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The Inhibition Effect of Alcoholic Extract of *Capsicum Annuum* (Chili Pepper) on The Growth of Barely Grains' Associated Fungi Species

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Abstract

In this research, isolation and identification of some fungal species that were associated with barley grains (obtained from Misurata Agricultural Research Center) were carried out, to study the effect of their secretions on the germination and growth of barely seedlings. The ethanolic extract of Chili pepper was also tested for its biological control on the germination and growth of the chosen fungal species. 10 fungal species belonging to 4 different genera were isolated and identified from barley grains, among these, two species were selected (*Aspergillus niger* and *Rhizopus stolonifer*) for further investigation. Both of the two fungal filtrates showed an inhibitory effect on the germination and length of radicles of barley grains and seedlings, results also showed that ethanolic extract of *Capsicum annuum* (Chili pepper) had a varied inhibitory effect on spore germination of the selected fungal species, the reason for inhibition of fungi treated with *C. annuum* extract may be due to the presence of different chemicals have inhibitory effects on fungi.

Keywords: Barley Grains - Fungal Filtrates - Ethanolic Extract - Isolation - *Capsicum Annuum* - Germination.

Introduction

Barley is one of the most important and most widespread cereal crops in the world, in terms of adaptation, especially in dry areas. This spread of the barley crop is due to its increased tolerance to unsuitable environmental conditions. Many scientists believe that barley is the oldest grain known to man and cultivated, and fossils of its grains have been found in many countries of the world that have ancient civilizations (El-Hashash & El-Absy, 2019; Kant *et al*., 2016).

During harvest, transportation, or storage, barley grains are infected with many fungi belonging to the genera *Alternaria*, *Aspergillus*, *Rhizopus*, *Penicillium*, *Trichoderma* and other fungi (Fleurat-Lessard, 2017; Nishimwe *et al.*, 2020). These fungi cause significant economic losses because of their impact on the vitality of the grain and reducing its germination rate, which leads to a reduction in agricultural production. When using such grains in agriculture, as well as the ability of some species of the fungi *Penicillium* and *Aspergillus* to produce mycotoxins. The most prominent and dangerous of these toxins are aflatoxins, which are considered among the most dangerous food pollutants present due to their carcinogenic impacts on humans and animals (Mohapatra *et al*., 2017).

The use of fungicides has played a role in combating fungi for many years, but many problems have arisen in their use, such as environmental pollution, the emergence of resistant strains, and others. Among the control methods that are currently used successfully to combat pathogens are plant extracts as promising alternatives to chemical resistance methods, as many of them have been proven

to be effective in resisting fungal, as it is cheap and safe to use, and do not leave any toxic residue on plants, in addition to being easy to obtain due to its abundant availability in nature (Assress *et al*., 2021; Goswami *et al*., 2018).

This study aims to:

- Isolation and identification of some fungi species associated with barley grains.
- Investigating the effect of the isolated fungal filtrates, on grains' germination rate and seedling development
- Testing the inhibitory effect of alcoholic extract of *C. annuum* (Chili pepper), on the growth of the investigated fungi species.

MATERIALS AND METHODS

Samples Collection

Samples of barley grains were brought to the Faculty of Science / Botany Department and obtained from the Agricultural Research Center in Misrata (*Hordeum vulgare*), the barley Misurata cultivar 04.

Isolation and identification of fungi associated with barley grains

A random sample of barley grains was selected from the quantity that was brought to the lab, and using sterile forceps near the flame, part of which was planted in Petri dishes containing the (PDA), each dish has 5 grains and has equal proportions, barley grain were lightly pressed using a forceps, so that most of its surface become in direct contact and immersed in the agar.

After the planting, all the dishes were placed in the incubator at a temperature of 25 ° C, after 7 days of incubation, the dishes were extracted and the developing fungi were identified around the grains using a complex optical microscope and special scientific references (Gupta *et al*., 2020).

