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RESEARCH

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Toxicity, joint action effect, and enzymatic assays of abamectin, chlorfenapyr, and pyridaben against the two-spotted spider mite *Tetranychus urticae*

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Abstract

Background: In the present study, the comparative toxicity of three different acaricides (abamectin, chlorfenapyr, and pyridaben) in technical and formulated forms was assessed on the eggs and adult females of a susceptible strain of *Tetranychus urticae*. Joint toxic effects of the tested acaricides were also performed against eggs and adults. In addition, the in vitro assay of the tested acaricides was evaluated against some target enzymes isolated from the adult females.

Results: The LC_{50} values against eggs by leaf-disk-dip technique were estimated to be 294.27, 1032.93, and 9550.54 mg/L for abamectin, chlorfenapyr, and pyridaben, respectively. However, the LC_{50} values were 783.06, 666.55, and 731.36 mg/L for the formulations Agromectin, Challenger, and Sanmite, respectively. Abamectin was found to be the greatest lethal to the adults (LC_{50} = 5.39 mg/L) followed by chlorfenapyr (LC_{50} = 106.51 mg/L) after 24 h of the treatment by slide-dip technique. Pyridaben was least toxic (LC_{50} = 690.23 mg/L). Agromectin (LC_{50} = 0.94 mg/L) followed by Challenger (LC_{50} = 73.65 mg/L) while the Sanmite was the lowest toxic one (LC_{50} = 1160.60 mg/L) against the adults. The results of joint toxic action proved that all combinations between the technical or formulated acaricides exhibited potentiation effect and the toxicity was increased significantly against eggs and adults of *T. urticae* compared to the individual pesticide. The activity of acetylcholinesterase (AChE), adenosine triphosphatase (ATPase), acid and alkaline phosphatases (ACP and ALP), carboxylesterase (CaE), gamma-aminobutyric acid transaminase (GABA-T), and glutathione-S-transferase (GST) isolated from adults treated with 0.025, 0.05, 0.1, 0.5, 1, and 5 mg/L were significantly inhibited compared to the control.

Conclusion: This study provides the theoretical basis for a rational application of abamectin, chlorfenapyr, and pyridaben mixtures in *T. urticae* control.

Keywords: Acaricidal activity, *Tetranychus urticae*, Joint toxic effect, Biochemical analysis

Background

The two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is considered one of the greatest common plant pests in the world and it is responsible for significant yield damages in numerous economically essential crops in many field and greenhouse conditions (Adesanya et al., 2021). The checklist of host plants attack by this mite includes more than 1200 species (Shukla, 2021), including food crops, vegetables, fruits, and ornamentals plants. Damage to plants can be classified as direct or indirect effects (Santamaria et al., 2020). The direct effects extend from small spots on the top side of the leaf due to chlorophyll depletion, webbing, and

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defoliation, even necrosis in leaves and small stems, or even plant death. Indirect effects of feeding may include a reduction in transpiration and photosynthesis and can lead to leaf color changing to yellow to white and often referred to as bronzing, causing loss of quality and yield or death of host plants (Park & Lee, 2002). In addition, mites can transmit some pathogenic fungi, bacteria, and viruses and inject systemic toxic substances into the leaf that interfere with vital processes.

Various strategies include biological and chemical treatments have been applied to control *T. urticae*, especially in protected crops (Badawy et al., 2018; El-Saiedy et al., 2008; Wang et al., 2018). A variety of commercially available acaricides have been used such as abamectin, bifenazate, chlorfenapyr, clofentezine, cyflumetofen, dicofol, etoxazole, fenpyroximate, hexythiazox, propargite, pyridaben, spiromesifen, and spiromesifen (Dekeyser, 2005; Van Leeuwen et al., 2015). However, mite control is being delayed due to the rapid development of resistance to many chemical classes of pesticides. Recently, there has been a tendency to mix acaricides to prevent multiple uses of a single acaricide and reduce the development of single acaricide resistance in mite populations (Jeppson et al., 2020). One of the most effective approaches for delaying the development of pesticide resistance is to use a mixture of pesticides (Shukla, 2020). Studies have indicated that the mixture of different acaricides such as chlorphenamidine, bifenthrin, propargite, hexythiazox, and abamectin has demonstrated a synergistic effect on Tetranychidae mites (Liang et al., 2018; Maciesiak & Olszak, 1999). Since most acaricides do not affect eggs, frequent application was required for approximately 10–14 days to control the egg stage. High reproductive potential shortens the life cycle and elective pressure led to the use of a formulated acaricide to control adults and eggs from *T. urticae*. Ismail et al. found that when applying a mixture of abamectin and spinosad at a median lethal concentration (LC_{50}), the mortality was 74%, the fertility decline was similar to abamectin alone and egg hatchability was lower than for either compound alone (Ismail et al., 2007). Although there have been many studies regarding the synergistic effect of acaricide mixtures, the synergistic mechanism based on enzyme detoxification of mixtures has rarely been studied (Carnesecchi et al., 2019; Della Vechia et al., 2021).

Most recent acaricides extend their action by disrupting respiratory processes or affecting growth and development. Among these products, abamectin, chlorfenapyr, and pyridaben are used as acaricides in Egypt. Abamectin is a low-activity neurotransmitter acaricide and is considered a chloride channel activator. It is a mixture of avermectin B1a (> 80%) and avermectin B1b (< 20%) isolated from the fermentation of the soil bacterium *Streptomyces avermitilis*. Abamectin is non-toxic to beneficial arthropods in the open field because of its short environmental stability, rapid absorption in treated plants, and rapid degradation of surface residues (Lasota & Dybas, 1991). Chlorfenapyr is a halogenated pyrrole compound and exactly a pro-insecticide (metabolized into an active pesticide after ingoing the host). At the biochemical level, it works by disrupting the production of adenosine triphosphate, specifically, "the oxidative removal of the *N*-ethoxymethyl group from the compound by the mixed-functional oxidases forming CL 303268. CL 303268 breaks down oxidative phosphorylation in mitochondria, which leads to disruption of ATP production, cell death, and ultimately death of the organism (Marcic, 2012). It is included in the Environmental Protection Agency (EPA) list as an alternative to organophosphorus pesticides. Pyridaben, a pyridazinone derivative, is an insecticide and acaricide that is permitted for practice in the EU and several other countries worldwide. The compound affects metabolism, inhibiting the electron transport chain in the mitochondria by binding with complex I at the coenzyme Q0 site. It exactly works to block mitochondria and prevent oxidation of isolated complex I with high strength (Dekeyser, 2005).

The availability of reliable baseline data on target mite exposure to acaricides is one of the most important prevalent factors in regulating the usage of acaricides. Furthermore, it is best to evaluate the effect of pesticides on eggs and adults to measure the efficacy of these products. Therefore, the aim of the current research was to compare the toxicity of abamectin, chlorfenapyr, and pyridaben in technical and formulated forms against the eggs and adults of *T. urticae* under laboratory conditions using different bioassays. The joint toxic effects were also evaluated, which can help prolong the service life of acaricides and provide a practical basis for effective control. To support the biological activity data, the in vitro assessment of the tested acaricides was tested against certain enzymes including acetylcholinesterase (AChE), adenosine triphosphatase (ATPase), acid and alkaline phosphatases (ACP and ALP), carboxylesterase (CaE), gamma-aminobutyric acid transaminase (GABA-T) and glutathione-S-transferase (GST) extracted from *T. urticae* females.

