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Interaction of agricultural drainage water cations with insecticide potency

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Abstract:

The insecticides potency for controlling target insects in field may affected by many cations which present in water used in insecticides preparation in the field. The toxicity of three types of insecticides lufenuron, lambda-cyhalothrin and tetramthrin and dimethoate. The LC_{50} values for each compound against the 4th instar larvae of cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) 72 hours was 0.001, 0.21 and 0.046 ppm. Six different types of cobalt, sodium, potassium, calcium, magnesium and manganese all in a chloride salt to study their toxicity in presence and absence of LC_{50} of tested insecticides to examine their interaction and interference with insecticides toxicity. Sodium and magnesium chloride had categorically decreased the toxicity of all tested insecticides at concentration 100, 0.01 ppm mortality decreased to 10, 40 and 36 % and 30, 30, 50 %, respectively. Inhibition percentage of total ATPase and AChE were investigated. Na^{+} counteracts effects of all tested insecticides with significant decrease in the levels of ATPase inhibition. Conversely, Mg^{2+} decrease in the levels of AChE inhibition. The toxicity and biochemical data had shown a very interrupted effect due to the presence of these cations due to their interference with the site of action of these pesticides.

Introduction

The cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is one of the most devastating agricultural lepidopterous pests. It can attack abundant economic field and vegetable crops over the year in Egypt (Kandil *et al.*, 2003). The efficacy of the pesticides to cause its toxic effect depends on surrounding condition in which the effect occurs (Pietrock and Marcogliese, 2003). Several abiotic characteristics of water and soil, such as temperature (Rotich *et al.*, 2004), pH (Boss and Mott, 1980), dissolved oxygen content (Panigrahi *et al.*, 2014), hardness (Persoone *et al.*, 1989), salinity (Huang and Brattsten. 2007), cations (Lo and Lee, 1989) and heavy metals (Broerse and van Gestel, 2010) may affect the toxicity of pesticides on organisms.

As a result of the increase in population and with increased demand for water. In Egypt, the increasing scarcity water with the expansion of the cultivation of new land in Sinai and Western Sahara. The reuse of agricultural drainage water provides an integral supplement to the water supply during the coming years. The recycling of this water influences the quality of water in flowing through Egypt's irrigation network. Therefore, the Government works to improve the quality of agricultural drainage water. Exploit it to the water irrigation deficit (Barnes, 2014). Major cations including Ca^{2+}, K^+, Mg^{2+}, Na^+ were listed in drains which may be attributed to high fertilizers, pesticides, other contaminants (Ezzat and Reham, 2012 and Nasr and Zahran, 2015).

ATPases plays an important role in the ionic transfer across the membrane that is a target of the neurotoxic effect of OPs and pyrethroid compounds (Nozdrenko *et al*., 2016 and Kakko *et al*., 2003). Cations facilitate neuromuscular excitability, enzymatic reactions and retention of membrane permeability. Cations counteracted effects of insecticides by promoting normal ATPase activities (Kiss and Fazekas, 1983). Wherefore cations affect the sensitivity of ATPases and AChE inhibition by pesticides. Moreover, it may interfere with the pesticides efficacy on the target and non-target organisms (Takano *et al.*, 1983; Shaker *et al*., 1987; Sarma *et al*., 2013; El-Alfy *et al.*, 2001 and Senger *et al*., 2011).

The aim of this work is to study the problem of contamination soil and agricultural drainage water with cations and know relationship of the pesticides efficacy and its toxicity to the pest studied.

Materials and methods

1. Insect rearing:

A laboratory strain (Lab) of cotton leafworm *S. littoralis* was obtained from Central Lab. of Pesticides, Agricultural Research Center (ARC) Cairo, Egypt that was reared under laboratory conditions for several years without exposure to insecticides. The colony was kept at a temperature of 27±2 ºC and 65±5 RH. (El-Defrawi *et al*., 1964). Larvae were reared on castor oil leaves (*Ricinus communis* L.), the 4th larvae selection for bioassays and biochemical assessments.

