Fungal Remediation of Nematocide with Fluopyram Active Ingredient in Different Concentrations with *P. Jensenii*

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Abstract

The use of chemicals such as insecticides, herbicides, fungicides and nematocides is common in agriculture and farmers nedd to use them to protect crops from pests, weeds and fungi. While these agents are necessary to control pests and weeds, they have often had undesirable consequences, such as harming non-target organisms, including aquatic species. Bioremediation is one of an usefulI method for eliminating negative effects of these kind of chemicals in receiving environments such as soil and water. In this study *Pencillium jensenii* soil fungi used as a bioremediator tool for bioremediate a fluopyram nematocide with important environmental parameters such as Chemical Oxygen Demand (COD) and Total Organic Carbon (TOC) in agiated culture conditions in certain time periods. According to the results of the study, *P. jensenii* performed high COD and TOC removal efficiencies against fluopyron nematocide as 88% for both parameters in 6 and 7 days respectively. This means, *P. jensenii* is a suitable tool for bioremdiate this kind of nematocides in agricultural activities.

Keywords: Bioremediation, Chemical oxygen demand, Nematocide, *P. jensenii*, Total organic carbon.

Research article
Received Date: 5 October 2023
Accepted Date: 8 November 2023

INTRODUCTION

Pesticides represent a global economic problem and an element of increasing human health concern due to adverse health and environmental problems that occur worldwide, especially affecting end consumers and producers. Pesticides are also important for consumers' acute risk assessment due to the variability of their residues (Prodhan et al., 2023). However, the use of pesticides can also cause environmental and health problems. Pesticides are chemicals, usually synthetic, that are toxic to their biological targets. There are various classes of pesticides, and they are generally classified according to the type of target pest and the way they are used. These classes include insecticides, nematocides, herbicides, fungicides, and rodenticides often used in the home and garden (Casida and Durkin, 2013).

In addition to all these, the effects of pesticides on the ecosystems of aquatic environments, which are the ultimate reservoir, have become a wide area of interest (Tan et al., 2021). These organic-based pollutants can enter receiving aquatic environments through different pathways. It is an undoubted fact that once pesticides enter aquatic environments, they will have a negative impact on aquatic organisms (Huang et al., 2022). Additionally, the use of pesticides may cause target pests to become resistant to pesticides (Tabashnik et al., 2010). Overuse or misuse of pesticides can have significant impacts on natural ecosystems. This can lead to loss of plant and animal species, groundwater pollution, decreased soil quality, and increased adverse effects on human health (Aktar et al., 2009). Today, many pesticide mitigation strategies, such as integrated pest and low input management, have been implemented at the farmland level. Such management strategies both reduce the amount of pesticides applied and aim to minimize the human and environmental health effects of pesticide use (Möhring et al., 2020).

Nematodes are thin, cylindrical parasitic worms that are known pests of plants. These worms can damage plant roots, inhibiting plant growth and causing yield loss. It can also be applied to some plant leaves. In this way, it protects the root system of plants and improves plant health.

Fluopyram is an active ingredient in an pesticide that is effective against nematodes. It affects the nervous system of nematodes and disrupts their vital functions. It can affect the movement and feeding abilities of nematodes by blocking the communication of nerve cells. Thus, fluopyram-containing nematocytes can exert a lethal effect against nematodes or suppress their ability to reproduce.

Its chemical name is 4-(2,6-difluorobenzyl)-1-(1-methyl-1H-pyrazol-4-yl)-5-oxymethyl-1H-pyrazol-3-carboxamide. This compound was developed to be effective against agricultural pests such as nematodes. Bioremediation is the use of specific microorganisms, plants or microbial enzymes to reduce or eliminate environmental pollution. This process uses biological mechanisms to metabolize or transform the pollutant. Various types of pollution involve a wide range of substances, including oil and petroleum products, heavy metals, and agricultural chemicals (such as pesticides and herbicides). The bioremediation methods explained were an effective and environmentally friendly technique because they result in the complete transformation of pesticides into a non-toxic product. Many of the researchers suggests that the bioremediation process can overcome the challenges and limitations of physical and chemical treatment for pesticide removal (Femina et al., 2023).

