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

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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. Bioassays were conducted with hexanic, acetonic 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 acetonic 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 $1.4{\times}10^8$ha grown globally with maize, ~$96{\times}10^6$ha are located in developing countries. Four countries account for more than half of the developing world's maize cultivation area (Pingali and Pandey, 2001): China ($26{\times}10^6$ha); Brazil ($12{\times}10^6$ha); Mexico ($7.5{\times}10^6$ha); and India ($6{\times}10^6$ha).

In a global ranking of direct human consumption of maize, Mexico is at the top, exceeded only by Bosnia-Herzegovina. In 2006, *per capita* maize consumption in Mexico was ~260kg/person, and the country dedicated ~$12{\times}10^6$ton of maize grain to direct human consumption. Combining human consumption with maize dedicated to livestock and national reserves, the total annual consumption of maize in Mexico hovers around $26{\times}10^6$ton. Of this amount, ~$20{\times}10^6$ton were produced in the country, and another 6-$7{\times}10^6$ton were imported, the latter being composed primarily of yellow maize for 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 EXTRACTOS DE SEMILLAS DE *Carica papaya* (L.) CONTRA EL GUSANO COGOLLERO *Spodoptera frugiperda* (J.E. Smith) (LEPIDOPTERA: 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, Amarilla y Hawaiana) incorporados a una dieta artificial para insectos. Se realizaron bioensayos con extractos hexánicos, acetónicos y metanólicos 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 INSETICIDA 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. Estudaram-se 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 metanólicos em concentrações de 10, 100 e 1000ppm. Todas as provas foram realizadas com larvas de primeiro estágio da lagarta militar do milho* Spodoptera frugiperda. *A variável resposta foi a mortalidade do inseto. Nos resultados ficou registrado que os extratos de sementes dos cultivares Maradol, Mamey e Amarela de* C. papaya*, seguidos pelos extratos de sementes do cultivar Havai, aplicados em concentrações de 10, 100 e 1.000ppm, 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 herbivores (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 *Prosopis 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, acetonic and methanolic 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 plantations 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 laboratory and fed individually on an artificial diet (Burton and Perkins, 1987) and kept in closed, cylindrical plastic containers, 3cm high by 3.5cm diameter. The larvae underwent 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 dampened in a 10% sugar solution to feed them. Mating and laying of eggs took place in these containers. For the tests, the second generation of neonate 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 lka Wearke electric grinder (model MF 10 Basic, GMBH & 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 lightly in the recipient and left in extraction for 72h at ambient temperature. After this time, the mix was vacuum filtered using Whatman® Nº 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 (Büchi 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 ±1ºC, 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).

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*

Extract	Mortality ±SE (%)		
	10ppm	100ppm	1000ppm
Maradol Hexane	50.6 ±3.1 c	38.7 ±3.1 c	44.0 ±4.3 cd
Acetone	73.6 ±2.1 a	63.5 ±2.8 a	71.7 ±3.4 a
Methanol	46.1 ±3.9 c	47.6 ±1.9 b	48.1 ±1.3 c
Mammee Hexane	50.1 ±2.8 c	51.1 ±3.5 b	57.0 ±1.0 b
Acetone	62.8 ±3.9 b	57.0 ±2.5 ab	48.7 ±1.6 c
Methanol	57.1 ±7.3 bc	51.2 ±5.0 b	52.6 ±4.2 bc
Yellow Hexane	50.3 ±2.9 c	59.7 ±1.4 a	56.1 ±1.1 b
Acetone	54.0 ±1.2 c	62.3 ±1.2 a	58.9 ±2.2 b
Methanol	54.7 ±1.6 c	42.3 ±1.5 c	33.1 ±1.9 e
Hawaiian Hexane	20.6 ±3.0 e	49.7 ±2.2 b	42.2 ±6.3 cd
Acetone	38.5 ±3.9 d	49.5 ±2.1 b	43.3 ±5.1 cd
Methanol	25.3 ±2.6 e	24.1 ±1.4 d	36.4 ±6.7 de
Control Hexane	8.9 ±0.1 f	8.9 ±0.1 e	8.9 ±0.1 f
Acetone	10.5 ±0.4 f	10.5 ±0.4 e	10.5 ±0.4 f
Methanol	9.6 ±2.3 f	9.2 ±2.3 e	9.2 ±2.3 f

