EXPLORING THE EFFICACY OF INDIGENOUS *TRICHODERMA ASPERELLUM* AND *T. HARZIANUM* FOR BIODEGRADING THIAMETHOXAM AND CHLORANTRANILIPROLE INSECTICIDES

ISMAIL, A. M.[1,2,3*] – AHMED, A. Y.[4,5] – EID, H. T.[4,5] – SALEH, R. A.[4,5] – HELMY, R. M. A.[6] – EL GANAINY, S. M.[1,3] – HAJJAR, M. J.[1] – MOHAMED, H. A.[7,8]

[1]*Department of Arid Land Agriculture, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al-Ahsa 31982, Saudi Arabia*

[2]*Pests and Plant Diseases Unit, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al-Ahsa 31982, Saudi Arabia*

[3]*Vegetable Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center (A.R.C.), Giza 12619, Egypt*

[4]*Department of Mycology Laboratories, Plant Pathology Research Institute, Agricultural Research Center (A.R.C.), Giza, Egypt*

[5]*Unit of Identification of Microorganisms, Plant Pathology Research Institute, A.R.C., Giza, Egypt*

[6]*Pesticides Residue and Environmental Pollution Department, Central Agriculture Pesticides Laboratory, A.R.C., Giza 12618, Egypt*

[7]*Seed-Pathology Research Department, Plant Pathology Research Institute, Agricultural Research Center (A.R.C.), Giza 12619, Egypt*

[8]*Date Palm Research Center of Excellence, King Faisal University, P.O. Box 420, Al-Ahsa 31982, Saudi Arabia*

**Corresponding author*
e-mail: amismail@kfu.edu.sa; ma.ah.ismail@gmail.com

(Received 17th Feb 2024; accepted 28th Jun 2024)

Abstract. The study investigated the insecticide tolerance and dissipation potential of five *Trichoderma* isolates, namely *T. asperellum* (4 isolates) and *T. harzianum* (single isolate), to thiamethoxam and chlorantraniliprole in treated liquid medium and tomato fruit. The isolates were identified using ITS gene amplification and sequencing regions. In vitro tolerance was assessed using a poisoned medium technique, with mycelial placed onto Potato Dextrose Agar (PDA.) media supplemented with commercial insecticides at recommended doses. Dissipation potential was determined using the isolates after independently inoculating them into PDB liquid media with Actara and Voliam Flexi. The results showed that the inhibitory effect of thiamethoxam and chlorantraniliprole was almost zero, and the five isolates were competent to reach 9 cm growth like the control after 96 h of incubation at 25°C ± 2°C. Mycelial fresh weights of *T. harzianum* EGY-T4 and *T. asperellum* EGY-T5 were significantly equal to their respective control without insecticide. *T. asperellum* EGY-T1 had the highest dissipation rate of 36% and 30% for thiamethoxam in Actara and Voliam Flexi when applied at the higher dose of 100 mg L^{-1}. The dissipation rate of thiamethoxam was recorded at 92.2% when applied in combination with *T. asperellum* EGY-T1, reducing the PHI from 7 to 4 days. Chlorantraniliprole was dissipated to the extent of 51.3 and 99.5% when applied in combination with *T. asperellum* EGY-T1 and *T. harzianum* EGY-T4, respectively, with residues reaching up to 0.12 and 0.21 mg kg^{-1} after one day.
Keywords: *Trichoderma, pesticides, biological, dissipation, residues, Actara, Voliam Flexi*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

Introduction

Pesticides have been employed in agricultural practices for a minimum of 80 years, and their utilization has been on the rise in recent decades. However, there is ongoing public apprehension regarding the potential health hazards associated with the application of pesticides. Pesticide use will rise as the global population reaches 8.5 billion by 2030 (Clark and Tilman, 2017). Neonicotinoid insecticides (N.I.s) have been widely used in the field of plant protection and have become one of the largest-selling insecticides (Bonmatin et al., 2015). Thiamethoxam is one of the N.I.s belonging to a subclass of nicotinyl compounds used for the control of a wide range of pests, including cuttlefish, aphids, white butterflies, and some species of cockroaches, including the Colorado cockroach (Tomizawa and Casida et al., 2005; Mohamed et al., 2022; Maienfisch et al., 2001). The structure of thiamethoxam is presented in *Figure 1a*. Thiamethoxam is present in formulations applied worldwide, with products registered in more than 130 countries, including the United States, Canada, Brazil, Australia, the European Union, India, and Russia (Hilton et al., 2016). A commercial formulation of thiamethoxam named Actara, 25% W.G., is developed by Syngenta and has been registered by the Egyptian Ministry of Agriculture and Land Reclamation under number 1003. Furthermore, Voliam flexi® 40% W.G. is a new commercial systemic insecticide with two active ingredients, thiamethoxam, and chlorantraniliprole, that have different target sites. This insecticide has been developed to control a wide range of serious pests on rice, fruits, vegetables, ornamental plants, and some field crops around the world (Mohamed et al., 2022). The structure of chlorantraniliprole is presented in *Figure 1b*.

Currently, pesticide remediation from agriculture fields is one of the important issues because such chemicals are very expensive and problematic as they form toxic chemicals by reaction of various organic and inorganic contents and elements present in soils (Niti et al., 2013). Various methods exist for the biodegradation of pesticides, which can occur under aerobic or anaerobic circumstances depending on the types of microorganisms involved. Furthermore, the bioremediation procedures can be categorized into three distinct groups based on the location of the remediation treatment: in-situ, ex-situ, or on-site. Only efficient microbial technology is useful for pesticide removal or degradation from agricultural soils. Microbial degradation (use of fungi, bacteria, actinomycetes, and viruses) can effectively remove pesticides from the contaminated soil (organochlorines, organophosphates, and carbamates) through enzymatic degradation (Porto et al., 2011). Biological degradation involves the use of effective microorganisms to degrade the complex pesticide into simple inorganic chemicals (Płaza, 2001). Moreover, this technology is less hazardous, environmentally friendly, economically viable, and socially acceptable (You and Liu, 2004). Among these microorganisms, species of the genus *Trichoderma* can transform xenobiotic agents into non-toxic compounds, e.g., pesticides such as dichlorvos, cyanide pollutants, and even heavy metals (He et al., 2014). Up until recently, research on Trichoderma's tolerance mechanisms for chemical pesticides has been focused on the molecular and physiological levels. Two theories about the processes of *Trichoderma* tolerance have emerged from these findings. The first is that chemical pesticides are unable to identify *Trichoderma* because of alterations at their target sites. The alternative theory proposes that *Trichoderma* can break down or metabolize specific chemical pesticides. The oxidation mechanism of *T. harzianum* was found to be capable of degrading organochlorine pesticides (Szpyrka et al., 2020). Therefore, the aim of the study was first to investigate the tolerance of *T. asperellum* and *T. harzianum* to active ingredients

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

thiamethoxam and chlorantraniliprole in solid PDA and liquid PDB media. Based on this, the objective of the present investigation was to evaluate the in vitro compatibility of two native species, *T. asperellum* and *T. harzianum*, with the insecticides, for which it was necessary to determine the dissipation potential of thiamethoxam and chlorantraniliprole by *T. asperellum* and *T. harzianum* in liquid medium and under greenhouse conditions.

