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Compatibility Of Funguses With Chemical Insecticides Used In Rice Cultivation

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Abstract: The integration of chemical and biological methods for pest control has become an indispensable strategy to reduce environmental pollution in recent years. For this reason, this work evaluated the compatibility of the biocontrol fungi Paecilomyces sp., Lecanicillium lecanii, and Trichoderma sp. in vitro conditions with four chemical insecticides used for pest control in rice crops in the department of Norte de Santander. The agrochemicals used were Actara® (0.01-0.05%), Engeo® (0.05-0.6%), Lorsban® (0.1-1.0%) and Numetrin® (0.01-0.4%). The tests were performed on PDA agar, where the growth inhibition, agrochemical effect on the concentration, and conidia germination were determined. The statistical results showed that the Lorsban® insecticide had the greatest inhibitory effect on mycelial growth for the three fungi evaluated and was classified as moderately toxic according to the OILB.

Keywords. Agrochemicals; biocontrol; contamination; Lecanicillium lecanii; Paecilomyces sp.; Trichoderma sp.

Introduction

Rice is one of the most cultivated cereals worldwide, constituting the most consumed staple food globally and the primary protein source for many people (Mondal et al., 2017). However, diseases-causing-microorganisms or insects have reached crop losses (Rakes et al., 2016) close to 12.2% (Rath et al., 2018), requiring the use of chemical fertilizers and pesticides to control them (Rakes et al., 2016). Currently, the indiscriminate use of agrochemicals on crops has negatively affected the environment, affecting soil biodiversity, food security, and agricultural sustainability (Rath et al., 2018; Meena et al., 2020). The use of entomopathogenic and antagonistic fungi as biological control agents on pest insects reduces the use of chemical pesticides because they are capable of producing enzymes such as chitinases, proteases, and glucanases that can degrade the insect cuticle and the cell wall of phytopathogenic fungi (Franco et al., 2011). For example, according to Liu et al., (2017), Trichoderma sp. can degrade organophosphorus agrochemicals and organochlorine pesticides highly used in agriculture. However, pathogenic, antagonistic, and degradative activity depends on the Phytopathogens strain, environmental conditions, and susceptibility. Therefore, one strategy to reduce agrochemical use, environmental impact, and variability in biological activity are entomopathogenic and antagonistic fungi with low concentrations of chemical pesticides. Therefore, the objective of this research was the compatibility assessment of Paecilomyces sp., Lecanicillium lecanii, and Trichoderma sp. under in vitro conditions with four different chemical insecticides used in rice crops to involve them in the integrated management of pests and diseases.

Materials and Methods

Strain reactivation

The biocontrol-fungi strains (Paecilomyces sp., Lecanicillium lecanii and Trichoderma sp.) were used, provided by the Cepario of Universidad Francisco de Paula Santander (UFPS). Reactivation was performed on Sabouraud agar, incubated at 27°C for eight days.

Compatibility assessment

The insecticides Numetrin® (Cypermethrin 200g/L), Engeo® (Thiamethoxan 14.1% w/v, Lambda-Cyhalothrin 10.6% w/v), Lorsban® (Chlorpyrifos 15% w/w), and Actara® (Thiamethoxam 25% w/w) were tested by subjecting them to UV light in a laminar airflow cabinet during 10 minutes to eliminate the presence of any microorganism. A standard solution of each product was prepared in amber flasks with sterile distilled water. In Potato Dextrose Agar (PDA), the amount corresponding to each pesticide solution was added, obtaining five test concentrations (see Table 1), taking two values below and two above the recommended field dose (Castellanos et al., 2015).

Table 1. Agrochemical treatments used for the three biocontrol fungi according to the field dose (dc = recommended field dose)

Fungi	Treatments			
Trichoderma sp.	Num 0.01%	Lor 0.1%	Eng 0.05%	Act 0.010%
Paecilomyces sp.	Num 0.05%	Lor 0.2%	Eng 0.1%	Act 0.015%
Lecanicillium lecanii	Num 0.1% (dc)	Lor 0.4% (dc)	Eng 0.2% (dc)	Act 0.025% (dc)
	Num 0.2%	Lor 0.8%	Eng 0.4%	Act 0.030%
	Num 0.4%	Lor 1%	Eng 0.6%	Act 0.050%
	Control	Control	Control	Control

