J. Crop Prot. 2021, 10 (3): 585-596

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_2-C(O) NH-NH P(O)(OC_6H_5) and OC_4H_3-C(O)NH-NHP(S)(OCH_3)$_2$) 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_{50} values, inhibition mechanisms, and inhibitory constant (Ki) of NH_2-C(O) NH-NH P(O)(OC_6H_5) and OC_4H_3-C(O)NH-NHP(S)(OCH_3)$_2$ 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_i obtained was 1.16 and 0.88 μM^{-1} min^{-1} 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 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 control (Rechcigl and Rechcigl, 2000), pesticides are still the main tools used for pest control (Casida and Quistad, 2004). Discovery and registration of new pesticides are lengthy

Handling Editor: Khalil Talebi-Jahromi

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Received: 28 September 2020, Accepted: 02 November 2021
Published online: 27 November 2021

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

Triton X-100, bovine serum albumin, acetylthiocholine iodide (ATCH), 5,5-dithiobis-2-nitrobenzoic acid (DTNB), alpha-naphthyl acetate (α-NA), beta-naphthyl acetate (β-NA), fast blue RR salt, Procainamide hydrochloride, Dimethyl sulfoxide (DMSO), Na_2HPO_4 (99%), NaH_2PO_4 (99%), Sodium dodecyl sulfate (SDS) and N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) were all from Sigma-Aldrich and Methanol was from, Merck. The ECH Sepharose 4B was purchased from Amersham Pharmacia 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 ± 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).

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.

NO.	Compounds	Mortality (%) (5000 mg/l)	Mortality (%) (2500 mg/l)	IC_{50} insect (mg/l)	IC_{50} Human (mg/l)	Ref.
1	NH-NHP(O)$(OC_6H_2)_2$	60	5	42.03	7.006	(Asadi *et al*., 2016)
2	$NH_2C(O)NH$-NHP(O)(Cl)(OC_6H_5)	60	5	69.4	9.886	(Asadi *et al*., 2016)
3	$NH_2C(O)$ NH-NH P(O)(Cl-OC_6H_4)(Cl)	20	-	68.65	2.831	(Asadi *et al*., 2016)
4	$NH_2C(O)$ NH-NH P(O)(C_6H_5)(Cl)	5	-	72.65	5.370	(Asadi *et al*., 2016)
5	NH_2-C(O) NH-NH P(O)(OC_6H_5)	100	90	15.02	4.020	(Gholivand *et al*., 2016)
6	NH_2-C(O) NH-NH P(S)$(OC_2H_5)_2$	5	-	35.78	7.140	(Gholivand *et al*., 2016)
7	NH_2-C(O) NH-NH P(S)$(OCH_3)_2$	40	-	43.90	2.340	(Gholivand *et al*., 2016)
8	NH_2-C(S) NH-NH P(S)$(OC_2H_5)_2$	80	20	65.39	35.52	(Gholivand *et al*., 2016)
9	C_2H_5-NHC(S)NH-NHP(S)$(OCH_3)_2$	75	10	165.00	127.10	(Gholivand *et al*., 2016)
10	C_2H_5-NHC(S)NH-NHP(O)$(C_6H_5)_2$	50	-	207.23	119.84	(Gholivand *et al*., 2016)
11	C_6H_5-NHC(S)NH-NHP(O)$(C_6H_5)_2$	80	30	130.19	110.88	(Gholivand *et al*., 2016)
12	OC_4H_3-C(O)NH-NHP(S)$(OCH_3)_2$	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)