Materials and methods

Pesticides, chemicals, and reagents

The technical grade of abamectin (> 95% purity) and a formulation of Agromectin (1.8% EC) were obtained from Syngenta Agro. (Giza, Egypt). Chlorfenapyr technical (95% purity) and a formulation of Challenger (36% SC) were obtained from Sumitomo Corporation (Cairo, Egypt). Pyridaben (> 95% purity) and a formulation of

Sanmite (20% WP) were obtained from DuPont (Cairo, Egypt). The chemical structures of these compounds are shown in Fig. 1.

Acetylthiocholine iodide (ATChI), 5,5-dithio bis (2-nitrobenzoic) acid (DTNB), adenosine triphosphate (ATP), 1-chloro-2,4-dinitrobenzene (CDNB), glutathione, *p*-nitrophenyl phosphate (*p*-NPP), Folin–Ciocalteu phenol reagent, alkaline copper reagent, dimethyl sulfoxide (DMSO), trichloroacetic acid (TCA), alpha-naphthyl acetate, Fast blue B salt dis sodium, α-ketoglutarate, 2-mercaptoethanol, sodium–potassium tartrate, β-nicotinamide adenine dinucleotide (β-NAD), Triton X-100, gamma-aminobutyric acid (GABA), bovine serum albumin (BSA), sodium dodecyl sulfate (SDS), and Tris hydrochloride (Tris HCL) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemicals and solvents were of analytical reagent grade and were purchased from El-Gomhoria for Pharmaceutical and Chemicals Co. (Alexandria, Egypt) and were used without further purification.

The tested spider mite

A population of *Tetranychus urticae* was reared on castor oil leaves bean, *Ricinus communis* L., at the Department of Pesticide Chemistry and Technology, Faculty of Agriculture, Alexandria University. Rearing conditions were 26 ± 2°C and 65 ± 5% RH with a light period of 12 L and 12 D during the year to avoid induction of diapause.

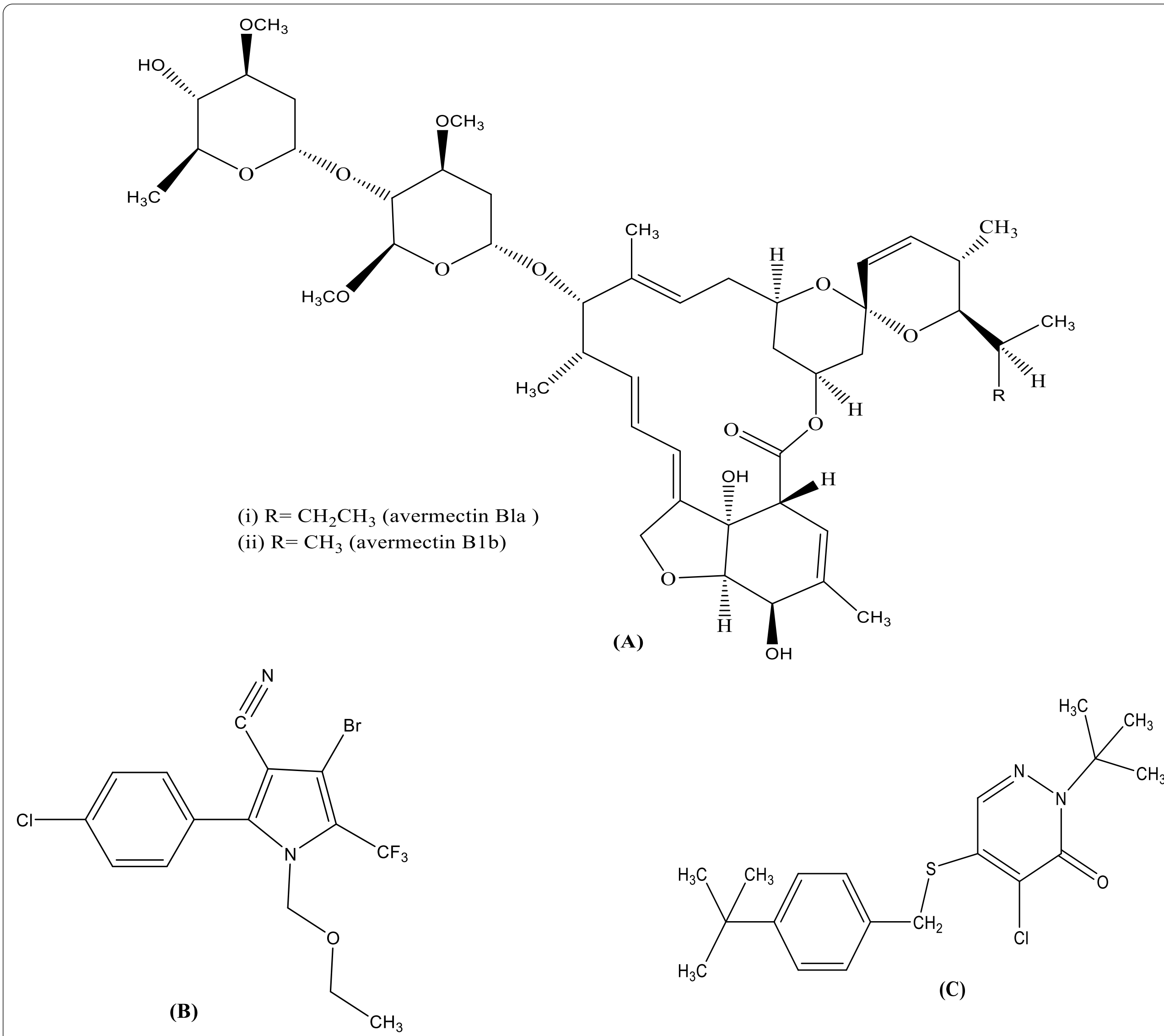

Fig. 1 Chemical structures of abamectin (**A**), chlorfenapyr (**B**) and pyridaben (**C**)

Water was changed every 2 days and fresh leaves were provided for feeding the different stages of the mite population. The population on leaves was always preserved in a glass rearing room (80 × 200 × 80 cm), which was covered with a wire net. Mites were continuously moved from old leaves to new leaves by inserting the old mite-infested castor-oil bean leaves onto the new leaves (Badawy et al., 2010). This strain was not exposed to any contaminants, including pesticides.

Bioassay techniques

Contact toxicity assay against eggs

The Leaf-disk-dip technique was used to determine the acaricidal activity of the tested acaricides (technical and formulations) against eggs of *T. urticae* (Siegler, 1947). The leaf discs were cut from caster-oil leaves and kept over wet cotton pads in a Petri dish (9 cm diameter). The discs were infested with 10 adult females that were allowed oviposit for 24 h on the upper surface of each leaf disc. After that, the females were removed and the eggs were counted under 10× of a stereomicroscope (Optica microscope, T1A, Italy). Technical acaricides (abamectin, chlorfenapyr, and pyridaben) were dissolved separately in DMSO and diluted by distilled water containing 0.05% Tween-80 to obtain the desired concentrations (10–1000 mg/L). However, the formulations of these acaricides were directly prepared in distilled water at a range of 1.0–100 mg/L. The discs attached with eggs were immersed in the test liquid solution for 5 s with gentle agitation. The tested units were kept together with untreated controls, in a holding chamber of about 25 °C and 95% RH. The results were recorded when the hatched mites in control have extended the deutonymphal phase, i.e., all the present eggs were hatched or nonviable. Hatched and un-hatched eggs were counted and the percentage of un-hatchability was calculated as follows:

$$\text{Un - hatchability (\%)} = (a/b) \times 100$$

where a is un-hatched eggs and b is the number of total eggs counted before treatment. The bioassay tests were accomplished in triplicate and the LC_{50} values were calculated according to probity analysis (Finney, 1971).