2. Insecticides and chemicals:

One insect growth regulators lufenuron (Wormatin, 5% EC) from Bayer Crop Science Jordan, one pyrethroid lambda-cyhalothrin 2.5% and tetramthrin 2.5% (Lambada plus 5 % EC) provided from El-Helb Pesticides and Chemicals Dumyat Al Jadidah, Dumyat, Egypt and one organophosphorus insecticides dimethoate (Belthethoate, 40% EC) provided from BR Agrotech Ltp India. Six different salts in chloride form (Cobalt and manganese) was obtained from Sigma Aldrich Co. and (Sodium, potassium, calcium and magnesium) chloride was obtained EL Nasr pharmaceutical chemicals Co., Egypt.

3. Toxicity of tested insecticides against 4th larvae of *Spodoptera littoralis*:

The leaf-dipping bioassay method was used to determine (LC_{50}) values of tested insecticides against 4th instar larvae of *S. littoralis.* Castor leaves were cut into discs (9 cm). Each disc was dipped in different concentrations of insecticides that prepared in distilled water for 10s. Treated and control discs were held vertically to allow excess dilution to drip off, and were air-dried for 2 hrs. Disc offered to ten larvae in each treatment with three replicates and kept under laboratory conditions (27 ±2 ºC and 65-70% RH.). Mortality counts were recorded after 24, 48 and 72 hr. of treatment.

4. Toxicity of tested insecticides in the presence of different cations concentrations:

For analysis of the effects of synergists or antagonist effect on toxicity of lufenuron lambda-cyhalothrin and tetramthrin and dimethoat in present of different salt solution of ($Cocl_2$, NaCl, KCl, $CaCl_2$, $MgCl_2$ and $MnCl_2$). Castor leaves discs treated with LC_{50} concentration of tested insecticides that prepared in distilled water containing 0.01,1,100 ppm of each salt solution. Number of dead larvae per each replicate was counted after 72 hr. of treatment. Mortality percentage was calculated and correcting for natural death according to Abbott equation (Abbott, 1925).

5. Enzyme preparation and AChE activity:

Fourth instar larvae of *S. littoralis* treated with LC_{50} of tested insecticides alone and with 100 ppm salt solutions of NaCl or $MgCl_2$, after 72hr. of bioassay test. One gm from treated and untreated larvae were homogenized in ice cold 40 mM Tris-HCl (pH 7.4) for 50 sec. then the homogenates were centrifuged at 5000 rpm for 15min at 4 ºC. The resulting supernatants were filtered and recentrifuged at 10,000 rpm for 30 min. The resulting supernatants were stored at (-20 ºC) for used as enzyme source. AChE activity determined according to method reported by (Ellman *et al.*, 1961), in total volume of three ml, 100µl of .01 M 5,5 dithio bis-(2-dinitrobenzoic acid) (DTNB) dissolved in 0.1 M phosphate buffer pH 7.4 , 30µl of 0.075 M acetylthiochoholine iodide (ATChI) and 50 µl of enzyme. The reactions were incubating at 37 ºC for 15 min. enzyme activity is measured spectrophotometrically as λ 412nm. The enzyme specific activity was computed as (ΔO.D. λ_{412} /mg protein/min). Inhibition percentage (I %) of AChE activity was calculated as follows:

Inhibition % = [1- $SA_{T/}SA_C$] ×100, where SA_C is specific activity of the enzyme in the control and SA_T is specific activity of the enzyme in the treatment.

