Crude Volatile Oil Derived from Sphaeranthus Indicus *Linn* (Asteraceae) Comparative Insecticidal Activity Against Agriculture Pests Spodoptera Litura and Plutella Xylostella

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**ABSTRACT:** The goal of this work is to compare the Larvicidal activity of plant-derived essential oils against Spodoptera litura and Plutella xylostella and eliminate the chemical burden in managing agricultural pests. Pest rearing, homology modelling, and molecular docking are among of the approaches used in this study. The G Power value is set at 0.8, with two sets of five sample sizes each. At 500 ppm, the death rate was highest in all instars: 93.1% (II instar), 85.2% (III instar), and 81.2% (IV instar). The death rate was lower at the lowest dose of 100 ppm across all larval instars. Both pests' second instar larvae were more vulnerable at 500 ppm and was considerably different from other treatment dosages. On third instar larvae, the fatal values (LC50 and LC90) were 213.17 and 421.88 ppm, respectively. When compared to other chemical compounds, essential oil extracted from Sphaeranthus indicus Linn (Asteraceae) has effective insecticidal action against agricultural pests Spodoptera litura and Plutella xylostella.

**Keywords:** Sphaeranthus indicus, Spodoptera litura, Plutella xylostella, Pest, Mortality, Novel Essential oils, Protein, Docking.

1 Introduction

Plants are attractive options for the treatment of insect pests due to their ecotoxicological properties, which include decreased toxicity to people, cheap, simple cultivation and degradation, as well as less environmental impact [1]. Furthermore, aromatic plants have demonstrated efficacy as insecticides, and their novel essential oils (EOs) frequently make up the bioactive component [2]. Novel essential oils have demonstrated high insect mortality rates, antifeedant and repellent qualities, and have been utilized successfully in a variety of applications, including fumigation [3]. Plant metabolites work by either interfering with GABA-gated chloride channels or having an impact on the octopaminergic neural system of pest insect pests. Other impacts include changes in behavior (attraction/repulsion) and contact toxicity during various phases of the target [4].

In the past few years, more than 1500 research and review articles were published in PubMed related to bio-active pesticides. The most cited article is [5] *Sphaeranthus indicus*
Linn belongs to the family Asteraceae. It is commonly found in the Indian subcontinent that had been traditionally used for treating various ailments. Recent scientific studies have shown that the methanolic extract of *Sphaeranthus indicus* Linn (Asteraceae) has significant insecticidal activity against two major agricultural pests, *Spodoptera litura* and *Plutella xylostella*. The extract was found to be effective in controlling the larvae of these pests, reducing their mortality rate by up to 77% [6]. Furthermore, the researchers noted that the extract was also able to reduce the hatching rate of the eggs of the pests by up to 68.4%. These findings suggest that the methanolic extract of *Sphaeranthus indicus* Linn (Asteraceae) can be an effective tool in combating the infestation of these pests in agricultural fields [7]. It is believed that the active component of the oil, anethole, is responsible for its insecticidal activity [8]. The oil has also been found to have anti-inflammatory and anti-bacterial properties. It can be used as an alternative to conventional chemical insecticides in the control of the aforementioned pests. *Spodoptera litura*, otherwise known as the tobacco cutworm or the Chinese cutworm, is an agricultural pest belonging to the family Noctuidae. It has now spread to many other parts of the world, including the United States, Europe, India, and Australia. The larvae of this species feed on the leaves of many crops, such as tobacco, cotton, and soybeans, as well as a wide variety of other plants. They are considered significant agricultural pests due to their voracious appetite and ability to cause damage to crops. Adult moths are light brown in color, with yellow-brown forewings and hindwings. The larvae are dark green to black in color, with white stripes running down their backs. They can reach up to two inches in length. The diamondback moth, also known as *Plutella xylostella*, is a species of moth that may be found all over the world. It is a significant agricultural pest that preys on cruciferous plants like broccoli and cabbage as well as a number of other vegetable crops. This species, which is endemic to Europe, has spread throughout the world due to its vast range of hosts, capacity to consume a wide range of plants, and quick adaptation to new settings. The larvae of this species have characteristic diamond-shaped markings on their backs and are often yellow-green in color. They are common in great numbers during the summer and can seriously harm crops. Little and gray adult moths with longitudinal bands of yellowish brown color.

