TOXICOLOGICAL EFFECT AND BIOCHEMICAL ANALYSIS OF EXTRACTS OF *Sapindus saponaria* Linnaeus AND *Piper hancei* Maxim AGAINST FRUIT FLY

LAN MUXIANG

SULTAN IDRIS EDUCATION UNIVERSITY

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LAN MUXIANG

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ABSTRACT

This research aimed to study *Sapindus saponaria* ethanol extract and *Piper hancei* petroleum ether extract for their toxicological effects on fruit flies (*Bactrocera dorsalis*) through contact toxicity, ingestion toxicity, and oviposition deterrence in the laboratory, alongside the field evaluation using male annihilation (MAT) and bait application (BAT) technologies. Compound profiling was conducted via HPLC-TOF-MS and GC-MS. In contact toxicity experiments, *P. hancei* extract (10 mg/mL) achieved 98.33% mortality in 36 hours, while *S. saponaria* extract (40 mg/mL) reached 75%. The lowest LC50 values at 36 hours were 3.77 mg/mL (*P. hancei*) and 26.20 mg/mL (*S. saponaria*). In ingestion toxicity tests, *P. hancei* extract showed a 91.41% mortality rate, while *S. saponaria* extract reached 89.69% at 72 hours. The lowest LC50 values at 72 hours were 2.11 mg/mL (*P. hancei*) and 42.09 mg/mL (*S. saponaria*). Oviposition deterrence was higher in *S. saponaria* extract (98.15%) than *P. hancei* extract (94.57%). Field evaluations showed that under MAT, *S. saponaria* extract had higher captures (16.68) but lower population suppression rate (PSR) and control effect (CE, 11.79%) than *P. hancei* extract (CE, 43.43%). In BAT, *S. saponaria* had higher captures (5.67) but lower PSR and CE (25.93%) than *P. hancei* (CE, 71.76%). Both extracts reduced fruit fly abundance over time. Phytochemical analysis revealed that *S. saponaria* extract contained saponins, glycosides, and triterpenoids, while *P. hancei* extract had alkaloids and volatiles with pesticidal properties. Overall, both plant extracts exhibited certain contact toxicity, ingestion toxicity, and oviposition deterrence against fruit flies, with *P. hancei* extract demonstrating superior effects. This suggests that both plants have potential as botanical pesticides.

KESAN TOKSIKOLOGI DAN ANALISIS BIOKIMIA EKSTRAK SAPINDUS SAPONARIA LINNAEUS DAN PIPER HANCEI MAXIM TERHADAP SERANGGA PEROSAK LALAT BUAH