Contact toxicity assay against adult females

The tested acaricides (abamectin, chlorfenapyr, and pyridaben), in technical and formulations, were assayed against adults females of *T. urticae* using the slide-dip technique (Dittrich, 1962). Microscope slides (75 × 25 mm) were prepared with strips of adhesive tape, which were applied to one side of the slide. Tape with two adhesive sides is necessary (e.g. Scotch Brand No.413 liner type, or No 665 without liner). Adult females of the tested mite of 2–5 days old were placed on their backs on the tape in rows, not closer than 10 mm to either end of the slide. Care was taken for no touching the adhesive surface when the protective covering was removed. A fine brush (No. 000 or finer, sable or squirrel hair) was used to place twenty females on their backs on an adhesive tape fixed on a glass slide. Technical acaricides were dissolved in DMSO and diluted by the solution of 0.05% Tween-80 in distilled water to obtain 0.1–1000 mg/L. However, the formulations of these acaricides were directly prepared in distilled water at a range of 0.01–100 mg/L. The slides containing mites were dipped in pesticide solutions, so the mites are immersed completely in the diluted toxicant and lightly agitated for 5 s to confirm whole wetting. When the slides were withdrawn, they should be placed on the edge of absorbent material and allowed to drain for 15 min. To achieve a uniform residue, it may be desirable to remove excess liquid by carefully blotting the slide, especially close to the lowest row of fixed mites. The treated slides were preserved in a chamber with an optimum environment (25 ± 2 °C and 95% RH). The slides were stored horizontally, rather than vertically, this resulted in better survival. After 24 h and 48 h of the treatment, mortality was noted by 10× stereomicroscope (Optica microscope, T1A, Italy). The adults unsuccessful to respond when prodded lightly with a fine brush were considered dead. Water, 0.05% Tween-80, and DMSO were considered as controls. If the mortality in the controls fell between 5 and 20%, Abbott's formula was applied to correct it (Abbott, 1925) and the LC_{50} values were calculated according to probity analysis (Finney, 1971). The bioassay tests were performed in triplicate.

Joint toxic effect of the tested acaricides

The joint toxic effect of the tested acaricides against eggs and adults of *T. urticae* (Koch) was assessed (Mansour et al., 1966). The expected LC_{25} value, which was obtained from the regression line, was tested in the pair combination (abamectin + chlorfenapyr, abamectin + pyridaben, and chlorfenapyr + pyridaben). Blends were tested against adults and eggs using the slide-dip technique and leaf-disc-dip, respectively. The observed mortality was recorded 24 h after treatment. The co-toxicity factor was calculated by the following equation:

$$\text{Co - toxicity factor} = \frac{(\text{OM} - \text{EM})}{\text{EM}} \times 100$$

where OM is the observed mortality (%) and EM is the expected mortality (%). A factor of + 20 or higher means strengthening or potentiation, a negative factor of 20 or less means antagonism and the value between 20 and + 20 means an additional effect (Mansour et al., 1966).

Biochemical studies

Preparation of homogenates and protein assay

Adult females of *T. urticae* (0.25 g) were homogenized in 20 mL of cold appropriate buffer based on the type of the enzyme using a Teflon-glass homogenizer. The homogenates were centrifuged at 5000–10,000 rpm and 4 °C for 10 min. The supernatant was used as enzyme extracts for enzymes assay. Total protein content was determined according to the Lowry method (Lowry et al., 1951) using Folin–Ciocalteu phenol reagent. The protein content of the sample was obtained by comparison with the BSA standard curve ($K_{\text{value}} = 0.0353$).

In vitro assay of enzymatic activities

The in vitro incubation of each enzyme extract (AChE, ATPase, ACP, ALP, GABA-T, CaE, and GST) was firstly performed for 30 min with a series of acaricide concentrations (0.001, 0.01, 0.025, 0.05, 0.1, 0, 0.5, 1, and 5 mg/L) that prepared in DMSO. The activity of the residual enzyme was determined colorimetrically using a specific method. Blanks without homogenate or substrate were used for the non-enzymatic activity.

AChE activity was assayed using a procedure of Ellman et al., (1961) using DTNB (10 mM) and ATChI (75 mM) as substrates and the absorbance was measured at 412 nm using UV/Visible Spectrophotometer (Unico 1200-Spectrophotometer).

ATPase activity was determined according to the method of Koch (1969) using ATP as a substrate. The protein was precipitated with TCA, then the protein-free filtrate was treated with acid molybdate solution and the phosphoric acid formed was reduced by the addition of ferrous sulfate reagent to produce the blue color. The resulting color was measured at 740 nm.

Activities of ACP and ALP were measured by the method of Bergmeyer (1967) using *p*-NPP as a substrate. The ACP assay medium consisted of 250 µL *p*-NPP in sodium acetate buffer (pH 4.0) and 100 µL of enzyme extract. The reaction mixture was completed up to 2 mL by the acetate buffer and the reaction was incubated for 10 min at 37 °C. The reaction was stopped by addition 400 µL of TCA (10%) and 500 µL NaOH (0.1 M). ALP assay medium consisted of 250 µL *p*-NPP in Tris–HCl buffer (pH 8.6) and 100 µL of the enzyme extract. The mixture was completed to the total volume of 2 mL by s Tris–HCl buffer and the reaction was incubated for 10 min at 37 °C. The reaction was stopped by addition 500 µL of Tris–HCl buffer. The yellow coloration resulting from *p*-nitrophenol (*p*-NP) in the determination of ACP and ALP was measured at 420 nm.

GABA-T activity was measured by the method of De Boer and Bruinvels (1977) with some modifications (Pandey & Singh, 1985). The reaction buffer contained 50 mM Tris–HCl (pH 8.5) and 100 µL of 2 mM α-ketoglutarate (pH 7), 100 µL of 2-mercaptoethanol (20 mM), and 20 µL of β-NAD (1.1 mM). The reaction was initiated by adding 200 µL (3 mM) of GABA. Incubation was carried out for 30 min at 25 °C and the absorbance was recorded at 340 nm.

The CaE activity was assessed based on Miller and Karn (1980) procedure using α-NA as a substrate with some modifications (Rabea et al., 2017). The enzymatic formation of α-naphthol was stopped by the addition of 25 µL of 0.3% Fast Blue B salt in a 3.5% SDS solution as a chromogenic agent. The solutions were further incubated for 15 min at 37 °C. The absorbance of the α-naphthol-Fast Blue complex was read at 555 nm.

The GST activity was determined using CDNB as a substrate and the absorbance was measured at 340 nm (Saint-Denis et al., 1998). One unit of the GST activity corresponded to the quantity of enzyme conjugating mg of glutathione per min.

Each enzyme activity was repeated three times and was expressed as OD mg protein^{-1} min. I_{50}, the concentration producing 50% inhibition of each enzyme activity was calculated according to the probit analysis (Finney, 1971).

