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ORIGINAL RESEARCH

Characterization of *Guazuma ulmifolia* Lam. seed gum and its effect on the activity of *Metarhizium anisopliae* (Metschn.) Sorokin on *Bemisia tabaci* Genn

Caracterización de la goma de semillas de *Guazuma ulmifolia* lam. y su efecto sobre la actividad de *Metarhizium anisopliae* (Metschn.) Sorokin en *Bemisia tabaci* Genn

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ABSTRACT

The present study was conducted to evaluate the functional properties of seed gum of *Guazuma ulmifolia* (GG) and its effects on the entomotoxic activity of the fungus *Metarhizium anisopliae* (MET) on whitefly nymphs (*Bemisia tabaci*). The GG was extracted from mature fruit seeds in distilled water at 60°C and precipitated in ethanol. The quantity of gum extracted from the seed of *G. ulmifolia* was 67.1 g/kg. This gum showed a dispersion in water of 79.6% and thermal stability. The rheological studies indicated that GG had a non-Newtonian behavior and high viscosity at low concentrations. The swelling index (SI) and water dispersion (W_{DIS}) of GG were determined at different temperatures. The apparent viscosity (µ) of GG was measured using an AR-2000 rheometer. An entomotoxic bioassay was carried out under greenhouse conditions using MET ($1x10^8$ conidia mL) suspended in aqueous solutions of GG at 0.2 and 0.5%. These solutions were sprayed on eggplant leaves infested with *B. tabaci* to evaluate their entomopathogenic effect. The use of GG improved the lethal effect of MET on *B. tabaci* nymphs by 44.4%. This increase in nymphal mortality caused by MET when combined with GG, suggest that GG could be a good alternative as a carrier and protective agent for entomopathogenic fungi used in pest control.

Keywords: fungi activity, nymph mortality, seed protection, tree species, viscosity.

RESUMEN

El presente estudio se realizó para evaluar las propiedades funcionales de la goma de *Guazuma ulmifolia* (GG) y su efecto en la actividad entomotóxica del hongo *Metarhizium anisopliae* (MET) sobre ninfas de mosca blanca (*Bemisia tabaci)*. La GG se extrajo de semillas de frutos maduros en agua destilada a 60°C y se precipitó en etanol. La cantidad de goma extraída de la semilla de *G. ulmifolia* fue de 67.1 g/kg, dicha goma mostró una dispersión en agua de 79.6% y estabilidad térmica. Los estudios reológicos indicaron que la GG tuvo un comportamiento no-Newtoniano y alta viscosidad a bajas concentraciones. El índice de hinchamiento (SI) y dispersión de agua (W_{DIS}) de la GG se determinó a diferentes temperaturas. La viscosidad aparente (µ) de la GG se obtuvo empleando un reómetro AR-2000. Se realizó un bioensayo entomotóxico usando MET ($1x10^8$ conidios mL) suspendido en diferentes soluciones de GG (0.2-0.5%) en agua. Dichas soluciones se rociaron sobre hojas de berenjena infestadas con *B. tabaci* para evaluar su efecto entomopatogénico. El uso de la GG mejoró el efecto letal de MET sobre ninfas de *B. tabaci* en un 44.4%. Este incremento en la mortalidad de ninfas por efecto de MET en combinación con GG, sugiere que GG podría ser una buena alternativa como agente portador y protector de hongos entomopatógenos usados en control de plagas.

Palabras clave: especie arbórea, hongo entomopatógeno, mortalidad de ninfas, protección de semillas, viscosidad.