Enzyme assays

Survived larvae, treated with diffident concentrations of selected compounds, were collected and transferred to the freezer (-80 ºC) 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 ºC. The supernatants were used for subsequent analyses. The AChE and esterase activities were measured according to the methods proposed by Ellman *et al*. and Van Aspern, respectively (Elman *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 µl) was added to a tube containing 140 µl of the buffer and 20 µl of DTNB and 40 µl 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 µl of supernatant was mixed with 112 µl phosphate buffer (pH 7.0), 25 µl substrate, and 50 µl fast blue RR salt (1 mM). The increase in absorbance was recorded kinetically at 450 and 540 nm due to the formation of α-naphthol and β-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. Enzyme purification was carried out using the method of Gao and Zhu (Gao and Zhu, 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_i, 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 pre-incubation of the enzyme in different concentrations of inhibitors at room temperature. The IC_{50}s and 95% confidence limits were determined by probit analysis using the POLO-PC software (LeOra Software. 1987). Activities of AChE in the presence of IC_{25} and IC_{50} concentrations of inhibitors were determined at the different concentrations of ATChI in addition to the fixed concentrations of DTNB. The K_m and V_{max} values and inhibitory constant, K_i, with and without inhibitors were estimated from Lineweaver–Burk plots.

Statistical analysis

LC_{50} 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 $p \leq 0.05$. 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_{HOMO} and E_{LUMO}), electrophilicity (ω), polarizability (PL, the charge difference between the atoms in functional groups) and the net atomic charges (Q). Furthermore, hydrophobic coefficient (logP), dipole moment (μ), 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 ($|r| < 0.5$) to log ($1/IC_{50}$) 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 among different Acephate-derived 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 had more insecticidal efficiency 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).

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

LC value	Compounds		
	5	12	Acephate
LC_{50}	886.11	1221.09	142.01
(mg/l) 95% confidence limits	(693.83-1133.44)	(947.16-1711.17)	(98.66-199.64)
Slope ± Standard error	2.76 ± 0.09	1.25 ± 0.12	1.722 ± 0.15

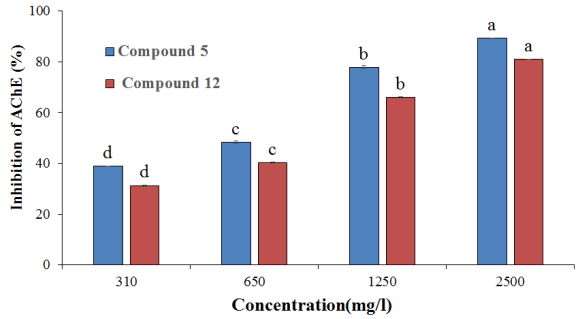

Figure 1 Inhibition percent of AChE from third instar larvae of *Xanthogaleruca luteola* treated with different concentrations of compounds #5 and 12.

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

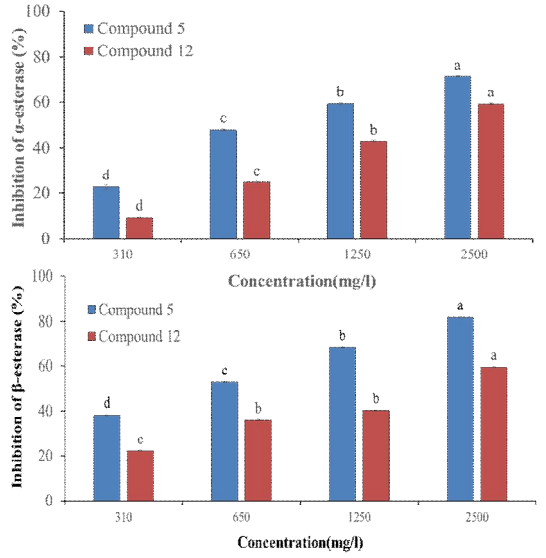

Figure 2 Inhibition percent of α- (A) and β- esterase (B) from third instar larvae of *Xanthogaleruca luteola* treated with different concentrations of compounds # 5 and 12.

