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# Synthesis and larvicidal efficacy of pyrazolopyrimidine derivatives conjugated with selenium nanoparticles against *Culex pipiens* L. and *Musca domestica* L. larvae

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ABSTRACT

The synthesized pyrazolopyrimidine derivatives conjugated with selenium nanoparticles were prepared via a reaction of pyrazolone **1** with aryl-aldehyde and malononitrile or 3-oxo-3-phenylpropanenitrile in the presence ammonium acetate or pipridine using an ultrasonic bath as a modified method in the organic synthesis for such materials. The structure of the synthesized compounds was elucidated through various techniques. All the synthesized pyrazolopyrimidines were used in the synthesis of selenium nanoparticles (SeNPs). These nanoparticles were confirmed using UV-spectra, Dynamic Light scattering and (TEM) techniques. The larvicidal efficiency;of the synthesized;compounds; was investigated against some strains such as *Culex pipiens;*and *Musca domestica* larvae. Bioassay test showed pyrazolopyrimide derivatives to exhibit an acceptable larvicidal;bioefficacy. The derivative (**3**) exhibited;the highest;efficiency for more than; lab strains of both species. Moreover, *C. pipiens* larvae were more sensitive towards the examined compounds than *M. domestica*. The field;strain displayed lower affinity for the 2 folds compounds. Some biochemical changes were tracked through analysis of insect main metabolites (protein, lipid and carbohydrate), in addition to measuring the changes in seven enzymes after treatment. Generally, there was a reduction in the protein, lipids and carbohydrates after treatment with all tested compounds. Moreover, a decrement was noticed for acetylcholine esterase and glutathione;S-transferase; enzymes. There was an increment in the acid;phosphatase; and alkaline phosphatase. In addition, there was elevation in Phenoloxidase level but it noticed the declination in both Cytochrome P450 and Ascorbate peroxidase activity after treatment both flies with derivatives of selenium-nanoparticles in both lab and field strain. Generally, the experiments carried out indicate that antioxidant and detoxification enzymes may play a significant role in mechanism of action of our novel nanocompounds. The cytotoxicity of the synthesized compounds and conjugated with SeNPs showed enhanced compatibility with human normal fibroblast cell line (BJ1) with no toxic effect.

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## 1. Introduction

The analogs of nitrogen-based heterocyclic compounds occupy an important category of therapeutic diets [1]. Pyrans, pyrazoles and pyrimidines serve as powerful building blocks and scaffolds for the development of novel heterocyclic compounds. Pyran ring constitutes a structural motif present in many natural products [2]. Pyrazoles are the most important structural units in bioactive compounds, a number of natural products, photochromic and insecticides [3,4]. Pyrimidine exhibits various biological efficiencies comprising antiviral, anti-inflammatory ones [5]. Pyrazolopyrimidines derivatives stood out in the development of novel compounds with biological activity [6]. Rift valley fever and west Nile are two mosquito borne arboviruses. They are mainly transmitted by Culex mosquitoes [7]. Similarly, the house fly; Musca domestica, is major scourge for humans. It emerges mainly with its disease vectoring capacities [8]. House flies are considered as transmitting tens of diseases to people. They comprise typhoid fever, dysentery, cholera and intestinal parasites [9]. The control of this fly is very difficult because of its large populations and a high fecundity [10]. There are various species of insects, including vector of diseases. They develop resistance against various commonly used insecticides and insect growth regulators [11]. Indeed, the resistance to insecticides rises gradually. It becomes the major barrier against vectors disease control [12].

One method to combat such resistance is to use novel chemicals with novel mode(s) of action. Nano-formulations are important for enhancing the solubility of poorly water-soluble compounds, producing stable formulations without the usage of toxic organic solvents [13]. Selenium (Se) is vital nonmetal. This element participates in cellular functions known as seleno-proteins [14]. Selenium nanoparticles (SeNPs) show vitality due to their minimal toxicity and bioavailability. Besides, these nanoparticles are able to interact with proteins. They will be biocompatible as organic/inorganic compounds [15–17]. SeNPs have anticancer efficiency [18]. There are biomedical applications of these nanoparticles. They comprise anticancer activity, and biosensor implementations Abdelhamid *et al.,* **(2023)**. Different studies discussed preparing selenium nanoparticles through different approaches. They include chemical reduction; and green synthesis [19].

Our work aims to estimate the efficiency of novel pyrazolopyrimidine derivatives (**2a**, **2b** and **3**). They are conjugated with SeNPs against lab; and field; strains of 3rd instar; larvae of *C. pipiens;*and 2nd instar larvae;of *M. domestica*. Moreover, it is proposed to investigate the biochemical changes of main metabolites (protein, carbohydrate and lipid) and four enzymes (acid &alkaline phosphatase, acetyl cholinesterase and glutathione S- transferase) in both treated larvae compared to the control ones which help to express the behavior of these novel formulations.

