**Influence of temperature on the efficiency of *Commiphora molmol* and Acetylsalicylic acid against *Culex pipiens* (Diptera: Culicidae)**

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**Abstract** The temperature has the greatest effect on the development and survival of mosquito larvae. The efficiency of insecticides relies not only on the active ingredient but also on the insect's environment. Variations in ambient temperature may thus impact the toxicity of the compounds to mosquitoes. The present study was undertaken to study the effect of temperature on *Culex pipiens* response to *Commiphora molmol* resin, Mirazid, crystalline and pharmaceutical form of acetylsalicylic acid. Bioassay tests were carried out on the 3rd instar larvae under laboratory conditions. The LC50 of the tested compounds were used to study the temperature-toxicity relationship, while the LC50 of Mirazid and pharmaceutical form of acetylsalicylic acid, as the most potent compounds, were used for further biochemical studies. Results indicated that, Mirazid was the most potent compound, with LC50 of 28.3 ppm followed by *C. molmol* resin with LC50 of 41.6 ppm while the crystalline acetylsalicylic acid was the least effective compound with LC50 of 799.7 ppm. The temperature has a considerable impact on *Cx. pipiens* larvae's susceptibility to the tested compounds. High temperature (36 0C) resulted in high mortality rate (72.88, 78.20, 58.8 and 62.12 % for of *C. molmol* resin, Mirazid, CASA and PASA, respectively. Moreover, there were significant changes in α- esterase, β–esterase and glutathione -S-transferase levels as well as the total protein content in the treated larvae due to the change in temperature. These findings show that temperature is an essential factor to consider in the management of *Cx. pipiens*.

**Keywords:** *Culex pipiens*, *C. molmol*, acetylsalicylic acid, Temperature-toxicity relationship

**Introduction**

*Culex pipiens* is common in Egypt. This mosquito spreads several human and animal illnesses, such as Elephantiasis, Rift Valley fever, St. Louis encephalitis, and West Nile fever (Vinogradova 2000). Chemical insecticides are the most widely used method of mosquito control (Elono *et al.* 2018; Sayed *et al*. 2018; Liu *et al*. 2019; Wilson *et al.* 2020; Wang *et al*. 2020; Mapossa *et al*. 2021; Kaura *et al*. 2022), but their indiscriminate use may lead to insecticide resistance, environmental pollution, and adverse effects on human health (Zayed *et al*. 2019). These obstacles necessitated the development of novel control strategies. Plants are a well-known source of pesticides, which have been used for pest control throughout human history. The plant produces these compounds as part of its natural fight against pest infestations (Howe and Jander 2008). Oleo-gum-resin derived from plants of the genus Commiphora (Burseraceae) is extensively used in traditional medicine to treat a variety of ailments (Grbi *et al*., 2018). Essential oils and botanical extracts from Commiphora-genus plants have been utilized as egg-laying inhibitors and larvicides against several mosquito species (Massoud and Labib 2000; Regnault-Roger *et al*. 2012; da Silva *et al*. 2015; Muturi *et al*. 2020). Actually, acetylsalicylic acid (ASA) is a synthetic derivative of salicylic acid, which is a major component of herbal extract found in the bark and leaves of the Salix tree. Several researchers have demonstrated that salicylic acid and *salix safsaf* extract have larvicidal effect against mosquito larvae (Mondal *et al.*, 2014) and *M. domestica*. (Mansour *et al*. 2011; Selem & El-Sheikh. 2015; Hasaballah *et al*.2021)

The most significant abiotic element influencing the survival and development of mosquito juvenile stages is temperature (Delatte 2009; Christiansen-Jucht *et al*., 2014). The efficiency of insecticides against their target is dependent not only on the active component but also on the insect's surroundings (Zayed *et al*., 2019) hence a change in the surrounding temperature might affect the toxicity of the molecule to ectothermic species. Temperature also influences proteins and enzymes, which play a significant role in the survival of several species (Imasheva et al. 1998).

The efficacy of the control program is heavily based on resistance management strategies. For the development of these strategies, it is vital to know the factors that influence resistance and to identify the mechanisms involved. The objective of this study is to investigate the larvicidal efficacy of *Commiphora molmol* oleo gum resin, Mirazid, and crystalline and pharmaceutical forms of acetylsalicylic acid against *Cx. pipiens*, as well as their temperature-toxicity relationship. In order to elucidate the metabolic processes of the organism at different temperatures, the most toxic substances were also utilized.

**2. Materials and Methods**

**2.1. Test Insects**

*Culex pipiens* population were collected from natural breeding sites in Shiblanga Village, Qalyubiyya Governorate, Egypt and reared for seven generations in the insectary of Medical Entomology, Faculty of Science, Benha University, Benha, Egypt, at (27 ± 2 °C, 12:12 h light/dark period, 75±5% relative humidity) (Zayed *et al.* 2019). White enamel pans (35 - 40 cm in diameter and 10 -12 cm in depth) employing de-chlorinated tap water were used for rearing larvae and fish food (Tetramin®) was used as food. The adult mosquitoes were reared in wooden cages (35×35×40 cm), and provided with a cotton piece soaked with 10% sucrose solution placed on the cage top and was periodically blood-fed. The eggs were collected and transferred to clean enamel pans.

**2.2. Compounds used**:

*Commiphora molmol* (myrrh) oleo gum resin was purchased from a local Market of Medicinal Plants, Agricultural Seeds, and Herbs, Dokki, Giza, Egypt, and Mirazid capsules 300 mg: A pharmaceutical form (myrrh derivative) was produced from Pharco Pharmaceutical Company, Egypt and was purchased from pharmacy, Cairo, Egypt (Fig. 1). Two formulations of Acetylsalicylic acid (ASA) were used: Crystalline acetylsalicylic acid (CASA) was purchased from El Nasr Co. for chemical, Cairo, Egypt, and Green Aspirin (Rivo 320 mg) tablet: A pharmaceutical form of acetylsalicylic acid (PASA) produced by The Arab Drug Company for Pharmaceutical & Chemical industries, 5 El Massan St. -Al amyria, Cairo, Egypt (Fig. 2).