1. INTRODUCTION

Gums or hydrocolloids are long chain polysaccharides, characterized by their property of forming viscous dispersions or gels in water. Natural gums can be found in gummy slimes from fermentation processes, seaweeds, plant exudates, plant extracts and seed flours (Pacheco-Aguirre *et al*., 2010). Gums has been used in industrial products as thickeners, stabilizers, emulsifiers, coatings, encapsulants and carriers. All these functional properties depend on their mechanical (texture) and flow (viscosity) properties (Milani & Maleki, 2012). Currently, research on non-conventional gums has opened new avenues on novel uses of these products and have replaced the use of synthetic gums (El Kader *et al*., 2008). Several studies has found promising results with gums from seeds of the Fabaceae *Leucaena leucocephala*, *Delonix regia*, *Adenanthera pavonina*, *Caesalpinia pulcherrima*, *Gleditsia triacanthos* and *Sophora japonica* (El Kader *et al*.,

2008; Pacheco-Aguirre *et al*., 2010; Cerqueira *et al*., 2009), the Malvaceae *Durio zibethinus* and *Guazuma ulmifolia* (Mirhosseini & Amid, 2012; Carrascosa & Lorenzo, 2013), the Brassicaceae *Lepidium perfoliatum* and *Alyssum homolocarpum* (Koocheki *et al*., 2009; Koocheki *et al*., 2010), and the Lamiaceae *Ocimum basilicum* (Hosseini *et al*., 2010). *G. ulmifoli*a is a tree that grows in tropical zones of Mexico, South America and in the Caribbean. This tree is considered a multipurpose tree which is used as ground cover and forage source in cattle production (Villa *et al*., 2009; Manríquez *et al*., 2011), as well as cosmetics and medicament (Berenguer *et al*., 2007; Lopes *et al*., 2009; Alonso & Salazar, 2008; Patel *et al*., 2012). Gum obtained from bark and leaves of *G. ulmifolia* has been use as strengthener and fixer of color in archaeological paintings (Ruiz, 2009; Carrascosa & Lorenzo, 2013). Moreover, the gum of *G. ulmifolia* seed has effects on the control of mosquito larvae through its attraction and retention (Gallardo, 1993), however the physicochemical characteristics such as viscosity, water dispersion, swelling index among others, of gum of *G. ulmifolia* have not been studied, which allows to be used more widely in control of infestation in horticultural crops.
Metarhizium anisopliae (MET) is an entomopathogenic fungus that has been widely used as a biological control agent. Several works have reported that MET is an excellent control agent against whitefly (*Bemisia tabaci*) infestation in horticultural crops. Faria & Wraight (2001) have reported that isolates of MET are highly virulent at nymphs of *B. tabaci*. Flores *et al*., (2012) indicated that MET is effective against the eggs, first, second and third instar nymphs, and pupae of *B. tabaci.* Norhelina *et al*., (2013) evaluated five MET strains against *B. tabaci* nymphs finding that strain GJ4 was highly infective to nymphs. A well know constraint in the use of biological control fungi is the effect of environmental factors such as solar ultraviolet radiation (UV-A and UV-B) and high temperatures (Rangel *et al*., 2004). These factors affect the conidia viability, which results in inactivation and germination inhibition (Ojeda *et al*., 2011; Herlinda *et al.*, 2018). To counteract these effects, the use of natural gums may be feasible as carrier and protective agent for microorganisms, which could be due to water blinding capacity and reflected in the viscosity and swelling index, among other physicochemical parameters, protecting them from extreme weather conditions, such as high temperature and UV radiation (Yin *et al*., 2019; Pacheco-Aguirre *et al*., 2016). The present work was carried out to evaluate the functional properties of seed gum from *Guazuma ulmifolia* and its application as carrier to enhance *MET* activity on the whitefly (*B. tabaci*) nymphs.

2. MATERIALS AND METHODS

2.1 Plant material

Guazuma ulmifolia fruits (Fig. 1) were collected in the municipalities of Merida and Conkal, Yucatan, Mexico. Dried ripe fruits were collected directly from the field, selecting healthy fallen fruits from six trees. The fruits were processed in the laboratory of Microbiology at the Tecnologico Nacional de México, Campus Conkal, Yucatan. These fruits were passed through a fodder crusher (TRF 300G TRAPP, Jaraguá do Sul/SC, Brasil) to break the husk and release the seeds. The seeds were cleaned sequentially through a 4 (4.76 mm), 8 (2.38 mm) and 16 (1.19 mm) mesh to remove all husk residues. The husk residues below the size of the seeds were removed by fluidized bed. Clean seeds were stored in polyethylene bags at 4 °C until use.