6. Enzyme preparation and ATPase activity:

One gram of treated and untreated of 4th instar larvae homogenized in 10 ml in solution (40 mM Tris–HCl, 320 mM sucrose, 1 mM EDTA, buffer pH 7.4). the homogenates were centrifuged at 5,000 rpm for 10 min at -4ºC. Supernatant was then recentrifuged at 17,000 rpm for 30 min at 4ºC. The formed pellets were resuspended in the buffer and stored at (-20 ºC) for use. Total ATPases activity was determined according to Koch *et al*. (1969), with slight modification by (Morshedy,1980) using Tris–HCl buffer instead of imidazole buffer. the enzyme source (100 µl) was mixed with a reaction mixture 850 µl contained 40 mM Tris–HCl pH 7.4, 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 5 mM ATP. The mixture was incubated for at 37 ºC 15 min. the reaction was stopped by adding

150 µl of TCA (30 % w/v). The hydrolysis Pi was measured according to (Taussky and Shorr, 1953) by adding 4 mL of fresh coloring reagent (5 g $FeSO_4$ in10 % Amm. Molybdate in 10 N H_2SO_4). The absorbance was measured at 750nm against blank using spectrophotometer. The enzyme activity was represented as inorganic (Pi µmole/mg protein/ h). Inhibition percentage of ATPases activity was calculated. The standard curve of Pi was made using KH_2PO_4 (concentrations from 10 to 100 µmol/ml). 4 ml of the coloring reagent was added to 1 ml of each concentration. The color was measured at 750 nm.

The protein content in prepared homogenates of larvae of *S. littoralis* was assayed spectrophotometrically by methods of (Lowry *et al*., 1951) using bovine serum albumin (BSA) as a standard protein.

7. Statistical analysis:

Data were subjected to one-way analysis of variance (ANOVA) using the IBM SPSS statistics version 25.0 software package. Mean separations were performed by Tukey-Kramer honestly significant differences (HDS) and the results were considered statistically significant when $P < 0.05$. The LC_{50}, their 95% confidence limits, slopes and (Chi) were calculated according to (Finney, 1971) using computerize Ldp-line program.

Results and discussion

1. Toxicity of tested insecticides against 4th larvae of *Spodoptera littoralis*:

Toxicity results of tested insecticides expressed as LC_{50} values are given in (Table, 1). Lambda was the highest toxic compound against fourth instar larvae of *S. littoralis* followed by lufenuron and dimethoate after 24hr. exposure period LC_{50} values was 0.041, 0.05 and 0.78%. The LC_{50} values after 48hr. was 0.0 19, 0.033 and 0.053% for lufenuron, lambda-cyhalothrin and tetramthrin and dimethoate. After 72hr. lufenuron was the highest effective insecticide followed by lambda-cyhalothrin and tetramthrin but dimethoate was the least effective compound LC_{50} values was 0.001, 0.021 and 0.046%, respectively. Furthermore, the confidence limits of LC_{50}, s after 72 hr. were not overlapped. Slopes of Ldp lines were greater for all insecticides except from lufenuron, which show a low slope in its Ldp lines1.8, 1.0, 0.9 after 24, 48,72hr. exposure period these low slopes probably reflect the heterogeneity of response to the lufenuron in these population. These finding agree with Maqsood *et al*. (2016) reported, lufenuron proved the most effective insecticide against *S. lituraiis* followed by chloropyrifos, spinethylin, acrinathrin, gamma cyhalothrin, emamectin benzoate, thiodicarb and flubendiamide. Also, Bakr *et al.* (2013) found *S. littoralis* was most susceptible to IGRs, chitin synthesis inhibitors lufenuron than molting hormone agonist tebufenozide. Lufenuron was the highest effective insecticides against the 2nd instar larvae of *S. littoral is* followed by methomyle and diple 2x (*Bacillus thuringiensis*) (Abdel-Aal and El- Shikh, 2012).

Table (1): Toxicity of tested insecticides against 4th instar larvae of *Spodoptera littoralis*.

