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Ability of *Aspergillus flavus* to degradation of herbicide Topik EC 100 (Clodinafop-propargyl)

Farah Abd-Alrazzaq Abd * and Ihsan Flayyih Hasan AI- Jawhari

Department of Biology, College of Education for Pure Sciences, University of Thi Qar, Iraq.

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Abstract

Aspergillus flavus was isolated from the soil field cultivated with wheat crop (*Triticum astevium L.*), which was treated with Topik EC 100 (Clodinafop-propargyl) herbicide in Al-Fuhud city, southeast of Thi-Qar Governorate-Iraq. soil samples were collected at a depth of (0-15) cm for a period of 17 weeks, and the fungus were isolated from the soil by dilution method, the pH, electrical conductivity, organic matter and soil texture were measured. the results showed that *Aspergillus flavus* was dominant in this study, where the percentage of appearance of *Aspergillus flavus* was 86%. the fungus was treated with the active ingredient Clodinafop-propargyl of Topik 100 EC herbicide in solid and liquid media (Mineral salt medium). the fungus showed its ability to grow in the medium containing the active substance. the results showed that *Aspergillus flavus* could biodegrade the active ingredient. the substances were measured by Fourier Transform Infrared spectroscopy and Gas chromatography- mass spectroscopy, the results showed that biodegradation percentage of the active ingredient Clodinafop-propargyl at a concentration 0.01 ppm by *Aspergillus flavus* was 80%.

Keywords: Biodegradation; Clodinafop-propargyl; Herbicides; Soil; Wheat

1. Introduction

Herbicides are still widely used all over the world, and are indispensable now, and in the future, and it will be difficult to produce enough food for the world's population, which is constantly increasing, without the use of chemical herbicides, and given that these herbicides are invalid and classified as toxic and dangerous, so there was, and still is a major concern in this regard, which relates to the safety, and quality of food, that humans eat after being sprayed with pesticides [1]. These toxic chemicals are produced in different parts of the world periodically or on a regular basis. these harmful substances or large amounts of toxic by-products resulting from their incomplete transformation accumulate in the ecosystem and affect the ecological balance. many microorganisms such as fungi can alone or in a synergistic manner contribute effectively to environmental remediation and achieve the goal of ecosystem balance [2]. Herbicides have major uses in the field of agricultural production, as they are used along with other pesticides for the purpose of controlling the main pests that pose a threat to the crop and reduce its essential productivity [3]. Therefore, these materials spread widely, which made manufacturers race to produce many of them according to different purposes.

The biodegradation process is considered one of the safest, and most effective processes, and treatments. It is also an environmentally friendly or ecofriendly technique for disinfecting polluted sites. This process is carried out by various organisms such as fungi, bacteria, and others in addressing the problem of pesticide residues, and heavy metal elements in the soil, and reduce the increase in pollution, and environmental problems faced by the organism [4].

Therefore, our current study aims to know the ability of *A. flavus* to biodegradation the active ingredient Clodinafop-propargyl of Topik EC100 herbicide in wheat fields.

* Corresponding author: Farah Abd-Alrazzaq Abd

2. Material and methods

2.1. Chemicals

All chemicals used in the current study were obtained from BDH and Merch.

The active ingredient Clodinafop-propargyl of Topik EC 100 herbicide was obtained from Nanjing Dulay (NJDULY)/China with a purity of 97%.

2.1.1. Media

Potato Dextrose (PDA): It consists of 200g potatoes, 20g dextrose and 15g agar dissolved in a liter of distilled water and then sterilized in autoclave at 121°C and pressure 15 psi for 20 minutes.

Liquid mineral salt medium: used for the growth of fungi [5] consisting of (K2HPO4, 1.71g; KH2PO4, 1.32g; NaNo3, 0.42g; Mg7H2O, 0.42g; CaCl2, 0.02g) the contents are dissolved in liters of distilled water and then sterilized in autoclave at 121°C and 15 psi pressure for 20 minutes.

2.1.2. Isolation of A. flavus

A. flavus was isolated from field soil samples treated with the herbicide Topik EC100 by using serial dilution [6]. fungal cultures were maintained by continuously purifying fungal species by transferring a disc of pure fungal colonies via a sterile cork borer into Petri dishes containing Potato medium (PDA), and chloramphenicol 250 mg/L, then incubating them in the incubator at 25 °C for 7 days.

