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Original Article

Potential Use of Microbial Product to Degrade Chlorpyrifos in Tea Cultivation Soil

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Abstract: Pesticides containing organophosphate (OP) compounds in general and Chlorpyrifos (CPF) in particular are commonly used to control insects and pests. However, they are highly toxic not only to insects and wild animals but also negatively impact the environment and human health. This study provides an assessment of the effectiveness of using microbial product (MP) to degrade CPF in a large- and small-scale tea cultivation environment. This microbial preparation is made from selected strains of *Methylobacterium populi* CNN2 and *Ensifer adhaerens* VNN3, chosen from previous research based on their high CPF degradation ability of almost 95% after 3 days of cultivation. The research results show that in some farming areas, residual CPF exists both in the soil and in agricultural products. With MP addition, the CPF content in tea decreased by 76.35% in a net house experiment scale and the efficacy of CPF treatment in the soil reached 72-76%. Additionally, the use of MP also increased the nitrogen-fixing microorganisms density in the large-scale experiment from 10^3 to 10^5 CFU/g, stimulating growth and productivity for tea plants. The CPF content in the experimental formula using MP was reduced by 11-16 times compared to the control.

Keywords: Microbial product; pesticides containing organophosphate, chlorpyrifos.

1. Introduction

Plant protection products (PPP) of organic phosphorus (OP) and specifically chlorpyrifos (CPF) are widely used in agriculture due to their effective pest control capabilities and diversity of species [1, 2]. However, residues of these PPPs have led to ecological pollution and

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affected human health through the food chain. CPF has been detected in groundwater, surface water, soil, sediments, and even in breast milk [3-5]. In tea-growing soil in Vietnam, CPF residue levels reached 28 mg/kg [6], many times higher than the maximum allowed by TCVN 15: 2008/BTNMT at 0.1 mg/kg of soil. The negative impacts of CPF entering the human body through the environment have been highlighted in various research studies, causing diseases such as cancer, cardiovascular issues, and disruptions in pregnancy. Therefore, the removal of CPF residues in the environment in general and agricultural soils, in particular, is crucial. There are many different physicochemical methods for treating CPF in contaminated environments, such as deep burial in the ocean, open pit burning, and improved oxidation processes. However, these processes lead to the formation of secondary pollutants with higher toxicity and the potential for accumulation of persistent substances that require very expensive, non-ecological and technically challenging treatment [7]. Therefore, the use of indigenous microorganisms to remove CPF from the environment has become the choice of scientists for its effectiveness, economy and environmental friendliness [8-10]. Among the solutions for handling residual PPPs, the use of microorganisms (MOs) is considered effective, safe, and environmentally friendly [11]. This article will focus on the research results of applying microbial product (MP) from selected MOs strains to address residual CPF in agricultural soils.

2. Research Methods

2.1. Sampling Method

- Tea sampling method: QCVN 01-28:2010/BNNPTNT [12].
- Vegetable sampling method: TCVN 9016:2011 [13].
- Soil sampling method: TCVN 7538-2:2005 (ISO 10381-2:2002 [14].

2.2. Microbiological Quantification Methods

- Microorganism quantification method - TCVN 4884-1:2015 (ISO 4833-1:2013) [15].
- The density of free nitrogen-fixing microorganisms is determined according to TCVN 6166: 2002 [16].
- The CPF extraction process is carried out according to the method described by Gan J [17] and Gao Y [18]. Specifically, 10 g of soil is shaken with 20 mL of methanol/water solvent (4:1, v/v) for 1 hour, then centrifuged at 10,000 rpm for 15 minutes to collect the supernatant. Repeat the extraction process twice, combine, and evaporate to dryness under vacuum to 15 mL. The solution is then acidified with HCl to pH ~1.0 and extracted with chloroform (30 mL) using a funnel. Subsequently, the organic phase is dried under vacuum and reconstituted with 10 mL of acetone.
- Method for determining CPF in soil using the EPA Method 8270D and Elshikh M.S [19].
- Method for determining CPF in tea product using EN 15662:2008 [20] and Mali H [21].

