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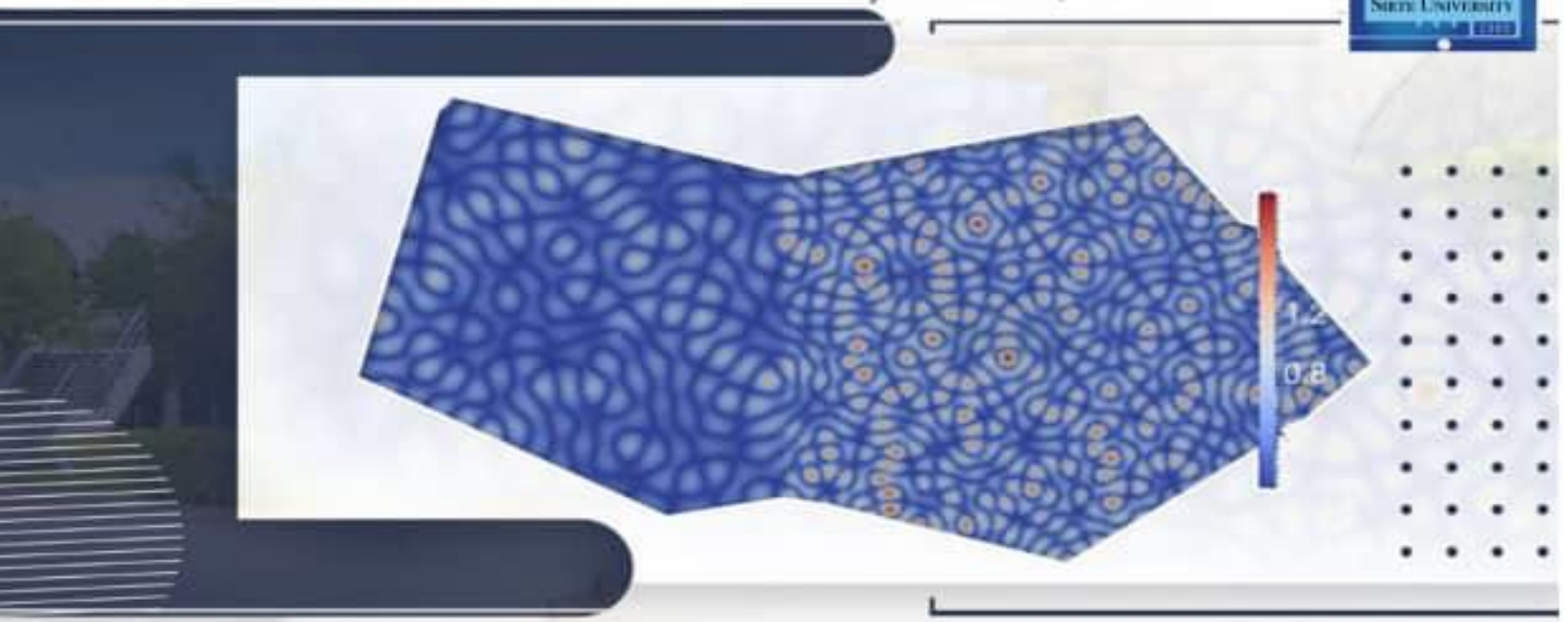

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Mycoflora Associated with Barely Grains (*Hordeum vulgare* L.) in the Eastern Parts of Libya

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A B S T R A C T

During the harvest season (2019-2020), local variety of barley seeds (*Hordeum vulgare* L.) were collected from three different locations, Almerj, Gerdina, and Sultan; situated in the eastern part of Libya. The present experiment was performed to identify, and compare natural mycoflora associated with barley seeds among these locations, also to evaluate sodium hypochlorite's application, as seed disinfectants against fungal contaminators. Barley seeds were surface disinfected with a 4% sodium hypochlorite for two minutes or washed only with deionized water (control) before plating on potato dextrose agar (PDA) medium. The following parameters were recorded and calculated: seed survival (G %), fungal frequency (F), isolation frequency (IF) and fungal relative abundance (RD). Four fungal species were identified as *Aspergillus niger* van Tiegh, *A. flavus* Link (ascomycetes), *Rhizopus stolonifera* (Ehrenb. :Fr.) Vuill (zygomycetes), and *Bipolaris australiensis* (Bugnicourt) (ascomycetes). The most predominant recovered species was *A. niger* followed by *B. australiensis*, *R. stolonifera*, and *A. flavus*, (18.5%), (9.67%) (2.84%), (4.65%), respectively. Results also showed Sultan had the highest seed germination, followed by Almerj and Gerdina, 64.5 %, 44.5%, and 20.5 %, respectively. Moreover, seed's pretreatment with sodium hypochlorite and seed's origin had no significant effect on frequency and relative abundance of fungi.

