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Aqueous Extracts of Species of the Genus *Campomanesia* (Myrtaceae) Affect Biological Characteristics of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae)

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Abstract

Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae) is an insect pest that causes great damage to *Brassica* cultures. It is necessary to develop alternative control methods, because this pest is resistant to many synthetic insecticides that are harmful to the environment. The objective of this study was to evaluate the effects of aqueous extracts of *Campomanesia adamantium*, *C. guazumifolia*, and *C. xanthocarpa* on the life cycle of *P. xylostella*. These aqueous extracts were prepared in a concentration of 10 g/mL and then applied on cabbage disks of 4 cm^2 to feed the larvae until they reached pupal stage. The disks were evaluated daily during the larval stage and replaced every 24 hours. The experiment consisted of ten replicates, each replicate containing five subsamples. The parameters evaluated were larval and pupal survival, pupal biomass, gender ratio, male and female longevity, number of eggs, fecundity, oviposition period and egg survival. The *C. xanthocarpa* extract increased larval stage and decreased pupal biomass and oviposition period. The *C. adamantium* extract decreased larval duration, pupal biomass, male longevity, and oviposition period. The *C. guazumifolia* extract decreased larval stage, male longevity, and oviposition period. The chemical composition of the extracts of *Campomanesia* species presented flavonoids such as quercetin, phenolic compounds, and tannins, and the highest retention time occurred in *C. adamantium*. Thus, the extracts of *Campomanesia* species were effective in decreasing and controlling the oviposition period of *P. xylostella*, probably because of the presence of flavonoids, which indicates a possible antioxidant potential and, therefore, the observed antibiosis.

Keywords: diamondback moth, botanical insecticides, myrtaceae, supression of oviposition

1. Introduction

The diamondback moth, *Plutella xylostella* (Linnaeus 1758) (Lepidoptera: Plutellidae), is an important microlepidoptera for agriculture, being considered the main pest of Brassicaceae cultivations (Furlong et al., 2013). The highest loss caused by this insect refers to management costs, which amount to more than one billion dollars annually (Zalucki et al., 2012), resulting in vegetable damages of up to 100%, which makes them inappropriate.

The use of pesticides is still the method most used by producers to control insects that cause agricultural damage, such as *P. xylostella*. These pesticides are used because they are practical, fast, and efficient in population control (Talekar & Shelton, 1993), but their use may select for more resistant individuals (Thuler et al., 2007), which would probably maximize the problem. Therefore, it is necessary to seek control alternatives for these harmful

insects. Some studies have shown that the use of insecticidal plants deserves to be highlighted because of their low toxicity, selectivity, and efficiency against numerous insect pests (Neves & Nogueira, 1996).

Studies involving plant extracts with medicinal properties have reported insecticidal properties such as mortality (Bandeira et al., 2013; Mishra & Singh, 2014; Peres et al., 2017) and feeding deterrence (Chandrashekharaiah et al., 2015; Couto et al., 2016); Deformities in adults, pupae and larvae of *Plutella xylostella* (Peres et al., 2017).

A recent study by Peres et al. (2017) shows evidence of the insecticide potential of Cerrado species. In this study, the aqueous extracts of three species were analyzed: *Alibertia edulis* (Rich.), *Alibertia intermedia* (Mart.), and *Alibertia sessilis* (Vell.) K. Schum. Treatments with *A. intermedia* and *A. sessilis* extracts resulted in the lowest oviposition period and number of hatched larvae. The harmful effects of these aqueous extracts on the life cycle of *P. xylostella* were attributed to flavonoids and other phenolic compounds present in *A. intermedia* and *A. sessilis*. According to the author, these aqueous botanical extracts have low toxicity when compared to synthetic insecticides and may be an effective approach to control *P. xylostella* populations.

