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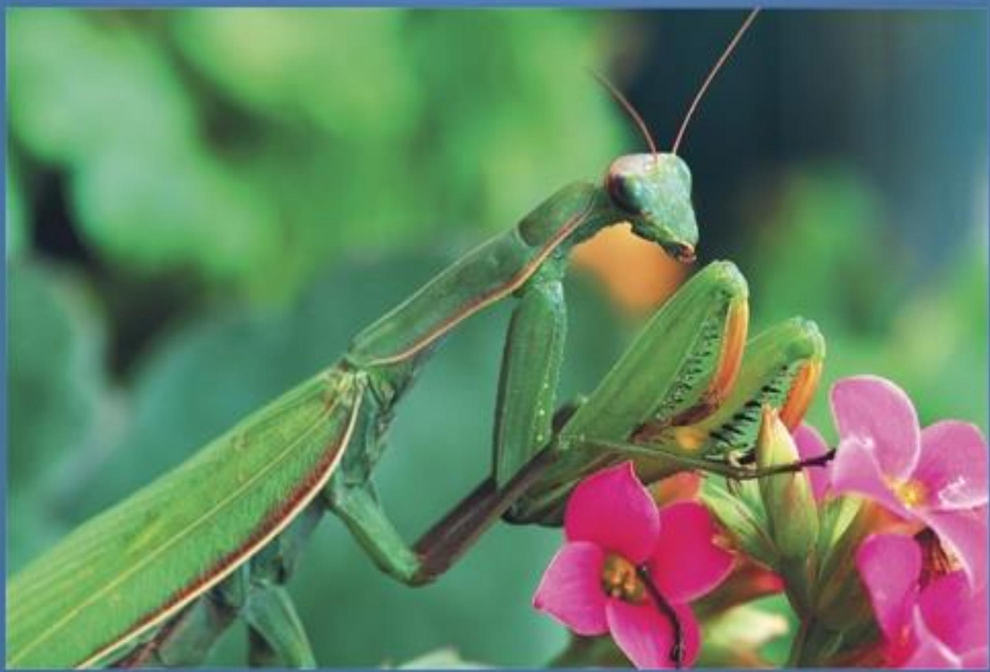

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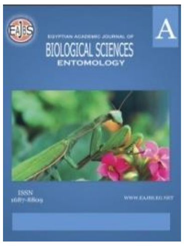

The Insecticidal Potential of *Azadirachta indica* and *Phaleria macrocarpa* Plant Extracts against Pineapple Mealybug, *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae)

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ABSTRACT

Crude extracts from neem, *Azadirachta indica*, and God's crown, *Phaleria macrocarpa*, were tested at two concentrations, 5% and 10% for the insecticidal activity against pineapple mealybugs, *Dysmicoccus brevipes* under laboratory conditions. In this study, synthetic insecticide; dimethoate and commercial neem were used as positive control while water was used as a negative control. All treatments were applied using the topical bioassay method in five replications. In 24 hours after treatment, no mortality was recorded in all treatments. Only after 48 hours, 52% and 28% mortality were recorded in the sample treated with 10% and 5% of *A. indica* extract. *Dysmicoccus brevipes* treated with 10% *A. indica* extract, showed 100% mortality at 72 hours while the sample treated with dimethoate reached 100% mortality at 120 hours after treatment. In conclusion, the 10% of *A. indica* extract showed higher potency in comparison to other treatments including dimethoate, commercial neem and *P. macrocarpa* extract.

INTRODUCTION

Dysmicoccus brevipes is a worldwide pest of pineapple, *Ananas comosus* and a vector of pineapple mealybug wilt (PMW) disease caused by a virus that is a serious threat to pineapple cultivation in the world. The disease causes severe dieback, reddening of leaves, wilting and collapse of mature plants (Sether and Hu 2002; Rohrbach and Johnson 2003). *Dysmicoccus brevipes* has a large global distribution of more than 126 countries according to Scalenet (2022). Despite the common name, pineapple mealybugs are identified as polyphagous pest as it causes damage to a wide range of crops, about 40 plant families including grapes, citrus, nuts, and banana (CABI, 2021). The small sap-sucking insect belongs to Pseudococcidae family under the suborder of Sternorrhyncha of Hemiptera. They can be found in colonies, especially in the protected and hidden area underneath the plant part or base area of the plant host (Luz and Santa-Cecília 2005). Severe infestation can progress up to the leaves, fruits, blosso cups, and crowns thus reducing the fruit quantity and quality by influencing plant development (González-Hernández *et al.*, 1999; Mau and Kessing 2007; Mamahit *et*

