Article

Control of guava cloves with plant extracts

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Abstract

Extracts made by alcoholic maceration, showed the greatest inhibition of the growth of the *P. clavispora* fungus with respect to water maceration or infusion. In bioassays with alcoholic extracts concentrated with rotary evaporator and filtered, extracts of jaral (*Cistus* sp.), olives (*Bidens odorata* Cav.), Mesquite (*Prosopis laevigata* Humb. & Bonpl. Ex Willd.), paradise (*Melia azedarach* L.), olive tree (*Olea europaea* L.), trompillo (*Solanum eleagnifolium* Cav.), lantana (*Lantana* sp.), rosemary (*Rosmarinus* sp.), rue (*Ruta graveolens* L.), venadilla (*Bursera simaruba* (L.) SARG.), cow tongue (*Rumex crispus* L.) and Australian eucalyptus (*Corymbia* (=*Eucalyptus) gummifera* (Gaertn.) Hill & Johnson) showed fungal growth reductions, greater than 90%. In the field, extracts of red eucalyptus plants (*Eucalyptus camaldulensis*) and Australian eucalyptus (*Corymbia gummifera*) showed less damage than chemical synthesis fungicides evaluated. The foregoing indicates that the use of these extracts is feasible in the control of the *P. clavispora* fungus, reducing the environmental impact.

Keywords: *Eucalyptus*, *Psidium guajava*, bioassays, field evaluation.

Reception date: December 2019
Acceptance date: March 2020

Introduction

The scab disease of the guava is a disease that affects both fruits and leaves of the guava tree (*Psidium guajava* L.), in the former dark brown circular lesions (such as a startled scab of the epidermis) with the appearance of a 'rusty nail', while asymmetrical reddish brown spots are observed on the leaves in the middle portion around the central rib, the tender shoots curl over their bundle and the terminal buds dry and fall (Nieto, 1996; González, 2002). The effects may exceed 70% in wild plants (Farfán, 2005) and around 14% in commercial orchards (Mishra and Prakash, 1986; González *et al*., 2008).

The causative agent is the fungi *Pestalotiopsis* (=*Pestalotia*) and *Neopestalotiopsis* (Keith *et al*., 2006; Solarte *et al*., 2017), the species that affect the guava are: *P. palmarum* (Cooke) Steyaert, *P. psidii* (Pat.) Mordue, *P. versicolor* (Speg.) Steyaert (Montiel, 1997) and *P. olivacea* Guba (Mishra and Prakash, 1986); *P. psidii* is considered the causative agent of scab disease in the guava producing area of Aguascalientes and Zacatecas (Nieto, 1996), while Serrano *et al*. (2018) found that *P. clavispora* is the causative agent in the area of Calvillo, Aguascalientes. Farr *et al*. (2008), mention that *P. psidii* affects guavas in other regions of the world (Burma, Ivory Coast, Ecuador, Hawaii, India, Malaysia, Mozambique, Nigeria, Puerto Rico, Taiwan, Tanzania, India, Venezuela, Zambia and Zimbabwe) and report effects on *Psidium guineense* in Venezuela, on *Psidium pomiferum* in Ecuador, on *Feijoa sellowiana* in Italy, on *Musa paradisiaca* in India and on *P. pomiferum* in India and Nepal.

For its control chemical synthesis fungicides are applied (Kasar *et al*., 2006; Prakash and Pandey, 2007; Ray *et al*., 2007). In Calvillo, Aguascalientes, Mexico, fungicides are applied on a scheduled basis, from July to October, because the conditions of temperature and high relative humidity favor their presence and damage (González *et al*., 2009), are performed from three to six applications of fungicides mixed or alternated with insecticides, due to the suspicion of a vector insect (*Monalonion* sp. Hemiptera: Miridae) detected in the area (Serrano *et al*., 2018). With 7 000 ha of guava in the area, more than 10 t of pesticides would be applied only to combat this disease, which could cause collateral damage to the environment.

