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The Effect on Okra Seed Germination using Filtrates of Isolated Pathogenic Fungi from Black Point Infected Wheat Grains

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ABSTRACT: The effect of eight fungi filtrates (isolated from infected black point wheat grains) was carried out in 2020 on healthy seeds of okra (*Abelmoschus esculentus*) at Baba Farid College, Bathinda, Punjab. The total four concentrations i.e. 25, 50, 75 and 100% of twelve day old culture filtrates of *Alternaria alternata, Aspergillus niger, Rhizopus* spp., *Helminthosporium*s spp., *Curvularia* spp., *Penicillium* spp. and *Fusarium* spp. were used for present studies. These overnight pre-soaked seeds of okra were planted in pots under green house condition. Total three replications were used and each pot was having 12 seeds. Further the germination percentage of okra seeds was recorded. The result revealed that there was significantly reduction in percentages seed germination was observed as the concentration was increased. The maximum reduction of germination was recorded at 100% concentration in which *Helminthosporium* spp. (38.8±0.6) was showing maximum germination followed by *Rhizopus* spp. (30.3±0.8), *Curvularia* spp. (28.3 ±0.6), *Alternaria alternate* (28.0±0.5), *Aspergillus niger* (22.2±0.8), *Fusarium* (21.3±0.6), *Penicillium* spp. (19.0±0.5), *Aspergillus flavus* (16.7±0.5). Therefore all above fungus were showing phytotoxic effect on germination of okra seeds. Hence, this study is more beneficial to the farmers and growers to assist and encourage replacing infected seeds to reduce the initial inoculums to minimise the amount of crop quality because it is seed borne and post-harvest disease.

Keywords: Black point, Culture filtrates, Okra seeds, Pathogenic fungi, Seed germination, Wheat

INTRODUCTION

Wheat is most important cereal crops in world (Gurjar *et al.,* 2016). It is cultivated on 31.6 million hectare area with total production of about 108 million metric ton (MMT) (GAIN, Global Agricultural Information Network 2021). It is basic component of food for humans and livestock. It is consumed throughout the world in many forms, such as Bread, Cookies, Cakes, Pies, Pastries, Cereals, Crackers, Pasta and Noodles. After China, India is the second largest producer of wheat in the world (Sharma and Sendhil, 2016). These productions were affected by many factors out of which about 20% of the global wheat production is lost due to diseases caused by Fungus, Bacteria, Virus, Phytoplasma and Nematodes (Agrios, 2005). The diseases cause reduction in the crop yield and quality of the grains (Tripathi *et al.,* 2013). Among many post-harvest diseases, Black point (BP) is considered as emerging problem in wheat (Kaur *et al.,* 2020). BP disease of wheat is also known as kernel smudge, predominately caused by *Alternaria, Cochliobolus, Fusarium, Cladosporium, Curvulavia, Penicillium, Aspergillus* and *Stemphylium* genera (Ramires *et al.,* 2018). These fungi in favourable conditions produce mycotoxins and remains in the seeds and soil.

Many researchers reported accumulation of many mycotoxins in Black point infected kernels (Jahani *et al.,* 2013; Desjardins *et al.,* 2007). The mycotoxins, not only causing productive and economic losses but also could lead to a serious risk for human and animal consumption, due to their toxic effects on several biological activities (Almoammar *et al.,* 2014; Arcella *et al.,* 2016; Divakara *et al.,* 2017).

Hence in the current studies was conducted to record the effect of cultural filtrate of black point associated fungi on germination of okra seeds.

MATERIALS AND METHODS

The present study was conducted at Baba Farid College, Bathinda, Punjab.

