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Effect of *Aspergillus flavus* on Seed Germination and Seedlings Growth of Barley and Some of Associated Weeds

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Effect of *Aspergillus flavus* on Seed Germination and Seedlings Growth of Barley and Some of Associated Weeds

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Abstract. The research was conducted during the 2021 growing season which included a series of experiments to investigate the effect the fungus Aspergillus flavus presence on germination and growth of barley and some barley weeds including annual ryegrass Lolium rigidum, darnel Lolium temulentum and yellow sweet clover Melilotus officinalis. Results of isolation and identification of fungi associated with seedlings roots of barley and the weeds showed that A. flavus was the most prevalent fungus associated with roots and surrounding soil recording the highest frequency among all the found fungi. In plastic pots, Aspergillus flavus was added to the potting soil and tested for its the effect on seed germination and seedlings growth of barley and weeds under study. The results showed after 20 days of planting that the fungus led to an increase in the percentage of germination and fresh weight and lengths of barley seedlings while the presence of the fungus decreased germination and seedlings growth of studied weeds with the highest negative impact on the darnel. Incubation at different temperatures were tested for their effects on A. flavus growth, and results showed that the fungus grows higher when incubated at relatively high temperatures (25-35°C), while the lowest growth was at 15 °C and the fungus did not grow at 10 °C.

Keywords. Bio-controlling fungi, Cereal, Herbicides, Weeds.

1. Introduction

Weeds associated with barley crop, such as annual ryegrass *Lolium rigidum*, darnel *Lolium temulentum* and yellow sweet clover *Melilotus officinalis*, is the most important problem that accompanies barley cultivation and causes high yield loss [1,2]. Although the use of traditional pesticides in the control of barley bushes is considered one of the important activities accompanying crop management to ensure high yield [3,4]. The concern accompanying the use of chemical pesticides in the control of the bush remains due to environmental pollution and the emergence of pesticide-resistant phases . Among the most promising alternative ways to control weeds is the use of bio-based fungicide with so-called mycoherbicides [5]. They are biological preparations for which fungi are their active substance and have the ability to control specific bushes with a level equivalent to that of chemical pesticides and can be applied in the form of sporophytes that spray directly on the weeds [6]. The fungus *Aspergillus flavus* is one of the most reliable fungi that can be used for this purpose. *A. flavus* is a widespread fungus with the ability to form vast numbers of asexual reproductive units. Some types are sexual phase and stone bodies which are more resistant to inappropriate environmental changes. The reason for the spread of the fungus in different environments is due to its ability to produce different enzymes, which enables it to exploit different food sources and endures different environmental conditions during its time tolerating drought conditions and a wide range of moisture tension. The surveys showed the spread of fungi in various soils of Iraq [7,8]. It was found that all fungal species belonging to the genus Aspergillus, namely *A. niger*, *A. fumigatus*, *A. terreus* were the most frequent species among the recorded fungi [9]. The aim of this study, therefore, was to investigate the effect the fungus *Aspergillus flavus* presence on germination and growth of barley and some barley weeds including annual ryegrass *Lolium rigidum*, darnel *Lolium temulentum* and yellow sweet clover *Melilotus officinalis*.

2. Materials and Methods

Seven days before the date of planting, random samples were taken from the field soil, and 10 samples per dunum were taken in a zigzag pattern from a depth of 5-25 cm. Samples of each dunum were combined as one representative sample and distributed in wooden plates (25 x 25 x 6 cm) perforated from below. The plates were sewed each with 20 seeds of barley or weeds under study. Irrigation was applied as needed until complete germination at which the germination percentage was calculated. After 20 days of germination, the number of healthy and rotten seedlings of barley and weeds was calculated and seedling length and weight were also recorded and compared among seed type treatments.

2.1. Isolation and Diagnosis of Fungi Associated With Seedlings of Barley Weeds

Five seedlings at age of 20-day were taken for both barley and weed seedlings under study and were superficially sterilized in 10% sodium hypochlorite for three minutes. The seedlings were then washed thoroughly with distilled water and then directly cultured in 9 cm Petri dishes on PSA medium, and incubated at 25 ± 2 C°). After 3-7 days of incubation, growing fungi colonies were examined and counted which then were purified and cultured in the same medium. Pure fungi cultures were observed under compound microscope and fungi were diagnosed according to [10]. The ratio of appearance and frequency of isolates was calculated according to the following equation.

2.2. Effect of Aspergillus Flavus on Seed Germination of Barley and Associated Weeds in A Pot Experiment

A sterile soil was used, which was divided into two parts. The first part was treated with fungus *Aspergillus flavus* previously grown on millet seeds at a rate of 6 g / kg soil, while the second part was not mixed with the fungus to represent control and according to the treatments. The soil was distributed in plastic pots with a diameter of 15 cm (3 kg). The seeds of barley, wheat, rouita and donkey were cultivated separately and with three replications for each type of seed at the rate of ten seeds / pot. The pots were placed in natural conditions and carefully watered, germination percentage was recorded and results were taken 20 days after planting by calculating the percentage of seed germination and rotting.

