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ORIGINAL ARTICLE

Growth, tolerance, and enzyme activities of *Trichoderma* strains in culture media added with a pyrethroids-based insecticide

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Endoglucanases

Abstract The application of pyrethroids and carbamates represents an environmental risk and may exert adverse effects on beneficial microorganisms such as *Trichoderma*, which contribute to the biocontrol of several fungal phytopathogens. This research evaluated the tolerance of several strains of *Trichoderma* to a selected culture medium contaminated with a commercial insecticide (H24®) composed of pyrethroids, permethrin and prallethrin, and carbamate propoxur, and determined the influence of this insecticide on the release of enzymes such as chitinases, peroxidases, and endoglucanases by a consortium of selected *Trichoderma* strains grown in liquid culture medium. Four out of 10 *Trichoderma* strains showed tolerance to 200 ppm (~48.3% of growth) of the commercial insecticide after 96 h of exposure to a contaminated solid medium. After eight days of growth in liquid culture, the insecticide enhanced extracellular protein content and peroxidase activities in the *Trichoderma* consortium but decreased both chitinase and glucanase activities. These fungal responses should be considered when implementing strategies that combine alternative pesticides and fungal biocontrollers for managing fungal phytopathogens.

PALABRAS CLAVE
Permetrina;
Praletrina;
Propoxur;
Peroxidasas;
Quitinasas;
Endoglucanasas

Crecimiento, tolerancia y actividades enzimáticas de cepas de *Trichoderma* en medios de cultivo adicionados con un insecticida a base de piretroides

Resumen La aplicación de piretroides y carbamatos representa un riesgo ambiental y puede ejercer efectos adversos sobre microorganismos benéficos, como el *Trichoderma*, que contribuyen al biocontrol de varios fitopatógenos. Por un lado, esta investigación evaluó la tolerancia de varias cepas de *Trichoderma* a un medio de cultivo sólido contaminado con un

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insecticida comercial (H24®) compuesto por piretroides (permetrina y praletrina) y carbamato propoxur; por el otro, determinó la influencia de este insecticida en la liberación de enzimas como quitinasas, peroxidasas y endoglucanasas por un consorcio de cepas seleccionadas de *Trichoderma* cultivadas en medio líquido. Cuatro de 10 cepas de *Trichoderma* mostraron tolerancia a 200 ppm (∼48,3% de crecimiento) del insecticida comercial después de 96 horas en un medio sólido contaminado. Tras ocho días de crecimiento en cultivo líquido, el insecticida aumentó el contenido de proteínas y la actividad peroxidasa del consorcio *Trichoderma*, pero redujo las actividades quitinasa y glucanasa. Estas respuestas fúngicas podrían ser consideradas al implementar estrategias para el biocontrol y el manejo de hongos fitopatógenos.

Introduction

After the prohibition of using the highly toxic and persistent dichloro diphenyl trichloroethane (DDT), alternate insecticides based on carbamates and pyrethroids (single or combined) began to be utilized for controlling insect pests[17]. However, these insecticides may also represent potential issues for human and environmental health[47]. Some carbamates such as propoxur or pyrethroids such as permethrin and prallethrin may have carcinogenic and mutagenic properties, and inflammatory effects in the stomach; in addition, they are also associated with alterations in functional sodium channels and poor motor development in children[25,30,44]. Furthermore, several studies reported the effect of carbamates and pyrethroids on beneficial bacteria and communities in the rhizosphere[10,35,36,38,47]. Therefore, research focused on improving strategies for integrated pest management, which includes the combination of organically-derived pesticides and the application of antagonist microorganisms to reduce chemical damage induced by insecticides in the environment[38].

Trichoderma genus includes worldwide ubiquitous fungi commonly found in soils and possesses a powerful and versatile enzymatic machinery (including cellulases, chitinases, peroxidases, and proteases, among others) which may degrade a wide range of organic recalcitrant compounds in soil[3,29,41]. Commonly, *Trichoderma* is one of the widest fungi directed to control plant pathogens since they produce enzymes with hydrolytic capacity and secondary metabolites related to processes such as antibiosis, space competition, plant growth improvement, and resistance against biotic and abiotic stresses[27,32,37]. Furthermore, *Trichoderma* spp. are tolerant to many agrochemicals and have the potential to degrade chemical pesticides because they possess specific enzymes to metabolize such compounds[8].

Therefore, the aims of this research were (1) to determine the tolerance of several strains of *Trichoderma* to solid culture medium contaminated with commercial insecticide H24® (composed of pyrethroids, permethrin and prallethrin, and carbamate propoxur) and (2) to evaluate the effect of this insecticide on the release of enzymes such as chitinases, peroxidases, and endoglucanases by selected *Trichoderma* strains grown in liquid culture medium.

Materials and methods

Microbiological materials

This research utilized ten strains of *Trichoderma*: Trich CP01 (*T. virens*), Trich CP03 (*T. koningii*), Trich CP04 (*T. viride*), Trich CP022 (*T. virens*), Trich CP023 (*T. koningii*), Trich CP037 (*T. virens*) Trich CP038 (*T. harzianum*), Trich CP056 (*T. viride*), Trich CP0X (*T. atroviride*), Trich CP0TGC (*T. viride*), and one strain of *Phanerochaete chrysosporium*-ATCC 34540 (CDBB 686) as referential fungus able to degrade toxic organic contaminants.

All *Trichoderma* strains are part of the microbial collection of the Microbiology Laboratory (Colegio de Postgraduados), which were reported as tolerant to crude oil, and to high concentrations of naphthalene, phenanthrene, and benzo[*a*]pyrene[2]. The strain of *P. chrysosporium*-ATCC 34540 (CDBB 686) was acquired from the CINVESTAV microbial repository and reported as tolerant and degrader of persistent organic pollutants, including insecticides[24].

Chemical reagents and culture media

The commercial insecticide H24® contains permethrin (360 mg/kg), propoxur (890 mg/kg) and prallethrin (50 mg/kg) as active ingredients; thus, the total of active ingredients yields up to 1300 mg/kg, which are dissolved in an organic solvent. This commercial insecticide is commonly applied for controlling flying and crawling insects that attack cotton crops due to its active ingredient permethrin. Potato dextrose agar (PDA) medium (BD Bioxon®) was prepared according to the manufacturer's specifications and different concentrations of the commercial insecticide (0, 50, 100, 150, and 200 ppm) were added to it.

The liquid culture consisted of a mineral medium (MM) prepared in accordance with Gao et al. with some modifications[13]. The MM contained (g/l): 1.0 K_2HPO_4, 0.5 KCl, 0.5 $MgSO_4{\cdot}7H_2O$, 0.01 $FeSO_4$, pH 7.0. The nitrogen source was meat peptone (CAS 91079-38-8, Merck), considering a nitrogen content of ∼12.5% and nitrogen in the insecticide (propoxur), the carbon source was sucrose and that carbon derived from the insecticide. The final C/N

ratio was ∼20/1, considering 100 ppm of active ingredients (permethrin, prallethrin, and propoxur) in commercial insecticide H24®. Both media were autoclaved at 121 °C for 15 min; afterwards, the H24® insecticide was added using filtration.

