

## Effect of the Magnetic Field Interacted with Two *Aspergillus* Species in Degradation of Some Chemical Pesticides

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**Abstract:** The aim of this study was to investigate the degradation of chemical compounds of five pesticides (Bayfidan EC, Bulldock EC, Oberon SC, Galaxy SL and Trayf EC) using the magnetic field interacted with two fungal species *Aspergillus flavus* and *A. terres*. Pesticides in the concentrated form were exposed to a magnetic field (0) for a period of 720 h and were used based on the recommended dose in two different culture media (PDA and PDB) to test the biodegradation level by the two fungi. The insecticide Bulldock was more toxic after exposure to the magnetic field and resulted in more inhibition rate on *A. flavus* than non-magnetized. Exposure to magnetic field did not differ from non-exposure in terms of *A. terres* growth on both Bulldock and Oberon culture media. The biodegradation of Trayf was slightly affected by magnetic field exposure while galaxy was not affected. Bayfidan degradation was affected by the magnetic field. This magnetized fungicide was more toxic to *A. terres*, causing an inhibition rate of 54.33%.

**Key words:** Investigate the degradation, chemical compounds, inhibition, non-magnetized, stimulation, PDB

### INTRODUCTION

In 2007, global sales of pesticides amounted to more than \$ 30 billion indicating that pesticides are still the most usable method in pest control although, the prior knowledge of the risks and damage caused by pesticides to the environment and public health. Alternative methods of pesticides in pest control such as biological control, agricultural practices, physical and genetic methods have led to the development of IPM programs and pest management systems which are striving to promote environmental means in pest control. To achieve food security, the world has begun of using modern and safe techniques to increase production because the continued use of pesticides to increase agricultural production has become a major harm to human health and serious environmental risk (Al-Malah and Al-Jubori, 2013). It is therefore, necessary to look for a strategy that will mitigate environmental decline while maintaining the agricultural production at satisfactory level quantitatively and qualitatively (Al-Adil and Khalid Mohamed, 2006; Dewan *et al.*, 1994). Researchers have increased the interest of using magnetized water in adapting the properties of brackish and fresh water and their use for agricultural purposes. The magnetization process is accompanied by a range of changes in the chemical and physical properties of water including increasing dissolved oxygen, reduction of surface tension, increased solubility of solids and electrical conductivity, soil nutrients availability, improve of the cell membrane permeability and low viscosity compared with normal

water. These changes in magnetized water will increase plant absorption leading to acceleration of vital processes and positively affects plant growth and development (Toledo *et al.*, 2008).

Magnetized water was also used for pesticides dilution and to study its effect on the technical specifications of the pesticides. Name year (Gorski and Wachowiak, 2004) found that dilution of Karate 2.5 EC and Winglofos 550 EC. Using magnetized water resulted in significantly effective control of the two-point red dot mite *Tetranychus urticae* Koch and the rice weevil *Sitophilus oryzae* L. Al-Mallah *et al.* (2012) reported that magnetized water resulted in an increase in the thickness of the separation layer of the pesticide emulsion to 10.75 mm for the Flash when stored at 55°C for three months followed by Cyrin with an average separation layer of 6.4 mm compared to 0.2 mm of the control. Using magnetized water in degradation of chemicals including chemical pesticides would be an favorable, clean and environmental friendly method to reduce pesticides residues hazards. This study, therefor was conducted to investigate the possible use of magnetic field and magnetized water to reduce environmental pollution and to control plant pathogens.

### MATERIALS AND METHODS

**Isolation and diagnosis of pesticides degrading fungi from soil and organic fertilizers:** Agriculture field soil and two organic fertilizers (rice and wheat compost from

Organic fertilizer center/Kufa) were used as well as five different pesticides including the fungicide Bayfidan EC, insecticides Bulldock EC and Oberon SC and herbicides Galaxy SL and Trayf EC at their commercial concentrations (without dilution). The soil and organic fertilizers were evenly distributed 300 mL plastic pots and the pesticides degrading fungi were isolated by two methods.

First, pots with soil or compost were saturated with the commercial concentration of each pesticide individually with three replicates each and stored in the laboratory condition. Three months later, the fungi were isolated from each pot using the dilution method (Salwan, 2015).