Growth inhibition determination

In Petri dishes with PDA medium, 0.5 cm diameter discs of each 10-day old strain culture plus the agrochemical concentration were seeded and the plates were incubated at 27°C in dark conditions. Finally, the biocontrol fungi colony radius was compared for each treatment with the control growth radius, seeded in a PDA medium without chemical insecticide, and incubated under the same conditions. The evaluation of day ten was taken into account for the analysis of the results. The colony growth inhibition percentage was determined considering the formula (1) provided by Gallego et al., (2014), where EUr is Experimental Unit Radius, and Cr is Control Radius.

$$\mathbf{I}\,(\%) = \frac{\{1-(\mathrm{EUr})\}}{\mathrm{Cr}} \mathrm{x}100 \quad (1)$$

Evaluation of the agrochemical effect on conidia concentration and germination

0.5 cm discs of each fungus were seeded in the specific culture media and incubated in dark conditions to stimulate mycelium formation at 27°C. The sporulation intensity was used as an indicator of the agrochemical effect since the disks were placed in 2 mL of sterile distilled water with 0.1% tween and shaken repeatedly. Counting was performed in the Neubauer chamber (Alcantara et al., 2020). Conidia germination was evaluated using an aqueous suspension of each fungus adjusted to 10^7 conidia/mL in a final volume of 10 mL of sterile distilled water, 100µL were added in Petri dishes with the specific culture media. Each box (treatment) was incubated at 27°C in dark conditions for 24 hours. Then, germination percentage was calculated using the formula (2) (Grijalbal et al., 2014).

$$\%\,\mathbf{Germination} = \frac{\mathrm{N^\circ\,GC}}{\mathrm{N^\circ\,GC} + \mathrm{N^\circ\,GNC}} \times 100 \quad (2)$$

Where GC is the germinated conidia, and GNC is the non-germinated conidia.

Determination of the CI-50

The CI-50 was determined ten days after the trial and the agrochemical toxicology was classified (see Table 2) according to the values of the fungus growth inhibition percent after ten days, using the International Organization for Biological Control scale (OILB) (Castellanos et al., 2015).

Table 2. Toxicity classification of the agrochemicals according to the OILB.

Growth inhibition (%)	Classification
< 30%	Harmless
30-75%	Slightly toxic
75-90%	Moderately toxic
>90%	Toxic

Determination of the T-value

The compatibility of each insecticide with the biocontrol fungus was calculated according to the T-value proposed by Alves et al., (1998), using the parameters percent growth inhibition and the effect on the ability to form spores through the formula (3).

$$\mathrm{T} = \frac{(20\,[\mathrm{CV}] + 80\,[\mathrm{ESP}])}{100} \quad (3)$$

The T variable represents the corrected value for product classification; CV is the vegetative growth percentage of the treatment with the control and, ESP is the treatment sporulation percentage concerning the control (Castellanos et al., 2015). T values are classified according to the established scale (Table 3).

Table 3. Classification of the compatibility according to T value

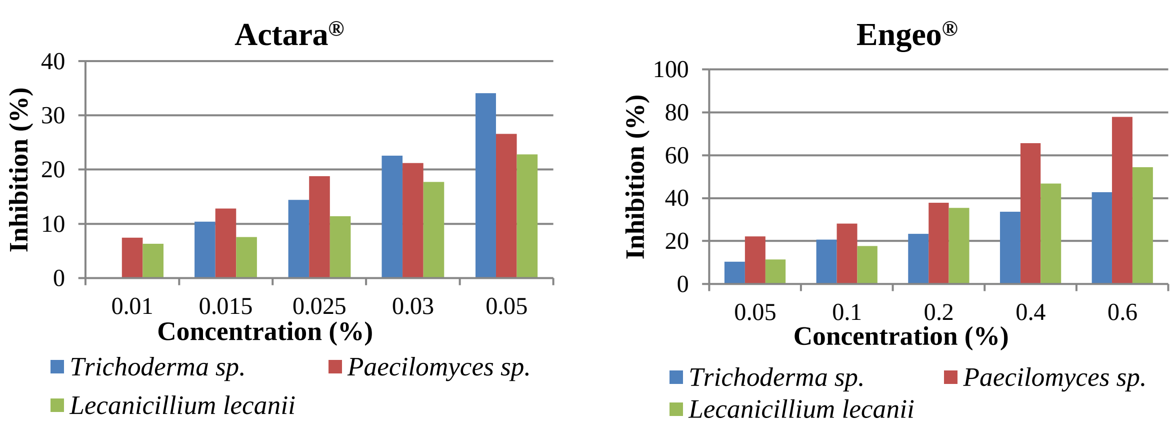
T value	Classification
0 to 30	Toxic
31 to 45	Very toxic
46 to 60	Moderately toxic
>60	Compatible