In this study, one of a soil fungi *P. jensenii* used as a new bioremediator tool for remediate COD and TOC value of the fluopyrom prepared for suggested agricultural using dosage in agiated culture conditions. The difference of this study from previous studies is that it is aimed at monitoring the bioremediation effect of *P. jensenii* soil fungus on fluopyram nematocide in agiated culture conditions daily with COD and TOC which are two important environmental pollution indicator parameters.

MATERIAL and METHOD

Preparing *Penicillium jensenii*

P. jensenii pure microorganism cultures used in the study were obtained from Munzur University Environmental Engineering laboratory. These fungi collected from agricultural soil and isolated and identified before. Pure cultures were propagated to carry out bioremediation activities. For this purpose, the cultures transferred to solid agar media in petri dishes were placed in a 27°C incubator. Then, they were enriched in Saboraud dextrose broth medium at the same temperature for bioremediation activities in liquid media (Cruikshank, 1972).

Preparing pesticide solutions

Fluopyram was prepared in 250 ml conical flasks as 250, 500, 750 and 1000 mg/L, based on the application dose of farmers and recommended in the usage recipe.

Removal of Fluopyram by Bioremediation Process

In the study, firstly, COD and TOC values of the environment that includes enriched *P. jensenii* fungi were monitored every 24 hours. When these values were equal to the concentration of the pesticide, 1 ml of enriched fungal samples taken from these environments (each 1 ml contains approximately 10^6 colony-forming individuals) were inoculated into fluopyrome media prepared at 250, 500, 750 and 1000 mg/L. Then, these pesticide-fungus mixtures were taken into a incubator and shaken at 160 rpm at 27^0C. The samples taken every 24 hours were filtered through a 0.45µm filter and the bioremediation activities of the *P. jensenii* fungus were followed by COD and TOC analyses. In the study, COD analyzes were performed with the open relux method as specified in standard method 5220B. HACH DRB 200 thermoreactor using Hach COD kits that measured in the range 0–1500 mg/l (Cat number: 23459-52) used for COD analysis while Shimadzu TOC-V device was used for TOC analyses. Standard Method 5310B High emperature burning method prefered for TOC analysis. In order to inhibit the activity of fungal colonies, one drop of 1N H_2SO_4 was added to the filtrates. experiments lasted 14 days in total. All of the COD and TOC analysis were performed three times and average value of these replications taken for consideration.

RESULTS AND DISCUSSION

Bioremediation Studies Results on the basis of COD and TOC

In the study, the bioremediation activity results based on COD and TOC shown by *P. jensenii* fungus on Fluopyram nematocytes at different concentrations are shown in Figures 1 and 3 on a concentration basis; on the basis of removal efficiency, it is shown in Figures 2 and 4.