2.1.3. The ability of A. flavus to grow in the solid medium treated with Clodinafop-propargyl.

The medium was prepared from Potato Dextrose Agar (PDA) in three conical flasks of 250 ml capacity, then autoclaved at 121 °C, and 15 psi pressure for 20 minutes, then the active ingredient Clodinafop-propargyl was added to the medium after a drop after the temperature of the medium reached to appropriate temperature, as it was added at two concentrations: (0.01, 0.004) ppm. the medium was poured into sterile Petri dishes, then the dishes were inoculated with *A. flavus* by transferring a disk with a diameter of 4 mm, and age of 7 days from a pure culture, through a sterile cork borer to the middle of each dish, then the dishes were incubated at 25 ° C for 7 days. this experiment was conducted by three replicates for each concentration. fungal growth rates were calculated by measuring colonial diameter.

2.1.4. The ability of A. flavus to grow in liquid mineral salts medium treated with Clodinafop-propargyl.

A solution of mineral salts was prepared for the growth of fungi, as it was distributed in conical flasks of 250 ml capacity, at a rate of 50 ml for each flask, then sterilized in an autoclave at a temperature of 121° C and a pressure of 15 pounds / inch for a period of 20 minutes, and after the temperature decreased to reach a suitable degree, the active ingredient Clodinafop-propargyl at two concentrations (0.01, 0.004) ppm were added, and the flasks were left without adding the substance for comparison, then the flasks were inoculated by disc transfer. 4 mm of 7-day-old fungal cultures of *A.flavus*, using a sterilized cork borer, then the flasks were incubated at a temperature of 25 ° C for 7 days, and this experiment was conducted with three replications for each treatment. the flasks were collected, and the liquid was filtered from flasks by filter paper Whattman No.1, then dried in an oven at a temperature of 50 °c for half an hour, after that the mycelium was weighed using a sensitive balance.

2.1.5. The ability of A. flavus to biodegradation Clodinafop-propargyl in mineral salts medium

The filtrate was collected from the flasks of the liquid medium inoculated with *Aspergillus flavus* by the method of [7] with some modifications to extract metabolites. the filter was transferred to a water bath at a temperature of 70 °C, and evaporated until the filter reached a volume of 1 ml. the samples were transferred to sterile test tubes with the addition of 1 ml of acetonitrile, and 1 ml of distilled water, and they were separated by a centrifuge (Gallenkamp) at a speed (10000 rpm) for 5 minutes, then transfer the filtrate to the 70°C water bath again until the sample volume is concentrated to 1 ml. the samples are then transferred to sterile bottle test tubes on which all required details are recorded, and kept in the refrigerator until analyzed by FTIR spectroscopy, and Gas chromatography-Mass spectroscopy (GC-MS).

2.2. Statistical analysis

All applications were analyzed using ANOVA In program SPSS (version 23.0).

3. Results and discussion

The results showed that the frequency of isolated *A.flavus* from soil treated with Topik EC100 herbicide reached to 86% (Table.1).

A.flavus was clearly resistant two concentrations of the active ingredient of the herbicide were used, in solid medium (PDA) as the diameter of the *A.flavus* colony reached to (8.5) cm in a concentration of (0.01,0.004) ppm respectively (Figure.1) , this result refer that *A.flavus* has the ability to use herbicides as a source of carbon and energy (Table .2), and this is consistent with what was found by[8] (Al-Nassar, 2021), that the colony diameter of *Aspergillus flavus* reached to (8.5) cm in all concentrations of Tribenuron Methyl herbicides, as it is consistent with (Nuaima, 2021) that the colony diameter of *A.flavus* reached to (8.5) at a concentration of (0.01) of Chevalier herbicide. While [7] mentioned that *A. flavus* achieved high resistance to Tribenuron Methyl herbicide, which inhibited the fungus in the soil by 35% at a concentration of (75) ppm.

The results showed that Clodinafop-propargyI was stimulated the dry weight of *A.flavus* at a concentration of (0.01) ppm, when compared to the control treatment (Table.3), and this is in accordance with what was indicated by [8] where she stipulated that Tribenurone Methyl it is a stimulator of *A. flavus* in all concentrations of this herbicide, many authors refers that the herbicide Fluazifop, which belongs to the same chemical group as the Topik herbicide , stimulated the dry weight of the *A.flavus* significantly, and the herbicide Haloxyfop, which belongs to the same chemical group, stimulated the dry weight of *A.flavus* and recorded very little inhibition at a concentration of 100 mg / liter [9]. [10] indicated that the active ingredient of the herbicide Chevalier stimulate *A.flavus* at a concentration of (0.01), while [7] indicated that the dry weight of mycelium of *A.flavus* increased in concentration (50) ppm in mineral salts medium treated with Tribenurone Methyl. the result showed that disappearance of a few peaks in the region (500 - 1500), and the appearance of wide peak in region (3000-3500), and this indicates the presence of an OH group (Figure.3) when compared with the standard active ingredient (Figure.2), which means the presence of acid, and the formation of a carboxylic acid. The result was indicate to ability of *A.flavus* to biodegrade the active ingredient of Topik in the mineral salts medium by using (FTIR) spectroscopy.