Insecticides	Time exposure (hrs.)	LC_{50} (%) [a]	Confidence limits (%) 95%		Slope[b] b ± S.E	χ^{2c}
			lower	Upper		
Lufenuron	24	0.050	0.041	0.063	1.80±0.21	**0.01**
	48	0.019	0.012	0.028	1.00±0.18	**2.44**
	72	0.001	0.0009	0.002	0.91±0.11	**5.20**
lambada-cyhalothrin and tetramthrin	24	0.041	0.039	0.043	6.72±1.43	**2.14**
	48	0.033	0.028	0.035	8.66±1.76	**5.10**
	72	0.021	0.019	0.023	3.50±0.25	**1.83**
Dimethoate	24	0.078	0.066	0.107	2.79±0.55	**0.62**
	48	0.053	0.049	0.058	4.28±0.55	**2.26**
	72	0.046	0.043	0.049	5.23±0.56	**0.04**

a The concentration that causes 50% mortality.
b Slope of the concentration–inhibition regression line ± standard error

2.Toxicity of tested insecticides in the presence of different cation concentrations:

All concentrations of cations have no effect on fourth instars larvae of *S. littoralis* after 72h exposure period. Sodium, Potassium and Cobalt decrease the toxicity of lufenuron to *S. littoralis*. Sodium chloride at 100 ppm significantly decreases lufenuron toxicity, the mortality decreases from 50% to 10% ($p < 0.05$). Calcium and manganese chloride did not significantly change the lufenuron toxicity. Magnesium chloride decreases the lufenuron toxicity at concentration 0.01 ppm, the mortality decreased to 30% (Figure, 1a). Sodium chloride reduces lambda-cyhalothrin and tetramthrin toxicity in significant at all tested concentrations. Cobalt, potassium and manganese chloride had no significant. Conversely, 0.01, 1 ppm magnesium and calcium chloride led to significantly decreased of lambda-cyhalothrin and tetramthrin toxicity (Figure, 1b). Cobalt and manganese chloride did not affect of dimethoate toxicity. Sodium and potassium chloride at 1, 100 ppm achieved significantly lessens on dimethoate toxicity. While, magnesium and calcium chloride are lessening dimethoate toxicity at 0.01ppm. This suggests that sodium chloride had categorically decrease toxicity of all tested insecticides at concentrations 1 and 100 ppm and magnesium chloride decreased insecticides toxicity at 0.01 ppm (Figure, 1c). The present results agreement with which found before that salinity decreased toxicity of beta-cypermethrin, acephate, temephos and atrazine (Wang *et al*., 2013; Huang and Brattsten, 2007 and Hall *et al*., 1994). Under isosmotic conditions, less mortality was observed in compared with hyperosmotic conditions (Song and Brown. 1998). While increases in the salinity led to a significant increase in the toxicity of insecticides toxicity (El-Alfy *et al*., 2001). Magnesium divalent cation have been found to reduce the toxicity of OP or pyrethroides and reduced mortality. It therefore gives ca-antidote to treat pesticide-poisoning (Pajoumand *et al.*, 2004; Singh *et al*., 1998 and Ajilore *et al*., 2018). Existence calcium reduced the toxicity of Deltamethrin. Ca^{+2} is known to be the antagonist of the nervous excitation or the phosphorylation inhibition or modification of the receptivity of the sodium channels which is the main known target of pyrethroids (Ghillebaert *et al*., 1996 and Matsumura, 1987). Therefore, cations levels can interfere with insecticides and increase or decrease its toxicities.

