Biopesticidal Activity of Lunumidella (*Melia dubia*) Leaf Extract

T.D.D. Kulawardhana, R.D.N. Debarawatta and G. Pamunuwa*

ABSTRACT

This study was conducted to investigate the pest controlling potential of two *Melia dubia* leaf extracts, namely aqueous extract (T1) and aqueous formulation prepared using the methanol extract (T2) to control pests such as thrips, mealy bugs, scale insects and caterpillars. Plant parts with pests were selected and the two prepared extracts were sprayed with a control (C). Five days after application, the Pest Mortality Percentage was recorded. The aqueous extract (T1) was applied for a period of four weeks with seven day intervals. The aqueous formulation prepared incorporating the methanol extract (T2) was applied in two concentrations as 50 g/L and 100 g/L. The aqueous formulation prepared using the methanol extract (T2) was highly effective against thrips, mealy bugs and caterpillars. The aqueous extract (T1) showed the highest Pest Mortality Percentage within two weeks of preparation but the activity decreased thereafter. The Pest Mortality Percentages resulted from the two methods were significantly different from each other and the higher percentage was shown by the aqueous formulation (T2). When the aqueous formulation (T2) was applied to the caterpillars in the cabbage cultivation, it showed a significant reduction in the number of caterpillars along with a reduction of the damage caused by the caterpillars to the cabbage leaves. The yields obtained from the treated beds with T2 were significantly higher than those obtained from the control beds. Even though the aqueous extract (T1) has short storage ability, it can be introduced to farmers as a biopesticide which can be prepared by their own for controlling pests in their crops. As the chemically extracted *Melia dubia* leaf powder which was used in the preparation of the aqueous formulation (T2) can be stored frozen for a long period retaining its bioactivity, there is great potential for improving it as a commercial product.

Keywords: Biopesticide, Leaf extract, *Melia dubia*, Pest mortality

INTRODUCTION

Lunumidella (*Melia dubia*) is a native tree to India and it is a fast-growing tree in the wet and intermediate zones of Sri Lanka. It is commonly known as Maha Neem or Forest Neem and belongs to the family Meliaceae. It is a widely grown tree species in India, Sri Lanka, Malaysia, Australia and Angola (Gopal et al., 2015). *Melia dubia* is commonly found in plains 750 m above sea level and grows well in areas with a rainfall of 625 mm to 875 mm. It needs a well-drained fertile soil with ample moisture supply to grow up to 25 m in height (Gopal et al., 2015). Fruits of *M. dubia* are ellipsoid and flowers are violet and white in color.
Leaves are bi-pinnate and long stalked. Leaflets are in two to five pairs and having an ovate to ovate lanceolate shape with a length of 4-8 cm (Anon, 2011).

*Melia dubia* consists of many primary and secondary metabolites including unsaturated fatty acids, linolenic acid, palmatic acid (Murugesan *et al.*, 2013), polyphenols, steroids, tetranortriterpenoids (Purushothaman, 1984), monoterpenes (Nagalakshmi *et al.*, 2001), alkaloids, carbohydrates, steroids, tannins, flavonoids, limonoids, saponins and glycosides (Valentina *et al.*, 2013).

Importantly, numerous compounds with pesticidal potency are present in different parts of *M. dubia*. Accordingly, many extracts of various parts of this plant show pesticidal properties. Refined bark consists 60-70% of toosandanin that can be used to control *Helicoverpa armigera* (Koul *et al.*, 2002) and that compound is a strong antifeedant, stomach poison and growth inhibitor against *Pieris rapae* larvae (Foon, 1987).

Limonoids from Meliaceae have the potential to control a variety of insect pests effectively without harming the environment (Carpinella *et al.*, 2002). Many different extracts of *M. dubia* have ovicides (Malarvannan *et al.*, 2009), larvicides (Karthikeyan *et al.*, 2014), growth inhibitors, antifeedants, stomach poisons and make moulting disorders and morphological defects of a number of pests (Bhuiyan *et al.*, 2001).

