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The toxicity of *Sapindus saponaria* and *Piper hancei* extracts against *Bactrocera dorsalis*

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Abstracts: Botanical extracts as alternative insecticides to chemical pesticides are widely studied globally. This study aims to evaluate the toxicity of ethanol extracts of *S. saponaria* and petroleum ether extracts of *P. hancei* against *B. dorsalis*. The two extracts were diluted into four different concentrations and applied to test tubes to form a pesticide film. Ten pairs of adult flies were introduced into the test tubes and kept for 2 hours before being transferred to the plastic box for further rearing. Mortality was observed and recorded at 3, 6, 12, 24, and 36 hours, and the corresponding LC50 values were analyzed. The results showed that the 80 mg/ml and 40 mg/ml *S. saponaria* extract achieved mortality rates (MR) of 96.67% and 76.67% within 36 hours, respectively, while concentrations below 20 mg/ml resulted in MR no higher than 30%. For *P. hancei*, the extracts of 20 mg/ml, 10 mg/ml, and 5 mg/ml caused MR of 100%, 98.33%, and 68.33% within 36 hours, respectively, while the 2.5 mg/ml extract resulted in a 30%. Notably, the 20 mg/ml *P. hancei* extract achieved a 91.67% MR within just 6 hours. The LC50 values of *S. saponaria* ranged from 151.89 mg/ml to 24.49 mg/ml from 3 to 36 hours, while those of *P. hancei* ranged from 12.90 mg/ml to 3.63 mg/ml. In conclusion, both botanical extracts demonstrated certain toxicity and exhibited a cumulative effect, while the *P. hancei* extract also showed strong knockdown effect. These results indicate that both plants have potential insecticidal activity, and further research on their detailed chemical constituents and mechanisms of action is required in the future.

Keywords: toxicity*, Sapindus saponaria*, *Piper hancei, Bactrocera dorsalis,* LC50

1.0 Introduction

Bactrocera dorsalis (Hendel) (Diptera: Tephritidae), is a highly invasive alien pest that originated from the tropical and subtropical regions of Asia, also known as the oriental fruit fly or fruit fly. It is considered a complex of destructive and persistent pests, with a wide host range, encompassing over 250 fruits and vegetables, causing a considerable impact on fruit production systems and commercialization, resulting in significant annual economic losses (Fiaboe et al., 2021; Jaffar et al., 2023). In recent times, the continuous expansion of *B. dorsalis* poses a significant threat to the commercial fruit industry, particularly in subtropical and tropical regions worldwide. This threat manifests in increased production and control costs, as well as the imposition of new quarantine restrictions (Aketarawong et al., 2014). Due to its rapid adaptability to new environments and its strong dispersal capacity, *B. dorsalis* can readily colonize new habitats and spread swiftly, often being transported within infested fruits or vegetables. Meanwhile, global transportation, commercial trade, and travel create new avenues for dispersal, facilitating the movement of *B. dorsalis* (Kriticos et al., 2013). Furthermore, *B. dorsalis* disrupts ecosystem function and biodiversity in invaded regions by displacing other species of *B. dorsalis* from their ecological niches (Aketarawong et al., 2007). Therefore, *B. dorsalis* has been consistently recognized as a high-risk pest and has been designated as a quarantine species by numerous countries (Khamis et al., 2009).

Researchers keep exploring the mechanisms driving the successful invasion of *B. dorsalis* and have developed effective management strategies. Following the invasion of *B. dorsalis* around the world, chemical control has been widely used to suppress the population density and reduce the damage of the pest. Insecticide pulverization has been considered more advantageous than other control techniques due to its rapid onset, persistence, and high efficiency (Mutamiswa et al., 2021). However, controlling this pest presents multiple challenges. The use of agrochemicals poses risks to human and ecosystem health, and endangers the survival of biocontrol agents (Majeed et al., 2025). Nowadays,

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concrete efforts have been made on environmentally friendly alternatives for synthetic insecticides, such as botanical pesticides.

Sapindus saponaria (also named *Sapindus mukurossi*), which is common in southeast Asia, have been revealed to have insecticidal activity (Menezes et al., 2023; Sochacki, & Vogt, 2022; Jesus et al., 2020; Sarma et al., 2019). *Piper hancei*, commonly used as a substitute for traditional Chinese folk medicine, was also revealed to be effective on *Musca domestica*, *Culex quinquefasciatus, Aedes albopictus*, and have good toxicity to *Spodoptera litura* (Ma et al., 2016; Dong & Xu, 2012). There have been no reports on the biological activity of these two plants against *B. dorsalis*. In this study, they were extracted with ethanol and petroleum ether, respectively, and used for conducting contact toxicity experiments on *B. dorsalis*. The objective of the current study was to evaluate the performance of two plant extracts, *S. saponaria* and *P. hancei,* at the toxicity of contact killing *B. dorsalis* through bioassays done in the laboratory. The mortality of the insects and the LC50 of the extracts were involved to evaluate the toxicity.