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al., 2010). Management of mealybugs has largely relied on using synthetic pesticides. Pre-treatment of pineapple sucker with an insecticide such as malathion and diazinon, is widely practiced for pests and disease prevention (Sipes 2000; Rohrbach and Johnson 2003; Dey *et al.*, 2018). Moreover, the application of imidacloprid, chlorpyriphos and quinalphos insecticides are widely used in pineapple planting areas to prevent and manage *D. brevipes* population and infestation throughout the cultivation period (Geiger and Daane 2001; Joy *et al.*, 2012). However, the excessive use of synthetic insecticides poses a risk of insect resistance, and chemical residue in the plant, harmful to the environment and human health as well. With the increase in awareness of using chemical pesticides, people are moving towards greener approaches such as botanical pesticides as an alternative to chemical pesticides. Botanical pesticides affect various insects in different ways depending on the physiological characteristics of the insect species as well as the type of insecticidal plant. Botanical pesticides are considered safe because they have low or no pesticide residue which is making them safe for humans, the environment, and the ecosystem. Recent studies have been focusing on the development of plant extracts as alternative control methods, which are cost-effective, environmentally friendly and capable of keeping insect pests at bay.

MATERIALS AND METHODS

All efficacy tests of *A. indica* and *P. macrocarpa* were conducted under laboratory conditions of the Insect Ecology Laboratory, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. The laboratory condition was maintained at a temperature of 27°C±2 with 60±5% relative humidity. Prior to the experiment, the sanitation process was conducted in the laboratory to ensure the absence of microorganism and other pests.

Sampling and Insect Culture: The mealybugs, *D. brevipes* were collected during the dry season from a pineapple field in Taman Kekal Pengeluaran Makanan (TKPM) Jalan Kebun, Klang, Selangor (2° 59' 17.7216" N 101° 30' 4.9428" E). The collection of mealybugs was conducted without causing loss to the pineapple plants. Live insects were carefully removed from the host plants' parts; fruits, leaves, and crowns using a fine brush and put into a small container. The collected individuals were maintained using pineapple crowns in the laboratory.

Preparation and Extraction of Plant Materials: Leaves of *A. indica* and *P. macrocarpa* were collected from an organic farm in Faculty of Agriculture, Universiti Putra Malaysia. The leaves were cleaned and oven-dried at 45°C for five days. After the leave was completely dried, a table type of smashing machine (FDS Model, Taiwan) was used to make the leave into powder form. The solvent extraction method (modified from Harpreet et al. 2011) was performed to extract the plant bioactive compounds with methanol used as a solvent. Following the methods, leaves powders were suspended in methanol for 48 hours and filtered through filter paper (Whatman No. 1). Crude extract of the plants was acquired by removing the solvent in the filtrate using a rotary evaporator that consist of rotavapor R-215, heating bath B-491 and vacuum pump V-700 (BUCHI, United Kingdom).

Mortality Bioassay of Crude Plant Extracts: 5% and 10% of *A. indica* and *P. macrocarpa* concentrations were prepared from the crude extracts, modified from Lira et al. (2019). Dimethoate (synthetic) and commercial neem (botanical) were used as positive controls, meanwhile, water was used as a negative control. For the mortality evaluation, mealybugs at the nymphs' stage were chosen. The topical application method was used, and the plant extract was directly applied to the back of the nymphs with a micropipette. Every treatment was conducted in five replications and the mortality of mealybugs was observed until five days after treatment (DAT). The experiment was conducted using a Completely Randomized Design (CRD), and data were analysed using ANOVA SAS with mean comparison subjected to Tukey HSD. The percentage of mortality of each treatment was calculated below:

