

#### **Research Article**

# Insecticidal potential of some Acephate derivatives and their quantitative structure-activity relationship (QSAR)

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Abstract: Organophosphates (OPs), one of the most important pesticide groups, are used worldwide to control pests. Acetylcholinesterase (EC 1.14.18.1) (AChE), an enzyme from insects' nervous systems, is the leading target site of this group of pesticides, such as Acephate. Inhibition of enzyme activity through Acephate-derived compounds can control both resistant and non-resistant pests to OPs. In this research, the toxicity of these compounds was assayed regarding the control of Xanthogaleruca luteola (Muller). Results of the in vivo screening test revealed that two derived compounds of phosphorhydrazides (PHA) (i.e., NH<sub>2</sub>-C(O) NH-NH P(O)(OC<sub>6</sub>H<sub>5</sub>) and OC<sub>4</sub>H<sub>3</sub>-C(O)NH-NHP(S)(OCH<sub>3</sub>)<sub>2</sub>) showed the most significant insecticidal potential. AChE was purified and isolated from the third instar larvae of elm leaf beetle, X. luteola, using affinity chromatography. IC<sub>50</sub> values, inhibition mechanisms, and inhibitory constant (Ki) of NH<sub>2</sub>-C(O) NH-NH P(O)(OC<sub>6</sub>H<sub>5</sub>) and  $OC_4H_3$ -C(O)NH-NHP(S)(OCH\_3)<sub>2</sub> as inhibitors were calculated for the purified AChE. These compounds inhibited acetylcholinesterase (AChE) and general esterases of third instar larvae of elm leaf beetle. These compounds, by mix inhibition mechanism, inhibited AChE enzyme, and K<sub>i</sub> obtained was 1.16 and 0.88  $\mu$ M<sup>-1</sup> min<sup>-1</sup> for NH2-C(O) NH-NH P(O)(OC6H5) and OC4H3-C(O)NH-NHP(S)(OCH3)2, respectively. QSAR study based on multiple linear regressions (MLR) and principal component analysis (PCA) showed that the non-descriptor net charge of the nitrogen atom influenced by the polarization of N-H group had the most significant effect on the insecticidal potential. Therefore, designing new compounds that control the N-H polarization of the nitrogen atom could be an excellent option to study insecticidal properties of Acephate-derived compounds.

Keywords: QSAR study, Xanthogaleruca luteola, insecticide derivatives, phosphoramidate

#### Introduction

Today, a wide range of agricultural products are being lost due to pests around the world. At the

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same time, a lot of effort is required to provide enough food for the growing human population. Although pesticides, biological control, and genetically manipulated plants can be mentioned as appropriate solutions for pest (Rechcigl and Rechcigl, 2000). control pesticides are still the main tools used for pest control (Casida and Quistad, 2004). Discovery and registration of new pesticides are lengthy

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and costly processes. Large numbers of molecules with the toxic ability to control pests are removed through screening programs. The safety of a limited number of these compounds can be evaluated by performing numerous tests to introduce them to the pesticide market (Sparks, 2013). A quantitative structure-activity relationship (QSAR) study can reveal the relationship between chemical structures and insecticidal properties. It can propose various strategies to reduce the direct costs of these materials (Benfenati, 2007). So far, some researchers have used QSAR to discover new pesticides, including Acetamiprid (Yamada et al., 1999), Thiamethoxam (Maienfisch et al., 1999), Etoxazole (Suzuki et al., 2002), spinosad (Thompson et al., 2001), Indoxacarb (Lahm et al., 2001), Sulfoxaflor (Zhu et al., 2011) and organophosphorus derivatives (Mudasir et al., 2013). The acetylcholinesterase, AChE, (EC 3.1.1.7), is one of the most important enzymes in the insect nervous system. Organophosphate (OP) and carbamate pesticides inhabit this enzyme and lead to permanent nerve stimulation, vibration, and insect death (Lee et al., 2015; Zolfaghari et al., 2019). Many types of research have been focused on the purification and characterization, and inhibition of this enzyme by different inhibitors in Apis mellifera L. (Guilbault et al., 1970), Schizaphis graminum Rondani (Gao and Zhu, 2001), Bombyx mandarina Moore (Lang et al., 2010) and Cimex letularius L. (Hwang et al., 2014).

OP insecticides have a wide range of different physical, chemical, and biological properties. In this research, due to these compounds' diverse and unique properties, the toxicity of Acephate-derived compounds (phosphorhydrazides (PHA)), as the best Acephate-derived compounds, was screened in the third instar larvae of elm leaf beetle. After that, AChE of the third instar larvae was purified, and the mechanisms of its inhibition under the influence of PHA compounds were studied. Finally, the QSAR models, in addition to the relationship between structure and insecticidal properties of PHA compounds, were evaluated to design new compounds with more toxicity on X. luteola.