Bioassay 1. Fungal growth and tolerance to increased concentrations of commercial insecticide

Agar disks of 5 mm in diameter with fungal mycelium were sown in Petri dishes containing solid PDA medium with or without the commercial insecticide (four replicates per treatment); all fungal cultures were incubated at 28 °C for 96 h. The growth diameter was measured every 24 h, for 4 days. The results were used to estimate the radial growth, radial growth rate, radial growth rate inhibition (%) (RGRI%), and the inhibitory dose 50 (ID_{50})[28,34]. The radial growth rate was calculated with the quadratic equation that was fitted to the dose–response curve for each strain. The radial growth rate was used to estimate the (ID_{50}) for each strain and the RGRI%[28,34]. The ID_{50} was also utilized as a reference to define a sub-ID_{50} without inhibiting the growth and further estimations[33].

Bioassay 2. Fungal protein content and induced peroxidase, chitinase, and glucanase activities in a liquid medium containing/contaminated with 100 ppm of commercial insecticide

Four tolerant strains of *Trichoderma* sp. (a consortium of *Trichoderma* sp.) selected from Bioassay 1, and *P. chrysosporium*-ATCC 34540 were used for evaluating the effect of a sub-ID_{50} of commercial insecticide H24® on the enzymatic activities of interest.

The initial fungal inoculum was adjusted for applying 1×10^6 spores ml of the *Trichoderma* consortium (adding the same number of spores per each fungal strain), and 1×10^6 spores ml of *P. chrysosporium*-ATCC 34540. Fungal cultures were maintained for 8 days at 200 rpm and 28 °C. Afterwards, protein analysis and enzyme tests were performed.

Protein content was determined with the Biuret method by using bovine albumin as standard[14]. The reaction mixture contained 100 μl of fungal supernatant and 1000 μl of Biuret reagent. This mixture was incubated for 30 min at room temperature (20–25 °C). Then, absorbance readings at 540 nm were taken by using a spectrophotometer (Synergy 2, Biotek®).

Non-specific peroxidase (POX, EC 1.11.1.7) activity was measured in 96-well microplates by mixing 20 μl of the fungal supernatant with 190 μl of phosphate buffer (50 mM, pH 7.0), 10 μl of guaiacol 1%, and 20 μl of H_2O_2 in phosphate buffer (50 mM, pH 7.0). Absorbance readings at 450 nm were taken from this mixture every 15 s for 5 min, by using a spectrophotometer (Synergy 2, Biotek®). POX activity was calculated using a molar extinction coefficient (ε) of 16.8/mM cm. One unit of POX activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol of tetraguaiacol per min at 25 °C and pH 7.0[7].

Chitinase activity (GlcNAc activity) was measured by using the method proposed by Vargas-Hoyos and Gilchrist-Ramelli, using N-acetyl-D-glucosamine (GlcNAc) as standard[22,42]. Thus, 500 μl of fungal supernatant was mixed with 100 μl of colloidal chitin in sodium citrate buffer (50 mM, pH 5.2), and incubated for 30 min at 40 °C; then, the mixture was cooled at ambient temperature (20–25 °C), and 2000 μl of dinitrosalicylic acid (DNS) were added, for further incubation at 90 °C for 5 min. The reaction mixture was cooled with iced water, and absorbance readings were measured at 540 nm in a Synergy 2, Biotek® spectrophotometer. One unit of GlcNAc activity was defined as the amount of the enzyme necessary to liberate 1 μmol/min of reducing sugar (glucose). Enzyme activity was expressed in units per mg of protein.

β-1,4-Glucanase activity (CMCase activity) was quantified by the DNS technique using D-glucose as standard[15,22]. The reaction mixture consisted of 500 μl of carboxymethyl cellulose (2%) in sodium citrate buffer (50 mM, pH 4.8) and 500 μl of fungal supernatant. The mixture was incubated for 30 min at 50 °C and cooled at room temperature. Then, 5000 μl of DNS solution was added and incubated at 90 °C for 5 min; the reaction was stopped by adding iced water. Absorbance readings were taken at 540 nm in a spectrophotometer (Synergy 2, Biotek®). One unit of CMCase activity was defined as the amount of the enzyme necessary to liberate 1 μmol/min of glucose, and the enzyme activity was expressed in terms of units per mg of protein.

Statistical analysis

Data were analyzed by one-way ANOVA and by the mean comparison test (Tukey, $\alpha = 0.05$). Data represents the values of the means ± standard error (SE), from four replicates for each treatment. Analyses were performed using SPSS software, version PASW 18 (IBM SPSS-IBM Corp).

Results

Bioassay 1. Fungal growth and tolerance to three concentrations of commercial insecticide

In general, all *Trichoderma* strains tolerated the presence of the commercial insecticide H24® when applied at 50, 100, and 150 ppm. Nevertheless, the strains CP01 (*Trichoderma virens*), CP04 (*T. viride*), CP038 (*T. harzianum*), CP056 (*T. viride*), and CP0TGC (*T. viride*) stopped their growth after 72 h of exposure to 150 ppm of the commercial insecticide (Figs. 1b–d). Only four *Trichoderma* strains (CP03 *T. koningii*, CP022 *T. virens*, CP037 *T. virens*, and CP0X *T. atroviride*) tolerated the application of the insecticide at 200 ppm (Fig. 1e). Fungal adaptation to the insecticide occurred after 24 h when all *Trichoderma* strains exposed to 50, 100, 150, and 200 ppm showed visible mycelial growth (Figs. 1b–e).

The growth rate of *Trichoderma* strains and *P. chrysosporium*-ATCC 34540 in a solid medium (PDA) containing 0, 50, 100, 150, and 200 ppm of active ingredients (permethrin, prallethrin, and propoxur) of the commercial insecticide was determined. The tested concentrations had a negative effect on the radial growth rate of all *Trichoderma* strains and *P. chrysosporium*-ATCC 34540 when compared to their respective control without insecticide.

