



ORIGINAL ARTICLE

# Insecticidal activity of *Muntingia calabura* extracts against larvae and pupae of diamondback, *Plutella xylostella* (Lepidoptera, Plutellidae)

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Cordycepin

**Abstract** The aim of the present study was to evaluate the insecticidal effects of hexane and ethanolic extracts of the flowers and fruits of *Muntingia calabura* against diamondback moth, *Plutella xylostella*. The leaf disc immersion methodology was carried out to assess the insecticidal effects on the larvae and pupae as well as duration of the larval phase following feeding of the first instar larvae of diamondback on the treated leaf discs for 72 h. All extracts were toxic to larvae and pupae of *P. xylostella*. The ethanolic extracts of the flowers and fruits of *M. calabura* were the most toxic against first instar *P. xylostella* larvae with LC<sub>50</sub> values of 0.61 µg mL<sup>-1</sup> and 1.63 µg mL<sup>-1</sup> respectively, followed by the hexane extract of the fruits (LC<sub>50</sub> = 5.5 µg mL<sup>-1</sup>) and flowers (LC<sub>50</sub> = 18.9 µg mL<sup>-1</sup>). All extracts were more toxic to *P. xylostella* larvae compared with cordycepin, the positive control which produced 100% mortality at 500 µg mL<sup>-1</sup> in 72 h. Fruit extracts were more active than the flowers in producing pupal mortality following 72 h of feeding of the first instar larvae on leaf discs treated with the extracts. Both the hexane and the ethanolic extracts of *M. calabura* fruits and flowers prolonged larval duration by ~2 days in some cases as compared with the control (7.2 days). These results suggest that *M. calabura* has potential for development as commercial insecticide for controlling *P. xylostella* due to its insecticidal effects.

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## 1. Introduction

*Plutella xylostella* (L.) (Lepidoptera, Plutellidae), commonly known as the diamond back moth, is one of the most important pests of cruciferous crops throughout the world, causing direct damage to cabbage, with losses as much as 100% (Castelo-Branco and Gatehouse, 2001). The synthetic insecticides have been the main control strategy for this pest (Sarfraz

and Keddie, 2005). The indiscriminate use of synthetic pesticide is harmful to the ecosystem due to its high degree of toxicity and affects not only the target pest but also beneficial species. The use of these insecticides has facilitated the emergence of generations of more resistant insects and caused the contamination of crops with toxic residues, thereby placing human health at risk (Oliveira et al., 1999). Medicinal plants have been investigated as an alternative to conventional insecticides, with reports of toxicity to a number of arthropods long before the advent of synthetic insecticides (Martins et al., 1994).

The search for insecticidal properties in medicinal plants has increased significantly in recent years and has become a promising field of research. Recent studies involving organic extracts from plants with medicinal properties have reported insecticidal properties such as mortality (Rani et al., 1999; Boiça-Júnior et al., 2005; Li et al., 2008) and feeding deterrence (Zhang et al., 2003; Liu et al., 2007) in *P. xylostella* larvae as well as repellence (Hou et al., 2002) and infertility in adults (Gu et al., 2004).

The potential of *P. xylostella* to cause harm, together with emerging populations that are resistant to synthetic insecticides (Bhattacharya et al., 2002; Sayyed et al., 2008), has led researchers world-wide to search for botanical insecticides in different genera of plant species for the control of this pest. *Muntingia calabura* is one of the many species from the family Tiliaceae used in folk medicine throughout the world. It is commonly known as the Jamaican cherry, Panama berry or Singapore cherry in English and Pau-seda or *calabura* in Brazil. *Muntingia calabura* is a native plant from the Antilles that was introduced in Brazil by the Instituto Agrônomo de Campinas in 1962 (Lopes-Moura et al., 1999). Although not native to South America, this plant has been widely cultivated and used in urban planning as a decorative tree on streets and public squares in different regions of Brazil. In folk medicine, infusions from the leaves were used to reduce gastric distress and prostate swelling (Morton, 1987). The flowers were used to make tea for headaches relief and early cold symptoms and have antiseptic and anti-spasm effects (Pio-Corrêa, 1987). The fruit is highly appreciated as an *in natura* food or used to make jams.