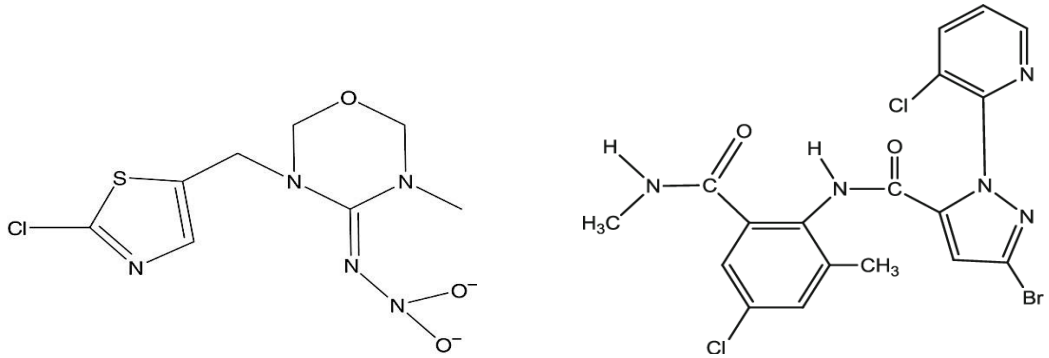

Figure 1. *Chemical structure of thiamethoxam (a); and chlorantraniliprole (b)*

Materials and methods

Isolation and molecular characterization of Trichoderma fungi

Trichoderma fungi (*Fig. 2*) were isolated in association with other fungi from soil and root samples from some Egyptian geographical locations in 2022. The isolates were stored on a PDA medium at 25°C and were initially described based on colony and conidia morphology using Barnett and Hunter's taxonomic criteria (Barnett and Hunter, 1972). Total genomic DNA. was extracted from five *Trichoderma* isolates using the Dellaporta procedure for genomic DNA. isolation (Dellaporta et al., 1983). The internal transcribed spacer region (ITS) of rRNA was sequenced and amplified using primer pairs ITS4 and ITS5 (White et al., 1990). The PCR reaction was carried out in a 25 µL reaction volume with 10 µL of PCR Master Mix (amaR OnePCR, GeneDirex, Inc.), 11 µL of ddH2O, 1.5 µL of each primer, and 1 µL of template DNA. The PCR products were cleaned and sequenced in both directions using the Macro-gene Inc. Sequencing Service in Seoul, Korea. The PCR amplification conditions were carried out following Haouhach et al. (2020) using a 2720 Thermal Cycler (Applied Biosystems, Foster City, California). To assign taxonomy, the BLASTn algorithm was performed using the NCBI GenBank database, comparing the queries to type specimens.

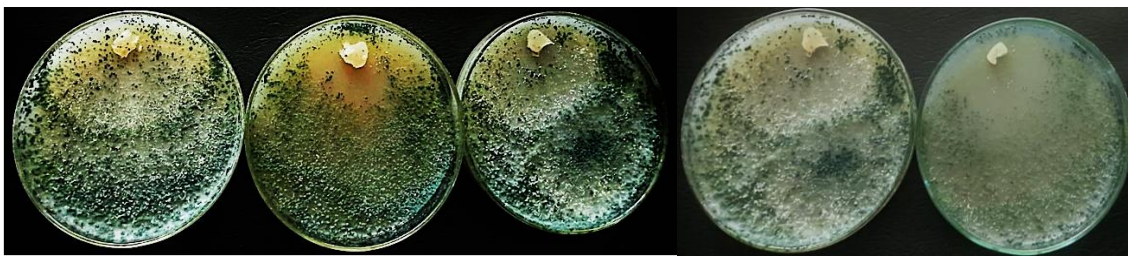

Figure 2. *Trichoderma species used in this study; T. asperellum (a, b, c and d), T. harzianum (e)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

Source of tested insecticides

The commercial insecticides Actara 25% WG **(**Thiamethoxam**)** and Voliam Flexi 30% SC **(**Thiamethoxam + Chlorantraniliprole**)** were obtained from the local Egyptian market and used throughout the study. The investigated insecticides were used at three endorsed dosages for laboratory and greenhouse experiments.

Insecticides tolerance of T. asperellum and T. harzianum

The tolerance of five *Trichoderma* isolates representing two species, *T. asperellum* and *T. harzianum*, in response to active ingredients thiamethoxam and the mixture of thiamethoxam + chlorantraniliprole, was determined using the poisoned PDA medium technique as described by Prasanna et al. (2002) and Jayaraman et al. (2012). The autoclaved PDA medium was supplemented with the active ingredients of Actara at doses 25, 50, and 100 mg L^{-1} and Voliam flexi at doses 12.5, 25, and 50 mg L^{-1} after cooling to approximately 45°C–50°C. The insecticide amended PDA medium was then poured into 90-mm Petri dishes (approx. 12ml in each) and allowed to solidify. A 5 mm mycelial plug was taken from fresh *Trichoderma* cultures (5 days old) and placed in the center of the Petri dishes. This was followed by incubating the inoculated plates for four days at 25°C ± 2°C with temporary intermitted exposure to light during the examination. This experiment was performed twice with three replicates (five plates per each) for each treatment as well as for the control. The experiments were terminated when *Trichoderma* growth in the control reached the border of the Petri dishes. The radial mycelial growth of *Trichoderma* was measured every 24 h for five days, and the inhibition rate was calculated using the formula given by Vincent (1947): Inhibition (%) = C - T/C × 100, where C the growth of *Trichoderma* in control plates, T = the growth of *Trichoderma* in treated plates.

Effect of the two insecticides on the fungal biomass of T. asperellum and T. harzianum

The fresh weight of the mycelial biomass of five *Trichoderma* isolates was determined on Potato Dextrose Broth (PDB) medium. Three conical flasks containing 200 ml of PDB amended with the three doses of each insecticide at the recommended dose were inoculated with 5-mm agar plugs from 5-day-old cultures of each *Trichoderma* isolate. Control flasks were treated with equal amounts of sterilized water. The conical flasks were incubated in darkness at 25°C ± 2°C with only intermittent exposure to light when they were examined. At the end of the incubation period, the mycelial mats were then filtered through Whatman No.1 filter paper, dried in an oven at 60°C, and weighed (Karpagavalli and Nannapaneni, 2020).

Biodegradation experiments

Biodegradation ability of T. asperellum and T. harzianum to insecticides under laboratory conditions

The biodegradation potential of *Trichoderma* isolates was investigated against the active ingredients of thiamethoxam and chlorantraniliprole under laboratory conditions using a Potato Dextrose Broth (PDB broth) medium. The conical flasks containing 200 ml of autoclaved PDB medium were amended with two insecticides, namely Actara at doses 25, 50, and 100 mg L^{-1} and Voliam flexi at doses 12.5, 25, and 50 mg L^{-1}. The insecticides amended media were inoculated with three 5-mm fresh mycelial discs of 5-day-old cultures

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

of each *Trichoderma* isolate and incubated for ten days on a rotary shaker (250 rpm) (Jayaraman et al., 2012). PDB media inoculated with only sterile agar discs were served as control. These experiments were done in the dark to avoid degradation by photolysis.

Biodegradation ability of T. asperellum EGY-T1 and T. harzianum EGY-T4 to insecticides treated tomato fruit under greenhouse conditions

Biodegradation ability by *Trichoderma* isolates was also evaluated against active ingredients thiamethoxam and chlorantraniliprole under greenhouse conditions. Tomato seedlings cv. Super strain B was planted in 30-cm-diameter plastic pots filled with a sterilized soil mixture of sand and peat moss (1:2 w/w). Tomato plants at 90 days of age were sprayed with two *Trichoderma* isolates at a concentration (1×10^6 spores/ml). Two hours later, plants were sprayed with thiamethoxam and chlorantraniliprole at the above-mentioned doses. Pots were kept in an air-conditioned greenhouse at 25°C ± 2°C and 60%–75% relative humidity. The pots were randomly placed in the greenhouse and irrigated and fertilized as needed. The experiment was accomplished two times in an entirely random block design; three replicates were used for each treatment, and each replicate contained ten plants. Tomato fruit was collected after 1 h, 1, 4, and 7 days of spray to detect and quantify the insecticide residues.