1 Introduction

Barley (*Hordeum vulgare* L.) has been a central and staple commodity crop in Libya. Barley usages include its grinded flour for making Bazine, a famous traditional Libyan cuisines, and bread. Furthermore, barley grains and hay are used extensively for feeding livestock, and malting. Libya's production of barley was significantly low compared with other neighboring countries' yields, 260 thousand tons in 2005, therefore, the country relies completely on importing barley seeds from foreign market (Elbeydi *et al*., 2007).

Barley seed quality and nutritional reduction can be substantially deteriorated by the presence of mycotoxins which are produced by filamentous fungi. In a study conducted on stored barley grains in Spain,

Aspergillus niger was reported to produce a list of toxins, for example, aflatoxin B1 (AFB1), B2 (AFB2), and ochratoxin A (OTA)(Mateo *et al*., 2011). Aflatoxin is considered the most studied fungal toxin that affect human health and livestock alike. Prolonged human consumptions of aflatoxin result in adverse implications on human wellbeing including liver damage and digestion difficulty (Sarma *et al*., 2017& Williams *et al*., 2004). Therefore, the current study aimed to isolate and identify natural barley seed mycoflora in three different locations; and whether or not presoaking seeds with sodium hypochlorite can reduce fungal contamination.

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2 Materials and Methods

2.1 Site description:

Three locations were selected to conduct the present study namely, Almerj, Gerdina, and Sultan, which were chosen based on their high yield per hectare.

2.2 Sampling:

Barley seeds were collected after the harvesting season (2019-2020), transported in plastic bags, and kept at laboratory temperature. From each site, we randomly collected 1.0 kg of seeds. Of 1.0 kg, four-hundred seeds were randomly chosen and divided equally into two groups (i.e., each group had 200 seeds) for further analysis. The first seed group rinsed thrice with only deionized water (untreated), while the second group was disinfected externally with 4% sodium hypochlorite , for a period of two minutes(treated). Seeds were dried out with paper towel prior to placing them on an artificial growth medium. Each seed treatment had ten plates, therefore, the total number of plates was 60 plates. The total number of barley seeds was 600 seeds. Following the rules of International Seed Testing Association(ISTA) on seed health, ten barley seeds were picked with sterilized forceps and transferred to sterilized Petri dish (90 mm,16 mm, Bibby Sterilin Ltd, UK) equidistantly containing potato dextrose agar (PDA) medium (Oxoid Ltd, UK), amended with amoxicillin (0.5 g/ L) to eliminate bacterial growth . The plates were incubated for one week at 25°C with 12 hours photoperiod cycle, and screened for their fungal compositions.

Seed germination percentage, fungal frequency, and relative abundance of fungi were calculated by the following formulas described by Marasas (1988):

Germination percentage

$$(G\%) = \frac{\text{Numebr of seeds germinated}}{\text{Total number ofseeds}} \times 100$$

Fungal frequency

$$(F\%) = \frac{\text{Number of seeds containing a particular fungi}}{\text{Total number of seeds}} \times 100$$

Relative abundance

$$(RF\%) = \frac{\text{Total number of a fungus on seed}}{\text{Total number of colonies of all fungi}} \times 100$$

2.4 Fungal identification:

A sterilized needle was used to obtain fungal fragment from Petri dish. Fungal tissues were placed on clean microscopic slide (90 mm,16 mm, Bibby Sterilin Ltd, UK) and stained with the commonly used lactophenol cotton blue stain. Fungal spores were photographed with a fixed camera attached to a compound microscope at 200 x magnification (Olympus microscope camera, Japan).

Fungal key identification texts such as(Dugan, 2006, Navi et al., 1999, & Robinson, 2011)were consulted for aiding in accurate identification.

2.3 Statistical analysis:

Complete randomized block design (CRBD) was used in the experiment. The investigated parameters were normally distributed with *Shapiro-Wilk* test. Differences in means and standard deviations were calculated with two-factorial ANOVA (*P* values <0.05). Means were compared using Fisher's protected LSD test. Data analyses were performed using R statistical software (R Core Team, 2019). Graphics illustrated here were done with ggplot – a package in R (Wickham, 2016).