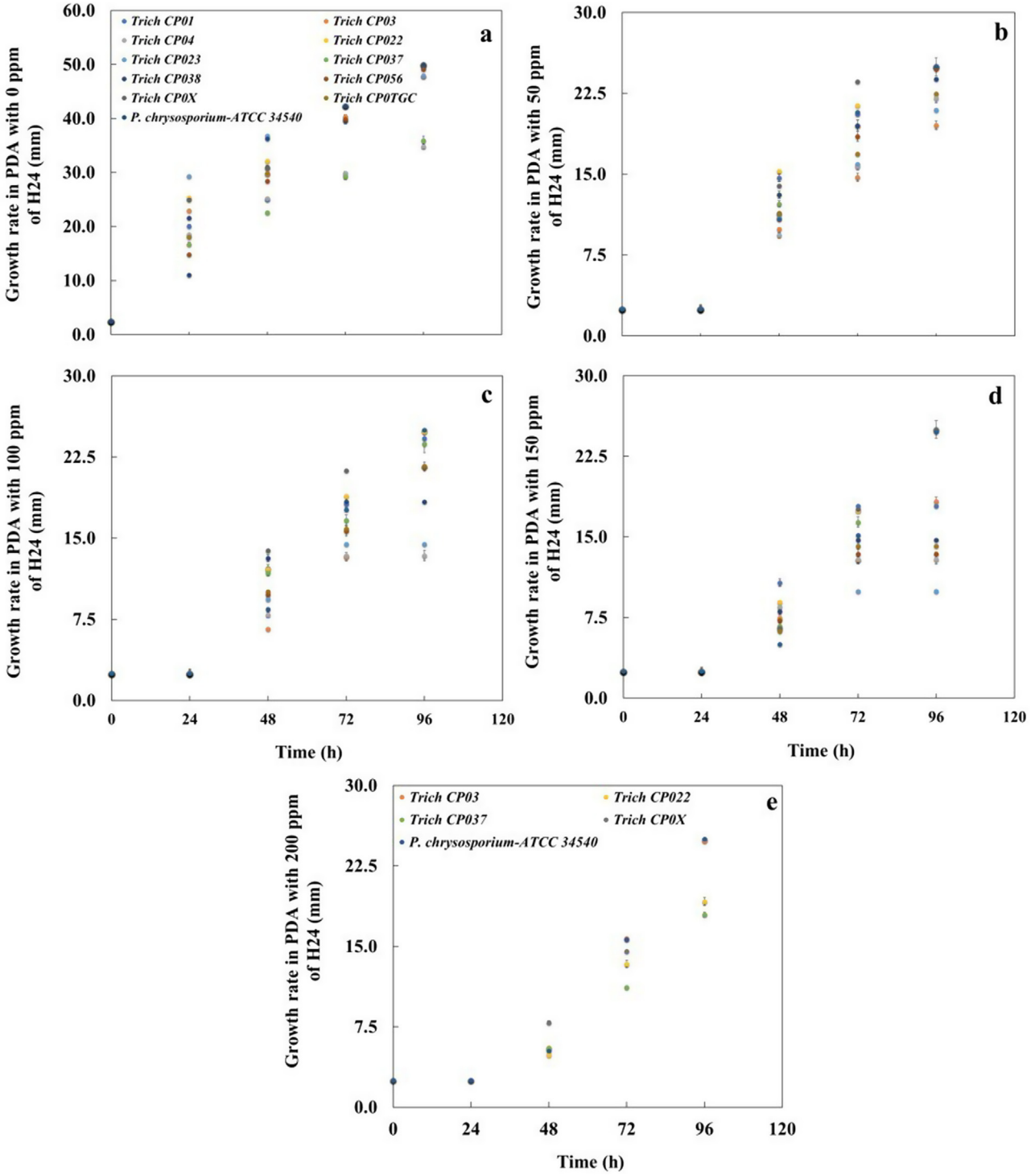

Figure 1 Growth rate of *Trichoderma* strains and *Phanerochaete chrysosporium*-ATCC 34540 in solid medium (PDA) with (a) 0 mg/l, (b) 50 mg/l, (c) 100 mg/l, (d) 150 mg/l, and (e) 200 mg/l of commercial insecticide H24® with three active ingredients (permethrin, prallethrin, and propoxur). Means ± standard error (n = 4).

The radial growth rate achieved at 150 ppm oscillated between ~0.50 and ~0.09 mm/h, while the radial growth rate for tolerant strains at 200 ppm was between ~0.53 and ~0.17 mm/h (Fig. 2a). Trich CP037 (*T. virens*) showed the lowest radial growth rate when exposed to 200 ppm of the commercial insecticide (Fig. 2).

Data from radial growth rate and radial growth rate inhibition (%) were used to obtain the inhibitory dose 50 (ID_{50})

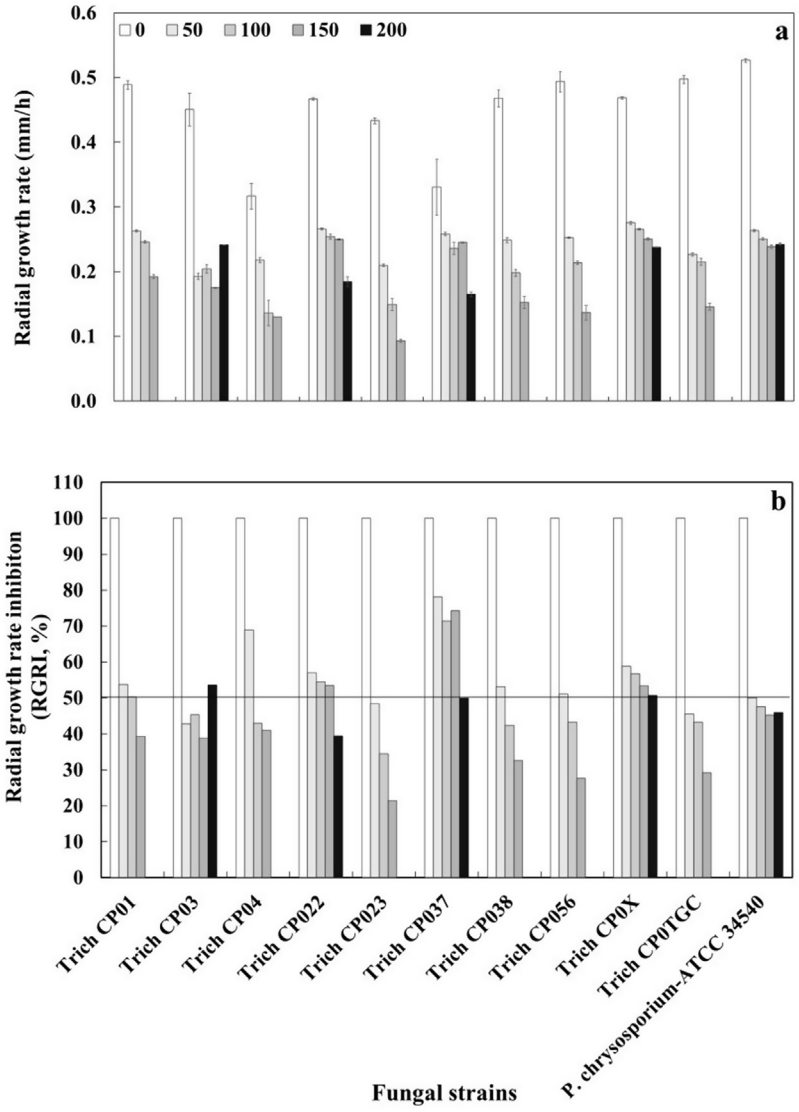

Figure 2 (a) Radial growth rate (mm/h) and (b) radial growth rate inhibition (RGRI%) of *Trichoderma* strains and *Phanerochaete chrysosporium*-ATCC34540 in solid medium (PDA) with 0, 50, 100, 150, and 200 mg/l of commercial insecticide H24® with three active ingredients (permethrin, prallethrin, and propoxur). Means ± standard error (n = 4).

of the commercial insecticide (Table 1). ID_{50} showed variations among all fungal strains. The values of the ID_{50} for those tolerant strains (Trich CP01; Trich CP04; Trich CP023; Trich CP038; Trich CP056; Trich CP0TGC) exposed to 150 ppm ranged from 77.27 to 107.66 ppm. Moreover, the values of the ID_{50} for those tolerant strains (Trich CP03; Trich CP022; Trich CP037; Trich CP0X; and *P. chrysosporium*-ATCC 34540) exposed to 200 ppm ranged between 131.64 and 219.04 ppm; of these strains, Trich CP037 showed the highest ID_{50} value (219.04 ppm) when compared to that from *P. chrysosporium*-ATCC 34540 (Table 1).