#### **Materials and Methods**

#### Chemicals

serum Triton X-100. bovine albumin. acetylthiocholine iodide (ATCH), 5.5dithiobis-2-nitrobenzoic acid (DTNB), alphabeta-naphthyl naphthyl acetate (α-NA), acetate ( $\beta$ -NA), fast blue RR salt, Procainamide hydrochloride, Dimethyl sulfoxide (DMSO), Na<sub>2</sub>HPO<sub>4</sub> (99%), NaH<sub>2</sub>PO<sub>4</sub> (99%), Sodium dodecyl sulfate (SDS) and N-ethoxycarbonyl-2-ethoxy-1,2dihydroquinoline (EEDQ) were all from Sigma-Aldrich and Methanol was from, Merck. The ECH Sepharose 4B was Amersham Pharmacia purchased from Biotech. Tetraethylammonium iodide (NEt4I) was purchased from Aldrich Chemical Company.

#### Synthesis

The synthesis of resveratrol analogs of Acephate has been reported previously (Asadi *et al.*, 2016; Gholivand *et al.*, 2016).

#### Sample preparation and Bioassay

larva and adults of X. luteola were collected from infected elm trees in the University of Guilan and then reared at least for three generations in the laboratory conditions (25  $\pm$ 2 °C with 16: 8 light: darkness and 60-70% relative humidity) (Bashari et al., 2014). In the beginning, for monitoring the toxicity of compounds, 5000 mg/l of each combination as selected concentrations were prepared using DMSO:water (1: 4 v/v), and third instar larvae were immersed in a prepared solution for 15 seconds. The mortality of tested insects was calculated after 24 h (Chauhan et al., 2013). According to the preliminary tests, four derived compounds (i.e., 5, 8, 11, and 12 in table 1) were selected for bioassays that could make more than 80% of mortality. Four concentrations (i.e., 310, 650, 1250, and 2500 mg/l) were applied to calculate each compound's LC50 values, which were estimated using POLO-PC software (LeOra, 1987).

NO.	Compounds	Mortality (%)	Mortality	IC <sub>50</sub>	IC <sub>50</sub>	Ref.
		(5000 mg/l)	(%) (2500 mg/l)	insect (mg/l)	Human (mg/l)	
1	NH-NHP(O)(OC <sub>6</sub> H <sub>2</sub> ) <sub>2</sub>	60	5	42.03	7.006	(Asadi et al., 2016)
2	NH <sub>2</sub> C(O)NH-NHP(O)(Cl)(OC <sub>6</sub> H <sub>5</sub> )	60	5	69.4	9.886	(Asadi et al., 2016)
3	NH <sub>2</sub> C(O) NH-NH P(O)(Cl-OC <sub>6</sub> H <sub>4</sub> )(Cl)	20	-	68.65	2.831	(Asadi et al., 2016)
4	NH <sub>2</sub> C(O) NH-NH P(O)(C <sub>6</sub> H <sub>5</sub> )(Cl)	5	-	72.65	5.370	(Asadi et al., 2016)
5	NH <sub>2</sub> -C(O) NH-NH P(O)(OC <sub>6</sub> H <sub>5</sub> )	100	90	15.02	4.020	(Gholivand et al., 2016)
6	NH <sub>2</sub> -C(O) NH-NH P(S)(OC <sub>2</sub> H <sub>5</sub> ) <sub>2</sub>	5	-	35.78	7.140	(Gholivand et al., 2016)
7	NH <sub>2</sub> -C(O) NH-NH P(S)(OCH <sub>3</sub> ) <sub>2</sub>	40	-	43.90	2.340	(Gholivand et al., 2016)
8	NH <sub>2</sub> -C(S) NH-NH P(S)(OC <sub>2</sub> H <sub>5</sub> ) <sub>2</sub>	80	20	65.39	35.52	(Gholivand et al., 2016)
9	C <sub>2</sub> H <sub>5</sub> -NHC(S)NH-NHP(S)(OCH <sub>3</sub> ) <sub>2</sub>	75	10	165.00	127.10	(Gholivand et al., 2016)
10	C <sub>2</sub> H <sub>5</sub> -NHC(S)NH-NHP(O)(C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub>	50	-	207.23	119.84	(Gholivand et al., 2016)
11	C <sub>6</sub> H <sub>5</sub> -NHC(S)NH-NHP(O)(C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub>	80	30	130.19	110.88	(Gholivand et al., 2016)
12	OC <sub>4</sub> H <sub>3</sub> -C(O)NH-NHP(S)(OCH <sub>3</sub> ) <sub>2</sub>	100	75	24.84	128.22	(Gholivand et al., 2016)
13	$OC_4H_3$ - $C(O)NH$ - $NHP(O)(OC_6H_5))_2$	65	5	217.18	118.21	(Gholivand et al., 2016)

**Table 1** Toxicity of Acephate derivative compounds on last instar larvae of *Xanthogaleruca luteola* (*in vivo* test) and their half-maximal inhibitory concentration ( $IC_{50}$ ) on AChE activity.