Percentage of mortality (%) = (Number of dead individuals) / (Total number of individuals) × 100)

RESULTS

The mortality of *D. brevipes* after being treated with 5% and 10% *A. indica* and *P. macrocarpa* methanolic extracts were tabulated in Table 1. Based on the results, zero mortality is recorded for all treatments after 24 hours of application (1 DAT). After 48 hours (2 DAT), both concentrations of *A. indica* recorded 28% and 52% mortality, respectively. Meanwhile, 4% of mortality was recorded in 10% *P. macrocarpa* extract and the commercial neem. At 3 DAT (72 hours), 72% and 100% mortality are recorded in each 5% and 10% of *A. indica* extract, whereas only 12% mortality was recorded amongst the nymphs that were treated with 10% of *P. macrocarpa* extract. During these hours, the percentage of mortality of neem and 10% *P. macrocarpa* extract showed no significant difference. As for dimethoate, the mortality of the mealybugs was 36% after 3 DAT. Interestingly, the mortality of *D. brevipes* treated with 5% of *A. indica* extract remained at 72% until the last day of the observation. 5% *P. macrocarpa* started to show its efficacy at 4 DAT (96 hours) with 16% mortality recorded. Meanwhile, 10% of the same plant extract recorded 28% of the mortality during similar hours after the treatment. The highest mortality recorded among the mealybugs treated with 5% and 10% of *P. macrocarpa* extract at the end of the observation were 28% and 32% each, respectively. At the end of the observation period, dimethoate recorded 100% of mortality.

Table 1: Percentage (%) mortality of *D. brevipes* when treated with 5% and 10% of *A. indica* and *P. macrocarpa* crude plant extracts against commercial neem, dimethoate and water at 24, 48, 72, 96 and 120 hours.

Treatment	**Concentration**	**Percentage (%) mortality of *D. brevipes***				
		24h	48h	72h	96h	120h
A. indica	5%	0^{a}	28^{b}	72^{b}	72^{a}	72^{b}
A. indica	10%	0^{a}	52^{a}	100^{a}	100^{a}	100^{a}
P. macrocarpa	5%	0^{a}	0^{c}	0^{d}	16^{bc}	28^{c}
P. macrocarpa	10%	0^{a}	4^{c}	12^{cd}	28^{bc}	32^{c}
Neem (commercial)	Recommended rate	0^{a}	4^{c}	20^{cd}	36^{b}	36^{c}
Dimethoate	Recommended rate	0^{a}	0^{c}	36^{c}	76^{a}	100^{a}
Water	-	0^{a}	0^{c}	0^{d}	0^{c}	0^{d}

Means with the same letters in a column indicate no significant difference at $p<0.05$

DISCUSSION

In this study, *A. indica* extracts show promising results in controlling *D. brevipes* and showed high efficacy towards the mealybugs as early as 2 DAT in comparison to other treatments. According to Campos *et al.* (2016) and Isman (2008), *A. indica* has the potential as a botanical insecticide and can cause mortalities in many insect groups including homopterous ones like mealybugs. Lira *et al.* (2019) reported that 5% and 10% of *A. indica* leaf extract caused intermediate mortality of *D. brevipes* at 72 hours after the contact while Memon *et al.* (2017) reported that high dose of neem extract can reduce the population of mealybug in 72 hours. Interestingly, the efficacy of the commercial neem on *D. brevipes* was significantly lower than the crude extract at the recommended dose. However, it is expected that the recommended dose of commercial neem which was 1% is not sufficient and in fact, have lower efficacy than 5% and 10% *A. indica* extract, resulted in low mortality of *D. brevipes*. It is suggested that a higher concentration of commercial neem might obtain the same results as the crude extract. According to Chen *et al.* (2018), neem contains a mixture of several compounds belonging to limonoid-type triterpenoids that are endowed with insecticidal properties. Among the compounds, azadirachtin that abundantly found and is considered the most active compound that demonstrated high potential for use against insect pests due to its high insecticide activities (Biondi *et al.* 2012), although other limonoids may contribute to the efficacy of neem insecticides (Boursier *et al.* 2011). Therefore, less efficacy of commercial neem suggested that other components present in the extract may also contribute to the insecticidal activity. In addition, Basavaraju *et al.* (2013) reported that 3% of neem oil significantly reduced the population of *D. brevipes*. Sahito *et al.* (2011) highlighted in their study that neem oil reduced the mealybug population up to the 7th day of application. According to Mamoon-ur-Rashid *et al.* (2012), neem oil at 25000 and 30000 ppm on Hibiscus leaves killed 53% and 60% 2nd instar cotton mealybug nymphs, respectively after 24 hours pre-treatment. This paralleled the findings of Merghem and Mohamed (2017) and Ali (2017). Therefore, it is important to have a sufficient dose of the active ingredient in the biopesticide such as *A. indica* to increase the efficacy against pests.