The genus *Campomanesia* (Myrtaceae) is typical of the Cerrado and has been studied by several authors because of its anti-inflammatory (Silva et al., 2016a) and antioxidant (Abe et al., 2014) properties, leading us to hypothesize that this genus is potentially insecticidal, as the literature shows that the plant extract contains many chemical substances, such as flavonoids, tannins and saponins (Markman, 2002), resulting in antibiosis with insects. Plants have two types of metabolites: primary and secondary. Primary metabolites respond for plant survival, having an active function in the processes of photosynthesis, respiration, and nutrient assimilation, whereas secondary metabolites are closely associated with defense strategies (Nass, 2007).

Studies have shown that the metabolite interference can inhibit food consumption, and morphological and physiological transformations in the pupal stage of some insects, demanding intense biochemical activity (Matias da Silva et al., 2017; Duffey & Isman, 1981; Isman & Duffey, 1982; Klocke & Kubo, 1991; Summers & Felton, 1994; Pan et al., 2016).

The objective of this study was to evaluate the insecticidal activity of aqueous extracts of *Campomanesia xanthocarpa* O. Berg, *C. guazumifolia* (Cambess.) O. Berg and *C. adamantium* (Cambess.) O. Berg on the biological cycle of *P. xylostella*.

2. Materials and Methods

2.1. Rearing of Plutella xylostella

The larvae and pupae of *P. xylostella* used in the experiment were from Insect-Plant Interaction Laboratory of the Faculty of Biological and Environmental Sciences at the Federal University of Grande Dourados, Mato Grosso do Sul, Brazil. These stock creation is maintained under constant temperature conditions (25±2 °C) and relative humidity (55±5%).

Cabbage disks of 8 cm in diameter were used as oviposition substrate in wet filter paper disks. This set was changed daily. The adults were fed with diluted honey (10%). After oviposition, the discs with the postures were placed in a sterile, transparent plastic container measuring 30 cm long × 15 cm wide × 12 cm high. The container contained leaves of organic cabbage (*B. oleracea* var. Acephala) to feed larvae until they entered the pupal phase. The leaves were previously sanitized with 5% sodium hypochlorite solution and washed in running water. The pupae were placed in cages until the emergence of adults.

2.2 Botanical Material

Leaves of *Campomanesia adamantium* (Cambess.) O. Berg, *Campomanesia guazumifolia* (Cambess.) O. Berg, and *Campomanesia xanthocarpa* O. Berg were collected at Fazenda Coqueiro (forest), in the municipality of Dourados, Mato Grosso do Sul (22°14′ S, longitude of 54°9′ W, and altitude of 452 m), in the period from 7:00 to 9:00. Authorization for collection of botanical material was granted by the Brazilian National Research Council (CNPq)/Council of Genetic Heritage Management (CGEN/MMA, number 010220/2015-1).

The species were identified based on comparison with exsiccate specimens from the Universidade Federal da Grande Dourados (UFGD) herbarium. They are registered under the following numbers: *Campomanesia adamantium*, DDMS 5695; *Campomanesia guazumifolia*, DDMS 5254; and *Campomanesia xanthocarpa*, DDMS 4644.

2.3 Preparation of Aqueous Extracts

Aqueous extracts of *C. adamantium*, *C. guazumifolia* and *C. xanthocarpa* were prepared by maceration from leaves that were collected, dried in a forced circulation oven for 3 days at a maximum temperature of 40 °C (±1 °C) and then ground in a mill to obtain fine powder.

In total, 10 g of the aforementioned powder was manually shaken with 100 mL of distilled water. The extracts were cooled at 10 °C for 24 h, and thereafter filtered through voile fabric, yielding extracts with a concentration of 10% (weight/volume).

2.4 Bioactivity of C. adamantium, *C. guazumifolia* and *C. xanthocarpa Aqueous Extracts Against P. xylostella*

The bioactivity of extracts of *Campomanesia adamantium*, *C. guazumifolia* and *C. xanthocarpa* were evaluated by means of immersion of 8 cm diameter organic cabbage (*B. oleracea* var. Acephala) in aqueous extracts for 1 min. The control consists of identical disks immersed in distilled water for 1 min. After the immersion, the discs were placed in trays with filter paper and at room temperature to remove excess moisture and, subsequently, transferred to Petri dishes. A newly hatched larva of *P. xylostella*, with up to 24 hours, was placed in each Petri dish that contended a cabbage disk treatmented and a filter paper disk moistened with 1 ml of distilled water. The tests were performed at a temperature of 25±2 °C, a RH of 55±5% and with photoperiod of 12 h.

