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Bioactivity of microencapsulated soursop seeds extract on *Plutella xylostella*

Bioatividade de extrato microencapsulado de sementes de graviola sobre *Plutella xylostella*

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ABSTRACT

*The aim of this study was to evaluate the bioactivity of microencapsulated extract from the soursop seeds, **Annona muricata** L. (**Annonaceae**), on diamondback moth, **Plutella xylostela** L. (Lepidoptera: **Plutellidae**). Microencapsulation was performed in a Mini Spray Dryer model B-290 using 50mL of ethanolic and hexanic extracts plus 150mL of ethanol and 150mL of ultrapure water, mixed with aerosil (first polymer) or arabic gum (second polymer). It was possible to microencapsulate the ethanolic extract of soursop seeds only by using the polymer arabic gum at 20%. The microencapsulated extract caused significant acute toxicity (LC_{50}=258mg L^{-1}) and chronic effects, especially reduction of larval viability and increased larval stage. We concluded that the microencapsulation of the ethanolic extract of soursop seeds can be a viable alternative for controlling diamondback moth with possible gains for the environment.*

Key words: *diamondback moth, **Annona muricata**, biology, LC_{50}.*

RESUMO

*O objetivo deste estudo foi avaliar a bioatividade do extrato microencapsulado das sementes de graviola, **Annona muricata** L. (**Annonaceae**), sobre a traça-das-crucíferas, **Plutella xylostella** L. (Lepidoptera: **Plutellidae**). A microencapsulação foi realizada em um Mini Spray Dryer modelo B-290 utilizando-se 50mL dos extratos etanólico e hexânico mais 150mL de álcool etílico e 150mL de água ultrapurificada, misturado com aerosil (primeiro polímero) ou com goma arábica (segundo polímero). Só foi possível microencapsular o extrato etanólico de sementes de graviola com a utilização do polímero goma arábica a 20%. O extrato microencapsulado causou significativa toxicidade aguda (CL_{50}=258mg L^{-1}) e efeitos crônicos, especialmente redução da viabilidade larval e aumento da duração do estágio larval. Conclui-se que a microencapsulação do extrato etanólico da semente de graviola pode ser uma alternativa viável no controle da traça com possíveis ganhos para o meio ambiente.*

Palavras-chave: *traça-das-crucíferas, **Annona muricata**, biologia, CL_{50}.*

INTRODUCTION

Among the factors contributing to reduce brassica yield worldwide is diamondback moth - ***Plutella xylostella*** (L., 1758) (Lepidoptera: ***Plutellidae***), a pest present in almost all producing regions and in all stages of the plant (TALEKAR & SHELTON, 1993). Its control is difficult because of its fast capacity of migration, easy adaptation to the environment, high fecundity and short life cycle, which provides a rapid increase in its resistance to chemical insecticides (CASTELO BRANCO et al., 2001).

The use of synthetic insecticides has been the most used way to control this pest, which requires a high number of applications, leading a resistant to the major groups of insecticides which include organochlorines, organophosphates, carbamates and pyrethroids (FURLONG et al., 2013).

The search for new insecticides, including the use of plants as natural insecticides, is an open, broad and continuous field of research

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(SCHMALTZ et al., 2005). The diversity of substances present in the flora remains appealing in the area of insect control, taking into account that only a small portion of the plants was investigated with such a purpose.

Among the plants that have potential for pest control are some species of ***Annonaceae*** family, which have already been investigated (ISMAN & SEFFRIN, 2014). One of these species is soursop (***Annona muricata*** L.), that has shown some insecticidal, nematicidal and bactericidal effects. Its seeds are a promising source for manufacturing plant extracts, mainly because it is a byproduct of the industrialization process of soursop pulp (HERNANDÉZ & ANGEL, 1997).

The ***P. xylostella*** larvae showed 100% mortality when exposed for up to 12 days in the ethanol extract of ***A. muricata*** (5mg mL^{-1}). The lower concentrations also affected the larval stage and its viability was reduced (TRINDADE et al., 2011).