Identification of fungus to be studied ecologically and physiologically

The study selected various fungi from barley grains samples for environmental and physiological studies due to their presence in most samples and representing different grades and families **Figure (1)**.

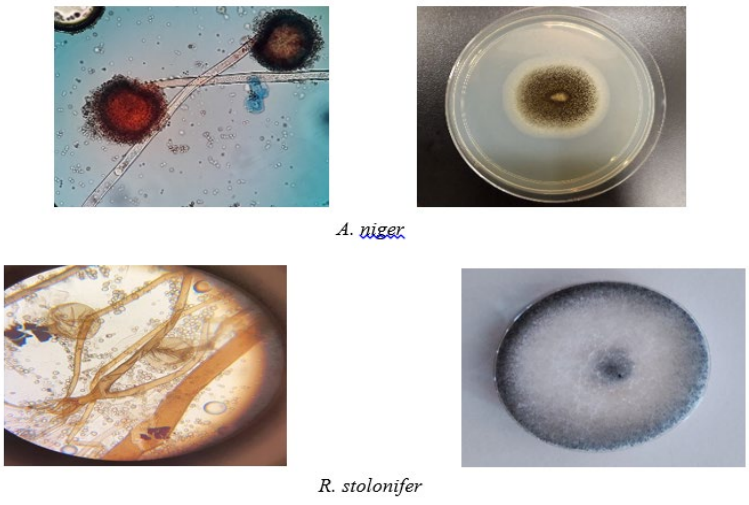

Figure:(1). Phenotypic (morphological and cultural), (Microscopical) characterization of some the isolates (*A. niger*, *R. stolonifer*) on PDA from barley grains.

Effect of fungi filterates on percentage of germination of barley grains and seedling growth:
Where 9 bottles containing each flask were equipped with 25 mL of PDB, Then, using the cutter, tablets were taken from the 9 mm PDA diameter containing *R. stolonifer, A. niger* for of 3-4 days, where each of the fungus was assigned 3 bottles. The three other vials were left without planting the fungus for comparison as in **Figure (2).**

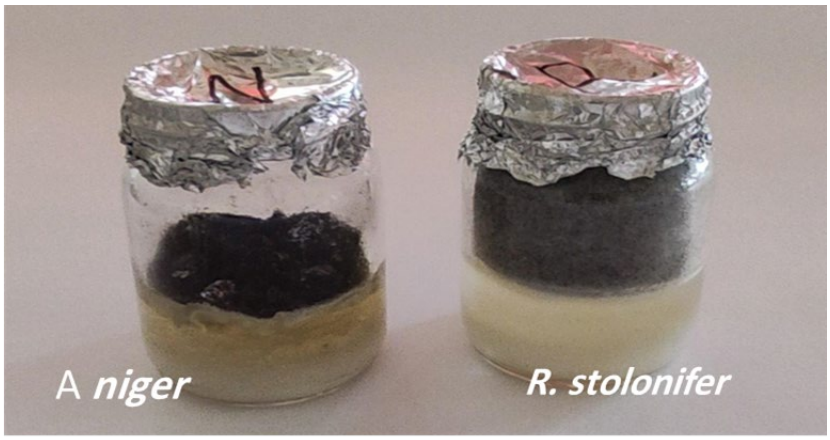

Figure:(2). Growth *A. niger, R. stolonifer* on PDB at 25 ° C After 10 days.