### 2 Materials and Methods

Molecular docking is performed using the Swiss model (Saves 6.0), Q-Mean (4.3) and Pyrx (4.0). The G Power value of the study is 0.8. The study was performed in vitro at the department of bioinformatics, Saveetha school of engineering, SIMATS. Different methods are used in the study, such as: plant harvesting pest rearing homology modelling. Each of these methods is performed using the websites mentioned below. The study was performed in three groups, each with 15 sample sizes.

**Plant Harvesting**

The leaves of the *Sphaeranthus indicus* linn were collected freshly from Virudhunagar district, Tamil nadu. Harvesting of Sphaeranthus indicus is best done during the flowering period, which occurs in late summer or early fall. The flowers should be picked before they begin to wilt, as they contain the most active components. The flowering tops can be picked off the plant and air-dried between filter paper sheets.

**Insect Rearing**

Creepy crawly raising is the method of keeping up a populace of bugs, for investigate or other purposes. Insect rearing can involve a variety of techniques, such as controlling the population size, providing food for them, and providing the right environmental conditions. It has also been used as a pest control tool in urban settings, as well as in agriculture and nature.. The larvae stages of *Spodoptera litura* and *Plutella xylostella* were collected and
brought to the laboratory for rearing purposes.

**Larvicidal Bioassay**

The food was not given to the larvae during this trial. After the exposure period of three-hour, the larval mortality was determined. The fatal values, LC50 and LC90 were determined by using Probit analysis (Minitab®17), as well as their 95% confidence intervals were determined.

**Statistical Analysis**

The Accuracy (%) comparison of insect mortality using the innovative adaboost algorithm and the decision tree algorithm were exhausted by IBM-SPSS 29.0.0.0(241). The identification of accuracy is the dependent variable in this research. Variance analysis was performed on mortality experiment data, and data were analyzed as the mean of five replications. By using the Minitab®17 application and the Tukey's multiple range test (significance at p 0.05). Among the plant extract and insect the significant differences were examined. By using the Minitab®17 programme, a probit analysis with 95% reliability interval determines the lethal concentrations required to kill 50% (LC50) of the larvae in 24 hours. **Homology Modeling**

Homology modeling is a method used to predict the three-dimensional structure of a protein from its primary sequence. It involves using the known structures of related proteins, or homologs, as templates, and applying sequence alignment algorithms to predict the corresponding 3D structure of the target protein. Protein sequence alignment algorithms are used to identify regions of homology between target and template proteins. The template protein structure can then be used to model the target protein. The protein information bank (PIB) did not possess the gem structure of glutathione s transferase omega 2, a protein of the species GSTO2 (Gulf spodoptera littura) [9]. SWISS Demonstrate workspace can be used to reconstruct the structure from the previous layouts in order to model the protein structure. [10]. The obtained structure was subjected to a refinement process in GROMOS and was then further confirmed using the SAVES the auxiliary examination confirmation server's meta server. The Spares program incorporates information from the computer program PROCHECK [11,12] 3D Confirm which are valuable for deciding the calibre of the protein structure (Bet 2014). It analyzes the stereochemical quality of turn, torsion point, and bond point extend in terms of the rate of positive zone buildups and the rate of non-proline, glycine, and other buildups. The structure's quality was at that point evaluated utilizing QMEAN. This protein structure confirmation strategy is perfect for assessing the creation and change of crystallographic models [13]

