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Diamide insecticides: efficacy, toxicity and analytical methods for residue monitoring in food samples

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Abstract

Anthranilic diamide is a new commercial group of insecticides that target insect ryanodine receptors, causing uncontrolled calcium ion release and depletion, which prevents muscular contraction. Due to the high advantages of diamide over other insecticide groups, it has been applied to control a variety of insect pests on a wide range of agricultural crops. The development of this group has encouraged agricultural producers to increase their reliance on it to protect crops from pest damage, resulting in increase residues in agricultural products. To ensure food safety, it is necessary to carefully inspect for diamide residues in food commodities. QuEChERS method for extraction and clean up of diamide insecticide residues was optimized to be used with a variety of analytical techniques, including HPLC-MS, GC-MS, and LC-MS. Recoveries in food samples ranged from 73 to 112% with limits of quantification 0.5 - 2 µg kg^{-1} and a half-life around 1.5 days. More advanced and sensitive methods were developed and optimized for detecting the amounts of diamide residues in food samples and in the related environmental matrices, to manage their impact on human health and environmental safety. Immunoassays, which depend on specific action and interaction between antigen and antibody, have been developed as a rapid and cost-effective technique for specific monitoring insecticide residues in various food and environmental samples. Advances in immunoassay convert enzyme-linked immunosorbent assay (ELISA) into a very useful tool in residue detection of diamide insecticides in food as addressed in this review article.

***Keywords**:* Diamides, bioefficacy, toxicity, immunoassay, residues, food safety

1. Introduction

Diamide is a new anthranilic group of insecticides developed by Bayer Crop Science that widely used in recent years [1-2]. Insecticides related to this group are effective against many insect orders; i.e. Lepidoptera, Dipteral, and Coleoptera [3-4]. Diamide insecticides have a novel mode of action, which target the ryanodine receptors (RyR) through binding this receptor and causing massive release of calcium ions from muscle cells due to activating calcium channels existing on RyR. This action eventually leads to keeping insect muscles continuously contracted and result in insect death at the end due to paralysis [5-6]. Two classes of synthetic insecticides related to diamide are widely used in pest control. The phthalic diamides (i.e., flubendiamide) and the anthranilic diamides (i.e., chlorantraniliprole, cyantraniliprole, and tetraniliprole) are the commercial insecticides that bind with insect RyR [7-8]. The insecticide, flubendiamide, was approved in the US in 2008 to control pests attacking grapes, corn, and cotton [9] and it was withdrawn in 2016 due to environmental concerns [10].

2. Bioefficacy and safety to natural enemies

Diamide insecticides are exceptionally effective against a wide range of pests in insect orders of Lepidoptera, Dipteral, Coleoptera, Hemiptera, and Isoptera. In the study of Luo et al. [11] and Liu et al. [12], the insecticidal activities of some synthesized chlorantraniliprole derivatives were evaluated using a lepidopteran harmful pest of crops worldwide, diamondback moth (*Plutella xylostella* Linnaeus).

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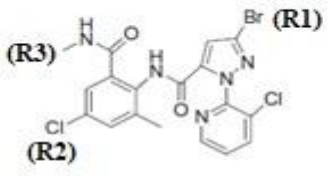
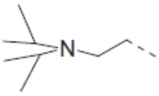
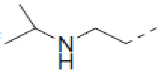
Data presented in Table (1) are summarizing the findings of the most effective derivatives comparing with the parent compound. The most effective derivative showed relative potency 0.63 comparing the parent compound chlorantraniliprole with LC_{50} of 2.4 mg L^{-1} comparing with 1.5 mg L^{-1} of chlorantraniliprole. Chemicals 8c, 8i, 8k, and 8l exhibited high pesticidal activities (> 60%). The initial association of the building blocks for the title compounds showed that the compounds of the cyano group (R_2 = CN) have low pesticidal action. The two groups of methylenes and the second amine in amide moiety were essential components for increasing the bioactivity.

Table 1 :Insecticidal activities, LC_{50} values, and relative potency of chlorantraniliprole and its derivatives 8c, 8i, 8k, and 8l against *Plutella xylostella*.