Natural insecticides generally have short persistence in the environment after use and during storage (CLOYD, 2004). Therefore, it is necessary to develop formulations that could improve their viability and facilitate their application by farmers. Among the formulations used there is the emulsion that consists in a mixture of two immiscible fluids, by using agitation or addition of homogenizers (BAJPAI & GIRI, 2002).

Another alternative is nanotechnology, process that has been applied mainly in the pharmaceutical and cosmetic industry to spread bioactive molecules more efficiently, facilitating penetration of these compounds into the human skin. For medicines is a widespread technique, but for cosmetics is a limited area because of its recent use (SCHMALTZ et al., 2005; BARIL et al., 2012). The application of nanotechnology in agricultural pest control is a very new approach of study, thus becoming a vast field to be explored (NEVES, 2008).

Thus, the microencapsulation process of soursop seed extracts could assure a more slow and controlled release of the active ingredients for the plants, thus enhancing its activity against pests and reducing the environmental impacts usually caused by synthetic products.

The aim of this study was to evaluate the bioactivity of microencapsulated extract from the soursop seeds, ***Annona muricata*** L. (***Annonaceae***) on the diamondback moth, ***Plutella xylostela*** L. (Lepidoptera: ***Plutellidae***).

MATERIALS AND METHODS

Insect rearing

The initial population of ***P. xylostella*** was derived of the Universidade Federal Rural de Pernambuco (UFRPE) and maintained at 26±2ºC, 60±10% relative air humidity and 12−hour photoperiod, according to the methodology established by MEDEIROS et al. (2003), and fed with leaves of cabbage, ***Brassica oleracea*** var. ***acephala*** DC. (***Brassicaceae***) cv. 'Georgia', produced in beds containing the commercial substrate Bioplant™ inside a green house. Experiments started from the third generation of insects in the laboratory.

Preparation of soursop seed extracts

Soursop seeds were obtained from the fresh waste of a processing fruit pulp company in Anadia, AL, Brazil, in 2012. Seeds were air-dried at 48ºC for 48 hours, triturated in a forage grinding mill (mesh size 2.5mm) to yield a fine, uniform powder, and stored at 4±2ºC.

To prepare the hexanic extract, 2.3kg of the seed powder were used with 5L of hexane [$CH_3(CH_2)4CH_3$] in a percolator for a period of 72 hours. After that, the same seed powder was used for the ethanolic extraction using 4.6L of ethanol (CH_3CH_2OH) for three periods of 72 hours. Excess of solvents was removed under reduced pressure at 50°C; and the crude residues were placed in desiccators to remove any remaining water and stored at 4±2ºC.

Microencapsulation

The microspheres were obtained by spray drying using Buchi® Mini Spray Dryer B -290 (Switzerland) with a nozzle of 1.0mm and a parallel flow pattern at an inlet air temperature of 180ºC, a feed rate of 10ml min^{-1} and aspirator at 100%, representing an air flow of 35m^3 h^{-1}. Two forms of microencapsulation were conducted: the first one by using 50mL of organic extract plus 150mL of absolute ethyl alcohol and 150mL ultrapurified water, mixed and then added with aerosil (polymer) calculated from the weight of solid contained in 1ml of the extract. The solid weight of the extract was performed by placing 1mL of the extract in an oven at 145°C for 4 hours and then weighed. The second one by performing the microencapsulation in the same way but replacing the aerosil for 20% gum arabic and mixed.

Determination of LC_{50} and LC_{99} of microencapsulated soursop seed extracts and the effect of LC_{50} on ***Plutella xylostella*** biology

As in the microencapsulation process some losses in the active ingredients may occur, it was conducted a test to establish the LC_{50} and LC_{99} using the concentrations 0.0 (control), 100, 250, 500, 1.000 and 2.500mg L^{-1} plus 10.000mg L^{-1} DMSO (Dimethyl sulphoxide) in distilled water.