2.3. Aspergillus Flavus Performance at Different Incubation Temperatures

Pure cultures of *Aspergillus flavus* were used to test the effect of incubation at different temperatures on fungal growth grown in standard PSA medium. A Cork borer was used to cut and transport 5mm of pure fugal mycelium to be placed in the center of the PSA dish. Three plates were placed in an incubator at temperatures of 10, 15, 20, 25 or 35 C°. After seven days of incubation, the fungal radial growth was measured for each incubation temperature and means were compared among each other.

3. Results and Discussion

3.1. Isolation and Diagnosis of Fungi Associated With Seedlings of Barley Weeds

The results of isolating the fungi associated with the roots of barley seedlings and weeds under study showed that there are several fungi with different rates of occurrence or frequency (Table 1). Most of the fungi were deficient fungi and found the fungus *Aspergillus flavus* at a higher frequency of

30.919% with significant differences from the fungi that follows *Rhizoctonia solani* with a frequency of 20.586%. The rest of the fungi appeared with a frequency between 14.786% for the fungus *Aspergillus niger* and the lowest frequency of *Aspergillus* spp 0.286%.

Table 1. Fungi associated with the roots of barley seedlings and weeds (ryegrass, darnel and yellow sweet clover) under study.

No.	Fungi	Frequency of appearance
1	*Alternaria alternate*	0.319
2	*Aspergillus flavus*	30.919
3	*Aspergillus niger*	14.786
4	*Aspergillus terreus*	4.586
5	*Aspergillus* spp.	0.286
6	*Fusarium solani*	1.319
7	*Fusarium virticilloides*	4.786
8	*Fusarium* spp.	5.753
9	*Penicillium expansum*	1.119
10	*Trichoderma* spp.	1.186
11	*Rhizoctonia solani*	20.586
	L.S.D. $_{(P \leq 0.05)}$	5.230

The presence of fungus *Aspergillus flavus* was the highest frequency among other fungi isolated in this study. This is often due to the ability of this fungus to grow in a wide temperature and humidity range and its adaptation to different environmental conditions that may be inappropriate for its growth. In addition to its ability to form reproductive units that tolerate environmental conditions, along with its rapid reproduction and spread, which has led to it being the most dominant fungi in soil and mousse [11].

It is clear from Table (2) that the highest germination percentage, soft weight and gestation length were in the treatment of barley seeds and *Aspergillus flavus*. While the fungus *A. flavus* completely prevented seed germination of yellow sweet clover and darnel and reduced germination and growth indicators of rigid ryegrass[12]. It is clear that the fungus affects the germination of barley seeds and increases the indicators of seedling growth compared to the

Table 2. The effect of *Aspergillus flavus* on barley seed germination and seedling growth in plastic pots 20 days post-planting.

Tratments	% germination	Fresh weight (mg)	Seedling length (cm)
Barley	90.00	40.29	5.167
Barley + *A. flavus*	98.00	95.73	9.000
Yellow sweet clover	90.67	26.42	3.133
Yellow sweet clover + *A. flavus*	0.00	0.00	0.000
Annual ryegrass	5.33	33.67	3.167
Annual ryegrass + *A. flavus*	21.67	7.95	2.677
Darnel	96.00	65.48	4.00
Darnel + *A. flavus*	00	00	00
L.S.D.$_{(P \leq 0.05)}$	7.15	12.37	2.11

negative effect of the same fungus on the bush seeds. It is evident from this that the *Aspergillus flavus* is used as a biological control agent for the bush and a growth regulator for barley, often because it produces some useful growth regulators for plants that increase the rate of germination and barley growth.

The results of Table 3 showed that the highest radial growth of *Aspergillus flavus* (9 cm) was at 35 °C while the lowest growth rate (2.3) was when incubating at 15 °C, noting that the fungus did not grow at 10 C°. It is clear that *A. flavus* can grow in different thermal ranges with better growth at relatively high temperatures. Similar temperatures are appropriate for barley growth in November. Therefore, at such temperature the fungus will be more successful and gives better results by increasing barley seedlings growth and reducing weeds before their seed production. This period is ideal for reducing weeds and thus increasing barley growth, which is also reflected in increased production [13].

Table 3. *Aspergillus flavus* performance at different incubation temperatures after 7 days of inoculation on PSA medium.

Temperature C°	*A. flavus* radial growth (cm)
10	0
15	2.3
20	4.3
25	6.2
30	7.6
35	9.00

metabolites of different fungal groups have been used as natural herbicides against various weed plants, In general, most of the successful fungal preparations in weed control were more effective and efficient on leguminous and broad-leaved weeds than on grassy weeds [14]. In case of fungi that are pathogenic to certain weed plants, there are specialized genes mostly involved with pathogenicity, expressing proteins with roles of plant cell degrading enzymes and VR proteins. It was also found that infection and pathogenicity were associated with the production of indole acetic acid [15], which is a plant hormone that is used in the manufacture of herbicides [16,17].

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