2.3. Evaluation Method of the Impact of the Preparation in CPF Treatment

Net house scale experiments:

The tea variety LDP 1 provided by the Tea Research and Development Center of the Northern Mountainous Agriculture and Forestry Science Institute; the experiment was arranged in a Randomized Complete Design (RCD), replicated three times in a net house. Analyze and evaluate the results of CPF decomposition after 60 days of research, the experimental formulas were arranged specifically as follows:

Formula 1: Control (no MP) + CPF (200 mg/m^2); Formula 2: MP 1 g/m^2 + CPF 200 mg/m^2; Formula 3: MP 2 g/m^2 + CPF 200 mg/m^2; Formula 4: MP 3 g/m^2 + CPF 200 mg/m^2; Formula 5: MP 5 g/m^2 + CPF 200 mg/m^2; Formula 6: MP 10 g/m^2 + CPF 200 mg/m^2.

The basis for choosing the dosage is QCVN 01-189/2021 of the Ministry of Agriculture and Rural Development on fertilizer management, microbial organic fertilizer [22].

Small scale experiment: was carried out in Hung Son commune, Anh Son district, Nghe An province, on 2 types of tea plants: Tea plants in the budding stage (3 years old): PH 8 variety; Commercial tea plants (19 years old): LDP1 variety; Control formula: No MP applied; Formula 1: Adding MP 100 kg/ha; Formula 2: Adding MP 200 kg/ha; Formula 3: Adding MP 300 kg/ha. Experimental layout method: Completely randomized block design (CRBD), replicated 3 times. Experimental plot area: 100 m^2/plot. Total experimental plot area is 1200 m^2.

Large scale experiment: conducted at the location and on 2 types of tea plants as in the small scale experiment: Experimental formulas: Control formula (CF): No MP applied; Experimental formula (EF): MP application at a dosage of 100 kg/ha. Experimental layout method: Completely randomized block design (CRBD), replicated 3 times. Experimental plot area: 1,000 m^2/plot, 3 repetitions. Total experimental plot area is 2 formulas x 2 locations (soil types) x 2 types of tea plants x 1,000 m^2 = 8,000 m^2.

2.4. Data Processing Methods

Use Excel software to manage data, calculate, and compare mean values. Data processing using IRISTAT 4.0 software.

Table 1. Analysis results of CPF content in tea, vegetables, and cultivation soil samples

Sample	Sample code	Analysis result (mg/kg)				
		CPF	Fenitrothion	Malathion	Parathion	Profenofos
Tea	TN1	1,125	ND	ND	ND	ND
	TN2	0,457	ND	ND	ND	ND
	NA1	0,372	ND	ND	ND	ND
	NA2	0,059	ND	ND	ND	ND
Tea soil	TN1	0,029	ND	ND	ND	ND
	TN2	0,007	ND	ND	ND	ND
	NA1	27,8	ND	ND	ND	ND
	NA2	16,9	ND	ND	ND	ND
	BL1	25,95	ND	ND	ND	ND
	BL2	7,63	ND	ND	ND	ND
	BL3	2,15	ND	ND	ND	ND
	BL4	18,44	ND	ND	ND	ND
	BL5	3,06	ND	ND	ND	ND
Cabba-ge	1	0,094	ND	ND	ND	ND
	2	0,053	ND	ND	ND	ND
Tomato	1	0,034	ND	ND	ND	ND
	2	0,032	ND	ND	ND	ND
Carrot	1	1,106	ND	ND	ND	ND
	2	0,783	ND	ND	ND	ND
Cabba-ge soil	1	0,025	ND	ND	ND	ND
	2	0,032	ND	ND	ND	ND
Tomato soil	1	0,025	ND	ND	ND	ND
	2	0,018	ND	ND	ND	ND

Note: ND- not detected.