There are literature reports on chemical and biological studies of the leaves and roots of *M. calabura*. Kaneda et al. (1991) and Su et al. (2003) studied the anti-tumour properties of organic extracts from the roots and leaves, respectively. The leaf extract also has antinociceptive properties against chemically or thermally induced noxious stimuli (Zakaria et al., 2006, 2007). Despite the studies published on the proven effectiveness of organic and/or aqueous extracts from the leaves and flowers of *M. calabura*, reports about the biological action of organic extracts from the fruits and flowers against *P. xylostella* are lacking. The chemical composition of *M. calabura* plant is already known and the leaves have been shown to contain flavonoids, chalcones, terpenoids and phenolic compounds (Preethi et al., 2012).

As part of a systematic study to evaluate the insecticidal potential of the medicinal flora that grows in Pernambuco (Brito et al., 2006; Pereira et al., 2008, 2009), the aim of the present study was to assess the insecticidal effect of hexane and ethanolic extracts from the fruits and flowers of *M. calabura* against *P. xylostella* larvae and pupae. Since *P. xylostella* larvae are very difficult pests to control it is important to control

them at an early stage to minimize damage to the crucifer production in many parts of the world.

## 2. Material and methods

### 2.1. Plant material

Flowers and fruits were collected from the *M. calabura* tree located in the campus of the Universidade Rural Federal de Pernambuco (UFRPE), Recife – Brazil in July 2008. The plant was identified by Dr. Carmen Zickel from Biology Department, UFRPE and a voucher sample was deposited in the Vasconcelos Sobrinho Herbarium, UFRPE (#2816).

### 2.2. Hexane and ethanolic extract preparation

Hexane and ethanolic extracts were prepared from the fruits and flowers of *M. calabura*. Fruits and flowers after collection were washed and oven-dried at 40 °C for 48 h and were ground in a mill and weighed separately. They were then transferred to glass beakers and hexane was added. Cold maceration was carried out for 72 h with 24 h intervals to ensure the extraction of a larger number of substances from the plant. The extract was filtered and evaporated at reduced pressure to minimize possible degradation of the chemical constituents at high temperatures. After removal of the solvent, crude hexane extract was obtained. The same procedure was repeated using ethanol as a maceration solvent to obtain crude ethanolic extract.

### 2.3. Insects rearing and colony maintenance

A colony of *P. xylostella* was maintained at the Laboratory of Insect Biology of UFRPE. *Plutella xylostella* was raised based on the method described by Barros and Vendramim (1999). The newly-emerged adults were sexed and placed in plastic cages with a sponge soaked in water to maintain proper humidity inside the cage. On the sponge was placed a disc of filter paper (8.0 cm diameter) and a leaf-disc of *Brassica oleracea* (8.0 cm diameter) to stimulate the oviposition of *P. xylostella*. Adults were fed with a 10% honey solution provided in polyurethane foam attached to a circular hole at the top of the cage.

Discs of kale leaves with the eggs were transferred to Petri dishes daily, where they remained until the hatching of the *P. xylostella*. The discs and the larvae were kept in rectangular plastic containers (60 × 30 cm) with organic kale leaves used as food. The larvae remained in these containers until pupation and the kale leaves were replaced daily with the fresh ones. These pupae were collected in test tubes sealed with plastic containing PVC (polyvinyl chloride) for air circulation. The pupae were kept under room temperature until emergence of adults, which were transferred to new cages (rectangular plastic containers; 60 × 30 cm).

### 2.4. Screening of insecticidal activity

A one-gram aliquot of hexane and ethanolic extract from the flowers and fruits of *M. calabura* was diluted in 19.9 mL of distilled water and 0.1 mL of Tween 80 as an emulsifier. The solution was mixed and filtered through a Whatman No. 1 filter

paper and served as a stock solution. Stock solution was diluted to the desired concentrations ranging from 0.25 to 30  $\mu\text{g mL}^{-1}$ . All experiments were conducted in the Laboratory of Insect Biology/Plant Resistance to Insects, Agronomy Department, UFRPE, between October 2008 and January 2009, at temperature =  $30 \pm 0.5$  °C, Relative humidity =  $67 \pm 2.0\%$  and photoperiod = 12 h.