Discs of 8cm in diameter were cut from cabbage leaves including midrib and immersed separately for 30s in the different solutions, and control discs were immersed only in 1% DMSO in distilled water. Discs were left to dry out at room temperature of 26±2ºC for 2 hours, after which they were placed in individual Petri dishes together with 12 newly hatched larvae. Dishes were sealed with PVC film and maintained at room temperature of 26±2ºC, 60±10% relative air humidity and 12h of photophase. As the cabbage discs became yellow, or were consumed by the larvae, they were replaced daily by newly treated discs. Larval mortality was evaluated on the third day of the experiment, when larvae left the leaf mines, and daily until the pupal stage.

In the insect biology experiment, to LC_{50} (258mg L^{-1}), when the larvae turned into pupae, they were individually placed in glass tubes until adult emergence and the duration and viability of larval, pupal stages and adults longevity were evaluated.

The experiment was carried out in a completely randomized design with six treatments and five replicates to determine the lethal concentrations of each extract. To determine the LC_{50} and LC_{99}, it was used Probit analysis performed with the software SAS version 9.0 (SAS Institute, 2002). In the biology experiment, data were submitted to analysis of variance and means were compared by Tukey test, at 5% of probability. Data of the larval viability were transformed into $\sqrt{x+0.5}$, with Assistat version 7.5 software (SILVA & AZEVEDO, 2009).

RESULTS AND DISCUSSION

Microencapsulation

The first process of microencapsulation of the organic extracts using aerosil was considered not efficient as no powder (microcapsules containing the active ingredient) was formed. In the second process, when aerosil was replaced by gum arabic, a powder was formed containing the microcapsules (25µ) only in the ethanolic seed extracts of soursop. The encapsulation efficiency was near 55%.

SHAHIDI & HAN (1993) suggest that encapsulation by spray-drying involves four stages: preparation of the dispersion or emulsion; homogenization dispersion; atomizing the emulsion, and dehydrating the atomized particles. The first stage consists of the formation of a fine stable emulsion of the active material solution. In this case, aerosil, which is an emulsion stabilizer (CORNEC, 1990), was not adequate.

However, the gum arabic, which is a product obtained by spontaneous drying of the exudates of ***Acacia senegal*** (L.) (***Fabaceae***), has wide application in the preparation of emulsions and suspensions, and is soluble in water (GABAS & CAVALCANTI, 2003).

The microencapsulation of hexanic extract was not possible as no powder was formed and maintained its liquid form after two attempts. It is believed that the hexanic extract had the presence of oils (ONIMAWO, 2002), which hinder its microencapsulation.

Determination of LC_{50} and LC_{99} of microencapsulated ethanolic extract of soursop seeds to ***Plutella xylostella***

The estimated lethal concentrations for microencapsulated ethanolic extract of soursop for CL_{50} (IC 95%) were 258mg L^{-1} (193-329mg L^{-1}) and for CL_{99} (IC 95%) were 3.081mg L^{-1} (1.944-6.291mg $L^{-1)}$ with slope (± EP) of 2.15 (±0.65) and (χ =17.5; df=18).

Similar results of the biotoxic action of microencapsulated extracts were also observed by MARCOMINI (2009), when assessing nanoformulations of neem on ***Spodoptera frugiperda*** (J.E. Smith, 1798) (Lepidoptera: ***Noctuidae***). The mortality rate was of 40-46% at concentrations of 0.64% (3.87mg AZ-A L^{-1}), as well there was weight reduction of caterpillars. MARCOMINI (2009) showed that the microencapsulation preserved the active principle of the extract and that a higher concentration leads to a greater mortality of the insects, as seen in this study. KANIS et al. (2012) reported that microencapsulated extracts of ***Copaifera*** sp. (***Leguminosae***) showed reduced activity on the first day, but the insecticidal action remained above 40% for 17 days. The lower activity on the first day of the experiment could be explained by the slow release of the microencapsulated active ingredient, unlike the pure extract, where the active ingredient is released at once. In the present study, the mortality of larvae

treated with microencapsulated extract was evenly distributed during the assessment, thus confirming the principle of slow release of the extract containing nano particles of the active principle.