3 Results

3.1 Barley seeds fungal compositions:

In this study, we identified four fungal species based on colony color, and conidial shape on growth medium. The species were *Aspergillus niger* (Fig.2.1), *A. Flavus* (Fig.2.2), *Biboplaris australiensis* (Fig.1.3), and *Rhizophus stolonifera* (Fig.2.4). *A. niger* was morphologically identified based on the presence of black colony color, and microscopic characters such as conidia and conidiophore (fig.2.1).

3.2 Effect of seed treatment and locations on seed germination (G%):

Sultan had the highest seed germination in comparison with Almerj and Gerdina. Moreover, seed germination was significantly higher in Almerj than in Gerdina (*P*< 0.05) as illustrated in Fig 1. Treated seeds with sodium hypochlorite had no effect on seed germination (*P* = 0.7781).

3.3 Effect of seed treatment and locations on fungal frequency (F%), and relative abundance (RD%):

In contrary to the seed germination, both treatment and location had no significant impact on F, and RD (Fig.3,

4.). It has been noted that *A. niger* was the predominant recovered species in the unsterilized treatment, but the species was not dominant in sterilized treatment. Moreover, *R. stolonifera* was higher in sterilized seeds than unsterilized seeds (Fig.3,4.).

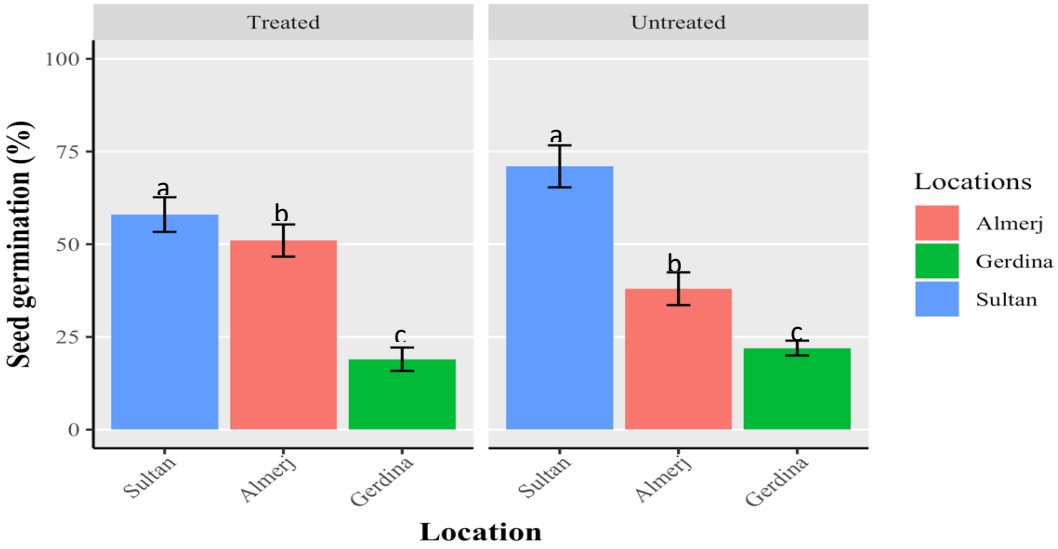

Fig.1. Effect of location and seed treatment on barley seed germination. Barley seeds were collected from three locations, Almerj, Gerdina, and Sultan. Seed treatments were barley seeds disinfected with 4 % NaClO, whereas untreated seeds were only rinsed with deionized water. Bars with the same or without letters are not statistically different according to a F-protected LSD (*P*=0.000002)

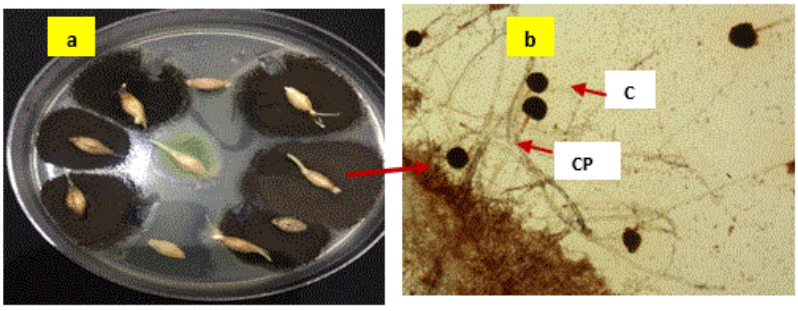

Fig.2.1 a) *A.niger* colony on PDA, b) microscopic photo of *A.niger*. Scale bar=200x**C**: conidia, **CP**: conidiophore.