ABSTRAK

Kajian ini bertujuan untuk mengkaji kesan toksikologi ekstrak etanol *Sapindus saponaria* dan ekstrak petroleum eter *Piper hancei* terhadap lalat buah (*Bactrocera dorsalis*) melalui ketoksikan sentuhan, ketoksikan pemakanan, dan penghalang oviposisi di makmal, serta penilaian lapangan menggunakan teknologi pemusnahan jantan (MAT) dan aplikasi umpan (BAT). Profil sebatian dilakukan menggunakan HPLC-TOF-MS dan GC-MS. Dalam eksperimen ketoksikan sentuhan, ekstrak *P. hancei* (10 mg/mL) mencapai kadar kematian 98.33% dalam 36 jam, manakala ekstrak *S. saponaria* (40 mg/mL) mencapai 75%. Nilai LC50 terendah pada 36 jam ialah 3.77 mg/mL (*P. hancei*) dan 26.20 mg/mL (*S. saponaria*). Dalam ujian ketoksikan pemakanan, ekstrak *P. hancei* menunjukkan kadar kematian 91.41%, manakala *S. saponaria* mencapai 89.69% dalam 72 jam. Nilai LC50 terendah pada 72 jam ialah 2.11 mg/mL (*P. hancei*) dan 42.09 mg/mL (*S. saponaria*). Penghalang oviposisi lebih tinggi pada *S. saponaria* (98.15%) berbanding *P. hancei* (94.57%). Penilaian lapangan menunjukkan bahawa dalam aplikasi MAT, *S. saponaria* mencatat tangkapan lebih tinggi (16.68) tetapi mempunyai kadar penindasan populasi (PSR) dan kesan kawalan (CE, 11.79%) yang lebih rendah berbanding *P. hancei* (CE, 43.43%). Dalam aplikasi BAT, *S. saponaria* juga mencatat tangkapan lebih tinggi (5.67) tetapi dengan PSR dan CE (25.93%) yang lebih rendah berbanding *P. hancei* (CE, 71.76%). Kedua-dua ekstrak mengurangkan kelimpahan lalat buah dari semasa ke semasa. Analisis fitokimia menunjukkan bahawa ekstrak *S. saponaria* mengandungi saponin, glikosida, dan triterpenoid, manakala ekstrak *P. hancei* mengandungi alkaloid dan sebatian meruap yang mempunyai sifat insektisida. Secara keseluruhan, kedua-dua ekstrak tumbuhan menunjukkan ketoksikan sentuhan, ketoksikan pemakanan, dan penghalang oviposisi terhadap lalat buah, dengan ekstrak *P. hancei* memberikan kesan yang lebih unggul. Ini menunjukkan bahawa kedua-dua tumbuhan berpotensi sebagai racun perosak botani.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BAT	Bait Application Technique
CE	Control Effect
CK	Control
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
EOs	Essential Oils
FAO	Food and Agriculture Organization of the United Nations
FIR	Fruit Infestation Rate
FOC	Flora of China
GC-MS	Gas Chromatography - Mass Spectrometry
HPLC-TOF-MS	High Performance Liquid Chromatography - Time of Flight - Mass Spectrometry
IPM	Integrated Pest Management
IPPC	International Plant Protection Congress
IRAC	International Insecticide Resistance Action Committee
LC50(90)	Lethal Concentration 50% (90%)
MAA	Mean of Adults Trapped after the Application
MAB	Mean of Adults Trapped before the Application
MAT	Male Annihilation Technique
ME	Methyl Eugenol

MIC	Minimum Inhibitory Concentration
MR	Mortality Rate
NIST	National Institute of Standards and Technology Database
NPV	Nuclear Polyhedrosis Virus
OECD	Organization for Economic Co-operation and Development
OMWW	Olive Mill Wastewater
ORR	Oviposition Reduction Rate
PSR	Population Suppression Rate
RSV	Rice Stripe Virus
PVY	Potato Virus Y
SRBSDV	Southern Rice Black Streaked Dwarf Virus
TCM	Traditional Chinese Medicine database
TMV	Tobacco Mosaic Virus
ToMV	Tomato Mosaic Virus
TuMV	Turnip Mosaic Virus
UV	Ultraviolet
WMV	Watermelon Mosaic Virus

APPENDIX LIST

CHAPTER 1

INTRODUCTION

1.1 Research Background

There is no dispute that agriculture remains a crucial tool for nation-building. It plays a vital role in ensuring food security, alleviating poverty, and conserving the essential natural resources that the present and future generations depend on for their survival and well-being (Udemezue, 2021). The Food and Agricultural Organization (FAO) of the United Nations has emphasized the need to increase global food production by 70% to meet the growing demand caused by the rapidly expanding global population (Kumar, 2012). Given that the global population is projected to reach 10 billion by 2050, increasing food production should be the top priority for all countries.