**Modeling and Refinement**

By employing the template ID 3wd6.1.A with a 39.91% sequence identity, homology modeling was successful in determining the structure of the GSTO2 protein (Figure 2).[14] Furthermore, we used PROCHECK, VERIFY 3D, and ERRAT to validate the structure's quality. According to the findings, the Ramachandran plot shows that 93.8% of the residues are in the most preferred region, 5.7% are in additional authorized regions, 0.5% are in generously allowed regions, and 0.0% are in the disallowed regions (Figure. 3). Verify3D secured 89.45% of the nuclear facilitate compatibility (Figure. 4A&4B), and the Z- score of the in general degree of the exactness of the structure from Qmean4 is 0.04 . The protein's by and large quality calculate was 99.5614% concurring to ERRAT (Figure. 5) [14]. In arrange to get it the perplexing conformations and intelligent of the useful movement, atomic docking was inevitably inspected for the clarified structure.[15,16]

**Molecular Docking**

Molecular docking is a molecular biology computational approach for foreseeing the favored introduction of one atom to another when they are reinforced together to make a steady complex. The strategy is utilized to appraise the official affinities of peptides,
proteins, and little atoms to a known 3D structure of a target. Docking may be utilized to do virtual screening on endless libraries of compounds, rate the discoveries, and give auxiliary speculations approximately how the ligands square the target, which is greatly valuable in lead enhancement. Atomic docking is performed between a protein (Glutathione S transferase) and a ligand, piperine. Glutathione S-transferases, once in the past known as protein, are a lesson of eukaryotic and prokaryotic stage II metabolic isozymes well perceived for their capacity to catalyse the oxidation of glutathione the conjugation of the diminished shape of glutathione to xenobiotic substrates for the reason of detoxification. Piperine is the central plant alkaloid disconnected from dark pepper (Flute player nigrum) and long pepper (Flute player longum) [17]Piperine has a few natural properties counting pain relieving, anticonvulsant, antitumor, and anti-inflammatory exercises. Pyrx is an open source tool developed by a team of scientists at the University of Toronto. It is freely available for use in academic and research settings. It has been used in a number of studies to predict binding sites for different molecules, including proteins, DNA, and small molecules. Pyrx uses Vina algorithms to dock protein and ligand.

3 Results

The larval toxicity of crude volatile oil obtained from Sphaeranthus indicus (Si-CVO) against the lepidopteran pest Sphaeranthus litura is dosage dependant in II, III, and IV instar larvae. At 500 ppm, the death rate was highest in all instars: 93.2% (II instar), 87.3% (III instar), and 84.21% (IV instar). The death rate was lower at the lowest dose of 100 ppm across all larval instars (Fig. 1A). Similarly, the toxicity of crude volatile oil produced from Sphaeranthus indicus (Si-CVO) against Plutella xylostella causes dose-dependent mortality in II, III, and IV instar larvae. All of the fatality rates were highest at 500 ppm. 93.1% (second instar), 85.2% (third instar), and 81.2% (fourth instar). The death rate was lower at the lowest dose of 100 ppm across all larval instars (Fig. 1B). Second instar larvae of both pests were more sensitive at 500 ppm (96.5%, F4,20=24.31, P<0.001), which was statistically different from other treatment dosages. On third instar larvae, the fatal values (LC50 and LC90) were 213.17 and 421.88 ppm, respectively.

The docking results from ligplot demonstrated that the piperine(ligand) associated with GSTO2 shows that there were no hydrogen bonds. The 3D structure of protein from the Swiss model was displayed (Fig. 2). Also, a Procheck-Ramacharan plot from SAVES 6.0 software was displayed (Fig. 3) and their 3D verification was performed (Fig. 4). The
protein's overall quality factor was performed using ERRAT (Fig. 5). There were 15 hydrophobic interactions present between GSTO2 protein and Piperine ligand (Fig. 6).

Fig. 2. 3D structure of protein from Swiss model.

Fig. 3. Procheck-Ramachanran plot from SAVES 6.0.