Statistical analysis

To estimate the parameters of a concentration-mortality line for each bioassay (leaf-disk-dip technique, slide-dip technique, and enzymatic assay), replicate data were collected and analyzed using the probit model in the IBM SPSS software version 25.0 (Statistical Package for Social Sciences, Chicago, IL, USA) (IBM 2017). The log dose-probit (Ldp) lines allowed the determination of LC_{25}, LC_{50}, LC_{95}, and I_{50} for the bioassays and enzymatic inhibition according to the probit analysis (Finney, 1971). The values were considered significantly different if the 95% confidence limits did not overlap.

Results

Contact toxicity against the eggs of *T. urticae*

The contact acaricidal activities (LC_{25} and LC_{50}) of the technical (abamectin, chlorfenapyr, and pyridaben) and formulated (Agromectin, Challenger, and Sanmite) acaricides against eggs of *T. urticae* by the leaf-disk-dip technique are presented in Table 1. The results of the technical compounds show that the LC_{25} values were 22.79, 26.40, and 838.21 mg/L for abamectin, chlorfenapyr, and pyridaben, respectively. However, the LC_{50} values were 294.27, 1032.93, and 9550.54 mg/L for abamectin, chlorfenapyr, and pyridaben, respectively. The data proved that abamectin had the highest toxicity against eggs, while pyridaben was the least effective acaricide.

Table 1 Acaricidal activity of technical (abamectin, chlorfenapyr, and pyridaben) and formulated (Agromectin, Challenger, and Sanmite) acaricides against eggs of two-spotted spider mite *T. urticae* by leaf-disk-dip technique

Acaricide	LC_{25}[a] (mg/L)	95% confidence limits		LC_{50}[a] (mg/L)	95% confidence limits		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
		Lower	Upper		Lower	Upper			
Abamectin	22.79	5.49	51.03	294.27	173.01	452.42	0.61 ± 0.09	− 1.50 ± 0.26	5.22
Chlorfenapyr	26.40	2.62	73.89	1032.93	562.75	2510.32	0.42 ± 0.09	− 1.28 ± 0.25	0.70
Pyridaben	838.21	192.29	1754.90	9550.54	4481.54	40,267.52	0.64 ± 0.06	− 2.54 ± 0.64	28.01
Agromectin	11.48	0.64	36.19	783.06	158.35	6852.69	0.37 ± 0.03	− 1.73 ± 0.08	31.34
Challenger	4.98	0.26	15.60	666.55	154.75	5374.64	0.32 ± 0.03	− 0.89 ± 0.08	8.82
Sanmite	5.02	1.57	11.99	731.36	389.22	1765.73	0.31 ± 0.03	− 0.89 ± 0.80	8.93

[a] Lethal concentration, 25% or 50%. The concentration value for a pesticide that is required to kill 25% or 50% of the members of tested eggs after a specified test duration

[b] Slope of the concentration-mortality regression line ± standard error

[c] The *y*-intercept of the regression line ± SE

[d] Chi-square goodness of fit test

The LC_{25} values of formulated acaricides (Agromectin, Challenger, and Sanmite) were 11.48, 4.98, and 5.02 mg/L for Agromectin, Challenger, and Sanmite, respectively. However, the LC_{50} values were 783.06, 666.55, and 731.36 mg/L, respectively. Challenger (a.i. chlorfenapyr) was the most active acaricide followed in the descending order by Sanmite (a.i. pyridaben) and then Agromectin (a.i. abamectin).

Contact toxicity against the adult females of *T. urticae*

The results of the acaricidal activity of technical and formulated acaricides against the adult females of *T. urticae* after 24 and 48 h by the slide-dip technique are indicated in Tables 2 and 3, respectively. The data are presented as LC_{25}, LC_{50} values, and their 95% confidence limits with other statistical parameters. The results of the technical acaricides indicate that the LC_{25} values were 0.33, 2.10, and 168.58 mg/L for abamectin, chlorfenapyr, and pyridaben, respectively after 24 h of the treatment. However, the LC_{50} values were 5.39, 106.51, and 690.23 mg/L for abamectin, chlorfenapyr, and pyridaben, respectively (Table 2). After 24 h of the treatment, the LC_{25} values were 0.046, 0.094, and 0.282 mg/L for abamectin, chlorfenapyr, and pyridaben, respectively. However, the LC_{50} values were 0.52, 6.33, and 37.64 mg/L for abamectin, chlorfenapyr, and pyridaben, respectively (Table 3).

The acaricidal activity of formulated acaricides (Agromectin, Challenger, and Sanmite) against adult females after 24 and 48 h are shown in Tables 2 and 3, respectively. The results after 24 h of exposure showed

Table 2 Acaricidal activity of technical (abamectin, chlorfenapyr, and pyridaben) and formulated (Agromectin, Challenger, and Sanmite) acaricides against adult females of two-spotted spider mite *T. urticae* after 24 h by slide-dip technique

Acaricide	LC_{25}[a] (mg/L)	95% confidence limits		LC_{50}[a] (mg/L)	95% confidence limits		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
		Lower	Upper		Lower	Upper			
Abamectin	0.33	0.128	0.67	5.39	3.25	8.19	0.62 ± 0.05	− 0.44 ± 0.08	22.11
Chlorfenapyr	2.10	0.761	4.31	106.51	62.27	203.51	0.39 ± 0.05	− 0.80 ± 0.08	9.26
Pyridaben	168.58	121.40	221.67	690.23	514.19	1009.66	1.11 ± 0.12	− 3.33 ± 0.26	7.61
Agromectin	0.054	0.006	0.108	0.94	0.39	1.82	0.475 ± 0.051	− 0.12 ± 0.07	1.91
Challenger	0.36	0.05	1.22	73.65	29.53	260.33	0.293 ± 0.053	− 0.54 ± 0.081	0.49
Sanmite	5.85	1.81	14.35	160.60	56.32	707.39	0.33 ± 0.031	− 0.949 ± 0.09	5.89

[a] Lethal concentration, 25% or 50%. The concentration value for a pesticide that is required to kill 25% or 50% of the members of tested eggs after a specified test duration

[b] Slope of the concentration-mortality regression line ± standard error

[c] The y-intercept of the regression line ± SE

[d] Chi-square goodness of fit test

Table 3 Acaricidal activity of technical (abamectin, chlorfenapyr, and pyridaben) and formulated (Agromectin, Challenger, and Sanmite) acaricides against adults of two-spotted spider mite *T. urticae* after 48 h by slide-dip technique

Acaricide	LC_{25}[a] (mg/L)	95% Confidence Limits		LC_{50}[a] (mg/L)	95% confidence limits		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
		Lower	Upper		Lower	Upper			
Abamectin	0.046	0.01	0.12	0.52	0.21	0.97	0.69 ± 0.07	− 0.15 ± 0.08	3.22
Chlorfenapyr	0.094	0.01	0.31	6.33	2.93	11.48	0.37 ± 0.45	− 0.79 ± 0.09	1.79
Pyridaben	0.282	0.04	0.89	37.64	19.64	74.48	0.32 ± 0.04	− 0.50 ± 0.08	2.62
Agromectin	0.036	0.013	0.137	0.561	0.25	1.013	0.69 ± 0.08	− 0.35 ± 0.08	8.17
Challenger	0.049	0.046	0.565	1.63	0.027	9.01	0.44 ± 0.05	− 0.09 ± 0.07	8.82
Sanmite	0.416	0.014	1.21	47.71	7.84	202.03	0.33 ± 0.03	− 0.55 ± 0.07	19.99

[a] Lethal concentration, 25% or 50%. The concentration value for a pesticide that is required to kill 25% or 50% of the members of tested eggs after a specified test duration

[b] Slope of the concentration-mortality regression line ± standard error

[c] The *y*-intercept of the regression line ± SE.