**2.3. Larvicidal bioassay**

Bioassay of *C. molmol* resin, Mirazid, CASA and PASA was performed to determine the median lethal concentration (LC50) of each compound against *Cx. pipiens* larvae.*C. molmol* resin, CASA and PASAwere dissolved inde-chlorinated tap water, while Mirazid was dissolved in Ethylene glycol (1 capsule/ml Ethylene glycol) and the resulting solution was diluted with water to a final 100 ml and this is considered as the stock solution. Six serial concentrations of each compound were prepared: 10, 20, 40, 80, 160 &320 ppm for larvae treated with *C. molmol* resin and mirazid and 200, 400, 800, 1200, 1600 & 2000 ppm for larvae treated with CASA and PASA. WHO (2005)technique was used for the larvicidal bioassays. Twenty five 3rd instar larvae of *Cx. pipiens* were transferred to each 250 ml beaker containing 200 ml of distilled water and test concentration. Water or ethylene glycol was used as negative control. Three replicates for each concentration were undertaken. All experiments were incubated at laboratory conditions (27±2 °C and 75±5% relative humidity). The mortality counts were recorded after 48 h of the exposure period.

**2.4. Effect of temperature on the larvicidal activity of the tested compounds**

To determine the relationship between post-treatment temperature and the larvicidal efficacy of the aforementioned compounds against *Cx. pipiens* larvae. Three groups per compound of twenty-five 3rd instar larvae were released in a glass beaker containing the LC50 of each compound, as determined earlier in this study (41.6, 28.4, 799.7, and 570.9 ppm for *C. molmol* resin, Mirazid, CASA, and PASA, respectively) (Table 3). Each group was incubated independently at 15, 26, and 36 degrees Celsius. In parallel, a control of untreated larvae was also done. Each experiment was conducted three times. The death rate was calculated 48 hours after exposure.

**2.5. Biochemical assay: -**

To determine the effect of temperature on the total protein and the activities of some detoxification enzymes of *Cx. pipiens* 3rd instar larvae, the predetermined LC50 of Mirazid and PASA, as the most toxic compounds were used (28.3ppm Mirazid and 570.9 ppm PASA). Larvae reared at different temperature degrees (15, 26 & 36 °C) for 48 hours and submitted to biochemical assays for α- and β-esterase, GST and total protein.

**2.5.1. Sample preparation**

After 48h of compound administration at each temperature, surviving *Cx. pipiens* larvae were collected and homogenized in distilled water using a cooled glass Teflon homogenizer (ST- Mechanic Preczyina, Poland). The homogenates were spun at 8000 rpm for 20 minutes at 4°C using a centrifuge that was chilled (6 MR, USA). The supernatant was frozen for future use while the deposit was discarded.

**2.5.2. Protein assay**

The method described by Bradford (1976) was used to determine the total protein of *Cx. pipiens* larvae. Bovine Serum albumin was used as the standard and Coomasie brilliant blue G-250 as the dye binding to proteins.

**2.5.3. Esterases Assay:**

Alpha- and beta-naphthyl acetate was utilized as substrates for alpha- and beta-esterases, respectively, according to the technique of Van-Asperen (1962). 5ml substrate solution (3x10-4M alpha-or beta-naphthylacetate, 1% acetone, and 0.1M phosphate buffer, pH7) was added to 20l of larval homogenate. To halt the enzymatic process, 1 ml of diazoblue color reagent (made by combining 2 parts of 1% diazoblue B and 5 parts of 5% sodium lauryl sulphate) was added to the mixture after precisely 15 minutes of incubation at 27 degrees Celsius. The blank included five milliliters of substrate solution and one milliliter of diazo blue colouring reagent. The color of alpha-naphthol was measured at 600 nm and that of beta-naphthol at 555 nm. The absorbance of a single larva compared to the absorbance standard curve for known alpha and beta-naphthol concentrations, respectively. 20 mg of alpha- or beta-naphthol is diluted in 100 cc of pH7 phosphate buffer to prepare standard curves (Stock solution). To dilute the stock solution, 10 milliliters of the buffer were added to 90 milliliters of the buffer. 0.1, 0.2, 0.4, 0.8- and 1.6-ml aliquots of the diluted solution containing 2, 4, 8, 16, and 32 ug of naphthol were pipetted into test tubes, and phosphate buffer was added to increase the volume to 5 ml. 1 ml of the reagent diazo blue was administered, and a UV spectrophotometer was used to measure the color produced in comparison to a blank. The enzyme's activity was reported as ug of alpha/beta naphthol produced per minute per mg of larval protein.

**2.5.4. Glutathione-S-transferase (GST) assay:**

GST activity was estimated following the procedure of Habig *et al*., (1974). One ml of potassium phosphate buffer (pH 6.5) and 100µl of the reduced glutathione (GSH) were added to 200µl of the homogenate. The reaction started by adding 25µl of the substrate solution, 1-chloro 2,4-dinitrobenzene (CDNB). The CDNB and GSH concentrations were changed to 1 mM and 5 mM, respectively. The mixes were incubated for five minutes at 30 C. By using an ultraviolet spectrophotometer, the change in absorbance level was recorded at 340 nm every 30 seconds for five minutes against a blank. Reaction mixture without the enzyme was used as blank, to determine the nano mole substrate conjugated/ min/ larva by using the molar extinction coefficient, 9.6/mM/cm.

**Statistical analysis:**

The probit analysis (Finney, 1971) was used to the mortality data to determine the LC50 of each compound. In the investigations on the link between temperature and toxicity, the Chi-square test (X2) was undertaken for each temperature range. Version 11.5 (SPSS 2007) of the Statistical Package for Social Science (SPSS) software was used for analyses, and the significance level was set at P< 0.05. The biochemical test data were evaluated using the statistical program Costat and one-way analysis of variance (ANOVA) (Cohort Software, Berkeley). When the ANOVA results were significant (P < 0.01), Duncan's multiple range test was employed to compare the means.

**Results**

**Larvicidal activity**

*Cx. pipiens* 3rd instar larvae were used to determine the toxicity of *C. molmol* resin, Mirazid, crystalline and pharmaceutical form of acetylsalicylic acid after 48 h exposure at laboratory conditions. Results in **Table (1)** demonstrated that, all tested larvae were more sensitive to Mirazid with mortality percent 100% at a concentration 160 ppm, while *C. molmol* resin achieved 100% mortality at 320 ppm. The results also revealed that, the mortality percentage was increased by increasing the concentrations and this increase was very highly significant ((p≤0.001).

**Table (2)** showed the mortality percentages of *Cx. pipiens* 3rd instar larvae due to the exposure to different concentrations of CASA and PASA. Toxicity was significantly (p≤0.001) enhanced by increasing the concentrations, and the PASA was most toxic, it induced 100% mortality at a concentration of 1600 ppm, while the CASA achieved 92.8% at the same concentration.