Fig. 4. 3D verify-SAVES 6.0
The accuracy values of Aedes aegypti and Culex are given (Table 1).

**Table 1.** Accuracy values of *Ae. aegypti* and Culex are mentioned in the below table.

<table>
<thead>
<tr>
<th>SI.No</th>
<th>Accuracy in %</th>
<th>S. litura</th>
<th>P. xylostella</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>67</td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
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<td>93.21</td>
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<tr>
<td>5</td>
<td>98.12</td>
<td></td>
<td>94</td>
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</table>

The Group Statistical analysis of *S. Litura* and *P. xylostella* by taking each of 5 variables are presented (Table 2).
Table 2. Group Statistical analysis of *S. Litura* and *P. xylostella* by taking each of 5 variables. Standard error mean for the *S. Litura* is 5.68823 and *P. xylostella* is 5.83732.

<table>
<thead>
<tr>
<th>Group Statistics</th>
<th>II Instar</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
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</thead>
<tbody>
<tr>
<td>ACC U R A C Y</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>S. Litura</em></td>
<td>10</td>
<td>84.9340</td>
<td>13.05265</td>
<td>5.83732</td>
<td></td>
</tr>
<tr>
<td><em>P. xylostella</em></td>
<td>10</td>
<td>81.8420</td>
<td>12.71927</td>
<td>5.68823</td>
<td></td>
</tr>
</tbody>
</table>

Followingly, the Independent sample test for significance and standard error determination was displayed (Table. 3).

Table 3. Independent sample test for significance and standard error determination. The significance value (2-tailed) is 0.001 (p < 0.05) which is statistically significant for 95% confidence intervals.

<table>
<thead>
<tr>
<th>Independent Samples test</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Si-CVO</td>
<td>Levene's Test for Equality of Variances</td>
<td>T-test of Equality of Means</td>
<td>95% of the confidence interval of the Difference</td>
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<td></td>
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<tr>
<td></td>
<td>F</td>
<td>Sig.</td>
<td>t</td>
<td>df</td>
<td>Sig (2-tailed)</td>
<td>Mean Difference</td>
</tr>
<tr>
<td>Equal variances assumed</td>
<td>.01</td>
<td>.90</td>
<td>-.379</td>
<td>8</td>
<td>&lt;.001</td>
<td>-3.09200</td>
</tr>
<tr>
<td>Equal variances not assumed</td>
<td>-.379</td>
<td>7.995</td>
<td>&lt;.001</td>
<td>-3.09200</td>
<td>8.15048</td>
<td>-21.88923</td>
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</table>

4 Discussion

The larval toxicity of crude volatile oil obtained from Sphaeranthus indicus (Si-CVO) against the lepidopteran pest Sphaeranthus litura is dosage dependant in II, III, and IV instar larvae. Similarly, Plutella xylostella larval toxicity of crude volatile oil obtained from Sphaeranthus indicus (Si-CVO) results in dosage dependent mortality against II, III, and IV instar larvae. The volatile oil derived from this plant has been found to be an effective insecticide against certain agricultural pests, such as *Spodoptera litura* and *Plutella xylostella*. The research work on novel essential oils provides evidence that the essential oil derived from Sphaeranthus indicus Linn (Asteraceae) showed substantial larvicidal activity against two economically challenging pests *Spodoptera litura* and *Plutella xylostella* in vitro compared with the chemical substances in existence. The efficacy of the essential oil suggests its potential to replace existing synthetic chemicals in agricultural pest management and minimize risk to health and the environment.

5 Conclusion

The results of this study also demonstrate the potential of novel essential oils as
biopesticides in the future and can play a vital role in naturally controlling the agricultural pest. Homology modeling and molecular docking performed in this study provides an intensive insight of the drug-protein interaction of essential oil against the insect pest. The hydrophobic interaction, hydrogen bond of the target protein and ligand has also been demonstrated using docking analysis.

References


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