Statistical Analysis

Analyses of variance were performed using ANOVAs, and a POST HOC analysis was used for the variables, and Tukey test was performed, with a confidence level of 95% (p = 0.05) for each biocontrol fungus.

Results and Discussion

In Figure 2, it was found that when the insecticide Actara® was applied at 0.05% concentration, it inhibited the growth of biocontrol fungi by more than 20%, where Trichoderma sp. presented the highest value of 34.07%. According to Dhanya et al., (2016), Trichoderma viride presents 100% compatibility with the insecticides imidacloprid, chlorantraniliprole, spinosad, acephate, and flubendiamide, as well as 15.5% inhibition with the insecticide thiamethoxam and 60.6% with quinalphos. On the other hand, when using Engeo®, the most susceptible fungus to its action was Paecilomyces sp. when using concentrations of 0.4 and 0.6 generating an inhibition of 65.67 and 77.91% respectively. In contrast, Trichoderma sp. showed the lowest index in the same concentrations. Likewise, in all the different concentrations of the insecticide Lorsban®, an inhibition percentage higher than 50% was reached, being Lecanicillium lecanii the most affected in 0.8 and 1 concentrations. Finally, Paecilomyces sp. was the most affected using Numetrin®, while Lecanicillium lecanii presented inhibition values lower than 25%.



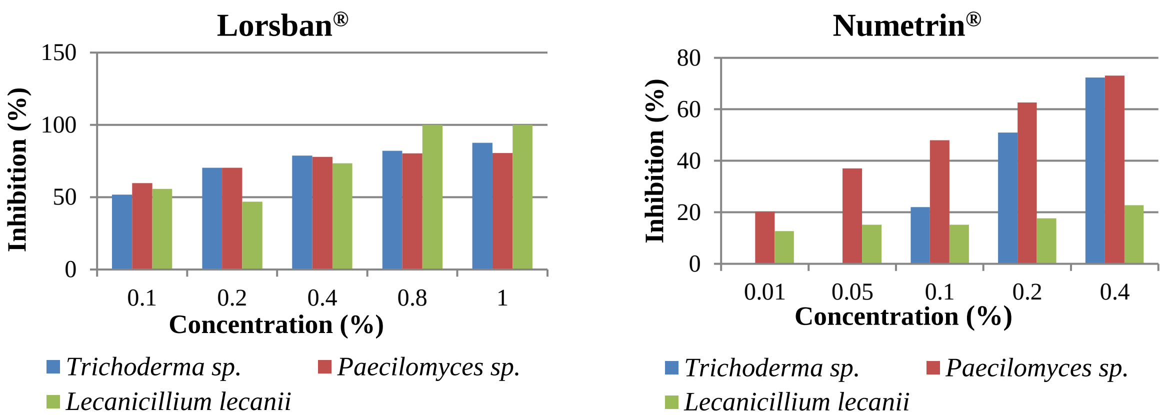


Figure 2. Inhibition percentages presented by the biocontrol fungi in the different agrochemicals.

Also, it was found that as the concentration of the insecticide increased, the inhibition percentage increased. The lowest inhibition percentages were observed when implementing the Actara® insecticide, where Lecanicillium lecanii was the least affected when using the maximum contaminant concentration. It was found that Trichoderma sp. obtained higher germination percentages (Fig. 3), indicating that it is probably using some secondary metabolite produced in vitro, which favors its development by assimilating more rapidly the medium compounds. However, Paecilomyces sp. presented the lowest germination percentages, mainly in the medium with Lorsban®, not exceeding 2%, accompanied by Lecanicillium lecanii with 31.57%, being the highest value reported in the medium with Numetrin®.