Table 1 Frequency of isolated *A.flavus* from soil treated with Topik 100 EC herbicide

Fungus name	**Numbers of samples in which the fungal type appeared**	**The percentage of appearance of the Species**
Aspergillus flavus	14	86%

Table 2 Colony diameted of *A.flavus* on solid medium treated with Clodinafop- propargyl

Fungus	**Concentration ppm**			**Mean**
	Control	**0.01**	**0.004**	
Aspergillus flavus	49.42±25.69	51.61±25.11	52.90±25.73	50.48

Table 3 Dry weight of *A.flavus* in mineralsalts medium treated with Clodinafop-propargyl

Fungus	**Concentration ppm**			**Mean**
	Control	**0.01**	**0.004**	
Aspergillus flavus	0.71±0.00	1.02±0.13	0.86±0.05	0.86

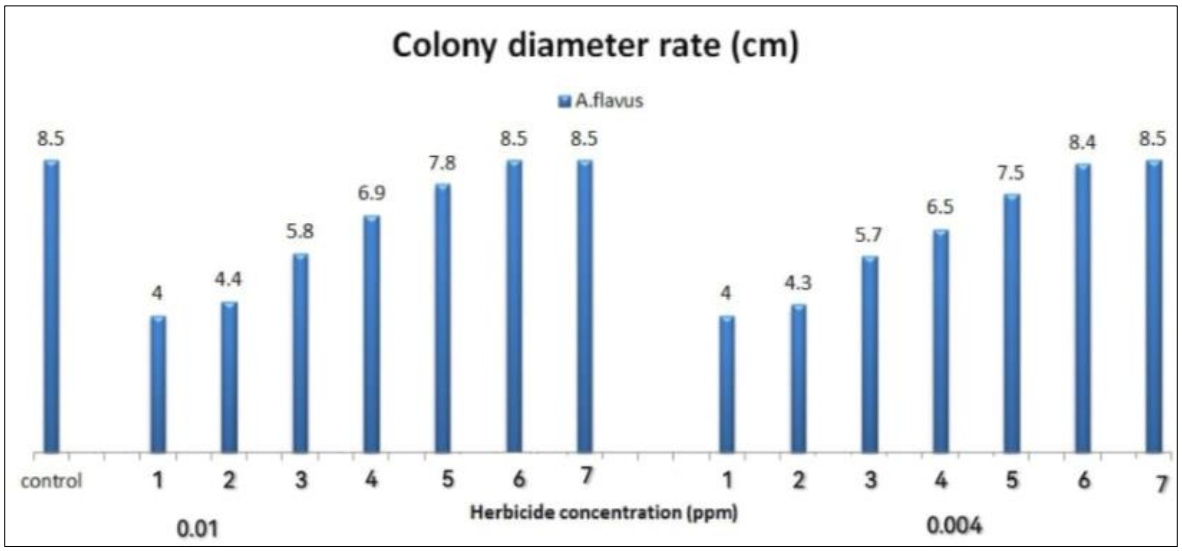

Figure 1 Effect of Clodinafop-propargyl on growth of A.flavus in solid media

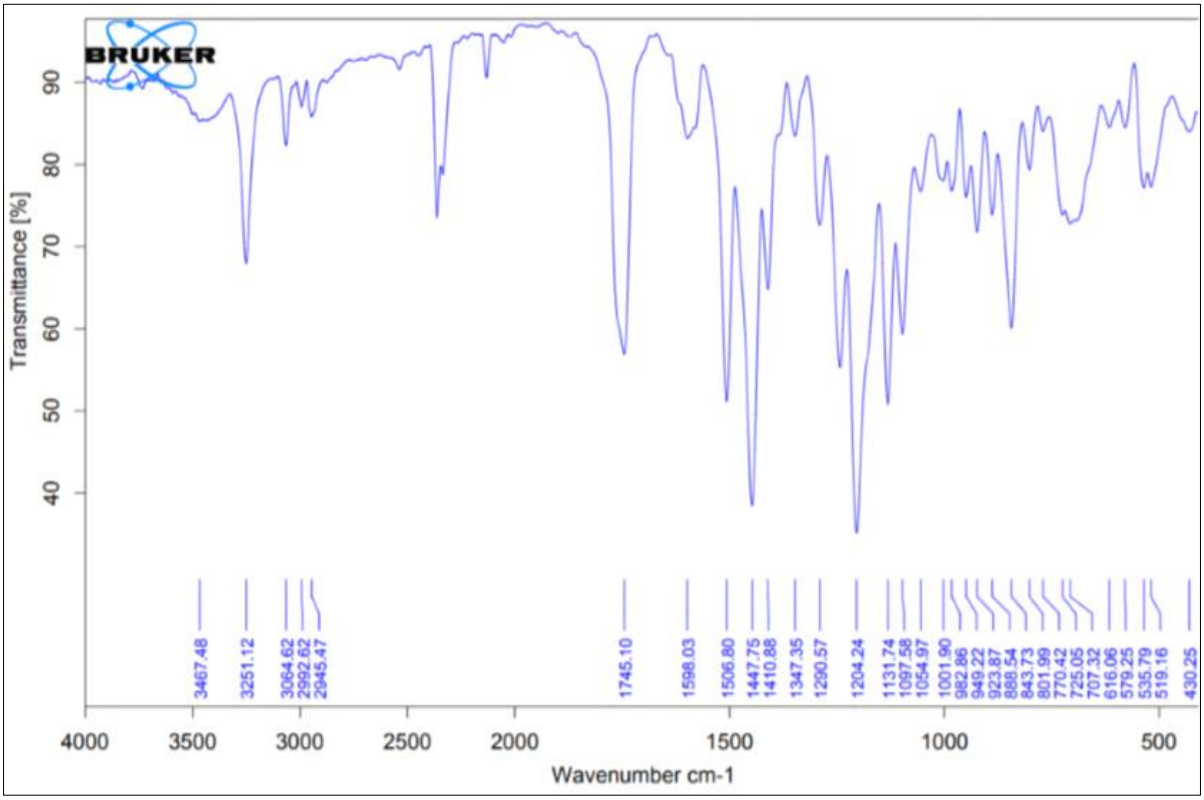

