LARVICIDAL EFFECTS OF *Murraya koenigii* EXTRACTS AGAINST THE LARVAE OF *Metisa plana*

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Abstract

Metisa plana is the most destructive pest in oil palm plantation area in Malaysia. It is a vigorous insect which feeding on the oil palm leaves. In Peninsular Malaysia, infestation of *M. plana* was estimated at about 4,904 ha. Its infestation has caused 33-40% yield losses. In this study, *Murraya koenigii* was chosen to evaluate the larvicidal activity against larvae of *M. plana.* The leaves immersed with different extracts at different concentration (50, 100, 200, 300, 400 and 500ppm) were provided as food source. The mortality rate was recorded every 6 hours for 3 days. The result revealed that all concentrations were effective to control the larvae. Hexane extracts which contains glycoside, terpenoid, phenol, alkaloid and tannin exhibited good potential as larvicidal as it demonstrated 100% mortality rate in the first 12 hours followed by chloroform extract which showed 100% mortality rate after 24 hours. Meanwhile, methanol leaves extracts at 50 ppm did not show any larvicidal activity against *M. plana*. However, *M. koenigii* aqueous leaves extract at 500 ppm recorded only 37% mortality rate after 36 hours. The results suggested that *M. koenigii* has potential for development as commercial insecticide for controlling *M. plana* due to its larvicidal effect.

Keyword: larvae, larvicidal, *Metisa plana*, Mortality rate, *Murraya koenigii*

Introduction

M. plana or commonly known as bagworm belonging to the family Lepidoptera and it has considered as one of the most destructive pests in oil palm (*Elaeis guinensis*) plantations. Based on the analysis of pest infestation towards 69 estates in Peninsular Malaysia, it is reported that bagworm is the primary pest that attacked more than 63, 955 ha of oil palm plantation. Cumulative analysis of area being infected by *M. plana* alone was about 4904 ha, 18,297 ha, followed by *Pteroma pendula*, 18,297 and mixed of *M. plana* and *Pteroma pendula* were about 14,607 ha (Ho, 2002). It was reported that *M. plana* can defoliate the whole palm canopy and consequently result in yield loss. After being infected, the yield was expected to decrease by 30% to 40% over the next two years (Ho, 2002; Hasber & Noor Hisham, 2012).

The life cycle of *M. plana* is short which range from 70 to 110 days (Khoo et al., 1991; Yap, 2005; Hamim et al., 2011). The species have seven larval instars and each larval instar is protected in a larval bag with different in size and colour (Hamim et al., 2011). Damage caused by *M. plana* started when larvae feed straight away upon being laid on the leaf surface and thus cause serious defoliation of oil palm leaves (Hamim et al., 2011). Chua et al. (2012) stated that understanding of the *M. plana* biology and life cycle is important for management of the pest.

To date, in controlling the *M. plana* infestation, the applications of synthetic insecticide were used. Various protective chemicals have been applied and increase day by day. Trichlorfon, lambda-cyhalothrin, cypermethrin emulsion concentrated (EC) and cypermethrin emulsion water based (EW) are among synthetic insecticide that can be used to control *M. plana* (Hasber et al., 2015). Nevertheless, it was mentioned that continuous use of chemical pesticides may toxic to environment and humans (Noorshilawati et al., 2015). Thus, bio-pesticides derived from natural sources to control pest such as *Metisa plana* is needed for environmental sustainability and human health. Plants were believed to have good anti-pest activities as plants contain secondary metabolites that can be used to control pest and disease (Noorshilawati et al., 2015).

In this study, *M. koenigii* leaves were selected for larvicidal activities as it has been reported to possess several bioactivities (Sumantha et al., 2014). It is extensively used as herb, spice, condiments and to treat various types of ailment in Indian traditional system (Harish et al., 2012). Scientific research had proven that *M. koenigii* have significant antimycobacterial, antidiarrhea and antibacterial activities. Preliminary phytochemical screening of *M. koenigii* indicated the presence of carbohydrate, protein, phytosterols, flavonoids, glycosides, tannins, alkaloids, anthraquinone and saponins in the leaf materials (Savitha & Balamurugan, 2014). The presence of various kind of phytochemical compound possesses the importance of *M. koenigii* which can be used to heal various ailments (Salna et al., 2011).