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

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-

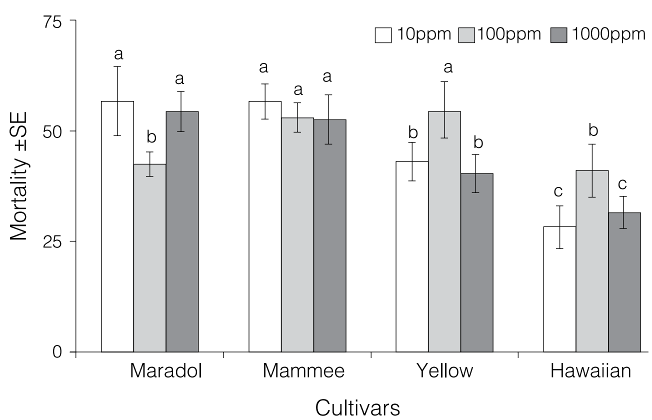

Figure 1. Effects of papaya cultivars on mortality of first larval stage of *Spodoptera frugiperda* fed with artificial diet treated with different concentrations of extracts from seeds.

tonic extracts from cultivars Maradol and Yellow, and hexanic from cultivar Yellow extracts were the most active (63.5, 62.3 and 59.7% mortalities, respectively) and with a statistically significant difference ($p<0.001$) with respect to the rest of the extracts.

At the 1000ppm concentration, the Maradol and Mammee cultivars were the most toxic, with no significant difference between them, causing mortality rates of 54.4 ±4.6 and 52.8 ±5.6% respectively (Figure 1). According to the extracts, the acetonic treatments caused the highest ($p<0.01$) mortality in the insect (Figure 2), with 45.9 ±1.2%. In the concentrations of 10, 100 and 1000ppm, various extracts showed significant mortality (>50%), among which the acetonic extract from cultivar Maradol was again the most toxic ($p<0.01$) for *S. frugiperda* larvae with 73.6 ±2.1, 63.5 ±2.8 and 71.7 ±3.4% mortality, respectively (Table I).

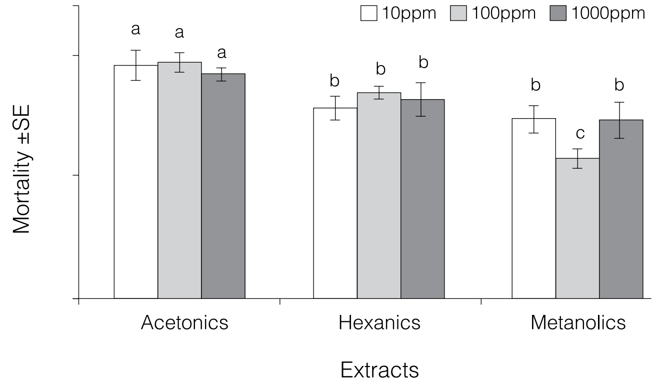

Figure 2. Effects of different concentrations of hexanic, acetonic and methanolic extracts from seeds of the *Carica papaya* on mortality of first larval stage of *Spodoptera frugiperda* fed with treated artificial diet.

Discussion

This study shows that various extracts of the *C. papaya* seed of the Maradol, Yellow and Mammee cultivars, and to a lesser extent of the cultivar Hawaiian, are toxic to *S. frugiperda* larvae, achieving corrected mortality percentages of between 50.0 and 73.6% in concentrations of 10, 100 and 1000ppm.

In previous studies with powdered *C. papaya* seeds, Figueroa-Brito (2002) reported 100% larval mortality at concentrations of 10, 15 and 20% with powder of the cultivar Mammee. The same happened with powder from the Maradol, Yellow and Hawaiian cultivars which, at the same concentrations, led to mortality in all the larvae after 24h (García, 2004). Franco *et al.* (2006) studied mortality related to time, noting that the powder of the four cultivars at 10 and 15% caused corrected mortality rates in the insect of over 90% after 72 and 96h respectively. In evaluating the insecticide effect of the 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, acetonic or aqueous 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 powdered) 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 acetone, 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 havanensis* (Jacq.) by López-Olguín (1994), of *Cabralea canjerana* (Vell.) and *Cedrela fissilis* Vell. by Rodríguez 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 insecticide effects on *S. frugiperda*, such as methanolic extracts of *Yucca periculosa* Baker (Torres *et al*., 2003), *Iostephane 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 cystecine from the latex of *C. papaya* against *Spodoptera litura* (F.) (Konno *et al.,* 2004), or cystein 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 phytogedunines or acetates of phytogedunines and compounds: ligedunin from *Cedrela* spp. (Céspedes *et al*., 2000), piplartin, 4′-desmetilpiplartin and cenocladamin (Dyer *et al*., 2003) and piperin from *Piper cenocladum* C. DC. (Batista-Pereira *et al*., 2006), and acetogenin from *Annona cherimolia* Mill. (Álvarez *et al.,* 2007) on *S. frugiperda*. 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 (Schmutterer, 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 semifield and in field conditions to obtain basic knowledge in order to establish their possible incorporation into the integrated management of the pest.

Conclusions

Hexane, acetone and methanol extracts of 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. The acetonic extracts of the Maradol and Mammee cultivars at 10ppm were the most effective.

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