In the area of Calvillo, the Institute of Health of the State of Aguascalientes has reported an increase in chronic renal diseases associated with the application of pesticides in the crop, observing overexposure to Malation and Cypermethrin in seven rural communities, concluding that 9% of the Study population is at moderate risk of progression to kidney damage. The increase in urinary excretion of sodium and phosphates was correlated with the serum concentrations of Cipermethrin (Mendoza *et al*., 2015).

Alternative strategies to control the disease are fruit bagging (Villamizar *et al*., 2003; Ramirez, 2005; Morera and Blanco, 2009), the use of antagonists (Gonzalez *et al*., 2008) and the application of botanical fungicides. In relation to the latter, the neem (*Azadirachta indica*), the purple basil (*Ocimum sanctum*), the eucalyptus (*Eucaliptus globulus* L.), the mint (*Menta piperita*) and the horsetail (*Equisetum arvense*) of the which *E. globulus* showed a control of 89% (Mishra, 2004; Parada, 2005; Quijada and Gómez, 2005). The control of plague organisms such as guava weevil (*Conotrachelus dimidiatus* Ch.) with botanical extracts has been explored with good results in the

Calvillo area; however, it has not been adopted since the evaluated plants (jicama or pepper) are not produced in the area, which implies a cost to obtain them, together with the elaboration work (González *et al*., 2008).

In the orchards associated with guava, wild plants coexist that can be used for the management of pest organisms. The objective of this study was to evaluate extracts of plants for regional use or present in the guava orchards of the producing area of Calvillo, Aguascalientes as an alternative for the management of the disease, considering its accessibility, low investment, easy processing and low environmental impact.

Materials and methods

Collection of biological material

Vegetable material was collected from 64 broadleaf species located in and around guava orchards, such as weeds or ruderal plants on perimeter fences. The material was transferred to the Plant Health Laboratory of the Pabellón Experimental Field (CEPAB), where it was dehydrated under the shade at room temperature (Rodríguez *et al*., 2012) afterwards, the samples (whole plant) were ground in a hammer mill (Hamilton Beach® Custom Grind™ Deluxe model), weighed and stored in 0.5 kg plastic bottles.

Preparation of extracts

Infusions. 10 g of plant material were weighed and placed in an aluminum container with 1 L of water, to which heat was applied until boiling for 5 min, they were filtered and stored in amber jars (Biswas *et al*., 2013). The solutions were filtered with sterile gauze, then with Whatman No. 42 paper and subsequently taken to a rotary evaporator to remove the solvent (Dhiman *et al*., 2011) until leaving a third of the total extract (the stock solution). Finally, they were sterilized with 0.45 µm micropore filters. Sterile solutions were stored in amber bottles protected from light and evaporation.

Aqueous and ethanolic macerates. The extracts were obtained by the method of maceration at room temperature (27 °C) with 70% water and alcohol, the maceration consisted of making 1:4 solutions (for each gram of plant material 4 ml of solvent were added), which they were left in maceration for a period of 7 days with manual agitation twice daily for 1 min. In water maceration, electropure and boiled water was used (Biswas *et al*., 2013). The same infusion procedure was followed to concentrate and store the macerates.

Bioassays of effectiveness against *Pestalotiopsis*

The two bioassays were performed in 9.0 cm diameter petri dishes, which were filled with 20 ml of sterile PDA medium + 150 µL of each extract, once the medium solidified the pathogen was inoculated by an agar disc of 0.6 cm in diameter with active growth of the fungus, it was incubated at a temperature of 27 °C until the negative control mycelium (PDA without extract) covered the entire plate (Baños *et al*., 2004). The percentage of inhibition of radial mycelial growth with respect to the negative control was measured by the formula: % inhibition of radial growth = [(control-treated)/control] X 100.

To know the effect of the best form of preparation of the extracts, three forms of preparation were considered a) macerated in water; b) macerated in 70% alcohol; and c) infusions. In the first instance, 10 plants were used for domestic use (Mexican cuisine), present in urban vegetation or as weeds in the area of Calvillo, Aguascalientess. A completely randomized design was used, with 13 treatments and three repetitions. They were compared with a commercial extract (Biogober®) and two commercial products (Fractal® and Cupravit®) (Table 1), the estimated variable was the percentage of inhibition.