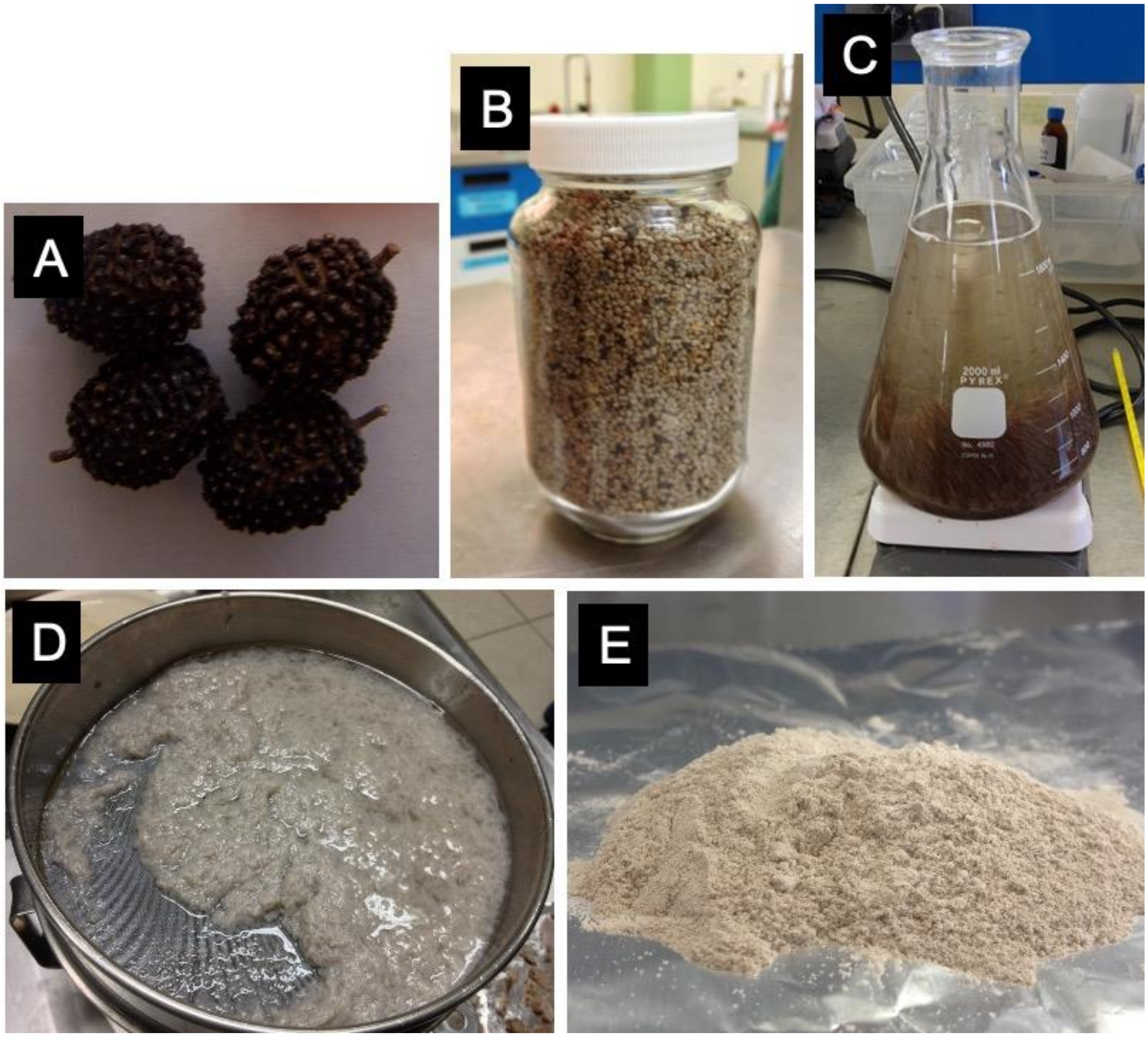

Fig. 1. Extraction process of *Guazuma ulmifolia* seed gum. A) Fruits, B) Seeds, C) Seed shaking, E) Gum precipitated with alcohol, F) Gum flour.