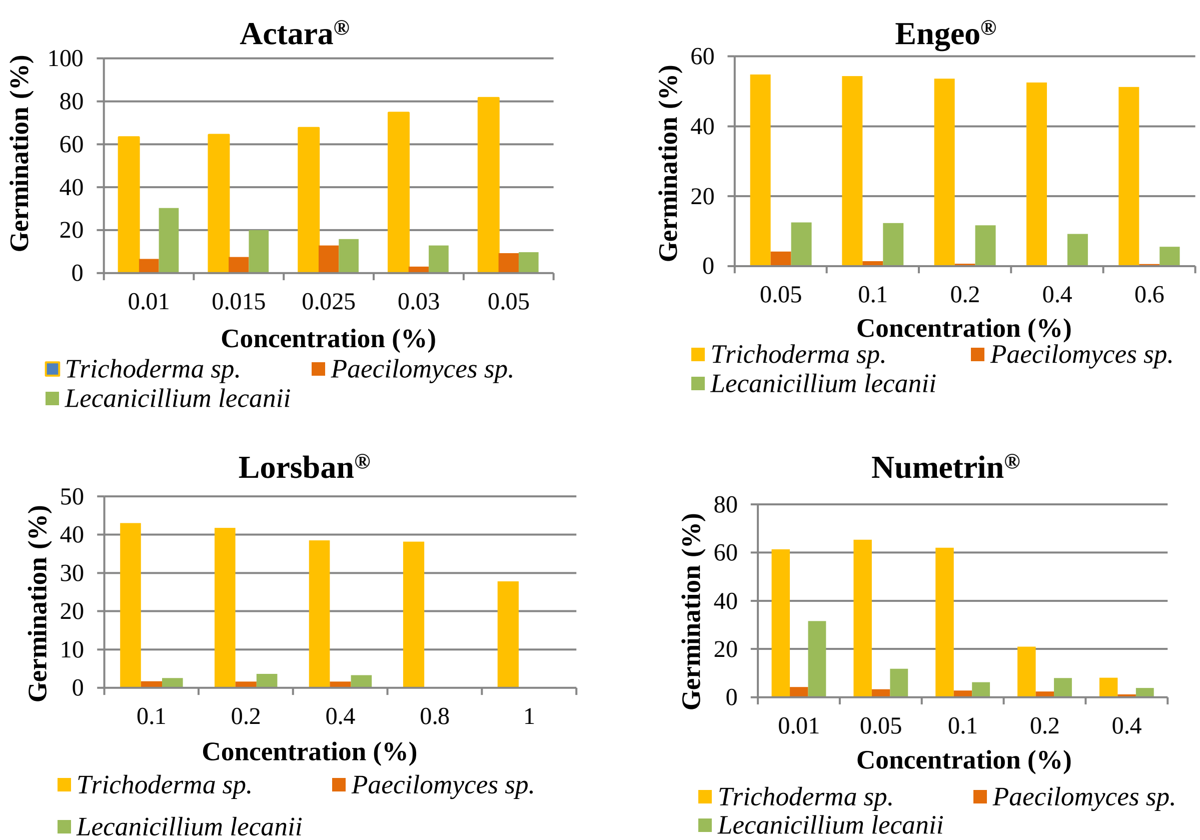


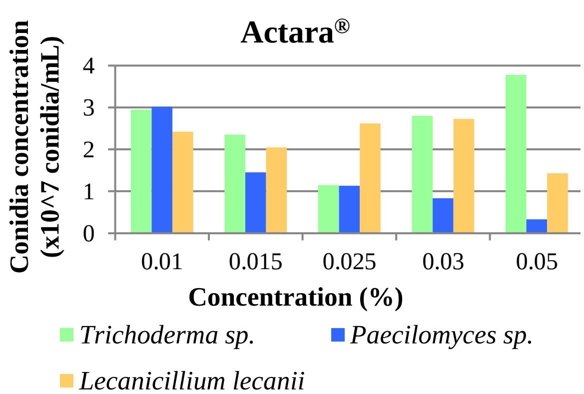
Figure 3. Germination percentages of the biocontrol fungi when implementing different concentrations of the agrochemicals.

