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Effect of *Alternaria* sp on seed germination in rapeseed, and its control with seed treatment

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The role of *Alternaria* sp on seed vigor of rapeseed, *Brassica napus,* was investigated. Seed samples were collected from rapeseed growing regions of Sindh province. The samples were processed for fungal recovery using blotter paper and agar plate methods. Both methods produced a number of parasitic and saprophytic fungi. Among the recovered fungi, *Alternaria* sp was predominant with 16% average infection. Similarly, effect of seed treatment with Topsin M. was examined in this experiment. It improved seed health significantly over inoculated seeds. The treated seeds showed greater germination (16.15%) with 83.69% more healthy seedling in comparison with inoculated seeds. Root and shoot systems were significantly improved in seedlings from treated seed compared to those from inoculated seeds. The results of this study suggest grading seed samples to reduce seed impurities and seed treatment (particularly with Topsin M) to eliminate seed borne pathogen, especially Alternaria from rapeseed to enhance production by the growers.

Key words: Seed health, seed borne fungi, *Alternaria*, seed treatment.

INTRODUCTION

Seed health is a key factor in sustainable crop production. Disease free plants ensure a healthy crop stand in the field. This provides better outputs at farm level. However, seed borne pathogens are known to affect seed health and its vigor. A number of crop diseases are disseminated to distinct areas through infected seeds (Kandhare, 2014). As a result, new diseases are easily established in those environments. Seed contamination with such pathogens occurs in many ways like direct infection on the field and improper storage conditions. High relative humidity, suitable temperature and higher moisture content enhance pests and diseases establishment in seeds. Infestation by these fungi has been observed in all parts of the seeds as it harms seed tissues externally or internally causing seed rot, necrosis and seedling diseases (Anwar et al., 1994; Al Kassim 1996; Rai et al., 2015).

Pre- and post-emergence seedling diseases contribute significantly to poor crop stands. Majority of plant pathogens that attack rapeseed are seed borne in nature.

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Particularly, *Alternaria brassicola, Alternaria alternate, Curvularia lunata* and *Fusarium* spp. are of great concern. *A. brassicicola* is the most common pathogen of most crops belonging to the same group as rapeseed along with *A. alternata* (Gupta and Chaudhary, 1994). Black spot in rapeseed due to *A. brassicicola* is a seed borne disease. *Alternaria* is known to produce a number of toxins which help it to overcome the host resistance. These toxins cause tissue necrosis with cell-wall degrading enzymes (Ahmad and Sinha, 2002; Cho et al., 2016; Zaheer et al., 2015).

Therefore, early defoliation, flower-bud abortion, and premature ripening due to severe infection of these diseases results in substantial yield losses (Seidle et al., 1995; Kumar et al., 2014). Under such circumstances, 20 to 30% losses in rapeseed yields have been observed in Canada (Mc Donald, 1959; KL et al., 1990). Apart from this effect, a number of seed-borne fungi including *Aspergillus flavus*, *A. ochraceus, Rhizopus, Mucor* and *Fusarium* spp. produce mycotoxins.

Consequently, these toxins not only reduce germination but also induce biochemical changes in seeds (Kandhare, 2015; Farid et al., 2017a; b). In oilseed crops, the presence of these fungi in seed deteriorates oil and sugar contents, and their quality. Oil seed crops are very important for edible oil production worldwide. But these have not gained much importance in Pakistan. Currently, due to increased demand of good quality edible oils, special focus is being given to exploit the potential of local and exotic rapeseed germplasm. Significant achievements at national level have been made in canola types with better oil content than the indigenous material. This has increased the demand for good quality and disease-free seed of these genotypes at farm level. The present investigation was carried out on the association of *Alternaria,* and other seed mycoflora with rapeseed.

MATERIALS AND METHODS

Collection of seed samples

Seed samples of rapeseed were collected from grain markets, and farmers' lots from rapeseed growing pockets of Sindh. The samples inspected were processed for the association of seed-borne fungi followed (ISTA, 2005) by visual/dry inspection of seeds, standard blotter paper and agar plate methods and seedling assay.

Visual/dry inspection of rapeseed seeds

1 kg seed of each sample was examined visually for the presence of impurities, broken seeds or any fruiting structure. Contaminated seeds were observed under stereomicroscope for the confirmation of these impurities.

Standard blotter paper method

Seeds of each sample were placed aseptically on three layers of water soaked blotter paper. The experiment was conducted under completely randomized design (CRD) with five replications. Five seeds were placed in each Petri plate (9 cm), and three petri plate of each sample per replication were used. The plates were incubated at 25±2°C for 7 days under fluorescent light (ISTA, 1993). After incubation, seeds were examined under stereoscopic microscope. The initial growth of each fungus was purified on potato dextrose agar (PDA) medium. Percentage infection for each fungus was calculated by counting infected seed out of the total plated seeds.