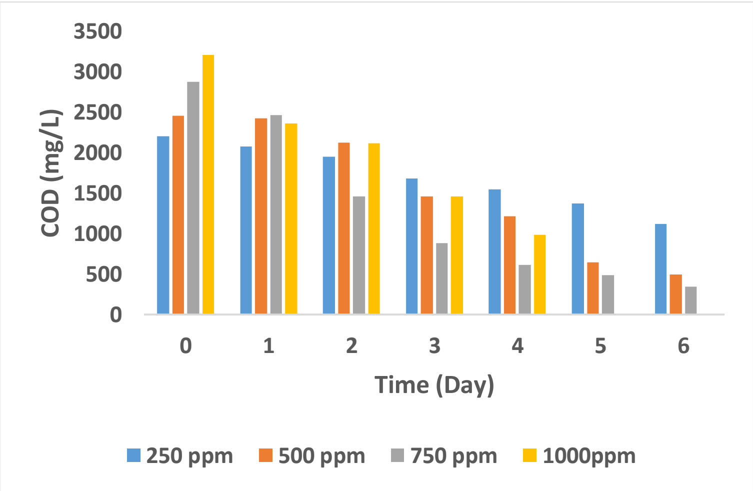
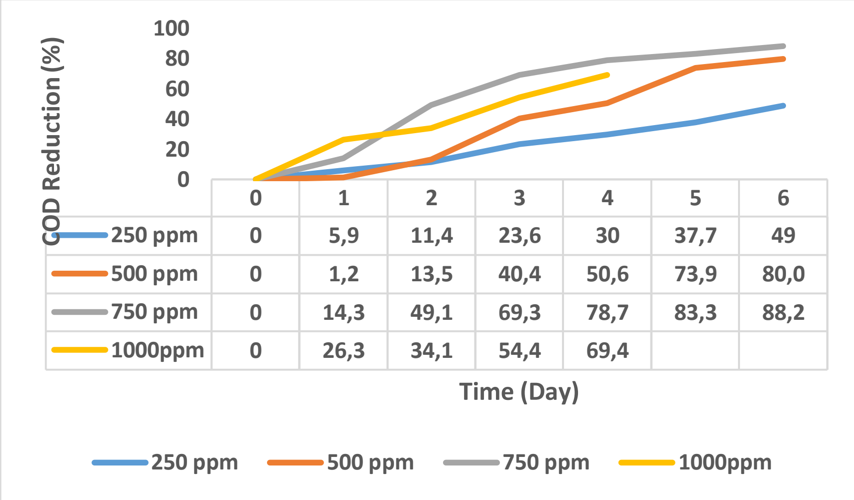


Figure 1. COD decrease based on concentration shown by *P. jensenii*



	0	1	2	3	4	5	6
250 ppm	0	5,9	11,4	23,6	30	37,7	49
500 ppm	0	1,2	13,5	40,4	50,6	73,9	80,0
750 ppm	0	14,3	49,1	69,3	78,7	83,3	88,2
1000ppm	0	26,3	34,1	54,4	69,4		

Figure 2. COD reduction efficiency (%) shown by *P. jensenii*

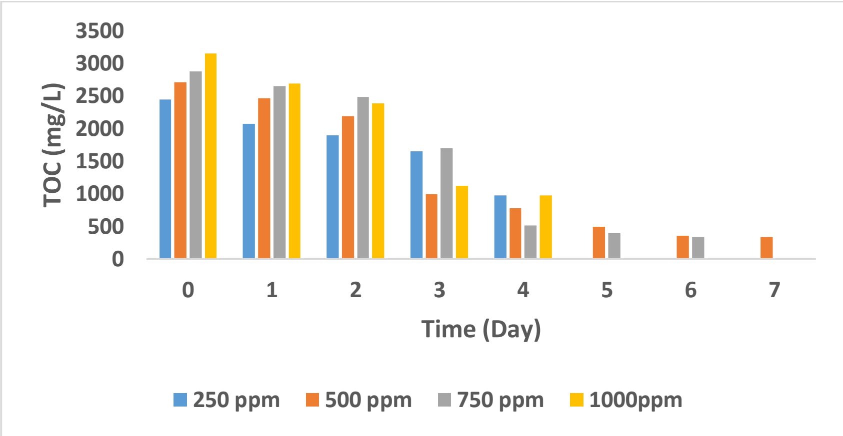
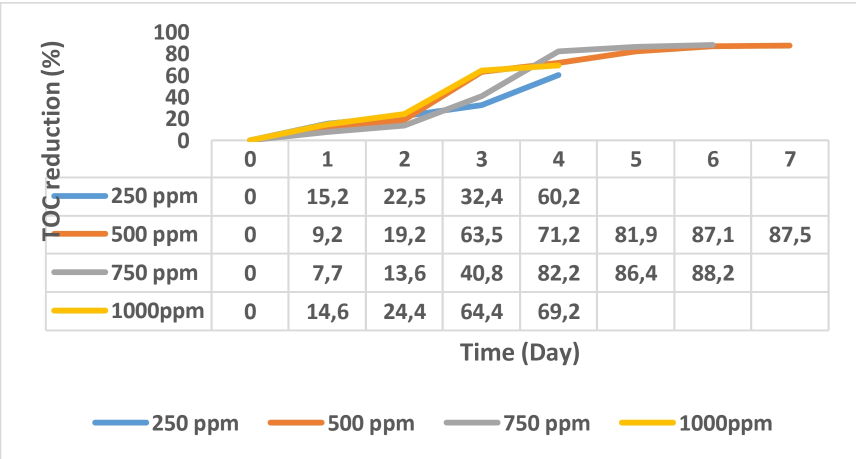