## 2. Materials and methods

### 2.1. Techniques

(in the supplementary file)

### 2.2. Synthesis of 2a, 2b and 3

(in the supplementary file)

### 2.3. Larvicidal investigations

(in the supplementary file)

### 2.4. Cytotoxic effect of synthesized compounds and loaded into SeNPs

(in the supplementary file)

## 3. Results and discussion

### 3.1. Chemistry

This work focuses on the synthesis of pyrazolopyrimidine derivatives (**2a**, **2b** and **3**) using sonication as a new technique as shown in Fig. 1 by the reaction of pyrazolone **1** with arylaldehyde and malononitrile or 3-oxo-3-phenylpropanenitrile in the presence of ammonium acetate or pipridine and evaluation of their larvicidal potency. The synthesis process is classified into: a- Synthesis of pyrazolopyrimidine derivatives [**2a, 2b & 3**] (in the supplementary file), b- In situ synthesis of selenium nanoparticles

#### 3.1.1. b- In situ synthesis of selenium nanoparticles in the presence of heterocyclic compounds 2a, 2b and 3 (Het-SeNPs)

This study aims to innovate a novel selenium nanoparticles (SeNPs) preparation method with the synthesized compounds **2a**, **2b**, and **3** as illustrated in Figure S1 (in the supplementary file). They exhibit appropriate low reducing effect in addition to high stabilizing properties during SeNP preparation. The glucose has reduced the $Se^{+}$ cation to $Se^{\circ}$ using ascorbic acid catalyst; which acts as an aldehyde to form SeNPs. It stabilizes the nano-structure of SeNPs [17,20]. The organic substrates loaded with; –NH; and -$NH_2$ are able to reduce Se ion.

Fig. 2 depicts the UV–VIS data of SeNPs. Selenium has an absorption peak at 460 nm. It suggests that SeNPs have resulted in appropriate surface plasmon resonance. Furthermore, UV–vis analysis of synthesized Se NPs was revealing a dark blue shift. A blue shift revealed a low in particle size and synthesized Se NPs compounds with high band gap, which highly initiated release the generation of ROS in NPs [21]. The same trend was observed with previous report in controlling *Aedes aegypti* with silver nanoparticles [22]. The new nanoparticles disperse without agglomeration.

Characterizing SeNPs either alone or; loaded onto; the synthesized heterocyclic compounds was displayed in Fig. 3. Fig. 3a illustrates selenium nanoparticles in spherical shapes. Minor aggregates are present. These nanoparticles are between 24 and 35 nm in size. Loading the prepared nanoparticles; onto the investigated compounds; (**2a**) (**2b**), and (**3**) demonstrates that SeNPs extend uniformly through both samples; as shown in Figs. 3**b** and 3**c**. These compounds prevented agglomerate formation in these particles. Nitrogen-based compounds stabilizing effect [14,15] assisted to form discrete nanoparticles in an appropriate distribution.

Particle size distribution of SeNPs was explored; using dynamic;light scattering (DLS) investigations as shown in Figure S3 (in the supplementary file). It shows how stable and uniform the size of the particle is [23]. In our study, we observed that the formulated nanoparticles have low size. It results in high uniformity of droplet size. Moreover; it provides long-term stability. Stabilizing these nanoparticles is ascribed to

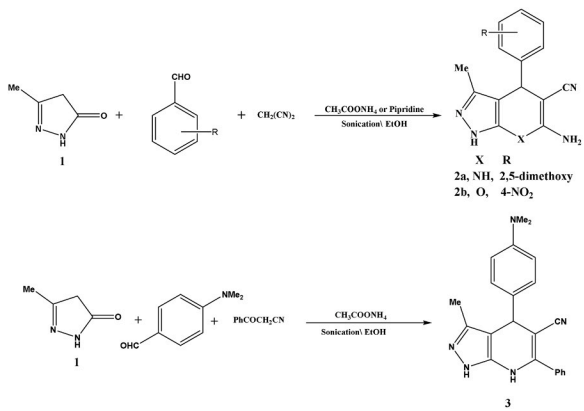


**Fig. 1.** Scheme route for synthesizing compounds **2a**, **2b** and **3**.

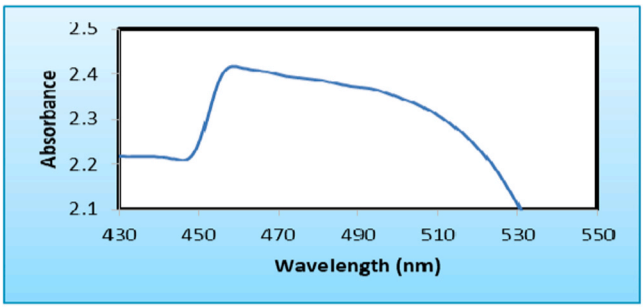


**Fig. 2.** UV-Vis spectrum of selenium nanoparticles.

the presence of surfactant. It provides a mechanical obstacle against aggregation [24].