Before the 19th century, most food in the world was produced organically by using organic fertilizers, and human and animal power. Subsequently, the rapid growth of the population demanded the application of modern technology in agricultural production systems to balance the need for food for human consumption and commercial purposes. Raising crop yields on an industrial scale requires the conscious application of traditional fertilizers and insecticides (Udemezue, 2021). Modern agriculture, which relies largely on the widespread use of exotic inputs such as hybrid seeds, chemical fertilizers, pesticides, and other aids to increase yields, has gone a long way towards alleviating world hunger over the last century, but this has not benefited modern agriculture and had a significant role as a protagonist during the green revolution. The negative side of these chemically synthesized fertilizers and pesticides not only got the limelight when the chemical residues started to accumulate in the soil, water, and products but also led to the emergence of several pests and diseases (Meena & Mishra, 2020). Pests, widely known mainly include fungi, bacteria, nematodes, weeds, rodents, and insects, which are considered important factors in determining the yield and quality of agriculture production (Melikuziyev et al., 2022; Udemezue, 2021). It was reported that about 30% loss of potential yield and 14% damage in agricultural storage production were created by pests, and major losses appeared in developing countries. It has then raised concern about sustainable development which was considered as the judicious exploitation of the environment for the benefit of both the present and future generations (Udemezue, 2021).

With a significant contribution to the national GDP made by local and international trade, fruit, and vegetable production remains one of the pivotal sub-sectors of the agriculture industry through employment creation, improved nutrition, and improved foreign exchange earnings (OECD/FAO, 2016; Weinberger & Lumpkin, 2007). The most common fruits consumed worldwide are apples, peaches, citrus, grapes, and exotic fruits typical for the cultivation region. Other fruits with a large production are exotic fruits like coffee, macadamia, pineapple, papaya, and mango (Fierascu, et al., 2020; Urbaneja, et al., 2020). Citrus, for example, is originally from Asia and produced in most Asian countries, including China, India, Indonesia, Iran, Japan, Korea, Malaysia. There are approximately 38% of the total area planted to citrus in the world, in which China is the world's greatest citrus producer with an estimated 2.5 million ha producing 36.6 million tons, followed by India, another large citrus producer with nearly a million hectares and production of over 7 million tons in 2015 (Urbaneja, et al., 2020). Peach, also the most important temperate fruit crop worldwide, global production is currently largely dominated by China (Luo et al., 2022). However, a descending trend has been registered over the recent years in several key producing peach countries, including China, USA, Spain, and Italy, mainly due to the increased cost and the reduced revenue for the farmer (Manganaris et al., 2022).

With the continuous expansion of planting scale and the increase of planting varieties, there are more and more kinds of diseases and pests. Fruit pests pose a significant threat to agricultural productivity and food security worldwide. For example, the invasion of new pests, *Drosophila suzukii*, has led to substantial economic losses in small and stone fruits (Asplen et al., 2015). The Tephritidae family is globally distributed, comprising over 500 genera and 5000 species (Scolari et al., 2021). Six genera of them, namely *Anastrepha*, *Bactrocera*, *Ceratitis*, *Zeugodacus*, and *Rhagoletis* harbor predominantly harmful species (Doorenweerd et al., 2018; White & Elson-Harris, 1992). These flies are notorious for causing substantial economic losses by infesting various commercial fruits and vegetables, leading to considerable losses in orchards. Mango (*Mangifera indica*), for example, is particularly vulnerable to these economically important pests (Sarwar et al., 2014). Therefore, significant effort is carried out in the risk analysis process and substantial investments for control (Clarke & Measham, 2022). Additionally, these flies are recognized as high-value quarantine or invasive agricultural pests internationally, characterized by their rapid spread, invasiveness, and destructive impact (Liu et al., 2019a). Therefore, it is crucial to monitor and estimate infestations accurately to develop effective integrated pest management strategies (Sarwar et al., 2014).