The larvicidal activity of *C. molmol* resin, Mirazid, CASA and PASA to *Cx. pipiens* revealed that, Mirazid was the most potent compound, with LC50 of 28.3 ppm **(Table 3**). This compound was about 1.47, 28.26, 20.17 folds as toxic as *C. molmol* resin, CASA and PASA, respectively. In contrast, CASA was the least toxic compound with LC50 of 799.7 ppm.

**Effect of temperature on the toxicity of the tested compounds**

As indicated from **Table (4),** *Culex pipiens* displayed low mortality rates when exposed to the tested compounds at low temperature, but the mortality rate jumped when exposed to the tested compounds when maintained at high temperatures. At 15 °C the recorded mortalities were 48.2, 50.1, 44.1 and 46.2 % for larvae treated with *C. molmol* resin, Mirazid, CASA and PASA respectively. At 26 °C the mortality increased by about 13.69, 17.56, 17.00 and 13.63 %, whileat 36 °C increased by about 51.20, 56.08, 33.3 and 34.46 % for *C. molmol* resin, Mirazid, CASA and PASA respectively.This increase was significant (P< 0.05) in case of *C. molmol* resin, Mirazid.

**Biochemical effects:**

The biochemical assay results of *Cx. pipiens* 3rd instar larvae treated with the LC50 of Mirazid and PASA after 48 h from application at different temperatures were presented in tables (5-8).

**Table (5)** showed the mean total protein in mg protein/g. fresh body weight. The mean total protein increases remarkably in the control group by increasing the temperature from 15 0C to 26 0C, it was 65.6 ± 2.2 mg/g.bw at 15 0C and reached to 87.4 ± 6.17 mg/g.bw at 26 0C, then decreased again to 68.2 ± 16.8 mg/g.bw at 36 0C. Results also revealed that the mean total protein estimated in the larvae treated with Mirazid was 45.0±6.9, 65.8±1.65 and 43.5±5.55 mg/g.bw, while in the larvae treated with PASA, the protein content was 55.2 ±4.8, 80.5±4.98 and 52.4±2.95 mg/g.bw at 15, 26 and 36 0C respectively. This means that the tested materials induce reduction in the mean total protein than the control at all the tested temperature degrees and these differences were statistically significant.

**Table (6)** represented α-esterase activity in µg α-naphthol/min/g.bw. The obtained data indicates that, the activity of α-esterase in the control group was slightly increased by increasing the temperature from 15 Co to 26 0C. On other hand, the temperature 36 0C decreased α-esterase activity. A highly significant decrease (p≤0.01) was observed in α-esterase activity at 15 0C when the larvaeof *Cx. pipiens* were exposed to Mirazid and PASA for 48h, it was 146±24.3 and 150±11.4 µg α-naphthol/min/g.bw in Mirazid and PASA treated larvae, respectively compared to 257±40.6 µg α-naphthol/min/g.bw in the control. The activity significantly increased at 26 0C, the mean α-esterase activity was 214±26.6and 249±32.4 µg α-naphthol/min/g.bwin Mirazid and PASA treated larvae, respectively. At 36 0C the activity decreased again till reached 165.7±13 and 188.7±3.1in Mirazid and PASA treated larvae, respectively compared to 209.3±5.13 µg α-naphthol/min/g.bwin the control. This means that the tested materials induce reduction in α-esterase level of treated larvae than the control at all temperature degrees.

Data in **Table (7)** showed that, β-esterase activity of *Cx. pipiens* 3rd instar larvae increased in the control group from 240±15.3 µg β-naphthol/min/g.bw at 15 0C to 338.3±11.6 µg β-naphthol/min/g.bw at 26 Co after 48h exposure, then decreased again at 36 0C, it was 246.3±20.7 µg β–naphthol/min/g.bw The mean β-esterase activities of larvae treated with Mirazid and PASA were 280.6±13and 299.7±17.6 µg β–naphthol/min/g.bw, respectively at temperature 15 0C. These values were significantly increased than controls by 16.67 and 24.87% after 48h exposure, respectively. Treatment with Mirazid and PASA induces significant reduction in β-esterase activities of treated larvae at temperatures 26 Co and 36 0C, the activities were 303.3±17.4 and 173.7±15.9 µg β–naphthol/min/g.bw for Mirazid treated larvae and 328.3±10.7 and 179±3.60 µg β–naphthol/min/g.bw for PASA treated larvae compared to 338.3±11.6and 246.3±20.7 µg β–naphthol/min/g.bw of the control, respectively.

According to **Table (8)** the level of GST increased in the control groups by increasing the temperature, the mean activities were 17.8±3.37, 23 ±5.1and 45.2±4.87 41m mole sub conjugated./min/g.bw at 15, 26 and 36 0C, respectively. The treated larvae exhibited elevation in the activity of GST at temperature 15 0C and by 42.13%and 72.47%, and at 26 0C by 39.56% and 119.56% for Mirazid and PASA treated larvae, respectively. On the other side, the temperature 36 0C showed significant decrease (p≤0.05) in GST activity of treated larvae. The recorded activities were 34.9±2.76 and 41.6±1.41m mole sub conjugated./min/g.bw in Mirazid and PASA treated larvae, respectively compared to 45.2±4.87 m mole sub conjugated./min/g.bw of the control.

**Discussion**

In our study we determined the toxicity of *C. molmol* resin, Mirazid, CASA and PASA against the 3rd larval instar of *Cx. pipiens*, as well as temperature-toxicity relationship of the tested compounds to *Cx. pipiens*.

*Cx. pipiens* larvae were shown to be vulnerable to the investigated materials, with Mirazid proving to be the most harmful. Myrrh resin consists of chemical compounds with poisonous characteristics (Ahamad *et al*., 2017). Muturi *et al*. (2020) discovered that many representatives of the Commiphora genus generate mosquito-repelling bioactive chemicals. The biochemical study of total proteins in *Cx. pipiens* larvae indicated that the observed toxicity was likely caused by the suppression of certain detoxifying enzyme activities (Massoud *et al*. 2001). Mirazid was more efficacious than *C. molmol* resin as a result of its higher myrrh volatile oil content (Massoud *et al*. 2012). Massoud and Labib (2000) and Habeeb *et al*. (2009) revealed that the oleo-resin extract and the essential oil of *C. molmol* were harmful to the larvae of *Cx. pipiens*. Other plant extracts and essential oils from the Commiphora genus have shown toxicity against mosquitoes (Baranitharan and Dhanasekaran 2014; da Silva *et al*., 2015; Muturi *et al*., 2020). Our findings demonstrate that acetylsalicylic acid isolated from *salix safaf* in either crystalline or pharmaceutical form is larvicidal against *Cx. pipiens*. Mondal *et al*. (2014) demonstrated larvicidal activity of salicylic acid and 3, 5-di nitro salicylic acid against *Cx. quinquefasciatus*, which is consistent with our results. Alvandy *et al.* (2014) found that *Salix alba* L. extract in various solvents had larvicidial effects on *Ephestia kuehniella* larvae and toxic effects on *Musca domestica* (Mansour *et al.,* 2011; Selem & El-Sheikh., 2015; Hasaballah *et al,* 2021)