Derivatives structure	Insecticidal activity (at 2 mg/L for 72 h)	LC_{50} (72 h)	Relative potency
 chlorantraniliprole	53.9	1.5	-
R^1=Br, R^2=Cl, R^3=  derivative (8c)	6.7	6.9	0.22
R^1=Br, R^2=Cl, R^3=  derivative (8i)	53.0	2.6	0.57
R^1=Br, R^2=Cl, R^3=  derivative (8k)	23.3	2.8	0.54
R1=Br, R^2=Cl, R^3=  derivative (8l)	41.9	2.4	0.63

The data were adapted from Luo et al. [11].
* The relative potency of derivatives is calculated as follow: LC_{50} of chlorantraniliprole / LC_{50} of each derivative.

It was reported that chlorantraniliprole had the lowest toxic effect when bioassayed on three biological control agents of *Cycloneda sanguinea*, *Orius insidiosus and Chauliognathus flavipes* comparing with other insecticides of pyrethroid, organophosphorous, and neonicotinoid groups [13]. Chlorantraniliprole showed also not to give repellent effect for the tested species when exposed to the surface of the treated filter papers, which indicates that this insecticide seems to be the least harmful for the beneficial arthropod species (Fig. 1) tested in their study. Data of Fig. 1 revealed that all insecticides had high negative effect on biological species tested except with chlorantraniliprole. Insecticides were reported to affect on feeding, repellent, predator efficiency, and reproductive behavior [13]. Also, cyantraniliprole bioassay results showed to be relatively safe to the one of the most beneficial biological control predator, coccinellids, found in the ecosystem of potato crops. On the other hand, cyantraniliprole tested at 75 and 90 g a.i. ha^{-1} on potato crop by foliar application showed high reduction of the sucking pest populations of whiteflies, thrips, and aphids as well as larvae of armyworm after two sprays [14].

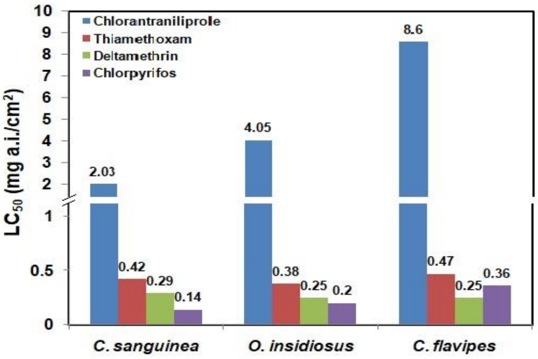


Fig. 1. Toxicity comparison of chlorantraniliprole with some other insecticides from different groups widely used worldwide [13].

Anthranilic diamide insecticides provide an alternative to pesticides of neonicotinoid and pyrethroid for pest control in selected vegetable plants, but with reduced risk of non-target effects and environmental concerns. There are many ways for delivering anthranilic diamides to vegetable crops. This might be done through seeds, foliar treatment, or in-furrow. In the case study of Schmidt-Jeffris and Nault [15], chlorantraniliprole and cyantraniliprole have been tested to control seedcorn worms, *Delia platur* (Meigen) and corn borer, *Austinia nubilalis* (Hübner) in snap beans. Chlorantraniliprole and cyantraniliprole have been tested as a treatment for seeds or in-furrow that reduced *D. platura* damage to the level equal to the standard seeds treatment of neonicotinoid. The two diamide insecticides used in all three ways of treatment greatly reduced the damage occurs by *A. nubilalis*, with foliar application that provided equal control effect as the standard pyrethroid treatment. Results from laboratory bioassays have shown that diamides provide protection potential up to 44 day after application, suggesting that chlorantraniliprole be distributed as a seed treatment has shown great promise control to vegetable pests. This also showed that the use of a single plant anthranilic diamide such as chlorantraniliprole, especially delivered as a seed treatment, can effectively treat both *D. Platura* and *A. nubilalis*, thereby reducing production costs and environmental risks [15].

Although anthranilic diamide insecticides showed high efficiency against insect pests, the resistance is a growing problem in many countries for this group (Fig. 2). I.e., Troczka et al. [16] studied the resistance of *Plutella xylostella* to diamides, which was the first record case of insect pest to develop resistance (Fig. 2). They clearly showed that mutations on the RyR target site are involved in conferring resistance and that there might be a metabolic component contributing to the resistant phenotypes to diamides. In other related studies, control failures of diamides have also been found in other lepidopteran and hemipteran insect pests [17]. The tomato leafminer, *Tuta absoluta*, exhibited high levels of diamide resistance (>2000 fold of resistance) [18], from 77 to 105 fold of resistance in the smaller tea tortrix *Adoxophyes honmai*, collected from Shizuoka Prefecture in Japan [19], and from 3.3 to 25.8 fold in the tobacco whitefly, *Bemisia tabaci*, from china [17].