### 2.5. Larval toxicity

The leaf disc immersion method was used to determine toxicity of larvae. Discs of kale leaf (8 cm diameter), were immersed for 10 s in 50  $\mu\text{l}$  of the different concentrations of the extract solutions: MCHFL (*M. calabura*-hexane extract of flowers), MCHFR (*M. calabura*-hexane extract of fruits), MCEFL (*M. calabura*-ethanolic extract of flowers), and MCEFR (*M. calabura*-ethanolic extract of fruits). Leaf-discs were air dried and transferred individually to Petri dishes (9 cm diameter) containing a filter paper disc (8 cm diameter) soaked in distilled water. Ten first instar larvae (< 12 h old) were introduced on the leaf discs. After placing lids on the Petri dishes they were wrapped in a Parafilm to avoid larval escaping. Concentrations varied from 0.25 to 30  $\mu\text{g mL}^{-1}$  for the extracts. The experimental design was entirely randomized, with five treatments including the control and four replications per treatment. Experiment was repeated twice. Mortality was assessed after 72 h of being feeding on the treated/control leaf discs. Cordycepin (500  $\mu\text{g mL}^{-1}$ ) was used as a positive control as it produced 94% mortality in third instar *P. xylostella* (72 h after treatment) at a concentration of 500  $\text{mg L}^{-1}$  (Kim et al., 2002). It is described as an insecticidal constituent of the fungus, *Cordyceps militaris* fruiting body (Kim et al., 2002). Although the exact mode of action of cordycepin is not known, it has been shown to act as a stomach poison rather than having a direct inhibitory effect on chitin synthesis (Kim et al., 2002).

### 2.6. Pupal toxicity

Mortality of the pupae was based on the method described by Park et al. (2002) with modifications. First instar larvae were reared on kale leaf discs treated with different concentrations of the extract solutions for 72 h after which the non-treated kale leaf discs were introduced into the cages and replaced with fresh ones every 48 h as described earlier until pupation. Control group was treated with water alone. We started off with approximately 35 larvae for each treatment to have at least 20 healthy larvae pupated/treatment, due to toxicity at higher concentrations.

Concentrations varied from 0.25 to 30  $\mu\text{g mL}^{-1}$  for the extracts. Experiment was repeated twice. The larvae that had transformed into the pupae were individually transferred to dishes to determine mortality within 72 h of being in pupation.

### 2.7. Larval duration

Larval duration was determined by rearing first instar larvae on kale leaf discs treated with different concentrations of the extract solutions for 72 h after which the non-treated kale leaf discs were introduced into the cages and replaced with fresh ones every 48 h until pupation as described earlier. Control

group was treated with water alone. We started off with approximately 35 larvae for each treatment to have at least 20 healthy larvae/treatment until pupation as the extracts were toxic at higher concentrations. Experiment was repeated twice. Larval duration was described as the number of days from hatching of the larvae to the pupal stage. Concentrations of the extracts varied from 0.25 to 30  $\mu\text{g mL}^{-1}$ .

### 2.8. Statistical analysis

Data obtained in these experiments were subjected to a One-Way Analysis of Variance using statistics software (Statistix 8, 2008). Where significant *F* values were found, a Tukey's multiple comparison test was used to test for significant differences between individual treatments.  $\text{LC}_{50}$  (lethal concentration producing 50% mortality) values with 95% confidence level were calculated with the Probit model using Polo-PC Software program (LeOra Software, 1987).

## 3. Results

### 3.1. Larval toxicity

The toxic effects of the *M. calabura* extracts varied depending on the part of the plant and type of solvent. The ethanolic extract of the flowers (MCEFL) was the most active followed by the ethanolic extract of the fruits (MCEFR), hexane extract of the fruit (MCHFR) and hexane extract of the flower (MCHFL) (Table 1).

$\text{LC}_{50}$  values (concentrations causing 50% mortality compared with the control) and the corresponding confidence intervals of the *M. calabura* extracts for larval mortality are displayed in Table 1. Cordycepin, the positive control, produced 100% mortality at 500  $\mu\text{g mL}^{-1}$  in 72 h.

Toxicity of the hexane and ethanolic extracts of the flower and fruit of *M. calabura* against first instar *P. xylostella* larvae, differed significantly from each other based on their non-overlapping confidence intervals of the  $\text{LC}_{50}$  values (Table 1). The ethanolic extracts were significantly more active than hexane extracts. Within the same extracts, the MCEFL ( $\text{LC}_{50} = 0.61 \mu\text{g mL}^{-1}$ ) was 4.3-fold more toxic than MCEFR ( $\text{LC}_{50} = 2.61 \mu\text{g mL}^{-1}$ ), while MCHFR ( $\text{LC}_{50} = 5.50 \mu\text{g mL}^{-1}$ ) was 3.4-fold more toxic than MCHFL ( $\text{LC}_{50} = 18.94 \mu\text{g mL}^{-1}$ ). Comparing the relative toxicities between them, MCEFL was 31.0-fold more toxic than MCHFL and 4.2 and 8.9 folds more toxic than MCEFR and MCHFR, respectively (Table 1).