2.2 Gum extraction

The seed gum (GG) extraction was carried out as documented by Capitani *et al*. (2015), with some modifications. Briefly, seeds were suspended in water (1:15 w/v, pH 7) and heated to 60 °C under constant agitation for 1 h. The suspension was then filtered sequentially through a 42 and 100 mesh (351 and 147 mm, respectively) to separate gum from seeds. This operation was repeated modifying relation seeds:water (1:5 p/v) and the gum collected in the filtrates were mixed and precipitated in pure ethanol (1:2, filtrate/ethanol), dried at 60 °C for 12 h in a circulating air oven (Imperial V Lab-Line Model 3476M, Boston, MA), and milled (Thomas-Wiley Laboratory Mill Model 4, Swedesboro, NJ) to an 80 mesh (173 mm) size with a proximate composition of 0.89 % protein, 10.47 % ash, 7.10 % fat, 2.93 % crude fiber, and 78.6 % total carbohydrates, determined according to AOAC International (1997).

2.2.1 *Swelling index and water dispersion*

Swelling index (SI) and water dispersion (W_{DIS}) were determined by using the method reported by Pacheco-Aguirre *et al.,* (2010), taking 40 ml of a 1% (w/v) dilution of GG extract in distilled water in four 50 ml centrifuge tubes. The tubes were heated separately in a water bath to 4 different temperatures (30, 50, 70 and 90°C) for 30 minutes. To prevent the gum swell sedimentation during heating, the tubes were constantly stirred and covered with plastic film to avoid water loss. After heating, the tubes were centrifuged (2,120g, 15 min) in an ultracentrifuge (Z-300K Hermle, Wehingen, Germany) and the pellet was recovered and weighed (W_p). Both the precipitated and the supernatant were dried at 105 °C for 4 h and dry solids were calculated for the precipitated (W_{ps}) and supernatant (W_s). SI was calculated (Equation (1)) as the ratio of the weight of the hydrated gum after centrifugation (g) to its dry mass (g).

$$SI = \frac{Wp}{Wps} \qquad \frac{(\text{hydrated gum (g)})}{(\text{dry gum in precipitate paste (g)})} \qquad (1)$$

The W_{DIS} is the percentage of dry mass in supernatant to the dry mass of whole gum sample (W_o):

$$W_{DIS} = \frac{Ws}{Wo} x100 \qquad \frac{(\text{soluble solids (g)})}{(\text{dry mass of whole gum sample (g)})} \qquad (2)$$

2.2.2 *Viscosity assay*

Viscosity (µ) of seed gum was determined by using the method reported by Pacheco-Aguirre *et al.,* (2010). This was carried out using an AR-2000 rheometer (TA Instrument) with a cone and plate geometry (20 mm diameter and 4° angle) and a shear rate range from 1 to 500 s^{-1}. To evaluate the viscosity of the GG, three temperature ramps (15, 25 and 45 °C) were used. Each analysis was done in triplicate.

2.3 Whitefly colony and bioassay

Adult whiteflies (*B. tabaci*) were originally collected from field-established habanero pepper (*Capsicum chinense* Jacq.) in Conkal, Yucatan, Mexico. The colony that has been maintained for three years was kept in entomological cages made of anti-aphid mesh with aluminum frame, in a greenhouse at 28 ± 6 °C with a natural photoperiod (L:D) of approximately 12:12 h. Insects in the cages were fed on 40 to 70 day-old *C. chinense*. These plants were grown in 2 L plastic pots containing peat moss (Cosmopeat®) and fertilized daily with triple 19 (NPK 19-19-19), 1 g/L (Chan *et al*., 2013).