Mealy bugs, thrips, scale insects and caterpillars are major pests causing harm to horticultural crops. Caterpillars reduce the plant parts by eating while others pierce and suck the cell sap hence forming malformed parts. Further, pests are responsible for transferring different plant diseases. Therefore, it is important to control the above pests in order to reduce the economic damage on crops. Different types of synthetic pesticides are used to control the pests in crops. However, those pesticides cause toxicity to non-target organisms, impose hazardous effects on ecosystems and develop resistance to pesticides among the exposed pests (Ntalli *et al.*, 2014).

Increasing environmental pollution and health hazards associated with the use of synthetic pesticides has resulted in renewed interest in the development and use of botanical pesticides for pest management. Therefore, this study was conducted to investigate the pest controlling potential of *M. dubia* leaf extract under laboratory conditions and in the field.

**MATERIALS AND METHODS**

**Experimental Site**

The study was carried out at the farm field of the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura situated in low country intermediate zone (IL1a) at an elevation of 30 m from mean sea level. The study was carried out from June to September, 2017.
**Preparation of the Aqueous Extract (T1) using M. dubia Leaves**

Mature *M. dubia* leaves were collected from the University premises and 1 kg of leaves was boiled with 16 L of water at 100 °C for 3 hours. After allowing it to cool to room temperature, it was filtered using a sieve to separate the leaf extract. A detergent was added as an emulsifier (1 mL of detergent (Teepol) in 1 L of water). The product was referred to as the aqueous extract.

**Preparation of the Aqueous Formulation (T2) using M. dubia Leaves**

*M. dubia* leaves (1 kg) was washed using water to remove dirt. It was dried at room temperature and kept in an oven at 80 °C for 48 hours. The dried leaves were ground using a mortar and pestle to get a powder. Leaf powder was mixed with 1.5 L of methanol (solvent) and it was kept in a mechanical shaker for 72 hours.

The solvent was evaporated using a rotary evaporator and the leaf extract was obtained as a powder. Aqueous formulations of two different concentrations were prepared using the powder from the methanol extract. The concentrations were 50 g/L and 100 g/L. Finally, the same detergent (Teepol) was added as an emulsifier (1 mL of detergent in 1 L of water). The product was referred to as the aqueous formulation.

**Quality Evaluation of the Extracts: Determination of Acid values**

Two milliliters of each leaf extract were dissolved in ethanol and titrated with 0.1 KOH using 1 mL of phenolphthalein indicator (Kovo, 2006). Acid value was calculated using the equation, Acid value = \( v \times c \times 56.1 / m \); where, \( v \) – volume of potassium hydroxide (mL), \( c \) – concentration of potassium hydroxide, \( m \) – mass of the test portion (g), and 56.1 – molar mass of potassium hydroxide.

**Field Layout**

Land was ploughed to a depth of 20 – 30 cm, and 12 raised beds (1×3 m) were prepared with 18 planting holes per each bed. The spacing 50×40 cm was maintained between and within rows. Drains with a width of 30 cm were prepared between beds to improve drainage. The experiment was conducted according to Completely Randomized Design (CRD) with six replicates.

**Crop Establishment and Maintenance**

Cabbage seeds (Variety Green Coronet) was sown in row seeding in a nursery, and nursery management practices were carried out according to the recommendations of the Department of Agriculture (DOA), Sri Lanka.

After 20 days from the seed sawing, the seedlings having four mature leaves were transplanted in the field. Each bed contained 18 plants. Two days before transplanting, organic fertilizer and inorganic fertilizer were incorporated into the soil according to the recommendations of the Department of Agriculture, Sri Lanka (Table 1).
Treatment Application for Controlling of Selected Pests under Laboratory Conditions

Plant parts with pests (thrips, scale insects, mealy bugs and caterpillars) were obtained and ten replicates were prepared with three plant parts per collecting container for the two treatments and control. Then, the *M. dubia* leaf extract was prepared using the boiling method (T1 – Aqueous extract) and solvent extraction (T2 – Aqueous formulation), and the preparations were sprayed separately as treatments T1 and T2 respectively. Water, which was the medium used in the preparation of the biopesticides T1 and T2, was sprayed as the control (C). Spraying was done using a hand sprayer.