The results revealed that when the temperature climbed, *Cx. pipiens'* sensitivity increased dramatically. Due to the greater irritation of mosquitoes at higher temperatures, there may be a more rapid absorption of the drug, a quicker knockdown, and a larger mortality rate (Hodjati and Curtis 1999). The increased chemical penetration into the bodies of *Cx. pipiens* larvae may have also contributed to the increased toxicity. At low temperatures, the toxicity of the compounds decreased, perhaps because the biotransformation process slowed down (Khan and Akram, 2014; Swelam *et al*., 2022). Our findings corresponded with those of El-Sayed and El-Bassiony (2016), Agyekum *et al*. (2021), and Salinas *et al*. (2021), who found that an increase in temperature is often associated with an increase in the toxicity of pesticides to several mosquito species.

To provide a reasonable explanation for the temperature-dependent variation in the reactivity of *Cx. pipiens* larvae to the utilized chemicals, biochemical experiments were done on the average total protein and three enzymes known to be involved in pesticide detoxification. Alpha-esterase, beta-esterase, and GST are the enzymes. Temperature influences not just pesticide components but also essential proteins and enzymes for the survival of a variety of species (Imasheva *et al*. 1998).

The present data showed that, the mean total protein increased by increasing the temperature from 15 to 26 0C, then decreased again at 36 0C and the tested materials induced reduction in the mean total protein than the control at all temperature degrees. It was possible that, Mirazid and PASA treatment led to decreased feeding (Tarigan *et al*. 2016). Consistent with our findings [Massoud](http://europepmc.org/search?page=1&query=AUTH:%22Massoud+AM%22&restrict=All+results) *et al*. (2001), Larson *et al*. (2010) and Dris *et al*. (2017) had reported a significant reduction in the mean total protein of the larvae of different mosquito species treated with plant extracts. Sonmez and Gulel (2008) and Zayed *et al*. (2019) evaluated the temperature impact on the total protein of the Bean Beetle *Acanthoscelides obtectus* and*Cx. pipiens* treated with different compounds and obtained comparable results.

Inhibition of enzyme activity is a well-known method for halting a vast variety of crucial physiological and biochemical processes (Zorlu *et al*., 2018). In our investigation, the activities of alpha- and beta-esterase were lower in treated than in untreated larvae at all temperatures. In response to temperature, the alpha- and -esterase activity rose from 15 Co to 26 0C, then declined at 36 0C. The insect's protein content may have decreased, which may account for the decline in activity (Oni *et al*., 2019). Fahmy and Amin (2019) reported a similar tendency, observing that the activity of alpha- and beta- esterases in the red palm weevil increased as the temperature climbed, until the ideal temperature was achieved, and then declined. Our findings are similar to those of several other studies using other mosquito species, such as those by Koodalingam *et al*. (2011), Lija-Escaline *et al*. (2015), Karthi *et al*.

At temperatures of 15 and 26 degrees Celsius, the examined compounds significantly increased GST activity in treated larvae, indicating that this enzyme plays a major role in detoxification. At 36 degrees Celsius, however, GST activity reduced. GST is an 85 percent protein-based enzyme that plays a critical role in the detoxification of hazardous substances that enter insect bodies, therefore the reduction in its activity produced by Mirazid and PASA may be connected to a decrease in the insect's protein content at 36 degrees Celsius. The reduction or stimulation of enzyme activity in insects exposed to entomotoxic plants may result in metabolic imbalance (Agra-Neto *et al*. 2015**)**. Results accord with Zayed *et al*. (2019), who showed a significant increase in GST activity in *Cx. pipiens* larvae and adults in response to a temperature rise from 20 to 30 degrees Celsius. Similar trend was observed by [Shahat](https://eajbse.journals.ekb.eg/?_action=article&au=287418&_au=Mohamed+A.M.+Shahat) *et al*. (2020), Sengodan *et al*. (2020) and Prakash *et al*. (2021).

**Conclusion**

This study explores the efficacy of *C. molmol* resin, Mirazid, CASA, and PASA in controlling *Cx. pipiens*. It was observed that larval mortality climbed as temperatures rose. High larval exposure temperatures modify the activity levels of alpha-esterase, beta-esterase, and GST enzymes, which play a crucial role in insect resistance to insecticides. It could be concluded that, the temperature plays a fundamental role not only on development and survival of mosquito larvae but also on the susceptibility to some compounds by affecting the level of detoxification enzymes which interfere with insect resistance.

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**References**

Ahmad SR, Al-Ghadeer A, Ali R, Qamar W, Aljarboa S (2017) Analysis of inorganic and organic constituents of myrrh resin by GC–MS and ICP-MS: An emphasis on medicinal assets. [Saudi Pharm J](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5506701/) 25(5): 788–794

# [Agra-Neto](https://www.researchgate.net/scientific-contributions/Afonso-Cordeiro-Agra-Neto-2035245382) AC, [Napoleão](https://www.researchgate.net/scientific-contributions/Thiago-Henrique-Napoleao-35532799) TH, [Pontual](https://www.researchgate.net/profile/Emmanuel-Pontual) E, [Santos](https://www.researchgate.net/scientific-contributions/Nataly-Diniz-de-Lima-Santos-35023879) NDL (2015) Effect of *Moringa oleifera* lectins on survival and enzyme activities of *Aedes aegypti* larvae susceptible and resistant to organophosphate. [Parasitol Res](https://www.researchgate.net/journal/Parasitology-Research-1432-1955) doi:[10.1007/s00436-013-3640-8](http://dx.doi.org/10.1007/s00436-013-3640-8" \t "_blank)

Alvandi S, Rafiei KZ, Nabaei SM (2014) Investigation on the larvicidial effects of *Salix alba* L. and *Pinus sylvestris* L. extracted in different solvents on larvae of flour moth *Ephestia kuehniela* (Zel.) (Lep. Pyralidae): [Journal of Entomological Research](https://www.sid.ir/en/Journal/JournalList.aspx?ID=13999)  6(2): 121 – 128