Table 1. Effect on the growth of the fungus *Pestalotiopsis* sp by plant extracts obtained in different forms.

Plant extracts		Extract type		
		Maceration		Infusion
Common name	Scientific name (Family)	Water	Alcohol	
Horse tail	*Equisetum arvense* (Equisetaceae)	68.33 ±1.52*	0 ±0	70.33 ±1.52
Red eucalyptus	*Eucalyptus camaldulensis* (Myrtaceae)	69.67 ±0.57	6 ±0	69 ±40
Australian eucalyptus	*Corymbia gummifera* (Myrtaceae)	53 ±3	3 ±2.64	63.67 ±3.78
Higuerilla	*Ricinus comunis* (Euphorbiaceae)	84 ±8.66	17.67 ±4.16	81.33 ±6.8
Olive	*Olea europea* (Oleaceae)	71 ±2.64	14.67 ±3.21	63 ±6.55
Oregano	*Origanum vulgare* (Lamiaceae)	30 ±1	0 ±0	63.33 ±5.13
Paradise	*Melia azedarach* (Meliaceae)	72.33 ±0.57	12.33 ±1.52	71.67 ±0.57
Rue	*Ruta graveolens* (Rutaceae)	24.33 ±3.51	0 ±0	42 ±4.35
Thyme	*Thymus vulgaris* (Lamiaceae)	21 ±6	11.33 ±5.03	25.67 ±3.51
Walnut	*Carya illionensis* (Juglandaceae)	45 ±3	11 ±1	69 ±2
		Commercial products		
Biogober	*Larrea tridentata* (Zygophyllaceae) + *Ricinus comunis* + citric acid	54.33 ±2.08		
Fractal	*Citrus aurantium* (Rutaceae)	13.67 ±2.51		
Cupravit	Copper oxychloride	12.67 ±1.52		
		Control		
Water	Electropurified	63 ±3.6		
Alcohol	70% ethyl alcohol		53.33 ±2.08	

*= mean growth in mm of three repetitions at 168 h after inoculation ± standard deviation.

In order to find plants from which to obtain a solution with an inhibitory effect on the growth of *Pestalotiopsis* sp., additionally the extracts obtained by the alcoholic maceration of 23 plants associated with the guava orchards were used, as well as the Australian eucalyptus and Copper oxychloride through a randomized block design with three repetitions with the percentage inhibition variable (Table 2).

Table 2. Effect on the growth of *Pestalotiopsis* sp. with plant concentrates associated with guava orchards.

Treatment		Colony growth Area (mm) ± dev. standard	Reduction (%)
Solanum eleagnifolium (Solanaceae)	Trumpet	88.42 ±29.18 e	97.58
Copper sulphate		123.02 ±40.42 e	96.65
Corymbia gummifera (Myrtaceae)	Australian Eucalyptus	143.3 ±57.15 e	96.07
Cistus sp. (Cistaceae)	Jaral	152.31 ±32.73 e	95.82
Lantana sp. (Verbenaceae)	Lantana	175.02 ±40.42 e	95.2
Rumex crispus (Polygonaceae)	Cow tongue	192.76 ±88.71 e	94.71
*Olea europea (*Oleaceae)	Olive	202.51 ±30 e	94.45
Rosmarinus officinalis (Lamiaceae)	Rosemary	202.79 ±55.83 e	94.44
Ruta graveolens Rutaceae)	Rue	203.19 ±11.65 e	94.43
Bursera simaruba (Burseraceae)	Venadilla	282.01 ±109.69 e	92.27
Melia azedarach (Meliaceae)	Paradise	297.93 ±93.88 e	91.83
Prosopis laevigata (Fabaceae)	Mesquite	349.08 ±266.96 e	90.43
Bidens odorata (Asteraceae)	Olives	356.96 ±230.25 de	90.21
Lepidium virginicum (Brassicaceae)	Bird chili	374.37 ±119.58 de	89.73
Acacia farnesiana (Fabaceae)	Huizache	404.38 ±120.08 de	88.91
Erodium cicutarium (Geraniaceae)	Lemongrass	456.89 ±303.13 de	87.64
Apium leptophyllum (Apiaceae)	Coriander	464.32 ±246.62 de	87.27
Ricinus comunis (Euphorbiaceae)	Higuerilla	577.28 ±354.16 de	84.17
Mimosa monancistra (Mimosoideae)	Garruño	891.66 ±162.24 de	75.55
Dysphania ambrosioides (Amaranthaceae)	Epazote	945.99 ±903 de	74.06
Carya illionensis (Junglandaceae)	Walnut	974.38 ±718.48 de	73.28
Nicotiana glauca (Solanaceae)	Giant	1270.32 ±270.48 bde	65.16
Malva sp. (Malvaceae)	Mallow	2071.76 ±1485.44 abcde	43.19
Leucaena leucocephala (Fabaceae)	Gouache	2711.92 ±833.24 abcde	25.63
Equ Equisetum arvense (Equisetaceae)	Horse tail	2943.73 ±957.07* abcde	19.28
Control without extract		3846.66 ±1440.9 abcd	0