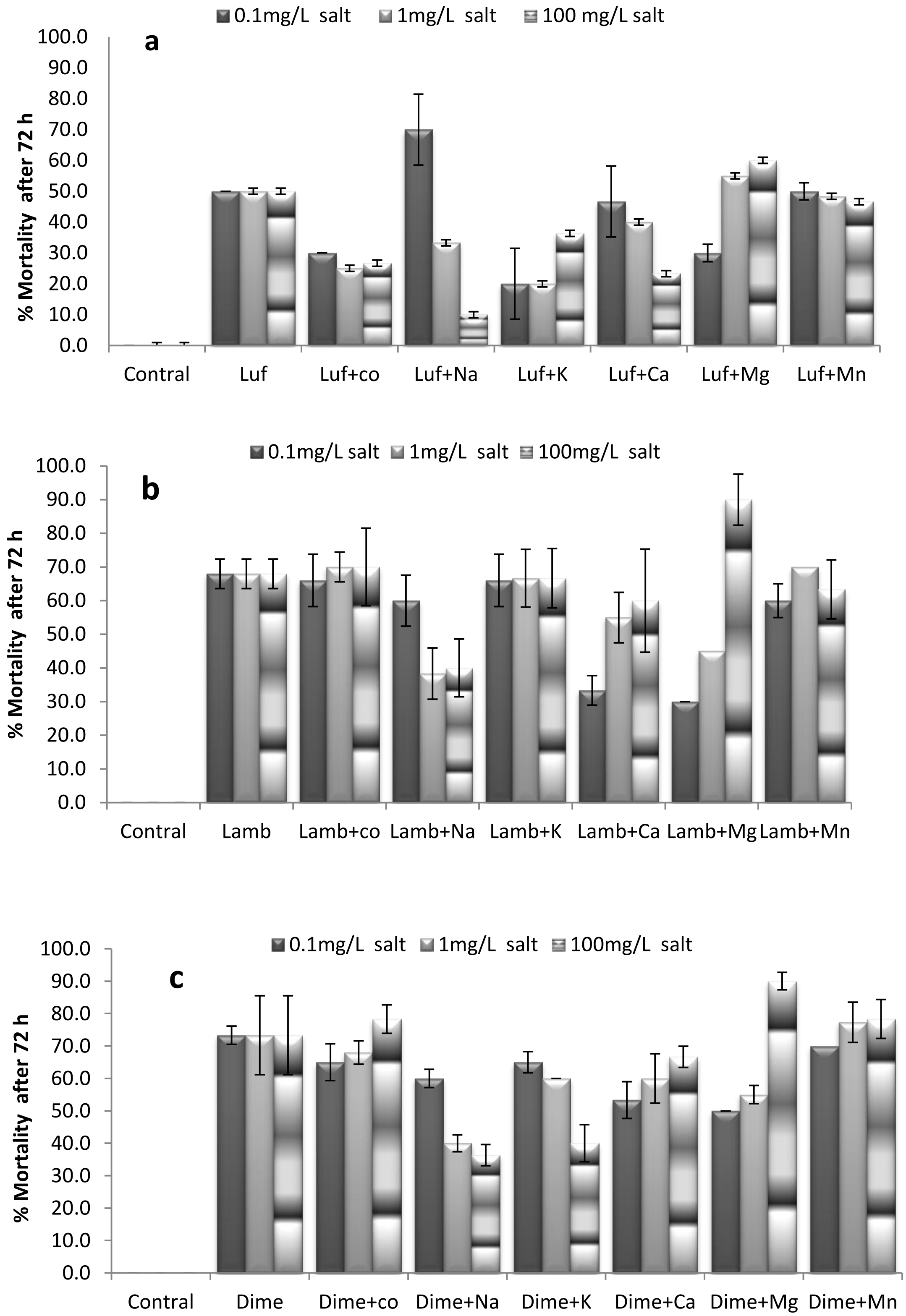

Figure (1): Effect of three concentrations of different cations on the potency of lufenuron lambda-cyhalothrin and tetramthrin and dimethoat against 4th instar larvae of *Spodoptera littoralis.*