After the completion of the transplantation, all the bottles were placed in the incubator at 25 ° C, ten days later the vials were removed from the incubator and the leachate was separated from the growth using sterile filter paper.

to investigate the impact of these fungal filtrates on the germination and growth of barley grains and seedlings, several clean and sterilized petri dishes, with sterilized filter paper. Each containing 5 grains of barley sterilized with 1.0 % hydrogen peroxide solution, each petri dish was sprayed with 10 mL from the filtration. Each fungus was assigned three replicates, for comparison nine dishes containing the same number of grains were irrigated with sterile distilled water, which was not planted with fungi. After finishing the irrigation, all the dishes were placed in the incubator at 25°C. Ten days later, the grain-containing dishes were extracted from the incubator and the developing and non-developing grains were calculated in each dish. The percentage of germination was calculated according to the following equation:

$$\text{Percentage of germination (\%)} = \frac{\textit{Number of developing seeds}}{\textit{Total number of seeds}} \times 100$$

In addition, the length of the radicles and coleoptiles was measured on the tenth day by using a ruler, and observations were made on the pathological symptoms that appeared on each dish. Three dishes were allocated for each period (Al Fadl & Al Haidari, 2012).

Effect of Ethanolic Extracts of *C. annuum* (Chili pepper) on the Growth of Isolations Fungi from barley Grains
Ten (10) grams of dried *C. annuum* (Chili pepper) were thoroughly washed with distilled water, and dried, then crushed by ceramic mortar to form powder. 100 mL of ethanolic was added. 2 mL of this extract was added after sterilization into the media before the hardening. Tablets from the rim of the colony of *R. stolonifer* and *A. niger* grown for 3-4 days, were transferred into the center of each of these dishes, then incubated under 25°C, A week later, each bipolar diameter was measured for each fungal colony, each of these was implemented triplicates (Al-Jawhari, 2012). The inhibition ratio was estimated according to the following equation:

$$\text{Inhibition ratio\%} = \frac{\textit{The average of the comparison} - \textit{Average transaction diameter}}{\textit{The average of the comparison}} \times 100$$

RESULTS AND DISCUSSION

Isolation and identification of some fungi on barley grains:

This study showed that barley grains were contaminated with a lot of types and numbers of fungal pathogens depending on the area produced. From barley grains, about 142 colonies were obtained from the Misrata Agricultural Research Center. Table (1) shows the number of colonies obtained from barley grains.

This may be due to the fact that the Misrata Agricultural Research Center has a mild and humid climate during the time of the formation of the saplings, allowing the moisture-loving fungi and temperate fungi to have a longer period of growth and reproduction for several generations and thus producing a large number of germs.

For those fungi isolated from the area of the Agricultural Research Center Misrata, amounted to 10 species belonging to four genus, the *Aspergillus* species were the most common species and represented by four different species, as well as the *Rhizopus*, which had a total of 40 colonies (*A. niger and R. stolonifer*) were selected to conduct some tests on them.

Table (1): Genera and fungal species isolated on PDA from barley grains at 25 ° C, after 7 days.

Name of fungi	Numbers of colonies on parley grains (center)
Aspergillus sp.	10
Aspergillus sp_1	2
Aspergillus flavus	6
Rhizopus stolonifer	40
Aspergillus niger	10
Rhizopus sp.	5
Fusarium sp.	1
Fusarium oxysporum	21
Penicillium sp_1	35
Penicilliun chrysogenum	12
Total number of colonies	142
Total number of species	10
Total number of genera	4

Effect of fungi filterates on percentage of germination of barley grains and seedling growth

Barley grains are contaminated while they are in the field or when they are stored and put on the market with many fungi that are likely to be secreted in the grains as they develop excretions or toxins that may affect their vitality.

To clarify the role of secretions of some fungi on barley grain has run out of this experiment, which showed that the results of all the tested fungi impact on the proportion of germination and development of seedlings resulting from them, but varying degrees depending on the type of pollinated fungi, The effect of these fungal filtrates on the percentage of germination of barley grains and the

development of seedling, From Table (2) and Figures (3,4,5), we note that the most common filtrate on the percentage of grain germination is the leachate of *A. niger*, This filtarets reduced the percentage of germination in irrigated grains was 0% after 10 days compared to germination rate of 98% in irrigated grains with non-fungal medium (comparative) or distilled water, It was ranked second in terms of the effect of the fungal filterate *R. stolonifer,* which also reduced the percentage of germination in irrigated grains to 20% after 10 days of incubation.