The second method was to immerse sterilized sticks (tooth picks) in the concentrated pesticides for each pesticide for 48 h. Three sticks were then embedded in each the pot of each type of potting soil mix with three replicates of each treatments. The pots were also stored in the laboratory condition for 3 months. Later, the sticks were pulled off and cultured in 9 cm Petri dishes with PDA to which the chloramphenicol was added at 250 mg/L. The dishes were incubated at  $25\pm 2^{\circ}\text{C}$ . After 3 days, the percentage of occurrence and frequency of each presented fungus was calculated (Dewan *et al.*, 1994). Present fungi were diagnosed in the graduate fungi laboratory in the Faculty of Agriculture, University of Kufa (Salwan, 2015). Since, the fungi *Aspergillus flavus* and *A. terreus* were the most prevalent (occurrence frequency) fungi, they were used in this study experiments.

**Pathogenicity test:** Fungi *Aspergillus flavus* and *A. terreus* were tested for their pathogenicity on

radish seeds in a Water Agar medium (WA). The 9 cm petri dishes were filled with WA and inoculated (cultured) with either *Aspergillus flavus* isolate #2 or *A. terreus* isolate #4 and incubated at  $25^{\circ}\text{C}$ . About 3 days after, the dishes were planted with 20 radish seeds each with three replicates for each fungus. Percentages of seed germination, seed rot, infected seeds and healthy seeds were calculated 7 days post planting (Carling and Leiner, 1986).

**Magnetic field effect on fungi (*A. terreus* and *A. flavus*) growth in PDA poisoned with pesticide (Bayfidan EC, Bulldock EC, Oberon SC, Galaxy SL and Trayf EC) medium:** Pesticides (Bayfidan, Bulldock, Oberon, Galaxy and Trayf) were placed in 25 mL tubes and exposed to the magnetic field (24 m T Tesla) for a period of 0 or 72 h (Fig. 1). About 15 mL of each pesticide was randomly placed in a metal container and magnetized on four sides. The control treatment was placed in a similar container but without magnets. After each magnetizing period, the recommended dose of each pesticide was taken and added to sterilized PDA medium and poured in Petri dishes. The dishes were then inoculated in the center with 0.5 cm disk of *A. terreus* or *A. flavus* cut from 7 days pure culture with three replicates each and incubated at  $25\pm 2^{\circ}\text{C}$ . Percent inhibition and/or stimulation was calculated according to Al-Malah *et al.* (1993).

**Magnetic field effect on fungi (*A. terreus* and *A. flavus*) growth in PDB liquid medium poisoned with pesticide (Bayfidan, Bulldock, Oberon, Galaxy and Trayf):** Potato Dextrose Broth liquid medium (PDB) was used in this test. The PDB medium was sterilized and distributed in 250 mL flasks with 100 mL each. The flasks were

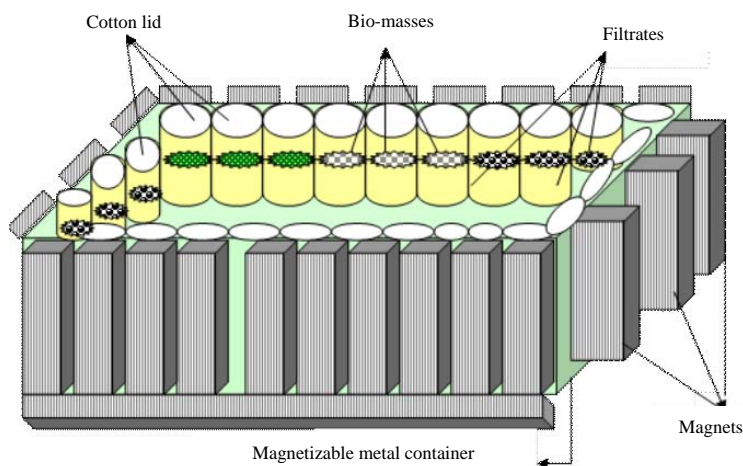


Fig. 1: A method to expose the bio-filtrates to the magnetic field

inoculated with three disks of *A. terres* or *A. flavus* and the in the pesticide (Bayfidan, Bulldock, Oberon, Galaxy and Trayf) was added to the medium. The treatments were PDB with or without exposure to magnetic field and PDB poisoned with a pesticide (Bayfidan, Bulldock, Oberon, Galaxy and Trayf) with or without exposure to magnetic field. All treatments were replicated three times for each fungus and each pesticide. Same number of flasks were not poisoned (no pesticide). All experimental flasks were incubated at  $25\pm 2^\circ\text{C}$  for 30 days with continuous exposure to the magnetic field (48 m M Tesla) and shaking every 2-3 days for even distribution. The biomass then was extracted with sterile forceps and placed on paper towels to remove the free water. Fresh weights were recorded and biomasses were placed in oven to dry at  $70^\circ\text{C}$  for 48 h and the final dry weight was recorded. Percent inhibition and stimulation were calculated as formerly mentioned (Salwan, 2015).