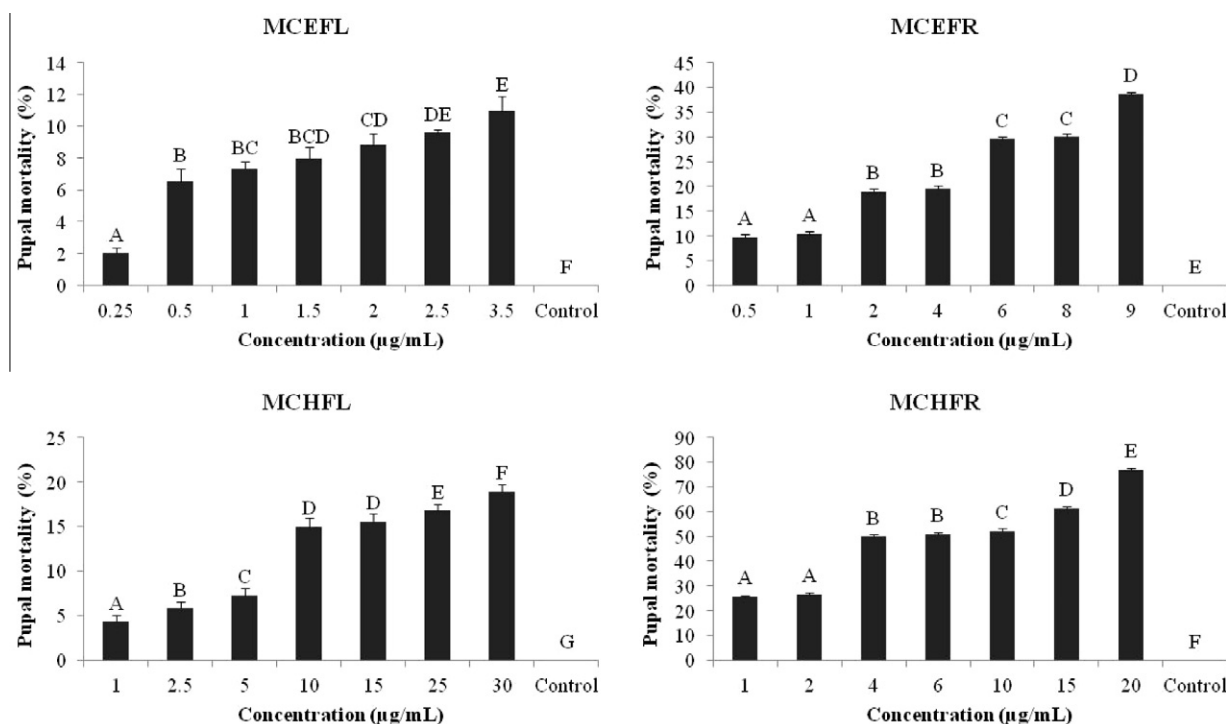
### 3.2. Pupal toxicity

The results of pupal toxicity following prior exposure of larvae to the extracts are displayed in Fig. 1. Treatment of the first instar larvae for 72 h with the hexane and the ethanolic extracts of *M. calabura* fruit (MCHFR) produced greater mortality of the subsequent pupae compared with the flower extracts. Pupal mortalities were 39% and 77% following prior exposure of the larvae to 9 and 20  $\mu\text{g mL}^{-1}$  of the ethanolic and the hexane fruit extracts respectively. Pupal mortalities were 11% and 18% following prior exposure of the larvae to 3.5 and 30  $\mu\text{g mL}^{-1}$  of ethanolic and hexane extracts of the flower respectively. Mortality of the flower extracts was

**Table 1** Lethal concentration (LC<sub>50</sub>) and toxicity ratio of hexane and ethanolic fruit and flower extracts of *M. calabura* on *P. xylostella* larvae following feeding on treated kale leaves for 72 h.