[Agyekum](https://pubmed.ncbi.nlm.nih.gov/?term=Agyekum%20TP%5BAuthor%5D) TP, [Botwe](https://pubmed.ncbi.nlm.nih.gov/?term=Botwe%20PK%5BAuthor%5D) PK,  [Arko-Mensah](https://pubmed.ncbi.nlm.nih.gov/?term=Arko-Mensah%20J%5BAuthor%5D) J, [Issah](https://pubmed.ncbi.nlm.nih.gov/?term=Issah%20I%5BAuthor%5D) I, [Acquah](https://pubmed.ncbi.nlm.nih.gov/?term=Acquah%20AA%5BAuthor%5D) AA, et al. (2021) A systematic review of the effects of temperature on *Anopheles* mosquito development and survival: implications for malaria control in a future warmer climate. Int J Environ Res Public Health 18(14): 7255 doi: [10.3390/ijerph18147255](https://doi.org/10.3390%2Fijerph18147255)

Baranitharan M, Dhanasekaran S (2014) Mosquito larvicidal properties of *Commiphora caudata* (Wight & Arn.) (Bursaceae) against *Aedes aegypti* (Linn.) *Anopheles stephensi* (Liston), and *Cx. quinquefasciatus* (Say). Int J Curr Microbiol App Sci 3: 262–268

Bradford MM (I976) A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal Biochem 72:248-254

Christiansen-Jucht CD, Parham PE, Saddler A, Koella JC, Basanez MG (2014) Temperature during larval development and adult maintenance influences the survival of *Anopheles gambiae* ss. Parasites & vectors 7(1): 489-500

da Silva RC, Milet-Pinheiro P, da Silva PCB, da Silva AG, et al. (2015): (E)-caryophyllene and α-humulene: *Aedes aegypti* oviposition deterrents elucidated by gas chromatography-electrophysiological assay of *Commiphora leptophloeos* leaf oil. Plos One 10: e0144586

Delatte H, Gimonneau G, Triboire A Fontenille D (2009) Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes albopictus*, vector of chikungunya and dengue in the Indian Ocean Journal of Medical Entomology 46: 33–41

Dris D, Tine-Djebbar F, Bouabida H, Soltani N (2017) Chemical composition and activity of an *Ocimum basilicum* essential oil on *Culex pipiens* larvae: Toxicological, biometrical and biochemical aspects. South African Journal of Botany 113: 362-369

[Elono](https://pubmed.ncbi.nlm.nih.gov/?term=Meyabeme+Elono+AL&cauthor_id=29757520) ALM,  [Foit](https://pubmed.ncbi.nlm.nih.gov/?term=Foit+K&cauthor_id=29757520)  [K](https://pubmed.ncbi.nlm.nih.gov/29757520/#affiliation-1),  [Duquesne](https://pubmed.ncbi.nlm.nih.gov/?term=Duquesne+S&cauthor_id=29757520) S,  [Liess](https://pubmed.ncbi.nlm.nih.gov/?term=Liess+M&cauthor_id=29757520) M (2018) Controlling *Culex pipiens*: antagonists are more efficient than a neonicotinoid insecticide. J Vector Ecol 43(1):26-35

El-Sayed SH, El-Bassiony GM (2016): Larvicidal, biological and genotoxic effects, and temperature-toxicity relationship of some leaf extracts of *Nerium oleander* (Apocynaceae) on *Culex pipiens* (Diptera: Culicidae). J Arthropod-Borne Dis 10(1): 1–11

Fahmy NM, Amin TR (2019) Partial kinetic analysis of haemolymph esterases from the red palm weevil; *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Curculionidae). Egypt Acad J Biolog Sci (C. Physiology and Molecular biology) 11(3): 169-180

Finney DJ (1971) Probit analysis. Cambridge Univ. Press, Cambridge, 333 pp.

Grbić LM, Unković N, Dimkić I, Janaćković P et al. (2018) Frankincense and myrrh essential oils and burn incense fume against micro-inhabitants of sacral ambients. Wisdom of the ancients. J Ethnopharmacol 219: 1–14

Habeeb SM, El-Namaky AH, Salama MA (2009) Efficiency of *Allium cepa* and *Commiphora molmol* as a larvicidal agent against fourth stage larvae of *Culex pipiens* (Diptera: Culicidae). American-Eurasian J Agric Environ Sci 5: 196–203

Habig WH, Pabst MJ, Jakoby WB (I974) Glutathione S-transferase the first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130-7139

Hasaballah AI, Selim TA, Tanani MA, Nasr EE (2021) Lethality and vitality efficiency of different extracts of *Salix safsaf* leaves against the house fly, *Musca domestica* L. (Diptera: Muscidae). African Entomology 29(2): 479-490

### [Hodjati MH, Curtis CF (1999) Effects of permethrin at different temperatures on pyrethroid](https://pubmed.ncbi.nlm.nih.gov/10608231/)-resistant and susceptible strains of Anopheles. Medical and veterinary Entomology 13: 415-422

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol 59: 41–66

Imasheva AG, Loeschcke V, Lazebny O (1998) Stress temperatures and quantitative variation in *Drosophila melanogaster*. Semantic Scholar 81(3): 246-253

Karthi S, Uthirarajan K, Manohar V, Venkatesan M, Chinnaperumal K, Vasantha SP, Krutmuang P (2020) Larvicidal enzyme inhibition and repellent activity of red mangrove *Rhizophora mucronata* (Lam.) leaf extracts and their biomolecules against three medically challenging arthropod vectors. Molecules 25(17): 3844-63

Kaur T, Walter NS, Kaur U, Sehgal R (2022) Different Strategies for Mosquito Control: Challenges and Alternatives. Mosquito-Research Advances in Pathogen Interactions, Immunity, and Vector Control Strategies. doi:10.5772/intechopen.104594

[Khan](https://pubmed.ncbi.nlm.nih.gov/?term=Khan%20HA%5BAuthor%5D) HAA, [Akram](https://pubmed.ncbi.nlm.nih.gov/?term=Akram%20W%5BAuthor%5D) W (2014) The effect of temperature on the toxicity of insecticides against Musca domestica L.: Implications for the effective management of diarrhea. PLoS One, 9(4): e95636. doi: [10.1371/journal.pone.0095636](https://doi.org/10.1371%2Fjournal.pone.0095636)