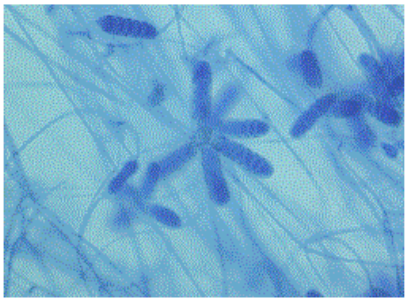

Fig.2.3 Microscopic photo of *B. australiensis* morphology.

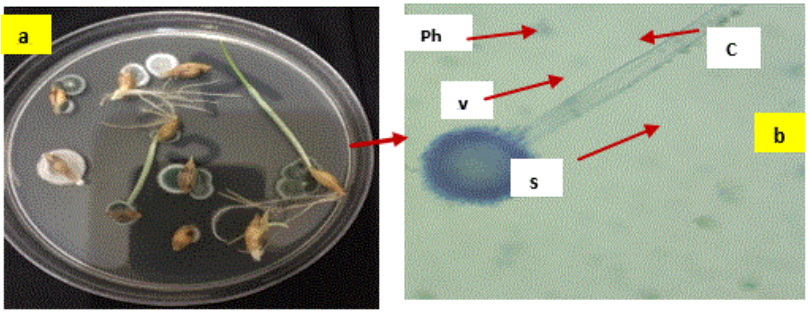

Fig.2.2 a) *A. flavus* colony on PDA, b) microscopic photo of *A. flavus*. Scale bar=200x **C**: conidia, **Ph**: phialides, **S**: stipe, **V**: vesicle.

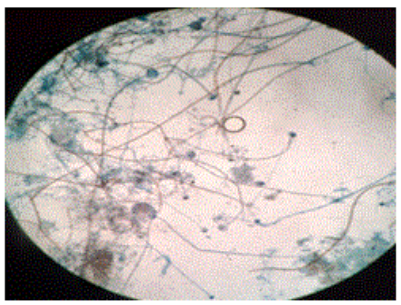

Fig.2.4 Microscopic photo of *R. stolonifera* morphology.

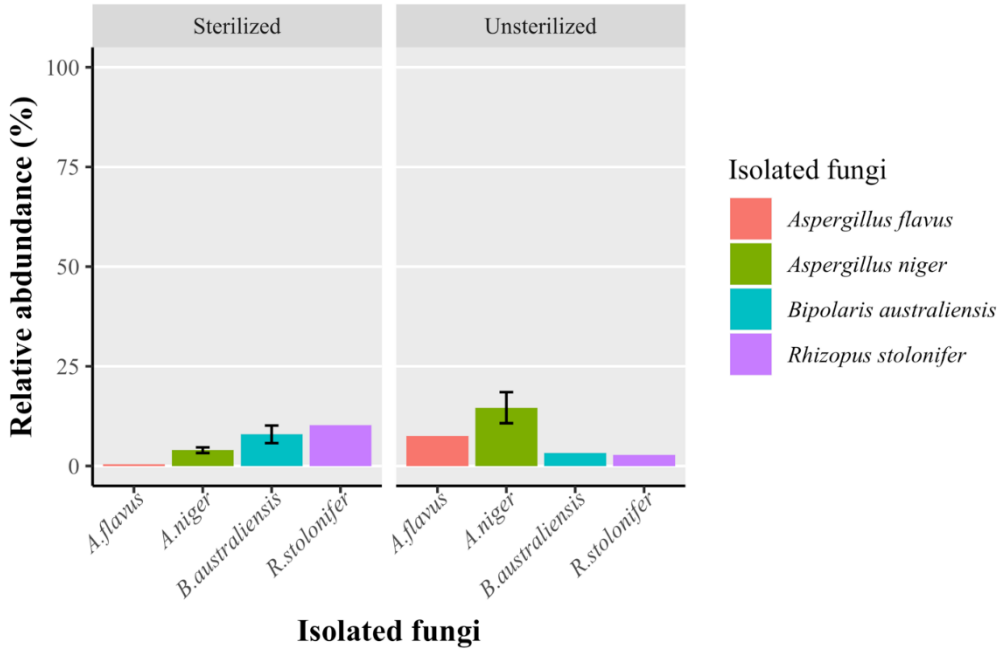

Fig. 3. Effect of location and seed treatment on relative abundance (RD). Barley seeds were collected from three locations, Almerj, Gerdina, and Sultan. Seed treatments were barley seeds disinfected with 4 % NaClO, whereas untreated seeds were only rinsed with deionized water. Bars with the same or without letters are not statistically different according to a F-protected LSD (*P*= 0.637).

