INSECTICIDAL ACTIVITY OF SEED EXTRACTS OF *Carica papaya* (L.) AGAINST THE FALL ARMYWORM *Spodoptera frugiperda* (J.E. SMITH) (LEPIDOPTERA: NOCTUIDAE)

Rodolfo Figueroa Brito, Arturo Huerta-de la Peña, Ignacio Pérez Moreno, Vicente Santiago Marco Mancebón and Jesús Francisco López-Olguín

SUMMARY

The present study shows that natural products from *Carica papaya* can be considered as a valid alternative to control pests in agriculture. The insecticide properties of the seed extracts of four cultivars of *C. papaya* (Maradol, Mammee, Yellow and Hawaiian) were added to an artificial insect diet. Bio-assays were conducted with hexanic, acetic and methanolic extracts at concentrations of 10, 100 and 1000ppm. All tests were performed with the first larval stage of *Spodoptera frugiperda*. The response variable was insect mortality. Extracts from seeds of the Maradol, Mammee and Yellow cultivars of *C. papaya*, followed by extracts from seeds of the Hawaiian cultivar, applied at concentrations of 10, 100 and 1000ppm, were toxic on *S. frugiperda* larvae (50-70% corrected mortality rate). The acetic extracts of the Maradol and Mammee cultivars at 10ppm were the most effective, causing mortalities of 73.6 and 62.8% of the larvae, respectively.

Introduction

Maize (*Zea mays* Linnaeus 1753, Gramineae) is one of the most important cereal crops in the world, contributing to the well-being of millions of poor farmers. It is a globally important crop and preferred staple food for more than a billion people in Sub-Saharan Africa and Latin America, where animal protein sources are not affordable by the common people. Of the 1.4×10^8ha grown globally with maize, ~96×10^6ha are dedicated to livestock feed from the USA (García-Raño and Keleman, 2007).

One of the factors of the losses in maize crop in Mexico and other maize-growing areas of the American Continent is pest insects, among which *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) is the most important. It attacks during all the stages of plant growth and it can cause yield reductions of up to 10% (Pingali and Pandey, 2001). This loss percentage is higher in tropical and subtropical regions of Latin America, with over 35% damage recorded in Colombia (Torres y Cotes, 2005) and 40% in Cuba (Fernández, 2002). In Mexico it causes crop losses from 20 to 100% (Del Rincón et al., 2006).

For control, either seed treatment with systemic insecticides or the application of granulated insecticides scattered on the ground are used. It has been shown that the indiscriminate use of these compounds leads to a major deterioration of the environment and can be harmful for the health of both producers and consumers, and also encourages the appearance of resistant pest populations, eliminating their natural enemies and facilitating a resultant growth in the impact of secondary pests, which results in a cost increase in the growing of maize.

KEY WORDS / Biological Activity / Caricaceae / Maize / Plant Extracts / Toxicity /

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ACTIVIDAD INSECTICIDA DE EXTRATOS DE SEMI ÍTAS DE CARICA PAPAYA (L.) CONTRA EL GUSANO COGOLLERO SPODOPTERA FRUGIPERDA (J.E. SMITH) (LEPIDÓPTERA: NOCTUIDAE)
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RESUMEN
Este estudio muestra que los productos naturales de Carica papaya pueden ser considerados como una alternativa válida para el control de insectos nocivos en la agricultura. Se estudiaron las propiedades insecticidas de extractos de semillas de cuatro cultivares de C. papaya (Maradol, Mamey, Amarillo y Hawaiana) incorporados a una dieta artificial para insectos. Se realizaron bioensayos con extractos hexánicos, acetónicos y metánolicos a concentraciones de 10, 100 y 1000ppm. Todas las pruebas se realizaron con larvas de primer estadio del gusano cogollero del maíz Spodoptera frugiperda. La variable respuesta fue la mortalidad del insecto. En los resultados se registró que los extractos de semillas de los cultivares Maradol, Mamey y Amarilla de C. papaya, seguidos por los extractos de semillas de el cultivar Hawaiana, aplicados en concentraciones de 10, 100 y 1000ppm, resultaron tóxicos en las larvas de S. frugiperda (50-70% de mortalidad corregida). Entre ellos, los extractos acetónicos de los cultivares Maradol y Mamey a 10ppm fueron los más efectivos al causar un porcentaje de mortalidad de 73,6 y 62,8% de las larvas, respectivamente.