Eggplant seedlings were planted in 2 L pots, filled with 0.4 kg of autoclaved soil mixture (% v/v): 70 % soils (the soil had medium-textured, with 0.93% N, and the total contents of P, K, Ca and Mg of 2.45, 3.5, 49.38 and 2.63 g kg^{-1}, respectively), 10 % commercial substrate (Cosmopeat®) and 20 % fine grave. Plants (30 cm height) were exposed to *B. tabaci* for 15 days at 28 ± 6 °C, as described by Chan *et al*., (2013). When eggs and nymphs were observed in the eggplant leaves, the biological activity of MET was evaluated as follows. Spores of MET were obtained from the Laboratory of Plant Health Services from Yucatan, Mexico. This MET strain has been used for microbial management of *Schistocerca piceifrons piceifrons* Walker (1870) and has also been lately used for *B. tabaci*. Conidia were harvested from 15-day fungal colonies grown in sabouraud dextrose agar (SDA) medium, suspended in sterile distilled water (0.05 % Tween 80) and filtered through five layers cheesecloth to remove mycelia mats and fragments of SDA (Chan *et al*., 2013). Concentrations of conidial suspensions were adjusted to 1 x 10^8 conidia mL with the use of a standard Neubauer chamber. Conidial suspensions (5 L suspension) were sprayed until running with a manual sprayer (Table 1) to the abaxial surface of *B. tabaci*-infested leaves. Seven days after MET was sprayed, leaves were excised and observed in a stereoscopic microscope (Leica EZ4) to evaluate nymphal mortality. Observation of nymphal mortality was carried out in sections of 8.58 cm^2 per leaf (Ruiz-Sánchez *et al*., 2011). The experiment was arranged in a completely randomized design using four replicates for each treatment (Table 1).

Table 1. Spore suspensions sprayed to *Bemisia tabaci* nymphs.

Treatment	Description
Control	Water (no fungal spore).
MET	*Metarhizium anisopliae* (1x10^8 conidia mL) in distilled water.
MET-GG 0.2 %	*M. anisopliae* (1x10^8 conidia mL) + seed gum of *G. ulmifolia* (0.2 % w/v) in distilled water.
MET-GG 0.5 %	*M. anisopliae* (1x10^8 conidia mL) + seed gum of *G. ulmifolia* (0.5 % w/v) in distilled water.

2.3.1 *Data analysis*

Analysis of variance and means comparison test (Tukey, $p< 0.05$) were performed by Statgraphics Centurion software (15.2.06 version). Prior to run, data of percent mortality were transformed to arcsin [y=arcsin(sqrt y/100)]) to fit normality of data..

3. RESULTS

3.1 Yield of seed and seed gum from *G. ulmifolia*

The yield of seeds was 148.5 g per kg of fruits. Gum extraction yielded 67.1 g per kg of seeds, with approximate contents of 0.89 % protein, 10.47 % ash, 7.10 % fat, 2.93 % crude fiber, and 87.19 % total carbohydrates. The seed gum of *G. ulmifolia* (GG) showed an increase in conductivity from 23 to 451 µS/cm when concentration increased from 0.5 to 1.0 % GG in water, respectively. The pH remains constant (pH 5.7) despite the increase in the concentration of GG.

3.1.1 *Swelling index and water dispersion*

The GG showed 79.6 ± 2.4 % of water dispersion (W_{DIS}) and 12.6 ± 4.8 g/g of swelling index (SI) at room temperature. When the temperature increased the GG-water system, the W_{DIS} and SI did not change significantly (Fig. 2). These results suggest that GG has thermal stability under increasing temperature.