Isolation and Identification of mycoflora associated with Black Point infected wheat:

Black Point kernel Seeds were surface sterilized with 1% sodium hypochloride (NaOCl) solution for 3 min, followed by several rinses in sterile distilled water and dried on filter paper (Mittal *et al.,* 1998). Agar Plate Method was used for detection of Black Point causing fungi. Potato dextrose agar (PDA) was used in this method for the isolation of mycoflora. Every five petriplates were containing 10 infected wheat seeds. These petri dishes were then incubated at 22 ± 2°C for 7 days under alternating cycle of 12 hours fluorescent light and constant darkness. Each experiment was repeated 3 times. After 7 days, these incubated Petri

dishes were examined under stereo-binocular microscope. The grown mycoflora was sub-cultured by using single spore technique on PDA slants and observed for fungal growth. Further identification of fungi was done with the help of keys, monographs and text provided by several authors (Pedro *et al.*, 2009). The frequency of the fungus was calculated by the following formula (Pathak & Zaidi, 2013):

$$\text{Frequency of the Fungus} = \frac{\text{No. of seeds containing a particular fungus}}{\text{Total seed used}} \times 100$$

Eight fungal species culture obtained from sub culture were grown in 250 ml of Erlenmayer conical flask contain 100 ml potato dextrose broth (PDB) for 12 days on rotary shaker (LM-450D) at 27±2°C. At the end of incubation period, cultures were filtered through Whatman filter paper no. 1 and centrifuged at 12,000 g to get cell-free filtrates.

Evaluation of phytotoxic effect on okra (*Abelmoschus esculentus*) seeds using filtrate obtained from isolated fungi associated with Black point:

To evaluate the phy-totoxic effect of above filtrates isolated from associated black point fungi the Okra seeds were used (El-Akkad, 1982).

These filtrates were diluted with distilled water to prepared 25, 50, 75 and 100 % concentration. Further 100 healthy surface sterilized okra seeds were suspended in each 50 ml of culture filtrates and incubated at 27±2°C for 24 hours. After this the seeds were then sown in sterilized plastic pots (7.5 cm × 6.5 cm), filled with autoclaved soil. Okra seeds soaked in un-inoculated PDB were served as control. About twelve seeds were grown in each pot. Three pots were used for each treatment. All the pots were maintained under the greenhouse conditions at 25°C for 2 weeks, after which the germination percentage was, calculated (Habib *et al*., 2011).

RESULTS AND DISCUSSION

A. Isolation and Identification of mycoflora associated with Black Point infected wheat

The result revealed that total of thirteen fungal species representing 8 fungal genera were isolated and identified with the help of keys, monographs and text provided by several authors (Pedro *et al.*, 2009). The culture of 8 fungal genera including including *Alternaria* spp. (Plate 1), *Aspergillus flavus* (Plate 2), *Aspergillus niger* (Plate 3), *Bipolaris sarokiniana (Helminthosporium sativum)* (Plate 4), *Curvularia lunata* (Plate 5), *Fusarium oxysporum* (Plate 6), *Penicillium* spp. (Plate 7) *Cochliobolus sativus* (Plate 8) and *Rhizopus stolnifer* (Plate 9) observed on PDA media.

The sub-cultured of the above selected fungi was done by using single spore technique on PDA slants which leads to growth of *Alternaria alternate* (Plate 10), *Aspergillus flavus* (Plate 11), *Aspergillus niger* (Plate 12), *Helminthosporium sativum* (Plate 13), *Curvularia lunta* (Plate 14), *Fusarium* spp. (Plate 15), *Pencillium* spp.(Plate 16), *Cochliobolus sativus* (Plate 17) and *Rhizopus* spp. (Plate 18).

These further identified was done by using following characterises: conidia with multicelled transverse and longitudinal septa of *Alternaria alternate* (Slide 1), *Alternaria tenius* (Slide 2) attach with mycelium, Spores of *Aspergillus flavus* attach with mycellium observed (Slide 3), *Aspergillus niger* (Slide 4), *Aspergillus fumigatus* (Slide 5), conidia of *Helminthosporium sativum* (Slide 6), *Curvularia luanata* (Slide 7), Mycelium of *Fusarium oxysporum* (Slide 8), conidia of *Fusarium oxysporum* (Slide 9), *Pencillium notatum* (Slide 10), *Pencillium chrysogenium* (Slide 11), Conidia of *Fusarium oxysporum* (Slide 12), Zygosporangium structure of *Rhizopus* also observed (Slide 13).