*M. dubia* aqueous extract prepared by the boiling method (T1) was stored at room temperature and the *M. dubia* methanol extract, used subsequently to prepare the aqueous formulation (T2), was kept frozen as a powder.

Treatment Application for Controlling of Selected Pests in Field Experiments

Eighteen beds were prepared and twelve beds were selected randomly for treatment application while six were demarcated for the control. In each bed, 10 plants were randomly selected and they were labelled for data recording. *M. dubia* aqueous formulation (T2) was applied as the treatment, and water, which was the medium used in the preparation of T2, was applied as the control (C).

*Melia dubia* aqueous formulation (T2) was applied to the beds with five-day intervals (Application 1, 2 and 3) according to completely randomized design (CRD). Numbers of live caterpillars were counted on each plant one day after application of T2.

The leaf area was measured visually from randomly selected two leaves from each plant and observations were indicated as per Table 2 both before and after the application of aqueous formulation (T2). Further, tap water, which was used as the medium in the preparation of T2, was applied as the control (C). Furthermore, the fresh weights of cabbage shoots per plant of both treated samples and control were measured after harvesting which was carried out after hundred days after transplanting from the nursery.

Statistical Analysis

Data obtained from ‘Pest Mortality Percentage’ (PMP) against two different concentration levels of aqueous formulation (T2) were analyzed by Student’s t test. Mean separation of the ‘Overall Effect of *M. dubia* Extracts on Controlling Pests in the Laboratory’ was done using the Tukey’s Test.

The mean values of ‘Number of Caterpillars in Cabbage Leaves’ and ‘Leaf Area of the Field Planted Cabbage Leaves by giving a Score for Remaining Leaf Area’ were analyzed using Kruskal-Wallis Test. Mean separation of the ‘Yield of Cabbage’ was done using the Tukey’s Test.
Table 1. Amounts of Fertilizer Applied as per Recommendation for Cabbage

| Fertilizer Application | Urea (g/3m² area) | TSP (g) (g/3m² area) | MOP (g/3m² area) |
|------------------------|-------------------|----------------------|------------------|
| BD                     | 33                | 82.5                 | 22.5             |
| TD1 (3 WAT)            | 33                | -                    | -                |
| TD2 (6 WAT)            | 33                | -                    | 22.5             |

*TSP- Triple Super Phosphate, MOP- Muriate of Potash, BD- Basal Dressing, TD- Top Dressing, WAT- Weeks After Transplanting*

Table 2. Scores of Remaining Leaf Area after the Caterpillar Damage of Cabbage Leaves

| Score | Leaf Area (%) |
|-------|---------------|
| 1     | 1 – 10%       |
| 2     | 11 – 20%      |
| 3     | 21 – 30%      |
| 4     | 31 – 40%      |
| 5     | 41 – 50%      |
| 6     | 51 – 60%      |
| 7     | 61 – 70%      |
| 8     | 71 – 80%      |
| 9     | 81 – 90%      |
| 10    | 91 – 100%     |

RESULTS AND DISCUSSION

*Quality Evaluation of M. dubia Leaf Extracts*

Two quality parameters, pH and acid value, were determined in both *M. dubia* leaf extracts: the aqueous extract (T1) and the aqueous formulation prepared by incorporating the powdered methanol extract (T2). The pH values of the aqueous extract (T1) and aqueous formulation (T2) were 4.35 and 4.36, respectively, which indicates that there is no any considerable variation of pH between the two types of preparations of *M. dubia* leaf extract. According to Jayathilaka et al. (2016), the pH value of *Azadirachta indica* leaf extract was 4.22. Therefore, it can be stated that the pH of plant extracts of same family are considerably acidic in nature. The acid values of the aqueous extract (T1) and aqueous formulation (T2) were also quite similar and they were 0.25 mL/g and 0.26 mL/g respectively.