[d] Chi-square goodness of fit test

clear differences between the tested acaricides and the highest effect was obtained with Agromectin (LC_{25} = 0.054 and LC_{50} = 0.94 mg/L) followed by Challenger (LC_{25} = 0.36 and LC_{50} = 73.65 mg/L) while the Sanmite was the lowest toxic one (LC_{25} = 5.85 and LC_{50} = 160.60 mg/L). After 48 h of exposure, the results confirmed that the same sequence was obtained with that the Agromectin was the highest acaricidal action (LC_{25} = 0.036 and LC_{50} = 0.561 mg/L) followed by challenger (LC_{25} = 0.049 and LC_{50} = 1.63 mg/L) while the Sanmite was the lowest toxic one (LC_{25} = 0.416 and LC_{50} = 47.71 mg/L).

Joint toxic effects against eggs and adult females of *T. urticae*

As shown in Table 4, the 24 h LC_{25} values of abamectin, chlorfenapyr, and pyridaben were 22.79, 26.40, and 838.21 mg/L, respectively against eggs using the leaf disc-dip technique. When these pesticides were mixed with the ratio of 1:1 of the LC_{25} value, the co-toxicity factors were 24.23, 28.35, and 45.63 for abamectin + chlorfenapyr, abamectin + pyridaben, and pyridaben + chlorfenapyr, respectively. According to the method of Mansour and others (Mansour et al., 1966), all combinations of the tested acaricides exhibited a potentiation effect and the toxicity was significantly increased against eggs compared to the individual compound. The joint toxic action of the formulated pesticides at LC_{25} levels of each acaricide indicated that the co-toxicity values for these mixtures were + 45.05, + 20.68, and + 7.42 for Agromectin + Challenger, Agromectin + Sanmite, and Sanmite + Challenger, respectively (Table 4). The first two combinations exhibited a potentiation effect however, the third mixture showed an additive effect.

Table 5 represents the joint toxicity of the technical and formulated mixtures at LC_{25} values of each

Table 4 Joint toxic effect of technical (abamectin, chlorfenapyr, and pyridaben) and formulated (Agromectin, Challenger, and Sanmite) mixtures at LC_{25} values of each acaricide against eggs of *T. urticae* by leaf-disk-dip technique

Acaricide		Conc. at LC_{25} (mg/L)		Observed mortality (%) at LC_{25}		Expected mortality (%)	Observed mortality (%)	Co-toxicity factor	Results
1	2	1	2	1	2	∑ (1 + 2)	∑ (1 + 2)	∑ (1 + 2)	∑ (1 + 2)
Abamectin	Chlorfenapyr	22.79	26.40	32.47	30.84	63.31	62.12	24.23	Potentiation
Abamectin	Pyridaben	22.79	838.21	32.47	28.82	61.29	64.17	28.35	Potentiation
Pyridaben	Chlorfenapyr	838.21	26.40	28.82	30.84	59.66	72.81	45.63	Potentiation
Agromectin	Challenger	11.48	4.98	35.24	31.02	66.26	72.53	45.05	Potentiation
Agromectin	Sanmite	11.48	5.10	35.24	29.12	64.36	60.34	20.68	Potentiation
Sanmite	Challenger	5.10	4.98	29.12	31.02	60.14	53.71	7.42	Additive

Co-toxicity factor = [(OM − EM)/EM] × 100, where: OM is the observed mortality (%) and EM is the expected mortality (%). A positive factor of 20 or higher means potentiation, a negative factor of 20 or lower means antagonism and the values between + 20 and − 20 indicate an additive effect

Table 5 Joint toxic effect of technical (abamectin, chlorfenapyr, and pyridaben) and formulated (Agromectin, Challenger, and Sanmite) mixtures at LC_{25} values of each acaricide against adult females of *T. urticae* by slide-dip technique

Acaricide		Conc. at LC_{25} (mg/L)		Observed mortality (%) at LC_{25}		Expected mortality (%)	Observed mortality (%)	Co-toxicity factor	Results
1	2	1	2	1	2	$\sum(1+2)$	$\sum(1+2)$	$\sum(1+2)$	$\sum(1+2)$
Abamectin	Chlorfenapyr	0.334	2.10	36.67	33.33	70.00	86.67	73.33	Potentiation
Abamectin	Pyridaben	0.334	168.58	36.67	30.00	66.67	83.33	66.67	Potentiation
Pyridaben	Chlorfenapyr	168.58	2.10	30.00	33.33	63.33	76.67	53.33	Potentiation
Agromectin	Challenger	0.054	0.361	36.67	30.00	66.67	90.00	80.00	Potentiation
Agromectin	Sanmite	0.054	5.85	36.67	26.67	63.34	86.67	73.00	Potentiation
Sanmite	Challenger	5.85	0.361	26.67	30.00	56.67	73.33	46.67	Potentiation

Co-toxicity factor = [(OM − EM)/EM] × 100, where: OM is the observed mortality (%) and EM is the expected mortality (%). A positive factor of 20 or higher means potentiation, a negative factor of 20 or lower means antagonism and the values between + 20 and − 20 indicate an additive effect

acaricide against adults of *T. urticae* using the slide-dip technique. The co-toxicity factors were + 73.33, + 66.67, and + 53.33 for abamectin + chlorfenapyr, abamectin + pyridaben and pyridaben + chlorfenapyr, respectively. In addition, the co-toxicity factors of formulated pesticides at LC_{25} levels of each acaricide were + 80.00, + 73.00, and + 46.67 for Agromectin + Challenger and Agromectin + Sanmite and Sanmite + Challenger, respectively. These findings demonstrated that all combinations of technical and formulated acaricides had a potentiation effect, and the toxicity against adults was greatly increased compared to the individual compound.

In-vitro enzymatic effect of technical acaricides

Effect on AChE

The data presented in Table 6 show the in vitro inhibitory effects of the technical abamectin, chlorfenapyr, and pyridaben on AChE isolated from the adult females of *T urticae*. The tested acaricides exhibited a high inhibitory effect on the enzyme compared to the untreated control. This was confirmed by calculating the half-maximal inhibitory concentration (I_{50}) for abamectin, chlorfenapyr, and pyridaben, which were 8.62, 578.09, and 140.74 mg/L, respectively. Abamectin was shown to be the most effective acaricide in inhibiting AChE activity, although chlorfenapyr was the least effective.

Effect on ATPase

The I_{50} values for abamectin, chlorfenapyr, and pyridaben against ATPase were 0.46, 13.86, and 8.11 mg/L, respectively. The ATPase was strongly inhibited by abamectin, followed by pyridaben, and then chlorfenapyr, as shown in these results.

Effect on ACP and ALP

The findings revealed that the three acaricides had strong inhibitory effects on both enzymes, with ACP inhibiting more than ALP (Table 6). For ACP, the I_{50} values were 0.008, 0.095, and 0.042 mg/L for abamectin, chlorfenapyr, and pyridaben, respectively. However, the I_{50} values were 0.061, 17.67, and 208.12 mg/L for abamectin, chlorfenapyr, and pyridaben, respectively against ALP. From these results, it can be concluded that the ACP and ALP were significantly inhibited by the abamectin compared to chlorfenapyr and pyridaben.