In this study, we used sonication in the synthesis of pyrazolopyrimidine derivatives **2a**, **2b** and **3** to reduce time and increase the yield, by the reaction of pyrazolone **1** with arylaldehyde and malononitrile or 3-oxo-3-phenylpropanenitrile in the presence ammonium acetate or pipridine. (FTIR) technique was used for affirming that hydroxyl, amine and carbonyl functional groups contributed in the formation of SeNPs. They are known as bioactive molecules capping their surface [25,26]. In Figure S4 (in the supplementary file), FTIR spectrum of SeNPs reveals shifts in the characteristic peaks. They comprise cyano groups (-C≡N) at 2196, 2189 and 2201 $cm^{-1}$ for **2a**, **2b** and, **3**, respectively. They can provide information about the strength of the interaction between molecules [27]. They denote that these biomolecules contributed to reduce and stabilize SeNPs [28]. $^{1}$H NMR for compound 2b as an example shows characteristic ab system for the para substitution at δ ppm 7.48 (d, 2 H, aromatic), 8.21 (d, 2 H, aromatic protons). The results of $^{1}$H NMR spectra of SeNPs showed the complex structure and heterogeneous nature of synthesized compounds (Figure S5) (in the supplementary file). The latter results were attributed to the proton NMR chemical shift (δ). It is influenced by; the closeness to electronegative atoms (O, N and halogens);and unsaturated groups (C═C, C═O). Mass spectrometry is an analytical tool. It regulates the mass-to-charge ratio (*m/z*) of one or more molecules in a sample. It can be used to estimate the suitable Mwt; of the sample components; as shown in Figure S4 (in the supplementary file).

### 3.1.2. Larvicidal bioassay

Table 1 illustrates the susceptibility of 3rd instar larvae;of laboratory and field *Culex pipiens;*strains to compounds **2a**, **2b**, and **3**. The greatest mortality percentage reached 88 % at a concentration of 0.5 ppm in compound **2b**, followed by 84 % and 70.66 % at the same concentration in both compounds 3 and 2a in a laboratory strain, respectively. While, the greatest mortality in the field strain reached 78.66 %, 72 %, and 70.66 % at compounds **3**, **2b**, and **2a** respectively. The toxicity of the three compounds increased gradually with increasing concentrations at

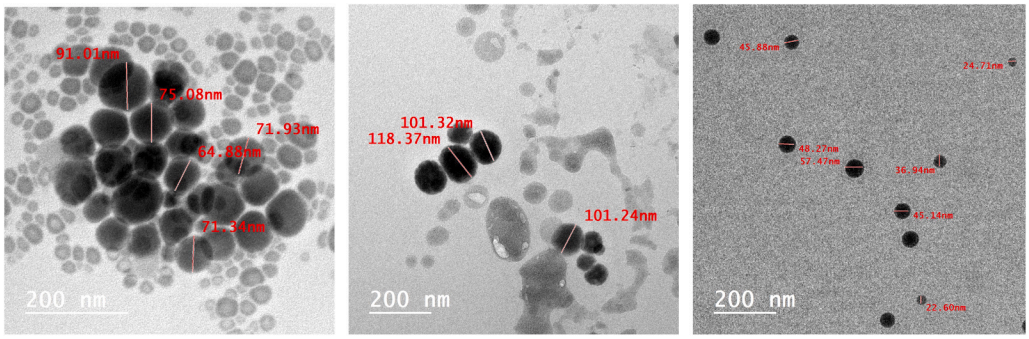


**Fig. 3.** a); b) and c) TEM of SeNPs; loaded onto (**2a**, **2b** and **3**) respectively.

**Table 1**
Larvicidal activity; of compounds; **2a**, **2b**, and **3** against 3rd larval instar of lab and field strains; *Culex pipiens*; 24 h post-treatment.

| Compound (ppm) | Percentage of Mortalities % | | | | | |
|---|---|---|---|---|---|---|
| | **2a** | | **2b** | | **3** | |
| | Lab | Field | Lab | Field | Lab | Field |
| Control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.1 | 20 | 18.66 | 24 | 30.66 | 37.33 | 20 |
| 0.2 | 45.33 | 36 | 52 | 36 | 48 | 42 |
| 0.3 | 58.66 | 42.66 | 65.33 | 45.33 | 57.3 | 49.3 |
| 0.4 | 61.33 | 50.66 | 81.3 | 56 | 74 | 72 |
| 0.5 | 70.66 | 70.66 | 88 | 72 | 84 | 78.66 |
| $LC_{25}$ | 0.112 | 0.14 | 0.106 | 0.096 | 0.075 | 0.126 |
| (FI at 95 %) | (0.08–0.139) | (0.103–0.170) | (0.084–0.126) | (0.056–0.130) | (0.047–0.100) | (0.099–0.150( |
| $LC_{50}$ | 0.254 | 0.327 | 0.191 | 0.285 | 0.182 | 0.247 |
| (FI at 95 %) | (0.219–0.292) | )0.283–0.388( | (0.168–0.214) | )0.237–0.348( | (0.147–0.213) | (0.218–0.277) |
| $LC_{90}$ | 1.202 | 1.639 | 0.587 | 2.230 | 0.967 | 0.883 |
| (FI at 95 %) | (0.864–2.057) | (1.099–3.228) | (0.496–0.743) | (1.291–6.402) | (0.707–1.625) | (0.696–1.26) |
| *Slope ± SE | 1.897± 0.246 | 1.830± 0.251 | 2.633±0.259 | 1.434±0.239 | 1.765±0.241 | 2.315±0.255 |
| *P* | 0.669 | 0.191 | 0.737 | 0.070 | 0.078 | 0.256 |
| $X^2$ | 1.558 | 4.755 | 1.269 | 7.071 | 6.805 | 4.055 |
| Relative potency | 1 | 1 | 1.33 | 1.15 | 1.40 | 1.32 |
| Toxicity index | 71.65 | 75.53 | 95.29 | 86.67 | 100 | 100 |