**Experimental design and data analysis:** Laboratory experiments were factorial based on Complete-Randomized-Design (CRD) with three replicates. Data were analyzed and Analysis of Variance (ANOVA) was performed. Means were compared with the Least-Significant-Difference (LSD) whenever appropriate at ( $p\leq 0.05$ ) (Gomez and Gomez, 1984).

**RESULTS AND DISCUSSION**

**Pathogenicity tests of studied fungi:** The results in Fig. 2 showed that the highest percentage of germination (100%) was in the control treatment and the lowest (65%)

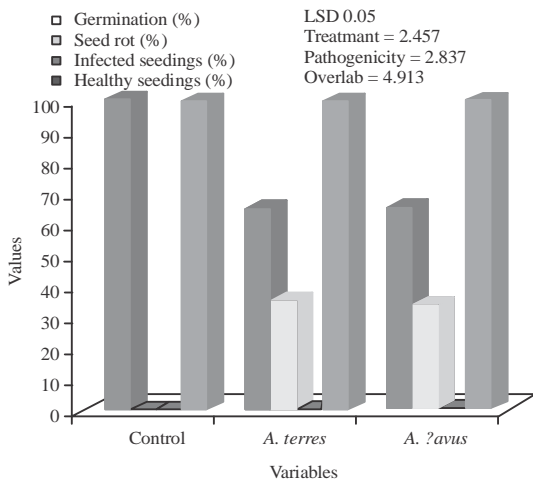


Fig. 2: Pathogenicity test of *A. terres* and *A. flavus* and their effect on radish seed germination, seed rot and seedlings health seven days after planting on WA in lab condition. Values are means of three replicates. Within treatment, bars with different letter are significantly ( $p\leq 0.05$ ) different

was in the treatment of *A. terres* in which significantly the highest percentage of rotting seeds (35%) was recorded compared to no seed rot in the control treatment. All treatments did not affect seedlings health, seedlings of all treatments were healthy.

The reason for the high germination rate of seeds with both fungi is that these fungi are non-pathogenic and their secretions are non-toxic and did not affect the germination of seeds (Dewan *et al.*, 1994). The increase in germination rate of radish seeds may be also due to these fungi secrete some substances that helps in seed outer shell degradation and accelerating seed germination. These substances may be enzymes such as cellulase or substances in the form of growth promoting such as Indol Acetic Acid (IAA) (Harman, 2000) or 2-carboxymethyl-3-n-hexyl malic acid (Mondal *et al.*, 2000).

**The effect of the magnetic field and fungi (*A. terres* and *A. flavus*) presence interaction on degradation of five pesticides (Bayfidan, Bulldock, Oberon, Galaxy and Trayf)**

**The insecticides Bulldock and Oberon:** The growth of the two fungi *A. flavus* and *A. terres* was affected by different type of insecticide and the exposure to magnetic field (Table 1). Compared to the radial growth of both fungi in the control, the highest percentage of inhibition was found to be in the treatment of *A. flavus* and the lowest was in *A. terres* treatment, 13.39 and 0.00%, respectively. The treatment of insecticide Bulldock with exposure to magnetic field resulted in the highest percentage of growth inhibition (12.09%) compared to the lowest inhibition rate (0.00%) from the control. For interaction treatment effect, the highest percentage of inhibition (24.18%) was found in the magnetized Bulldock interacted with the *A. flavus*. Regardless of exposure to magnetic field, the radial growth of *A. terres* was not effected by Bulldock nor by Oberon (Table 1).

Table 1: Effect of exposure to the magnetic field and the presence of fungi, *A. flavus* and *A. terres* in degrading chemical compounds of two different types of pesticides in the PDA medium, measured by percent inhibition/stimulation of fungal growth

Pesticide type	Stimulation/inhibition (%)		
	<i>A. falavus</i>	<i>A. terres</i>	Average
Control	8.31 <sup>(0.00)</sup>	9.00 <sup>(0.00)</sup>	0.00
Regular M. Bulldock	6.66 <sup>(19.85)-</sup>	9.00 <sup>(0.00)</sup>	9.92
Magnetized M. Bulldock	6.3 <sup>(24.18)-</sup>	9.00 <sup>(0.00)</sup>	12.09
Regular M. Oberon	7.04 <sup>(15.28)-</sup>	9.00 <sup>(0.00)</sup>	7.64
Magnetized M. Oberon	9.00 <sup>(7.66)+</sup>	9.00 <sup>(0.00)</sup>	3.83
Average	13.394	0.00	
LSD <sub>0.05</sub>	Pesticide = 8.55 Fungi = 5.41 Overlab = 12.10		