In addition, ID_{50} was used for a proposed sub-ID_{50} for those fungal strains that tolerated 200 ppm of the commercial insecticide. This sub-ID_{50} was utilized for Bioassay 2 to determine the enzyme activities from a *Trichoderma* sp. consortium and *P. chrysosporium*-ATCC 34540.

Bioassay 2. Fungal growth, protein content, and induced peroxidase, chitinase, and glucanase activities in a liquid medium containing 100 mg/l of commercial insecticide

The *Trichoderma* consortium [Trich CP03 (*T. koningii*), Trich CP022 (*T. virens*), Trich CP037 (*T. virens*), and Trich CP0X (*T. atroviride*)], and *P. chrysosporium*-ATCC 34540, were exposed to 100 ppm of the active ingredient of the commercial insecticide. After eight days, either the *Trichoderma* sp. consortium or *P. chrysosporium* grew and released proteins and enzymes into the contaminated liquid culture (Figs. 3 and 4).

In the present study, we observed that the growth of the *Trichoderma* consortium and *P. chrysosporium* was not affected by the active ingredients contained in the commer-

Table 1 Inhibitory dose 50 (ID_{50}) of commercial insecticide H24® with three active ingredients (permethrin, prallethrin, and propoxur) on the growth rate of ten strains of *Trichoderma* sp. and *Phanerochaete chrysosporium*-ATCC 34540 grown on solid culture medium.

Fungal strain	ID_{50} (mg/l) of commercial insecticide
Trich CP01	104.34 ± 1.47
Trich CP03	131.64 ± 15.80
Trich CP04	107.66 ± 3.56
Trich CP022	143.76 ± 3.45
Trich CP023	77.27 ± 0.99
Trich CP037	219.04 ± 3.16
Trich CP038	91.59 ± 2.15
Trich CP056	87.32 ± 4.58
Trich CP0X	167.10 ± 8.82
Trich CP0TGC	85.58 ± 0.32
P. chrysosporium-ATCC 34540	134.61 ± 1.34

Active ingredients in the commercial product (H24®) are permethrin, prallethrin, and propoxur. Means ± standard error (n = 4).

cial insecticide (Fig. 3a). However, in the absence and presence of insecticides, the biomass of the *Trichoderma* sp. consortium was significantly higher (~7-fold) than that of *P. chrysosporium*.

The strain of *P. chrysosporium*-ATCC 34540 produced more protein than the *Trichoderma* consortium in the absence or presence of the insecticide. The insecticide significantly influenced the protein production in the *Trichoderma* consortium and *P. chrysosporium*, whose protein content increased by ~1.3-fold under the contaminated culture (Fig. 3b).

On the other hand, without the insecticide, the *Trichoderma* consortium had significantly higher (~1.8-fold) POX activity than *P. chrysosporium*, reaching levels of ~6000 U/μg protein when compared to *P. chrysosporium* (~3250 U/μg protein) (Fig. 3c). Under insecticide contamination, POX activity increased in both fungal cultures; for the *Trichoderma* consortium, an increase of POX was achieved (~1.4-fold), whereas for *P. chrysosporium* the increase of POX was about ~2.1-fold than that of the respective control in the absence of the insecticide. Overall, the POX activity of the *Trichoderma* consortium was slightly higher (~1.2-fold) but not significant, as determined for *P. chrysosporium* (Fig. 3c).

The chitinase activity (GlcNAc activity) for the *Trichoderma* consortium was higher (~1.2-fold) than that achieved for *P. chrysosporium*, either in the absence or presence of the insecticide (Fig. 4a). Overall, the application of the insecticide caused a 25% decrease in the GlcNAc activity of both fungal cultures; however, the GlcNAc activity in the *Trichoderma* consortium was always significantly higher (~1.2-fold) than that in *P. chrysosporium* (Fig. 4a).

In the absence of the insecticide, the CMCase activity for the *Trichoderma* consortium was significantly higher (~1.4-fold) than the CMCase activity achieved by *P. chrysosporium*. The insecticide significantly decreased the CMCase activity

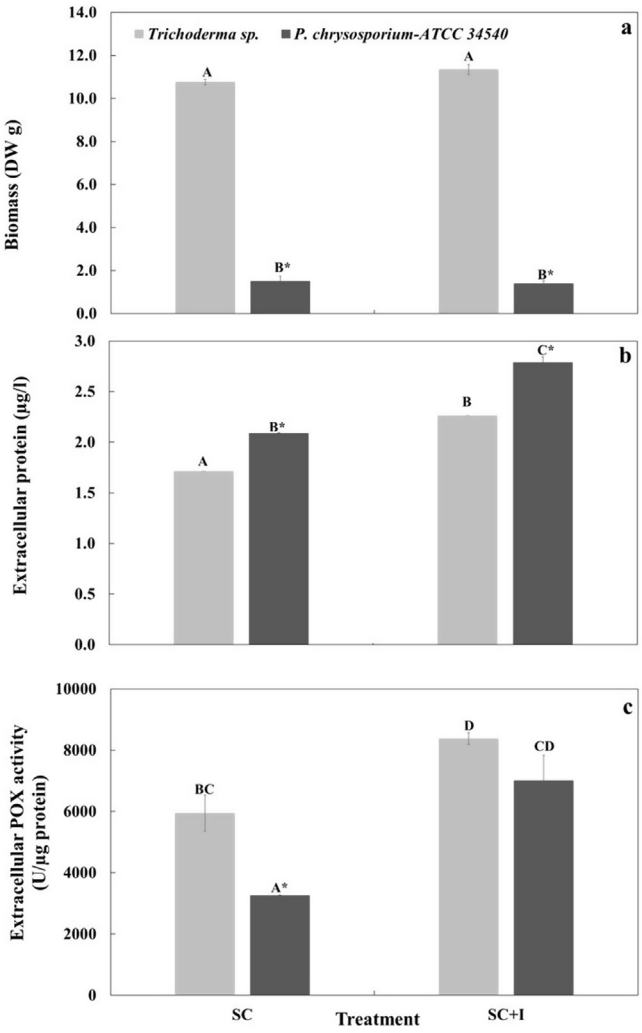

Figure 3 (a) Biomass (DW g), (b) extracellular protein content (μg/l), and (c) extracellular peroxidase activity (POX activity, U/μg protein) of *Trichoderma* sp. and *P. chrysosporium*-ATCC 34540 in liquid culture in the absence (SC) and presence (SC + I) of sub-ID_{50} (100 mg/l) of commercial insecticide H24® with three active ingredients (permethrin, prallethrin and propoxur), after 8 days in liquid culture. Different letters indicate significant differences among means for medium culture; asterisks indicate significant differences among means for microorganisms (Tukey, $\alpha = 0.05$). Means ± standard error (n = 4).

of both fungal cultures; overall, the enzyme activity of the *Trichoderma* consortium was significantly higher (~1.3-fold) than that of *P. chrysosporium* (Fig. 4b).