2.0 Material and Methods

2.1 Reagents and Instrument

The reagents and instrument used in this research are listed in Table 1 and Table 2.

Table 1: Reagents

Name	Specification	Manufacturer
Petroleum ether	Analytical grade	Tianjin Kangkede Technology Co., Ltd
Ethyl acetate	Analytical grade	Tianjin Kangkede Technology Co., Ltd
Ethanol	Analytical grade	Tianjin Kangkede Technology Co., Ltd
Methanol	Analytical grade	Tianjin Damao Chemical Reagent Factory
Acetone	Purity ≥ 99.5%	Tianjin Damao Chemical Reagent Factory

Table 2: Instrument

Name	Manufacturers
Biological incubator	Shaoguan Taihong Medical Instrument Co., Ltd
Centrifuge	Eppendorf AG (5804R)
Electro-heating incubator	Shanghai Boxun Industrial Co., Ltd
Electronic balance	Sedolis Scientific Instruments (Beijing) Co., Ltd
Electro-thermostatic water bath	Shanghai Yiheng Scientific Instrument Co., Ltd
Grinder	Zhejiang Yongkang Sufeng Industry and Trade Co., Ltd
Rotary evaporator	Tokyo Physicochemical Equipment Co., Ltd

2.2 Insects

B. dorsalis were obtained from a colony established from infested papaya and reared for two generations at the Life Science College, JiaYing University, GuangDong province, China, following the method described by Vargas et al. (2014) and He et al. (2020). Adults of fruit fly were allowed to emerge inside the gauze cages (45 cm×45 cm ×45 cm) with a 1:3:1 mixture of yeast extract, honey, and water. All experimental flies were held in a laboratory maintained at 27 ± 1 ℃, and 70% ± 5% relative humidity (RH), and a photoperiod of 12:12 (L:D) h. All fruit flies were tested after reaching sexual maturity.

2.3 Extracts

Based on the previous experiments, the aril of *S. saponaria* and plant of *P. hancei* were extracted with ethanol and petroleum ether, respectively, using Soxhlet apparatus. Subsequently, for *S. saponaria*, the original solution of ethanol extract of 80 mg/ml was diluted 2, 4, and 8 times to 40 mg/ml, 20 mg/ml, and 10 mg/ml. For *P. hancei*, the original solution of petroleum ether extract of 20 mg/ml was diluted 2, 4, and 8 times to 10 mg/ml, 5 mg/ml, and 2.5 mg/ml. In all, 10 extract concentrations were treated (including the control).

2.4 MR and LC50

The study was carried out by contact method according to Wang et al. (2013) and Hu et al. (2022) to evaluate toxicity of botanical extracts against *B. dorsalis*. Each test tube was dripped with 1ml extract to make the pesticide film by drying. Twenty adult flies (Female: Male = 1: 1) were introduced into the test tube. After maintaining for 2 hours, the flies were transferred into a plastic box to rear as before the experiment. The MR were recorded after 3 h, 6 h, 12h, 24 h, and 36 h. Correspondingly, the LC50 were analyzed to evaluate the toxicity. Three replicates of each extract concentration treatments were evaluated. In all, 600 adult flies were tested. The MR was calculated following the formula below.

$$MR = [(MRT - MRC) / (1 - MRC)] \times 100\% \quad (1)$$

Where the MRT represents MR of flies of treatment, while MRC represents MR of control.

2.5 Statistical Analysis

The mean mortality of *B. dorsalis* with all concentrations were compared using a one-way analysis of variance (ANOVA). Means were compared using the Duncan's multiple range test ($p < 0.05$). LC50 values were analyzed with Probit model. All statistical analyses were conducted using SPSS 27.0.

3.0 Results

3.1 The Mortality Rate (MR)

3.1.1 *S. saponaria* Extracts

The meanmortality rates at different concentrations of ethanol extract and observation times showed significant differences ($F=825.39$, $p < 0.001$). Also, the mortality differed significantly from observation times ($F = 333.83$, $p < 0.001$). Meanwhile, there was a significant interaction between the concentration and the observation time ($F = 56.68$, $p < 0.001$).