As for the effect of filtrates fungi along the radicles and coleoptiles, the effect of *A. niger* was clear, some of the irrigated grains were formed after 10 days radicles were small and distorted brown the average length is 0.0 cm, while the coleoptiles were also short, the average length of 0.0 cm after 10 days of incubation, *R. stolonifer* filterate came in second place in terms of effect, as the irrigated grains were made up of the radicles and coleoptiles with a mean length of 0.5 and 1.5 cm respectively after 10 days compared to the irrigated grains with non-fungal medium (the comparison), the average length of the radicles and coleoptiles was 4.5 cm and 10.5 cm respectively during 10 days of incubation, This is consistent with the finding (Garuba *et al*., 2014).

This study was conducted to investigate the 7-day fungal effects filtrates of *A. niger* and *Penicillium Chrysogenum* isolated from corn seeds on germination ratio and seedling growth. Results showed that the seed germination ratio treated with fungal filtrates *A. niger* and *Penicillium Chrysogenum* (65.33%,79.67% respectively) were less than the control (100%).

It is obvious that the germination of barley grains and the development of their seedlings were more influenced by the filtrate of *A. niger*, this may be due to the fact that this fungus is known for its production of aflatoxins, which is likely to have been produced in the liquid medium in which it grew, reducing the germination rate of irrigated grains, This is consistent with the findings (Kabak *et al.*, 2006), which indicated that aflatoxin poisons may inhibit or reduce the germination ratio of grains and seeds of many crops and reduce the growth rate of their seedlings, and the effect of this leachate on the growth and development of the radicles and coleoptiles produced from irrigated grains(Abbas *et al*., 2009).

Results also indicated that, *R. stolonifer* has a deleterious effect on barley grains germination and seedling development, but the effect was mild on grain germination and evolution of the radicles and coleoptiles, where the cause of the lack of the effect of this leachate that it contains toxins or substances inhibiting growth, but it is of another type that differs in their chemical qualities from the toxins produced by *A. niger,* and some are likely to be a disincentive to produce naturally occurring Gibberellins produced by the embryo during germination leading to a shortening of the radicles and coleoptiles from the irrigated grains, This is consistent with the findings of the researchers (Finneseth, 2010; Mondéjar-López *et al*., 2022).

Table (2): Effect filtrates of tested fungi on the percentage of parley germination and growth of seedlings after 10 days.

Fungal filterates	Percentage of germination (Germination rate)	Length the radicles/cm	Length the coleoptiles/cm
A. niger	0 %	.00	.00
R. stolonifer	20 %	0.5	1.5
Control	98 %	4.5	10.5

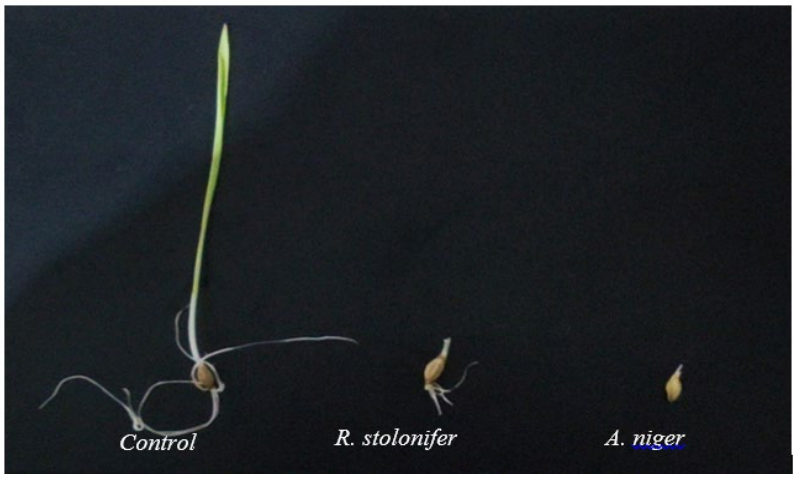

Figure:(3). Effect filtrates of tested fungi on the percentage of barley germination and growth of seedlings after 10 days.