Discussion

Many microorganisms grow in the presence of pesticides, and this ability is influenced by chemical, physical, biochemical, and environmental conditions, and also depends on the amount and type of pesticides[36]. The initial fungal response to contaminants reflects the initial adaptation to stressful cultural conditions and/or to contaminated environments[20].

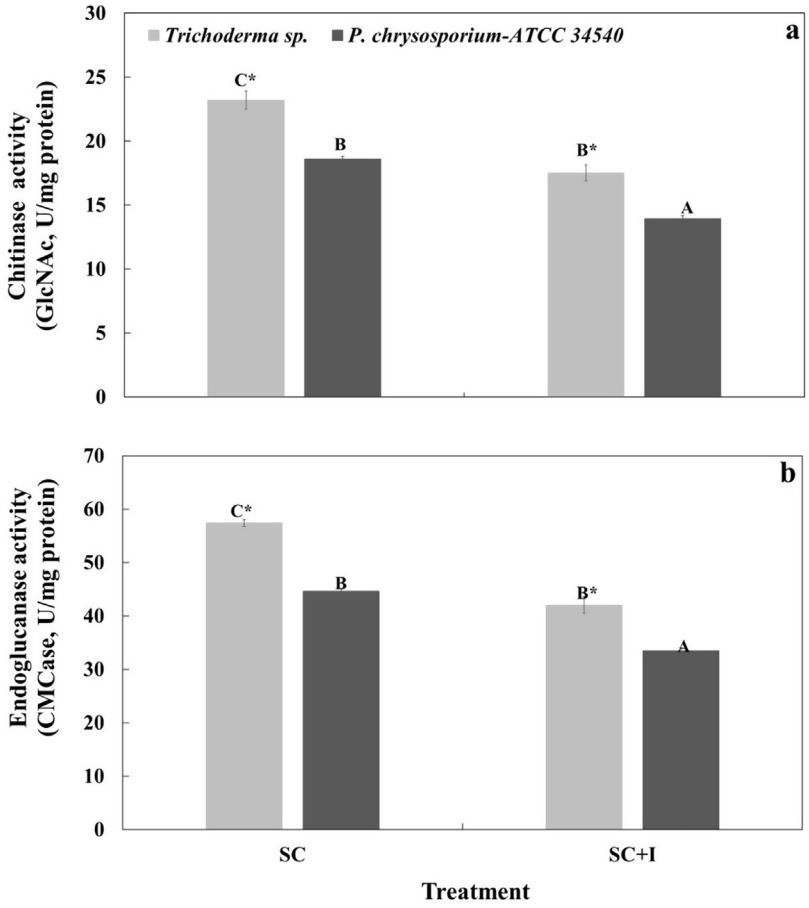

Figure 4 (a) Chitinase activity (GlcNAc, U/mg protein) and (b) endoglucanase activity (CMCase, U/μg protein) of *Trichoderma* sp. and *P. chrysosporium*-ATCC 34540 in liquid culture in the absence (SC) and presence (SC + I) of sub-ID_{50} (100 mg/l) of commercial insecticide H24® with three active ingredients (permethrin, prallethrin, and propoxur), after 8 days in liquid culture. Different letters indicate significant differences among means for medium culture; the asterisk indicates significant differences among means for microorganisms (Tukey, $\alpha = 0.05$). Means ± standard error (n = 4).

Synthetic pyrethroids (cypermethrin, deltamethrin, permethrin, and others) and carbamates such as propoxur may reduce the growth of bacteria and filamentous fungi such as *T. viride*, *T. harzianum*, and *P. chrysosporium* strains[1,10,23,36,47]. Conversely to our results, Schumacher and Poheling did not find negative effects of permethrin on the growth of *Metarhizium anisopliae*[35]. On the other hand, pyrethroids such as allethrin (50 mg/l) did not affect the growth of *Fusarium proliferatum* CF2[4]. Consistently to our results, Deng et al. observed that pyrethroids such as β-cypermethrin (100 mg/l) did not affect the final biomass produced by *Aspergillus niger* YAT; however, its radial growth was delayed[10].

The mixture of pyrethroids, β-cypermethrin, deltamethrin, fenvalerate, and α-cyhalothrin (100–1000 mg/l), reduced the growth in *T. viride* and *P. chrysosporium*[36]. In soils, the application of cypermethrin and chlorpyrifos alone and in combination dramatically decreased both bacterial and fungal populations[39]. The latter indicates that microorganisms require a certain period of adaptation to toxic contaminants to produce those necessary molecules for tolerating such compounds[1]. In the present study, the commercial product has a mixture of two pyrethroids (permethrin, prallethrin) with carbamate (propoxur), and this combination exerts certain toxicity which significantly delayed the growth of all fungal strains. This effect could be due to the synergy between carbamates and pyrethroids by which the toxicity to microbes may increase[17].

Some studies have demonstrated the ability of *Trichoderma* strains for tolerating and growing in the presence of organic compounds such as petroleum hydrocarbons and pyrethroids[2,6,36]. Furthermore, these fungi may use organic molecules as carbon and energy source since they have the enzymes necessary to perform such metabolism[18,45]. The fungus identified as part of the genus *Cladosporium* was reported as tolerant to pyrethroids, including β-cypermethrin, deltamethrin, bifenthrin and permethrin (100 mg/l)[8]. Other studies include fungi such as *Aspergillus oryzae* and *Cunninghamella elegans*, exposed to pyrethroids, cyhalothrin and 3-phenoxybenzoic acid, an intermediate in the degradation of permethrin[31,49].

Consortium cultures are better than individual cultures because there are complementary physiological and biochemical functions among microorganisms, i.e., while some microorganisms perform specific enzymatic activity, other microorganisms can perform some different enzyme activities, by which all the involved organisms may be benefited[40]. Enzyme activities include superoxide dismutases (SOD), peroxidases (POXs), catalases (CAT), chitinases, glucanases, and many others.

In nature, microorganisms coexist in consortia and interact with each other to transform organic materials[26,40]. Artificial and natural microbial consortia are being studied for assessing tolerance, removal, and degradation of inorganic and organic compounds[40]. However, little attention is given to filamentous fungi and their tolerance to insecticides as accounted for bacteria[9]. Moreover, research about fungal consortia exposed to pyrethroids and carbamates utilized as substitutes for DDT is scant. Furthermore, many fungi may remove or degrade inorganic and organic compounds from polluted systems through several biochemical processes which include antioxidant molecules and enzymes such as POXs. In this regard, POXs have antioxidant activity and are involved in the detoxification of reactive oxygen species (ROS) such as H_2O_2 accumulation in cells because these enzymes oxidize H_2O_2 and are involved in the detoxification processes of organic pollutants[19,46]. Other extracellular fungal POXs (lignin peroxidase, manganese peroxidase, and versatile peroxidase VP) are involved in the removal and degradation of pollutants such as polycyclic aromatic hydrocarbons (PAHs), dye-based textile effluents, polychlorinated biphenyls, fungicides, and pesticides[46].