JALALIZAND et al. (2013) studied the mortality effect of silver nano particles on adult two-spotted spider mites, ***Tetranychus urticae*** (Koch, 1836) (Acari: ***Tetranychidae***). They showed different effects on mites as they increased the concentrations at different time interval. GAVANJI et al. (2013) studied the comparative acaricidal efficacy of sulfur and nano sulphur and concluded that nano sulfur is a product effectively active against ***T. urticae***. Sulfur is an adulticide and nymphicide, but it was revealed that sulfur in nano scale shows a better inhibitory effect on mite populations.

Effect of LC_{50} of microencapsulated ethanolic extract of soursop seeds on ***Plutella xylostella*** biology

The larval duration (df=1, F=8.6512, P=0.0087) and viability (df=1, F=14.2372, P=0.0013) were significantly different at 0.05% of probability by Tukey test. The other parameters pupal viability (df=1, F=1.9867, P=0.1777) and duration (df=1, F=3.8954, P=0.0658) and adult longevity (df=1, F=1.6855, P= 0.2124) did not differ (Table 1).

The effect on the biology of insect was observed only in the larval stage. The larval viability was only 38.00% over a period of 8.91 days, well below the control that were 83.00% and 7.94 days, respectively (Table 1), showing that the microencapsulated treatment maintained for a longer period of time its ability to affect the development of ***P. xylostella***. In the same way, the development of ***Tuta absoluta*** (Meyrick, 1917) (Lepidoptera: ***Gelechiidae***) was also reported to be seriously affected by nanoformulation (NC40 powder) of aqueous solution of neem (FERREIRA et al., 2012). The treated larvae completed their development, in 16.1 days (control 12.2 days) and reduced the weight of the pupae to 23mg (control 27.9mg).

MARCOMINI (2009) studying the performance of six microencapsulated nanoformulations of neem reported that larval and pupal viability of ***S. frugiperda*** was not affected, but its larval duration, like in this research was significantly extended with a diet containing such nanoformulations.

The same technology has also been used to control other groups of insects like in the research of CARVALHO et al. (2015) who evaluated the efficacy of 19 nanoformulations of neem derivatives in controlling by systemic action nymphs of ***Bemisia tabaci*** (Genn., 1889) biotype B (Hemiptera: ***Aleyrodidae***). Two nanoformulations (NC-2 and NCL5L6-1) were believed to cause similar mortality to the commercial neem oil. The systemic action of the oil and nanoformulations depends on environmental conditions in which they are enforced and nanoformulations can be bioactive for 30 days after application.

CONCLUSIONS

The use of gum arabic was effective in the microencapsulation process of soursop seed extract. The estimated lethal concentrations were 258mg L^{-1} and 3.081mg L^{-1} for the CL_{50} and CL_{99}, respectively. The microencapsulated soursop seeds extract affect the larval duration and viability of ***P. xylostella***.

Table 1 - Mean ±SE of larval and pupal viability and duration and adult longevity of ***Plutella xylostella*** treated with ethanolic seed extract of ***Annona muricata*** .

	Larval viability (%)	Larval duration (days)	Pupal viability (%)	Pupal duration (days)	Adult longevity (days)
Control	83.00 ±4.46 a	7.94 ±0.21 a	83.94 ±5.09 ns	3.92 ±0.06 ns	3.21 ±0.12 ns
Micro.	38 .00 ±7.56 b	8.91 ±0.46 b	68.54 ±8.76 ns	5.27 ±0.23 ns	2.76 ±0.32 ns
F	14.2372	8.6512	1.9867	3.8954	1.6855
P	0.0013	0.0087	0.1777	0.0658	0.2124

Means followed by the same letter in columns are not statistically different by Tukey test (P≤0.05).
ns= Not significant.
SE = Standard error.
F = Test statistic F.
P = value of P.

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