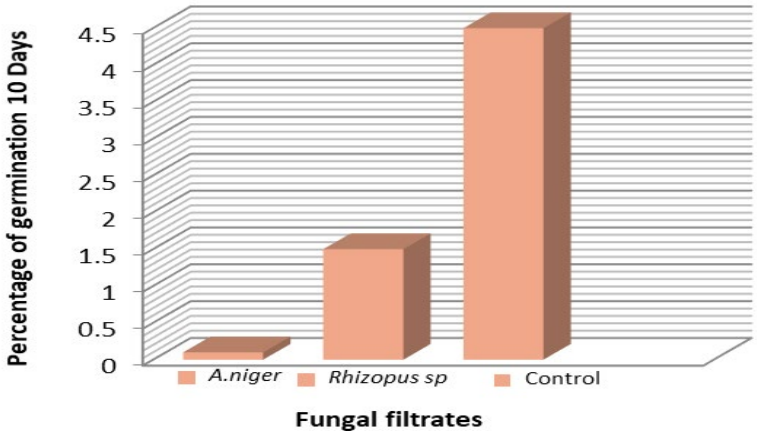

Figure:(4). Effect filtrates of tested fungi on the percentage of barley germination and growth of seedlings after 10 days.

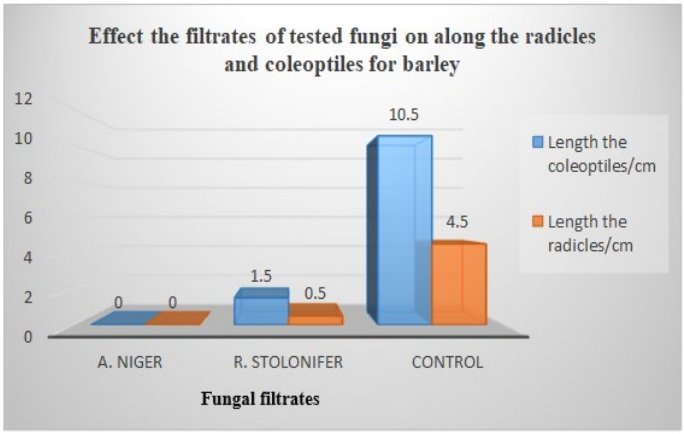

Figure:(5). Effect of the filtrates of tested fungi on along the radicles and coleoptiles for barley (10 days).

Effect of ethanolic extract of *C. annuum* (Chili pepper) on tested fungus associated with barley grains

The effect of ethanolic extract of *C. annuum* (Chili pepper) on the growth of irradiated fungi of parley grains is shown in tables (3-4). Ethanolic extract of *C. annuum* significantly inhibited the growth of tested fungi isolated from barley grains. (*R. stolonifer & A. niger*) where the diameter of the colony was 8.5 and 0.75 cm respectively compared to the treatment of the control 8.1 and 9 cm. The growth rate of *R. stolonifer* filling the dish with a note that no sporangium are created, (The ethanolic extract of *C. annuum* prevents the *R. stolonifer* to produce its spores), while the growth rate of *A. niger* when treated with the extract of *C. annuum* was 0.75 cm compared to the control of this fungus.