Values are mean of three replicates. Numbers in brackets are representing the percent inhibition/stimulation of each treatment over the control

Table 2: Effect of exposure to the magnetic field and the presence of fungi, *A. flavus* and *A. terres* in degrading chemical compounds of two different types of pesticides in the PDA medium, measured by percent inhibition/stimulation of fungal growth

Pesticide type	Stimulation/inhibition (%)		Average
	<i>A. flavus</i>	<i>A. terres</i>	
Control	8.31 <sup>(0.00)</sup>	9.00 <sup>(0.00)</sup>	0.00
Regular M. Galaxy	1.92 <sup>(76.89)</sup>	3.46 <sup>(61.55)</sup>	69.22
Magnetized M. Galaxy	2.3 <sup>(72.32)</sup>	3.31 <sup>(63.22)</sup>	67.77
Regular M. Trayf	1.63 <sup>(80.38)</sup>	1.43 <sup>(84.11)</sup>	82.24
Magnetized M. Trayf	1.25 <sup>(84.95)</sup>	0.83 <sup>(90.77)</sup>	87.86
Average	62.90	59.93	61.41
LSD <sub>0.05</sub>	Pesticide = 1.204	Fungi = 0.762	Overlab = 1.703

Values are mean of three replicates. Numbers in brackets are representing the percent inhibition/stimulation of each treatment over the control

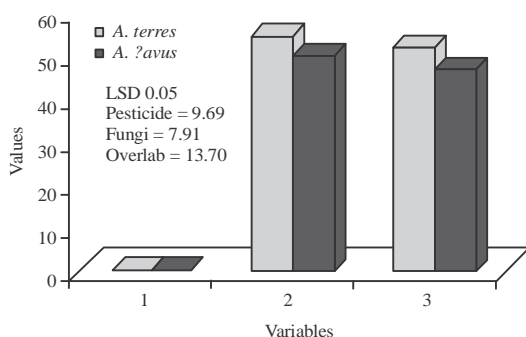


Fig. 3: Effect of exposure to the magnetic field and the presence of fungi, *A. flavus* and *A. terres* in degrading chemical compounds of Bayfidan pesticides in the PDA medium, measured by percent inhibition/stimulation of fungal growth tests on PDB liquid medium

**The herbicides Galaxy and Trayf:** In case of the herbicides treatments, results (Table 2) showed that there was a significant difference in the percentage of inhibition among fungi tested, that the highest percentage of inhibition was found in the treatment of *A. flavus* and the lowest was recorded in the treatment of *A. terres*, 62.90% and 59.93%, respectively. The highest percentage of inhibition was always in relation with the herbicides exposed to magnetic field, especially in the Galaxy treatment. The interaction between fungi and exposure to the magnetic field also affected the inhibition rates of fungi growth. The highest percentage of inhibition (90.77%) occurred in magnetized galaxy treatment interacted with *A. terres* compared to the control treatments of both fungi.

**The fungicide Bayfidan:** The results shows significant differences in the percentage of inhibition between the two fungi where treated with the fungicide Bayfidan (Fig. 3). *A. terres* was significantly affected by

the fungicide with inhibition rate of 35.4% compared to *A. flavus* which was less affected showing inhibition rate of 32%. The inhibition rates were affected by the direct pesticide treatment but not by exposing to the magnetic field. The highest percentage of inhibition (54.33%) was in the treatment of not magnetized Bayfidan on *A. terres* compared to the control treatment.

The mechanism of magnetic field in pesticide degradation is not yet known but it is believed that the magnetic field works on T. This is in line with mohassel findings (Mohassel *et al.*, 2009) that the use of magnetized water reduced the surface tension and increased the particle separation of the spray solution and increased the proportional efficiency of targeted control. The process of magnetization of the water through the conditioning of water properties based on magnetic technique lead to polarize water molecules with each other and randomly rearrange water molecules. This process will increase water quality and helps to remove undesirable odors from water such as sulfur and chlorine (Russel, 1987).