*Laboratory Application of Aqueous Extract (T1) for Controlling of Selected Pests*

According to Figure 1, the lowest PMP was observed in scale insects while the highest PMP was observed in caterpillars every
Thrips, that exhibited the second highest PMP, showed a considerably higher value than mealy bugs.

The Pest Mortality Percentage increased with time up to the second week and it started to decrease thereafter. Therefore, a deterioration of the pest controlling ability of the M. dubia aqueous extract (T1) with time can be recognized. Thus, the extract can be used up to two weeks after preparation to have a higher pest controlling potential.

Gopal et al. (2015) reported that M. dubia leaf extracts possess larvicidal activity and Carpinella et al. (2002) reported that limonoid from M. dubia shows an antifeedant activity and growth regulating activity against Spodoptera species. Therefore, it can be stated that the M. dubia aqueous extract obtained by the boiling method (T1) is much suitable against caterpillars showing consistency with the above mentioned findings.

The boiling method is an easy way to prepare a biopesticide from M. dubia. Further, the cost of preparation is very low. The needed raw materials for the preparation of aqueous extract can be found from the environment. Therefore, the preparation of the aqueous extract using the boiling method (T1) is much suitable for ordinary farmers to control the pests in their crops. Another advantage is the ability to keep the prepared biopesticide (T1) for two weeks without reducing its pest controlling ability.

Laboratory Application of Aqueous Formulation (T2) for Controlling of Selected Pests

According to the obtained results, the Pest Mortality Percentage (PMP) of selected pest types (thrips, mealy bugs, scale insects and caterpillars) at the two tested concentrations - 50 g/ L and 100 g/ L - of T2 were different. A high pest mortality percentage (> 80%) was shown by mealy bugs, thrips and caterpillars against both concentrations, while the lowest pest mortality (approximately 25%) was shown by scale insects (Table 3).

The reason for the lowest PMP shown by the scale insects could be their resistant body outer cover that makes it difficult to penetrate biopesticides into the pest’s body. Ntalli et al. (2014) reported that the methanol extract can decrease the heartbeat and cause malformations of fat body and mid gut cells in Spodoptera species. Koul et al. (2002) reported that the methanol extract of M. dubia can reduce the growth of Helicoverpa armigera. Accordingly, the extracts, in particular the aqueous formulation (T2), showed a much higher pest controlling ability against the selected pests except scale insects.

There was no significant difference in PMP between the two concentrations used in this study for controlling thrips and caterpillars (Table 3). Figure 2 shows the effect of application of the Melia dubia extracts on caterpillars, whereas figure 3 shows its effect on thrips.
Bio-pesticidal Activity of Lunumidella Leaf Extract

**Figure 1.** Pest Mortality Percentage (%) of the aqueous extract (T1).

**Table 3.** Mean of Pest Mortality Percentage (PMP) against two different concentration levels of aqueous formulation (T2)

| Factor   | Thrips  | Mealy bugs | Scales  | Caterpillars |
|----------|---------|------------|---------|--------------|
| CE 1     | 85.21<sup>a</sup> | 88.69<sup>b</sup> | 25.60<sup>b</sup> | 88.33<sup>a</sup> |
| CE 2     | 87.24<sup>a</sup> | 96.67<sup>a</sup> | 29.34<sup>a</sup> | 95.00<sup>a</sup> |
| Control  | 1.38<sup>b</sup> | 0.43<sup>c</sup> | 0.33<sup>c</sup> | 0.00<sup>b</sup> |

*Means with same superscripts are not significantly different at 0.05 level. PMP- Pest Mortality Percentage, CE 1- Concentration 50 g/L, CE 2- Concentration 100 g/L.