Materials and Methods

Plant Extraction

M. koenigii leaves were washed air-dried and ground into powder form. Then, dried *M. koenigii* leaves were then extracted with different solvent and distilled water yielding hexane, choloroform, methanolic and aqueous extract. For solvent extraction: The extraction of *M. koenigii* leaves was successively extracted using hexane, chloroform and methanol sequentially using method adopted by modification from previous studies (Savitha & Balamurugan, 2014; Eman et al., 2015; Abdulelah et al., 2015). For aqueous extraction: The samples were mixed with distilled water and stirrer for five minutes. The mixtures were filtered with muslin cloth and were centrifuged (3000 rpm/min) for ten minutes. The crude extract was dried then kept for future used (Savitha & Balamurugan, 2014).

Metisa plana Rearing

The larvae of *M. plana* were collected from middle and lower fronds of oil palm tree and placed into container covered with muslin cloth for ventilation. The larvae were maintained at 28°C ± 2. Fresh leaflets were provided every day as sources of foods.

Larvicidal Activity

Six different concentrations consist of 50ppm, 100ppm, 200ppm, 300ppm, 400ppm and 500ppm for each solvent viz. hexane, choloroform, methanol and aqueous *M. koenigii* extract were used as treatment.

The fresh leaflets were immersed in different extracts with different concentrations for 10 minutes. The leaflets were then placed into petri dish for evaluation of larvicidal activity. The second instar larvae were selected for the larvicidal activity as it has the most vigorous feeding habit in which its size enlarges more than 100%.

The leaflets soaked with DMSO and methanol (1:1), methanol 50% and distilled water were used as control. The mortality rates were observed and recorded at six hours interval for 36 hours.

Phytochemical screening

Phytochemical screening of *M. koenigii* leaves extracts were done to perceive the presence of compounds such as alkaloids, flavonoids, glycoside, phenol, tannins, terpenoids, and saponins. The phytochemical screening was conducted based on modified methods described previously (Savitha & Balamurugan, 2014; Malahubban et al., 2013; Ade-Ajayi et al., 2011).

Test for alkaloids: Leaves extracts were added with 2% H_2SO_4 and then was filtered before added a few drops of Mayer's reagent. Orange red colour proved the presence of alkaloid in leaves. Test for flavonoid: Leaves extracts were added with a few drops of 1% ammonia solution. The appearance of yellow coloration shows the presence of flavonoids in leaves extracts. Test for glycoside and terpenoids: About 2mL of acetic anhydride and 50% of concentrated H_2SO_4 were added to the leaves extracts. The presence of glycoside was indicated through formation of brown ring while red coloration determined the presence of terpenoid. Test for phenol: A few drops of ferric chloride were added into the extracts. The formation of bluish black indicates the presence of phenol. Test for tannins: 5g of leaves extract was added with 20ml distilled water. The mixtures were then boiled and then filtered. A few drops of 0.1% ferric chlorides were added into the mixtures and the appearance of brownish green or blue-black coloration indicated the present of tannins in leaves extracts. Test for saponin: The leaves extract was shaken vigorously with water. The formation of foamy showed the presence of saponin.

Result and Discussion

The Figure 1 shows that *M. koenigii* hexane leaves extract promising good larvicidal activity against *M. plana*. All concentrations showed more than 70% of mortality rate after 6 hours exposure. While, there was no mortality rate recorded in control test. The good larvicidal activity may be responsibled by the presence of tannin, terpenoids and other compounds.