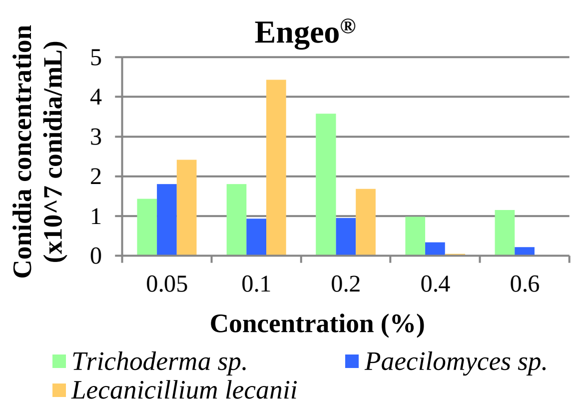
Trichoderma sp. obtained the highest percentages generating a significant difference compared to the other strains, identifying that the Actara® insecticide produced more than 50% germination. According to Wu et al. (2017), Trichoderma asperellum strain GDFS1009 presents a high mycelium production and sporulation capacity. Likewise, it secretes glucanase, chitinase, and protease enzymes to degrade the pathogenic fungal walls; as well as several species of Trichoderma can secrete different secondary metabolites, such as isonitrile, dicetopiperazines, and sesquiterpenes, to tolerate toxic environments.

Trichoderma sp. produced the highest quantity of conidia in the media supplemented with Actara® and Numetrin® being $3.78x10^7$ the highest concentrations. The latter ratifies that this fungus can produce some metabolite to assimilate faster the toxic compound, agreeing with Jaiswal et al., (2017). Based on the mentioned report, the fungus Trichoderma viride can tolerate and bioremediate chlorpyrifos since it possesses the opd gene and the enzymes Methyl parathion hydrolase (MPH), OPenylenediamine Dihydrochloride (OPD) and, Mevalonate Pyrophosphate Decarboxylase (MPD) responsible for the insecticide hydrolysis, employing it as the only carbon and energy source.

Likewise, Lecanicillium lecanii was the only one capable of generating conidia greater than $2x10^7$ in the medium with Lorsban®, which is curious since its germination percentage with this same insecticide was the lowest. According to Hanan et al., (2020), Lecanicillium lecanii can secrete bioactive compounds with insecticidal activity such as abamectin and spinosad.

Finally, Paecilomyces sp. obtained the highest value in Actara® being $3.02x10^7$, while its lowest values were observed with the agrochemicals Engeo® $0.217x10^7$ and zero values in Lorsban®. According to Gallego et al., (2014), "in a toxic medium, the fungus could be making a reproductive effort to increase the production of conidia as an escape strategy against the presence of a toxicant in the medium." Likewise, Van den Brule et al., (2019) state that spores are stress-resistant cells essential for the colonization of different ecosystems by dispersing through the air.





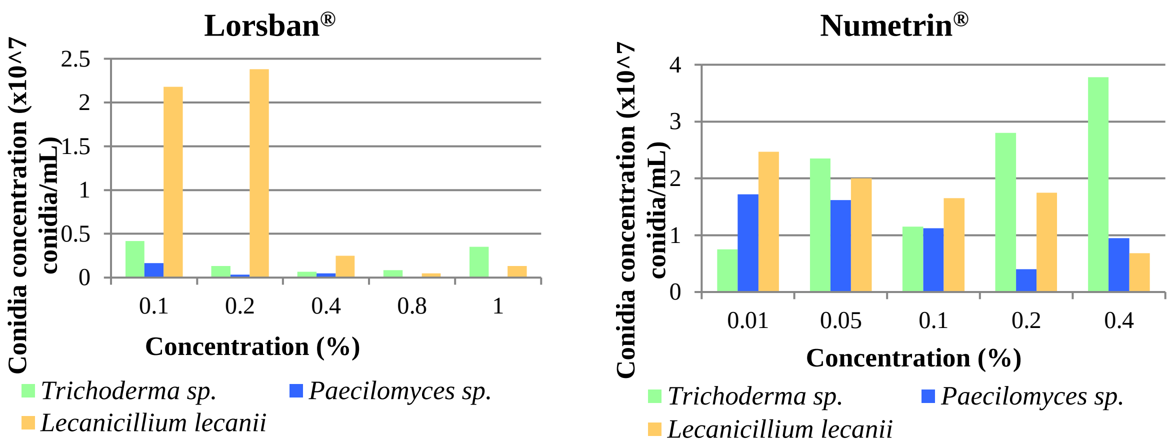


Figure 4. Conidia concentration reported on the different insecticides by biocontrol fungi.