To determine the duration of the larval stage, the larvae were monitored until reaching the pupal stage. The first mortality assessment was performed 48 hours after confinement, as the larvae can remained in the parenchyma of the cabbage disk this period. Subsequent evaluations were performed daily and cabbage discs were replaced every 24 hours by new discs immersed in the given treatment. The evaluation of the larval stage consisted of counting the number of dead individuals and replacing the cabbage leaf discs. Pupal survival was determined by isolation of individual pupae in 24 or 96—well ELISA plates (Biomerica). The pupae were weighed 24 hours later and observed until adult emergence.

During the experiment the following biological parameters were evaluated: duration and survival of the larval and pupal stages, pupal biomass (Bel Mark Analytical Balance—0.001 g), female and male longevity, number of eggs, fecundity [oviposition period (days) = Period between the first and last stance] and egg survival (Figure 1).

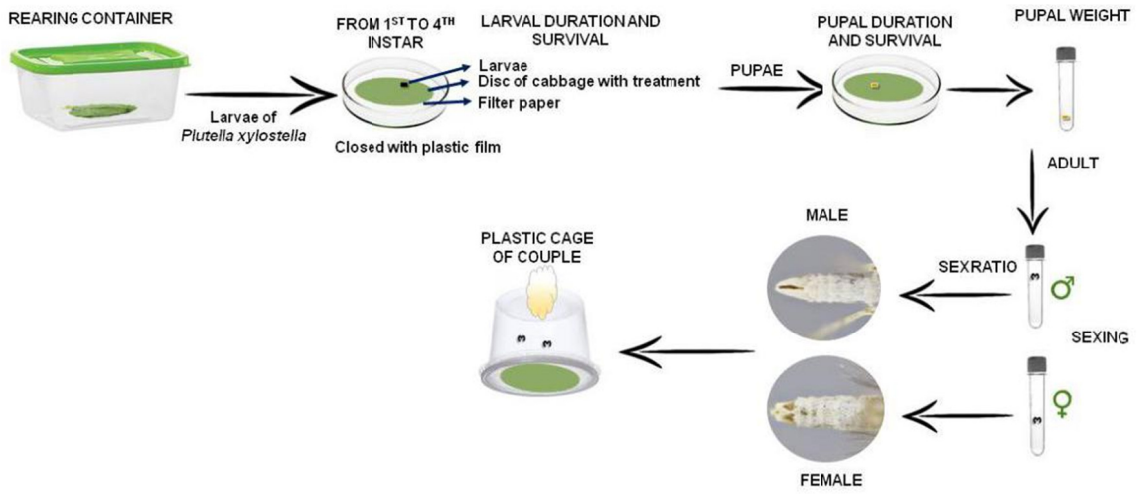

Figure 1. Schematic representation of the methodology used for evaluation of biological parameters (based on Matias da Silva, 2017)

In the reproductive phase, five couples (n = 5) of each treatment were kept separately in plastic cages containing disks of organic kale and disk of filter paper moistened as substrates of oviposition. Adults were fed 10% diluted honey. The number of eggs was evaluated daily along with the oviposition substrate exchange and larval hatching was observed (Figure 2).