Figure 2 Clodinafop-propargyl (standard)

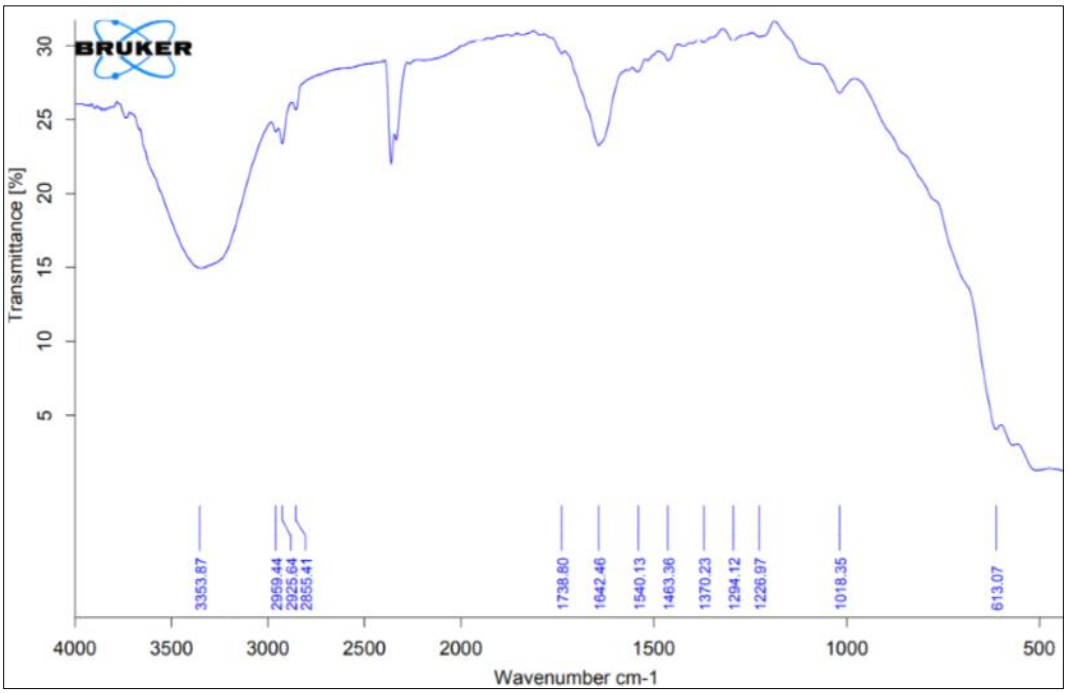

Figure 3 Transformations of Clodinafop-propargyl by *A.flavus* using the (FTIR) spectroscopy

4. Conclusion

Aspergillus flavus showed its ability to biodegrade the active ingredient of Topik 100 EC herbicide in solid and liquid medium at two different concentrations, (0.01 and 0.004) ppm of clodinafob. these data in this study improved our knowledge of herbicide residues, and the behavior of this fungus in soil treated with these herbicides, and how fungi decompose pesticide residues in the environment, as well as these can be used organisms to remove herbicide residues now, and in the future.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare no conflict of interest of regarding the publication of this paper.

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