ATIVIDADE INSECTICIDA DE EXTRATOS DE SEMENTES DE CARICA PAPAYA (L.) CONTRA A LAGARTA-DO-CARTUCHO SPODOPTERA FRUGIPERDA (J.E., SMITH) (LEPIDÓPTERA NOCTUIDAE)
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RESUMO
Este estudo mostra que os produtos naturais de Carica papaya podem ser considerados como uma alternativa válida para o controle de insetos nocivos na agricultura. Foram estudadas as propriedades inseticidas de extratos de sementes de quatro cultivares de C. papaya (Maradol, Mamey, Amarelo e Havai) incorporados a uma dieta artificial para insetos. Realizaram-se bioensaios com extratos hexânicos, acetônicos e metânolicos em concentrações de 10, 100 e 1000ppm. Todas as provas foram realizadas com larvas de primeiro estágio da lagarta militar do milho Spodoptera frugi-perda. A variável resposta foi a mortalidade do inseto. Nos resultados ficou registrado que os extratos de sementes dos cultivares Maradol, Mamey e Amarilla de C. papaya, seguidos pelos extratos de sementes do cultivar Hawaiana, aplicados em concentrações de 10, 100 e 1000ppm, resultaram tóxicos nas larvas de S. frugiperda (50-70% de mortalidade corrigida). Entre eles, os extratos acetônicos dos cultivares Maradol e Mamey a 10ppm foram os mais efetivos ao causar uma porcentagem de mortalidade de 73,6 e 62,8% das larvas, respectivamente.

It is therefore important to foster the implementation of integrated pest management, improving the processes of sampling and monitoring as a basis for correct decision-making and promoting the rational integration of other alternative tools, so as to reduce the adverse effects mentioned previously. One of such tools is the use of products from vegetable sources. Plants contain a wide diversity of secondary metabolites, some of which exert an important role in their defense against pathogens and herbivo-vores (Ware and Whitaker, 2004). In various cases, it has been demonstrated that the use of products of plant origin reduces the application of synthetic agrochemicals with a resulting lower level of resistance development among pest populations, thanks to their differing modes of action and a greater respect for beneficial insect fauna (Bahena et al., 2003) due to their selectivity. Figueroa-Brito (2002) evaluated the effects of powders from different plants applied as 15% of an artificial diet of S. frugiperda. The author noted that the leaves of Pithecellobium dulce Benth (Fabaceae) and Crescentia alata H.B.K (Bignoniaceae) and the seeds of Jacaratia mexicana A. DC. (Caricaceae) acted as a deterrent, and that the leaves of Prospis juliflora (Sw) DC. (Fabaceae) and the seeds of Carica papaya (L.) (Caricaceae) and Bromelia hemisphaerica Lam. (Bromeliaceae) proved toxic for first instar larvae of the pest. In addition, powder from C. papaya cultivar Mammee seeds (Figueroa-Brito, 2002; Figueroa-Brito et al., 2002a,b), as well as those of the Maradol, Yellow and Hawaiian cultivars (Franco et al., 2006) in concentrations of 10, 15 and 20% were highly toxic and caused 100% mortality rates of larvae of S. frugiperda in less than 96h. Based on these antecedents, the aims of the present study were: a) to evaluate the toxic effect of hexanic, acetic and methano-lic extracts of seeds of the Mammee, Maradol, Yellow and Hawaiian cultivars of C. papaya upon neonate larvae of S. frugiperda, and b) to compare the relative toxicity of the different cultivars and the concentrations of the extracts.