* Slope of the concentration-inhibition; regression line ± standard error; (X2) Chi-square value; LC values in ppm (95 % C.I.); with the lower and the upper limit; LC values = Lethal concentrations values; 95 % C.I. = Ninety-five percent confidence limit.

both strains. Hence; the larvicidal activity of the SeNPs; may result from the denaturation of sulfur-containing proteins; or phosphorous-containing compound like DNA. It drives to denaturating organelles and enzymes. So; it reduces the cellular membrane permeability. It is followed by a decrease in the synthesis of ATP. Finally, it conducts to losing the cellular function [29]. Bioassay studies merges between the laboratory bioassay;and the field conditions. They can define the tolerance and resistance ratio. They reside a consistent log concentration and probit mortality relationship [30]. Our results show that the tested compounds are more effective against the lab strain; than the field population of *C. pipiens* larvae. Consequently, the tolerance or diminishing the sensitivity of the field strain of *C. pipiens* larvae might be due to traditional insecticide application. It detects the cross resistance and natural variations [31]. The mosquito might be affected by agricultural practices; pesticides; and natural xenobiotics in mosquito breeding sites. They allow cross-resistance to different insecticides [32].

Based on the $LC_{50}$ values, **3** (0.182 ppm) was the most potent compound. It is accompanied by compound **2b** (0.191 ppm). The least active compound was achieved by compound **2a** (0.254 ppm) for a laboratory strain. For the field strain, the most effective compound was compound **3** (0.247 ppm) followed by compound **2b** (0.285 ppm) then compound **2a** (0.327 ppm). Relative potencies of 1, 1.33, and 1.40, at compounds **2a**, **2b**, and **3**, respectively are noticed for laboratory strain and 1, 1.15, and 1.32 at compounds **2a**, **2b**, and **3**, respectively for field strain. Interestingly, a relevant work has been reported by Abdelhamid *et al.,* **(2022).** It showed that the difference in the cytotoxicity of triazole derivatives (N-heterocyclic compounds) conjugated with selenium nanoparticles may be related to the changes in their interaction mechanism. They may result due to the existence of various biomolecules on their surfaces. The low slope values showed the tested population of;*Culex pipiens;* larvae to be homogenous. This result may be due to the change in liability toxicant penetration to inside larva. These results agree with previous relevant ones [33,34].

Calculations for 3rd instar larvae of laboratory and field *Musca domestica* strains to compounds **2a**, **2b**, and **3** has been represented in table S1 (in the supplementary file). The greatest percentage of mortality reached 88 % at a concentration of 2.5 ppm in compound **3**, followed by 81.3 % and 73.33 % at the same concentration in both compounds **2b** and **2a** in a laboratory strain, respectively. While, the greatest mortality in the field strain reached 81.33 %, 66.66 %, and 60 % at compounds **3**, **2a**, and **2b**, respectively. The toxicity of the three compounds increased gradually with increasing concentrations at both strains. Similar observations were noticed by [35]**.** They explored that the percentage of ceasing the adults of *Aphis nerii* augmented upon raising the nanoparticle content, along with treating lesser grain borer with some nanoparticles [36]**.** The reason of insecticidal effect may be due to the small particles size. It is able to penetrate the cell membrane leading to cell death. This consequence resulted from the oxidative stress generated by SeNPs [37]. Moreover, during feeding, the nanoparticles may have entered the digestive system of house fly causing DNA damage and cell death.

Based on the $LC_{50}$ values, **3** (0.182 ppm) is the most potent compound. It is pursued by compound **2b** (0.191 ppm). The least efficiency was achieved by compound **2a** (0.254 ppm) for a laboratory strain. For the field strain, the most effective compound was **3** (0.257 ppm) followed by compound **2b** (0.285 ppm) then compound **2a** (0.327 ppm). In this work, a medium concentration against the third instar larvae of *M. domestica* tissue homogenates is shown. It may be due to the inhibitory effect of SeNPs on the DNA repair system. It might have reacted directly with the larval DNA destroying it via the oxidative stress. Such findings cope with other studies which dealt with the impact of weed plant extracts and metabolites against larvae of *M. domestica* to fragment and destroy DNA [38]. Relative potencies for laboratory strain of 1, 1.18, and 1.47 for compounds **2a**, **2b**, and **3**; respectively. Field strain showed 1.01, 1, and 1.34 for compounds **2a**, **2b**, and **3**; respectively. Low slope values indicated that the tested population of *Musca domestica* larvae is homogenous [39] observed the same results after treatment larvae with carbon quantum dot nanoparticles.