Effect on GABA-T

GAPA-T content was significantly reduced and the I_{50} values were 0.42, 6.41, and 19.80 mg/L for abamectin, chlorfenapyr, and pyridaben, respectively (Table 6). Abamectin was the highest active compound against GAPA-T followed by chlorfenapyr and then pyridaben.

Effect on CaE

The I_{50} values were 29.99, 91.68, and 11.09 mg/L for abamectin, chlorfenapyr and pyridaben, respectively against CaE (Table 6). The enzyme activity was significantly inhibited by pyridaben followed by abamectin however, chlorfenapyr was the lowest active compound. CaE plays a major role in the detoxification of numerous endogenous and exogenous agrochemicals through hydrolyses. However, in the present study, the CaE activity was decreased with different concentrations of abamectin, chlorfenapyr, and pyridaben.

Effect on GST

The data indicated that the tested acaricides showed an inhibitory effect on GST compared to the untreated control and the I_{50} values were 0.043, 82.82, and 67.67 mg/L

Table 6 The in-vitro effect of technical abamectin, chlorfenapyr, and pyridaben on AChE, ATPase, ACP, ALP, GABA-T, CaE, and GST activities isolated from the adult females of two spotted spider mites *T. urticae*

Enzyme	Acaricide	I_{50}[a] (mg/L)	95% confidence limits		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
			Lower	Upper			
AChE	Abamectin	8.62	2.99	17.80	0.86 ± 0.06	− 0.81 ± 0.11	23.07
	Chlorfenapyr	578.09	276.40	1609.90	0.30 ± 0.04	− 0.80 ± 0.09	4.16
	Pyridaben	140.74	57.70	388.41	0.46 ± 0.05	− 0.99 ± 0.10	14.44
ATPase	Abamectin	0.46	0.17	0.93	0.72 ± 0.08	0.24 ± 0.11	6.24
	Chlorfenapyr	13.86	5.34	28.04	0.61 ± 0.05	− 0.69 ± 0.10	13.42
	Pyridaben	8.11	0.89	17.36	0.65 ± 0.05	− 0.51 ± 0.10	29.04
ACP	Abamectin	0.008	0.001	0.06	0.53 ± 0.10	1.10 ± 0.14	0.38
	Chlorfenapyr	0.095	0.01	0.36	0.42 ± 0.06	0.423 ± 0.10	4.25
	Pyridaben	0.042	0.65	3.16	0.70 ± 0.11	0.21 ± 0.05	3.58
ALP	Abamectin	0.061	0.005	0.25	0.33 ± 0.09	1.16 ± 0.14	0.52
	Chlorfenapyr	17.67	7.93	32.95	0.94 ± 0.06	1.17 ± 0.12	21.41
	Pyridaben	208.12	98.76	560.22	0.76 ± 0.05	− 1.75 ± 0.13	29.51
GABA-T	Abamectin	0.42	0.13	0.92	0.62 ± 0.07	0.24 ± 0.12	9.34
	Chlorfenapyr	6.41	0.36	23.99	0.39 ± 0.05	− 0.32 ± 0.09	19.74
	Pyridaben	19.80	10.19	32.24	1.34 ± 0.09	− 1.74 ± 0.15	21.59
CaE	Abamectin	29.99	14.848	54.73	0.85 ± 0.06	− 1.26 ± 0.11	18.48
	Chlorfenapyr	91.68	61.94	136.10	0.55 ± 0.05	− 1.08 ± 0.10	8.49
	Pyridaben	11.09	2.39	28.87	0.58 ± 0.05	− 0.61 ± 0.09	23.07
GST	Abamectin	0.043	0.003	0.209	0.41 ± 0.06	0.56 ± 0.11	2.87
	Chlorfenapyr	82.82	66.47	102.47	1.19 ± 0.08	− 2.28 ± 0.16	4.71
	Pyridaben	67.67	45.36	98.95	1.33 ± 0.09	− 2.43 ± 0.18	10.34

[a] Half maximal inhibitory concentration

[b] Slope of the concentration-inhibition regression line ± standard error

[c] The *y*-intercept of the regression line ± SE

[d] Chi-square goodness of fit test

for abamectin, chlorfenapyr, and pyridaben, respectively (Table 6). Abamectin was the most active one however, chlorfenapyr and pyridaben were the least effective acaricides.

Discussion

Several researchers have reported the toxicity of various pesticides against the eggs and adults of *T. urticae*. For example, Weidong proved that the LC_{50} values of technical chlorfenapyr and abamectin were ranged between 0.122 and 7.656 mg/L against eggs of *T. urticae* (Weidong, 2002). Another study confirmed that the LC_{50} value of abamectin was 8.7 mg/L against eggs (Van Pottelberge et al., 2009). Kumari et al. reported that there was a significant difference between chlorfenapyr, dicofol, fenpyroximate, hexythiazox, propargite, and spiromesifen against eggs of *T. urticae* using the spray method at the recommended concentration (Kumari et al., 2017). Based on the observations of the tenth day, they found that the ovicidal activity of spiromesifen (100% mortality) was followed by dicofol (7.78% mortality) and hexythiazox (6.67%). In addition, nearly no action on hatching was found in both abamectin and chlorfenapyr treatments (0.54% non-hatchability). However, all eggs treated by propargite were hatched. Sato et al. reported that eggs younger than 72 h were more sensitive than other stages to spiromesifen and that the egg-laying was considerably affected (Sato et al., 2011). The eggs of *T. macfarlanei* and *T. truncatus* were reported to be highly susceptible to spiromesifen, hexythiazox, and chlorfenapyr at the LC_{50} level (Ullah & Gotoh, 2013). The authors found that the LC_{50} of chlorfenapyr for *T. truncatus* was approximately 10 times (492 mg/L) higher than the LC_{50} for the recommended concentration (50 mg/L). In addition, the eggs of both strains were highly susceptible to all the tested acaricides, because the LC_{50} values were lower than the recommended concentrations for the tested acaricides. Reports on the ovicidal action of abamectin are conflicting too. For example, it did not affect *T. urticae* eggs in a wide range of concentrations (Kumar & Singh, 2004), whereas Salman reported that abamectin was highly toxic for eggs at all ages but did not affect mite fertility

(Salman, 2007). Given acaricide formulations, Ismail and others reported that Vapcomic 1.8% EC (formulation of abamectin) caused 87% mortality on egg hatching of *T. urticae* at 2.5 mg/L (Ismail et al., 2007). Also, Hosny and others found that LC_{50} of abamectin (1.8% EC) was 1.05 mg/L and LC_{50} of chlorfenapyr (36% SC) was 168.11 mg/L against eggs of *T. urticae* (Hosny et al., 2010).

From the obtained data after 24 and 48 h of the treatment with technical compounds, abamectin showed the highest acaricidal activity against the adult females followed by chlorfenapyr while pyridaben was significantly less toxic compound. The results obtained by He et al. proved that LC_{50} values of abamectin ranged from 0.012 to 0.147 mg/L against adults of *T. cinnabarinus* (He et al., 2009). On the contrary, Van Pottelberge et al. found that the LC_{50} values were 0.4 mg/L for abamectin and 156 mg/L for pyridaben against *T. urticae* adults (Van Pottelberge et al., 2009). Herron and Rophail found that the LC_{50} of chlorfenapyr and pyridaben were 0.54 and 0.29 μg/L, respectively against Russell Fox Pressured (field strain of *T. urticae*) (Herron & Rophail, 2003). Devine et al. found that the LC_{50} of pyridaben was 0.6 mg/L against adults of *T. urticae* (Devine et al., 2001).