Agar plate method

Potato dextrose agar medium was used for fungal recovery from rapeseed seeds. Media and glassware were sterilized at 121°C, and 15 lbs psi for 15 min. About 15 ml of sterilized PDA medium was poured in each Petri dish (9 cm) under aseptic conditions. 5 seeds of each sample were placed directly in each Petri dish containing nutrient media under aseptic conditions. The Petri plates were incubated at 25±2°C for 7 days. The experiment was conducted under CRD with five replications. Each fungus was identified on the basis of spore/ conidia formation under a compound microscope according to Booth (1971), Ellis (1971), Barnett and Hunter (2003), Subramanian (1971), Larone (2002) and Schell (2003) in addition to the use of various keys and pictograms.

Seed inoculation

Inoculum of *Alternaria* was prepared for pathogenicity test. Seeds of the test cultivars were dipped in the conidial suspension (10^6 conidia ml^{-1}) of each fungus. These seeds were sown in earthen pots containing sterilized soil. One hundred seeds of each sample were sown in earthen pots with three replications using completely randomized design. Untreated seeds sown in earthen pots served as control. Isolations were also made from the seeds that failed to germinate, and infected seedlings to re-isolate the fungus.

Effect of fungicides dressing on seed-borne mycoflora of rapeseed seeds

Composite seed samples of each rapeseed sample were treated with thiophenate methyl (active ingredient Topsin-M) at 2.5g/ Kg seed. The treated seeds were sown in earthen pots containing sterilized soil under greenhouse condition. The seeds without fungicides treatment served as control. Germination in treated and non treated seeds was recorded after 7 days. Similarly, the germination % rotted seeds, abnormal seedlings and root shoot length were calculated.

RESULTS

Association of various seed borne fungi with rapeseed seeds

The presence of seed impurities such as fruiting bodies, dead and dry seeds were examined visually using magnifying glasses for estimation. All the samples showed the presence of these impurities. However, the overall proportion of seed debris was significantly higher than the broken seeds. Straw, dust particles, and seeds of other crops were the main debris in all samples.

These were less in sample numbers 17 and 18 while

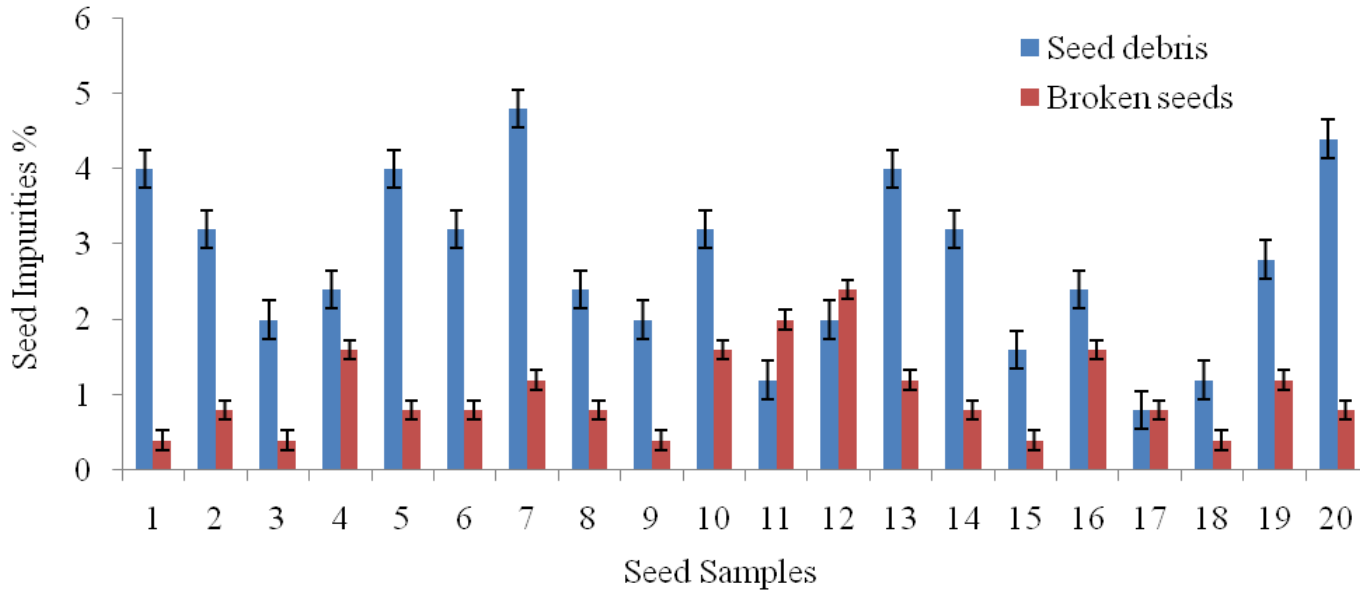

Figure 1. Association of impurities in seed samples.