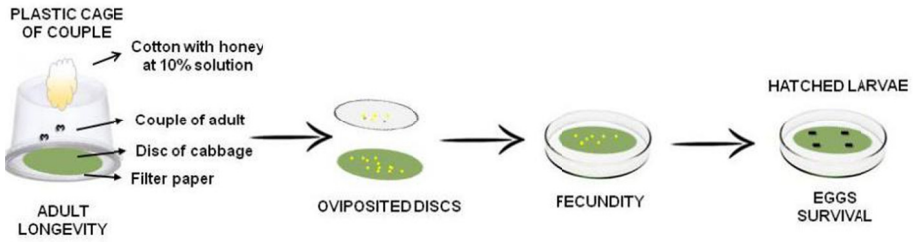

Figure 2. Schematic representation of the methodology used for evaluation of biological parameters of the reproductive phase of *Plutella xylostella* (based on Matias da Silva, 2017)

The experimental design was completely randomized, with each treatment consisting of ten replicates, each containing five subsamples, totaling 50 larvae/treatment. Data on larval and pupal survival were transformed in arccosine of $\sqrt{x/100}$; and data on larval and pupal duration, male and female longevity, and fecundity were transformed in arccosine of $\sqrt{x + 0.5}$. The results were submitted to analysis of variance, and the means were compared using Tukey test ($P \leq 0.05$) and SANEST software (version 3.0).

2. Results

Larval duration of *P. xylostella* decreased when the *C. adamantium* treatment was applied but the results did not differ from those of the control group or the *C. guazumifolia* treatment. The *C. xanthocarpa* treatment resulted in the longest larval duration. All caterpillars treated with plant extract showed a decrease in larval survival, differing from the control group. There was no difference between the control group and *C. guazumifolia* for pupal duration. However, the extracts of *C. adamantium* and *C. xanthocarpa* showed a decrease in number of days of 4.99 and 4.21 days, respectively, when compared to the other extract. As for pupal survival, there was a higher mortality in the *C. xanthocarpa* extract, with emergence of 47.79%, while there was no significant difference in the other treatments, with survival above 92%. There was an increase in larval stage in the *C. xanthocarpa* extract resulting in an increase of 5.22 mg in pupal biomass. There was decreased larval duration in the *C. adamantium* treatment, which affected pupal biomass (Table 1).

Table 1. Duration (days) and survival (%) of larval and pupal stages, and pupal weight (mg) of *Plutella xylostella* treated with aqueous extracts of *Campomanesia* species (25±2 °C, 70±5 RH, 12 h photophase)

	Duration larval (days)	Survival larval (%)	Duration pupal (days)	Survival pupal (%)	Weight pupal (mg)
Control	7.96±0.14 ab	96.46±2.60 a	6.25±0.14 ab	99.89±1.00 a	4.80±0.00015 a
	n=50	n=50	n=47	n=46	n=46
C. adamantium	6.55±0.26 b	60.98±6.29 b	4.99±0.55 bc	97.55±6.67 a	2.50±0.00028 b
	n=50	n=50	n=30	n=28	n=28
C. guazumifolia	7.95±0.48 ab	69.21±6.70 b	6.66±0.41 a	92.39±0.41a	5.06±0.00018 a
	n=50	n=50	n=33	n=29	n=29
C. xanthocarpa	8.22±0.21 a	58.40±6.29 b	4.21±0.19 c	47.79±7.43 b	5.22±0.00026 a
	n=50	n=50	n=28	n=10	n=10
Value of F	F=6.15	F=9.42	F=10.3333	F=14.7065	F=32.0563
	P=0.0021	P=0.00022	P=0.00014	P=0.00002	P=0.00001
	GL=3	GL=3	GL=3	GL=3	GL=3
C.V. (%)	6.2%	23.7%	9.7%	22.4%	16.2%

Note. *Means followed by distinct letters in the column differ from each other at a significance level of 5% probability when compared using the Tukey test. n = number of insects. CV = coefficient of variation.

There was no gender ratio difference among treatments (F = 0.8634; df = 3; P = 0.52864). Longevity in the adult stage increased for males and decreased for females in the *C. xanthocarpa* treatment. There was no difference among treatments regarding number of eggs. However, results of egg survival show that the extracts of *C. adamantium*, *C. guazumifolia*, and *C. xanthocarpa* impair egg hatching, with respective 51, 50, and 26% hatch rates while the control group showed 89% survival rate. As for fecundity, there was no significant difference among treatments (Table 2).