B. Evaluation of phytotoxic effect on okra (Abelmoschus esculentus) seeds using filtrate obtained from isolated fungi associated with Black point

The phytotoxic effect on okra revealed that all eight fungal filtrates (Plate 19) extracted from Black point associated fungi significantly reduced seed germination when compared with control (Plate 20). *Helminthosporium* spp. (38.8±0.6) was showing maximum germination followed by *Rhizopus* spp. (30.3±0.8) *Curvularia* spp. (28.3 ±0.6), *Alternaria alternate* (28.0±0.5), *Aspergillus niger* (22.2±0.8), *Fusarium* spp. (21.3±0.6), Penicillium spp. (19.0±0.5), *Aspergillus flavus* (16.7±0.5) at 100% germination. At 25 percent the maximum germination was observed in *Helminthosporium* spp. (75.0±1.0), followed by *Curvularia* spp. (72.0±0.5), *Penicillium* spp. (61.6±0.8), *Alternaria alternate* (58.0±0.0), *Fusarium* spp. (56.3±0.8), *Aspergillus niger* (54.3±0.3), *Rhizopus* spp. (53.0±0.5), and *Aspergillus flavus* (50.0±1.0), respectively (Table 1). Therefore result revealed that there was gradual decrease in the germination percentage as the concentration of the filtrate increased from 25, 50, 75 and 100 percent. Hence all above fungus were showing phytotoxic effect on germination of okra seeds.

Many research scientists in past also reported the toxic effect of fungi. Ismaiel and Papenbrock (2015) reported the presence of *Fusarium* and *Pencillium* spp. which produces fungal toxin. The toxic effects of mycotoxins on plants by interference with chlorophyll synthesis was also reported (Jeswal and Kumar, 2015; Divakara *et al.*, 2017). Similar result was observed in present study where after the treatment of okra seeds by the filtrate of *Aspergillus flavus* and *Aspergillus niger* causes chlorosis (Plate 21). Shirurkar and Wahegaonkar (2012) reported the toxic product of *Aspergillus flavus* as Aflatoxin.

Kabak *et al.*, (2006) identified 300 to 400 mycotoxins from 100 species of fungi that can infect plants and produce mycotoxins. Among these genera, *Fusarium*, *Alternaria*, *Aspergillus*, *Penicillium*, *Cochliobolus* and *Bipolar issorokiniana* are especially worrisome since they are able to produce mycotoxins. These toxic secondary metabolites can accumulated in colonized tissues (Perrone *et al.*, 2007).

These fungal species and production of mycotoxins contaminate harvested seeds causing losses of agricultural commodities 3.71-12.49% per annum in many zones of the world (Koteswara, 2014).