The presence of tannin in *M. koenigii* hexane leaves extracts showed direct toxicity effect leading to inhibition of larvae or insect growth and eventually death (Ibanez et al., 2012). Besides, terpenoid screened in *M. koenigii* hexane leaves extracts have been identified as major repellent against the ant, *Nasius niger* (Junker et al., 2011)**.** According to previous study (Anna et al., 2014), it was also proved that *M. koenigii* hexane leaves contain non-polar compounds which had significant effect on feeding activity of *Spodoptera litura* and another study by Paranagama et al. (2002) confirmed that the extracts also showed contact toxicity and fumigant toxicity against *Callosobrunchus maculatus.*

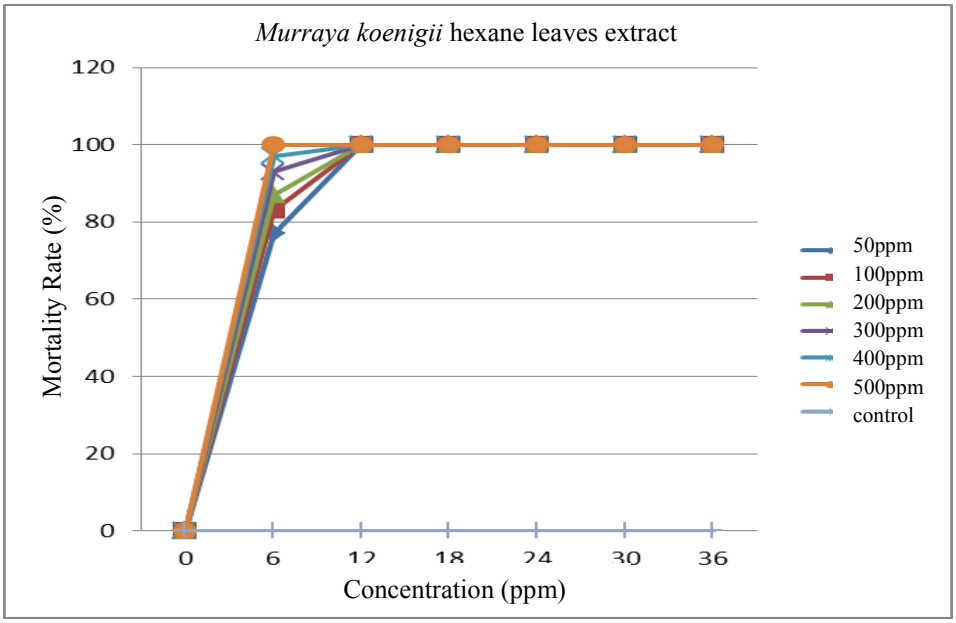

Figure 1. Larvicidal effect of *M. koenigii* hexane leaves extracts against *M. plana*

All concentrations of *M. koenigii* chloroform leaves extracts showed 100% mortality rate after 24-hour exposure (Figure 2). The larvicidal effect of *M. koenigii* chloroform leaves extracts against *M. plana* showed that the mortality rate was increased with increasing concentration. It took about 24 hours for 50 ppm of *M. koenigii* chloroform leaves extracts to show 100% mortality rate compared to 500ppm which only took 6 hours.

Alkaloid present in *M. koenigii* chloroform leaves extracts has an important role in bioassay activity (Argah et al., 2011). The terpenoids' present also can act as antifeedant, growth disruptor and possesses toxicity toward insect (Khalid et al., 1989).