#### **Enzyme assays**

Survived larvae, treated with diffident concentrations of selected compounds, were collected and transferred to the freezer (-80 °C) to be used as a source of enzymes. Then treated larvae were homogenized in cold phosphate buffer containing 0.1% Triton X-100 using a hand-held glass homogenizer and centrifuged at 13,000 rpm for 15 min at 4 °C. The supernatants were used for subsequent analyses. The AChE and esterase activities were measured according to the methods proposed by Ellman *et al.*, 1961; Van Aspern, 1962).

AChE activity was determined at room temperature in phosphate buffer (50 mM; pH 7.0) in the presence of DTNB and acetylthiocholine iodide (ATChI) as the substrate. The supernatant (40  $\mu$ l) was added to a tube containing 140  $\mu$ l of the buffer and 20  $\mu$ l of DTNB and 40  $\mu$ l ATCH. The concentration of thiocholine obtained from the catalyzed reaction was measured by the method according to Elman *et al.* (1961). Absorbance was measured at 412 nm with a Microplate Reader Model Stat Fax® 3200 (Awareness Technology Inc.). For esterase assay, 12.5  $\mu$ l of supernatant was mixed with

112  $\mu$ l phosphate buffer (pH 7.0), 25  $\mu$ l substrate, and 50  $\mu$ l fast blue RR salt (1 mM). The increase in absorbance was recorded kinetically at 450 and 540 nm due to the formation of  $\alpha$ -naphthol and  $\beta$ -naphthol, respectively.

#### **Enzyme Purification**

The synchronized third instar larvae were homogenized in 50 mM phosphate buffer, pH 7.0, containing 0.1% Triton X-100.

The homogenate was centrifuged twice at 13000 rpm for 30 min. The supernatant was used as the source of enzyme for purification purification. Enzyme was carried out using the method of Gao and Zhu Zhu, (Gao and 2001) with minor modification (Sharifi et al., 2016).

#### Mechanism of enzyme inhibition

To investigate the mechanism of inhibition, the effects of  $IC_{50}$  and  $IC_{25}$  concentrations of each inhibitor were investigated on the purified enzyme, and then kinetic parameters were determined. The impact of these two inhibitory concentrations on the purified AChE, measured  $K_m^{app}$  and measured  $V_{max}^{app}$ were conducted using different substrate concentrations (10, 5, 2.5, 1.25 and 0.625)

mM). The inhibitory constant, K<sub>i</sub>, and linear equation inhibitory constant were calculated separately for a combination of  $K_m^{\ app}/\ V_{max}^{\ app}$ versus every concentration of inhibitors (Eisenthal and Danson, 2002). The effects of different concentrations of inhibitors were surveyed on the purified AChE activity. AChE activity was measured, as described above in the enzyme assay section, after 15 min of preincubation of the enzyme in different concentrations of inhibitors at room temperature. The IC<sub>50</sub>s and 95% confidence limits were determined by probit analysis POLO-PC (LeOra software using the Software. 1987). Activities of AChE in the presence of IC<sub>25</sub> and IC<sub>50</sub> concentrations of inhibitors were determined at the different concentrations of ATChI in addition to the fixed concentrations of DTNB. The  $K_m$  and V<sub>max</sub> values and inhibitory constant, K<sub>i</sub>, with and without inhibitors were estimated from Lineweaver-Burk plots.

#### Statistical analysis

LC<sub>50</sub> values and 95% confidence intervals were calculated from probit regressions using the POLO-PC computer program. Data were analyzed using one-way analysis of variance (ANOVA) (SAS Institute, 2002) by Tukey's test when the probability  $p \le 0.05$ . To identify the effect of physicochemical parameters on the AChE inhibition activity, QSAR equation was performed according to the method described by Hansch and Fujita (Hansch and Fujita, 1964). The stepwise multiple linear regression procedures (MLR method) were performed to select the descriptors by the software package SPSS 16.0. The electronic and structural descriptors were obtained by either quantum chemical calculations or theoretical and experimental studies. The electronic descriptors or; consisted of the energy of the frontier orbital (E<sub>HOMO</sub> and  $E_{LUMO}$ ), electrophilicity ( $\omega$ ), polarizability (PL, the charge difference between the atoms in functional groups) and the net atomic charges (Q). Furthermore, hydrophobic coefficient (logP), dipole moment ( $\mu$ ), and