*= Colony growth mean of the fungus *Pestalotiopsis* sp., followed by the same letter are not statistically different using 0.05% Tukey.

Field experiment

The experiment was carried out in the locality 'Las Moras' (Mesa Grande, Calvillo, Aguascalientes; 21° 47' 09.4'' and 102° 43' 17.7'' with an altitude of 1 827 meters above sea level), where an infestation of the immature fruit disease (<2 cm in diameter) and a tree with the presence of nymphs and adults of the bed bug *Monalonion* sp. (Hemiptera: Miridae), considered this as the disease vector.

Once the trees were selected, all the affected fruits were eliminated and the treatments were randomized considering a randomized block design with nine treatments and three repetitions, with the following treatments: 1) Biogober® (BG) a commercial product based on extracts of governor at doses of 10 cc L^{-1} of water; 2) *Eucalyptus camaldulensis* (EER) red eucalyptus extracts at a dose of 50 ml of infusion (40 g of foliage in 1.5 L of water) per 1 L of water; 3) Excerpt from governor *Larrea tridentata* (EG) in prepared infusion and same doses as the EER; 4) Australian eucalyptus extract *Eucalyptus gummifera* (EEA), prepared and same doses as treatment 2.5) Cupravit® copper oxychloride (CU) at a dose of 5 g L^{-1} of water; 6) Metalaxyl + Chlorothalonil with trade name Ridomil Gold® (RG) at a dose of 5 g L^{-1} of water; 7) Mancozeb (MZ) at 5 g L^{-1} of water; 8) water only; and 9) Malation insecticide (M) at a dose of 3 cc L^{-1} of water.

To improve the emulsion and the adhesion to the plant, 5.0 cc of Tamis® and 3 drops of Tween 20® per liter of water were added to all the preparations. The application of the treatments began when most of the fruits were approximately 2 cm in diameter and three applications of the chemicals and six of the botanists were made. At the end of the applications, all the fruit present in each tree was counted and all the affected fruits were harvested. The incidence was determined (healthy fruits - damaged fruits) and the severity determined with the following scale 0 = no damage, 1= slight 1-3 nails <10% damage, 2= moderate 5 -7 nails around 15 to 20% of damage, 3= strong> 10 nails and 40-60% damage and 4= very strong> 70% (González *et al*., 2009).

The data obtained were transformed to a weighted average of the infection (Townsend and Heuberger, 1943) using the following formula: $PI= [\sum(n*v)/CM*N]*100$. Where: PI= weighted average of infection, n= number of fruits for each class on the scale, v= numerical value of each class, CM= major category, N= total number of fruits in the sample. For their analysis, the data were transformed to a square root of X+0.5 to decrease the variance and were analyzed using the SAS JMP program and the differences between means were compared using 0.05% Tukey. To determine the biological effectiveness of the treatments, Abbott's formula (1987) was applied.

$\%E= [IT-It/IT]*100$, where:%E= percentage of effectiveness, IT= control infection, It= treatment infection.