Materials and Methods
Collection and rearing of S. frugiperda
S. frugiperda larvae were collected from maize planta-
tions in Yautepec, Morelos, Mexico, during July 2005. Using entomological brushes and tweezers, 153 larvae at different stages were collected from the maize plants (~15 days of growth). The larvae were then taken to the labora-
tory and fed individually on an artificial diet (Burton and Perkins, 1987) and kept in closed, cylindrical plastic con-
tainers, 3 cm high by 3.5 cm diameter. The larvae under-
grew pupation in these same vials and when they emerged as adults, they were placed in brown paper containers with a volume of 3 liters, containing a 10cm-diameter plastic Petri dish with cotton wool damped in a 10% sugar solution to feed them. Mating and lay-
ing of eggs took place in these containers. For the tests, the second generation of neo-
nate larvae were used.
**Plant extracts**

*C. papaya* fruits were acquired from different markets. The Yellow and Hawaiian cultivars in the state of Oaxaca, and the Mammee and Maradol cultivars in the state of Morelos. The seeds were separated by cultivar and left to dry in the shade for 15 days. Once dry, the seeds were ground and sieved with an Ika Werke electric grinder (model MF 10 Basic, GBMB & Co., Germany) using a 0.25mm mesh. From the powder obtained, 500g of each cultivar were weighed out and placed in 2 liter Erlenmeyer flasks with 1.5 liters of hexane for a first maceration. The mixture obtained was stirred in the recipient and left in extraction for 72h at ambient temperature. After this time, the mix was vacuum filtered using Whatman® N° 5 filter paper. The same procedure was followed using either acetone or methanol as a solvent (Figueroa-Brito, 2002). The hexane, acetone or methanol, as applicable, were removed from the solutions obtained by reduced pressure distillation using a rotavapor (Buchi model R-114), so as to obtain the hexanic, acetonic and methanolic extracts of each cultivar, and these were dried in a laminar flow cabinet, to be used in the bioassays.

**Bioassay**

For the preparation of 250g of artificial diet, the following components proposed by Burton and Perkins (1987), were used: beans (30g), wheat germ (13.75g), brewers' yeast (8.75g), ascorbic acid (0.87g), sorbic acid (0.27g), methyl parahydrobenzoate (0.55g), formaldehyde at 10% (2.5ml), water for beans (116ml) and water for agar (90ml). These ingredients were mixed with the hexanic, acetonic or methanolic extract of *C. papaya* seeds of each cultivar to reach concentrations of 10, 100 and 1000ppm. Controls consisted on only the artificial diet and 1ml of hexane, acetone or methanol. Diet ingredients and the concentrated extracts were mixed following the method suggested by Franco et al. (2006). Of the prepared mixture, 5ml were placed in cylindrical plastic containers measuring 3cm high by 3.5cm diameter.

Once the diet-extract or diet-solvent gelled, a neonate larva was placed in each container with the aid of a fine, camel-hair brush. This process was repeated 3 times, using 30 larvae for each replication, and the containers were arranged in a totally random way in a breeding chamber (Precision Incubator 818, model FFU20FC4CW0 18, Electrolux Home Products, USA) at 27 ±1°C, 60 ±5% relative humidity and photoperiod of 12:12h. Mortality was recorded for the life span of the larvae, which the test lasted, and was corrected according to the Abbot method (Abbot, 1925). The mortality data were transformed to arcsine before carrying out ANOVA at a significance level of 5% (α=0.05). The mortality (%) means were compared using the Tukey test (Steel et al., 1997), in two ways: 1) mortality means of each cultivar, in order to know which of them, in general, was the most toxic; and 2) by comparing all the extracts, in order to know which were more active. Raw data is presented and the mortality data is corrected data. Statistical analysis was performed using SigmaStat (2004).

**Results**

In the absence of *C. papaya*, mortality in the case of the controls with hexane and methanol was <10% and with the acetone control it was 10.5% (Table I). At a concentration of 10ppm, the Maradol and Mammee cultivars were the most active with an average larval mortality rate of 56.7% in both cases, showing a significant difference compared to the Yellow and Hawaiian cultivars, which also showed significant differences (p<0.001) between themselves (Figure 1). Regarding the extracts, the mean for mortality obtained with acetone (47.8 ±3.2%) was significantly greater (p<0.001) than the means in those with methanol and hexane, without there being significant differences between the latter two (Figure 2). The three extracts from Yellow and Mammee cultivars, and hexanic and acetonic extracts from cultivar Maradol, were the most toxic ones, as they caused mortality rates ≥50%. In particular, the acetonic extract from cultivar Maradol was the most active one (p<0.001), with a corrected mortality of 73.6 ±2.1% (Table I).