molecular volume (Mv) was used as the structural descriptors. The toxicity of phosphorhydrazide analogues is expressed in  $\log (1/IC_{50})$  as an anticholinesterase activity. The descriptor values are related to toxicity using MLR analysis. MLR of descriptors selected to biological activity gives rise to the problem of multicollinearity. This problem can be solved by using the principal component analysis (PCA). These linear combinations form a new set of variables, main components (PCs), mutually orthogonal. The first PC contains the greatest variance, and the second new variable includes the second largest variance, and so on. The variable selection in this PCA study was performed by using Fisher's weights. The descriptors with higher correlation coefficient and lower correlation ( $|\mathbf{r}| < 0.5$ ) to log (1/IC<sub>50</sub>) were selected to carry out stepwise MLR analysis and to optimize the QSAR equation (Schuurmann et al., 2008). All quantum chemical calculations were carried out by using the Gaussian 03 program package (Frisch et al., 2005).

#### Results

### Screening of acephate-derived compound toxicities on *X. luteola*

In the current research, the insecticidal efficiency of Acephate-derived compounds against *X. luteola* is presented in tables 1 and 2.

The screening test results showed that different Acephate-derived among compounds, two compounds (i.e., numbers 5 and 12) have favorable insecticidal efficiency and inhibitory effect against AChE. Hence, bioassay tests were performed on the target pest using two mentioned compounds. Results of bioassays showed that compound 5 more insecticidal efficiency had than compound 12.

The activity of AChE and some detoxified enzymes such as alpha- and beta-esterase were assayed on the survived larvae after 24 hours of exposure to each concentration of selected compounds (Figures 1 and 2).

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**Table 2** Susceptibility of last larval instar of *Xanthogaleruca luteola* to Acephate derivative compounds 24 h after exposure.

L C velue	Compounds										
LC value	5	12	Acephate								
LC <sub>50</sub>	886.11	1221.09	142.01								
(mg/l) 95% confidence limits	(693.83-1133.44)	(947.16-1711.17)	(98.66-199.64)								
Slope $\pm$ Standard error	$2.76\pm0.09$	$1.25 \pm 0.12$	$1.722 \pm 0.15$								



**Figure 1** Inhibition percent of AChE from third instar larvae of *Xanthogaleruca luteola* treated with different concentrations of compounds #5 and 12.

\*Different letters (a–d) indicate significant differences in relative activity (Tukey's test, P < 0.05).



**Figure 2** Inhibition percent of  $\alpha$ - (A) and  $\beta$ - esterase (B) from third instar larvae of *Xanthogaleruca luteola* treated with different concentrations of compounds # 5 and 12.

\*Different letters (a–d) indicate significant differences in relative activity (Tukey's test, P < 0.05).

As shown in figure 2, inhibition of AChE picked up significantly with increasing concentration of compounds. Compound No. 5 showed higher inhibitory potential on purified AChE than compound No. 12 (Table 3).

Purification of AChE of the third instar larvae of X. luteola is the first step to study and determine the type of inhibitory mechanism of the enzyme (Sharifi et al., 2016). In the second step, calculations of  $IC_{50}$ and IC<sub>25</sub> values of selected compounds, as inhibitors, on the purified AChE were performed, and kinetic parameters were determined for each of them by Hyper software. Furthermore,  $K_i$  was evaluated for each chosen compound concerning the kinetic parameters (Table 4).

#### **QSAR** analysis

The biological activity of phosphorhydrazide (PHA) compounds and their physicalchemical properties, log (1/IC<sub>50</sub>) value are considered as dependent variables. However, the quantum chemical properties are considered as an independent variable. The relationships between dependent and independent variables were used to design QSAR equation (Table 5).

QSAR equation is presented in the following steps according to descriptors as obtained by related software.

(1)

$$\begin{split} p(IC_{50}) = & 4.10 \pounds Q_{\mu} + 168.45 | Q_{N(\mu)} + 0.27 \& Q_{N(\beta)} - 0.29 \Im PL_{p=X} + 51.3852 PL_{\lambda \in H(\alpha)} \\ & + 11.479 PL_{\lambda \sim H(\beta)} + 60.500 \pounds_{HOMO} + 84.542 \pounds_{LUMO} + 0.89 \& 0 - 0.289 \mu + 0.89 \& Logp + 0.005 M \sim 82.98 \mu + 0.89 \& Logp + 0.80 \& Logp$$

 $n = 13; R^2 = 0.863; S_{reg} = 0.47; F_{statistic} = 0.573; r = 0.721$ 

**Table 3** Inhibitory effect ( $IC_{50}$  values) of different inhibitors on crude extract and purified AChE from *Xanthogaleruca luteola*.