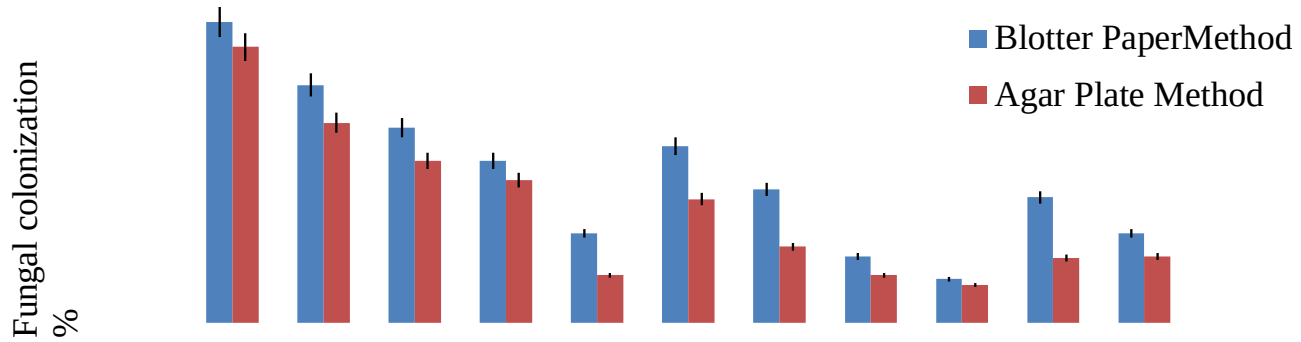

Figure 2. Fungal colonization with seed samples of Rapeseed

their percentage was higher in samples 1, 7, 13 and 20. Broken seeds and mummies were observed in all samples. These may result during postharvest or some other physical damages (Bux et al., 2013). These are not only the cause of disease spread but also provide a good shelter for overwintering and survival of pathogens (Figure 1).

Blotter paper and agar plate methods were used for the recovery of *Alternaria* from seed samples. The results revealed that its colonization was significantly higher in blotter paper (Figure 2). In order to find and examine the maximum fungal population, seed blotter paper method was seen to be very effective (Elwakil and Ghoneem, 1999; Walcott, 2003). This provided excellent conditions for mycelial growth and sporulation of several fungi (Neergaard, 1979). Morphological characteristics were identified according to Larone (2002) and Schell (2003). Apart from *Alternaria*, a number of other fungi were also observed. The present results are also in conformity with Saleh et al. (2003), who have reported the prevalence of various seed borne fungi in rapeseed. *Alternaria* sp is responsible for leaf spot disease in oil seeds and also for loss of seed viability (Malaker et al., 2008).

Effect of culture filtrate of *Alternaria* and fungicide dressing on seed health of rapeseed

The results of this study revealed significant differences in seed germination, number of abnormal seedling and root shoot length in inoculated and treated seeds. The seedlings of the treated seeds (Table 2) were healthy

Table 1. Effect of culture filtrate of *Alternaria* on seed health of rapeseed.