In the adjusted model for the inhibitory concentration of insecticides on Trichoderma sp. it was observed that Numetrin® and Actara® are compatible with the antagonist, presenting a CI-50 of 0.23 and 0.11%, respectively, values higher than the recommended dose in the field, which suggests that both insecticides can be used in combination with this biocontrol fungus (see Table 4). Research conducted by Castellanos et al., (2015) evaluated the in vitro effect of commercial pesticides on Trichoderma harzianum strain A-34, where the insecticide cypermethrin at 2000 mg/L concentration obtained an inhibition of 61% and a sporulation of $4.5x10^7$, classifying it with the toxicity of harmless OILB.

Table 4. IC-50 values of insecticides for the fungus Trichoderma sp.

Item	CI-50%
NUMETRIN®	0.23*
LORSBAN ®	0.06**
ENGEO®	1.35**
ACTARA®	1.35**

* Significant for p<0.05; ** Highly significant for p<0.01

For the development of the biocontrol fungus Paecilomyces sp., the insecticides Numetrin® and Engeo® presented an IC-50 higher or equal to the field dose, being considered compatible or slightly toxic, where the Actara® insecticide obtained an IC-50 of 0.34%, being the inhibitory concentration of 50% of the fungus mycelial growth (see Table 5).

Table 5. IC-50 values of insecticides for the fungus Paecilomyces sp.

Item	CI-50%
NUMETRIN®	0.09**

LORSBAN ®	0.02*
ENGEO®	0.22**

* Significant for $p<0.05$; ** Highly significant for $p<0.01$

Finally, the insecticides Numetrin®, Engeo®, and Actara® presented an inhibitory concentration of 1.52, 0.48, and 0.11%, respectively, higher than the field applied dose. The latter suggests that the field doses must be higher for inhibiting 50% of Lecanicillium lecanii mycelial growth than the applied ones. The above can be observed in Table 6. In a study conducted by Walkunde et al., (2019), they studied the efficacy of biopesticides and chemical insecticides against wheat aphid (R. padi), finding that the treatment composed of Lecanicillium lecanii 1.15% WP @ 2.0 kg/ha fb. thiamethoxam 25 WG @ 50.0 g/ha was the most promising by registering 10.07 amount of aphids/shoot/plant on average.

Table 6. IC-50 values of insecticides for the fungus Lecanicillium lecanii.

Item	CI-50%
NUMETRIN®	1.54**
LORSBAN ®	0.09**
ENGEO®	0.48*
ACTARA®	0.11**

* Significant for $p<0.05$; ** Highly significant for $p<0.01$

In the trials with Trichoderma sp. the Lorsban® insecticide was the only product that presented a moderate toxicity level concerning the mycelial growth inhibition percentage at the field dose, the Numetrin® insecticide was classified as harmless with a percent growth inhibition of 22.03%, being less than 30%, according to the OILB scale. When evaluating the compatibility of the insecticides Chlorpyrifos 50% + Cypermethrin 5% at concentrations in the interval of 55-220 ppm on the fungus T. harzianum, it was found that the insecticides affected its growth even at the lowest concentration allowing a slow radial growth the first five days (Siddhartha et al., 2017). The main ingredient of Lorsban® insecticide is chlorpyrifos, being highly toxic and persistent in the environment; however, the microorganisms Trichoderma harzianum and Trichoderma sp. possess the ability to mineralize the aliphatic, aromatic, and heterocyclic compounds present in the insecticide, achieving their assimilation (Neelam and Kusum, 2020).

In Paecilomyces sp. the insecticides Numetrin®, Lorsban®, and Engeo® showed some level of toxicity, generating a growth inhibition of 48.05, 77.91, and 37.91%, respectively. Furthermore, the insecticides Lorsban® and Engeo® were slightly toxic to Lecanicillium lecanii, inhibiting mycelial growth by 73.41 and 35.44%. Finally, according to the compatibility analyses, the insecticide Lorsban® was classified as very toxic for each of the biocontrol fungi, reporting the lowest T-values, as shown in Table 7. The above agrees with a study conducted by Gonçalves et al., (2019), in which they evaluated the fungi toxic effects of the agrochemicals chlorantraniliprole, flubendiamide, Etofenprox, and thiamethoxam. They implemented the experiments on soybean and rice crops using the fungi Beauveria bassiana and Metarhizium anisopliae, observing that Etofenprox and thiamethoxam were moderately compatible with the tested fungi.