Chemical analysis

Extraction and purification of insecticides

QuEChERS method was used as described by Anastassiades et al. (2003), of which 10 g of well-homogenized tomato samples were placed in a tube containing 10 ml of acetonitrile, shacked well horizontally for 1 min, then vortexed for 1 min, and centrifuged for 5 min at 3000 g. The supernatant was separated and cleaned by salts. After that, the supernatant was centrifuged at 3000 g for 5 min, the acetonitrile layer was removed, and 1 ml of the higher layer was filtrated with a filter of 0.22 μm and transferred into glass vials.

Method validation

The linearity of the calibration curve was evaluated using the correlation coefficient (R2) for the peak areas against the concentrations in the range of standard solutions in terms of microliters at variable concentrations, with the intervals 0.01, 0.1, 0.5, 1, 2, 5 and 10 mg/kg for injected solutions. R2 should be greater than 0.99. The percentage maximum difference in the response factor (R.F., %) should be lower than 20%. The standard solutions were randomly injected in triplicate. The limit of detection (L.O.D.) was determined to evaluate the sensitivity. The limit of quantitation (L.O.Q.) of the method was established as the lowest fortification level that achieves a recovery percentage of 80–120 with an RSD (Relative standard deviation) lower than 20% (Li et al., 2017). Matrix-matched calibration standards verified calibration curve linearity at five concentrations.

Quantification of insecticides using high-performance liquid chromatography (HPLC)

HPLC was used. A diode array detector (DAD)-equipped Agilent 1260 system (Agilent Technologies, Santa Clara, California). An Agilent Eclipse plus C18 column

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

(4.6 × 250 mm, 5 mm) was used to inject 50 μL at 0.5 mL/min for thiamethoxam and 0.8 mL/min for chlorantraniliprole at 35°C. Thiamethoxam and chlorantraniliprole were dissolved in acetonitrile and methanol (70:30%) and 60:40%, respectively. Quantification was done at 254 nm for thiamethoxam and 230 nm for chlorantraniliprole using a calibration curve with five analytical standards from 0.01 to 10 mg kg^{-1} for each pesticide.

Statistical analysis

All obtained data were analyzed utilizing analysis of variance (ANOVA) among treatments. Means were compared via least significant differences (LSD) at 5% level for laboratory experiments and 5% for greenhouse experiments utilizing M-Stat software v. 7.0.1 (Wisconsin, USA).

Results

Isolation and molecular characterization of Trichoderma fungi

Five *Trichoderma* isolates, representing two species, were recovered from different soil rhizospheres and roots. The generated sequences were edited and corrected where required and subjected to the Blastn sequence against the GenBank database. Based on a mega blast search of NCBI's GenBank nucleotide database, isolate ITS sequence closest hits GenBank, closest hits using isolates' ITS sequences EGY-T1, EGY-T2, EGY-T3 and EGY-T5 had highest similarity 100% to *T. asperellum* (GenBank Accessions; MT133310, OR911936, MT529846 and MT529837), respectively. Further, the closest hit using the ITS sequence of the isolate EGY-T4 had 90% similarity to *T. harzianum* GenBank Accessions; MZ681867). The sequences were deposited in GenBank under accession numbers OQ355363, OQ355565, OQ355648, OM757839, and OQ357568 for the obtained isolates EGY-T1, EGY-T2, EGY-T3, EGY-T4, and EGY-T5, respectively.

Effect of insecticides on the Mycelial growth of T. asperellum and T. harzianum

The results shown in *Figure 3* revealed that *T. asperellum* and *T. harzianum* were able to tolerate and grow in a PDA medium supplemented with two insecticides, thiamethoxam and chlorantraniliprole, at the three doses. However, thiamethoxam and chlorantraniliprole revealed an inhibitory effect on the colony of *T. asperellum* and *T. harzianum* isolates at 24, 48, and 72 h of inoculation, exhibiting a significant decrease in their growth when compared to their respective control without insecticide (*Fig. 3a, b*). At 96 h of inoculation, the inhibitory effect of the thiamethoxam and chlorantraniliprole was almost zero, and the five isolates were able to reach 9 cm growth like that observed in the control (*Fig. 3a, b*). This indicated that there was no drastic reduction of radial growth at the recommended dose for field application.

Effect of insecticides on the fungal biomass of T. asperellum and T. harzianum

According to the results highlighted in *Figure 4*, all the tested isolates of *T. asperellum* and *T. harzianum* were able to grow in the presence of thiamethoxam and chlorantraniliprole in PDB liquid medium and produced fresh weight (g) lower than or significantly ($P < 0.05$) equal to their respective control without insecticide (*Fig. 4a, b*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

According to the statistical analysis, the mycelial fresh weights (g) of *T. harzianum* EGY-T4 and *T. asperellum* EGY-T5 were significantly ($P < 0.05$) equal to their respective control without insecticide. However, the dry weight (g) of all *Trichoderma* isolates was significantly ($P < 0.05$) reduced in response to thiamethoxam and chlorantraniliprole three tested concentrations (*Fig. 5a, b*).

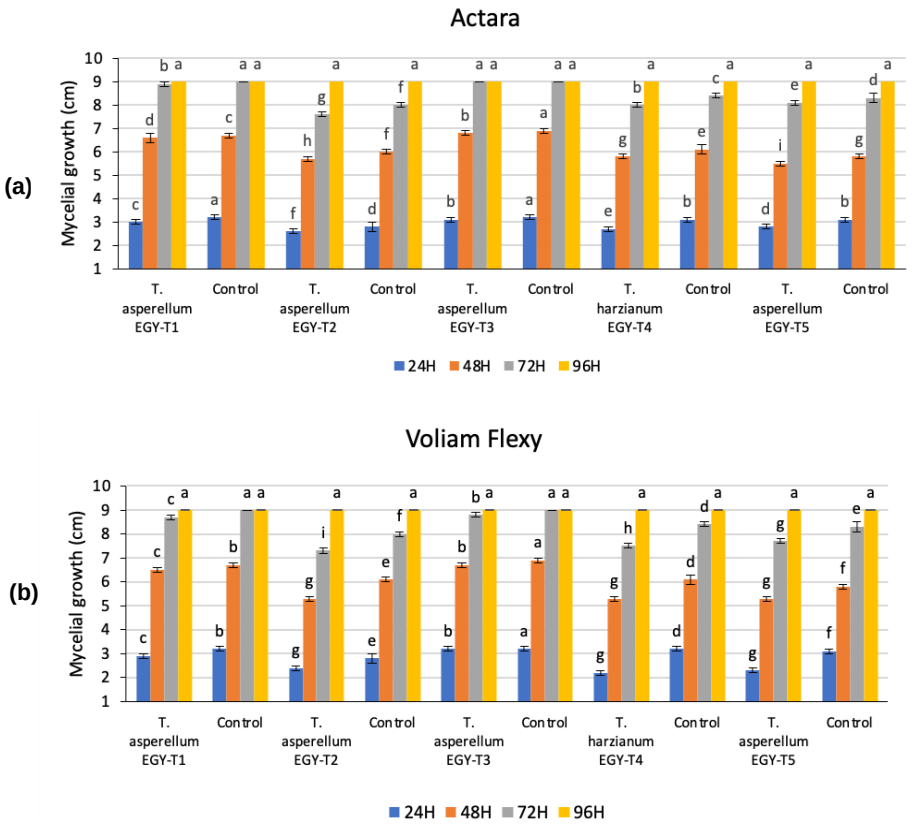

Figure 3. *Mycelial growth of Trichoderma species on PDA medium in response to active ingredients of the two insecticides Actara (Thiamethoxam) (a); and Voliam Flexi (thiamethoxam + chlorantraniliprole) (b). The values expressed within columns represent the mean of three repetitions ± standard deviation. Bars marked by different letters differ significantly ($p < 0.05$) as revealed by the LSD test*