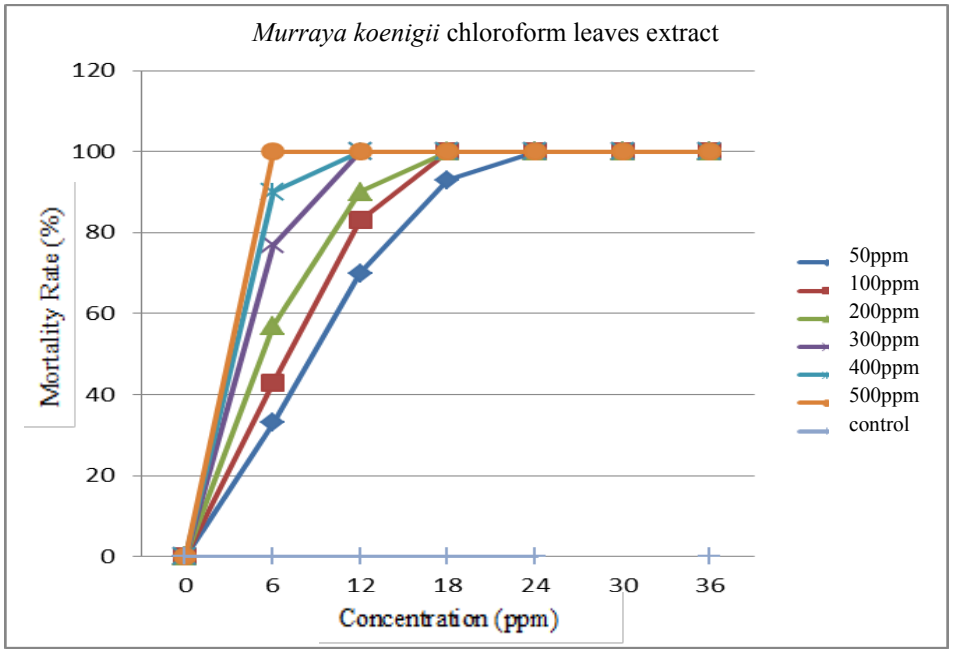

Figure 2. Larvicidal effect of *M. koenigii* chloroform leaves extracts against *M. plana*

As less compounds was present in *M. koenigii* methanol leaves extracts, lower mortality rate were recorded in the larvicidal test as compared to *M. koenigii* hexane and chloroform leaves extracts. The highest mortality rate (97%) was recorded at 500 ppm concentration after 36 hours exposure (Figure 3). Meanwhile, both 50 ppm and control test did not record any mortality rate. Alkaloids which have great toxic effect against pathogens and predators (Helio & Arthur, 2015) were absence in the *M. koenigii* methanol leaves extracts.

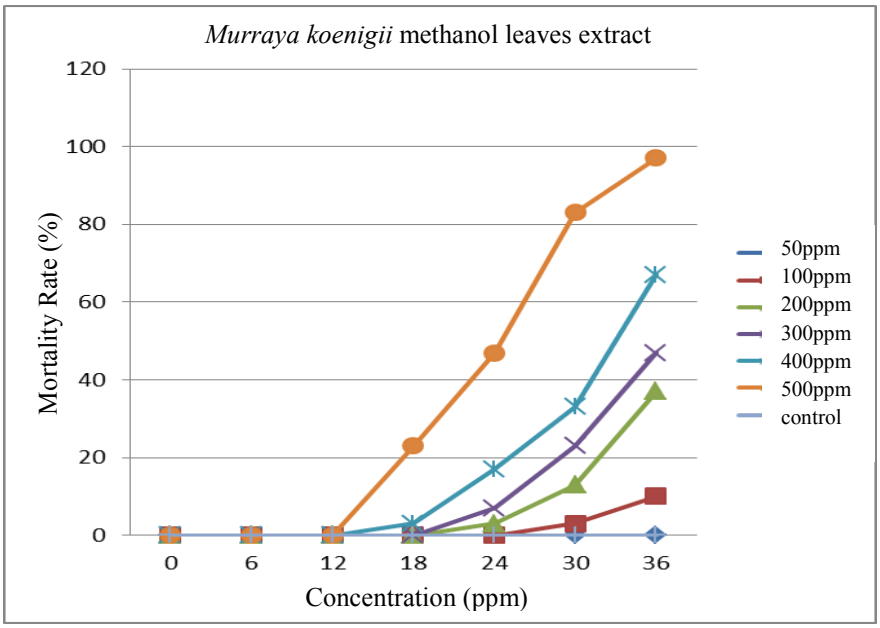

Figure 3. Larvicidal effect of *M. koenigii* methanol leaves extracts against *M. plana*