Treating 3rd instar larvae of lab and field strain of *Culex pipiens* with compounds **2a**, **2b**, and **3**; acid phosphatase activity increased significantly compared with the control sample was showed in table S2 (in the supplementary file). The corresponding changes were (+120 %, +53.6 %), (+80.18 %, +42.9 %), and (+50.15 %, +21.4 %) respectively in lab and field strain in that order, compared with the control. Detoxification enzymes in insects have a vital effect in the defense mechanism;towards strange structures [40]. An enzyme that is lysosomal is acid phosphatase. It is abundant in degraded tissues, intestines, detached organs, and Malpighian tubules [47]. This enzyme breaks down trans-phosphorylation events and orthophosphate esters. It supplies a pool of phosphate needed to synthesis higher energy chemicals such as adenosine triphosphate (ATP), ATPase, and genetic DNA or RNA [41].

Alkaline phosphatase activity of *Culex pipiens* larvae treated with compounds **2a**, **2b**, and **3** significantly increased; the corresponding changes were (+19.78 %, +14.6 %), (+8.31 %, +9.5 %), and (+13.53 %, +6.3 %) respectively. Alkaline phosphatase exists in intestinal epithelial tissues. It provides phosphate ions from mononucleotides and ribo-nucleoproteins to various metabolic processes. Moreover, it is efficient in tissues with active membrane transport; such as intestinal epithelial cells, hemolymph;and Malpighian tubules [42]. In disagreement with [43]**,** alkaline and acid phosphatases showed a decrement in *Culex pipiens* larvae treated with alumina nanoparticles compared to the control samples. The high efficiency of alkaline phosphatase activity corresponds to the increased alkaline phosphatase activity reported by [44]. The latter was recorded for treated larvae of *Cx pipiens* with clove oil (*Syzygium aromaticum)*. The results may be attributed to developmental disturbance Shekari *et al.;* **(2008)** [45] also pointed to the contribution of this enzyme in the detoxification process. The efficiency of AChE was diminished upon comparison with the control group. It showed changes of (-4.68 %, −7.5 %), (-6.98 %, −12 %), and (-11.65 %, −19.4 %) respectively. The AChE enzyme is an efficient biomarker for in vitro neurotoxicity estimation [46]. Inhibiting the AChE enzyme in the contracted muscles could drive to paralysis [47]. So, in the recent assay, AChE inhibition lowers the normal respiration of larvae. Geneally, the relevant studies demonstrate that the synthesized SeNPs from N-heterocyclic compounds exhibit promising neurotoxic activity. This result as the same as that obtained by [48] who found that treated fourth instar larvae of *Aedes aegypti* with synthesized $CeO_2$ attend to significantly decreased in acetylcholinesterase activity.

The activity of the GST enzyme decreased upon treating larvae with compounds **2a**, **2b** and **3**. The corresponding changes were (-1.3 %, −8.5 %), (-5.47 %, −17 %) and (-8.96 %, −19.8 %) respectively. In contrast to our results; [49] detected an increase in GST activity in *Culex pipiens* larvae [50]. In agreement with other results, an aqueous extract from *Lumnizera racemosa* flower buds and ZnO nanorods exhibited a close mitigation in the GST effectivness in *Ae. aegypti* larvae [51]**.** Some chemicals may change the efficacy of GST. According to our study, the activity of the GST enzyme was significantly decreased. SeNPs might have interfered in a redox reaction [52]. Simultaneously, these nanoparticles have led to some metabolic disorders with delaying growth and deteriorating the mosquito larvae Pratt **(1963).**

Fig. 4 demonstrates some biochemical variations in the whole-body tissue of both lab and field strain of *Culex pipiens* larvae (protein, lipid, and carbohydrate). The greatest reduction of biomolecules compared to control was recorded in protein (34.8 %, 30.3 %), lipids (24.99 %, 30.4 %), and carbohydrates (41.10 %, 28.6 %) when applying compound 3, respectively. On the other hand, compound 2b decreased the protein (26.1 %, 18.7 %), lipids (18.8 %, 28.7 %) and carbohydrates (34.24 %, 21.9 %), respectively. Meanwhile, compound **2a** showed lowest effect on biomolecules reduction; protein decreased by (23.2 %, 14.2 %), lipid decreased by (9.4 %, 21.7 %) and carbohydrates decreased by (20.6 %, 12.4 %); respectively. Our results agreed with [49] who used Se Nanoparticles Based on *Achillea fragrantissima*