Compound	IC <sub>50</sub> Crude (mg/l) 95% confidence intervals	IC <sub>50</sub> Purified AChE (mg/l) 95% confidence intervals
Acephate	5.85 (4.76-8.46)	1.93 (0.91-3.04)
5	15.02 (12.34-16.41)	5.75 (3.81-6.71)
12	24.84 (26.12-36.56)	9.14 (6.14-10.05)

Table 4	Effect	of $IC_2$	5 and	IC <sub>50</sub>	of Aceph	ate c	derivatives	on	AChE	kinetic	parameters	purified	from	Xantho	ogaleruca
luteola.															

Compound	Concentration (ppm)	${{K_m}^{app}}$ $\mu M$	V <sub>max</sub> <sup>app</sup> μmol/min/mg protein	K <sub>i</sub> μM <sup>-1</sup> min <sup>-1</sup>	Inhibition type	
Control	-	$60.19 \pm 1.32$	$52.40 \pm 1.18$	-	-	
Acombata	$I_{25} = 0.65$	$125.01 \pm 2.95$	$17.65 \pm 2.91$	$6.02 \pm 0.51$	Mixed	
Acephate	$I_{50} = 2.30$	$74.99 \pm 2.81$	$8.02 \pm 1.46$	$0.93 \pm 0.51$	0.51 Mixed	
5	$I_{25} = 1.21$	$102.10\pm3.56$	$41.86 \pm 2.20$	$1.162 \pm 0.16$	Mixed	
5	$I_{50} = 5.98$	$84.75\pm2.84$	$16.43 \pm 1.87$	$1.102 \pm 0.10$	WIIXCu	
12	$I_{25} = 2.85$	$142.75 \pm 2.35$	$33.79 \pm 1.35$	$0.88 \pm 0.24$	Minad	
12	$I_{50} = 7.94$	$97.38\pm2.58$	$12.06 \pm 1.22$	0.88 ± 0.24	Mixeu	

Table 5 Quantum-chemica	l and theoretical	descriptors for	<sup>c</sup> compounds	s computed	by Gaussian	03 program
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No	Electron	Electronic								Hydrophobic		Steric	log (I/L.)
INO.	Qp	$Q_{N\left(\alpha\right)}$	$Q_{N\!\left(\beta\right)}$	P <sub>P-X</sub>	$P_{N\text{-}H(\alpha)}$	$P_{\text{N-H}(\beta)}$	EHOMO	ELUMO	ω	μ	logP	Mυ	$-\log(1/150)$
1	2.35	-0.829	-0.672	-3.45	1.228	1.239	-0.244	-0.019	0.07	6.886	0.04	126.643	-1.62356
2	2.16	-0.812	-0.495	-3.21	1.268	0.903	-0.268	-0.039	0.10	8.146	0.19	150.991	-1.84136
3	2.16	-0.812	-0.408	-3.21	1.238	0.904	-0.263	-0.044	0.10	6.926	1.03	179.817	-1.83664
4	1.98	-0.823	-0.496	-3.05	1.246	0.899	-0.280	-0.059	0.13	3.093	0.26	171.836	-1.86124
5	2.44	-0.80	-0.485	-3.48	1.214	0.854	-0.246	-0.028	0.08	7.067	1.37	245.822	-1.17664
6	1.95	-0.797	-0.486	-2.54	1.208	0.858	-0.231	-0.022	0.07	7.246	1.25	161.607	-1.55364
7	1.95	-0.797	-0.486	-2.54	1.209	0.858	-0.234	-0.022	0.07	7.007	1.35	127.581	-1.64246
8	1.97	-0.798	-0.450	-2.61	1.222	0.852	-0.238	-0.031	0.08	9.156	2.15	182.117	-1.81531
9	1.95	-0.792	-0.461	-2.60	1.215	0.859	-0.236	-0.029	0.08	8.664	3.12	145.030	-2.21748
10	2.04	-0.799	-0.463	-3.16	1.212	0.857	-0.235	-0.051	0.11	14.551	4.96	255.997	-2.47322
11	2.04	-0.796	-0.455	-3.15	1.210	0.855	-0.234	-0.053	0.11	12.077	6.63	286.695	-2.11691
12	1.95	-0.793	-0.447	-2.60	1.213	0.850	-0.255	-0.063	0.13	9.669	5.35	140.240	-1.3955
13	2.45	-0.796	-0.447	-3.58	1.219	0.850	-0.256	-0.064	0.13	11.011	7.42	199.641	-2.3378