Seed sample	% Germination	Rotten seeds	Abnormal seedlings	Root length(cm)	Shoot length(cm)
1	77.00^{BCDEF}	6.6667^{CDE}	8.67^{EF}	4.33^{CD}	4.33^{ABCDEF}
2	77.67^{BCDE}	7.3333 ^{CDE}	12.00^{BC}	4.00 ^{CDE}	4.33^{ABCDEF}
3	74.67^{EFGHI}	8.00^{BCDE}	10.67^{CDE}	2.67^{FGH}	3.00^{EF}
4	78.00^{ABCD}	6.67^{CDE}	12.67^{ABC}	3.33^{DEFG}	3.67^{CDEF}
5	73.67^{GHI}	8.33^{BCDE}	12.00^{BC}	2.33 ^{GH}	3.33^{DEF}
6	79.33^{AB}	5.00^{E}	14.33^{A}	3.67^{DEF}	4.67^{ABCDEF}
7	75.33^{DEFGHI}	7.00^{CDE}	14.00^{AB}	3.33^{DEFG}	3.00^{EF}
8	76.67 ^{BCDEFG}	9.67^{ABC}	12.00^{BC}	2.00^{H}	2.67^{F}
9	74.67^{EFGHI}	8.67^{ABCD}	10.67^{CDE}	6.00^{B}	5.00^{ABCDE}
10	79.33^{AB}	9.67^{ABC}	9.67^{DE}	3.67^{DEF}	2.67^{F}
11	79.00^{ABC}	11.33^{AB}	7.00^{F}	3.00^{EFGH}	3.33^{DEF}
12	72.67^{I}	12.00^{A}	12.67^{ABC}	7.33^{A}	5.33^{ABCD}
13	75.67^{DEFGHI}	8.67^{ABCD}	11.00^{CD}	6.00^{B}	4.0^{BCDEF}
14	76.00^{CDEFGH}	7.67^{CDE}	14.00^{AB}	3.67^{DEF}	4.33^{ABCDEF}
15	77.00^{BCDEF}	7.00^{CDE}	11.00^{CD}	6.00^{B}	6.33^{A}
16	81.0^{A}	5.67^{DE}	9.00^{DEF}	2.67^{FGH}	3.00^{EF}
17	76.67^{BCDEFG}	8.33^{BCDE}	11.00^{CD}	5.00^{BC}	6.00^{AB}
18	75.33^{DEFGHI}	11.33^{AB}	12.67^{ABC}	7.33^{A}	5.67^{ABC}
19	73.00^{HI}	12.00^{A}	13.33^{AB}	5.00^{BC}	5.33^{ABCD}
20	74.33^{FGHI}	7.00^{CDE}	12.33^{ABC}	4.00 ^{CDE}	4.00^{BCDEF}
S^{E}	1.5951	1.7826	1.1304	0.6498	1.0853
LSD	3.2239	3.6028	2.2846	1.3133	2.1934

with proper root and shoot. No abnormal symptoms were observed. However, in inoculated seeds (Table 1), less seed germination and more abnormal seedlings with reduced root and shoot system were observed. Seed mycoflora is reported to reduce seed germination thus causing pre- and post- emergence seedling death. Shrestha et al. (2000) in a pathogenicity study observed symptoms of *Alternaria* in rapeseed seedlings. Similarly, the infection deteriorates seed health and reduces germination (Ismail et al., 2004; Anwar et al., 1994; Elwakil and Ghoneem 1999). Nowadays, chemical seed treatment is very common worldwide due to its wide spectrum ability to control plant diseases (Nameth, 1998). In our farming system, this could not get much importance, there is need to educate farmers about seed treatment.

DISCUSSION

The present work revealed the occurrence of various impurities in all collected samples. Among the twenty rapeseed samples, only a few showed fewer impurities (Figure 1). Presence of such impurities affects seed health and marketing. Among the methods of fungal detection from rapeseed blotter paper method, agar plate was found to be very effective. *Alternaria* was the most predominant fungus with an average infection of 16% in all samples among the isolated fungi.

The location of *Alternaria* in seed is very important. It mostly resides in seed coat and sporulates in the hilum Region (Shrestha et al., 2003; Walcott 2003). Seed infection transmits *Alternaria* to seedlings. Plant pathogenic *Alternaria* sp produces host specific toxins, and use secondary metabolites in disease initiation, apart from toxins, certain lipase and cell wall-degrading enzymes contribute significantly in its pathogenesis (Chao, 2016; Rathod, 2012).

Seed borne fungi are responsible for mycotoxin production in seeds, and induce biochemical changes resulting in damaged seed contents (Swami and Alane, 2013). As a result, quality of oil contents in seed is affected (Wani et al., 2012).

The role of *Alternaria* in seed germination was also investigated in this study. Reduced seed germination was observed in all samples. The important symptom found were seed rot, lesion on seedling and reduced root shoot system. Successful re-isolations were made from infected seedlings and rotted seeds. These results are in conformity with Shrestha et al. (2005) who observed that lesions on cotyledonary and first true leaves were due to seed-borne inoculum. Adverse effects of mycotoxins which inhibited the growth of seedlings have been reported by Howlett (2006). Impact of fungicides treatment on fungal population was also investigated in this study.

Significant difference was noticed in seed germination

Table 2. Effect of fungicide dressing on seed health of Rapeseed.