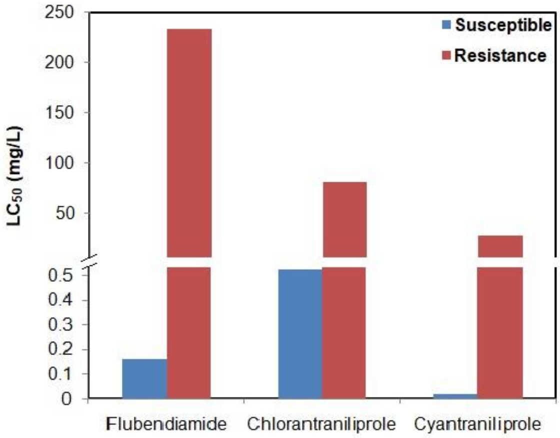


Fig. 2. Levels of resistance developed to diamide insecticides in lepidopteran pests.

3. Toxicity of diamides

The selective toxicity of different insecticides against target and nontarget organisms are necessary factor for their application [20]. Diamide insecticides are used in a variety of applications (foliar, seeds, and soil treatments) to control a large number of crop pests due to their low risk [21]. For the wide application and inappropriate use, these insecticides pose a considerable impact on the ecological environment (plant development and nontarget organisms) [22]. Reports showed that insecticides of diamide, i.e. flubendiamide, severely affected the honey bees antennal neurons through damaging the calcium homeostasis [4]. Chlorantraniliprole, the first insecticide of diamide, and cyantraniliprole showed to inhibit growth rate, weight, and reproduction of earthworms, *Eisenia fetida*, by increasing the level of reactive oxygen species (ROS), malondialdehyde (MDA) content, and causing biomacromolecule damage [12, 23]. Cui et al. [24] evaluated the acute and chronic toxicity of three diamides (flubendiamide, chlorantraniliprole, and cyantraniliprole) to *Daphnia magna*. Their results showed that acute exposure to the three insecticides caused a significant increase in ROS, increase in catalase (CAT) activity, and a significant decrease in activities of antioxidant enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD). The effect of biochemical measurements was consistent with the down-regulated transcription of sod and gpx antioxidant genes. Severe developmental abnormalities in embryos of *D. magna* such as curved tail spine, under-developed second antennae, and abnormal body region are suggested to be induced by diamides. They also stated that chronic exposure to the three tested diamides could cause lethal and sub-lethal effects on daphnids, indicating that even low levels of diamide insecticides might cause considerable ecological risks to aquatic ecosystems.

Despite that the chronic toxicity studies of diamide insecticides against beneficial insects and nontarget organisms are important for their safety. The acute toxicity of tetraniliprole, as a diamide insecticide, was investigated by Ma et al. [22] against *E. fetida* for testing its safety. They concluded that selective toxicity of tetraniliprole was higher than 4000 when calculated between *E. fetida* as a nontarget and *Agrotis ipsilon* as a target organism, suggesting the beneficial of this insecticide as a good candidate for integrated pest management. Bogan [14] tested the phytotoxicity of chlorantraniliprole on potato plants and showed the safety of this insecticide on the tested crop as it did not cause any noticeable phytotoxic symptoms.

4. Residue of diamide in/on food samples

The limit of detection (LOD) and the limit of quantification (LOQ) are considered the lowest concentrations of pesticides for their confident identification and quantification, respectively. I.e., the LOD and LOQ for chlorantraniliprole were determined as 0.005 mg/kg and 0.02 mg/kg, respectively which were generally considered satisfactory for the analysis of the analyte. Although, diamide insecticides have low toxic effects unless they are overused and overexposed. In many countries, the maximum residue limits (MRLs) of diamide insecticides in different agro-products are documented. The MRLs of diamide insecticides except for tetrachlorantraniliprole in mushrooms are 10 μg kg^{-1} have been regulated in European Union (EU), cyantraniliprole in tuber vegetables, garlic, and onions are 50 μg kg^{-1}, while those of chlorantraniliprole in potatoes and cereals (except rice) are 20 μg kg^{-1} [25-26].