*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_{25} 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 physical-chemical properties, log ($1/IC_{50}$) 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)

$$p(IC_{50}) = 4.105Q_P + 168.451Q_{N(\alpha)} + 0.278Q_{N(\beta)} - 0.293PL_{P=X} + 51.3852PL_{N=H(\alpha)} + 11.479PL_{N-H(\beta)} + 60.500E_{HOMO} + 84.542E_{LUMO} + 0.899\omega - 0.289\mu + 0.891Logp + 0.005Mv - 82.98$$

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

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

Compound	IC_{50} Crude (mg/l) 95% confidence intervals	IC_{50} 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_{25} and IC_{50} of Acephate derivatives on AChE kinetic parameters purified from *Xanthogaleruca luteola.*

Compound	Concentration (ppm)	K_m^{app} µM	V_{max}^{app} µmol/min/mg protein	K_i $\mu M^{-1}min^{-1}$	Inhibition type
Control	-	60.19 ± 1.32	52.40 ± 1.18	-	-
Acephate	I_{25} = 0.65	125.01 ± 2.95	17.65 ± 2.91	6.93 ± 0.51	Mixed
	I_{50} = 2.30	74.99 ± 2.81	8.02 ± 1.46		
5	I_{25} = 1.21	102.10 ± 3.56	41.86 ± 2.20	1.162 ± 0.16	Mixed
	I_{50} = 5.98	84.75 ± 2.84	16.43 ± 1.87		
12	I_{25} = 2.85	142.75 ± 2.35	33.79 ± 1.35	0.88 ± 0.24	Mixed
	I_{50} = 7.94	97.38 ± 2.58	12.06 ± 1.22		

Table 5 Quantum-chemical and theoretical descriptors for compounds computed by Gaussian 03 program.

No.	Electronic									Hydrophobic		Steric	log (I/I_{50})
	Q_p	$Q_{N(\alpha)}$	$Q_{N(\beta)}$	P_{P-X}	$P_{N-H(\alpha)}$	$P_{N-H(\beta)}$	EHOMO	ELUMO	ω	µ	logP	Mυ	
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 $0.5 \leq r$), R^2 determination coefficient, S_{reg} standard deviation (optimal value $0.5 \leq S_{reg}$), 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)

$$p(IC_{50}) = 1.287Q_P + 62.125Q_{N(\alpha)} - 0.101PL_{P=X} - 9.032PL_{N-H(\alpha)} + 3.566PL_{N-H(\beta)} + 32.187E_{HOMO} - 20.881E_{LUMO} - 0.041\mu - 0.155Logp + 20.24$$

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

Table 6 VIF[a] values of experimental and theoretical QSAR equations.

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

[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 hydrazides (P(O,S)-NH-NH-C(O,S)) 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 3-dimensional 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 to Aldridge's classification, carboxylesterases belong to the group of β-esterase. 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_{50} values are different when exposed to crude extract and purified AChE.

Tested synthesized compounds (i.e. No. 5 and 12) decreased V_{max}^{app} values of purified AChE compared to the control; in contrast, vice versa, K_m^{app} 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 more affected by electron descriptors, $Q_{N(a)}$□E_{HOMO}□E_{LUMO}□$PL_{N\text{-}H(a)}$, than by structural descriptors, log P > μ. 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 on the interaction of phosphorhydrazides 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 chemical-physical 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 $PL_{N\text{-}H(a)}$ with r = -0.802 and $PL_{N\text{-}H(a)}$ 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 $PL_{N\text{-}H}$ 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 $Mv < \mu < Qc < Qp < E_{HOMO} < PL_{N\text{-}H}$. The relationship between the internal matrix of this parameter and phosphorus atom's net charge, in addition to the compound's 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)

$$p(IC_{50}) = -0.646\mu + 3.477Q_P + 1.378Q_C + 57.272PL_{N-H} - 25.476E_{HOMO} - 0.033Mv + 67.019$$

$$n = 13; R^2 = 0.955; R^2_{Adj} = 0.925; S_{reg} = 0.331; r = 0.20;$$

$$F_{statistic} = 31.970; q^2 = 0.955; P < 0.0001$$

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 non-dependent descriptors are $Q_{N(a)} > E_{HOMO} > E_{LUMO} > PL_{N\text{-}H(a)}$. The internal matrix of descriptors shows that the most dependence is between the nitrogen atom's net charge and $PL_{N\text{-}H}$. 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 = X represents interesting electron and structural properties to evaluate chemical-physical descriptors and their relationship to biological activity. Phosphorhydrazides compound's toxicity as the least human's 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