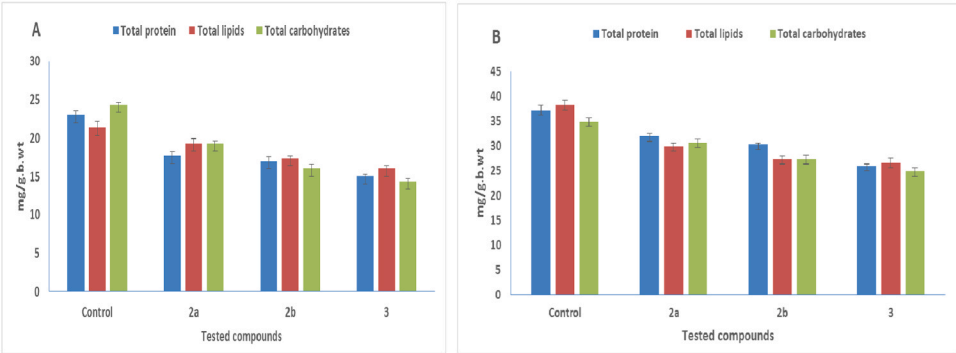


**Fig. 4.** Effect of compounds **2a**, **2b**, and **3** on biomolecules availability in the lab strain (A) and field strain (B) of *Culex pipiens* 3rd instar larvae.

(Forssk.) extract against *Culex* larvae. Generally, there is a noticed decrease in protein levels for the treated larvae. The decrease in protein contents of the treated samples might be due to protein connect with foreign compounds such as tested nanoparticles or might be due to protein changed into simple amino acids to release energy [53].

Total proteins, lipids and carbohydrates indicate the health situation in insect's body. Considering these 3 essential variables guides to know the effect of employed nanoparticles on the physiology of insects. Changing these biochemical parameters was observed by several researchers when they applied many kinds of nanoparticles to the same insect [49]. Lipids as a carbon source build up a part of the cell membrane and cuticle, providing a considerable metabolic energy. In the current study, SeNPs showed a declination in the total lipids when compared with the normal insects. This reduction can be referred to the collapse of lipids which are employed in growth [54]. Carbohydrates contribute in regulating the male and female reproductive systems. Metal nanoparticles are capable of bonding with (S) proteins and (P) in DNA. In this case, they decrease the membrane permeability leading to cell death [55].

Data in Table 2 show that when 3rd instar larvae of lab;and field strains of *Musca domestica;* were treated with compounds **2a**, **2b** and **3**; the acid phosphatase activity significantly increased compared with the control group. The corresponding changes were (+51.77 %, +43.8 %), (+85 %, +27 %), and (+48 %, +12.5 %) respectively. Alkaline phosphatase;activity of *Musca domestica* larvae of lab and field strains treated with compounds **2a**, **2b**, and **3** significantly increased; the corresponding changes were (+15 %,+9 %), (+5.4 %, +7.8 %), and (+4.3 %, +4.5 %) respectively. Acid and alkaline phosphatases;share in the hydrolytic cleavage;of phosphoric acid esters. They can adjust the acid-alkali balance [56]. These enzymes are involved in metabolic and cellular signaling processes [57].

The efficacy of AChE diminished remarkably in contrast with the control group. Corresponding changes were (-11.7 %, −3.1 %), (-28.4 %, −6.2 %), and (-43.4 %, −11.4 %) respectively. This enzyme is the main neuro-transmitter for hydrolyzing the acetylcholine into acetate and choline. It is released from pre-synaptic neuron to a synaptic cleft. It binds with the receptor to pass $Na^+$ across this channel. It contributes in synthesizing acetylcholine with Acetyl CoA Oehmichen & Besserer **(1982)** [58]. Our results in this work cope with those of Parthiban *et al.,* **(2019)** [59]. They noticed that as the larvae of *Aedes* are subjected to the synthesized silver nanoparticles (AgNPs), the activity of AChE declines. It is followed by liberating a small amount of acetylcholine compared to the control larva. AgNPs link to the receptor of AChE dropping the release of these enzymes. Hence, this enzyme cannot hydrolyze the liberated acetylcholine which gathers at the postsynaptic terminal junction. So, sodium channel opens with a continuous stimulation in the nervous system of the investigated mosquito. A disturbance in the neuro-muscular system known as ataxia takes place leaving dead larvae [60].

GST enzyme showed a noticeable decrease in its efficiency in treated larvae. The corresponding changes were (-5.2 %, −6 %), (-8.4 %, −12.5 %) and (-14.2 %, −16.4 %) respectively. In other opinion, El Gohary *et al.,* **(2021)** [44] revealed increasing in GST enzyme activity in treated *culex* larvae with ***Syzygium aromaticum***

Nano- Formulation. This work presents close results to Abdel-Gawad **(2018)** [61]. Glutathione S-transferase enzyme diminished after feeding larvae of house Fly with $LC_{50}$ *Moringa oleifera*/metal nanoparticles. These enzymes catalyze the hydrolysis of ester- and amide- compounds. They are in charge of detoxifying or activating the metabolism of different xenobiotics [52]. In compliance with Ranson *et al.* **(1997)** [62], GST enzymes are connected with the resistance of insecticide. Moreover, the declination in the activity may be sue to a poor defense mechanism in the detoxification of SeNPs. Such behavior can points to the larvicidal feature of SeNPs [61].