Residue analysis of diamide insecticides has been determined in many food and environmental samples, which is a necessary procedure to the food safety and quality. Residues of chlorantraniliprole in/on tomato fruits were determined by Malhat et al. [27]. They found that residue of chlorantraniliprole was decreased from 2.3 to 0.1 mg/kg when determined after spray directly (0 time) and after 15 days of spray, respectively with a 95% reduction. While this insecticide was found in the soil after spray in a concentration of 4.5 mg kg^{-1} and decreased to 0.16 mg kg^{-1} after 15 days of spray with 96% decrease. The dissipation pattern of chlorantraniliprole on cauliflower and the waiting period for the safety to the consumers were studied by Kar et al. [28]. They used quick, easy, cheap, effective, rugged, and safe (QuEChRS) method for the samples extraction and cleanup with estimating residues of chlorantraniliprole using high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS). They observed initial deposits of chlorantraniliprole 0.18 and 0.29 mg kg^{-1} after applications of chlorantraniliprole (Coragen 18.5 SC) for three times at recommended (RD) and double the recommended (2RD) doses (9.25 and 18.50 g a.i. ha^{-1}), respectively. The residues were found to be less than the MRL of 2.0 mg kg^{-1} stated by the Codex Alimentarius Commission, which dissipated below LOQ of 0.10 mg kg^{-1} after 3 and 5 days at both tested dosages.

Dissipation and persistence behaviour of insecticides combination (Chlorantraniliprole 9.26 % and λ-cyhalothrin 4.63 % ZC) in/on pigeonpea were studied through a field trial at RD and 2RD of 30 and 60 g a.i. ha^{-1}. Ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) for chlorantraniliprole was used for the quantitative analysis and QuEChERS method was validated for its accuracy, precision, and sensitivity. The results showed that chlorantraniliprole persisted up to 30 days at RD and 2RD with the half-lives ($t_{1/2}$) of chlorantraniliprole ranged from 4.95 to 5.78 days at RD and 2RD in pigeonpea, respectively. Residues of the insecticide were below LOQ when measured from the soil on the 30th day [29].

Cyantraniliprole is an o-amino-benzamide insecticide formed by substituting the 4-halo group of the former anthranilic diamide chlorantraniliprole with a cyano group [30]. Analytical method studies for cyantraniliprole residue have been reported in several samples including field crops, vegetables, and environmental materials [31-33]. The fate of cyantraniliprole in rice field ecosystem was studied to detect the residue of this insecticide in rice straw, paddy water, brown rice, and paddy soil [34]. The residues of cyantraniliprole in brown rice were lower than 0.05 mg kg^{-1} after 14 days of pre-harvest interval with a MRL of 0.1 mg kg^{-1} and dosage of 100 g a.i. ha^{-1}, which could be considered safe to human beings and animals. The average recoveries of cyantraniliprole ranged from 79.0% to 108.6%, LOQ were 18, 2.8, and 4.3 mg kg^{-1} for rice straw, paddy water, and brown rice, respectively. Their results showed that the $t_{1/2}$ of cyantraniliprole was 3.2 and 4.9 days in rice straw and paddy water, respectively. In experiments carried out on chrysanthemum [35] and ornamental snapdragon [36] under greenhouse conditions, cyantraniliprole was found to be under MRL on chrysanthemum after 21 days of spray with a RD for 2 times with t1/2 of 10 days (Table 2).

Table 2.Residue levels of chlorantraniliprole, cyantraniliprole, and tetraniliprole in different plant samples.

Parameters	Diamide insecticides						
	chlorantraniliprole			cyantraniliprole			tetraniliprole
	pigeonpea	tomato	cauliflower	rice	chrysanthemum	snapdragon	maize
Residue (mg kg^{-1})	1.289-0.026[a] (97.98%)[*]	2.308-0.1[b] (95.66%)	0.18-BDL[c] (100%)	0.041-0.012[d] (70.7%)	0.4-0.15[f] (62.5%)	23.8-6.7[h] (71.8%)	0.92-0.01[i] (98.9%)
$T_{1/2}$ (days)	5.78	3.3	1.36	3.2[e]	10	15 - 56	7.15
Waiting period (WP)/PHI (days)/BMRL	WP=3.65	PHI=8	PHI=3	PHI= 14	BMRL=21[g]	-	-
Sample type for residue	pod	fruit	cauliflower curd	brown rice	plant	leaves	leaves
Reference	Kansara et al. [29]	Malhat et al. [27]	Kar et al. [28]	Zhang et al. [35]	Gong et al. [36]	Huynh et al. [37]	Ma et al. [22]

[a] from 1 to 30 days of application.