Results and discussion

In vitro effect of the form of preparation of plant extracts on *Pestalotiopsis* sp.

Alcoholic maceration (Table 1) showed the greatest inhibition of fungus growth, compared to water maceration and infusion (7.6 mm), even lower than chemical treatments (Fractal and Copper oxychloride) with a growth of 13.67 and 12.67 mm. The higuerilla and olive extracts

showed no outstanding effect. The commercial Biogober extract showed no significant effects in reducing fungus growth that were similar to the control with alcohol.

Regarding the bioassay of alcoholic extracts concentrated with rotary evaporator (Table 2), some extracts showed effects of fungus growth reduction greater than 90% (jaral, olives, mesquite, paradise, olive, trompillo, lantana, rosemary, rue, bandage, tongue of cow and Australian eucalyptus), similar to the copper treatment that showed an average reduction of 96.65%. The extracts with the lowest effect on the growth of the fungus were: horsetail, huache and mallow.

Evaluation of extracts in commercial gardens

The trees under treatment had an average of 1 502 tree fruits (range from 585 to 3 729), in the establishment of the experiment an average of 47 fruits with nail incidence was found, with a range of 0 to 361 fruits damaged per tree (average incidence of 3%).

Table 3 shows the average number of fruits damaged by tree, where it was observed that only the treatment with the insecticide Malathion was statistically different from the rest of the treatments. The treatments with the least number of affected fruits were Malathion and the Australian and red eucalyptus (5, 10 and 11 fruits damaged per tree representing 0.5, 0.4 and 1% damage respectively), while those with the highest number of affected fruits were: the governor and the control with water with 91 and 65 damaged fruits representing 5.3 and 4.6% damage respectively.

Table 3. Effectiveness of plant extracts for the control of *Pestalotiopsis clavispora* in the field.

Treatments	Fruits affected by nails		PI**	Effectiveness***
	Number	(%)		
Biogober® (BG)	28 a*	2.2	0.886	51.38
Red eucalyptus (EER)	11.3 a	1	0.376	79.35
Governor (EG)	91.3 a	5.3	2.031	-11.49
Australian eucalyptus (EEA)	10.7 a	0.4	0.071	99.05
Preventive fungicide (CU)	23.7 a	1.6	0.283	84.47
Curative fungicide (RG)	23.3 a	3.2	1.327	27.14
Preventive fungicide (MZ)	22.3 a	1.9	0.653	64.15
Insecticide (M)	5.3 b	0.5	0.154	91.55
Absolute control	65.7 a	4.6	1.822	

*= means of fruits damaged by tree, means followed by the same letter are not statistically different by means of Tukey at 0.05%; **= percentage of infection according to Townsend and Heuberger, 1943; ***= effectiveness of treatments according to Abbot's formula, 1987.

The chemical synthesis fungicides as a group showed similar damages among them with 22 damaged fruits, which is lower than that of Biogober with 28 damaged fruits (23, 23 and 22 fruits respectively for CU, RG, MZ and BG). When the data of percentage of damage with respect to the total of fruits present per tree are observed, the fruits damaged in the treatments with governor and the control with water represented 5.3 and 4.6%, whereas, in the Australian eucalyptus, red and the Malation they represent 0.4, 0.5 and 1%.

Regarding the severity (Figure 1), only the treatments of Malathion and Australian eucalyptus did not show fruits with very strong damages. In the control and in the governor's statement there were more than 10 fruits with strong damages and more than 25 fruits with medium damages. Red eucalyptus, Australian eucalyptus and Malathion presented less than 5 fruits with light damage. In general, light damage prevailed in all treatments except for Mancozeb and Ridomil fungicides where light damage predominated or there was no trend.