[Koodalinga](https://www.researchgate.net/profile/Arunagirinathan-Koodalingam)m A, [Mullainadhan](https://www.researchgate.net/scientific-contributions/Periasamy-Mullainadhan-38610485) P, [Munusami](https://www.researchgate.net/profile/Arumugam-Munusami) A (2011) Effects of extract of soap nut *Sapindus emarginatus* on esterases and phosphatases of the vector mosquito, *Aedes aegypti* (Diptera: Culicidae). [Acta Tropica](https://www.researchgate.net/journal/Acta-Tropica-0001-706X) 118(1):27-36

Larson RT, Lorch JM, Pridgeon JW, Becnel JJ, Clark GG, Lan Q (2010) The biological activity of α-mangostin, a larvicidal botanic mosquito sterol carrier protein-2inhibitor. [Journal of Medical Entomology](https://www.researchgate.net/journal/Journal-of-Medical-Entomology-1938-2928) 47(2):249-57

# Lija-Escaline J, Senthil-Nathan S, Thanigaivel A Pradeepa et al. (2015) Physiological and biochemical effects of botanical extract from Piper nigrum Linn (Piperaceae) against the dengue vector Aedes aegypti Liston (Diptera: Culicidae). [Parasitology Research](https://link.springer.com/journal/436) 114: 4239–4249

Liu H, Xie L, Cheng P, Xu J et al. (2019) Trends in insecticide resistance in *Culex pipiens* pallens over 20 years in Shandong, China. Parasites & Vectors 12(167): 1-9

Mansour SA, Bakr RFA, Mohamed RI, Hasaneen NM (2011) Larvicidal activity of some botanical extracts, commercial insecticides and their binary mixtures against the housefly, *Musca domestica* L. The Open Toxinology Journal 5(1): 1-14

Mapossa AB, Focke WW, Tewo RK, Androsch R, Kruger T (2021) Mosquito-repellant controlled release formulations for fighting infectious diseases. Malaria Journal 20(1):165. doi: 10.1186/s12936-021-03681-7

[Massoud](https://www.researchgate.net/scientific-contributions/A-M-Massoud-2029570826) A M,  [Labib](https://www.researchgate.net/scientific-contributions/I-M-Labib-34249782) I M (2000) Larvicidal activity of *Commiphora molmol* against *Culex pipiens* and *Aedes caspius* larvae. [Journal of the Egyptian Society of Parasitology](https://www.researchgate.net/journal/Journal-of-the-Egyptian-Society-of-Parasitology-0253-5890) 30(1):101-15

Massoud AM, Labib IM, Rady M (2001) Biochemical changes of *Culex pipiens* larvae treated with oil and oleo-resin extracts of Myrrh *Commiphora molmol*. [Journal of the Egyptian Society of Parasitology](https://www.researchgate.net/journal/Journal-of-the-Egyptian-Society-of-Parasitology-0253-5890)  31 (2): 517-529.‏

Massoud AM, Shalaby HA, El Khateeb RM, Mahmoud MS, Kutkat MA (2012) Effects of Mirazid ® and myrrh volatile oil on adult *Fasciola gigantica* under laboratory conditions. Asian Pacific J of Trop Biomed 2(11): 875-884

Mondal RP, Ghosh A, Chandra G (2014) Mosquito larvicidal potential of salicylic acid and 3, 5-di nitro salicylic acid against filarial vector *Culex quinquefasciatus*. Journal of Mosquito Research 4 (1): 21-26

Muturi EJ, Hay WT, Doll KM, Ramirez JL, Selling G (2020) Insecticidal activity of *Commiphora erythraea* essential oil and its emulsions against larvae of three mosquito species. Journal of Medical Entomology 57(6): 1835–1842

Oni, M O, Ogungbite OC, Oguntuase SO (2019) Inhibitory effects of oil extract of green Acalypha (*Acalypha wilkesiana*) on antioxidant and neurotransmitter enzymes in *Callosobrucus maculatus*. The Journal of Basic and Applied Zoology 80:47 <https://doi.org/10.1186/s41936-019-0116-0>

# [Prakash](https://sciprofiles.com/profile/1459436) P, Gayathiri E, Manivasagaperumal R, Krutmuang P (2021) Biological activity of root extract *Decalepis hamiltonii* (Wight & Arn) against three mosquito vectors and their non-toxicity against the mosquito predators. Agronomy*,*11(7), 1267; <https://doi.org/10.3390/agronomy11071267>

Regnault-Roger C, Vincent C. and Arnason JT (2012) Essential oils in insect control: low-risk products in a high-stakes world. Annu Rev Entomol 57: 405–424

Salinas WS, Feria-Arroyo TP, Vitek CJ (2021) Temperatures influence susceptibility to insecticides in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) mosquitoes. Pathogens 10(8):992. doi: 10.3390/pathogens10080992

[Sayed](https://www.researchgate.net/profile/Rehab-Sayed-4) R, [R. S. Abdalla](https://www.researchgate.net/scientific-contributions/Ragaa-S-Abdalla-2119367901), [Salwa Rizk](https://www.researchgate.net/profile/Salwa-Rizk-2), [T. S. El sayed](https://www.researchgate.net/scientific-contributions/Tammy-El-sayed-2165797749) (2018) Control of *Culex pipiens* (Diptera: Culicidae), the vector of lymphatic filariasis, using irradiated and non-irradiated entomopathogenic nematode, *Steinernema scapterisci* (Rhabditida: Steinernematidae). [Egyptian Journal of Biological Pest Control](https://www.researchgate.net/journal/Egyptian-Journal-of-Biological-Pest-Control-2536-9342) 28(1): doi:[10.1186/s41938-018-0070-z](http://dx.doi.org/10.1186/s41938-018-0070-z" \t "_blank)

Selem GS, El-Sheikh EA (2015) Toxicity and biochemical effects of Neem Azal T/S, willow (*Salix aegyptiaca* L.) and chaste berry (*Vitex agnus-Castus* L.) on house fly, *Musca domestica* L. (Diptra: Muscidae). Journal of Biopesticides 8(1): 37-44

|  |  |
| --- | --- |
| Sengodan K, Karthic U, Vinothkumar M, Manigandan V, Kamaraj C, Prabhakaran V, Patcharin K (2020) Larvicidal enzyme inhibition and repellent activity of red mangrove *Rhizophora mucronata* (Lam.) Leaf extracts and their biomolecules against three medically challenging arthropod vectors.Molecules 25(17): 3844.doi:[10.3390/molecules25173844](http://doi.org/10.3390/molecules25173844)  Shahat MA, El-Sheikh TM, Hammad KM, Hasaballah AI, Shehata AZ (2020) Effect of some plant extracts on the biochemical parameters, AChE and GST activities of the mosquito, *Culex pipiens* L. (Diptera: Culicidae). Egyptian Academic Journal of Biological Sciences, E. Medical Entomology & Parasitology, 12(2), 69-80‏ [Sonmez](http://ascidatabase.com/author.php?author=Evrim&last=Sonmez) E, [Gulel](http://ascidatabase.com/author.php?author=Adem&last=Gulel) A (2008) Effects of different temperatures on the total carbohydrate, lipid and protein amounts of the bean beetle, *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). Pakistan Journal of Biological Sciences 11 (14): 1803-1808 |  |