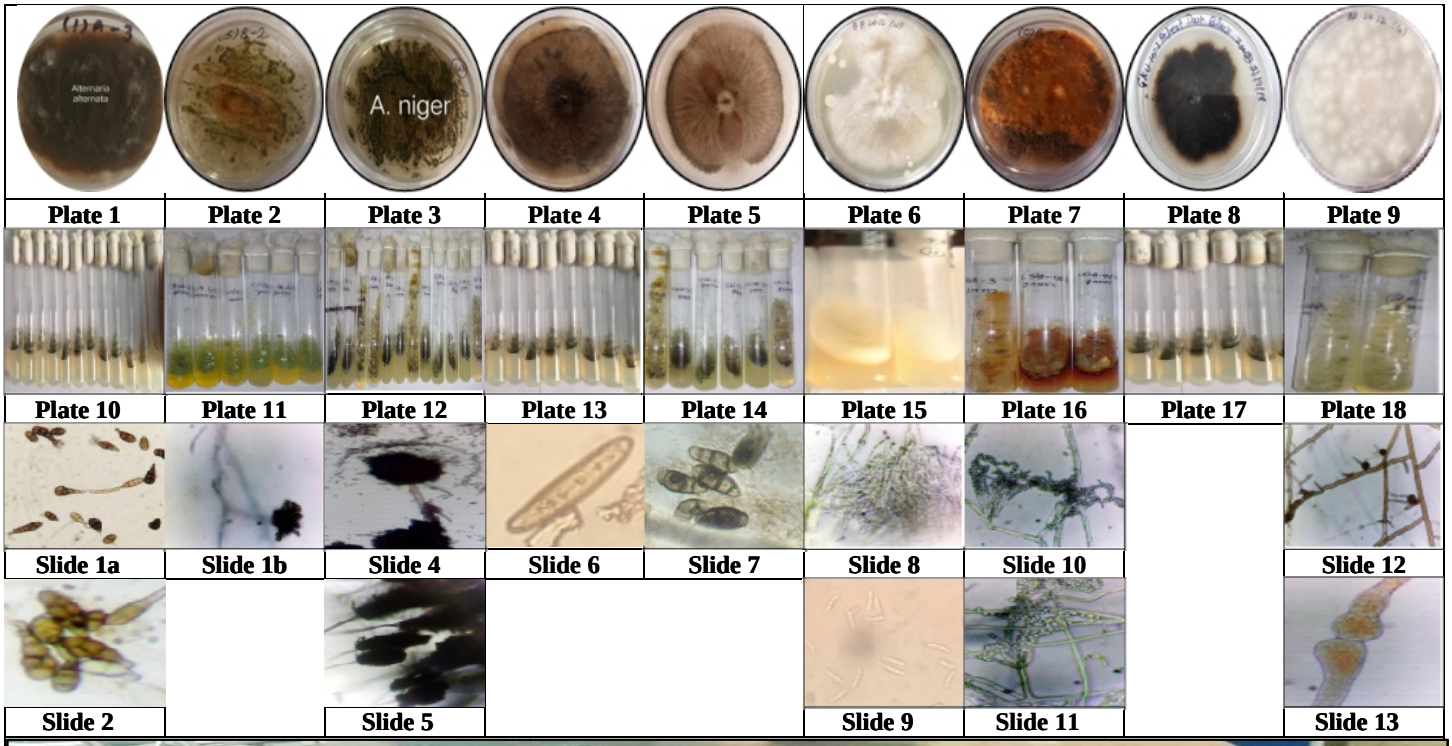

Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9
Plate 10	Plate 11	Plate 12	Plate 13	Plate 14	Plate 15	Plate 16	Plate 17	Plate 18
Slide 1a	Slide 1b	Slide 4	Slide 6	Slide 7	Slide 8	Slide 10		Slide 12
Slide 2		Slide 5			Slide 9	Slide 11		Slide 13

Plate 19

Plate 20: Effect of okra seeds in various concentrations of culture filtrates of seed -borne fungi on the percentage of seed germination.

Plate 21: Mycotoxin bioassay using okra seedlings, the leaves infected with fungal have chlorosis.

Table 1: Effect of okra seeds in various concentrations of culture filtrates of seed -borne fungi on the percentage of seed germination (2019- 2020).

Fungal cultural filtrate	Seed germination (%)				
	0.0	25	50	75	100
Aspergillu sniger	97.2±0.3	54.3±0.3	44.6±0.8	39.6±0.3	22.2±0.8
Fusarium spp.	97.2±0.3	56.3±0.8	46.6±0.3	25.0±0.5	21.3±0.6
Pencillium spp.	97.2±0.3	61.6±0.8	50.0±0.5	28.0±0.0	19.0±0.5
Rhizopus spp.	97.2±0.3	53.0±0.5	43.0±0.0	25.0±0.5	30.3±0.8
Aspergillus flavus	97.2±0.3	50.0±1.0	41.3±0.6	19.0±0.0	16.7±0.5
Curvularia spp.	97.2±0.3	72.0±0.5	53.0±0.5	36.1±0.6	28.3±0.6
Alternaria alternate	97.2±0.3	58.0±0.0	47.3±0.3	28.0±0.5	28.0±0.5
Helminthosporium spp.	97.2±0.3	75.0±1.0	55.6±0.5	41.7±0.3	38.8±0.6

CONCLUSION

The present findings are significant are common in agricultural products like cereals, pulses, oil seeds, feeds (Fakhrunnisa *et al*., 2006). The current results are positively correlated with Narasimha Rao *et al.*, (2006), reported reduction in the germination when treated with isolated filtrate of different pathogen. Consumption of wheat invaded by these myco-toxins indicates a potential risk for contamination and hazardous for human health (Gautam and Bhadauria, 2009; Masiello *et al.*, 2020). However, consumers demand food and feed productions with a high qualitative standard to guarantee health of both human and other animals.

Hence, this present finding is more significant and beneficial to the farmers as well as to the grower which should assist in managing risk and will encourage them for replacement of infected seeds to reduce the initial inoculums to minimise the amount of crop quality.

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