Dissipation of thiamethoxam and chlorantraniliprole by T. asperellum and T. harzianum in liquid medium

The rate of dissipation of thiamethoxam and chlorantraniliprole in sterilized PDB medium, incubated with the *Trichoderma* isolates, was determined by HPLC analysis of samples collected after14 days incubation period (*Tables 1, 2,* and *3*). *T. asperellum* EGY-T1 was found to have the highest ability of degradation of thiamethoxam in Actara and Voliam Flexi insecticides at the higher dose of 100 mg, with values reaching 36% and 30%, respectively. Similarly, *T. harzianum* EGY-T4 revealed a degradation rate of 30% of the active gradient thiamethoxam in both Actara and Voliam Flexi insecticides at the higher dose of 100 mg (*Tables 1,* and *2*). By contrast, the rest of *T. asperellum* isolates revealed variable degradation rates of thiamethoxam ranging between 2%–16%. Furthermore, *T. asperellum* EGY-T1 also showed the highest degradation rate of 26% for chlorantraniliprole at the higher dose of 50 mg, followed by

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

T. harzianum EGY-T4 which achieved a higher degradation rate of 20% for chlorantraniliprole at the dose of 25 mg (*Table 3*). The rate of degradation of chlorantraniliprole by the rest of the isolates was very low (*Table 3*).

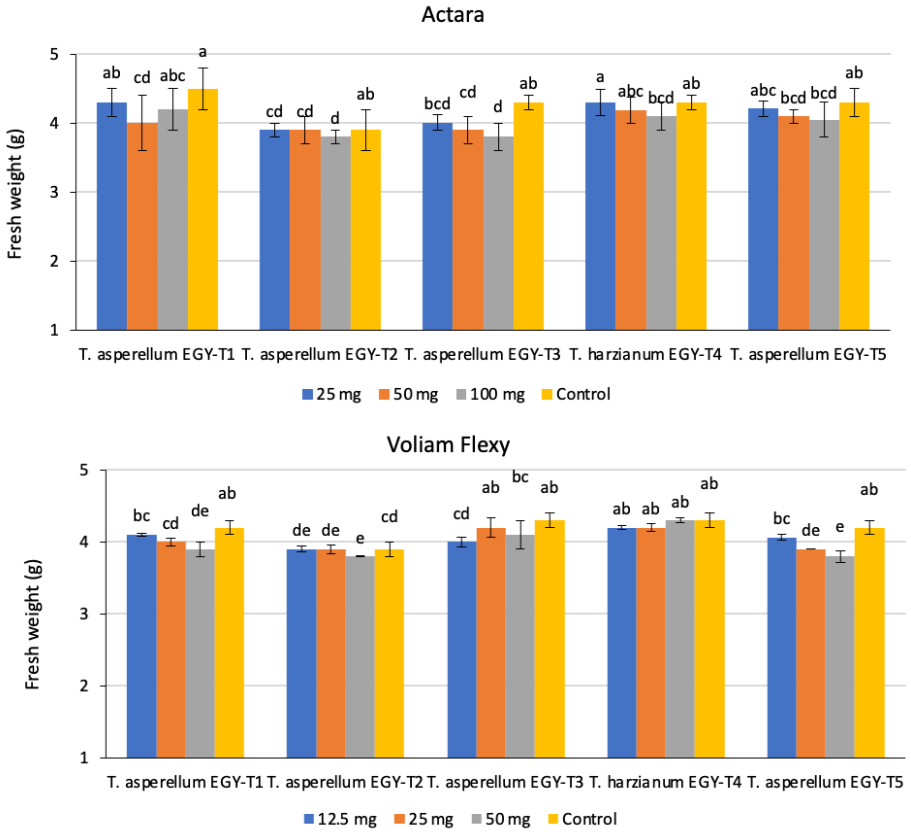

Figure 4. *Fresh weight (g) of fungal biomass of Trichoderma species in response to different doses of the active ingredients of the insecticides Actara (Thiamethoxam) (a); and Voliam Flexi (thiamethoxam + chlorantraniliprole) (b). The values expressed within columns represent the mean of three repetitions ± standard deviation. Bars marked by different letters differ significantly ($p < 0.05$) as revealed by the LSD test*

Table 1. *Dissipation of thiamethoxam in the commercial insecticide Actara by T. asperellum and T. harzianum in liquid media*

Dose	Actara-Thiamethoxam					
	25 mg		50 mg		100 mg	
Isolates	Residues (mg)	Dissipation (%)	Residues (mg)	Dissipation (%)	Residues (mg)	Dissipation (%)
T. asperellum EGY-T1	20.75 d	17%	40 c	20%	64 e	36%
T. asperellum EGY-T2	24 c	4%	48.5 a	3%	98 a	2%
T. asperellum EGY-T3	24.5 b	2%	48 b	4%	84 c	16%
T. harzianum EGY-T4	18.75 e	25%	36 d	28%	67 d	33%
T. asperellum EGY-T5	24.75 a	1%	48.5 a	3%	95 b	5%
L.S.D.	0.034		0.051		0.057	

S.E. = Standard Error. The values expressed within columns represent the mean of three repetitions ± standard error. Means within columns have the same letter are not significantly different ($p<0.05$) according to LSD test