Based on calculated fungal fresh and dry biomasses, growth inhibition or stimulation of fungi tested was affected by the pesticide type (Table 3). Generally, the highest rate of growth inhibition was found in the *A. terres* treatments and the lowest rate was observed in the *A. flavus* treatments. Results (Table 3) shows that the highest rate of inhibition (35.58%) was found in the *A. flavus* treatment while *A. terres* was less affected with an inhibition rate of 33.32%. The rate of inhibition was affected by the pesticide type. Among all pesticides tested, Trayf resulted in the highest inhibition rate (82.43%) compared to lowest rate (0.00) resulted from Oberon treatments. Moreover, Oberon resulted in growth stimulation where interacted with both fungi tested on the PDB media.

Although, fungi differed in their growth depend on the pesticide type, this may not give a specific explanation on the level of biological degradation of a pesticide by the fungal isolates. But some other biodegradation indicators such as change in color and odor can be reliable. Generally, the dense growth of a fungus in a toxic environment can indicate the biodegradation ability of that fungus, especially with the increase in hyphal growth which increases the contact area between the fungus cells and the pesticide molecules. This will accelerate the intake process of the pesticide compounds into the fungus body and may also increase the enzymes secretion and consequently increases the level of biodegradation (April *et al.*, 2000; Bennet *et al.*, 2002; Johnsen *et al.*, 2005; Al-Adil, 2006). This is consistent with former studies in which a positive correlation has been found

Table 3: Effect of exposure to the magnetic field and the presence of fungi, *A. flavus* and *A. terres* in degrading chemical compounds of five different types of pesticides in the PDB medium, measured by fresh and dry weight of fungal biomass

Pesticide type	Fungal biomass fresh weight (mL) from medium treated with magnetized pesticide		Fungal biomass dry weight (mL) from medium treated with magnetized pesticide		Average
	<i>A. flavus</i>	<i>A. terres</i>	<i>A. flavus</i>	<i>A. terres</i>	
Control	0.273 <sup>(0.00)</sup>	0.226 <sup>(0.00)</sup>	0.043 <sup>(0.00)</sup>	0.043 <sup>(0.00)</sup>	0.00
Bulldock	0.223 <sup>(18.31)-</sup>	0.190 <sup>(15.92)-</sup>	0.033 <sup>(23.25)-</sup>	0.040 <sup>(6.97)-</sup>	14.92
Oberon	0.406 <sup>(32.75)+</sup>	0.256 <sup>(11.71)+</sup>	0.046 <sup>(6.52)+</sup>	0.043 <sup>(0.00)</sup>	12.74
Galaxy	0.086 <sup>(68.49)-</sup>	0.056 <sup>(75.22)-</sup>	0.004 <sup>(90.69)-</sup>	0.002 <sup>(95.34)-</sup>	56.74
Trayf	0.203 <sup>(25.64)-</sup>	0.060 <sup>(73.45)-</sup>	0.023 <sup>(46.51)-</sup>	0.008 <sup>(81.39)-</sup>	82.43
Bayfidan	0.236 <sup>(13.55)-</sup>	0.190 <sup>(15.92)-</sup>	0.023 <sup>(46.51)-</sup>	0.036 <sup>(16.27)-</sup>	23.06
Average	25.66	32.03	35.58	33.32	
LSD 0.05	Pesticide = 6.33		Treatment = 8.55		Overlab = 15.22

Values are mean of three replicates. Numbers in brackets are representing the percent inhibition/stimulation of each treatment over the control

between growth increase and biodegradation of hydrocarbon compounds (Rudd *et al.*, 1996; April *et al.*, 2000; Okerentugba and Ezeronye, 2003).

Our results showed a sharp reduction in the biomass final dry weight, especially where fungi were cultured in the pesticide-containing media compared with the control treatment. This decrease in biomass of fungal body may be due to the high content of body fluid in the fungal hyphae resulting from increased surface area of the fungal filaments. This means the increase in contact between the pesticide molecules and fungus cells which accelerates the process of in taking these compounds into the fungus body and storing them in liquid form (April *et al.*, 2000; Bennet *et al.*, 2002; Johnsen *et al.*, 2005).

While the higher values of the dry biomass in the control treatment may be due to the lower amount of nutrients available in the medium which were depleted faster than those of the pesticide-containing medium. This led to fungus shorter life cycle and resulted in spore formation (reproduction units). These spores, generally have higher dry biomass weight that the other body fragments.

### CONCLUSION

However, the fungal growth on the PDB respond differently when a pesticide differed. Magnetized Oberon used in PDB medium resulted in growth stimulation of both fungi, the biomass fresh weight was increased by 11.7 and 32.75 mg for *A. flavus* and *A. terres*, respectively. While biomass dry weight was increased 6.52 mg in case of *A. flavus* on the magnetized Oberon-PDB medium.

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