Treated with Topsin.M (2.5g/kg seeds)					
Seed samples	**% germination**	**Rotted seeds**	**Abnormal seedlings**	**Root length (cm)**	**Shoot length(cm)**
1	94.33 ^{A}	4.33 ^{E}	1.00 ^{D}	7.33 ^{AB}	12.0 ^{ABCD}
2	91.00 ^{DEFG}	5.67 ^{CDE}	2.00 ^{C}	7.00 ^{AB}	12.0 ^{ABCD}
3	90.33 ^{DEFGH}	8.33 ^{AB}	2.00 ^{C}	7.67 ^{AB}	11.33^{ABCDE}
4	89.67 ^{FGH}	9.33 ^{A}	1.00 ^{D}	7.33 ^{AB}	10.00^{DE}
5	90.67 ^{DEFGH}	7.33 ^{ABCD}	2.00 ^{C}	7.33 ^{AB}	9.67^{E}
6	91.67 ^{BCDEF}	7.67 ^{ABCD}	0.67 ^{E}	7.67 ^{AB}	11.00^{BCDE}
7	91.67 ^{BCDEF}	8.00 ^{ABC}	1.00 ^{D}	7.33 ^{AB}	9.67 ^{E}
8	92.00 ^{BCDE}	6.00 ^{BCDE}	2.00 ^{C}	7.67 ^{AB}	11.67^{ABCDE}
9	92.33 ^{ABCD}	6.33 ^{BCDE}	3.00 ^{B}	7.67 ^{AB}	12.33^{ABC}
10	93.333 ^{ABC}	6.00 ^{BCDE}	1.00 ^{D}	7.33 ^{AB}	13.00 ^{AB}
11	88.67 ^{H}	7.00 ^{ABCD}	3.00 ^{B}	6.67 ^{B}	11.33^{ABCDE}
12	89.00 ^{GH}	7.67 ^{ABCD}	3.00 ^{B}	8.00 ^{AB}	12.33^{ABC}
13	90.00 ^{EFGH}	5.33 ^{DE}	4.00 ^{A}	7.33 ^{AB}	10.67 ^{CDE}
14	92.00 ^{BCDE}	5.67 ^{CDE}	1.00 ^{D}	6.67 ^{B}	10.33 ^{CDE}
15	90.67 ^{DEFGH}	6.33 ^{BCDE}	3.00 ^{B}	7.67 ^{AB}	10.33^{CDE}
16	93.67 ^{AB}	6.33 ^{BCDE}	1.00 ^{D}	8.67 ^{A}	10.67^{CDE}
17	93.667 ^{AB}	6.33 ^{BCDE}	1.00 ^{D}	8.00 ^{AB}	10.67^{CDE}
18	93.333 ^{ABC}	7.00 ^{ABCD}	2.00 ^{C}	8.00 ^{AB}	12.00 ^{ABCD}
19	91.33 ^{CDEF}	7.00 ^{ABCD}	2.00 ^{C}	7.33 ^{AB}	12.00^{ABCD}
20	89.67 ^{FGH}	7.67 ^{ABCD}	2.00 ^{C}	7.00 ^{AB}	13.33 ^{A}
SE	1.1255	1.2472	0.1054	0.8756	1.0853
LSD	2.2746	2.5207	0.2130	1.7696	2.1934

Table 3. Comparison of seed treatment and seed inoculation**.**

Seed health parameter	Treated	Inoculated	% improvement
Germination %	91.45	76.35	16.15
Rotted seed	6.89	8.4	17.97
Abnormal seedlings	1.88	11.53	83.69
Root length	7.48	4.26	43.04
Shoot length	11.32	4.19	62.98

and seedling health of treated and untreated seed (Table 3). The seeds treated with Topsin M showed maximum germination with healthy seedlings. These results are in accordance with Bhuiyan et al. (2013) and Sharma et al., (2015) who have observed better seed germination in fungicide treated seed. Sinha and Prasad (1981) reported less seed germination as a result of *Alternaria* infection. In order to reduce such infection, improving seed treatment is an effective method (Anjorin and Mohammad, 2014; Naher et al., 2016).

This does not only reduce seed borne infections but also protects the emerging seedlings from fungal infection (Kamal and Verma, 1987). As a result, pre- and post- emergence seedling mortality are reduced. According to Chilkuri and Giri (2014), seedling blight was due to seed borne fungi infection. The present work highlights the association between *Alternaria* and rapeseed. From the aforementioned, it can be concluded that blotter paper is an effective method in detecting seed borne infection such as *Alternaria*.

Seed borne fungi reduces seed germination (as is evident from the present results) therefore, seed treatment is recommended. It is also suggested that awareness must be created among farmers about seed treatment so that they can protect their crops from these pathogens. Foliar application of fungicides is an important component of disease management in plants. Therefore, judicious use of these fungicides especially, Topsin M, is recommended to overcome *Alternaria* diseases in rapeseed crop.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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