Kumari et al. proved that abamectin was the most toxic to the adults (LC_{50} = 0.39 mg/L) by spray method followed by fenpyroximate (LC_{50} = 5.67 mg/L), spiromesifen (LC_{50} = 12.53 mg/L), chlorfenapyr (LC_{50} = 32.24 mg/L), propargite (LC_{50} = 77.05 mg/L), and dicofol (LC_{50} = 146.65 mg/L) however, hexythiazox was the least toxic acaricide (Kumari et al., 2017). The LC_{50} values of chlorfenapyr and abamectin against *T. urticae* were 59.34 and 1.50 mg/L, respectively (Vásquez & Ceballos, 2009). Sato et al. reported LC_{50} of abamectin as 0.17 and 58.10 mg/L against susceptible and resistant strains of *T. urticae*, respectively and it was observed that the resistance ratio at LC_{50} reached 342 fold (Sato et al., 2005).

The results obtained by Kwon and others reported that the LC_{50} values of Vertemic (1.8% EC) (formulated abamectin) were 9.238 and 7.09 mg/L after 24 and 48 h of exposure, respectively against adults of *T. urticae* (El Kady et al., 2007). Also, the results obtained by Ismail et al., proved that the LC_{50} value of Vapcomic (1.8% EC) was 0.34 mg/L against adults of *T. urticae* (Ismail et al., 2007) which was higher than the LC_{50} (0.0135 mg/L) reported by Salman (Salman, 2007). Consequently, Arain found that the LC_{50} values of Sanmite 15% EC (formulated pyridaben) were 29.85 and 11.34 mg/L after the second and third day of the treatment against adults of *T. urtica*e (Arain, 2015). In addition, Hosny et al. proved that the LC_{50} of abamectin (1.8% EC) was 0.03 mg/L and 25.69 mg/L for chlorfenapyr (36% SC) against *T. urticae* adults (Hosny et al., 2010).

The combined effects and synergistic reactions of chemicals in mixtures are an area of great interest for both public and regulatory establishments. The key concern is whether certain chemicals can improve the influence of further chemicals so that they have a greater effect than expected. In general, the synergistic effect of the binary pesticide mixture is dependent on the type of pesticide, the mixed ratio, and the biochemical interactions among components with diverse types of action (Kim et al., 2014). The present study demonstrated that the mixtures of abamectin, chlorfenapyr, and pyridaben exhibited a potentiation effect and significantly increased the toxicity against adults and eggs of the tested mite compared to the individual compound. Therefore, this result suggests that these pesticide combinations could be potentially and simultaneously applied in the field.

Several studies reported that formamidine acaricides (amitraz and chlordimeform) effectively synergize toxic action of certain pyrethroids (deltamethrin, permethrin, cypermethrin, and phenothrin) and neonicotinoids (imidacloprid, thiamethoxam, and dinotefuran) in some insect species (Ahmed & Matsumura, 2014; Ahmed et al., 2015) and mites such as *T. urticae* (El-Sayed & Knowles, 1984). However, inappropriate pesticide combinations would cause an antagonistic effect. Garcia Mari et al. evaluated the toxicity of dicofol and tetradifon are usually applied together in Spanish Citrus orchards in the proportion 6:16 to complement their action on the citrus mites *Panonychus citri* (McGregor) and *T. urticae* Koch (Garcia Mari et al., 1988). By egg spraying, the acaricides affect as much the eggs as the larvae hatching from those eggs, and even dicofol caused higher mortality in *P. citri* larvae. The overall ovi-larvicide activity was similar in both acaricides, producing 100% mortality at rates between100 and 200 mg/L a.i. At field rates, tetradifon showed as light adulticide activity on *T. urticae* and does not affect females of *P. citri*. Dicofol caused a 100% mortality in both mite species. As the mixed contained a higher rate of dicofol, both the ovi-larvicide and adulticide activity against *P. citri* and *T. urticae* was accomplished by dicofol. Ahn et al. reported that the flufenoxuron and fenbutatin oxide mixture was effective against all stages of *T. urticae* in laboratory and field studies (Ahn et al., 1993). In addition, flufenoxuron only and mixed with alpha-cypermethrin was extremely active against immature stages, but was less active on the eggs and nontoxic to the adults. Ismail et al. reported that when a combination of spinosad and abamectin was examined at LC_{50}, the mortality rate was 74%, the reduction of fertility was similar to abamectin alone and the egg-hatching level was lower by either compound against eggs of *T. urticae* (Ismail et al., 2007). Zhonghua et al. found that the LC_{50} values were 0.01 mg/L and

1311.81 mg/L for abamectin and rubble seed oil alone, respectively against adults of *T. cinnabarinus* (Boisduval) (Zhonghua et al., 2006). However, the combination of abamectin-rubble seed oil had significant additive effects on *T. cinnabarinus*, and the ratio of 1:99 of abamectin and oil was the greatest noteworthy with a co-toxicity coefficient of 293.90 against adults. Etheridge and Phillips found that the insecticide mixtures exhibited higher toxicity against adults of *T. cinnabarinus* than the individual product (Etheridge & Phillips, 1976). To extend the service life of bifenazate, the co-toxicity of bifenazate and propargite against *T. urticae* was evaluated and the results exhibited that with a 5:1 mass ratio, respectively, the co-toxicity factor was 137.5, which presented a synergistic toxicity (Liang et al., 2018). Wang et al., (2015) reported that among the different mixture ratios of bifenazate and bifenthrin, the 1:1 ratio showed the highest synergistic toxicity to *T. urticae*, as the co-toxicity coefficients of 24- and 48-h treatment were 204 and 221, respectively. In addition, different acaricide mixtures were applied against of *T. urticae* and enhanced the toxicity against eggs and adults (Kim et al., 1993).

Previous research studies on the mechanisms of action of various acaricides have revealed that the primary targets in mites are specific enzymes, receptors, or channel sites at which they initiate specific associations with physiological changes. AChE, ATPase, GABA receptors, octopamine receptors, voltage-gated sodium channels, and glutamate-gated chloride channels are examples of these targets (Jeschke, 2021; Van Leeuwen et al., 2010). In addition, some detoxifying enzymes such as GST, CaE, and cytochrome P450 are involved in the detoxification of pesticides and other xenobiotics in insects and mites (Wu & Hoy, 2016).

Evidence has appeared that decreased AChE activity is not limited to organophosphorus and carbamate pesticides only, but various other classes of pesticides are also involved in reducing AChE (Frasco et al., 2005). The present study demonstrated that the tested compounds significantly inhibited AChE compared to the untreated control. However, the AChE from the resistance strain of *T. urticae* was insensitive to some organophosphorus pesticides such as monocrotophos, demeton-S-methyl, paraoxon-ethyl, chlorpyrifos-oxon, and the carbamate carbofuran (Kwon et al., 2010a). In addition, the AChE in the resistant strains was 34- to 380-fold fewer sensitive than AChE for a susceptible German strain, *T. urticae*, as demonstrated by conservative micro titer plate assays (Stumpf et al., 2001).