Extracts	<i>n</i>	df	Slope (CI 95%) <i>Plutella xylostella</i> larvae	LC <sub>50</sub> (µg mL <sup>-1</sup> ) (CI 95%)	χ <sup>2</sup>	TR <sub>50</sub>
MCEFL	600	4	2.76 (2.57–2.95)	0.61 (0.45–0.79)	9.34	–
MCEFR	600	4	2.43 (2.27–2.58)	2.61 (2.09–3.21)	6.03	4.23
MCHFL	600	4	1.45 (1.30–1.59)	18.94 (13.23–33.92)	6.27	30.72
MCHFR	700	4	2.27 (2.12–2.42)	5.50 (4.40–6.81)	10.60	8.91

*n* = Number of insects tested, df = Degrees of freedom, χ<sup>2</sup> = Chisquare, CI = Confidence Interval, TR = Toxicity ratio calculated according to Robertson and Presley (1992). MCEFL = ethanolic extract from flowers, MCEFR = ethanolic extract from fruits, MCHFL = hexane extract from flowers, MCHFR = hexane extract from fruits of *M. calabura*. Cordycepin, the positive control, produced 100% mortality at 500 µg mL<sup>-1</sup> in 72 h.



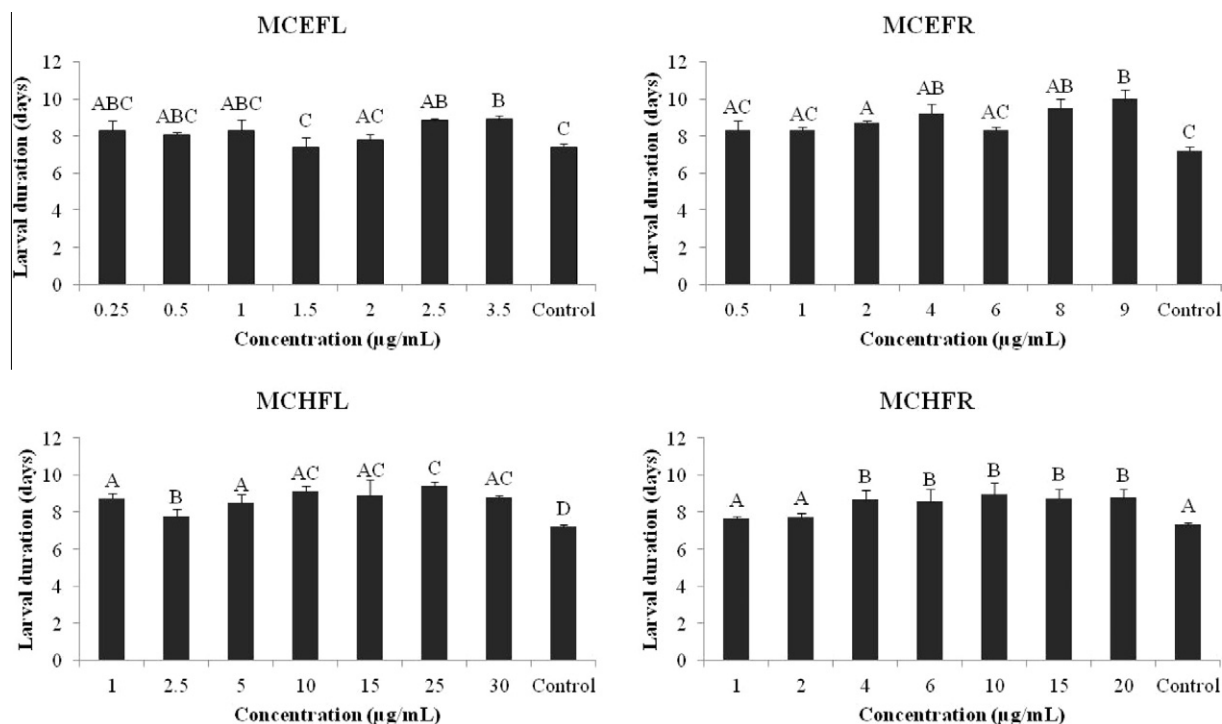
**Figure 1** Pupal mortality of *P. xylostella* following larval feeding on kale leaf discs treated with different concentrations of *M. calabura* extracts. Mortality of the pupae was assessed within 72 h in the pupal stage. Means ( $\pm$ SE) followed by the same letters are not significantly different from each other (Tukey's test;  $P = 0.05$ ).