*Bactrocera dorsali*s (Diptera: Tephritidae), also known as the oriental fruit fly or fruit fly, stands as one of the most destructive pests affecting fruit cultivation worldwide (Aketarawong et al., 2014). Its voracious appetite and widespread presence have led to significant economic losses in agriculture, involving small-scale farmers and large commercial enterprises. The fruit fly, originally native to Asia, has become a substantial concern for plant quarantine authorities due to its wide distribution across Asia, the Pacific Rim, and various other countries and regions. This highly invasive species has expanded its habitat beyond its native range and is now prevalent in numerous countries spanning multiple continents (Guo, 2015; Hou et al., 2006; Wan et al., 2012), can harm more than 300 kinds of vegetables, fruits, and flowers in 46 families, including mango, papaya, citrus, banana, loquat, guava, and peach in south China (Zhu et al., 2022). At present, chemical control is taken as the main management measure, combined with agricultural control measures such as bagging, and clearing of fallen fruit, as well as sexual attractant trapping method (Zhang & Li, 2007).

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Chemical pesticides play a significant role in modern agriculture, with their use being prevalent in various farming systems globally and contributing to current agriculture to fulfill the needs of a rising population. Uses of pesticides are not limited to agriculture, but they are also used to control domestic pests, disease insect vectors, and home gardening (Rani et al., 2021). For many years, chemical pesticides have been widely used in the world because of their high efficiency, fast effect, low cost, and convenient use, and have been in a monopoly position in plant protection for a long time (Wang, 2007). According to the evaluation of FAO after the investigation, around 40% of the increase in grain output after the 1950s was attributed to the research development and promotion of chemical pesticides, which shows the great contribution of chemical pesticides to agricultural production safety (Zhang, 2020).

However, the widespread and intensive use of chemical pesticides raises concerns about their potential toxic effects on human health and the environment. Studies have highlighted the toxic effects of chemical pesticides on human beings, with formulations containing numerous active substances (Alvarenga et al., 2022). Furthermore, the exposure of pesticide applicators, farm workers, and consumers to these toxic pesticides has been associated with an increased risk of cancer, emphasizing the urgent need for the decreased use of chemical pesticides and safer and eco-friendly alternatives (Pandey, 2023). In 2002, the European Commission issued a ban on the sale of 320 pesticides containing toxic chemicals, which also involved more than 60 kinds of pesticides being produced, sold and used in China (Wang, 2007). Since January 2007, five highly toxic pesticides, including methyl sulfur phosphorus, sulfur phosphorus, long-acting phosphorus, and phosphoramine, have been completely banned in China (Wang, 2007).

On the other hand, while chemical pesticides have been the mainstay of pest control in agriculture, their effectiveness has been indicated to have reached a limit, beyond which further reduction of pest population levels is not possible (Frey & Mani, 1992). Studies have also shown that the predominant use of chemical pesticides may not always provide complete and expected control effects of certain pests and can interfere with biological control methods (Dara et al., 2013). The intensive use of chemical pesticides, despite their inherent hazards, is a common practice in agricultural sectors that are confronting rapid change, such as in Ethiopia (Negatu et al., 2016). This reliance on chemical pesticides is further emphasized by the fact that they account for the vast majority of pesticides used in pest management, especially in developing countries, while bio-pesticides only have a small proportion (Huang et al., 2022). The limitation of pesticides underscores the need to explore integrated approaches that combine the use of chemical pesticides with bio-pesticides to achieve faster and cost-effective as well as eco-friendly pest control, in agriculture systems (Abraha et al., 2021). It is believed that pesticides in the 21st century should be “biologicall y reasonable pesticide” or “environmentally harmonious pesticides", which are characterized by high efficiency on pests, bio-safety on humans and livestock and other non-target organisms, and hav e little impact on environmental quality (He et al., 2003).

1.2 Problem Statement

After 30 years of rapid development, China's fruits have achieved "type diversification", and most of them are produced by themselves. The output of several major fruits ranks first in the world, especially citrus and apple, and citrus output accounts for about one-third of the world's output (Deng, 2018). According to the data from FAO, the planting area and yield of five main fruits, including apple, orange, banana, pear, and grape, have been increasing continuously, and the annual yield of apple, orange, and pear has ranked first in the world for many years (Wu, 2022). As fruit production scales up, disease and pest management have emerged as critical constraints on its development. Inadequate management practices and improper control methods frequently result in outbreaks of mites, aphids, scale insects, whiteflies, and fruit flies. These outbreaks pose a significant risk to orchard integrity and productivity (Huang, 2020; Shu, et al., 2020). The main common pests of these fruits mainly include *Bactrocera minax*, *Panonychus citri*, *Phyllocnistis citrella,* and *Bactrocera dorsalis* (Wang et al., 2019). Of them, *B. dorsalis* is one of the species that can harm many fruits, causing significant economic losses.