ATPase (EC 3.6.1.3) catalyzes the decomposition of ATP into ADP and a free phosphate ion (Kielley, 1961). It also has a role in nerve impulse conduction through nerve fibers by regulation of ion exchange (Na, K, and Mg). Our results are in agreement with several studies, which confirmed that the ATPase in *T. urticae* was sensitive to most of the acaricides and other pesticides. For example, Desaiah et al. found that the tricyclohexylhydroxytin (Plictran[R]) was an unresolved inhibitor of Mg^{2+}ATPase of spider mite homogenate (in vitro) and the I_{50} was 6.2×10^{-10} M (Desaiah et al., 1973). However, chlorbenside, chlorfenethol, and ovotran were less effective. Plictran at a greater concentration (2×10^{-7} M) was also more effective on Na^+, K^+-ATPase of mite homogenate as compared to chlorfenethol, chlorbenside, and ovotran. The results obtained by Xu and others showed that the acaricide abamectin significantly increased the ATPase activity in the resistance strain of *T. cinnabarinus* (Boisduval) by 1.43-fold to that in control (Xu et al., 2016). However, the ATP content in bifenazate treated mites declined progressively between 0 and 4 h after exposure, similarly to mites treated with the complex I inhibitor fenpyroximate in *T. urticae* (Van Leeuwen et al., 2006a).

Phosphatases are classified into acid phosphatase (ACP, EC 3.1.3.2) and alkaline phosphatase (ALP, EC 3.1.3.1) (Jansson et al., 1988). Both enzymes are metalloenzymes, involved in various metabolic processes, such as permeability, growth and cell differentiation, protein synthesis, absorption and transport of nutrients, and gonadal maturation (Jiang et al., 2012). The current data are in agreement with several studies, which confirmed that ACP and ALP in mites and other pests were sensitive, in vivo and in vitro, to most acaricides and other pesticides (Afify et al., 2012; Carvalho et al., 2013).

GABA is found commonly in most prokaryotic and eukaryotic organisms and can be transferred to astrocytes for catabolism by GABA-T, which converts GABA into a succinate aldehyde (Lee et al., 2011). It is a key inhibitory neurotransmitter in the central nervous system of various vertebrates and invertebrates including mites and insects (Schousboe & Waagepetersen, 2007). High levels of GABA were documented in mites resistant to abamectin in a previous study (Zhu et al., 2010), though the principal mechanism of the GABA accumulation in abamectin-resistant mites was not clear. The results of Xu et al. indicated that the GABA content in abamectin resistant strain of *T. cinnabarinus* was significantly increased (Xu et al., 2017). The authors found that the reductions in activity and mRNA expression of GABA-T were responsible for GABA accumulation in mites and the abamectin-treated individuals had a considerably higher GABA content than those untreated (1.52-fold). On the contrary, the results of abamectin did not show significant differences in GABA levels compared with abamectin-resistant individuals that were not treated with abamectin (Zhu et al., 2010).

CaE activities in *T. urticae* were not enough to account for the extremely high level of abamectin resistance (Kwon et al., 2010b). The present study proved that the concentrations of abamectin, chlorfenapyr and pyridaben that exceed 0.005 mg/L significantly inhibited CaE. However, concentrations lower than this led to activation of the enzyme indicating that the acaricides were being detoxified. Van Pottelberge et al. reported that the enzymes especially P450 monooxygenases and GST could be involved in the metabolic detoxification of spirodiclofen acaricide in *T. urticae* strains (Van Pottelberge et al., 2009). Also, deltamethrin, fipronil, and spinosad decreased the activity of CaE (Carvalho et al., 2013). The data of Van Leeuwen et al. study reported that the inhibitors of esterases such as chlorfenapyr showed remarkable inhibition of CaE in the adult females of *T. urticae* (Van Leeuwen et al., 2004).

GST is a detoxifying enzyme that catalyzes the coupling of a diversity of electrophilic substrates to the thiol group of glutathione resulting in less toxic forms and appears to contribute to cellular protection against oxidative stress (Hayes et al., 2005). Increased levels of GSTs have been associated with higher resistance to a wide variety of insecticides. In the present study, an increase in GST activity was found at low concentrations (≤ 0.01 mg/L) of chlorfenapyr and pyridaben (data not shown), strongly suggesting the induction of oxidative stress by these acaricides. However, abamectin inhibited the enzyme up to 0.001 mg/L. Yorulmaz and Ay reported that the sensitivity of susceptible and resistant strains of *T. urticae* to abamectin acaricide was investigated in terms of the enzyme activities of esterase, GST, and monooxygenase (P450). No substantial change in esterase activities was identified for the abamectin-resistant strain paralleled to the susceptible strain (Yorulmaz & Ay, 2009). After 12 cycles of exposure to a susceptible strain of *T. urticae* to chlorfenapyr, the resistance ratio was found to be 580 based on the LC_{50}s (Van Leeuwen et al., 2004). The authors reported that the synergistic experiments with *S,S,S*-tributylphosphorotrithioate, piperonyl butoxide, and diethylmaleate, which are inhibitors of esterases, monooxygenases, and GST respectively, suggested a major role of esterases in the resistance to chlorfenapyr. Moreover, in another study, the same authors showed that the GST activities to chlorfenapyr were not considerably different between strains of *T. urticae* (Van Leeuwen et al., 2006b).

Conclusion

In the present study, the acaricidal activity of three different acaricides (abamectin, chlorfenapyr, and pyridaben) in technical and formulated forms was examined on the eggs and adults of a laboratory strain of *T. urticae*. Abamectin exhibited the highest toxicity to the eggs and adults compared to other technical compounds. Among the acaricide formulations, Challenger was the best ovicidal whereas Agromectin was the most toxic to the adults. The joint toxic effects of the tested acaricides confirmed that the acaricide mixtures at LC_{25} values of technical or formulations exhibited a potentiation effect and the toxicity was increased significantly against eggs and adults compared to the individual pesticide. In addition, this study showed that the activity of AChE, ATPase, ACP, ALP, CaE, GABA-T, and GST were significantly inhibited in vitro at high levels of the tested pesticides. However, compounds especially chlorfenapyr and pyridaben at low concentrations (≤ 0.01 mg/L) activated GST and CaE indicating that a detoxification process has occurred. The current study showed that these acaricides (abamectin, chlorfenapyr, and pyridaben) could alternatively be used for the effective and sustainable management of mites.

Abbreviations

AChE: Acetylcholinesterase; ATPase: Adenosine triphosphatase; ACP: Acid phosphatase; ALP: Alkaline phosphatase; CaE: Carboxylesterase; GABA-T: Gamma aminobutyric acid transaminase; GST: Glutathione-S-transferase (GST); *T. urticae*: *Tetranychus urticae*; LC_{50}: Median lethal concentration; EPA: Environmental protection agency; EC: Emulsifiable concentrate; SC: Suspension concentrate; WP: Wettable powder; ATChI: Acetylthiocholine iodide; DTNB: 5,5-Dithio bis (2-nitrobenzoic) acid; ATP: Adenosine triphosphate; CDNB: 1-Chloro-2,4-dinitrobenzene; *p*-NPP: *p*-Nitrophenyl phosphate; DMSO: Dimethyl sulfoxide; TCA: Trichloroacetic acid; BSA: Bovine serum albumin; RH: Relative humidity.

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Authors' contributions

MEIB designed research work, analyzed results obtained, participated in manuscript writing, proofreading and sentence correction. MSM carried out the experiments and contributed to the statistical analysis of the results. MMK contributed research design and manuscript writing. All authors have read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article. In addition, the related datasets are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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