3. Research Results

3.1. Survey of Residual CPF in Agricultural Soil

The study collected samples to analyze and evaluate residual CPF in tea, vegetables cultivation soils in specific locations in Thai Nguyen, Bac Ninh, Nghe An, and Lam Dong provinces as follows:

- Tea and tea cultivation soil samples were collected in Hung Son commune, Anh Son district, Nghe An province (Coordinates 18°44'43" to 19°03'56" North; 105°17'56" to 105°41'9" East). Sample code: NA.
- Tea and tea cultivation soil samples were also collected in Phu Thinh commune, Dai Tu district, Thai Nguyen province (Coordinates 21°39'51" to 21°39'51" North; 105°34'51" to 105°35'4" East). Sample code: TN.
- Tea and tea cultivation soil samples were also collected in Loc Tan commune, Bao Lam district, Lam Dong province (Coordinates 11°35'43" to 11°35'45" North; 107°44'35" to 107°44'38" East). Sample code: BL.
- Vegetable and vegetable cultivation soil samples were collected in Que Vo district, Bac Ninh province (Coordinates 21°5'55" to 21°5'17" North; 106°7'18" to 106° 8'25" East).

The analysis results show that CPF is detected in both tea, vegetables, and cultivation soil samples. The CPF residue in some tea cultivation soil samples is significantly higher than in the products (sample NA1 is 75 times higher). This result indicates that pesticides containing CPF are widely used by farmers, especially in the tea-growing areas of Anh Son district, Nghe An. Other studies by various authors have also shown the extensive use of CPF for agricultural and industrial crops, including tea [2, 23].

3.2. Evaluating the Ability of Microbial Product to Degrade CPF

Several studies have focused on evaluating the ability of CPF degradation in contaminated soil environments by microorganism groups [24-26], investigating the degradation mechanism [27], and evaluating the activity and application of individual or combined microorganisms in CPF residue treatment [25, 28]. However, there is limited research on the use of preparations from native microbial strains with the potential to degrade CPF. This paper presents preliminary research results on the prospect of applying biological solutions in soil remediation, contributing to sustainable agriculture.

3.2.1. Introduction to CPF-degrading Microbial Product

From the study by the author group published [1], it was found that among 20 soil samples from tea-growing regions in Vietnam, four strains of bacteria capable of degrading CPF were isolated. Among them, three potential strains identified as *Methylobacterium populi* (CNN2), *Ensifer adhaerens* (VNN3), and *Acinetobacter pittii* (CNN4) were selected to produce CPF-degrading microbial product. Among these 3 strains, *E. adhaerens* (VNN3) showed the highest CPF degradation ability, followed by *M. populi* (CNN2) CPF with the highest degradation potential, reaching almost 95% after 3 days of incubation, followed by CNN4. The selected strains used for product production have high biological safety, can coexist and do not inhibit some beneficial strains. After production, the product quality complies with TCVN 6168-2002. The product reaches a useful cell density of >10^9 CFU/g after production and can maintain quality of >10^8 CFU/g after 9 months of storage. The biological activity of the microbial strains is stable. Biological activity testing results show that besides CPF degradation capability, the microbial strains in the product also have the ability to degrade poorly soluble phosphate compounds, enhancing effectiveness when applied in practice. In a study by Abraham et al, it was also shown that MOs degrade CPF while also having the ability to dissolve phosphorus, providing stimuli for plant growth [29].

3.2.2. CPF Degradation Capability of Microbial Products (Mp) on A Net House Scale

The experiment was set up with formulas incorporating MP ranging from 1, 2, 3, 5, 10 g/m^2 of soil, and CPF added to the soil at a dosage of 200 mg/m^2 of soil. The results of CPF content analysis in the soil and tea leaves in the experimental formulas after 60 days of fertilizing MP are presented in Table 2.

Table 2. Influence of MP Dosage on Residual CPF in Soil and Tea Leaves

No	Formula	CPF in soil (mg/kg)	CPF in tea leaves (mg/kg)
1	Control	3,68	1,46
2	MP 1 g/m^2 + CPF*	2,25	1,03
3	MP 2 g/m^2 + CPF*	1,87	0,324
4	MP 3 g/m^2 + CPF*	1,7	0,309
5	MP 5 g/m^2 + CPF*	1,15	0,284
6	MP 10 g/m^2 +CPF*	0,87	0,249
CV (%)		5,9	6,6
LSD 0,05		0,63	0,391
CPF* - CPF 200 mg/m^2			