In recent years, due to the ongoing expansion of trade, the increasing scale of fruit and vegetable production, the diversification of plant species, and the warmer climate, the occurrence of *B. dorsalis* and the economic threat has become increasingly severe. Based on surveys conducted in Guangdong, Fujian, Zhejiang, and other regions, the yield loss attributable to fruit fly in non-bagged orchards can reach as high as 80%. Even in orchards where bagging is practiced, fruit fly can still reduce crop yield by 20% (Yi et al., 2021). Furthermore, *B. dorsalis* has also been found to harm peaches, dates, pomegranates, and apples in the provinces north of the Yangtze River, but it has not been determined whether it can survive the winter in the north (Sun et al., 2017). The results of national epidemic surveillance in recent years showed that the epidemic area of the pest is expanding, and has been rapidly spreading and rampant year by year in

South China and Southwest China (Liang et al., 2003), harming more than 250 kinds of cultivated fruit and vegetable crops such as citrus, grape, fig, and pepper. It has affected the production of fruit and vegetable production areas and has become one of the factors restricting the sustainable and stable development of fruit tree production (Guo et al, 2022).

For instance, reports indicated that orchards situated near urban areas of Guangzhou experienced damage rates exceeding 40% on *Psidium guajava* (guava) crops. Particularly during the favorable growth periods of fruit fly, the damage rates on guava fruits are notably higher. In certain locations, the damage rates on mature guava fruits can even escalate to 80% to 90% (Huang & Han, 2005). According to the investigation, melons and fruit vegetables in Chengjiang Town, Meizhou, Guangdong Province, have been under the influence of fruit flies since 2000. *Averhoa carambola*, *Zizyphus mauritiana*, and other crops have experienced damage exceeding 95% (Zhang et al., 2008). In peach orchards, the typical fruit damage rate ranges from 20% to 35%, with severe cases reaching up to 60% (Yang et al., 2011).

The control measures for the fruit fly in China include agricultural techniques, monitoring and trapping, quarantine, and chemical control. Despite the intensive employment of integrated management techniques, chemical control remains the primary method for controlling populations of this pest. Insecticides like malathion, trichlorfon, endosulfan, abamectin, spinosad, β-cypermethrin, and pyrethroids demonstrate moderate effectiveness against fruit fly in the majority of orchards (Liu et al., 2019a). When applied at a concentration of 1:1000 combined with 3% brown sugar, trichlorfon was reported to result in a reduction of the fruit fly population by around

90% compared to the untreated control group. The control effect reached 71.9% when using chlorpyrifos in a recent field trial (Pan et al., 2014). Combining ME with malathion or dichlorvos in a ratio of 10:1 was shown to offer effective control of *B. dorsalis* (Wu, Li, et al., 2015). Other field experiments have shown that spraying malathion solutions can offer substantial control over *B. dorsalis*. To target overwintering adults emerging in spring, the entire soil surface of infested orchards is sprayed with insecticides like diazinon, phoxim, and isazofos, effectively eliminating larvae and pupae in the soil (Xu et al., 2009).