[b] from 1 to 15 days of application.

[c] from zero to 5 days of application. BDL= below detectable limit.

[d] from 7 to 21 days of application with 150 g a.i. ha^{-1} for 3 spraying times.

[e] $t_{1/2}$ for the rice straw.

[f] from 7 to 21 days of application with 8.6 kg ha^{-1} for 3 times.

[g] BMRL= below the maximum residue limit.

[h] from 15 to 56 days of application with 250.1 mg L^{-1}.

[i] from 5 to 35 days after germination of the treated seeds with 4.8 g a.i. kg^{-1} seed.

[*] Values in parenthesis are showing the percentage of reduction in residual level.

For snapdragon as a plant model, dissipation and transformation of cyantraniliprole was determined. Huynh et al. [35] found that the dissipation of this insecticide was depending on doses (high *versus* low dose) and the method of application (foliar spray *versus* soil drench). Over 8 weeks of treatments with a high-dose foliar application resulted in insecticide residue of 6.7–23.8 µg g^{-1} foliar fresh weight, while the residue was varied from 0.8 to 1.4 µg g^{-1} in the soil drench treatment (Table 2). The correlation between toxicity and residues of tetraniliprole as a seed dressing to control the black cutworm *A. ipsilon* in the maize seeding stage and its safety to *E. fetida* was investigated by Ma et al. [22]. Results indicated that the maximum residual concentration of tetraniliprole detected in the soil (5.86 mg kg^{-1}) during the entire exposure period, which was considerably lower than the LC_{50} value of tetraniliprole estimated against *E. fetida* (>4000 mg/kg). The recorded residue was 0.01 mg kg^{-1} in maize after 35 days of germination (Table 2).

5. Methods of diamide insecticides detection

Diamide (chlorantraniliprole, cyantraniliprole and flubendiamide) determination was reported using a number of analytical methods in several crops, vegetables, and environmental materials [37-40]. For successful determination, good extraction and cleanup processes are needed for a variety of samples. Scenario of the procedure may include: a weight from 1 to 3 kg of the sample (crop or vegetable) was homogenized by a laboratory homogenizer. Samples can be stored in a freezer at -20°C until analyses at early basis. An aliquot (5-10 g) of fine homogenized samples were weighed into a 50 mL Teflon centrifuge tube. For fortified samples, appropriate volumes of the pesticide concentration solutions were spiked with each sample for studying the recovery rate. The tubes should be vortexed for 1 min to distribute the insecticide evenly, and then the tubes need to be left at room temperature for 1 h to equilibrate and allow the pesticide to interact with the sample matrix before the extraction step. Then extraction was done using different solvents, i.e. mainly it was performed with the use of 10 mL of acetonitrile (typically with 10 g homogenized sample) by shaking the tubes for 2-10 min. For partitioning, buffer salt mixture (1 g NaCl and 4 g anhydrous MgSO4) was added in tubes and shaken for 5 min., then tubes were centrifuged at 4000 rpm for 10 min. A volume of 2 mL portion of supernatant was decanted into a 10 mL centrifuge tube containing an amount of sorbent (20 mg PSA, 2.5 mg graphitized carbon blacks (GCB), and 30-50 mg C18) and 150 mg anhydrous MgSO4 for cleanup. Tubes were intensively vortexed for 1 min before centrifugation at 4000 rpm for 5 min. By this step, the supernatants were transferred into an injection vial and ready for HPLC/GC/LC-MS/MS analysis.