Our results concur with those findings in white-rot fungi such as *Polyporus tricholoma*, *Cilindrobasidium leave*, and *Deconica citrispora* that increased POX activity (especially MnP) when exposed to paraquat, a widely applied herbicide in agriculture whose chemical structure resembles that of lignin[5]. In addition, *P. chrysosporium* can degrade a wide variety of organic pollutants due to the activity of non-specific extracellular POXs[24]. Furthermore, non-ligninolytic fungi such as *Aspergillus*, *Fusarium*, and *Trichoderma* may transform environmental pollutants such as PAHs, pesticides, and dyes, releasing POX enzymes[21]. Zhao et al. proposed that *A. oryzae* M4 uses some POXs in the presence of NADPH and O_2, for degrading 3-phenoxybenzoic acid, a subproduct of permethrin degradation[48]. *Trichoderma asperellum* H15 was exposed to PAHs with 3–5 rings (phenanthrene, pyrene, and benzo[*a*]pyrene), and the fungal POX activity increased after 4 days of exposure[46]. Many POXs were identified in *T. asperellum*, including cytochrome C peroxidases, catalases, glutathione peroxidase, and dye-decolorizing peroxidases[46]. Most fungi, including *P. chrysosporium* and *Trichoderma* sp., are studied for degrading or removing PAHs, organochloride pesticides, organophosphates, carbamates, and pyrethroids such as β-cypermethrin and deltamethrin, by means of the oxidative effects of POXs[11,12,24,43]. In our results, the POX activity detected in the *Trichoderma* consortium was higher than that of *P. chrysosporium*, which may be explained in part, by the fact that the *Trichoderma* consortium was integrated by four fungal strains, and thus, all strains may have released more POX enzymes.

The studies of POXs involved in either the removal or degradation of pyrethroids such as permethrin and prallethrin, and carbamates such as propoxur, are still little explored. Our results suggest that the *Trichoderma* consortium and *P. chrysosporium* increase their POX activities for tolerating the commercial insecticide in liquid culture. However, further studies are needed to identify specific POXs that participate during this process, and to determine whether these enzymes act on both the degradation and detoxification of insecticides and byproducts, as well.

On the other hand, chitinases play a key role in the transformation of chitin and are widely distributed in nature with a wider range of biotechnological applications, including the biocontrol of fungal phytopathogens, harmful insects, bioconversion of chitin wastes, to single-cell protein, biopesticides, among others[37]. Moreover, chitinases are important for maintaining the balance of carbon and nitrogen ratio in ecosystems[27,32,37]. Regarding chitin degradation, the chitinase family includes three enzymes that act separately, (1) endochitinases that recognize *o*-glycosyl bonds between chito-saccharide residues for catalysis and produce multimers of oligosaccharides, especially diacetylchitobiose; (2) exochitinases that release soluble low molecular weight dimers, and (3) chitobiose that hydrolyses diacetylchitobiose to N-acetyl-glucosamine (GlcNAc)[41]. Both bacteria and fungi use chitin as a carbon and energy source, and the production of chitinolytic enzymes is related to carbon sources in synthetic culture media[32,41].

Chitinases produced by *Trichoderma* correspond to enzymes that function as biological control agents and are responsible for the lysis and degradation of fungal cell walls and insect cuticles[16]. Furthermore, the exposure of *Paenibacillus* sp. to pyrethroids (cypermethrin) at concentrations recommended for field applications, caused the total inhibition of chitinase production, whereas insecticides such as methyl parathion and endosulfan significantly decreased (30–40%) the activity and stability of chitinases[38]. Organic molecules such as PAHs, with four or more aromatic rings, inhibited chitinase activities from *Aeromonas hydrophila* subsp. *anaerogenes* A52 and from *T. harzianum*[29,41]. To our knowledge, GlcNAc-activity has not been reported as being involved in the removal or degradation of organic pollutants such as pyrethroid and carbamate insecticides; however, this enzyme may serve as a biomarker for assessing detoxification processes of systems polluted with these compounds.

Endoglucanases (EGs) represent a group of dynamic cellulases that randomly attack internal *O*-glycosidic bonds of the cellulose chain, releasing glucan chains with different lengths, to generate new reducing and non-reducing ends; thus, EGs are the most important cellulases that contribute to the hydrolysis of cellulose[50]. EGs have many biotechnological applications for the industry, including animal food, textiles, laundry, pulp and paper, brewery and wine, food, and agriculture, among others. These enzymes are globally marketed from fungi such as *Aspergillus* and *Trichoderma*[3].

Overall, most studies are limited to reporting the degradation percentages of insecticides but not emphasizing the effects of insecticides on microbial biomolecules. The production of a high number of enzymes, and the optimal activity of these enzymes may depend on culture media and microorganisms. The use of pyrethroids and carbamates increased as a result of the prohibition of DDT, and these

insecticides can disturb agricultural soils and may lead to several issues related to environmental and human health pollution. Furthermore, these organic chemicals may exert toxic effects on microorganisms and higher organisms. The present study highlights that a commercial organic pesticide based on pyrethroids and carbamates may inhibit both the growth of filamentous fungi and the activity of important fungal enzymes involved in either chitin or cellulose degradation.

Overall, increasing the concentrations of the active ingredient of the commercial insecticide H24® decreased the radial growth rate in ten strains of *Trichoderma* sp. and *P. chrysosporium*. Four prominent strains of *Trichoderma* [*T. koningii* (Trich CP03), *T. virens* (Trich CP022), *T. virens* (Trich CP037), and *T. atroviride* (Trich CP0X)] were able to tolerate 200 ppm of this commercial insecticide. These four fungal strains grew in a liquid culture medium contaminated with 100 ppm of the commercial insecticide and showed increased protein production and high POX enzyme activity. Moreover, this commercial insecticide had negative effects on chitinase and endoglucanase activities derived from the *Trichoderma* consortium; therefore, it should be considered when using these microorganisms in combination with organic insecticides addressed to integrated pest management, and for assessing tolerance, detoxification, or degradation of organic insecticides based on permethrin, prallethrin and propoxur, for instance.

Conflict of interest

The authors declare that they have no conflicts of interest.