< 8% at the lowest concentrations. A One-Way ANOVA on pupal mortality following feeding of the first instar larvae on the leaf discs treated with the extracts for 72 h showed significant  $F$  values ( $F_{7,23} = 88.3$ ;  $p < 0.05$  for MCEFL,  $F_{7,23} = 1128$ ;  $p < 0.05$  for MCHFL,  $F_{7,23} = 1834$ ;  $p < 0.05$  for MCEFR,  $F_{7,23} = 7848$ ;  $p < 0.05$  for MCHFR). Mortality in the subsequent pupae following feeding on the extracts was significantly greater than the control pupae (Tukey's test,  $P < 0.05$ ) (Fig. 1) at all concentrations.

### 3.3. Duration of larval phase

Larval duration varied with the extracts. A One-Way ANOVA on larval duration following feeding of the first instar larvae on

the leaf discs treated with the extracts for 72 h produced significant  $F$  values ( $F_{7,23} = 7.68$ ;  $p < 0.05$  for MCEFL,  $F_{7,23} = 42.6$ ;  $p < 0.05$  for MCHFL,  $F_{7,23} = 10.6$ ;  $p < 0.05$  for MCEFR,  $F_{7,23} = 29.8$ ;  $p < 0.05$  for MCHFR). Larval duration of the treated larvae was significantly greater than the control for most of the concentrations of ethanolic and hexane extracts of the fruits and the flowers (Tukey's test;  $P = 0.05$ ). Comparing the effects of the different concentrations of *M. calabura* ethanolic extracts on larval duration, although there was a lack of dose response, larval duration of the treated larvae was significantly greater than the control at some concentrations (2.5 and 3.5 µg mL<sup>-1</sup> of MCEFL, 2.0, 4.0, 8.0 and 9.0 µg mL<sup>-1</sup> of MCEFR) (Fig. 2). Comparing *M. calabura* hexane extracts, both MCHFL (larval duration 7.8–9.3 days) and MCHFR (larval duration 8.7–9.4 days)



**Figure 2** Larval duration of *P. xylostella* following larval feeding of kale leaf discs treated with the *Calabura* extracts at different concentrations ( $n = 20$ ). Means followed by the same letters are not significantly different from each other (Tukey's test;  $P = 0.05$ ).

produced a significant increase in larval duration at all concentrations (Tukey's test;  $P = 0.05$ ) except for the two lowest concentrations of hexane extract of fruits (MCHFR) compared with the control (larval duration 7.2 days) (Fig. 2).

#### 4. Discussion

All extracts including the hexane and ethanolic fruit and flower extracts of *M. calabura* were toxic to *P. xylostella* larvae. Prior feeding of the first instar larvae on extracts treated leaf discs for 72 h also had an effect on subsequent pupae as well as prolongation of the larval duration. Our results have demonstrated that *M. calabura* extracts are more toxic than other organic and aqueous extracts from different plant species that have been evaluated for toxicity against *P. xylostella* larvae (Kodjo et al., 2011; Boiça-Júnior et al., 2005; Li et al., 2008; Trindade et al., 2011).

In the present study, ethanolic extract of flowers (MCEFL) of *M. calabura* was the most active against *P. xylostella* larvae with a  $LC_{50}$  of  $0.61 \mu\text{g mL}^{-1}$ . Our extracts seem to be more active than some of the other extracts reported previously against *P. xylostella*. Kodjo et al. (2011) tested aqueous extracts of the leaf, roots and seed as well as the oil emulsion of *Ricinus communis* (castor bean) against diamondback moth. A 10% oil emulsion of *R. communis* produced 100% mortality in third instar diamondback moth larvae using contact and ingestion bioassays. Whereas, aqueous extracts were less active producing 71%, 52% and 50% mortalities following feeding on cabbage leaf discs treated with 20% of seed, leaf and root extracts of *R. communis* respectively (Kodjo et al., 2011). Dursban, a positive control used in the same study produced 88% mortal-

ity at 5%. This shows that our extracts were more active than *R. communis* extracts, oil emulsion as well as dursban (positive control). Uma et al. (2009) tested the insecticidal effects of stem and leaf extracts of 4 *Euphorbia* species against diamondback moth. Mortality rates were 7% and 43% for second instar *P. xylostella* larvae following 96 h feeding on mustard leaf discs treated with 20% alcoholic leaf extracts of *Euphorbia nivula* and *E. pulcherrima* respectively. Mortality rates were 7% and 10% following 96 h feeding on mustard leaf discs treated with 20% alcoholic stem extracts of *E. antiquorum* and *E. tirucalli* respectively (Uma et al., 2009). Boiça-Júnior et al. (2005) used 10% aqueous extracts of *Enterolobium contortisilioidium* (fruits), *Nicotiana tabacum* (leaves), *Sapindus saponaria* (fruits) and *Trichilia pallida* (twigs) to achieve a 100% *P. xylostella* larval mortality following feeding on treated kale (*B. oleracea* var. *acephala*) leaves. Mortality was 100% in *P. xylostella* larvae following feeding on kale leaf discs treated with crude ethanolic extract of *Annona muricata* at  $5 \text{ mg mL}^{-1}$  for 12 days. Larval duration was also increased by 2.6 days at this concentration (Trindade et al., 2011).