PSA has an important role in the cleanup process as it presents a strong ion-exchange capacity and can interfere with sample compounds and effectively remove chemical as polyphenols, fatty acids, catechins, some organic acids and sugars from samples through hydrogen bond interactions [41-43]. The ability of PSA to purify pigments is limited. However, multi-walled carbon nanotubes (MWCNTs) strongly adsorb and remove the pigments as they exhibit a nano-scale hollow tubular structure, a large specific surface area, stability, durability, and inexpensive [44]. Accordingly, a mixture of PSA and MWCNTs might be used as the sorbent for the efficient dispersive SPE cleanup process with focusing on the amount of the sorbent as a key factor that influences the cleanup performance and the recovery rate. For example, using a too-small amount would result in a weak purification, whereas a too-large amount would exert a satisfactory purification with a recovery rate that is lower than that required for determination.

The highly accurate and sensitive analytical methods for the detection of diamide insecticides in different samples were generally based on high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS) [45-49]. The chlorantraniliprole levels in environmental and food samples (fruits and vegetables) are often analyzed with high performance liquid chromatography (HPLC) methods [50-51], with LOD and LOQ of 0.8 μg kg^{-1} and 1.6 μg kg^{-1}, respectively [52]. Recovery of diamide insecticides were studied in mashroom using three fortified concentrations, which showed good recovery rates (Fig. 3) [49]. However, the reported traditional methods require expensive instruments, highly qualified persons, and complicated procedures in sample pretreatment. It was attractive to develop rapid methods to detect the insecticide residues in various matrices other than HPLC-MS. Immunoassays and in particular enzyme-linked immunesorbent assays (ELISAs) comparing with instrumental methods are more simple, rapid, and cost-effective based on specific action between antigen and antibody that can be used in monitoring plenty of samples in the field and for high throughput detection. The good features of ELISA convert this method into a very powerful tool for agrochemical residue analysis [53].

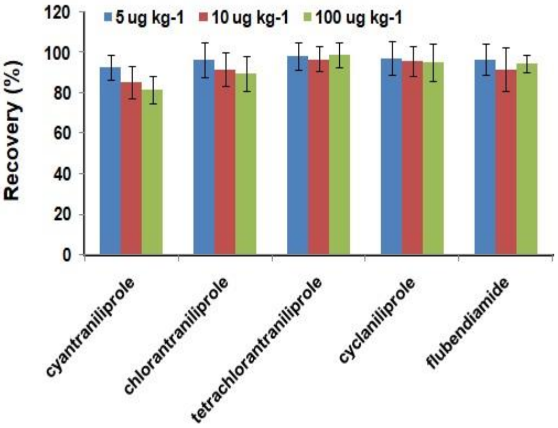


Fig. 3: The mean percentage of recovery of diamide insecticides from different samples of mushroom at three spiked concentrations (modified from [49]).

Many factors can affect the ELISA performance as ionic strengths, solvents, pH values, and concentrations of the assay buffer. Organic solvents are often used in general extraction procedures such as methanol, acetone, and DMSO. Despite that methanol is the most suitable organic solvent in extracting organic substances from different food samples, it was reported to have many effects on ELISA procedures. For instance, the assay buffer containing 10% methanol did not alter the IC_{50} value [54], while other studies showed that methanol caused a little effect on the ELISA performance [55-57]. In contrast, the lowest IC_{50} was recorded in the imidaclothiz icELISA when buffer containing 10%

methanol was used [58]. In the study of Cui et al. [59], when the methanol concentration was changed from 5 to 40% the Amax value of the icELISA was increased notably and a significant increase of the IC_{50} value from 2.44 to 12.3 ng mL^{-1} was noticed due to the addition of methanol, indicating ~8 fold decrease in sensitivity. The same phenomenon was also obtained for diniconazole in an ELISA [60].

To investigate the specificity of the developed ELISA for diamide insecticides, Liu et al. [61] used flubendiamide, cyantraniliprole, and 3 flubendiamide analogs that were synthesized in their laboratory to test the cross-reactivity of the developed assay. Their results showed IC_{50} and the cross-reactivity of each molecule with cyantraniliprole. However, no cross-reactivity with flubendiamide was obtained due to distinct differences between the chemical structures of both insecticides.

Develop immunoassays for pesticides detection have been extensively studied over the past decades using polyclonal and monoclonal antibodies (pAbs and mAbs). Advances in this field have led recently to produce nanobody (Nb)-based immunoassays, which proved to be a great method in the detection of environmental contaminants including insecticides in complicated matrices [62–65]. Nb has some advantages over traditional antibodies such as nano-scale size (~15 kD), thermal stability, water-solubility, ease of genetic modification, and cost of production that makes camelid single-domain antibodies (VHH) is referred to as Nb [66]. To enhance Nbs as analytical tools for fast detection, improve their analytical sensitivity, increase the specificity, broaden their application range, the high availability of molecular tools allows for the gene engineering of Nbs to meet these characteristics. Nbs can be stored in many forms including stable proteins, plasmids, and bacterial stabs, or they can be archived as the primary sequence then easily re-synthesized.