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References

1. Anusha J, Kavitha PK, Louella CG, Chetan DM, Rao CV. A study on biodegradation of propoxur by bacteria isolated from municipal solid waste. Int J Biotechnol Appl. 2009;1:26–31, http://dx.doi.org/10.9735/0975-2943.1.2.26-31.
2. Argumedo-Delira R, Alarcón A, Ferrera-Cerrato R, Almaraz JJ, Peña-Cabriales JJ. Tolerance and growth of 11 *Trichoderma* strains to crude oil, naphthalene, phenanthrene and benzo[a]pyrene. J. Environ. Manage. 2012;95:S291–9, http://dx.doi.org/10.1016/j.jenvman.2010.08.011.
3. Bhat MK. Cellulases and related enzymes in biotechnology. Biotechnol. Adv. 2000;18:355–83, http://dx.doi.org/10.1016/S0734-9750(00)00041-0.
4. Bhatt P, Zhang W, Lin Z, Pang S, Huang Y, Chen S. Biodegradation of allethrin by a novel fungus *Fusarium proliferatum* strain CF2, isolated from contaminated soils. Microorganisms. 2020;8:593, http://dx.doi.org/10.3390/microorganisms8040593.
5. Camacho-Morales RL, Gerardo-Gerardo JL, Guillén KN, Sánchez JE. Producción de enzimas ligninolíticas durante la degradación del herbicida paraquat por hongos de la pudrición blanca. Rev. Argent. Microbiol. 2017;49:189–96, http://dx.doi.org/10.1016/j.ram.2016.11.004.
6. Castellanos GL, Lorenzo NME, Lina MB, Hernández PR, Guillen SD. Efecto *in vitro* de plaguicidas comerciales sobre *Trichoderma harzianum* cepa A-34. Rev Fac Cien Agra. 2015;47:185–96 https://bdigital.uncu.edu.ar/objetos_digitales/7675/14-castellanos-fca47-2.pdf
7. Chance B, Maehly AC. Assay of catalases and peroxidases. Meth Enzymol. 1955;2:764–75, http://dx.doi.org/10.1002/9780470110171.ch14.
8. Chen S, Hu Q, Hu M, Luo J, Weng Q, Lai K. Isolation and characterization of a fungus able to degrade pyrethroids and 3-phenoxybenzaldehyde. Bioresour. Technol. 2011;102:8110–6, http://dx.doi.org/10.1016/j.biortech.2011.06.055.
9. Chen S, Zhan H. Biodegradation of synthetic pyrethroid. In: Arora PK, editor. Microbial metabolism of xenobiotic compounds. Microorganisms for sustainability. Springer; 2019. p. 229–44 https://link.springer.com/book/10.1007/978-981-13-7462-3
10. Deng W, Lin D, Yao K, Yun H, Wang Z, Li J, Zou L, Han X, Zhou K, He L, Hu X, Liu S. Characterization of a novel β-cypermethrin-degrading *Aspergillus niger* YAT strain and the biochemical degradation pathway of β-cypermethrin. Appl. Microbiol. Biotechnol. 2015;99:8187–98, http://dx.doi.org/10.1007/s00253-015-6690-2.
11. Ferreira JA, Varjani S, Taherzadeh MJ. A critical review on the ubiquitous role of filamentous fungi in pollution mitigation. Curr Pollut Rep. 2020;6:295–309, http://dx.doi.org/10.1007/s40726-020-00156-2.
12. Gangola S, Joshi S, Kumar S, Pandey SC. Comparative analysis of fungal and bacterial enzymes in biodegradation of xenobiotic compounds. Smart Bioremediat Technol. 2019: 169–89, http://dx.doi.org/10.1016/B978-0-12-818307-6.00010-X.
13. Gao L, Sun MH, Liu XZ, Che YS. Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of several biocontrol fungi. Mycol Res. 2007;111:87–92, http://dx.doi.org/10.1016/j.mycres.2006.07.019.
14. García-Espejo CN, Mamani-Mamani MM, Chávez-Lizárraga GA, Álvarez-Aliaga MT. Evaluación de la actividad enzimática del *Trichoderma inhamatum* (BOL-12 QD) como posible biocontrolador. J Selva Andina Res Soc. 2016;7:20–32 http://www.scielo.org.bo/pdf/jsars/v7n1/v7n1_a04.pdf
15. Ghose TK. Measurement of cellulase activities. Pure Appl. Chem. 1987;59:257–68, http://dx.doi.org/10.1351/pac198759020257.
16. González I, Infante D, Martínez B, Arias Y, González N, Miranda I, Peteira B. Induction of chitinases and glucanases in *Trichoderma* spp. strains intended for biological control. Biotecnol Apl. 2012;29:12–6 https://www.medigraphic.com/pdfs/biotecapl/ba-2012/ba121b.pdf
17. Hernández FA, Parrón T, Tsatsakis AM, Requena M, Alarcón R, López-Guarnido O. Toxic effects of pesticide mixtures at a molecular level: their relevance to human health. Toxicology. 2013;307:136–45, http://dx.doi.org/10.1016/j.tox.2012.06.009.
18. Kim H, Kim D-U, Lee H, Yun J, Ka J-O. Syntrophic biodegradation of propoxur by *Pseudaminobacter* sp. SP1a and *Nocardioides* sp. SP1b isolated from agricultural soil. Int Biodeter Biodegr. 2017;118:1–9, http://dx.doi.org/10.1016/j.ibiod.2017.01.024.
19. Koksal Z, Kalin R, Gulcin I, Ozdemir H. Inhibitory effects of selected pesticides on peroxidases purified by affinity chromatography. Int J Food. 2018;21:385–94, http://dx.doi.org/10.1080/10942912.2018.1424197.
20. Kurniati E, Arfarita N, Imai T, Higuchi T, Kanno A, Yamamoto K, Sekine M. Potential bioremediation of mercury-contaminated substrate using filamentous fungi isolated from forest soil. J Environ Sci. 2014;26:1223–31, http://dx.doi.org/10.1016/S1001-0742(13)60592-6.