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

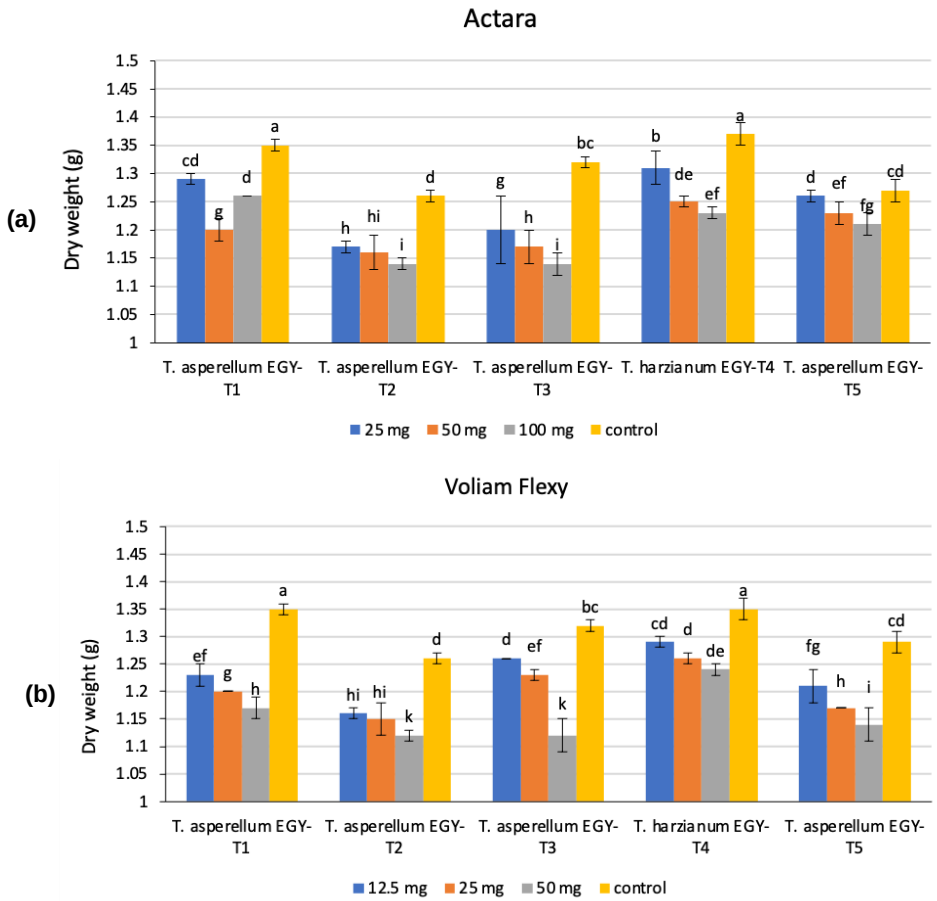

Figure 5. *Dry weight (g) of fungal biomass of Trichoderma species in response to different doses of the active ingredients of the insecticides Actara (Thiamethoxam) (a); Voliam Flexi (thiamethoxam + chlorantraniliprole) (b). The values expressed within columns represent the mean of three repetitions ± standard deviation. Bars marked by different letters differ significantly ($p < 0.05$) as revealed by the LSD test*

Table 2. *Dissipation of thiamethoxam in the commercial insecticide Voliam Flexi by T. asperellum and T. harzianum in liquid media*

Dose	**Voliam Flexi-Thiamethoxam**					
	12.5 mg		**25 mg**		**50 mg**	
Isolates	**Residues (mg)**	**Dissipation (%)**	**Residues (mg)**	**Dissipation (%)**	**Residues (mg)**	**Dissipation (%)**
T. asperellum EGY-T1	8.88 d	29%	17 e	32%	35 d	30%
T. asperellum EGY-T2	12.25 a	2%	24.25 a	3%	48.5 b	3%
T. asperellum EGY-T3	12.0 b	4%	23 c	8%	46 c	8%
T. harzianum EGY-T4	8.5 e	32%	19 d	24%	33.5 e	33%
T. asperellum EGY-T5	11.88 c	5%	24 b	4%	49 a	2%
L.S.D.	0.044		0.042		0.042	

S.E. = Standard Error. The values expressed within columns represent the mean of three repetitions ± standard error. Means within columns have the same letter are not significantly different ($p<0.05$) according to LSD test

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

Table 3. *Dissipation of chlorantraniliprole in the commercial insecticide Voliam Flexi by T. asperellum and T. harzianum in liquid media*

Dose	Voliam Flexi-Chlorantraniliprole					
	12.5 mg		25 mg		50 mg	
Isolates	Residues (mg)	Dissipation (%)	Residues (mg)	Dissipation (%)	Residues (mg)	Dissipation (%)
T. asperellum EGY-T1	11.0 d	12%	20.5 d	18%	37 e	26%
T. asperellum EGY-T2	12 b	4%	23.75 c	5%	46.5 b	7%
T. asperellum EGY-T3	11.75 c	6%	24 b	4%	46 c	8%
T. harzianum EGY-T4	10.38 e	17%	20 e	20%	42 d	16%
T. asperellum EGY-T5	12.25 a	2%	24.25 a	3%	48 a	4%
L.S.D.	0.053		0.033		0.057	

S.E. = Standard Error. The values expressed within columns represent the mean of three repetitions ± standard error. Means within columns have the same letter are not significantly different ($p<0.05$) according to LSD test

Method validation

Calibration curves were set for each compound by injections in triples (n = 3) for five concentrations, i.e., 0.01 to 10 mg kg^{-1} (*Fig. 6a, b*). Outstanding linearities of the calibration curves were obtained for thiamethoxam with regression coefficient (R2) 0.9992 and 0.9922 for chlorantraniliprole (*Fig. 6a, b*). The mean recoveries of thiamethoxam and chlorantraniliprole were within 51.3%–99.5%, and the RSD values ranged from 1.02% to 14.9%. These results suggested that the proposed method was reliable for the determination of thiamethoxam and chlorantraniliprole.

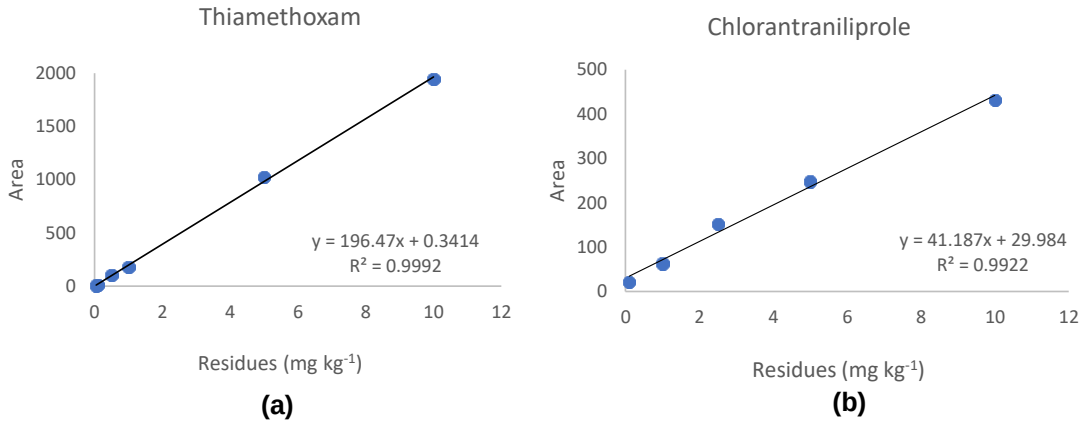

Figure 6. *Calibration curve was made with five concentrations ranging from 0.01 to 10 mg kg^{-1} for thiamethoxam (a); and chlorantraniliprole (b)*

Dissipation ability of T. asperellum EGY-T1 and T. harzianum EGY-T4 to insecticides treated tomato fruit

The dissipation pattern of thiamethoxam and chlorantraniliprole applied at recommended doses is shown in *Tables 4, 5,* and *6*. The dissipation rate of thiamethoxam (Actara) when applied alone recorded 97.2%, showing residues of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

0.006 mg kg^{-1} on day 7 of application. Its dissipation was recorded at 90.2% when applied in combination with *T. asperellum* EGY-T1, showing residues of 0.11 mg kg^{-1} on day 4. On the other hand, a higher dissipation rate of 99% of thiamethoxam (Actara) was obtained by *T. harzianum* EGY-T4, showing residues 0.01 mg kg^{-1} on day 4. Additionally, the amount of thiamethoxam (Voliam Flexi) dissipated by 94.3% when combined with *T. harzianum* EGY-T4 on day 4, with residues reaching 0.09 mg kg^{-1}. Interestingly, the results in *Table 6* indicated that the residues of chlorantraniliprole were dissipated to an extent of 51.3 and 99.5% after one day when applied in combination with *T. asperellum* EGY-T1 and *T. harzianum* EGY-T4, respectively. Both isolates decreased the residues of chlorantraniliprole up to 0.12 and 0.21 mg kg^{-1} after one day. Applying *T. asperellum* EGY-T1 and *T. harzianum* EGY-T4 in combination with thiamethoxam and chlorantraniliprole in tomatoes reduced the safe interval to four and one day, respectively.