Figure 3. TOC decrease based on concentration shown by *P. jensenii*



	0	1	2	3	4	5	6	7
250 ppm	0	15,2	22,5	32,4	60,2			
500 ppm	0	9,2	19,2	63,5	71,2	81,9	87,1	87,5
750 ppm	0	7,7	13,6	40,8	82,2	86,4	88,2	
1000ppm	0	14,6	24,4	64,4	69,2			

Figure 4. TOC reduction efficiency (%) shown by *P. jensenii*

When the results of the bioremediation activity of the *P. jensenii* fungus on the Fluopyram nematocyte are examined, it is seen that the removal efficiency on a COD basis at the end of the 6th day is 49, 80 and 88.2%, respectively, in 250, 500 and 750 mg/L fluopyram environments. At the end of the 4th day, the removal efficiency in the 1000 mg/L environment was found to be 69.4%. Since increases in the amount of COD were noticeable at the end of these days, the experiment was completed at the end of the 6th day for the 250, 500 and 750 mg/L environments, and at the end of the 4th day for the 1000 mg/L environment. As can be seen from Figure 1, the removal amounts on a COD basis increased from 2200 mg/L to 1120 mg/L at the end of the 6th day in a 250 mg/L environment; In environments of 500 and 750 mg/L, there is a decrease from 2450 and 2870 mg/L to 490 and 340 mg/L, respectively, during the same period. The removal amount in the 1000 mg/L environment is from 3200 mg/L to 980 mg/L at the end of the 4th day (Figure 1 and 2).

When the removal results of the study based on the TOC parameter were examined, the removal in the medium containing 250 mg/L fluopyrome increased from 2440 mg/L to 970 mg/L with an efficiency of 60.2% at the end of the 4th day; In a 500 mg/L environment, from 2710 mg/L to 340 mg/L at the end of the 7th day, with a removal efficiency of 87.5%; At the end of the 6th day in an environment of 750 mg/L, it increased from 2870 mg/L to 340 mg/L with an efficiency of 88.2%; Finally, in the 1000 mg/L environment, it decreased from 3150 mg/L to 970 mg/L at the end of the 4th day, with an efficiency of 69.2%. The reason why the study was terminated on days 4, 7, 6 and 4, respectively, in environments of 250, 500, 750 and 1000 mg/L is that there is an increase in the TOC value at the end of these days, as well as in the COD parameter (Figure 3 and 4).

The reason for these increases seen in both COD and TOC values after certain days is thought to be that after the fungus breaks down the pesticide in its environment, it competes with its own intracellular activities and dead fungal cells increase the COD and TOC values in the environment.

Considering the decrease in the TOC parameter, striking differences with COD are observed in terms of both timing and removal efficiencies. This key difference is that COD is greater than TOC. It is generally known that COD determines the amount of energy required to chemically decompose organic material (in this case it represents the organic load from both pesticides and the medium). TOC, on the other hand, covers all carbon-containing substances originating from the medium containing pesticides and microorganisms in the system.

This study is about the use of *P. jensenii* fungus obtained from agricultural soils in the bioremediation of Fluopyram nematocyte. In many studies in the past, microorganisms isolated from activated sludge of pesticide producing companies were used. However, this study, unlike others, involves isolating the *P. jensenii* fungus directly from agricultural fields and using it in bioremediation studies. This may mean that the resistance of these microorganisms to pesticides and their bioremediation capacity may be naturally higher, given the fact that agricultural environments are exposed to pesticides. Therefore, this study may be an important step to better understand the bioremediation potential of microorganisms obtained from agricultural fields.