Although chemical control has brought significant benefits, such as rapidly suppressing pests and diseases, saving time and costs, and increasing crop yields, it has also brought obvious drawbacks. The excessive dependence on chemical pesticides has resulted in numerous adverse outcomes, such as environmental pollution, health risks, negative effects on beneficial animals such as bees, earthworms, and termites, and even the emergence of pesticide resistance among pest populations (Baidoo et al., 2017; Tan et al., 2019). To address these adverse effects, there has been an increasing focus on exploring alternative methods of pest control. Integrated pest management (IPM) strategies have been suggested as a more sustainable approach, to reduce reliance on chemical pesticides and mitigate their adverse effects (Umina et al., 2015). Moreover, biological control and the utilization of biopesticides have become more prominent due to the constraints and negative repercussions associated with chemical control (da Silva et al., 2022; Tozlu et al., 2019).

Biopesticides encompass a diverse range of industrially produced agents of biological origin designed to control populations of insects, weeds, and disease-causing microorganisms (Wani et al., 2020). They can be categorized into three main classes, namely microbial pesticides, plant-incorporated protectants, and botanical pesticides (Manda et al., 2020). Botanical pesticides, derived from plants, have gained attention as an alternative to chemical pesticides due to their potential benefits in pest control. Before the advent of synthetic pesticides, they were extensively employed in both subsistence and commercial farming for thousands of years. Botanical pesticide compounds serve as repellents, attractants, antifeedants, and growth inhibitors (Ngegba et al., 2022). When these compounds are extracted using suitable solvents and/or combined with necessary pesticide adjuvants, they transform into botanical pesticides. The widespread use of synthetic pesticides, owing to their potency and effectiveness against devastating crop diseases, led to a decline in the application of plant-based products. However, in recent times, the excessive reliance on synthetic pesticides has posed a significant threat to environmental safety and human health. Botanical pesticides are experiencing a resurgence in popularity in organic farming due to their safe profile for crop consumption, and consumers are increasingly willing to pay premium prices for organic produce (Ngegba et al., 2022).

There are rich plant resources in China, with more than 2000 kinds of wild plants or Chinese herbal medicines distributed in more than 20 provinces, including a variety of biopesticide plants that have been comprehensively studied at home and abroad from the aspects of plant species, active components, action mechanism, etc. (Zhang et al., 2013). Many of them have pesticide effects, such as *Melia azedarach*, *Houttuynia cordata*, *Polygonum orlentale*, *Cinnamomum camphora*, *Litsea cubeba, Evodia lepta*, *Euphorbia hirta, Sapindus sa*yonara, *Piper longu*, *Piper hancei*, and others (Dong & Xu, 2012; Qi et al., 2018; Tang & Chen, 2004). Even only in the mountainous area of Northeastern Guangdong province, such as in Meizhou city, there are more than 200 species of plants with potential insecticidal or pesticidal activity that have been investigated and classified (Lan et al., 2022). Therefore, effectively harnessing local plant resources and developing potential botanical pesticides is highly advantageous for agricultural production.

Sapindus saponaria and *Piper hancei* are common native wild plants in Meizhou. They have been proven to possess potential pesticide activity and exhibit certain toxic effects on insects. *S. saponaria* is common in southern China. The study on *S. saponaria* as bio-pesticide mainly focused on the control of yellow tea thrips (*Scirtothrips dorsalis*), *Pomacea canaliculata*, *Aedes aegypti*, *Aedes albopictus*, *Bactrocera cucurbitae*, *Coptotermes formosanus*, *Thysanoplusia orichalcea*, *Plutella xylostella*, *Lipaphis erysimi* and others (Hu et al., 2022; Lu et al., 2010). Research on *P. hancei* primarily concentrated on *domestica*, *Aedes albopictus*, *Culex quinquefasciatus, Spodoptera litura*, *Thrips hawaiiensis* and *Brontispa longissima* (Dong et al., 2011; Dong & Xu, 2012; Ma et al., 2016). However, there are few reports on the pesticide activities of the two plant extracts against *B. dorsalis*. Based on the investigation of bio-pesticide plant resources in Meizhou and the compilation of the list of bio-pesticide plants, two common wild plants, *S. saponaria* and *P. hancei*, will be selected to evaluate the toxicity effect against *B. dorsalis* both in the laboratory and in the field, and to identify the chemical compounds with potential toxicity, and provide scientific basis and theoretical support for further development and utilization of bio-pesticides.