Table 4. *Dissipation ability of T. asperellum EGY-T1 and T. harzianum EGY-T4 to thiamethoxam (Actara) in treated tomato fruit*

Time (days)	Residues in fruits ± SE					
	Thiamethoxam	**RSD%**	**Thiamethoxam + *T. asperellum* EGY-T1**	**RSD%**	**Thiamethoxam + *T. harzianum* EGY-T4**	**RSD%**
1 h	1.55 ± 0.1	6.5	1.20 ± 0.10	8.3	1.24 ± 0.05	4.56
1	0.85 ± 0.03	3.6	0.30 ± 0.04	12.4	0.21 ± 0.02	7.16
4	0.23 ± 0.003	6.5	0.11 ± 0.005	4.9	0.01 ± 0.001	8.33
7	0.006 ± 0.00	10.8	nd	-	nd	-
Dissipation %	97.2		90.2		99	
PHI (days)	7		4		4	
MRL (mg kg^{-1})	0.2					

S.E. = Standard Error. The values expressed within columns represent the mean of three repetitions ± standard error. Means within columns have the same letter are not significantly different ($p<0.05$) according to LSD test

Table 5. *Dissipation ability of T. asperellum EGY-T1 and T. harzianum EGY-T4 to Thiamethoxam (Voliam Flexi) in treated tomato fruit*

Time (days)	Residues in fruits ± SE					
	Thiamethoxam	**RSD%**	**Thiamethoxam + *T. asperellum* EGY-T1**	**RSD%**	**Thiamethoxam + *T. harzianum* EGY-T4**	**RSD%**
1 h	2.3 ± 0.06	4.9	1.95 ± 0.02	1.02	1.7 ± 0.05	5.88
1	1.5 ± 0.04	2.4	0.85 ± 0.03	3.5	0.7 ± 0.03	3.4
4	0.28 ± 0.01	3.5	0.25 ± 0.02	8	0.09 ± 0.001	1.5
7	0.005 ± 0.00	9.08	0.007 ± 0.00	7.5	nd	
Dissipation %	98		96.9		94.3	
PHI (days)	7		7		4	
MRL (mg kg^{-1})	0.2					

S.E. = Standard Error. The values expressed within columns represent the mean of three repetitions ± standard error. Means within columns have the same letter are not significantly different ($p<0.05$) according to LSD test

Discussion

Trichoderma species are tolerant to many agrochemicals and have the potential to degrade chemical pesticides because they possess a specific enzymatic system to

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

degrade and metabolize such compounds, which are considered toxic substances for the environment (Asemoloye et al., 2019). This study compared the effectiveness of five isolates of *Trichoderma* belonging to two species, *T. asperellum*, and *T. harzianum*, to degrade thiamethoxam and chlorantraniliprole. Trichoderma isolates were confidently identified to species level based on amplification and sequencing of ITS gene region. BLAST queries using ITS resulted in high similarity to known species *T. asperellum* and *T. harzianum*. The results of our study revealed that all Trichoderma isolates tested have different levels of fungicide tolerance and removal or dissipation potential. Our findings revealed that thiamethoxam and chlorantraniliprole revealed an inhibitory effect on the colony of *T. asperellum* and *T. harzianum* isolates at 24, 48, and 72 h of inoculation, exhibiting a remarkable decrease in their growth when compared to their respective control without insecticide. However, Prasanna et al. (2002) found that thiamethoxam 70WS had no inhibitory effect on the growth of *T. harzianum* after 72 h and up to 1% concentration. Similarly, Thiruchchelvan et al. (2013) indicated that the inhibitory effect of chlorantraniliprole on *T. harzianum* growth at the recommended dose was almost zero after three days. Furthermore, Singh et al. (2012) showed that imidacloprid and some other insecticides were compatible with *T. harzianum*. Moreover, Madhavi et al. (2008) reported that *T. harzianum* and T. viride showed high compatibility with imidacloprid. Rangathswamy et al. (2011) demonstrated that thiamethoxam and some other insecticides are highly compatible with zero percent inhibition of growth on Trichoderma spp. Additionally, Silva et al. (2018) found that imidacloprid did not inhibit the mycelial growth of *T. asperellum* and *T. asperelloides*.

Table 6. *Dissipation ability of T. asperellum EGY-T1 and T. harzianum EGY-T4 to Chlorantraniliprole (Voliam Flexi) in treated tomato fruit*

Time (days)	Residues in fruits ± SE					
	Chlorantraniliprole	**RSD%**	**Chlorantraniliprole + *T. asperellum* EGY-T1**	**RSD%**	**Chlorantraniliprole + *T. harzianum* EGY-T4**	**RSD%**
1 h	0.87 ± 0.01	1.7	0.25 ± 0.02	8.2	0.65 ± 0.03	4.6
1	0.36 ± 0.03	8.4	0.12 ± 0.04	12.3	0.21 ± 0.02	9.5
4	0.13 ± 0.01	13.3	nd	nd	0.003 ± 0.0003	14.9
7	nd	nd	nd	nd	nd	
Dissipation %	64		51.3		99.5	
PHI (days)	4		1		1	
MRL (mg kg^{-1})	0.6					

S.E. = Standard Error. The values expressed within columns represent the mean of three repetitions ± standard error. Means within columns have the same letter are not significantly different ($p<0.05$) according to LSD test

Our results revealed that the mycelial fresh weights (g) of *T. harzianum* EGY-T4 and *T. asperellum* EGY-T5 were not affected by the presence of thiamethoxam and chlorantraniliprole after 96 h. Consistently to our results, Mendarte-Alquisira et al. (2023) indicated that the commercial insecticide (H24®) contained pyrethroids, permethrin, and carbamate propoxur, which had no effect on the biomass of the *Trichoderma* sp. after eight days in liquid culture. Conversely to our results, Schumacher and Poheling (2012) did not find negative effects of permethrin on the growth of *Metarhizium anisopliae*. On the other hand, pyrethroids such as allethrin (50 mg/L) did not affect the growth of *Fusarium proliferatum* CF2 (Bhatt et al., 2020).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

Consistently to our results, Deng et al. (2015) 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. Additionally, Jayaraman et al. (2012) stated that *T. viride* and *T. harzianum* and its consortium were able to grow in a fungal culture medium in the presence of chlorpyrifos, and they demonstrated an increase in the level of biomass and protein production.