The highest PMP was shown by mealy bugs and scales at the higher concentration level (CE 2; i.e. 100 g/L) of the aqueous formulation T2. Therefore, the *M. dubia* aqueous formulation (T2) can be used at a concentration of 50 g/L effectively to control thrips and caterpillars while it can be used at a concentration of 100 g/L to control mealy bugs and scales. As thrips, mealy bugs and caterpillars are a common problem to most horticultural crops and as the *M. dubia* leaf extracts are effective in controlling them, these extracts show potential as biopesticides against such pests.

**Overall Effect of M. dubia Extracts on Controlling Pests in the Laboratory**

According to the obtained results, there was no significant difference of PMP between the aqueous extract (T1) and aqueous formulation (T2) (Table 4). However, there was a significant difference between the control (C) and the aqueous formulation (T2) such that the aqueous formulation (T2) was more effective, while there was no any significant difference between the control(C) and aqueous extract (T1) at 95% confidence interval.
Figure 2. (A) Caterpillars damaging cabbage leaves; (B) Deadly appearance of caterpillars after the application of *Melia dubia* extract

Figure 3. (A) Thrips before the application of *Melia dubia* extract; (B) Dead thrips after the application of the extract

The control recorded the lowest PMP while the aqueous formulation (T2) showed the highest PMP, and the difference of the values was significant. Sasidharan *et al.* (2011) reported that methanol is a polar solvent and it can be used to extract organic hydrophilic compounds in plants. Thus, it could be expected that the aqueous formulation of *M. dubia* (T2) contains much more organic hydrophilic compounds than the *M. dubia* aqueous extract (T1), thus contributing to its higher pest controlling potential.
The solid methanol extract can be stored for a long period in a deep freezer preventing quality deterioration and the biopesticide (i.e. aqueous formulation (T2) can be prepared immediately before application). Due to the higher pest controlling ability of the aqueous formulation (T2) prepared through the solvent extraction of M. dubia leaves, it may be improved as a commercial product which may be used to control many major pests that damage horticultural crops.

Table 4. Overall Pest Mortality Percentage

| Treatment | Pest Mortality Percentage |
|-----------|--------------------------|
| T1        | 47.28 ab                 |
| T2        | 74.47 a                  |
| C         | 9.06 b                   |

Means with same superscripts are not significantly different at 0.05 level. PMP - Pest Mortality Percentage, T1- Aqueous extract, T2- Aqueous formulation, C – Control

Effect of M. dubia Extract on Controlling Caterpillars in Cabbage Cultivated Field

According to Table 5, the number of caterpillars in the cabbage cultivated beds was significantly different before and after each of the three applications of the aqueous formulation (T2) carried out at five-day intervals. Interestingly, the probability values of the number of caterpillars due to the three applications of the treatment (T2) were less than 0.05, and therefore, there is a significant effect on controlling caterpillars due to the aqueous formulation (T2) at every application.

Senthilkumar et al. (2012) reported that M. dubia leaf extract was effective against teak defoliator Hyblaeapuera. Toosendanin, which is a limonoid occurring in the bark, possesses insect growth inhibitory activity mainly against lepidopteran pests (Koul et al., 2000). Accordingly, the aqueous formulation (T2) of M. dubia possesses the biopesticide potential to control the caterpillars.

Effect of M. dubia Extract on Leaf Area Scores and Yield of Cabbages

The probability values of leaf area in the treated beds were not significantly different after the application of the aqueous formulation (T2) as the probability values were greater than 0.05. This result indicates that the leaf area of the cabbage plants treated with M. dubia aqueous formulation (T2) remained almost the same without getting reduced. This effect could be due to the reducing of the number of caterpillars by the application of the aqueous formulation (T2) as shown in Table 6. Therefore, M. dubia aqueous formulation (T2) may have the potential to be used to protect cabbages against damage caused by caterpillars.