For example, a mAb-based ELISA for the selective detection of a diamide insecticide, cyantraniliprole, in pakchoi (bok choy) has been developed which was specific to this insecticide [67]. While Nbs recognizing both cyantraniliprole and chlorantraniliprole were raised in Xu et al. [68] study to develop an ELISA for the detection of both diamide insecticides in bok choy and soil. Due to that chlorantraniliprole and cyantraniliprole have low toxicity to human beings; they are permitted to be sprayed on bok choy close to the time of harvest, resulting in the possibility of increase the insecticide residues in bok choy in this vegetable plant. Accordingly, the MRLs of chlorantraniliprole and cyantraniliprole in Brassica vegetables are 2 mg kg^{-1} in China [25]. For the desired characteristics, Xu et al. [68] reported that Nb-based ELISA could provide a rapid alternative to instrumental methods for the rapid detection of diamide insecticides in soil and bok choy. They conducted a comparison for the accuracy of Nb-based ELISA and HPLC for diamide detection and their results showed a good correlation of HPLC with those of Nb-based ELISA for cyantraniliprole (R_2 = 0.864) and chlorantraniliprole (R_2 = 0.997) (Fig. 4). Thus, the resulting Nb-based ELISA proved to be a valid method for detecting two diamide insecticides in both soil and bok choy samples.

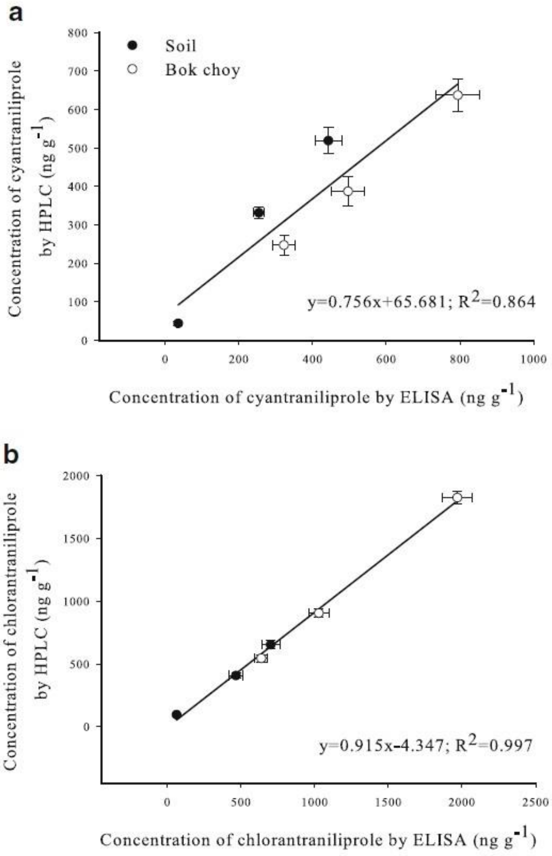


Fig. 4: Correlation between ELISA and HPLC for detection of cyantraniliprole (a) and chlorantraniliprole (b) in soil and bok choy samples (transferred from [68]).

6. Dietary risk assessment of diamide insecticides

Existing pesticide residues in food commodities and drinking water cause serious health problems. In Ethiopia's drinking water sources, pesticides were detected and showed to cause side effects on human health depending on the amount consumed and the acute reference dose. The continuous/chronic exposure to pesticides causes cumulative effects, which subsequently may cause harm to human health [69]. Chronic exposure to pesticides has been associated with health effects such as hormone disturbance, neurological disorders, reproductive aberrance, cancer, and cardiorespiratory symptoms [70].

As there are a few studies on health risk of consumption of the contaminated drinking water with pesticides, for this reason Elfikrie et al. [71] calculated non-carcinogenic risks using hazard quotient (HQ) that was used to determine non-carcinogenic health risks as when $HQ < 1$ means no significant risk and when $HQ > 1$ indicates significant health risk. The HQ was calculated from the following equation [72].