By observing the figures (6-7), the extract of the *C. annuum* is the superior effect, because it contains some active compounds such as saponins, phenols and alkaloids, It is known that these compounds have a disincentive effect for many pathogens (Agrios, 1985; Gülçin, 2005), This is consistent with what he found (Abdel Mohsen, 2011) In the testing of the efficiency of some plants, including *C. annuum* in the protection of the plant sun flower from the injury of fungus *Macrophomina Phaseolina* cause of rotting fever, with a reduction rate of fungal infection 53.1%. The reason for inhibition of fungi treated with chili extract is due to the presence of alkaloids Capsorubin, Dihgdro, Capsiain, Capsiacin, which may have an effect on these fungi, This is consistent with what he found (Tewari & Nayak, 1991) which found that the extract of this plant inhibits the growth of pathogenic fungi of rice, namely *Cochlibeolas migabeanus , Pyricularia oryzae , Rhizoctonia solani.* The reason for the inhibition of fungi treated with *C. annuum* extract may be due to the presence of different chemicals that have inhibitory effects on fungi, containing alkaloids and glycosides and the soap that melt easily in organic solvents and dissolved in water and which have an anti-fungal growth effect, This is consistent with what he found (Jiratko & Vesela, 1992), Which found that this extract inhibits the growth of *R. Solani*.

Table (3): The inhibitory effect of ethanolic *C. annuum* extract on *A. niger* at 25 ° C for one week.

Type of acetone extract	Average colony diameter (cm)	Percentage of inhibition%
C. annuum	0.75	90.3%
Control	8.1	0%

Table (4): The inhibitory effect of ethanolic *C. annuum* extract on *R. stolonifer* at 25 ° C for one week.

Type of acetone extract	Average colony diameter (cm) *	Percentage of inhibition%
C. annuum	8.5	5.5%
Control	9.0	0%

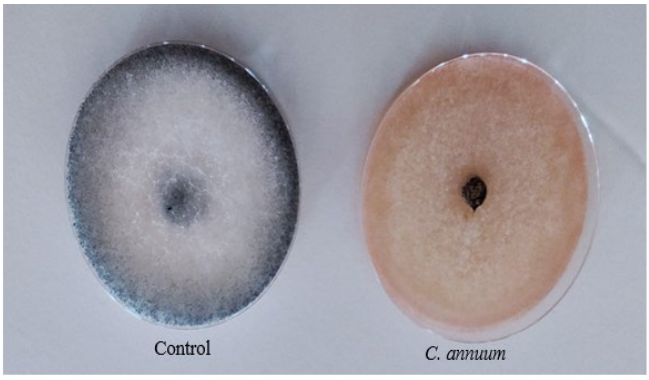

Figure:(6). Effect of ethanolic extract of *C. annuum* on growth *R. stolonifer* at 25 ° C for one week.

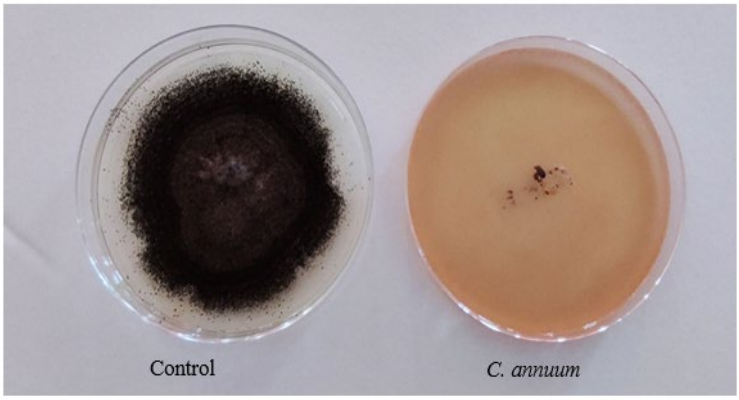

Figure:(7). Effect of ethanolic extract of *C. annuum* on growth *A. niger* at 25 ° C for one week.

CONCLUSION

The tested fungi and their secretions had an effect on the germination and the length of the radicles and coleoptiles for seedlings of barley grains. All the ethanolic extract tested of the *Capsicum annuum* (Chili pepper) showed a disincentive effect on the germination of the spores of the contaminated fungi for barley grains at varying rates.

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