The cabbage yield, in terms of average shoot weight per plant after application of the aqueous formulation (T2) was $0.153 \pm 0.106$ g while the control (C) exhibited $0.104 \pm 0.058$ g average shoot weight per plant. Therefore, there was a significant difference of cabbage yield between the cabbage plants treated with M. dubia aqueous formulation (T2) and the control (C) at 95 % confidence level. Cabbage plants in the control experiment were severely damaged by caterpillars and as a
Table 5. Effect of application of aqueous formulation on number of caterpillars

| Application of aqueous formulation (T2) | Mean | Probability value |
|----------------------------------------|------|-------------------|
| Application 1                          |      |                   |
| Before                                 | 4.6  | 0.000             |
| After                                  | 1.2  |                   |
| Application 2                          |      |                   |
| Before                                 | 1.0  | 0.000             |
| After                                  | 0.1  |                   |
| Application 3                          |      |                   |
| Before                                 | 0.1  | 0.034             |
| After                                  | 0.0  |                   |

Significantly different at p< 0.05 level. n = 60. T2 – Aqueous formulation

Table 6. Effect of application of aqueous formulation on leaf area

| Application of aqueous formulation (T2) | Mean Score of Leaf Area | Probability Value |
|----------------------------------------|-------------------------|-------------------|
| Application 1                          | 6.9                     | 0.083             |
| After                                  | 6.6                     |                   |
| Application 2                          | 6.6                     | 0.159             |
| Before                                 | 6.6                     |                   |
| After                                  | 6.6                     |                   |
| Application 3                          | 6.6                     | 0.321             |
| Before                                 | 6.6                     |                   |
| After                                  | 6.6                     |                   |

Significantly different at p< 0.05 level. n = 60. T2 – Aqueous formulation

result, the shoot weight per plant was reduced. The yield obtained from the plots treated with the aqueous formulation (T2) was significantly high due to controlling of caterpillars by the *M. dubia* methanol extract. Therefore, it can be assumed that the aqueous formulation (T2) prepared using the methanol extract of *M. dubia* may be used to reduce the damage caused by caterpillars to the crops in cultivations.

CONCLUSION

The methanol extract of *M. dubia* leaves can be used much more effectively than the aqueous extract to control thrips, mealy bugs and caterpillars. The aqueous formulation, which was prepared using the methanol extract of *M. dubia* leaves, helps to prevent the damages caused by caterpillars in the crop cultivations to a great extent. Therefore, the yield can be secured as it prevents the reduction of the leaf area through reducing the number of caterpillars. *M. dubia* aqueous formulation applied cabbage plants gave a significantly higher yield compared to the control. As the *M.*
dubia methanol extract powder can be stored for a long time in the frozen state, it may be possible to develop that extract as a commercial product. Hence, the farmers could prepare the biopesticide (i.e. aqueous formulation (T2)) easily from the frozen product, when it is needed. Even though the aqueous extract (T1) cannot be kept for a long period, it can be easily prepared by farmers at the onset of pests infestation. In sum, both the aqueous and methanol extracts of M. dubia leaves show high pesticidal activity while the methanol extract shows better activity. Also, the methods of preparation of the aqueous extract (T1) and aqueous formulation from the powdered methanol extract (T2) are relatively simple. Furthermore, it can be suggested that the utilization of the aqueous extract (T1) may be beneficial for small scale cultivations while the utilization of the aqueous formulation (T2) may be beneficial for relatively large-scale cultivations.

ACKNOWLEDGEMENT

Financial assistance given by the research grant Agri/D/4/1/1/II by the Department of Agriculture, North Western Province, Sri Lanka is greatly appreciated. The authors wish to express their gratitude to Prof. N.R. Abenayake and Mr. K.H.M. Indika Karunarathne for the statistical analysis of data. Sincere gratitude is extended to academic and non-academic staff of the Department of Horticulture and Landscape Gardening for their assistance given during the study.

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