$HQ = ADD/RfD$

Where, ADD is the average daily dose (mg kg^{-1} day^{-1}), and RfD is the reference dose (mg kg^{-1} day^{-1}). While, ADD is defined by the following equation [72]:

$ADD = C \times IR \times EF \times ED/BW \times AT$

Where C is the concentration of pesticide (mg L^{-1}), IR is ingestion rate (L day^{-1}), EF is exposure frequency (days $year^{-1}$), ED is exposure duration (years), BW is body weight (kg) and AT is averaging time (days).

The study of Elfikrie et al. [71] focused on the chronic exposure to target compounds including chlorantraniliprole via ingestion of finished water from drinking water treatment plant (DWTP). The chronic health risk (non-carcinogenic) assessments were focused on four pesticides (imidacloprid, tebuconazole, propiconazole and buprofezin) detected in the final stage of treatment plant or in finished water. The HQ and hazard index (HI) for non-carcinogenic health risks are studied in their investigation, which showed that buprofezin was having the highest HQ (1.85×10^{-4}) among kindergarten children while tebuconazole showed the lowest HQ (5.09×10^{-6}) among elderly group. They summarized their findings that all HQ values of four pesticides tested were <1 indicating that no significant chronic health risk can be obtained from daily ingestion of finished water from DWTP. Also, they showed that all HI values for the four pesticides were <1 indicating non-significant risk of exposure to the mixture of four target pesticides. They concluded that elderly group showed the lowest HI value (7.72×10^{-5}), while the highest HI value (2.94×10^{-4}) was recorded with the kindergarten children as the children and toddlers have the highest exposure due to their high consumption rate of water per kg bodyweight compared to adults [73].

RQ was estimated for the residues determined directly on the treated pigeonpea plants sprayed with an insecticide containing a combination from chlorantraniliprole and λ-cyhalothrin at 30 and 60 g a.i. ha^{-1} [29]. Their results indicated that dietary risk assessment (RQ <1), which suggested that the application of the combination at RD is safe for the consumers. In the same study, the estimated daily intake (EDI) was calculated for chlorantraniliprole and λ-cyhalothrin residue by multiplying the average food consumption rate (g day^{-1}) with the product of pesticide concentration (mg kg^{-1}) divided by the mean body weight of different groups of Indian consumers (kg) [29]. Also, they assessed the long-term risk assessment of intakes compared to pesticide toxicological data by calculating the RQ, dividing the EDI by the relevant acceptable daily intake (ADI) expressed in mg kg^{-1} bw day^{-1}. They concluded that the consumption of pigeonpea sprayed with a product containing chlorantraniliprole and λ-cyhalothrin and waiting for a period of 9 days before harvest are safe as their RQ values are less than one [74].

Risk assessment not only calculated for human but also for beneficial organisms. In the study of Ma et al. [22], the potential risk of tetraniliprole, as seed treatment, to earthworms was estimated by the PECsoil_SFO_China (xls) model [75]. This was done because the extensive use of pesticides are reported to cause a severe decline in earthworms and damage related ecological systems [76-77]; therefore, risk assessment of pesticides is needed for earthworms before their use [78]. The Ma et al. [22] results of RQ showed a low-tier risk assessment as the highest RQ calculated for tetraniliprole seed treatment to earthworms at the tested concentrations was 2.8×10^{-3} (<1), which was evaluated as acceptable.

7. Conclusion

Diamide group is considered highly effective against target pests and a relatively safe group of insecticides. This group is a widely used group currently due to its good characteristics. Although it showed high efficacy against pests compared with other groups of insecticides, some insects have developed > 2000 fold of resistance toward its insecticides. Also, considerable impacts on non-target organisms were recorded such as affecting the antennal neurons of the honey bees and damaging biomacromolecules and growth rates of earthworms. To ensure its safety on human beings, residue analysis has been done using traditional techniques (HPLC-, GC-, and LC- MS/MS) that need expensive instruments and higher costs. More advanced, cost-effective, and sensitive methods (immunoassays) were applied in diamide detection. To keep environment and human safety, acute and chronic toxicity studies of diamide are still needed as well as more accurate, fast, and sensitive methods for monitoring its residues in food commodities are critical.

8. Conflicts of interest

The authors declare that they have no conflict of interests.

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