Furthermore, it should be emphasized that, according to the Flora of China (FOC, 2007), the alternative scientific name for *Sapindus saponaria* L. is *Sapindus mukorossi* Garetn or *Sapindus abruptus* Lour. Now it has been revised and accepted as *Sapindus saponaria* L. Therefore, in terms of literature compilation, these three scientific names for this plant are classified as the same plant species of *S. saponaria.* Likewise, the alternative scientific name for *Piper hancei* is *Piper matthewii* or *Chavica leptostachya*. However, in literature and documentation, the commonly encountered name remains the current scientific name *P. hancei* in use.

1.3 Research Gap

Through a comprehensive literature review, it has been observed that various wild plant resources, including *S. saponaria* and *P. hancei*, have been explored as potential botanical pesticides, demonstrating toxic effects on certain insect species. However, several research gaps remain and need to be addressed to further enhance their efficacy and application.

1. China boasts an exceptionally rich diversity of wild plant resources, including medicinal, ornamental, and edible plants. Meizhou, with its varied terrain and favorable climatic conditions, is particularly abundant in plant species. However, research on pesticide plants in this region remains limited. This study aims to fill this gap by providing scientific evidence to explore the potential of Meizhou’s plant resources for botanical pesticide development.

2. As common wild plants in the Nanling region, *S. saponaria* and *P. hancei* have been investigated as potential botanical pesticides, exhibiting certain insecticidal and antifungal properties. However, their efficacy has been tested on only a limited range of insects and pests, with no reported studies specifically targeting fruit flies. This study represents a novel attempt to explore their potential in controlling this highly destructive and economically significant pest.

3. Previous studies have shown that *S. saponaria* is rich in saponin compounds, while *P. hancei* contains abundant alkaloids and volatile compounds. However, research on other bioactive compounds in these plants remains limited. This study conducts a comprehensive analysis and identification of extracts from *S. saponaria* and *P. hancei*, aiming to deepen the understanding of their chemical composition, characteristics, and functions. The findings will provide valuable insights for the further development and utilization of these two plant species.

1.4 Research Aim

This study aims to investigate the toxicological effects and biochemical compounds of extracts from *S. saponari*a and *P. hancei* against fruit flies (*B. dorsalis*) under both laboratory and field conditions. The findings will provide scientific evidence for assessing their potential as botanical pesticides and exploring their development as sustainable alternatives to chemical pesticides.

1.5 Research Objectives

There are a total of four research objectives as follows.

1. To identify the most effective solvent extract of *S. saponaria* and *P. hancei* from four different solvents, namely petroleum ether, ethyl acetate, ethanol, and methanol, against fruit fly.

2. To study the indoor toxicological effect of *S. saponaria* and *P. hancei* extracts with different concentrations against fruit flies, in terms of contact toxicity, ingestion toxicity, and oviposition deterrence in the laboratory based on the result of the identification of the most effective solvent extracts.

3. To evaluate the field toxicological effect of *S. saponaria* and *P. hancei* extracts against fruit flies, by applying MAT using the hanging trapping method and BAT using the cage trapping method in the orchard.

4. To identify the biochemical compounds with potential pesticide activity from the most effective solvent extracts of *S. saponaria* and *P. hancei* based on the identification of the most effective solvent extracts.

1.6 Research Scope

This research consists of four main studies. The preliminary study involves two wild plants, *S. saponaria* and *P. hancei*, both of which are extracted with four different organic solvents, namely petroleum ether, ethyl acetate, ethanol, and methanol. These extracts are then used to assay the contact toxicity under the laboratory conditions on adults of fruit flies by analyzing their mortality rates (MR). This aims to determine the most effective solvent extract with the highest MR against fruit flies, which will be used for subsequent research.