It has been understood that microorganisms in bioremediation systems can remain active throughout the duration of bioremediation activities (Lin et al., 2015). This shows that bioremediation can be an effective and alternative method to reduce environmental pollution.

It is recommended to use microorganisms to reduce the toxic effects of pesticides. After pesticides are released into the receiving environment, their negative effects on contaminated areas can also be investigated with environmental parameters such as COD, TOC and Biological Oxygen Demand (BOD_5).

Biodegradation of pesticides is related to pesticide residues, application time and the activities of microorganisms. Erguven and Yıldırım (2019) stated that *Methylobacterium radiotolerans* and *Microbacterium arthrosphaerae* strains completed Imidacloprid bioremediation after 18 days. While the COD parameter was determined as 52, 96 and 99%, respectively, with 20, 40 and 80 ml bacterial consortia, the BOD_5 rates were 88, 79 and 50%, respectively, in the same period. has been recorded.

Many researchers argue that certain microorganisms can develop tolerance to pesticides. In another study, *B. cereus, B. subtilis, B. melitensis, P. aeroginosa, P. fuorescence* and *S. marcescens* consumed 46-72% of chlorpyrifos as the sole carbon source in a sedimentary environment after three weeks. It has been found that they can decompose (Lakshmi et al., 2008).

The removal of herbicide residues in the bioremediation process depends on the activities and nutritional needs of the microorganism (Field et al., 1995; Erguven and Yıldırım, 2016). Penicillium species have been shown to exhibit metabolic activity on herbicides and similar micropollutants and alleviate the toxic effects of these chemicals by lignin degradation by 76% in 15 days and 94% in 30 days (Siripong et al., 2009). In the literature, it has been documented that endosulfan has a biodegradation capacity of up to 96% in studies in aerobic and anaerobic facilities using mixed bacterial populations (Kumar and Philip, 2006).

Góngora-Echeverría et al. (2020) examined the biodegradation of glyphosate in pure strains and microbial consortia. These studies have shown that the inoculation method is effective for the bioremediation of polluted agricultural soils. *Pseudomonas nitroreducens*, *Ochrobactrum sp*., their consortium and *Pseudomonas citronellolis* strain ADA-23B, showed more than 90% removal of glyphosate herbicide in agricultural fields. Erguven and Yildirim (2016) investigated the bioremediation rate of chlorsulfuron herbicide and evaluated its capacity to reduce COD. It has been determined that *B. simplex, B. muralis, M. luteus, M. yunnanensis* and *C. tetani* strains provide 70% to 93% removal within 4.5 days. Belal and Elkhateeb (2014) conducted a study on the bioremediation of pendimethalin and examined this process with the bacterial species *Pseudomonas putida*. It was determined that this bacterium eliminated all pendimethalin at a concentration of 100 µg/ml within one month. Later, in another study conducted with *Phanerochaete chrysosporium*, Belal and Elkhateeb (2014) determined that pendimethalin at a concentration of 100 mg/L was removed by 56%. This process achieved a removal success rate of 75% after 7 days, and 85% and 95% after 14, 21 and 28 days.

In this study, a bioremediation process was carried out on nematocytes with the active ingredient fluopyram under shaking culture conditions using *P. jensenii* fungus. In the study, we focused on the removal of two important environmental parameters such as COD and TOC, as much as possible. According to the results obtained from the study, it is recommended that *P. jensenii* fungus can effectively remove the herbicide containing the active ingredient Fluopyram on the basis of COD and TOC and that this fungus can be used in bioremediation activities on such nematocytes.

CONCLUSION

This study is about the bioremediation activities of *P. jensenii* fungus on fluopyram nematocides at concentrations recommended for use in agricultural areas, based on COD and TOC parameters.

In the light of the data obtained from the study, it offers a biological treatment method recommendation to eliminate nematocyte residues to farmers who frequently use or are considering using fluopyram in the agricultural sector. Additionally, bioremediation activities were examined with COD and TOC parameters instead of high-cost chromatographic methods to monitor this bioremediation process.

The findings reveal that the *P. jensenii* fungus has the capacity to successfully eliminate nematocysts. Therefore, the use of this mushroom is recommended.

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