For assaying insecticidal efficiency of 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 electron distribution 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 حشره‌کشی برخی از مشتقات آسفات و ارتباط کمّی ساختار و فعالیت آن‌ها (QSAR)

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چکیده: ارگانوفسفره‌ها (OP) یکی از مهم‌ترین گروه‌های آفت‌کش هستند که به‌صورت گسترده در سراسر جهان برای کنترل آفات مورد استفاده قرار می‌گیرند. مکان هدف اصلی این نوع آفت‌کش‌ها، مانند حشره‌کش آسفات، آنزیم استیل کولین استراز (EC1.14.18.1) از سیستم عصبی حشرات است. مهار این آنزیم به‌واسطه ترکیبات مشتق شده از آسفات می‌تواند آفات مقاوم و غیرمقاوم به ارگانوفسفره‌ها را کنترل کند. بنابراین در این پژوهش، سمّیت این دسته از ترکیبات روی سوسک برگ‌خوار نارون (Muller) *Xanthogaleruca luteola* ارزیابی شد. آزمایش غربال‌گری نشان داد که دو ترکیب از مشتقات فسفرهیدرازی (یعنی: NH_2-C(O) NH-NH P(O)(OC_6H_5) و OC_4H_3-C(O)NH-NHP(S)(OCH_3)$_2$) بیش‌ترین پتانسیل حشره‌کشی را نشان دادند. بررسی اثرات این ترکیبات در شرایط زنده (*in vivo*) از طریق تیمار لارو سن سوم سوسک برگ‌خوار نارون مورد ارزیابی قرار گرفت. آنزیم استیل‌کولین‌استراز لارو سن سوم سوسک برگ‌خوار نارون با به‌کارگیری کروماتوگرافی میل ترکیبی خالص‌سازی شد. مقادیر IC_{50} ، مکانیسم مهار و ثابت بازدارندگی (Ki) مهارکننده‌های NH_2-C(O) NH-NH P(O)(OC_6H_5) و OC_4H_3-C(O)NH-NHP(S)(OCH_3)$_2$ روی آنزیم خالص شده استیل‌کولین‌استراز محاسبه گردید. این ترکیبات با مکانیسم مهار مخلوط این آنزیم را مهار کرده و K_i آن‌ها به‌ترتیب برای NH_2-C(O) NH-NH P(O)(OC_6H_5) و OC_4H_3-C(O)NH-NHP(S)(OCH_3)$_2$ ۱/۱۶ و ۰/۸۸ بر میکرومولار بر دقیقه به‌دست آمد. معادلات QSAR این ترکیبات براساس روش‌های آماری رگرسیون خطی چندمتغیره (MLR) و آنالیز مؤلفه‌های اصلی (PCA) نشان داد که اتم نیتروژن با بار خالص غیراحیا که تحت تأثیر قطبیت گروه N-H قرار دارد، بیش‌ترین تأثیر را بر پتانسیل حشره‌کشی دارد. بنابراین طراحی ترکیبات جدیدی که قطبیت گروه N-H روی اتم نیتروژن را تحت تأثیر قرار می‌دهند، می‌توانند گزینه مناسبی برای بررسی خواص حشره‌کش ترکیبات مشتق شده ازحشره‌کش آسفات باشند.

واژگان کلیدی: مطالعه QSAR، *Xanthogaleruca luteola*، مشتقات حشره‌کشی، فسفردی آمید