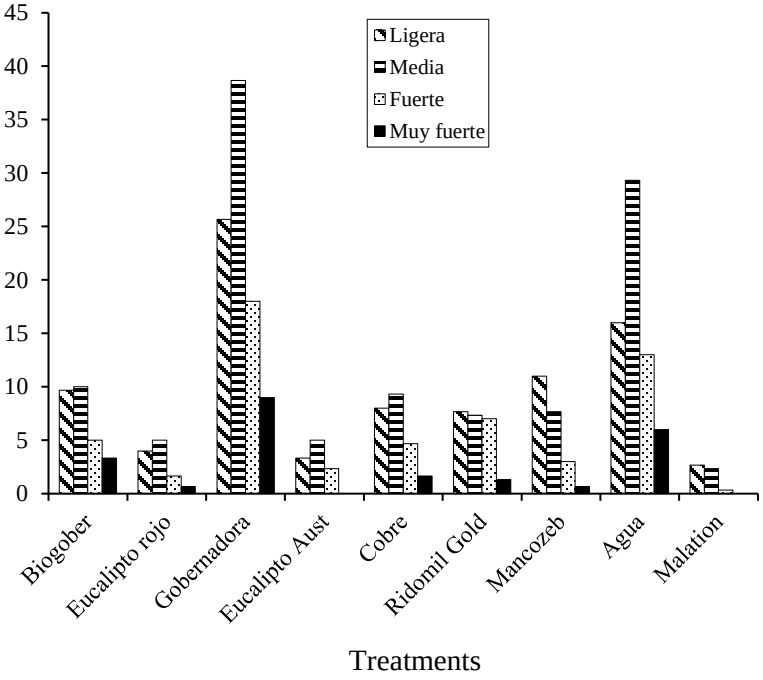

Figure 1. Degree of severity in the damages caused by *Pestalotiopsis* sp., to guava fruits under field conditions, Aguascalientes in 2016.

The percentage of infection (PI) (Table 3) in general was low. It was 0.071 in the Australian eucalyptus (EEA) followed by the Malathion and red eucalyptus (0.154 and 0.376 respectively) and the highest percentage was observed in the governor extract (EG) with 2.031, even higher than the control with water (1.822). Of the chemical synthesis fungicides, the one with the lowest PI was the Copper-based product (CU with 0.283). The best treatments for disease control were EEA, Malathion, CU and ER with efficiencies of 99, 91, 84 and 79%.

The results are consistent with the indications of Parada (2005); Quijada and Gomez (2005) on the effectiveness of eucalyptus extracts in the control of the guava clove caused by *Pestalotiopsis* spp. and reinforce the use of the ethanolic extracts of this plant in the fight against plant diseases (Baños *et al*., 2004; Alzate *et al*., 2009; Cazar *et al*., 2014), as well as it was observed that when applying insecticides against the vector the damages caused by the disease are reduced, reinforcing the signaling of the presence of a vector of the disease in the region (Serrano *et al*., 2018).

Conclusions

In the bioassays, plants associated with guava orchards were found that can be used to control the disease of the guava clove, from which we can mention the extracts of jaral, olives, mesquite, paradise, olive, trompillo, rosemary, rue, venadilla, cow tongue and eucalyptus. At the field level, extracts of red eucalyptus (*Eucalyptus camaldulensis*) and Australian eucalyptus (*Corymbia* (=*Eucalyptus*) *gummifera*) plants showed similar damage percentages to the Malathion insecticide and lower than those observed with the application of chemical synthesis fungicides evaluated.

Extracts of red eucalyptus at a dose of 50 ml of infusion (40 g of foliage in 1.5 L of water) per 1 L of water applied on six occasions are considered an option for disease control without the use of chemical synthesis. The handicraft extracts of governor (*Larrea tridentata*) showed no efficient control of the disease while the commercial formulation based on governor was only slightly greater damage than that of synthetic fungicides. The Malathion insecticide was one of the best treatments in the control of this disease, which shows the relevance of vector control (*Monalonion* sp.). The use of plant extracts represents an alternative in the control of *Pestalotiopsis clavispora* with a lower environmental impact.

Acknowledgments

To the PhD program in Biotechnology in Agricultural Processes of the National Technological Institute of Mexico (El Llano Aguascalientes Technological Institute) for allowing me to carry out the research work and to CONACYT for the financial support of the 2018 01NACF 03186 scholarship. To Mr. Joaquín Velasco Luevano for providing us with the facilities for the development of the experiment in your orchard.

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