3. Biochemical studies:

The specific activity and inhibition percentage of both total ATPase and AChE in case of insecticides in present and absent of sodium and magnesium chloride summarized in (Table, 2). The presence of sodium and magnesium ions with the insecticides reduces the inhibition of ATPase caused by those insecticides. Sodium and magnesium chloride decrease lufenuron inhibition from 67.55% to 20.20 and 50.78%. As well as, ATPase inhibition achieved highly significant between lambda-cyhalothrin and tetramthrin in absent or present both cations. Sodium and magnesium decrease enzyme inhibition from 64.06% to 29.14, 42.22%. Applied dimethoate with sodium or magnesium cations decreases ATPase inhibition approximately half less than dimethoate alone. Sodium and magnesium cations show a decrease of ATPase inhibition, which due to the acting mechanism of cations as a cofactor or as a ligand or effect proteins through a variety of mechanisms (Clark, 1958). This finding is like the earlier reports, Magnesium reactivates the membrane Na^{+}-K^{+}-ATPase and antagonist the direct toxic inhibitory effect of organophosphates on Na^{+}\ K^{+}-ATPase (Kiss and Fazekas, 1983 and Ajilore *et al*., 2018). Sodium, potassium, calcium, cobalt, magnesium, manganese showed a decreased of ATPase inhibition (Shaker *et al*., 1987). The presence of some salts such as Sodium and Magnesium chloride may be equivalent to the shortage of those ions. Which, effect on membranes permeability due to exposure to insecticides and reduced their toxicity to the target pests.

Table (2): In vitro inhibition of acetylcholinesterase and total adenosine triphosphatase isolated from 4th instar larvae of *Spodoptera littoralis* by selected two cations on potency some insecticides.

Insecticide LC_{50} (%)	Total ATPase		AchE	
	Specific activity ± S.E*	Inhibation ±S.E(%)	Specific activity ±S.E**	Inhibation ± S.E(%)
Control	14.80 ± 3.80^{a}	00.00 ± 0.00^{b}	$9.76\times10^{-3}\pm2.07\times10^{-3\,a}$	00.00 ± 0.00^{c}
Sodium chloride	7.37 ± 0.56^{b}	50.18 ± 3.81^{a}	$5.77\times10\text{-}^{3}\pm5.40\times10^{-4\,ab}$	40.87 ± 5.52^{a}
Magnesium chloride	7.70 ± 0.52^{b}	47.96 ± 3.54^{a}	$6.77\times10\text{-}^{3}\pm3.50\times10^{-4b}$	30.60 ± 3.57^{b}
Lufenuron	4.80 ± 0.13^{b}	67.55 ± 0.87^{a}	$3.10\times10^{-3}\pm3.32\times10^{-4c}$	68.21 ± 6.47^{a}
lufenuron+ sodium chloride	11.81 ± 1.61^{a}	20.20 ± 10.84^{b}	$8.34\times10^{-3}\pm1.03\times10^{-3\,a}$	14.53 ± 10.59^{c}
lufenuron+magnesium chloride	7.29 ± 0.99^{b}	50.78 ± 6.69^{a}	$6.22\times10^{-3}\pm3.86\times10^{-6b}$	36.22 ± 0.04^{b}
Lambada	5.32 ± 0.36^{c}	64.06 ± 2.40^{a}	$3.81\times10^{-3}\pm8.89\times10^{-4b}$	60.99 ± 9.10^{a}
lambada+ sodium chloride	10.49 ± 0.56^{a}	29.14 ± 3.79^{c}	$5.99\times10^{-3}\pm2.60\times10^{-4a}$	46.95 ± 6.82^{ab}
lambada+magnesium chloride	8.55 ± 0.27^{b}	42.22 ± 0.71^{b}	$5.92\times10^{-3}\pm2.61\times10^{-4\,a}$	39.30 ± 2.67^{b}
Dimethoate	4.88 ± 0.19^{b}	63.70 ± 4.51^{a}	$3.86\times10^{-3}\pm6.17\times10^{-5\,c}$	60.42 ± 0.63^{a}
Dimethoate + sodium chloride	9.73 ± 1.69^{a}	34.22 ± 1.71^{b}	$4.62\times10^{-3}\pm3.31\times10^{-b}$	56.03 ± 0.00^{b}
Dimethoate +magnesium chloride	9.27 ± 3.10^{a}	37.35 ± 11.41^{b}	$7.10\times10^{-3}\pm1.98\times10^{-3a}$	27.61 ± 1.16^{c}

*** Specific activity (Pi µmole/mg protein/ hr). ** Specific activity (ΔOD/mg protein/min).**

Means followed by the same letter are not significantly different (Tukey test, $p < 0.05$).