Citing to the data of table 5, equation (1) is given by 12 variables and 13 molecules. In this equation, n represents the compound number, r correlation coefficient (optimal value  $0.5 \le r$ ),  $R^2$  determination coefficient,  $S_{reg}$  standard deviation (optimal value  $0.5 \le S_{reg}$ ), and F statistic Fisher coefficient. Equation (1) has an elevated determination coefficient ( $R^2$ ) and low standard deviation ( $S_{reg}$ ), suggesting that this equation might be favorable. However, other equation characteristics such as huge correlation coefficient and VIF > 10 showed a high correlation error for many non-dependent variables. As the Primary component's analysis (PCA) is innovated for reducing or choosing the best variables, (PCA) can be used to solve the problem (Table 6). Standard equation might be obtained by eliminating variables with the same unit and remote samples,. Final equation (2) was obtained with nine variables, with  $R^2 = 0.942$ ,  $S_{reg} = 0.381$  and VIF < 10. (2)

$$\begin{split} p(lC_{50}) &= 1.287 Q_{p} + 62.125 Q_{N(\alpha)} - 0.101 PL_{P-X} - 9.032 PL_{N-H(\alpha)} + 3.566 PL_{N-H(\beta)} + 32.187 E_{H0MO} - 20.881 E_{L0MO} - 0.041 \mu - 0.155 Logp + 20.24 \end{split}$$

n = 13;  $R^2 = 0.949$ ;  $S_{reg} = 0.194$ ,  $F_{statistic} = 4.657$ , r = 0.358

**Table 6** VIF<sup>a</sup> values of experimental and theoreticalQSAR equations.

Independent variables	Eq. 1	Eq.2	
$Q_{\mathrm{P}}$	55.052	1.260	
$Q_{\rm N(\alpha)}$	249.906	8.729	
$Q_{N(\beta)}$	2.733	-	
$PL_{P=X}$	102.043	6.283	
$PL_{N-H(\alpha)}$	129.159	8.233	
$PL_{N-H(B)}$	58.766	9.442	
E <sub>HOMO</sub>	856.231	5.857	
$E_{\text{LUMO}}$	854.956	7.456	
ω	674.882	-	
μ	347.843	7.158	
LogP	421.257	9.286	
Mv	13.653	-	

 $\frac{1}{a}$  VIF =  $1/(1 - R_i^2)$ ; where, Ri is the multiple correlation coefficient of the ith independent variable on all of the other independent variables.

#### Discussion

Over the recent years, mono phosphoramide (P(O,S)-NH-NH-C(O,S))hydrazides was synthesized that showed valuable properties such as anti-tumor, pesticide, anti-bacterial, anti-fungal in addition to acetylcholinesterase enzyme inhibitory effect. Despite the similarity of these categories of compounds, from the 3dimensional structure aspect to the primary skeleton of Acephate derivatives (P(O)-NH-C(O)), their insecticidal properties have not yet been studied (Gholivand et al., 2016). The screening results in the present work showed that out of more than 25 compounds of Acephate derivatives that were examined, thirteen had insecticidal activity between 5 and 95% (Table 1), the other compounds had no activity. In comparison, the compounds numbered 5 and 12 had the best insecticidal effect among the thirteen compounds. Results of bioassay tests confirmed that Acephate insecticide inhibited AChE activity more than that of the two mentioned compounds. According Aldridge's classification, to carboxylesterases belong to the group of βesterase. These enzymes have serine amino acids in their active site, inhibited by paraoxon (Vaughan and Hemingway, 1995). Selected compounds used in this research belong to phosphorus compounds, and these compounds are also able to inhibit esterase enzymes. Results showed a dose-response-related manner between concentrations of compound and reduction of esterase activity. Generally, esterases metabolize enzymes in the insect's body, which play a significant role in poisonous detoxifying materials (Jakoby and Habig, 1980). These enzymes hydrolyze the acetylthiocholine iodide as a substrate for AChE. Organophosphates can inhibit their activity. That is why IC<sub>50</sub> values are different when exposed to crude extract and purified AChE.

Tested synthesized compounds (i.e. No. 5 and 12) decreased V<sub>max</sub><sup>app</sup> values of purified AChE compared to the control; in contrast, vice versa, K<sub>m</sub><sup>app</sup> values significantly increased. These results suggest that type of inhibitory mechanism of AChE was due to mixed inhibitors. Results also showed that among different screened compounds, the toxicity of Acephate showed the highest  $K_{i}$ , which was 5.98- and 7.88-folds higher than that of compounds No. 5 and 12, respectively. However, between the two newly synthesized compounds (i.e., No. 5 and 12),  $K_i$  of compound No. 5 was 1.32-fold higher than compound No. 12. Thus, we can hope to explore a new insecticide by changing the structure of compound No. 5.