Swelam ES, [Abdel-Rahman](https://www.sciencedirect.com/science/article/abs/pii/S1878818122000044#!) HR, [Mossa](https://www.sciencedirect.com/science/article/abs/pii/S1878818122000044#!) AT, [Ahmed](https://www.sciencedirect.com/science/article/abs/pii/S1878818122000044#!) FS (2022) Influence of temperature on the toxicity of fipronil to *Spodoptera littoralis* (Boisd.) Lepidoptera: Noctuidae). [Biocatalysis and Agricultural Biotechnology](https://www.sciencedirect.com/journal/biocatalysis-and-agricultural-biotechnology) [Volume 39](https://www.sciencedirect.com/journal/biocatalysis-and-agricultural-biotechnology/vol/39/suppl/C), <https://doi.org/10.1016/j.bcab.2022.102277>

Tarigan S, Dadang I, Harahap SI (2016) Toxicological and physiological effects of essential oils against *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Callosobruchus maculatus* (Coleoptera: Bruchidae). Journal of Biopesticides 9(2): 135–147

Terrie LC (1984) Induction of detoxification enzymes in insects. Annual Review of Entomology 29: 71–88

Van-Asperen K (I962) A study of house fly esterase by means of sensitive colorimetric method. Journal of Insect Physiology 8: 401-416

Vinogradova EB (2000) *Culex pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control. Sofia, Moscow. 250 p.

[Wang](https://pubmed.ncbi.nlm.nih.gov/?term=Wang%20Y%5BAuthor%5D) Y, [Cheng](https://pubmed.ncbi.nlm.nih.gov/?term=Cheng%20P%5BAuthor%5D) P,  [Jiao](https://pubmed.ncbi.nlm.nih.gov/?term=Jiao%20B%5BAuthor%5D) B, [Song](https://pubmed.ncbi.nlm.nih.gov/?term=Song%20X%5BAuthor%5D) X et al. (2020) Investigation of mosquito larval habitats and insecticide resistance in an area with a high incidence of mosquito-borne diseases in Jining, Shandong Province. PLoS One 15(3): e0229764. doi: [10.1371/journal.pone.0229764](https://doi.org/10.1371%2Fjournal.pone.0229764)

WHO (2005) Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization: Geneva, Switzerland

Wilson AL, Courtenay O, Kelly-Hope LA, Scott TW et al. (2020) The importance of vector control for the control and elimination of vector-borne diseases. PLoS Neglected Tropical Diseases 14(1):e0007831. doi: 10.1371/journal.pntd.0007831

Zayed AB, Mostafa AA, Moselhy WA, Mahmoud HI, Hassan SH (2019) Influence of temperature change on the growth and susceptibility of the common house mosquito, *Culex pipiens* in Egypt to some insecticides. International Journal of Ecotoxicology and Ecobiology 4(2): 42-50, doi: 10.11648/j.ijee.20190402.11

Zorlu T, Nurullahoğlu ZU, Altuntaş H (2018) Influence of dietary titanium dioxide nanoparticles on the biology and antioxidant system of model insect, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae).  [Journal of the Entomological Research Society](https://www.entomol.org/journal/index.php/JERS/issue/view/20%283%292018) 20(3): 89-103

**Table 1** Larvicidal activity of *C. molmol* resin and Mirazid against 3rd instar larvae of *Cx. pipiens*, 48 h post-treatment at laboratory conditions

|  |  |  |
| --- | --- | --- |
| Conc.(ppm) | Mortality % ± S.E | |
| *C. molmol* resin | Mirazid |
| Control | 0.8±0.80e | 0.8±0.80f |
| 10 | 12.0±2.53e | 23.2±2.94e |
| 20 | 28.8±3.44d | 36.8±3.20d |
| 40 | 45.6±4.66c | 56.8±6.50c |
| 80 | 70.4±7.33b | 82.4±5.31b |
| 160 | 88.0±5.37a | 100±0.00a |
| 320 | 100±0.00a | 100±0.00a |
| P value | 0.000 \*\*\* | 0.000 \*\*\* |
| LSD0.05 | 12.15 | 10.38 |

Means followed by the same superscript letter within a column are not significantly different (P>0.05), \*\*\* very highly significant (p≤0.001), LSD = least significant difference S.E= Standard Error

**Table 2** Larvicidal activity of crystalline and pharmaceutical form of acetylsalicylic acid against 3rd instar larvae of *Cx. pipiens* 48 hours post-treatment at laboratory conditions

|  |  |  |
| --- | --- | --- |
| Conc.(ppm) | Mortality % ± S.E | |
| CASA | **PASA** |
| Control | 0.8±0.80f | 1.6±0.98f |
| 200 | 11.2±2.33e | 12.0±3.79e |
| 400 | 22.4±4.83d | 27.2±3.88d |
| 800 | 39.2±1.50c | 60.0±3.35c |
| 1200 | 56.0±4.00b | 90.4±2.71b |
| 1600 | 92.8±4.45a | 100±0.00a |
| 2000 | 100±0.00a | 100±0.00a |
| P value | 0.000 \*\*\* | 0.000 \*\*\* |
| LSD0.05 | 8.99 | 7.66 |

Means followed by the same superscript letter within a column are not significantly different (P>0.05), \*\*\* very highly significant (p≤0.001), LSD = least significant difference S.E= Standard Error

**Table 3** Relative efficiency of *C. molmol* resin, Mirazid, CASA and PASA to *Culex pipiens* 3rd instar larvae after 48 h exposure at laboratory conditions

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compounds | LC50 | 95% Confidence limits  (ppm) | | Slope | χ2 (d.f.) |
| Lower | Upper |
| *C. molmol* | 41.6 | 32.5 | 50.7 | 1.08 | 2.01 (3) |
| Mirazid | 28.3 | 24.2 | 32.7 | 3.02 | 2.20 (3) |
| CASA | 799.7 | 698.5 | 980.9 | 0.06 | 3.06 (3) |
| PASA | 570.9 | 490.5 | 651.3 | 2.24 | 1.06 (4) |