Biochemical variation in the tissue of both lab and field strain of *Musca domestica* larvae (protein, lipid, and carbohydrate) are shown in Fig. 5. The greatest reduction of biomolecules compared to control was recorded in protein (-42.9 %, −31.7 %), lipids (-40 %, −24.6 %), and carbohydrates (-38.5 %,-16 %) when applying compound **3**, respectively. On the other hand, compound 2b decreased the protein (-33.3 %, −31.7 %), lipids (-37.8 %, −18.5 %) and carbohydrates (-28.2 %, −11.2 %), respectively. Meanwhile, compound 2a showed the least effect on biomolecules reduction. The protein decreased by (-23.8 %, −11.7 %), lipid decreased by (-26.7 %, −10.8 %) and carbohydrates

**Table 2**
Effect of $LC_{50}$ values of compounds;**2a**, **2b**, and **3**; on the activity of acid phosphatase, alkalinephosphatase, acetylcholinesterase; and Glutathione-S-transferases; in lab and field strain of *Musca domestica* larvae.

| Compounds | Acid phosphatase mU/mg protein | | Alkaline phosphatase mU/mg protein | | AChE ug AchBr/min/ mg protein | | GST µmol/min/mg protein | |
|---|---|---|---|---|---|---|---|---|
| | Lab | Field | Lab | Field | Lab | Field | Lab | Field |
| Untreated | 9±0.58$^a$ | 16±0.577$^a$ | 93±0.58$^a$ | 111.66±6.00$^a$ | 20±2.88$^a$ | 32.33±1.20$^a$ | 405.66±2.96$^a$ | 497±1.15$^a$ |
| **2a** | 8.66±0.33$^b$ | 23±0.577$^b$ | 107±1.15$^b$ | 121.66±6.00$^b$ | 17.66±0.88$^b$ | 31.33±0.33$^a$ | 384.66±3.17$^b$ | 467±1.15$^b$ |
| **2b** | 16.66±0.33$^c$ | 20.33±0.88$^c$ | 98±3.51$^c$ | 120.33±7.66$^b$ | 14.33±0.33$^c$ | 30.33±0.33$^a$ | 371.66±4.40$^c$ | 434.66±1.45$^c$ |
| **3** | 13.33±1.66$^d$ | 18±0.577$^d$ | 97±0.58$^c$ | 116.66±6$^c$ | 11.33±0.88$^d$ | 28.66±0.33$^b$ | 348±4.04$^d$ | 415.66±2.02$^d$ |

Means bearing different scripts; are different from control; at $p < 0.05$; Mean ± Standard error.