In our study, *Trichoderma* isolates showed significant differences during the study on the degradation of thiamethoxam and chlorantraniliprole in a liquid medium. According to our laboratory results, *T. asperellum* EGY-T1 exhibited the highest dissipation rate of thiamethoxam and chlorantraniliprole after 14 days. However, under greenhouse conditions, the highest dissipation rate of the two insecticides was obtained by *T. harzianum* EGY-T4, which reduced the PHI period from 7 to 4 days. The residues of thiamethoxam and chlorantraniliprole in tomato fruit were much below the maximum residue limit (MRLs) set by the European Union for thiamethoxam, 0.2 mg kg^{-1} and chlorantraniliprole 0.6 mg kg^{-1}. These findings indicate that the thiamethoxam residues and PHI period then decreased with the help of *T. asperellum* EGY-T1 and *T. harzianum* EGY-T4, creating a hypothesis on the potential of *Trichoderma* on the removal of thiamethoxam and chlorantraniliprole from tomato fruit. Likewise, Escudero-Leyva et al. (2022) demonstrated that *Trichoderma* isolates had degraded chlorothalonil after 14 days of incubation with a value that reached 89%. There are several reports of *T. atroviride*, *T. harzianum* sensu lato, *T. koningii*, and *T. viride* sensu lato, capable of degrading e.g., alachlor, endosulfan, methyl-parathion, monochlorobenzene, neonicotinoids, and pentachlorophenol, among others (Cheng et al., 2017; He et al., 2014; Nykiel-Szymanska et al., 2018; Escudero-Leyva et al., 2022). Furthermore, *T. viride* has been reported to degrade DDD (Ortega et al., 2011) and chlorpyrifos (Mohapatra et al., 2021). However, there are no reports yet available on the use of *T. asperellum* to remediate chlorantraniliprole, and this study represents the first attempt to illustrate the potential capacity of *T. asperellum* to remediate chlorantraniliprole.

The process of pesticide degradation entails the whole breakdown of an organic chemical by pesticide-degrading microorganisms into inorganic components. Although we did not illustrate the mechanisms behind the degradation process in our study, it is possible to speculate that the isolated *T. asperellum* and *T. harzianum* degraded thiamethoxam and chlorantraniliprole both in the media and in tomato plants by releasing intracellular or extracellular enzymes, which acted upon these insecticides, converting it into simpler forms of organic molecules. In these processes, fungi and bacteria are involved in producing intracellular or extracellular enzymes, including hydrolytic enzymes, peroxidases, oxygenases, etc. (Van Herwijnen et al., 2003). For example, marine-derived *Trichoderma* sp. (CBMAI 932) demonstrated to be capable of utilizing chlorpyrifos as a sole nutrient source by hydrolyzing it in distilled water (Alvarenga et al., 2015). The fungus was able to degrade 72% of the applied chlorpyrifos in media and reduce the concentration of 3,5,6-trichloro-2-pyridinol, and the metabolite formed by the enzymatic hydrolysis of chlorpyrifos. However, Choudhury et al. (2019) proposed that the herbicide Topramezone was degraded by *Trichoderma* sp. through various biochemical reactions, viz. demethylation, desulfonylation followed by hydroxylation of the herbicides, alkyl hydroxylation, hydrolysis of the carbonyl group of ketones, methoxylation, and hetero ring hydroxylation.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

Conclusions

The results of our study revealed that all tested *Trichoderma* isolates have some level of insecticide tolerance and removal or biodegradation potential. In this study, indigenous *Trichoderma* isolates were able to tolerate and prevail under the presence of insecticides, generating more questions related to the mechanisms and effects of agrochemicals for the microorganisms living in agroecosystems. Our results revealed that thiamethoxam and chlorantraniliprole had no inhibitory effect on mycelial growth as well as fresh weights (g) of *T. harzianum* EGY-T4 and *T. asperellum* EGY-T5 after 96 h, and exhibited equal growth to control. The isolates *T. harzianum* EGY-T4 and *T. asperellum* EGY-T1 were highly effective in reducing the residues of both insecticides below the maximum residue limit (MRLs) set by European Union for thiamethoxam, 0.2 mg kg^{-1} and chlorantraniliprole 0.6 mg kg^{-1}, leading to a reduction of PHI period from 7 to 4 days. The combination of pesticide tolerance, removal, and ecotoxicity assays with biocontrol or bioremediation microorganisms and interactions between agrochemicals should be considered in the development of sustainable agriculture strategies. Effective and indigenous microbial consortia contribute significantly to the removal of toxic pesticides. The use of an effective and indigenous microbial consortium as a tool for pesticide degradation on the treated crops needs to be adopted in practice at a larger scale.

Funding. This research was funded by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia, grant number (GRANT2318).

Acknowledgements. This research was funded by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia, for supporting this research for work through grant number GRANT2318.

Conflict of interests. The authors declare no conflict of interests.

REFERENCES

[1] Alvarenga, N., Birolli, W. G., Nitschke, M., de O'Rezende, M. O., Seleghim, M. H. R., Porto, A. L. M. (2015): Biodegradation of chlorpyrifos by whole cells of marine-derived fungi *Aspergillus sydowii* and *Trichoderma* sp. – Journal of Microbial and Biochemical Technology 7: 133-139.

[2] Anastassiades, M., Lehotay, S. J., Tajnbaher, D., Schenck, F. J. (2003): Fast and easy multiresidue method employing acetonitrile extraction/partitioning an "dispersive solid-phase extraction" for the determination of pesticide residues in produce. – Journal of the Association of Official Analytical Chemists 86: 412-431.

[3] Asemoloye, M. D., Jonathan, S. G., Ahmad, R. (2019): Degradation of 2, 2-Dichlorovinyl dimethyl phosphate (dichlorvos) through the rhizosphere interaction between Panicum maximum Jacq and some selected fungi. – Chemosphere 221: 403-411. DOI: 10.1016/j.chemosphere.2019.01.058.

[4] Barnett, H. L., Hunter, B. B. (1972): Illustrated Genera of Imperfect Fungi. – Burgess Publishing Company, Clayton, NC.

[5] Bhatt, P., Zhang, W., Lin, Z., Pang, S., Huang, Y., Chen, S. (2020): Biodegradation of allethrin by a novel fungus *Fusarium proliferatum* strain CF2, isolated from contaminated soils. – Microorganisms 8: 593. http://dx.doi.org/10.3390/microorganisms8040593.

[6] Bonmatin, J. M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E. A. D., Noome, D. A., Simon-Delso, N.,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

Tapparo, A. (2015): Environmental fate and exposure; neonicotinoids and fipronil. – Environ. Sci. Pollut. Res 22: 35-67.

[7] Cheng, Z., Li, C., Kennes, C., Ye, J., Chen, D., Zhang, S., et al. (2017): Improved biodegradation potential of chlorobenzene by a mixed fungal-bacterial consortium. – Int. Biodeterior. Biodegrad 123: 276-285. DOI: 10.1016/j.ibiod.2017.07.008.

[8] Choudhury, P. P., Singh, A., Singh, R. (2019): Biodegradation of topramezone by a *Trichoderma* isolate in soil. – Journal of Asian-Pacific Weed Science Society (1)1: 43-54.

[9] Clark, M., Tilman, D. (2017): Comparative analysis of environmental impacts of agricultural production systems, agricultural input efficiency, and food choice. – Environ. Res. Lett. 12: 064016.

[10] Dellaporta, S. L.; Wood, J.; Hicks, J. B. (1983): A Plant DNA. Minipreparation: Version II. –Plant Mol. Biol. Report. 1: 19-21.

[11] Deng, W., Lin. D., Yao, K., Yun, H., Wang, Z., Li, J., Zou, L., Han, X., Zhou, K., He, L., Hu, X., Liu, S. (2015): Characterization of a novel β-cypermethrin-degrading *Aspergillus niger* YAT strain and the biochemical degradation pathway of β-cypermethrin. –Appl. Microbiol. Biotechnol 99: 8187-98, http://dx.doi.org/10.1007/s00253-015-6690-2.