21. Marco-Urrea E, García-Romera I, Aranda E. Potential of non-ligninolytic fungi in bioremediation of chlorinated and polycyclic aromatic hydrocarbons. New Biotechnol. 2015;32:620–8, http://dx.doi.org/10.1016/j.nbt.2015.01.005.
22. Miller GL. Use of dinitrosalysilic acid reagent for determination of reducing sugar. Anal. Chem. 1959;31:426–8, http://dx.doi.org/10.1021/ac60147a030.
23. Mohamed NA, Radwan MA. Impact of pesticides on *Trichoderma harzianum* and on its possible antagonistic activity against *Fusarium oxysporum* under *in vitro* conditions. Asian J Agri Biol. 2017;5:291–302 https://www.asianjab.com/wp-content/uploads/2017/12/OA-AJAB-2017-07-092_OK.pdf
24. Mohapatra D, Rath SK, Mohapatra PK. Bioremediation of insecticides by white-rot fungi and its environmental relevance. In: Prasad R, editor. Mycoremediation and environmental sustainability. Fungal Biology. Cham: Springer; 2018., http://dx.doi.org/10.1007/978-3-319-77386-5_7.
25. Neta G, Goldman LR, Barr D, Apelberg BJ, Witter FR, Halden RU. Fetal exposure to chlordane and permethrin mixtures in relation to inflammatory cytokines and birth outcomes. Environ. Sci. Technol. 2011;45:1680–7, http://dx.doi.org/10.1021/Fes103417j.
26. Niehaus L, Boland I, Liu M, Chen K, Fu D, Henckel C, Chaung K, Espinoza MS, Dyckman S, Crum M, Dedrick S, Shou W, Momeni B. Microbial coexistence through chemical-mediated interactions. Nat Commun. 2019;10:2052, http://dx.doi.org/10.1101/358481.
27. Okongo NR, Puri AK, Wang Z, Singh S, Permaul K. Comparative biocontrol ability of chitinases from bacteria and recombinant chitinases from the thermophilic fungus *Thermomyces lanuginosus*. Biosci Bioeng. 2019;127:663–71, http://dx.doi.org/10.1016/j.jbiosc.2018.11.007.
28. Ono MA, Itano EN, Mizuno LT, Mizuno EH, Camargo ZP. Inhibition of *Paracoccidioides brasiliensis* by pesticides: is this a partial explanation for the difficulty in isolating this fungus from the soil? Med. Mycol. 2002;40:493–9, http://dx.doi.org/10.1080/mmy.40.5.493.499.
29. Ooki Y, Kumemura M, Itoh M, Korenaga T. Inhibitory analysis of the effect of polycyclic aromatic hydrocarbons on the activity of chitinase by means of liquid chromatography–mass spectrometry of chitin oligosaccharides. Anal. Bioanal. Chem. 2007;387:2641–4, http://dx.doi.org/10.1007/s00216-007-1157-7.
30. Ostrea EM Jr, Reyes A, Villanueva-Uy E, Pacifico R, Benitez B, Ramos E, Bernardo RC, Bielawski DM, Delaney-Black V, Chiodo L, Janisse JJ, Ager JW. Fetal exposure to propoxur and abnormal child neurodevelopment at 2 years of age. Neurotoxicology. 2012;33:669–75, http://dx.doi.org/10.1016/j.neuro.2011.11.006.
31. Palmer-Brown W, de Melo Souza PL, Murphy CD. Cyhalothrin biodegradation in *Cunninghamella elegans*. Environ. Sci. Pollut. Res. 2019;26:1414–21, http://dx.doi.org/10.1007/s11356-018-3689-0.
32. Ray L, Panda AN, Mishra SR, Pattanaik AK, Adhya TK, Suar M, Raina V. Purification and characterization of an extracellular thermo-alkali stable, metal tolerant chitinase from *Streptomyces chilikensis* RC1830 isolated from a brackish water lake sediment. Biotechnol Rep (Amst). 2019;21:e00311, http://dx.doi.org/10.1016/j.btre.2019.e00311.
33. Rojas-Loria CC, Favela-Torres E, González-Márquez H, Volke T. Role of glutathione and glutathione S-transferase in lead tolerance and bioaccumulation by *Dodonaea viscosa* (L.) Jacq. Acta Physiol Plant. 2014;36:2501–10, http://dx.doi.org/10.1007/s11738-014-1623-8.
34. Sain SK, Monga D, Kumar R, Nagrale DT, Hiremani NS, Kranthi S. Compatibility of entomopathogenic fungi with insecticides and their efficacy for IPM of *Bemisia tabaci* in cotton. J Pestic Sci. 2019;44:97–105, http://dx.doi.org/10.1584/Fjpestics.D18-067.
35. Schumacher V, Poehling H-M. *In vitro* effect of pesticides on the germination, vegetative growth, and conidial production of two strains of *Metarhizium anisopliae*. Fungal Biol. 2012;116:121–32, http://dx.doi.org/10.1016/j.funbio.2011.10.007.
36. Sethi S, Mathur N, Bhatnagar P. Growth response of microbial population exposed to synthetic pyrethroids. Int J Environ Sci. 2013;4:42–53, http://dx.doi.org/10.6088/ijes.2013040100005.
37. Singh G, Arya SK. Antifungal and insecticidal potential of chitinases: a credible choice for the eco-friendly farming. Biocatal Agric Biotechnol. 2019;20:101289, http://dx.doi.org/10.1016/j.bcab.2019.101289.
38. Singh AK, Ghodke I, Chhatpar HS. Pesticide tolerance of *Paenibacillus* sp. D1 and its chitinase. J. Environ. Manage. 2009;91:358–62, http://dx.doi.org/10.1016/j.jenvman.2009.09.001.
39. Srinivasulu M, Ortiz DR. Effect of pesticides on bacterial and fungal populations in Ecuadorian tomato cultivated soils. Environ Process. 2017;4:93–105 https://link.springer.com/article/10.1007/s40710-017-0212-4
40. Tzintzun-Camacho O, Loera O, Ramírez-Saad HC, Gutiérrez-Rojas M. Comparison of mechanisms of hexadecane uptake among pure and mixed cultures derived from a bacterial consortium. Int. Biodeterior. Biodegradation. 2012;70:1–7, http://dx.doi.org/10.1016/j.ibiod.2012.01.009.
41. Ulhoa CJ, Peberdy JF. Effect of carbon sources on chitobiose production by *Trichoderma harzianum*. Mycol Res. 1993;97:45–8, http://dx.doi.org/10.1016/S0953-7562(09)81111-8.
42. Vargas-Hoyos HA, Gilchrist-Ramelli E. Producción de enzimas hidrolíticas y actividad antagónica de *Trichoderma asperellum* sobre dos cepas de *Fusarium* aisladas de cultivos de tomate (*Solanum lycopersicum*). Rev Mex Mic. 2015;42:9–16 http://www.scielo.org.mx/pdf/rmm/v42/v42a3.pdf
43. Verma JP, Jaiswal DK, Sagar R. Pesticide relevance and their microbial degradation: a-state-of-art. Rev Environ Sci Biotechnol. 2014;13:429–66, http://dx.doi.org/10.1007/s11157-014-9341-7.
44. WHO (World Health Organization). Task Group on Environmental Health Criteria for Permethrin, United Nations Environment Programme, International Labour Organisation, World Health Organization, and International Program on Chemical Safety. d-Permethrin. Geneva: World Health Organization; 1990 https://apps.who.int/iris/bitstream/handle/10665/38949/9241542942-eng.pdf?sequence=1andisAllowed=y
45. Yang J, Feng Y, Zhan H, Liu J, Yang F, Zhang K, Zhang L, Chen S. Characterization of a pyrethroid-degrading *Pseudomonas fulva* strain P31 and biochemical degradation pathway of D-phenothrin. Front Microbiol. 2018;9:1003, http://dx.doi.org/10.3389/fmicb.2018.01003.
46. Zafra G, Moreno-Montaño A, Absalón AE, Cortés-Espinosa DV. Degradation of polycyclic aromatic hydrocarbons in soil by a tolerant strain of *Trichoderma asperellum*. Environ. Sci. Pollut. Res. 2014;22:1034–42, http://dx.doi.org/10.1007/s11356-014-3357-y.
47. Zhang Ch, Jia L, Wang S, Qu J, Li K, Xu L, Shi Y, Yan Y. Biodegradation of beta-cypermethrin by two *Serratia* spp. with different cell surface hydrophobicity. Bioresour. Technol. 2010;101:3423–9, http://dx.doi.org/10.1016/j.biortech.2009.12.083.
48. Zhao J, Jia D, Chi Y, Yao K. Co-metabolic enzymes and pathways of 3-phenoxybenzoic acid degradation by *Aspergillus oryzae* M-4. Ecotoxicol. Environ. Saf. 2019;189:109953, http://dx.doi.org/10.1016/j.ecoenv.2019.109953.

49. Zhu Y, Li J, Yao K, Zhao N, Zhou K, Hu X, Zou L, Han X, Liu A, Liu S. Degradation of 3-phenoxybenzoic acid by a filamentous fungus *Aspergillus oryzae* M-4 strain with self-protection transformation. Appl. Microbiol. Biotechnol. 2016;100:9773–86, http://dx.doi.org/10.1007/s00253-016-7847-3.

50. Zou G, Bao D, Wang Y, Zhou S, Xiao M, Yang Z, Wang Y, Zhou Z. Alleviating product inhibition of *Trichoderma reesei* cellulase complex with a product-activated mushroom endoglucanase. Bioresour. Technol. 2021;319:124119, http://dx.doi.org/10.1016/j.biortech.2020.124119.