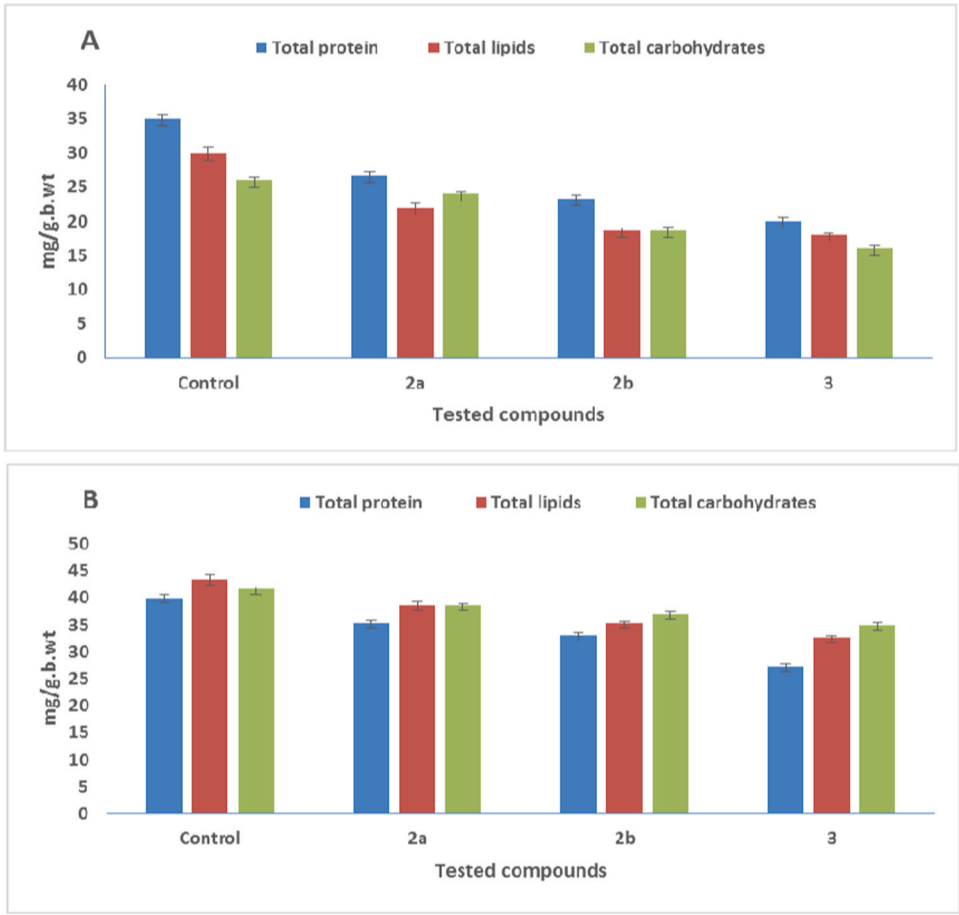


**Fig. 5.** Effect of compounds **2a**, **2b**, and **3** on biomolecules availability in the lab strain (A) and field strain (B) of *Musca domestica* 3rd instar larvae.

decreased by (-7.7 %, −7.2 %), respectively. The decrease in total protein led to protein breakdown into free amino acids. The insect was trying to interact physiologically with the impact of the $H_2O_2$ via generating defense proteins [63]. In nanomaterial-stressed organisms, attaining the energy needs is influenced by the decline of proteins and other nutrients caused by nanomaterials [64].

Data in Table S3 (in the supplementary file) showed that when 3rd instar larvae of lab and field strains of *Culex pipiens* were treated with compounds **2a-SeNPs**, **2b-SeNPs** and **3-SeNPs**; Cytochrome P450 (CYPs) activity significantly decreased compared with the untreated ones. The corresponding changes were (-27.27 %, −18.18 %), (-25.02 %, −0.09 %) and (-22.77 %, −7.73 %) respectively. Our results confirmed with **Suwanchaichinda and Brattsten (2002)** [65] after treatment of *Aedes albopictus* Mosquito Larvae with benzothiazole (BZT) and its derivatives. The results suggest that Cytochrome P450 detoxify nano-insecticides and thus cause insecticide tolerance in the mosquito larvae Generally, mode of action are needed to support insecticide resistance management, Cytochrome P450 inhibitors should be considered because of their high effectiveness for insect selectivity, their well-known mechanism of action and the ease of design and testing [66]. (in the supplementary file)

Data in Table S4 (in the supplementary file) indicated that when 3rd instar larvae of lab and field strains of *Musca domestica* were treated with compounds **2a-SeNPs**, **2b-SeNPs** and **3-SeNPs**; level of Cytochrome P450 activity was significantly decreased compared with the untreated ones. The corresponding changes were (-22.07 %, −13.19 %), (-17.42 %, −7.74 %) and (-11.92 %, −4.65 %) respectively. Our results confirmed the action of Cytochrome P450 enzymes which are an important metabolic system involved in the catabolism and anabolism of xenobiotics and endogenous sustances. Monooxygenase-mediated metabolism is a common mechanism by which insects become resistant to insecticides by the many insect species and insecticides affected [67]. Cytochrome P450 is also a hemoprotein which acts as the terminal oxidase in monooxygenase systems. Monooxygenases are unusual that they able to oxidize widely substrates and able to produce a ring array of reactions [68]. (in the supplementary file)

### 3.2. Cytotoxicity

The cytotoxicity was performed for the synthesized compounds (**2a, 2ab** and **3**) and the loaded with the nano-selenium (Het-SeNPs) against human normal fibroblast cell line (BJ1). The results were indicated in table S5 (in the supplementary file).

## 4. Conclusions

Pyrazolopyrimidine derivatives (**2a**, **2b**, and **3**) and conjugated with selenium nanoparticles (SeNPs) were synthesized. They were applied as larvicidal agents against *Culex pipiens* and *Musca domestica*. These compounds and their metal nanoparticles showed high effectiveness against lab strain than field strain of larvae of both insects. The current experiments revealed that SeNPs synthesized from N-heterocyclic compound attributed to a disruption in detoxification process, metabolism. These materials presented considerable neurotoxic efficiency. It is noticed that there are significant biochemical changes in whole body tissue of the employed strains of the tested pests. In a clearly way, resistance could be suppressed by using our nanoformulations which suggesting that cytochrome P450 (CYP)-mediated detoxification was a major mechanism of resistance. Decreased penetration was also demonstrated as a mechanism of resistance. Thus, ecofriendly SeNPs

may be used in insecticidal investigations as efficient larvicidal agents.

## CRediT authorship contribution statement

**Alya M. Alotaibi:** Resources, Formal analysis. **Nawaa A.H. Alshammari:** Methodology, Data curation. **Shaimaa M Farag:** Investigation, Conceptualization. **Wafa A.H. Alkherb:** Methodology, Conceptualization. **Zouhaier Aloui:** Data curation, Investigation, Methodology. **Ahmed Ali El-Sayed:** Writing – original draft, Supervision, Project administration, Conceptualization. **Fahad M Almutairi:** Supervision, Project administration, Investigation. **Nancy M. El-Shourbagy:** Visualization, Validation, Formal analysis.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.colsurfb.2024.114040.

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