In the present study, *P. macrocarpa* extracts show low efficacy against *D. brevipes* and have no significant difference with neem botanical insecticide. This result was unexpected because there were few reports of the potential of *P. macrocarpa* on other pests although there was no report of its insecticidal potential against mealybug. Roonjho *et al.* (2022) reported that 1000 mg/L concentration of *P. macrocarpa* extract eliminated aphid population under laboratory conditions after 72 hours and has a strong repellent effect against *A. gossypi*i. Moreover, Nugroho and Kesetyaningsih (2013) highlighted the larvacidal activity of *P. macrocarpa* against *Aedes aegypti.* In 2006, Iskandar et al. pointed out the insecticidal activity of *P. macrocarpa* against *Culex sp*. larvae. According to Napiah and Murti (2022), *Phaleria macrocarpa* contains chemical compounds like saponin, alkaloid and flavonoid that have potential toxicity against the insect. Saponins are a widely studied class of bioactive compounds that shows growth inhibition, feeding deterrent, cytotoxic and mortality activities against many insect pests (Chaieb, 2010; Singh and Kaur, 2018). Moreover, it can cause mortality in target pests by reducing their food intake and disturbing the food movement in insect gut due to less digestibility and toxicity of the food eaten (Adel *et al.*, 2000; Singh and Kaur, 2018). Many reports highlighted the ability of saponins as pesticides which can directly disturb the reproduction and growth of insect pests, as well as act as a deterrent or repellent (Mokhtar, 2016; Singh and Kaur, 2018; Roonjho *et al.*, 2020). Taylor *et al.* (2004) reported that saponin can also form complexes with cholesterol which cause ecdysial failure and cellular toxicity in insects. Therefore, a test of *P. macrocarpa* against mealybug can be further investigated with higher concentration.

This study also showed a different trend of mortality by dimethoate that showed mortality which was observed after 72 hours of the treatment. Similarly, Akhtar *et al.* (2018) reported that mealybugs remained alive for three days in an experiment against all concentrations of insecticide. The possible reason for this condition was that the mealybug has developed resistance against the insecticide. Mealybugs can rapidly develop resistance to insecticides (Afzal *et al.*, 2015) therefore insecticides may vary in their control efficacy (Saeed, 2007). In accordance with our finding, Su and Wan (1988) have reported that dimethoate causes 93 – 100% mortality of *Planococcus citri* infesting grapevine. Nevertheless, dimethoate also contributed to the mortality of 80% of cotton mealybug, *Phenacoccus solenopsis* (Suresh *et al.,* 2010; Sanghi, 2015), custard apple, *Planococcus pacifius* (Shukla and Tandon, 1984), enset root mealybug *Paraputo* spp (Bekele 2001), and citrus mealybug, *Planococcus citri* (Bentley and Martin, 2002; Hoffmann, 2009; Ghanim and Elgohary, 2015). However, unlike the present study, Biswas *et al.* (2015) reported that the mortality of mealybug can be observed as early as within 24 hours after the treatment with 96% of mortality.

Conclusion

From the present study, it was observed that the topical application of *A. indica* crude extract at 5% and 10% concentration in the laboratory conditions showed excellent insecticidal potential against *D. brevipes*. Results from the analysis concluded that *A. indica* crude has the potential in suppressing the population of *D. brevipes*.

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