The highest AChE inhibition 68.21% was found with lufenuron. However, sodium and magnesium chloride decreased this inhibition to 14.53, 36.22%, respectively. Magnesium was the most cation decreases inhibition of lambda-cyhalothrin and tetramthrin from 60.99% to 46.95% followed by sodium 46.95%. It can be noted that highest enzyme inhibition with dimethoate was 60.42%. The enzyme inhibition decreased to 27.61 % when magnesium chloride applied with dimethoate, sodium decreased enzyme inhibition unremarkable. Magnesium was most effective than sodium in reducing enzyme inhibition achieved by tested insecticides (Table, 2). These results are agreement with (Shaker *et al*., 1987) AChE Inhibition may be explained by its ability to inhibit acetylcholine and to antagonize the effects of insecticide (Pajoumand *et al*., 2004). OPs insecticides react with AChE by nucleophilic reaction of the serine hydroxyl functional group. Magnesium divalent cation is combined with serine hydroxyl group reduces the pKa of serine gives higher basicity to serine that in turn results in decreased reactivity and nucleophilicity of the serinic hydroxyl group for nucleophilic reaction with OPs and hence favors the reaction of serine with OP, resulting in a more polar extractable reaction product (Shetab-Boushehri *et al*., 2012). Also, Smissaert (1981) explanted that, activation of Acetylcholinesterase by Monovalent Na^+ by associated specific binding of the Na ions with the anionic subsite of the catalytic center reduces the reactivity of the (AChE). Low ionic strength, monovalent (Na^+, K^+) and divalent ($Ca2^{+,}$ $Mg^{2+)}$ metal ions enhanced the AChE enzymatic activity (Hofer *et al*., 1984). The activity of AChE was improved by monovalent and divalent cations whereby the activation caused by second group is much greater than caused by first group (Nachmansohn, 1940). This explains why magnesium chloride reduces AchE inhibition caused by both lambda-cyhalothrin and tetramthrin, dimethoate greater than reduced caused by sodium chloride.

In conclusion, this study results indicated that several actions play an essential role in the biochemical characteristic of living organisms. Cations interfere with enzymes activity i.e., acetylcholinesterase and adenosine triphosphatase activity. This may indicate that most cations are present in the cell body in a certain balance concentration and change of these balance equilibrium causes a toxic effect to the cell. Cations interfere with pesticide actions by effect their conjugations manner occur more harmful effect to non-target or decrease their effect by causing insensitivity to the pesticide targets at a certain concentration. It is clear from the above using agriculture contaminated water in preparing and dilution of pesticides will affect its potency in controlling different pests in the field applications. Subsequently, Government constructs more recycling drainage water stations before directing it back into the Egypt's irrigation network for reuse in agriculture.

It is concluded that Tthe toxicity of lufenuron, lambda-cyhalothrin, tetramethrin, and dimethoate against cotton leafworm *S. littoralis* has been seriously affected by presence of cation in water that used in insecticides dilution. Sodium and magnesium chloride had categorically decreased the toxicity of lufenuron, lambda-cyhalothrin and tetramthrin, and dimethoate. Sodium counteracts effects insecticides with significant decrease in the levels of ATPase inhibition. Magnesium decreases the levels of AChE inhibition. Therefore, cations showed a very discontinuous effect due to their interference with the site of action of these pesticides.

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