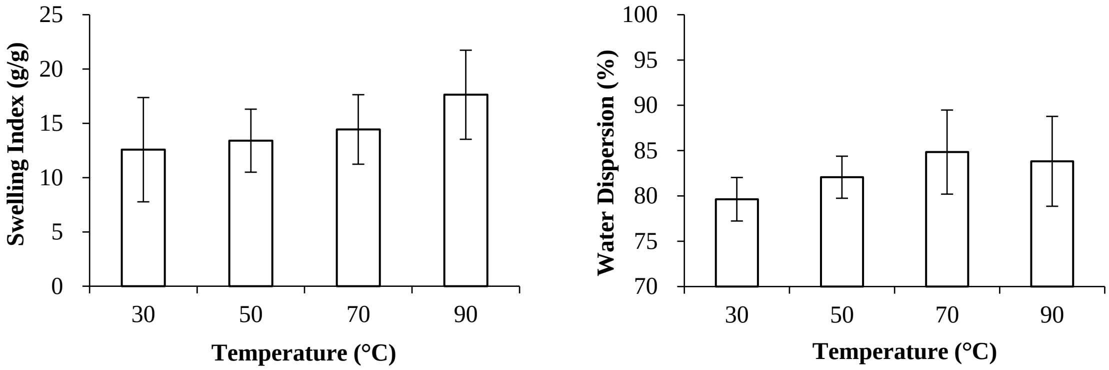

Fig. 2. Swelling index (g water/g-gum) and water dispersion (%) of *G. ulmifolia* gum.

3.1.2 *Viscosity assay*

Looking for possible technological applications of the polymeric matrices, we analyzed their viscosities response at steady state under the application of a variable shear stress. The GG viscosities to 0.5 and 1.0 % (pH 5.7 dispersion) in all temperatures exhibited a non-Newtonian behavior, as their apparent viscosities decreased when the strain rate (g) increased (Fig. 3a). The data were fitted to an Ostwald-de Waele model ($\sigma = k \cdot y^n$) and determined the consistency index (k) and flow behavior index (n). The coefficient of determination (R^2) for GG at 0.5 % and 1.0 % were 0.9921 and 0.9869, respectively.