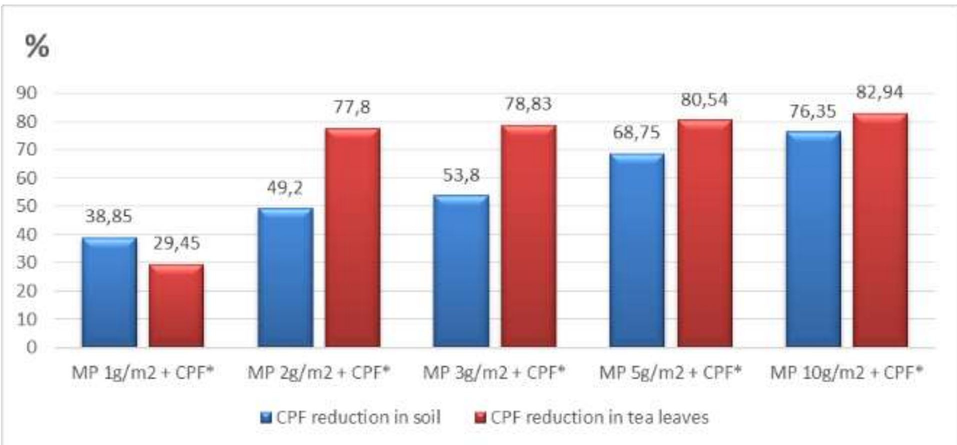

Figure 1. MP dosages and CPF degradation efficiency.

The results show that MP with fertilization dosages ranging from 1-10 g/m^2 all have the ability to degrade CPF.

Effectively reducing CPF absorption in tea leaves by 77-82%, that equivalent in 4 formulas (N^{o}3 to 6). However, the highest CPF degradation efficiency in soil was 76.3% with a fertilizer dosage of 10 g/m^2. Therefore, the preparation was used at a dosage of 10 g/m^2 for subsequent experiments to evaluate the effectiveness of the product with different levels of CPF contamination.

3.2.3. CPF Treatment Capability of MP on A Small Scale

The study was conducted in Hung Son commune, Anh Son district, Nghe An province - a mountainous commune in Anh Son district, western Nghe An province, which is one of the key areas for tea cultivation in Nghe An province with an area of over 2,500 ha. The amount of CPF was analyzed in tea planting soil before the experiment and the different formulas of MP on two types of tea: 1-3 years old (budding) and 19-year-old (commercial). The doses of MP were 100, 200 and 300 kg/ha.

Conducting an analysis of residual chlorpyrifos in tea planting soil shows that during the budding period, no residual CPF was detected (<0.002). This can be explained by the fact that local farmers did not use any pesticides during this cultivation period.

In commercial stage of tea plantations, CPF has been detected in all formulas at different concentrations of MP. Specifically, in the control formula - not using the product, the CPF content is 0.042 mg/kg. However, in the experimental formula - using MP at doses of 100-300 kg/ha, the results show that the CPF content decreases to only 0.011-0.012 mg/kg (equivalent to a reduction of 72-76%).

Table 3. The impact of MP on the residual amount of CPF in tea planting soil in Anh Son, Nghe An

Tea production stages	Formula	CPF residual amount (mg/kg)
Budding	Control: No MP	<0,002
	MP: 100 kg/ha	<0,002
	MP: 200 kg/ha	<0,002
	MP: 300 kg/ha	<0,002
Commercial	Control: no MP	0,042
	MP: 100 kg/ha	0,012
	MP: 200 kg/ha	0,011
	MP: 300 kg/ha	0,011

Table 4. Density of microorganisms in tea soil

Tea production stages	Formula	Free N fixing microorganism
Commercial	control: No MP	$3,3 \times 10^3$
	MP: 100 kg/ha	$0,4 \times 10^4$
	MP: 200 kg/ha	$5,9 \times 10^4$
	MP: 300 kg/ha	$6,2 \times 10^4$
Budding	control: no MP	$1,4 \times 10^3$
	MP: 100 kg/ha	$1,2 \times 10^4$
	MP: 200 kg/ha	$6,0 \times 10^4$
	MP: 300 kg/ha	$6,4 \times 10^4$

Analysis of microbial density in tea plantations in various formulas is recorded in the following Table 4.