Table 2. Longevity of male and female adults, fecundity, egg survival (%), and oviposition periods of *Plutella xylostella* treated with aqueous extracts of *Campomanesia* species (25±2°C, 70±5 RH, 12 h photophase)

	Longevity of male (days)	Longevity of female (days)	Fecundity	Egg Survival (%)	Oviposition periods (days)
Control	18.60±2.04 ab	12.60±1.36 ab	204.00±16.28 a	0.89±0.0037 a	11.40±1.29 a
	n=5	n=5	n=5	n=5	n=5
C. adamantium	13.60±2.20 b	10.40±2.13 ab	168.00±55.97a	0.51±0.14 b	9.40±1.88a
	n=5	n=5	n=5	n=5	n=5
C. guazumifolia	17.40±2.87 b	14.80±1.68a	250.60±13.96 a	0.50±0.016 b	12.60±1.63 a
	n=5	n=5	n=5	n=5	n=5
C. xanthocarpa	32.00±5.71 a	7.80±0.199 b	224.80±29.19 a	0.26±1.100 b	7.20±0.37 a
	n=5	n=5	n=5	n=5	n=5
Value of F	F=5.1498	F=3.8652	F=1.0920	F=9.1136	F=2.7980
	P=0.01111	P=0.02921	P=0.38184	P=0.00122	P=0.07292
	GL=3	GL=3	GL=3	GL=3	GL=3
C.V. (%)	38.7%	29.9%	35.2%	35.5%	31.1%

Note. *Means followed by distinct letters in the column differ from each other at a significance level of 5% probability when compared using the Tukey test. n = number of insects. CV = coefficient of variation

Phenolic compounds, flavonoids, and tannins were present in the extracts of all three species, with higher quantities in *C. adamantium*. The flavonoid quercetin was the only one present in the chromatogram (Figure 3).

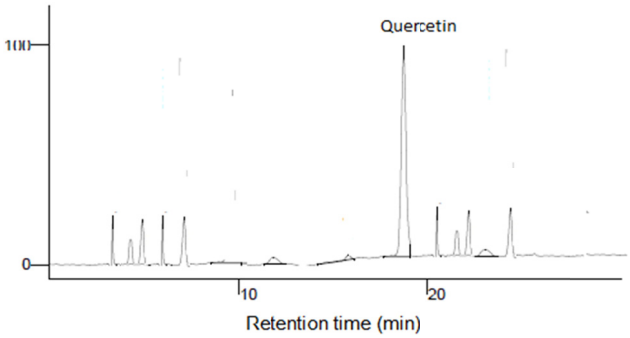

Figure 3. Chromatograms obtained from aqueous extract samples das tres espécies de *Campomanesia*

Regarding the retention time, the highest retention time was for the *C. adamantium* extract (Table 3).

Table 3. Chemical composition of extracts from *Campomanesia* species

Samples	Retention time (18.33 min) of Quercetin	Phenolic Compounds (mg/mL) (mg/g±SD)	Flavonoides (mg/mL) (mg/g±SD)	Taninos (mg/mL) (mg/g±SD)
C. adamantium	14.6±0,2	368.7±0.2	88.5±0.1	1.5±0.1
C. guazumifolia	11.3±0.1	342.7±0.4	81.4±0.2	1.3±0.1
C. xanthocarpa	9.68±0.1	293.4±0.7	67.7±0.2	1.2±0.1

Note. M = Média SD = Standard Desviation.

3. Discussion

The species of *Campomanesia* used in the present study show plausible results for their applicability to control *P. xylostella* populations. The literature shows the effects of plant substances on insects, such as reduction in food preference, mean number of eggs, and mean number of hatched larvae, in addition to oviposition suppression (Fonseca et al., 2018), insecticidal action (Amoabeng et al., 2014; Peres et al., 2017), oviposition inhibition (Torres et al., 2006), growth regulation (Koul, 2012), and repellent action (Koul, 2008), or effects that only distance

insects from plants, such as feeding inhibitors (Couto et al., 2016; Koul, 2005, 2008). In this line of study the genus *Campomanesia* interfered in the biological characteristics of *P. xylostella*.