The first study (Study 1) involves preparing extracts of *S. saponaria* and *P. hancei* with different concentrations based on the solvent extracts selected with the highest toxicity from the preliminary study. These extracts with three different concentrations are then used to conduct research on contact toxicity, ingestion toxicity, and oviposition deterrence against fruit flies under laboratory conditions. The mortality rates (MR) and LC50 values are calculated and analyzed to evaluate the contact toxicity and ingestion toxicity, while the oviposition reduction rate (ORR) is used to test the oviposition deterrence.

The second study (Study 2) aims to evaluate the toxicity of the most effective solvent extracts from *Sapindus saponaria* and *Piper hancei* against fruit flies in a peach orchard. The orchard is divided into three experimental blocks: the *S. saponaria* block (S.S block), the *P. hancei* block (P.H block), and the control block (CK block). To assess the efficacy of the plant extracts, the male annihilation technique (MAT) and bait application technique (BAT) are separately applied in each block. Meanwhile, the fruit fly population abundance is continuously monitored throughout the study. Subsequently, the population suppression rate (PSR) of fruit flies in each block is analyzed to evaluate changes in abundance. Finally, before harvest, the fruit infestation rate (FIR) and control effect (CE) are assessed in each block to determine the overall effectiveness of the treatments.

The final study (Study 3) involves the identification of the solvent extracts of *S. saponaria* and *P. hancei* with the highest toxicity. For the *S. saponaria* extract, HPLC-TOF-MS technology is used to analyze the compounds it contains. For the *P. hancei* extract, in addition to HPLC-TOF-MS, GC-MS technology is also utilized to analyze and identify its volatile compounds. Then, based on the characteristics and functions of the compounds identified, the components will be classified and corroborated by their pesticide activity observed in the experiments conducted previously and in the literature.

1.7 Research Significance

This study not only enhances the understanding of the toxicological and control effects of *S. saponaria* and *P. hancei* extracts against fruit flies but also provides a comprehensive analysis of their biochemical compounds. The findings offer objective and scientific evidence to support the development and utilization of these two plants as potential botanical pesticides.

1.8 Hypothesis

The extracts of *S. saponaria* and *P. hancei* demonstrate notable pesticidal activity against fruit flies and contain varying proportions of bioactive compounds with insecticidal properties.

1.9 Thesis Organization

This thesis consists of five chapters. Chapter 1 is dedicated to introducing the research background, problem statement, research gap, research aim, research objectives, research scope, research significance, and hypothesis. Chapter 2 provides an overview of *B. dorsalis*, including its biological characteristics, life cycle, behavioral traits, damage characteristics, and control methods. Following that, an overview of biopesticides was provided, which focuses particularly on botanical pesticides and the concept, types, and action mechanisms of plant secondary metabolites. It also elaborates on their insecticidal effects, then describes plant species with pesticide activity (classified taxonomically to the level of family), and finally introduces an overview of *S. saponaria* and *P. hancei*, focusing on their biological characteristics and research progress. Chapter 3 focuses on detailing the research methods involved in the thesis. This mainly includes research design, methods, identification of the most effective extract solvents of *S. saponaria* and *P. hancei* against fruit fly, indoor toxicology experiments which mainly involve assays for contact toxicity, ingestion toxicity, and oviposition deterrence of *S. saponaria* and *P. hancei* extracts against fruit fly, field toxicology evaluation which includes the application of Male Annihilation Technique (MAT) and Bait Application Technique (BAT), and finally, the identification of compounds of most effective solvent extracts of *S. saponaria* and *P. hancei*. Chapter 4 explains the research results and their discussion. The results consist of the solvent extracts with the most effective toxicity against fruit fly, the results of indoor toxicology experiments on contact toxicity, ingestion toxicity, and oviposition deterrence conducted with the most effective solvent extracts, the results of field toxicity evaluation produced by MAT and BAT application, and the identification of biochemical compounds from the effective solvent extracts of these two plants. Chapter 5 presents the conclusions drawn from the study, the contribution, and future recommendations.