The results show that the use of MP helps increase the density of free-living nitrogen-fixing microorganisms. The density of free-living nitrogen-fixing microorganisms in formulas using the product fluctuates around 10^4 CFU/g, higher than the control formula (10^3 CFU/g).

Using the product at doses of 100 kg/ha, 200 kg/ha, and 300 kg/ha has quivalent efficiency in treating CPF and increasing the density of nitrogen-fixing microorganisms. To minimize investment costs for users, consider selecting the product dosage at 100 kg/ha to continue evaluating the effectiveness of CPF treatment on a larger scale.

3.2.4. Capability of CPF Treatment of Microbial Product in A Large Scale

Through the data Table 5, it is shown that the soil samples in both the commercial and budding tea plantations had detected CPF at values of 0.064 and 0.042 mg/kg, respectively, before the experiment. However, when analyzing the CPF content in the soil after the experiment in both control formulas, the CPF residues decreased compared to the initial levels by 11 to 16 times.

This reduction could be due to the CPF residues being washed away or naturally decomposed under the influence of weather factors (rain, sunlight, wind, etc.) [30]. In the experimental formulas using MP, the remaining CPF residue is very low (<0.002 mg/kg). This result further confirms the effectiveness of the microorganisms in the product in reducing CPF residues in tea planting soil.

Table 5. Capability of CPF treatment of MP in tea planting soil in Nghe An

Tea production stages	Formula	CPF analysis result (mg/kg)
Commercial	Before experiment	0,064
	Control	<0,032
	Experiment	<0,002
Budding	Before experiment	0,042
	Control	<0,022
	Experiment	<0,002

Table 6. Density of microorganism in tea planting soil in Nghệ An

Tea production stages	Sample	Free N fixing microorganism (CFU/g)
Budding	Control	$1,3 \times 10^3$
	Experiment	$4,7 \times 10^5$
Commercial	Control	$5,9 \times 10^3$
	Experiment	$7,1 \times 10^5$

The results from Table 6 indicate that the use of MP helps increase the density of beneficial microorganisms in tea planting soil. Specifically, the density of free N fixing microorganism in formulas using the product all exceeded 10^5 CFU/g, higher than the control formula (around 10^3 CFU/g). This research result shows that besides reducing CPF in tea planting soil, the microbial product also has a synergistic effect in promoting the development of the microbial population in tea soil, especially the group of beneficial free-living nitrogen-fixing microorganisms [31].

4. Conclusions and Recommendations

4.1. Conclusions

At the net house scale, with a dosage of $10 g/m^2$ of MP, the highest CPF treatment efficiency is achieved at 76.3%.

On a small scale, the CPF treatment efficiency of microbial products in soil reaches 72-76%, and the use of these products helps increase the density of free-living nitrogen-fixing microorganisms to 10^4 CFU/g.

On a large scale, in experimental formulas using microbial products, the remaining CPF residue is very low (<0.002 mg/kg), confirming the role of microorganisms in the product in effectively reducing CPF residues in tea plantations. Besides reducing CPF, microbial products also promote the development of microbial populations in tea soil, especially beneficial free-living nitrogen-fixing microorganisms.

Initial research at different scales has shown the role of MP in reducing CPF residues in tea-growing soil, improving the density of beneficial microorganisms, thereby opening up an environmentally friendly solution for treating agricultural soil pollution, enhancing soil health, and aiming for a green and sustainable agriculture.

4.2. Recommendations

Recent research has shown that some environmental conditions such as humidity, pH, and temperature affect the CPF degradation rate of microorganisms. Specifically, humidity of

60%, pH of 6, and temperature of 37 °C are the optimal conditions that bring high efficiency in CPF treatment [32]. However, to fully assess the applicability of MP in CPF pollution treatment, this study will continue to consider the effects of other factors such as rainfall, soil properties, cultivation conditions, and so on, on the effectiveness of MP in reducing pesticide residues.

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