[12] Escudero-Leyva, E., Alfaro-Vargas, P., Muñoz-Arrieta, R., Charpentier-Alfaro, C., Granados-Montero, M. D. M., Valverde-Madrigal, K. S., Pérez-Villanueva, M., Méndez-Rivera, M., Rodríguez-Rodríguez, C. E., Chaverri, P., Mora-Villalobos, J. A. (2022): Tolerance and biological removal of fungicides by *Trichoderma* species isolated from the endosphere of wild Rubiaceae plants. – Front. Agron. 3: 772170. DOI: 10.3389/fagro.2021.772170.

[13] Haouhach, S., Karkachi, N., Oguiba, B., Sidaoui, A., Chamorro, I., Kihal, M., Monte, E. (2020): Three new reports of *Trichoderma* in Algeria: *T. atrobrunneum*, (South) *T. longibrachiatum* (South), and *T. afroharzianum* (Northwest). – Microorganisms 8: 1455.

[14] He, X., Wubie, A. J., Diao, Q., Li, W., Xue, F., Guo, Z., Zhou, T., Xu, S. (2014): Biodegradation of neonicotinoid insecticide, imidacloprid by restriction enzyme mediated integration (R.E.M.I.) generated *Trichoderma mutants*. – Chemosphere 112: 526-530. DOI: 10.1016/j.chemosphere.2014. 01.023.

[15] Hilton, M. J., Jarvis, T. D., Ricketts, D. C. (2016): The degradation rate of thiamethoxam in European field studies. –Pest Management Science 72(2): 388-397.

[16] Jayaraman, P., Naveen Kumar, T., Maheswaran, P., Sagadevan, E., Arumugam, P. (2012): In vitro studies on biodegradation of chlorpyrifos by *Trichoderma viride* and *T. harzianum*. –J. Pure Appl. Microbiol 6: 1465-1474.

[17] Karpagavalli, S., Kumar, N. K. (2020): Interaction of fungicides on the growth of *Trichoderma viride*. –Journal of Pharmacognosy and Phytochemistry 9(4): 922-924.

[18] Li, C., Liu, R., Li, L., Li, W., He, Y., Yuan, L. (2017): Dissipation behavior and risk assessment of butralin in soybean and soil under field conditions. –Environ. Monit. Assess 189: 476.

[19] Maienfisch, P., Angst, M., Brandl, F., Fischer, W., Hofer, D., Kayser, H., Widmer, H. (2001): Chemistry and biology of thiamethoxam: a second generation neonicotinoid. – Pest Management Science 57(10): 906-913.

[20] Mendarte-Alquisira, C., Alarcón, A., Ferrera-Cerrato, R. (2023): Growth, tolerance, and enzyme activities of Trichoderma strains in culture media added with a pyrethroids-based insecticide. – Revista Argentina de Microbiología 6(1): 79-89. https://doi.org/10.1016/j.ram.2023.06.004.

[21] Mohamed, I. A., Hamed, M., Abdel-Tawab, H. S., Mansour, S., Soliman, H. A., Lee, J. S., Sayed, A. E. D. H. (2022): Multi-biomarkers approach to assess the toxicity of novel insecticide (Voliam flexi®) on Clarias gariepinus: from behavior to immunotoxicity. – Fish and Shellfish Immunology 125: 54-64.

[22] Mohapatra, D., Rath, S. K. and Mohapatra, P. K. (2021): Evaluating a preparation of malathion-tolerant *Aspergillus niger* MRU01 for accelerated removal of four

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770

organophosphorus insecticides. –Journal of Chemical Technology and Biotechnology 96(6): 1603-1610.

[23] Niti, C., Sunita, S., Kamlesh, K., Rakesh, K. (2013): Bioremediation: an emerging technology for remediation of pesticides. –Research Journal of Chemistry and Environment 17: 4.

[24] Nykiel-Szymanska, J., Bernat, P., Słaba, M. (2018): Potential of *Trichoderma koningii* to eliminate alachlor in the presence of copper ions. –Ecotoxicology and Environmental Safety162: 1-9. DOI: 10.1016/j.ecoenv.2018.06.060.

[25] Ortega, S. N., Nitschke, M., Mouad, A. M., Landgraf, M. D., Rezende, M. O. O., Seleghim, M. H. R., Sette, L. D., Porto, A. L. M. (2011): Isolation of Brazilian marine fungi capable of growing on DDD pesticide. – Biodegradation 22: 43-50.

[26] Płaza, G. R. A. Ž. Y. N. A., Ulfig, K., Hazen, T. C., Brigmon, R. L. (2001): Use of molecular techniques in bioremediation. –Acta Microbiol Pol 50(3-4): 205-218.

[27] Porto, A. M., Melgar, G. Z., Kasemodel, M. C., Nitschke, M. (2011): Biodegradation of Pesticides. – In: Stoytcheva, M. (ed.) Pesticides in Modern World. Pesticides Use and Management. – InTech, London. DOI: 10.5772.17686.

[28] Prasanna, A. R., Nargund, V. B., Bheemanna, M., Patil, B. V. (2002): Compatibility of Thiamethoxam with *Trichoderma harzianum*. –Journal of Biological Control 149-152.

[29] Ranganathswamy, M., A. K., Patibanda. G. S., Chandrashekar, S. B., Mallesh, D., Sandeep, H. B., Halesh, K. (2011): Compatibility of *Trichoderma* isolates to selected insecticides in vitro. –Asian Journal of Bio Science 6(2): 238-240.

[30] Schumacher, V., Poehling, H-M. (2012): In vitro effect of pesticides on the germination, vegetative growth, and conidial production of two strains of *Metarhizium anisopliae*. – Fungal Biol 116: 121-32. http://dx.doi.org/10.1016/j.funbio.2011.10.007.

[31] Silva, M. A. F., Moura, K. E., Moura, K. E., Salomão, D., Patricio, F. R. A. (2018): Compatibility of *Trichoderma* isolates with pesticides used in lettuce crop. –Summa Phytopathologica 44(2): 137-142.

[32] Singh, P. V., Srivadtava, S., Shrivastava, K. S., Singh, B. H. (2012): Compatibility of different insecticides with *Trichoderma harzianum* under in vitro condition. – Plant Pathology Journal 11(2): 73-76.

[33] Szpyrka, E., Podbielska, M., Zwolak, A., Piechowicz, B., Siebielec, G., Słowik-Borowiec, M. (2020): Influence of a commercial biological fungicide containing *Trichoderma harzianum* Rifai T-22 on dissipation kinetics and degradation of five herbicides in two types of soil. –Molecules 25(6): 1391.

[34] Thiruchchelvan, N., Mikunthan, G., Thirukkumaran, G., Pakeerathan, K. (2013): Effect of insecticides on bio-agent *Trichoderma harzianum* Rifai under in vitro. –American-Eurasian J. Agric. Environ. Sci 13 (10): 1357-1360.

[35] Tomizawa, M., Casida, J. E. (2005): Neonicotinoid insecticide toxicology: mechanisms of selective action. –Annu. Rev. Pharmacol. Toxicol 45: 247-268.

[36] Van Herwijnen, R., Van de Sande, B. F., Van der Wielen, F. W. M., Springael, D., Govers, H. A. J., Parsons, J. R. (2003): Influence of phenanthrene and fluoranthene on the degradation of fluorine and glucose by *Sphingomonas* sp. strain LB126 in chemostat cultures. –FEMS Microbiol Ecol 46: 105-111.

[37] Vincent, J. M. (1947): Distribution of fungal hyphae in the presence of certain inhibitors. – Nature 159: 850.

[38] You, M., Liu, X. (2004): Biodegradation and bioremediation of pesticide pollution. – Chin J Ecol 23: 73-77.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2204_37553770