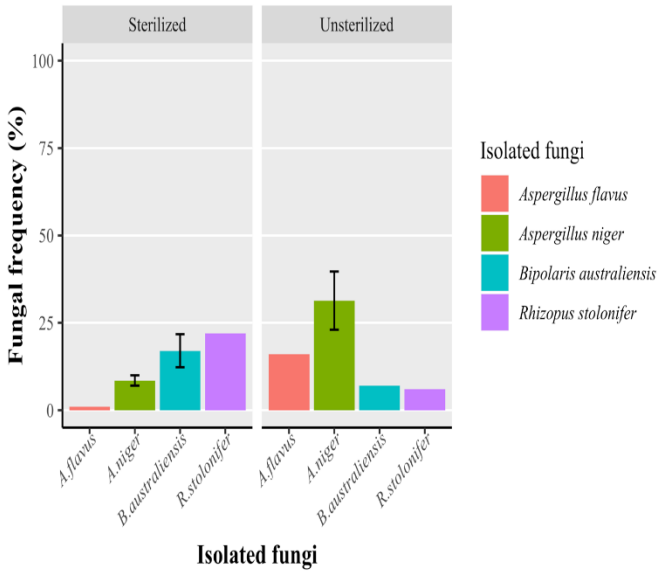

Fig. 4. Effect of location and seed treatment on fungal frequency (F%). Barley seeds were collected from three locations, Almerj, Gerdina, and Sultan. Seed treatments were barley seeds disinfected with 4 % NaClO, whereas untreated seeds were only rinsed with deionized water. Bars with the same or without letters are not statistically different according to a F-protected LSD (*P*= 0.637)

4 Discussion

The current experiment was carried out to determine mycoflora compositions of barley seeds collected from three locations in the eastern part of Libya. The present study revealed that local barley seeds are contaminated with A. niger, A. flavus, R. stolonifera, and B. australiensis. Secondary fungal metabolite secreted by species of Aspergillus can lead to aflatoxicosis syndrome on human health (Sarma et al., 2017& Williams et al., 2004).Toxins produced by these fungi varies according to the surrounding environment and the isolate type that secrets them (Blumenthal, 2004). Further studies are needed to identify the fungal toxins types being produced under the Libyan conditions, and to measure toxin's quantity for food safety consumptions.

Our findings were in line with Abubakr findings, in which A. niger, A. flavus were successfully isolated from barley seeds in the western part of Libya (Maryam, 2017). In Tunisia, Aspergillus was the most prevalent recovered species from barley seeds (Jedidi et al, 2018). Similarly, our finding matched Elham, and Modhi (2015) who stated that both A. niger, and A. flavus are the prevalent isolated species on external barley seeds in different areas of Saudi Arabia. Likewise, Hashem (1990) reported the presence of A. niger, A. flavus and B. australiensis on barley seeds in Saudi Arabia. Likewise the fungal species isolated in this study, A. niger, A. flavus, Bipolaris and Rhizopus stolonifera have been recovered from Triticum aestivum seeds (wheat) (Adhikari et al., 2016). Moreover, all isolated fungal species, except B. australiensis, reported in this study were also found on barley grains in Pakistan (Fakhrunnisa, and Ghaffar, 2006). In contrast to our finding, Ramadan et al (2013) isolated and identified different fungal genera on barley seeds, and these fungi were Alternaria, Aureobasidium, Cladosporium, Drechslera, Penicillium , Rhizoctonia and Stemphylium. We speculate that this discrepancy in results could be due to cultivar's type, agronomic practices or meteorological conditions.

In reference to seed germination, seeds collected from Sultan surprisingly exhibited the highest germination rate compared with the other locations, Almerj and Gerdina.

Disinfecting seeds with sodium hypochlorite, in the present study, had no effect on fungal frequency or fungal relative abundance compared with unsterilized seeds, irrespective of the place where seeds had been collected. Our results were contradictory to Abduhu et al., 2018 who found presoaked okra seeds (Abelmoschus esculentus L.) with sodium hypochlorite decreased the incidence of fungi, yet increased okra seed germination.

5 Conclusions

The present study confirmed the presence of four fungal species *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifera*, and *Bipolaris australiensis* that were isolated externally from barley grains. The results support previous studies' findings documenting the presence of these fungi on barley seeds. Seed germination was statistically higher in Sultan compared with the other locations. Barley seeds disinfected with sodium hypochlorite did not affect fungal frequency or fungal relative abundance compared with non-disinfected seeds. For better identification and accuracy, future research should apply molecular approaches to determine barley seed biomes.

Conflict of interest: The author declares that there are no conflicts of interest

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