#### QSAR of X. luteola

The purpose of QSAR studies is to use the information of the quantitative property to understand the important qualitative properties affecting the biological function of compounds, for example, insecticidal activity, so the number of these compounds could vary in different equations (Clarancia et al., 2018; Gholivand et al., 2021). In equation 2, inhibition, the potential is by affected electron more descriptors.  $Q_{N(a)} = E_{HOMO} = E_{LUMO} = PL_{N-H(a)}$ , than by structural descriptors, log P >  $\mu$ . Comparing the correlation coefficients,  $Q_{N(a)}$  (+ 62.125) with intercept (+ 20.24) confirms that the nitrogen atom's net charge in the group  $Q_{N(a)}$  has the most influence the interaction of phosphorhydrazides on derivatives by acetylcholinesterase enzyme in the tested insect. This descriptor's symbol positive (+) suggests that the inhibition level increases by increasing the nitrogen atom's net charge. The lower intercept value than the independent variable coefficient in this equation means the same equation has chosen the favorable chemicalphysical descriptor. Understanding relations in this correlation matrix can be a way to make an internal communication between the compound's independent variables and the potential for AChE inhibition. In table 6, a regression coefficient more significant than 0.70 indicates a close relationship between variables. So, there is an internal relationship between  $Q_{N(a)}$  and  $PL_{N-H(a)}$ with r = -0.802 and  $PL_{N-H(a)}$  and  $E_{HOMO}$  with r = -0.912. Thus, the nitrogen atom's electron properties (a) affect the polarization of a link between nitrogen and in turn, hydrogen, and this link control molecular orbitals.

### Comparison of AChE-QSAR model in human and X. *luteola* enzymes

QSAR studies on purified AChE of humans indicated that priorities to electron variables influence than structural and polarization parameter  $PL_{N-H}$  and had been determined as the most effective chemical-physical property in Acephate derivatives on their inhibition potential (Gholivand et al., 2016). Order of influence of electron variables is expressed as  $Mv < \mu < Qc < Qp < E_{HOMO} < PL_{N-H}$ . The relationship between the internal matrix of this parameter and phosphorus atom's net charge, in the compound's addition to orbital characteristics, confirm that polarization of N-H quantitatively changes 'high control by phosphorus atom' net charge and molecular

orbital energy and shows little relationship with carbon atom' net charge (Eq. 3). (3)

$$\begin{split} \mathbf{p}(\mathrm{IC}_{50}) &= -0.646\,\mu + 3.477 Q_P + 1.378 Q_C + 57.272 P L_{N-H} - 25.476 E_{HOMO} \\ &- 0.033 M\nu + 67.019 \\ n &= 13; R^2 = 0.955; R_{Adj}^2 = 0.925; S_{reg} = 0.331; r = 0.20; \\ F_{\mathrm{statistic}} &= 31.970; q^2 = 0.955; P < 0.0001 \end{split}$$

Results of equations QSAR on AChE of *X. luteola* mentioned above shows that the most important descriptor with nitrogen atom's net charge and effects of other nondependent descriptors are  $Q_{N(a)} > E_{HOMO} > E_{LUMO} > PL_{N-H(a)}$ . The internal matrix of descriptors shows that the most dependence is between the nitrogen atom's net charge and  $PL_{N-H}$ . By comparing these two equations, we can infer that the same primary factors influence the human's acetylcholinesterase enzyme and the insects' acetylcholinesterase enzyme.

Results showed that NH-P(O) moiety from Acephate C(O)-NH-P(O) skeleton has a more important role in the interaction with an acetylcholinesterase enzyme active site than C(O)NH moiety. In contrast, N-H functional group interaction is more affected by electron properties P = 0 than C = 0, which is affected by hydrophobic properties. Even so, in this part, by adding another N-H functional group to Acephate structure and distributing N-H electron effects between two phosphorylation and carbonyl groups, design and synthesis of new pesticides will be done. So that, these synthesized pesticides would have the most insecticidal property and the least side effect on humans. For this purpose, phosphorhydrazides compounds with total structure (X) P-NH-NH-C(X) are prepared. In these derivatives, the presence of two nucleophilic nitrogen atoms N-N linked to functional groups P = X and C = Xrepresents interesting electron and structural evaluate chemical-physical properties to descriptors and their relationship to biological Phosphorhydrazides activity. compound's as the least human's toxicity acetylcholinesterase enzyme inhibitor was

measured on elm leaf beetle, and compound with number 12 had the highest insecticide level. Because of the low inhibitory effect of these compounds on human acetylcholinesterase enzyme ( $IC_{50} = 128.22$ mM); this compound can be introduced as an insecticide (Gholivand *et al.*, 2016).