At a concentration of 100ppm, the Yellow and Mammee cultivars were the most toxic for the insect, with no significant mean mortality difference (Figure 1) noted between them (54.7 ±6.3 and 53.1 ±3.3%, respectively). Of the extracts, those obtained with acetone were the most active (p<0.005), leading to 48.5 ±2.1% mortality (Figure 2). Various extracts caused a significant mortality rate of over 50% (Table I). The ace-

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**TABLE I**

**MORTALITY (±SE) OF FIRST LARVAL STAGE OF Spodoptera frugiperda FED WITH ARTIFICIAL DIET CONTAINING DIFFERENT CONCENTRATIONS OF HEXANIC, ACETONIC AND METHANOLIC EXTRACTS FROM SEEDS OF FOUR CULTIVARS OF Carica papaya**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Mortality ±SE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10ppm</td>
</tr>
<tr>
<td>Maradol Hexane</td>
<td>50.6 ±3.1 c</td>
</tr>
<tr>
<td>Acetone</td>
<td>73.6 ±2.1 a</td>
</tr>
<tr>
<td>Methanol</td>
<td>46.1 ±3.9 c</td>
</tr>
<tr>
<td>Mammee Hexane</td>
<td>50.1 ±2.8 e</td>
</tr>
<tr>
<td>Acetone</td>
<td>62.8 ±3.9 b</td>
</tr>
<tr>
<td>Methanol</td>
<td>57.1 ±3.7 bc</td>
</tr>
<tr>
<td>Hawaiian Hexane</td>
<td>50.3 ±2.9 c</td>
</tr>
<tr>
<td>Acetone</td>
<td>54.0 ±1.2 c</td>
</tr>
<tr>
<td>Methanol</td>
<td>54.7 ±1.6 c</td>
</tr>
<tr>
<td>Maradol Methanol</td>
<td>20.6 ±3.0 e</td>
</tr>
<tr>
<td>Acetone</td>
<td>38.5 ±3.9 d</td>
</tr>
<tr>
<td>Methanol</td>
<td>25.3 ±2.6 e</td>
</tr>
<tr>
<td>Control Hexane</td>
<td>8.9 ±0.1 f</td>
</tr>
<tr>
<td>Acetone</td>
<td>10.5 ±0.4 f</td>
</tr>
<tr>
<td>Methanol</td>
<td>9.6 ±2.3 f</td>
</tr>
</tbody>
</table>

Means in the same column followed by different letters are significantly different (Tukey test, p<0.05). SE: Standard error.

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**Figure 1. Effects of papaya cultivars on mortality of first larval stage of Spodoptera frugiperda fed with artificial diet treated with different concentrations of extracts from seeds.**
of the four cultivars in the form of extracts in this study, lower mortality rates than those reported for seed powder were obtained. This can be explained by the fact that the high toxic activity of the C. papaya seeds in powder form may be due to a possible synergy, in which the active components act in a complementary way on S. frugiperda.

Despite these lower concentrations, in extract the seeds of C. papaya continue to exercise high toxic effects on larvae of S. frugiperda. Figueroa-Brito (2002) evaluated hexanic, aceton and methanolic extracts of leaves, seeds and flowers of C. papaya cultivar Mammee in fresh and powder form, at concentrations of 5, 10, 15 and 20%, applied to maize leaf discs on S. frugiperda larvae and the results showed that the acetonic extracts of the seeds (fresh and powder) were the most active, causing between 50 and 100% mortality in the insect at all the concentrations tested. In the current study, a similar effect was obtained with the acetonic extracts of the Maradol Yellow and Mammee cultivars, which were the most active ones, causing corrected mortality percentages of 50-70%, the most effective of them all proving to be the acetonic extract of the Maradol cultivar. These results may be due to the fact that when the different cultivars tested, especially Maradol and Mammee, are mixed with aceton, the most active components are extracted.