The increased duration of the larval stage induced by the extract of *C. xanthocarpa* is important for increasing the exposure time to natural enemies and the mean time of each generation, thus, decreasing overall population growth (Torres et al., 2001). Torres et al. (2006) corroborate that the duration of the larval stage is increased because of growth inhibitors or toxic substances present in the extracts.

The *C. xanthocarpa* extracts resulted in decreased larval and pupal survival and pupal duration, probably because the compounds present in the extracts act for longer in the middle intestine of *P. xylostella*. Among these compounds are some flavonoids such as quercetin, phenolic compounds, and tannins. The quercetin present in aqueous extracts of *Campomanesia* influences insect biology. Gazzoni et al. (1997) evaluated the effect of different quantities of rutin and quercetin on the biology of velvetbean caterpillars, *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) and reported that both quercetin and rutin caused an increase in the number of days of the total cycle (third instar to adulthood) and gradually increased the mortality rate of caterpillars. Peres et al. (2017) verified that quercetin present in *Alibertia sessilis* and *A. intermedia* influences the biology of *Plutella xylostella* causing deformity. Silva et al. (2016b) verified that the rutin flavonoid negatively affected the biology of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) by prolonging the larval development time, reducing the weight of larvae and pupae and decreasing the viability of the pupae.

Another interesting feature is that the *C. xanthocarpa* extract increased larval duration, consequently increasing pupal biomass, whereas the *C. adamantium* extract decreased larval duration and pupal biomass. Therefore, pupal weight is directly related to the insect performance in the larval stage (Maroneze & Gallegos, 2009).

The extracts of *C. adamantium* and *C. guazumifolia* decreased the longevity of males in the couples evaluated, but the extract of *C. xanthocarpa* increased longevity by 13.4 days. There was no significant difference among treatments for females, except for *C. xanthocarpa*, which decreased longevity by 4.8 days.

There was no significant difference in number of eggs in all treatments, but there was a decrease in egg survival in the three *Campomanesia* extract treatments. However, *C. xanthocarpa* presented a better result, with only 26% of the total number of eggs hatching. This decrease may be related to the quantity and quality of nutrients absorbed during the larval stage, since these parameters may influence the number of ovarioles per ovary and consequently decrease egg production (Costa et al., 2004). This is very important in the field, because this decreased egg survival will decrease the number of individuals in the next generation, and fewer caterpillars will cause less damage to crops (Maroneze & Gallegos, 2009). According to Peres et al. (2017), the aqueous extract of *Alibertia sessilis* increased larval duration, which resulted in larval mortality or late pupal mortality.

The qualitative analysis carried out by Abe et al. (2014) on *C. xanthocarpa* showed the presence of flavonoids, anthraquinones, steroids and/or triterpenoids, anthocyanin heterosides, saponins, tannins, amino groups, and fixed acids. The presence of flavonoids, tannins, and saponins (Markman, 2002) has been reported in leaf extracts. Quercetin, myricitrin, and rutin are among the flavonoids described (Schmeda-Hirschmann, 1995).

In a recent study, Peres et al. (2017) reported that aqueous extracts of the genus *Alibertia* increase larval duration and decrease pupal duration, pupal biomass, and fecundity. The authors related these effects to the presence of the flavonoids rutin, quercetin, and luteolin.

Quercetin is present in species of *Campomanesia* and affects insect biology. Peres et al. (2017) reported morphological changes in the biology of *P. xylostella* when treated with aqueous extracts of *A. sessilis* and *A. intermedia*.

We concluded that all *Campomanesia* extracts tested resulted in modification of biological characteristics of *P. xylostella*, indicating effectiveness in controlling and decreasing future generations of this insect. Therefore, further studies on these species should be conducted, including a detailed isolation of substances and new tests using the extracts.

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