#### Conclusion

assaying insecticidal efficiency of For Acephate-derived compounds, acetylcholinesterase enzyme of third instar larvae of elm leaf beetle was purified. Then, the inhibition mechanisms of screening compounds were studied on the purified enzyme after calculating kinetic parameters. Among different Acephate-derived compounds, two (i.e., No. 5 and 12) showed favorable insecticidal properties by inhibiting the AChE enzyme. The results obtained from QSAR equations showed nitrogen atom's net charge and N-H group polarization as very crucial factors. So, the elimination and adding N-H group strategy was used to investigate compounds' insecticidal properties. Also, the addition of N-H functional group and its distribution electron among two phosphorylation and carbonyl groups was used as a second strategy. According to the high insecticidal properties of this group of derivatives, in addition to low inhibition on human's AChE enzyme compared to Acephate-derived; the compounds No. 5 and 12 can be introduced as favorable and efficient insecticides.

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### مطالعه پتانسیل حشره کشی برخی از مشتقات آسفات و ارتباط کمّی ساختار و فعالیت آنها (QSAR)

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**چکیده:** ارگانوفسفرهها (OP) یکی از مهمترین گروههای آفتکش هـستند کـه بـهصـورت گـسترده در سراسر جهان برای کنترل آفات مورد استفاده قرار می گیرند. مکان هدف اصلی ایـن نـوع آفـت کـش.هـا، مانند حشره کش آسفات، آنزیم استیل کولین استراز (EC1.14.18.1) از سیستم عصبی حشرات است. مهار این آنزیم بهواسطه ترکیبات مشتق شده از آسفات می تواند آفات مقاوم و غیرمقاوم به ارگانوفسفرهها را کنترل کند. بنابراین در این پژوهش، سمّیت این دسته از ترکیبات روی سوسک بر گخوار نارون (Muller) Xanthogaleruca luteola (Muller) ارزیابی شد. آزمایش غربال گری نشان داد که دو تركيب از مشتقات فسفرهيدرازي (يعني: NH<sub>2</sub>-C(O) NH-NH P(O)(OC<sub>6</sub>H<sub>5</sub>) و NH<sub>2</sub>-C(O) NH-NH P(O) (NHP(S)(OCH<sub>3</sub>)2 بیش ترین یتانسیل حشره کشی را نشان دادند. بررسی اثرات این ترکیبات در شرایط زنده (in vivo) از طریق تیمار لارو سن سوم سوسک بر گخوار نارون مورد ارزیابی قرار گرفت. آنزیم استیل کولیناستراز لارو سن سوم سوسک بر گخوار نارون با به کار گیری کروماتوگرافی میل ترکیبی خالصسازی شد. مقادیر IC<sub>50</sub> ، مکانیسم مهار و ثابت بازدارندگی (Ki) مهار کنندههای -NH<sub>2</sub>-C(O) NH OC<sub>4</sub>H<sub>3</sub>-C(O)NH-NHP(S)(OCH<sub>3</sub>)<sub>2</sub> NH P(O)(OC<sub>6</sub>H<sub>5</sub>) روى آنزيم خالص شده استيل كوليناستراز محاسبه گردید. این ترکیبات با مکانیسم مهار مخلوط این آنزیم را مهار کرده و K<sub>i</sub> آنها بهترتیب برای NH-NH P(O)(OC<sub>6</sub>H<sub>5</sub>) و NH-NHP(S)(OCH<sub>3</sub>)<sub>2</sub> و NH<sub>2</sub>-C(O) NH-NH P(O)(OC<sub>6</sub>H<sub>5</sub>) بر میکرومولار بر دقیقه بهدست آمد. معادلات QSAR این ترکیبات براساس روش های آماری رگرسیون خطی چندمتغیرہ (MLR) و آنالیز مؤلفہ های اصلی (PCA) نے شان داد کے اتم نیتروژن با بار خالص غیراحیا که تحت تأثیر قطبیت گروه N-H قرار دارد، بیش ترین تأثیر را بر یتانسیل حسره کسی دارد. بنابراین طراحی ترکیبات جدیدی که قطبیت گروه N-H روی اتم نیتروژن را تحت تأثیر قرار مے دهنـد، می توانند گزینه مناسبی برای بررسی خواص حشره کش ترکیبات مشتق شده از حشره کش آسفات باشند.

واژگان کلیدی: مطالعه QSAR، *Xanthogaleruca luteola م*شتقات حشره کشی، فسفردی آمید