The Duncan test results of the mortality were given in Table 3. In the 3rd hour and the 6th hour, the mortality at 80 mg/ml were significantly higher than others ($F = 59.67$, $p < 0.001$; $F = 73.08$, $p < 0.001$). By the 12th h, there were significant differences among 80mg/ml, 40 mg/ml and 20mg/ml extracts ($F = 105.72$, $p < 0.001$). Then till the 24th h and the 36th h, the mortality at each concentration both differed from each other significantly ($F = 233.17$, $p < 0.001$; $F = 613.20$, $p < 0.001$). The mortality of the control (0 mg/ml) remained at 0. Obviously, the mortality of fruit flies increased across the concentrations and the observation time. The *S. saponaria* extract of 80 mg/ml showed the strongest contact toxicity against *B. dorsalis* (Table 3.1).

Table 3: Mortality of *B. dorsalis* exposed to *S. saponari*a extracts at the interaction of concentration and observation time

Treatment	Concentration	Mortality (%)				
		3h	6h	12h	24h	36h
Ethanol extract	80mg/ml	20.33a	38.33a	58.33a	81.67a	96.67a
	40 mg/ml	3.33b	10.00b	25.00b	41.67b	76.67b
	20 mg/ml	1.67b	6.67bc	13.33c	20.00c	30.00c
	10 mg/ml	0b	1.67c	6.67cd	11.67d	15.00d
Control	0mg/ml	0b	0cd	0d	0e	0e
F		59.67**	73.08**	105.72**	233.17**	613.20**
p		<0.001	<0.001	<0.001	<0.001	<0.001

Notes, Different letters in a column indicate significant differences using Duncan's multiple range test ($p < 0.05$). Asterisks signify a significant difference at level 0.01.

3.1.2 *P. hancei* Extracts

Mortality rates at different concentrations and observation times indicated significant differences ($F = 1196.99$, $p < 0.001$). Also, the mortality differed significantly from different observation times ($F = 153.46$, $p < 0.001$). Meanwhile, there was a significant interaction between the concentration of the extract and the observation time ($F = 22.71$, $p < 0.001$).

The Duncan test results of mortality were given in Table 4. When at 3rd hour, there were significant differences among 20 mg/ml, 10 mg/ml and other concentrations ($F = 118.66$, $p < 0.001$). In the 6th hour, the mortality differed significantly from each other ($F = 221.13$, $p < 0.001$). By the 12th and 24th hours, the mortality of treatments both differed significantly from each other ($F = 259.79$, $p < 0.001$; $F= 669.80$, $p < 0.001$). Till the 36th hour, the mortality of 20 mg/ml and 10 mg/ml were close to 100%, significantly higher than others ($F = 309.55$, $p < 0.001$). No mortality was observed in the control (0 mg/ml). Therefore, the mortality caused by *P. hancei* petroleum ether extract escalated with the increase in concentration and observation time. And the extract with 20 mg/ml indicated the highest toxicity against *B. dorsalis* (Table 3.2).

Table 4: Mortality of *B. dorsalis* exposed to *P. hancei* extracts at the interaction of concentration and observation time

Treatment	Concentration	Mortality (%)				
		3h	6h	12h	24h	36h
Petroleum ether extract	20 mg/ml	78.33a	91.67a	98.33a	100.00a	100a
	10 mg/ml	28.33b	51.67b	71.67b	90.00b	98.33a
	5 mg/ml	8.33c	15.00c	25.00c	43.33c	63.33b
	2.5 mg/ml	3.33c	6.67d	13.33d	21.67d	30.00c
Control	0 mg/ml	0c	0d	0e	0e	0d
F		118.66**	221.13**	259.79**	669.80**	309.55**
p		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Notes, Different letters in a column indicate significant differences using Duncan's multiple range test ($p < 0.05$). Asterisks signify a significant difference at level 0.01.