It is worth pointing out that the acetonic extracts of the Maradol and Mammee cultivars at 10ppm caused mortality rates of 73.6 and 62.8% respectively. These concentrations are significantly lower than those tried by Figueroa-Brito (2002), who found that the acetonic extract of seeds of the Mammee cultivar at 5% led to a 100% mortality.

There are many studies of toxicity carried out with other plants that reflect a similar behavior on S. frugiperda (50-100% mortality), such as those with macerations of Trichilia havanaensis (Jacq.) by López-Olguín (1994), of Cabralea canjerana (Vell.) and Cedrela fissilis Vell. by Rodriguez and Vendramim (1996), of Azadirachta indica (A. Juss.) and Cedrela odorata L. by Rodríguez and Vendramim (1997), and of Melia azedarach L. and Trichilia pallida Sw. by Rodríguez and Vendramim (1998). Many of the Meliaceae species continue to be active in the form of ethanolic extract, as in the case of M. azedarach (Mikolajczak et al., 1989) and of aqueous extracts, as occurs with A. indica (Mikolajczak and Reed, 1987) and T. pallida (Bogorni and Vendramim, 2005). In addition to these plants, there are other plants whose extracts have been reported as having insecticidal effects on S. frugiperda, such as methanolic extracts of Yucca periculosa Baker (Torres et al., 2003), Ilosephane heterophylla (Cav.) Benth. ex Hemsl. (Figueroa-Brito et al., 2006), acetonic extracts of P. dulce (Figueroa-Brito et al., 2007) and aqueous extracts of Ricinus communis L. (Trujillo and García, 2001) and Trichilia pallens C. DC. (Bogorni and Vendramim, 2005).

Knowledge of biologically active plant products which do not cause damage to the environment, with new biodegradable structures and which preserve biodiversity, contributes to the development of less damaging strategies than the use of chemical insecticides. Such is the case of papain and cysteine from the latex of C. papaya against Spodoptera littura (F.) (Konno et al., 2004), or cysteine protease inhibitors like papain on Coleoptera and Hemiptera insects, as well as on phytopathogen nematodes (Blanco-Labra and Aguirre, 2002). Other research has shown a similar effect to that of the C. papaya seeds; such is the case with the mixture of epiperic phytoecdunines or acetates of phytoecdunines and compounds: ligidunin from Cedrela spp. (Céspedes et al., 2000), piplartin, 4´-desmetilipi- plartin and cenocladamin (Dyer et al., 2003) and piperin from Piper ceno cladum C. DC. (Batista-Pereira et al., 2006), and acetogenin from Annona cherimolia Mill. (Álvarez et al., 2007) on S. frugi perda. These compounds do not affect its predators and parasitoids (Bahena et al., 2003), as is the case with Doru taeniatum (Dohrn) and Ectatomma ruidum Roger (Schn guter, 1990), and similarly in Chrysoperla carnea (Stephen) and Trichogramma spp., with NeemAzal-T/S, PC 05, Blank and PC Blank products (El-Wakeil et al., 2006; Aggarwal and Brar, 2006).

Considering the results obtained in this study together with the experimental data already gathered in the literature, it would be of interest to continue with further studies with the purpose of fractioning and purifying the compounds found.
in the seeds of the tested cultivars of C. papaya, in order to study whether they continue to be effective against S. frugiperda in the form of pure compounds or their mixtures, as has already been shown with the mixtures of compounds from the seeds of the Maradol, Yellow and Hawaiian cultivars (Figueroa-Brito et al., 2002a,b). It is also necessary to carry out evaluations of these mixtures and pure compounds in semi-field and in field conditions to obtain basic knowledge in order to establish their possible incorporation into the integrat-ed management of the pest.

Conclusions

Hexane, acetone and metha-nol extracts of seeds of the Maradol, Mammee and Yel-low cultivars of C. papaya, followed by extracts from seeds of the Hawaiian cultivar, applied at concentrations of 10, 100 and 1000ppm, were toxic on S. frugiperda larvae. The acetonic extracts of the Maradol and Mammee cultivars at 10ppm were the most effective.

REFERENCES