Likewise, according to Rakes et al., (2016), for the rice crop treatment, the agrochemicals Bim® 750 BR + Actara® 250 WG, Alterne® + Bim® 750 BR + Priori® 250 CS + Actara® 250 WG are highly compatible, reducing environmental contamination and generating more significant efficiency.

Table 7. Toxicity and compatibility classification of agrochemicals on biocontrol fungi.

	Toxicity (OILB)					
	Trichoderma sp.		Paecilomyces sp.		Lecanicillium lecanii	
Insecticide	Growth inhibition (%)	Classification	Growth inhibition (%)	Classification	Growth inhibition (%)	Classification
NUMETRIN®	22.03	Harmless	48.05	Slightly toxic.	15.18	Harmless
LORSBAN ®	78.7	Moderately toxic	77.91	Moderately toxic	73.41	Slightly toxic.
ENGEO®	23.33	Harmless	37.91	Slightly toxic.	35.44	Slightly toxic.
ACTARA®	14.44	Harmless	18.8	Harmless	11.39	Harmless
	Compatibility					
	Trichoderma sp.		Paecilomyces sp.		Lecanicillium lecanii	
Insecticide	T value	Classification	T value	Classification	T value	Classification
NUMETRIN®	66.878	Compatible	62.122	Compatible	58.124	Moderately toxic
LORSBAN ®	9.472	Very toxic	9.435	Very toxic	9.090	Very toxic
ENGEO®	30.058	Very toxic	64.19	Compatible	61.672	Compatible
ACTARA®	61.552	Compatible	55.856	Moderately toxic	62.774	Compatible

The main agrochemicals used in rice cultivation for pest control are Numetrin®, Engeo®, Lorsban®, and Actara®. Thiamethoxam (TMX) is a neonicotinoid insecticide extremely toxic to some organisms (Khaldoun et al., 2017; Perine et al., 2021). On the other hand, the insecticide chlorpyrifos has a destructive effect on soil microbial diversity (Kumar et al., 2017; Meena et al., 2020).

Therefore, biological control is a natural alternative for crop pest management, replacing the high concentrations in which agrochemicals are used in the field (Castellanos et al., 2015). Several studies ratify Trichoderma as an ecological, safe, and effective biological control agent in different crops (Thapa et al., 2020; Sood et al., 2020). Also, it is widely used in rice crops due to its ability to tolerate salinity, absorb nutrients, fertilize the soil, promote plant growth and cellulose degradation, high auxin production, and phosphate solubilization. Therefore, Trichoderma is considered a possible biofertilizer for rice crops by improving its yield and mitigating stress, becoming an excellent biopesticide (Debnath et al., 2020). Likewise, the genus Paecilomyces can produce secondary metabolites such as paeciloxazine with insecticidal action generating direct and/or indirect effects on crops (Dai et al., 2020), where the authors Moreno et al., (2020), found that Paecilomyces variotii can promote root and shoot development, increasing stem length and diameter by 30.2% and 15.7%, respectively.

Conclusions

Biological control is a natural alternative for pest management in crops, replacing the high concentrations in which agrochemicals are used in the field. The reactivated fungi showed the typical characteristics of the genera Paecilomyces, Lecanicillium and Trichoderma. Furthermore, the insecticides Actara® and Numetrin® showed the least inhibitory effect on the mycelial growth of the three biocontrol fungi, classifying them as harmless. In contrast, the Lorsban® treatments showed values ranging from 50% to 100% of mycelial growth inhibition on each biocontrol fungus, reporting the lowest germination percentages, indicating that the active ingredient of this insecticide, known as chlorpyrifos, could affect the development of fungi that did not present the metabolites responsible for their degradation and/or assimilation. Based on this study, it can be concluded that the different biocontrol fungi in combination with low concentrations of the insecticides Actara® and Numetrin® can be used in integrated management of pests and diseases in rice crops to reduce the excessive use of agrochemicals that, due to accumulation, contaminate soils, water tributaries, and the air.

Conflicts of interest: All authors declare that there is no conflict of interest that could jeopardize the validity of the results presented.

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