3.1.3 The LC50

The LC50 values of two botanical extracts against *B. dorsalis* were shown in Table 5. The LC50 of *S. saponaria* were 151.89 mg/ml, 123.59 mg/ml, 71.38 mg/ml, 38.28 mg/ml, and 24.49 mg/ml at each observation time. They are totally higher than those of *P. hancei*, which were correspondingly 12.90 mg/ml, 8.99 mg/ml, 6.55 mg/ml, 4.70 mg/ml, and 3.63 mg/ml (Table 3.3). Also, the LC50 values of both botanical extracts decreased as the observation time extended. Specifically, the LC50 value of *S. saponaria*. extract was the highest at the third hour, reaching 151.89, indicating the weakest toxicity effect. At 36 hours, the LC50 value was 24.49, indicating the strongest toxicity effect. Similarly, the LC50 value of *P.hancei* extract was also the highest at the third hour, at 12.90, representing the lowest toxicity potency, while at 36 hours, the LC50 value was the lowest, at 3.63, showing the highest toxicity efficiency. Moreover, at each observation time, the LC50 value of *P. hancei* is significantly lower than that of *S. saponaria*, indicating that *P. hancei* has a much higher toxicity to *B. dorsalis* than *S. saponaria* (Figure 1). Overall, both the *S.saponaria* and *P. hancei* extracts demonstrate a certain toxicity against *B. dorsalis* and illustrate cumulative effect, while the *P.hancei* also possesses rapid knockdown properties.

Table 5: LC50 values of *S. saponaria* and *P. hancei* extracts at the interaction of concentration and observation time

Extract	OT (h)	Regression Equation	LC50 (mg/ml)	95% Confidence Interval	chi-square
S.saponaria	3	y = - 6.03+2.77x	151.89	104.43 - 419.14	6.64
	6	y = - 4.32+2.06x	123.59	86.89 - 247.33	6.52
	12	y = - 3.63+1.96x	71.38	55.36 - 106.02	4.49
	24	y = - 4.02+2.54x	38.28	32.44 - 46.18	9.10
	36	y = - 4.48+3.23x	24.49	21.25 - 28.16	4.96
P.hancei	3	y = - 3.54+3.19x	12.90	11.13 - 15.40	11.35
	6	y = - 3.18+3.33x	8.99	7.85 - 10.39	8.05
	12	y = - 2.78+3.40x	6.55	5.72 - 7.50	9.31
	24	y = - 2.42+3.60x	4.70	4.09 - 5.36	6.25
	36	y = - 2.21+3.95x	3.63	3.14 - 4.12	5.62

Notes: OT = Observation Time.

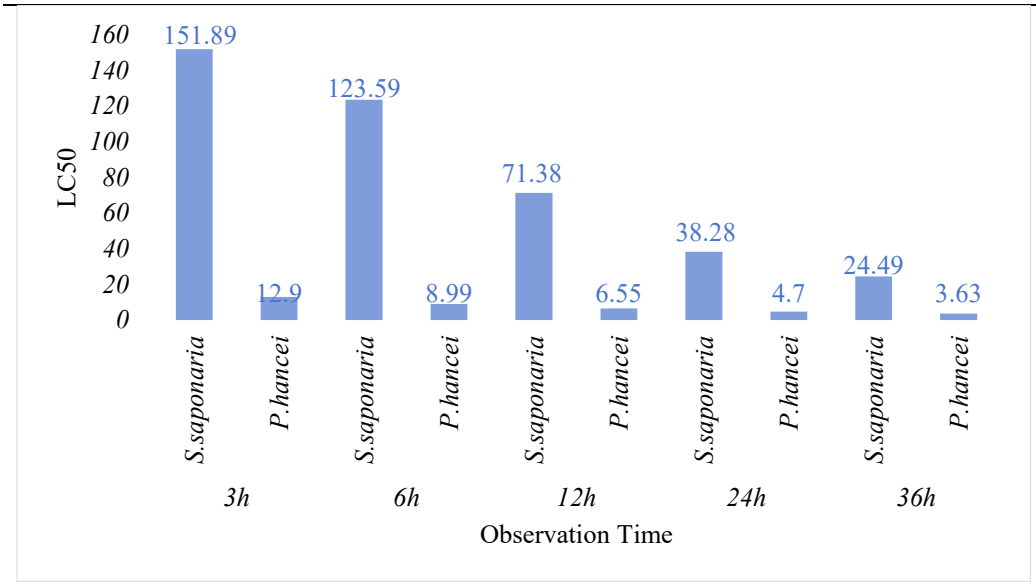

Fig. 1: The toxicity as LC50 values of different plant extracts at different observation time

4.0 Discussion

Botanical extracts have been used as biopesticides globally. The present study demonstrated that both *S. saponaria* ethanol extract and *P. hancei* petroleum ether extract exhibited significant contact toxicity against *B. dorsalis*, albeit with distinct temporal patterns and potency profiles. The *S. saponaria* extract displayed a time-dependent mortality response, with efficacy increasing significantly over prolonged exposure periods. The *P. hancei* extract manifested dual characteristics: a time-dependent effect coupled with rapid knockdown activity, achieving substantial mortality within short-term exposure windows. Notably, the LC50 values of *P. hancei* were consistently lower than those of *S. saponaria* extract across all observation times, indicating superior toxicological potency. These findings align with recent investigations into plant-derived insecticides but also reveal novel mechanistic insights specific to the management of *B. dorsalis*.