*X2*: Chi-square value; *d.f.*: degree of freedom

**Table 4** Temperature-toxicity relationship of *C. molmol* resin, Mirazid, CASA and PASA to *Culex pipiens* larvae, previously treated with the LC50 of each compound

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Compound | Temperature | | | | | |
| 15 °C | | 26 °C | | 36 °C | |
| M %±SE | χ2 (d.f.) | M %±SE | χ2 (d.f.) | M %±SE | χ2 (d.f.) |
| *C. molmol* | 48.2±3.66 | 2.27 (4) | 54.8±4.85 | 2.91 (4) | 72.88±3.20 | 11.14\*(4) |
| Mirazid | 50.1±3.20 | 0.06 (4) | 58.9±3.27 | 7.76 (4) | 78.20±2.94 | 30.04\*(4) |
| CASA | 44.1±1.52 | 4.01 (4) | 51.6±2.17 | 0.07 (4) | 58.8±1.26 | 2.70 (4) |
| PASA | 46.2±3.35 | 2.53 (4) | 52.5±3.88 | 2.07 (4) | 62.12±4.29 | 9.04 (4) |

M%: mortality %; SE: standard error; *X2*: Chi-square value; *d.f.*: degree of freedom

**Table 5** Effect of temperature on the total protein content of *Cx. pipiens* 3rd instar larvae treated with Mirazid and PASA after 48h exposure

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Tested  Material | Mean total protein content (mg/g body weight) | | | | | |
| At 15 0C | | At 26 0C | | At 36 0C | |
| Mean±SD | Change% | Mean±SD | Change% | Mean±SD | Change% |
| Control | 65.6±2.2a | - | 87.4±6.17a | - | 68.2±16.8a | - |
| Mirazid | 45.0 ±6.9b | -31.40 | 65.8±1.65b | -24.71 | 43.5±5.55b | -36.21 |
| PASA | 55.2±4.8c | -15.89 | 80.5±4.98a | -7.89 | 52.4±2.95c | -23.16 |
| P value | 0.007\*\* | - | 0.036\* | - | 0.006\*\* | - |
| LSD(0.05) | 10.01 | - | 9.35 | - | 7.517 | - |

Means within a column and followed by the same letter are not significantly different (p >0.05), SD; standard deviation, p; probability, LSD; least significant difference, **\***; significant (p≤0.05), **\*\***; highly significant (p≤0.01)

**Table 6** Effect of temperature on α-esterase activity of *Cx. pipiens* 3rd instar larvae treated with Mirazid and PASA after 48h exposure

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Tested  Material | α-esterase activity (µg α-naphthol/min/g body weight) | | | | | |
| At 15 0C | | At 26 0C | | At 36 0C | |
| Mean±SD | Change% | Mean±SD | Change% | Mean±SD | Change% |
| Control | 257±40.6a | - | 257.3±39.6a | - | 209.3±5.13a | - |
| Mirazid | 146±24.3b | - 43.19 | 214±26.6 a | - 16.83 | 165.7±13b | -20.83 |
| PASA | 150±11.4b | - 41.63 | 249±32.4 a | - 3.23 | 188.7±3.1b | -9.84 |
| P value | 0.0041\*\* | - | 0.319NS | - | 0.003\*\* | - |
| LSD(0.05) | 55.14 | - | 67.29 | - | 17.186 | - |

Means within a column and followed by the same letter are not significantly different (p >0.05), SD; standard deviation, p; probability, LSD; least significant difference, **NS**; non significant, **\*\***; highly significant (p≤0.01)

**Table 7** Effect of temperature on β-esterase activity of *Cx. pipiens* 3rd instar larvae treated with Mirazid and PASA after 48h exposure

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Tested  Material | β-esterase activity (µg β-naphthol/min/g body weight) | | | | | |
| At 15 0C | | At 26 0C | | At 36 0C | |
| Mean±SD | Change% | Mean±SD | Change% | Mean±SD | Change% |
| Control | 240±15.3**a** | - | 338.3±11.6a | - | 246.3±20.7a | - |
| Mirazid | 280.6±13b | 16.92 | 303.3±17.4b | -10.35 | 173.7±15.9b | -29.47 |
| ASA | 299.7±17.6b | 24.87 | 328.3±10.7a | -2.96 | 179±3.60b | -27.32 |
| P value | 0.03\* | - | 0.05**\*** | - | 0.009**\*\*** | - |
| LSD(0.05) | 29.44 | - | 28.58 | - | 30.46 | - |

Means within a column and followed by the same letter are not significantly different (p >0.05), SD; standard deviation, p; probability, LSD; least significant difference, **\***; significant (p≤0.05), **\*\***; highly significant (p≤0.01)

**Table 8** Effect of temperature on GST activity of *Cx. pipiens* 3rd instar larvae treated with Mirazid and PASA after 48h exposure

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Tested  Material | GST activity (m mole sub conjugated/min/g body weight) | | | | | | |
| At 15 0C | | | At 26 0C | | At 36 0C | |
| Mean±SD | | Change% | Mean±SD | Change% | Mean±SD | Change% |
| Control | 17.8±3.37a | - | | 23 ±5.1a | - | 45.2±4.87a | - |
| Mirazid | 25.3±3.8ab | 42.13 | | 32.1±4.46b | 39.56 | 34.9±2.76b | -22.78 |
| PASA | 30.7±2.9b | 72.47 | | 50.5±6.4c | 119.56 | 41.6±1.41a | -7.96 |
| P value | 0.0075\*\* | - | | 0.0122\* | - | 0.0233\* | - |
| LSD(0.05) | 9.969 | - | | 7.781 | - | 6.64 | - |

Means within a column and followed by the same letter are not significantly different (p >0.05), SD; standard deviation, p; probability, LSD; least significant difference, **\***; significant (p≤0.05), **\*\***; highly significant (p≤0.01)

|  |  |
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**Fig. 1** *Commiphora molmol* oleo gum resin (a) and Pharmaceutical form (myrrh derivative) Mirazid (b)

|  |  |
| --- | --- |
| C:\Users\Dr.Abla\Pictures\bs.tif  a |  |
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**Fig. 2** Salix tree **(a)** Crystalline acetylsalicylic acid (b) and pharmaceutical form of ASA Rivo or Green Aspirin (c)