M. koenigii aqueous leaves extracts also showed low larvicidal effect on *M. plana* as shown in Figure 4. There was no effect at the first 18 hours of exposure in each concentration. The highest mortality rate (37%) only recorded after 36 hours exposure at 500ppm concentration. 100 ppm, 200 ppm and 300 ppm concentration extracts show no mortality rate until 30 hours of exposures and increasing up to 7%, 10% and 13% respectively 6 hours later. The low mortality rate may be due to the absence of many secondary metabolites as shown in phytochemical screening test (Table 1).

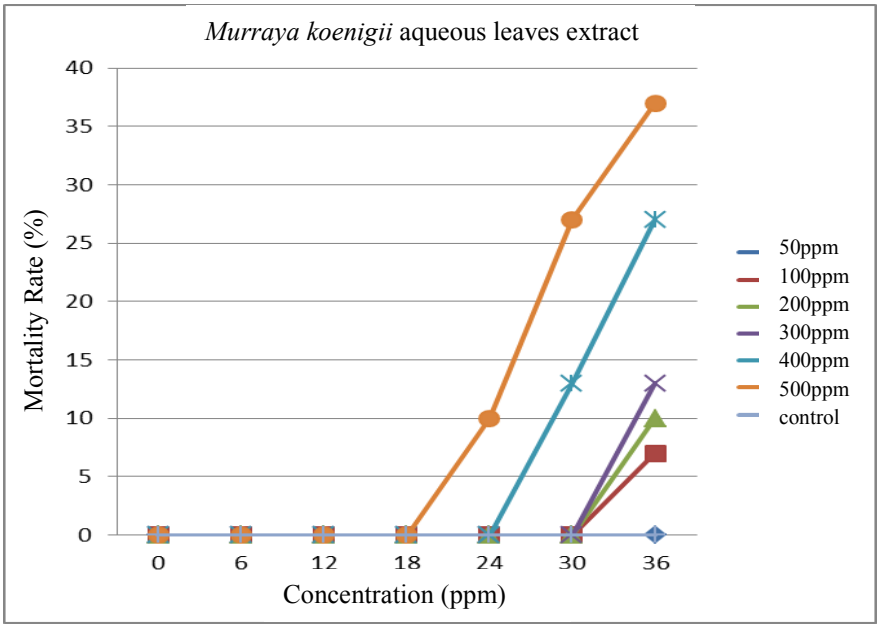

Figure 4. Larvicidal effect of *M. koenigii* aqueous leaves extracts against *M. plana*

Plants are one of important sources which contain various kinds of secondary metabolites responsible to protect themselves and human against disease (Abu Bakar et al., 2014). Phytochemical screening of *M. koenigii* hexane leaves extracts revealed that crude extracts contain glycisode, terpenoid, phenol, alkaloid and tannin. Table 1 shows that *M. koenigii* hexane and chloroform leaves extract contains various compounds. Terpenoids present in both extracts plays important role in plant defense against pest (Cheng et al., 2007).

Table 1. Secondary metabolites present in the *M. koenigii* leaves extracts

Compound	Hexane	Chloroform	Methanol	Aqueous
Alkaloid	+	+	-	+
Flavonoid	-	-	-	-
Glycoside	+	+	+	+
Phenol	+	-	+	-
Tannin	+	+	-	-
Terpenoid	+	+	-	-
Saponin	-	-	-	-

Conclusion

The study concludes that *M. koenigii* hexane and chloroform leaves extract exhibited effective larvicidal activity against *M. plana*. As it is effective in controlling the larvae of *M. plana*, it can be promoted as biopesticide. The application of biopesticides in plantation sector can help in protecting the environment and human health and thus reducing the management cost.

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Conflict of interests

Author hereby decl ares that there is no conflict of interests with any organization or financial body for supporting this research.

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