Studies have revealed that *S. saponaria* exhibited the strongest toxicity against pests. For instance, it produced great mortality on *O. coffeae* adults exposed to the tea leaves solution prepared with the plant extract (Handique et al., 2017). Eddaya et al. (2013) found that aqueous solutions with varying concentrations of *S. mukorossi* pericarp extract significantly affected the survival, consummation, growth, and development of larvae, and delayed larval development by 1 to 2 days, as well as reduced the larval weights by 7 to 68% (Eddaya et al., 2013). The inhibitory effect is probably linked to the presence of cardiotonic saponins and heterosides, known for their deleterious action in organisms (Menezes et al., 2023). In this study, as the exposure time increased, the mortality rate of *B. dorsalis* also increased, particularly at higher concentrations (40 mg/ml and 80 mg/ml), implying a strong accumulative effect or time-dependent effect. The gradual increase in mortality suggests that bioactive compounds, such as triterpenoid saponins - a hallmark of Sapindus species - may act through cumulative physical damage to the insect cuticle, leading to desiccation and delayed mortality. *S. saponaria* extract has been proven to exhibit insecticidal properties, including increased mortality, reduced consumption, weight loss, delayed development, and lower fecundity. It demonstrates a potent and rapid action against a wide variety of pests. (Roopashree & Naik, 2019). The precise mechanism behind these effects remains largely unclear, though it is believed to involve a combination of multiple activities. Potential mechanisms identified in the literature include repellent or deterrent effects, reduced food intake through the digestive system, inhibition of sterol assimilation, interference with the ecdysteroid receptor complex, membrane-permeabilizing properties, and apoptosis-inducing activity (Roopashree & Naik, 2019).

Similarly, the insecticidal activity of *Piper hancei* has been repeatedly confirmed. For instance, Dong and his team have conducted multiple studies on its toxicity to various insects, including the Egyptian mosquito (*Aedes aegypti*) and housefly (*Musca domestica)* (Ma et al., 2016; Dong & Xu, 2012). In contrast, in current study, the *P. hancei* extract displayed both cumulative and rapid knockdown effects, a dual mode of action rarely reported for Piperaceae-derived insecticides. The rapid mortality observed within 6 - 12 hours post-exposure suggests the presence of neurotoxic or acetylcholinesterase-inhibiting compounds, such as piperamides or phenylpropanoids, which are characteristic of Piper species (Lan et al., 2024). In particular, the unique isobutyl amide and 2E,4E-diene structural units contained in the fatty-chain amides are essential groups for these compounds to exert their insecticidal activity (Xin et al., 2016). Recent metabolomic studies have identified isoquinoline alkaloids in *P. hancei* as potent agonists of insect nicotinic acetylcholine receptors (nAChRs), potentially explaining its fast-acting properties (Lan et al., 2024).

The significantly lower LC50 values of *P. hancei* compared to *S. saponaria* indicate that *P. hancei* extract is significantly more toxic than *S. saponaria* extract, with lower LC50 values across all concentrations and time points. This

suggested that *P. hancei* may be a more potent option for pest control. It further corroborated findings by Kraikrathok et al. (2013), who documented superior insecticidal efficacy of Piperaceae extracts against Diptera due to synergistic interactions between alkaloids and lignans (Kraikrathok et al., 2013).

5.0 Conclusion

In conclusion, both *S. saponaria* and *P. hancei* extracts exhibited significant insecticidal activity against *B. dorsalis*, with distinct modes of action. The differential bioactivity profiles of these botanicals highlight their complementary potential in integrated pest management (IPM). While *S. saponaria* is effective over longer exposure times, *P. hancei* provides rapid knockdown effect and higher overall toxicity. Further phytochemical characterization and detailed mechanisms underlying these effects are required to identify the specific compounds responsible for these effects and assess their safety toward non-target organisms - a critical consideration for developing eco-friendly biopesticides (Damalas & Koutroubas, 2020).

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Conflict of Interest

The authors declare no conflicts of interest.

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