toria	*B. campestris*	14.0	5.7	+ve	nd	nd
41. Toria composite	*B. campestris*	0.2	nd	nd	nd	nd
42. Tower	*B. napus*	nd	nd	nd	nd	nd
43. Wester	*B. napus*	9.1	4.3	+ve	nd	+ve
44. Altex	*B. napus*	4.6	20.5	+ve	nd	nd
45. Torch	*B. campestris*	27.5	7.7	+ve	nd	nd
46. Unknown Variety of	Brassica	2.6	20.7	nd	nd	+ve
47. Unknown Variety of	Brassica	7.5	nd	+ve	nd	nd
48. Unknown Variety of	Brassica	4.9	6.1	nd	nd	nd
49. Unknown Variety of	Brassica	17.6	nd	+ve	+ve	nd
50. Unknown Variety of	Brassica	2.0	22.9	nd	+ve	nd
51. Unknown Variety of	Brassica	6.3	0.8	nd	nd	+ve
52. BM-I	*B. juncea*	nd	nd	nd	nd	nd
53. P-53	*B. juncea*	11.1	10.5	+ve	+ve	nd
54. R.D-80	*B. hybcea*	18.0	nd	nd	nd	nd
55. China sarsoon	*B. chinesis*	15.2	18.6	nd	nd	nd
56. Varuna	*B. juncea*	6.5	2.6	+ve	+ve	+ve
57. ORI-50-6	*B. juncea*	nd	nd	nd	nd	nd
58. S-262	*B. campestris*	nd	nd	nd	nd	nd
59. S-28	*B. napus*	0.8	0.11	+ve	nd	nd

*Only qualitative standards were available. Tea=Tenuazonic acid. AME= Alternariol Monomethyl Ether. AOH Alternariol. ATX= Altertoxin. +Ve= Presence detected but could not be quantified because standards were not available. nd=Not detected.

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