Torres et al. (2001) obtained the same mortality rate for *P. xylostella* larvae by using 10% aqueous extracts from the seeds and bark of *Azadirachta indica* A. Juss. and *Aspidosperma pyriforme* Mart. Recently, Li et al. (2008) demonstrated 91.7% larval mortality rate by using acetone fraction of the chloroform extract of *Xanthium sibiricum* at a concentration of  $50 \text{ g/L}$ , making this extract 15-fold less toxic than the MCEFL extract. Rani et al. (1999) achieved a 100% mortality rate of *P. xylostella* larvae with an ethanolic extract from the twigs of *Melia azadirach* at concentrations  $> 7.5\%$ , which are much higher than the concentrations used in our study.

Plant extracts are mixtures of several compounds. Blends are more effective than individual compounds in terms of forestalling and diluting resistance (Feng et al., 1995) and habituation (Akhtar et al., 2003) for long-term use. A possible explanation is that the activity of extracts may be attributed to more than one compound resulting either from the synergistic interaction between major compounds or their interaction with minor compounds (Akhtar et al., 2012a).

All extracts produced a significant increase in larval duration at most of the concentrations compared with the control. The mean number of days on *M. calabura* ethanolic fruit extract was 8.9 days, followed by the ethanolic extract of the flowers (8.6 days) and the hexane extracts of the fruits (8.5 days) and the flowers (8.3 days). This shows that both the ethanolic extracts were more active than the hexane extracts in prolongation of the larval period. This is consistent with the toxicity data against *P. xylostella* larvae. Similar findings have been described by Boiça-Júnior et al. (2005) and Torres et al. (2001). A plausible explanation for prolonged larval phase may be reduction in larval feeding possibly due to phagocytosis-inhibiting substances in the extracts (Hernandez and Vendramim, 1997).

Our experiments have shown that extracts were less toxic to the pupae following feeding of the larvae on leaf discs treated with the extracts for 72 h. Prior treatment of the larvae with the hexane and the ethanolic fruit extracts produced greater pupal mortality than flower extracts. Although, MCEFL was the most active extract against larvae, it was not very active against subsequent pupae. Larval duration was also significantly prolonged following prior feeding of the larvae on leaf discs treated with the extracts compared with the control. Increased larval phase had practically no influence over mortality in the pupal phase in our study conducted in the laboratory. However, increased larval phase in the field will expose the insects to predators, parasitoids and diseases which will ultimately result in an increase in their mortality rate before reaching the pupal stage (Akhtar et al., 2010, 2012b). Some of the treated larvae that would survive the larval phase may also die as pupae due to the long lasting effects of the extracts. Although the exact mode of action is not known, it seems like the extracts are acting as stomach poison. The results of the present study suggest that the *M. calabura* extracts can be used to protect crops against the extensive damage caused by *P. xylostella* larval feeding. Since the plant is easily grown and widespread, resource availability should not be an issue and the crude extract of *M. calabura* can be used as a crop protectant for local use in Brazil and many other regions of the world.

## 5. Conclusions

Our study demonstrates that efficacy of the extract varies with different parts of the plant and the type of organic solvent used in the preparation of the extract. The ethanolic extract of the flowers (MCEFL) was the most active toxicant followed by the ethanolic extract of the fruits (MCEFR), hexane extract of the fruit (MCHFR) and hexane extract of the flower (MCEFL) to first instar larvae of *P. xylostella*. Prior feeding of the larvae with the extracts had some long term toxic effects on subsequent pupae. Fruit extracts were more active than the flower extracts in this respect. All extracts produced a

significant increase in larval duration at most of the concentrations compared with the control. These organic extracts have potential for development as botanical insecticides. However further studies are needed, especially to evaluate the cost/benefit ratio regarding its use in the integrated pest management schemes and its effects on non-target organisms.

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