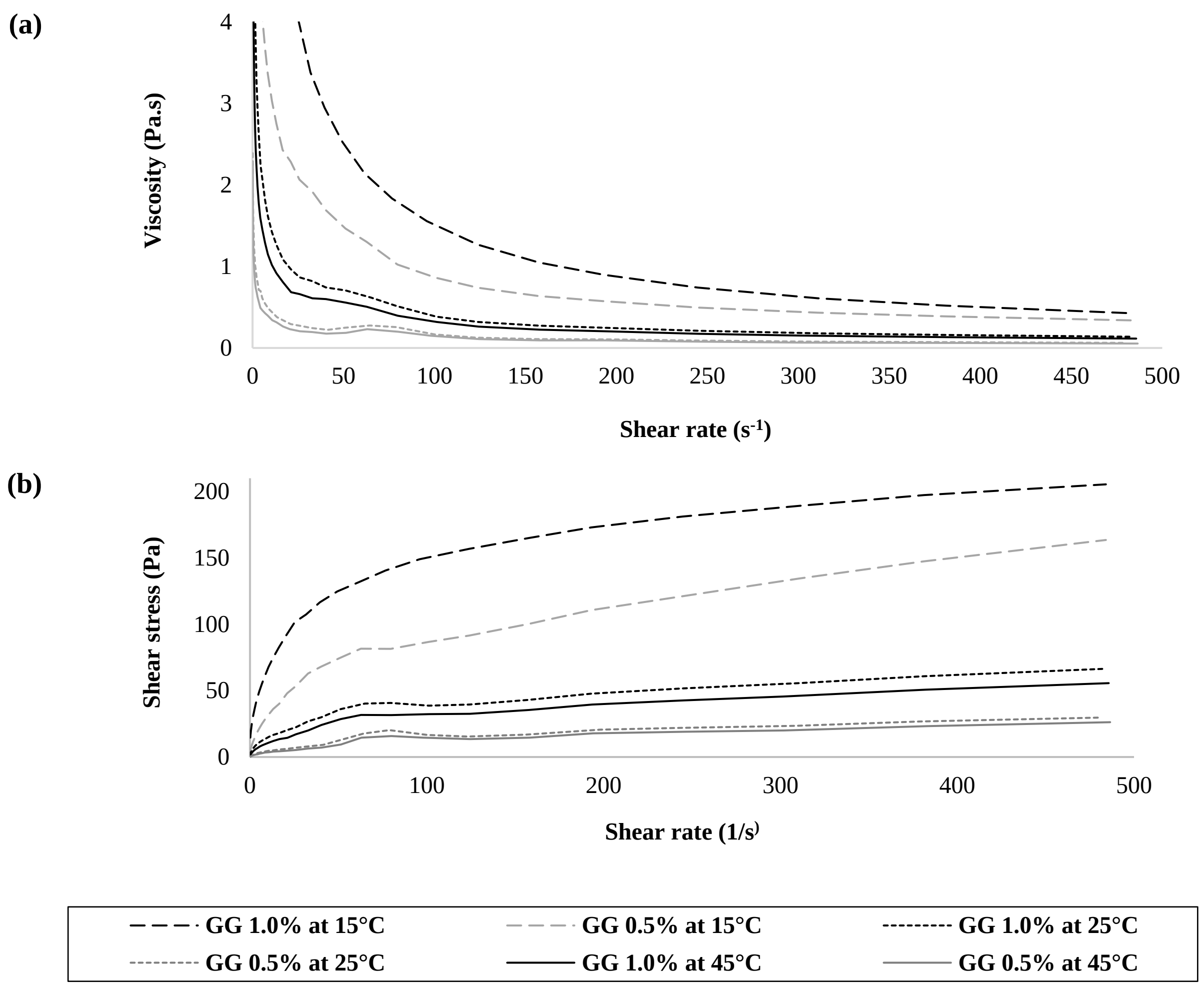

Fig. 3. Rheological behavior of *G. ulmifolia* gum (GG) at 15, 25 and 45 °C. (a) Viscosity curve, (b) flow curve.

At both GG concentrations viscosity decrease when temperature increased. The increase of shear rate (Fig. 3b) for two used GG concentrations showed a similar behavior of stress deformation at 25 and 45°C. The shear stress was affected by GG concentration at the afore mentioned temperatures, having a minor shear stress in GG at 0.5 %.

The consistency index (k) and the flow behavior index (n) indicate that both GG concentrations (0.5 and 1.0 %) behave as shear thinning fluid (pseudoplastic). The decrease of GG concentration also modified significantly ($F_{1,2}$= 2030, p<0.0001) the consistency index (Table 2). The increase in temperature affected significantly ($F_{2,2}$= 112, p<0.0001) the GG consistency, with a total decrease up to 40 % for 1.0 % GG. On the other hand, the decrease of GG concentration improved significantly the fluidity up to 20 % (Table 2). The GG fluidity increased significantly almost in all cases with the high temperatures ($F_{2,2}$=79, p<0.0001) and GG concentration ($F_{1,2}$=1350, p<0.0001), except in 45°C with an opposite pattern (Table 2).

Table 2. Consistency and flow behavior index of *G. ulmifolia* gum (GG) at 15, 25 and 45 °C

GG Conc.	Consistency index (Pa·s) 15 °C	25 °C	45 °C	Flow behavior index (n) 15 °C	25 °C	45 °C
0.5%	1.54±0.12Aa	1.38±0.07ABa	0.99±0.08Ba	0.48±0.01Aa	0.49±0.01Aa	0.52±0.01Ba
1.0%	6.60±0.12Ab	5.11±0.28Bb	3.95±0.29Cb	0.37±0.01Ab	0.40±0.01Bb	0.42±0.01Cb

Different capital letters indicate significant differences among temperatures, and different lowercase letters indicate differences between GG concentrations (Pairwise contrast Tukey, P≤0.05).

3.2 Effect of GG on the entomotoxic activity of *Metarhizium anisopliae* (MET)

For the bioassay two GG concentrations (0.2 % and 0.5 %) were used in the conidial suspension of MET that was sprayed to *B. tabaci*-infested leaves. These leaves had an average density of 5.4 ± 2.0 nymphs cm^2. Nymphal mortality (Fig. 4) showed significant differences ($F_{5,7}$=19.5, P<0.001) among MET treatments. The treatment MET-GG 0.5 % showed the highest activity on *B. tabaci* nymphs, recorded mortality was 91.1 ± 8.9 %. Nymphal mortality caused by MET-GG 0.2 % was not significantly different (Pairwise contrast Tukey, P≥0.05) to that caused by MET-bare spore (49.2 %; Fig. 4).

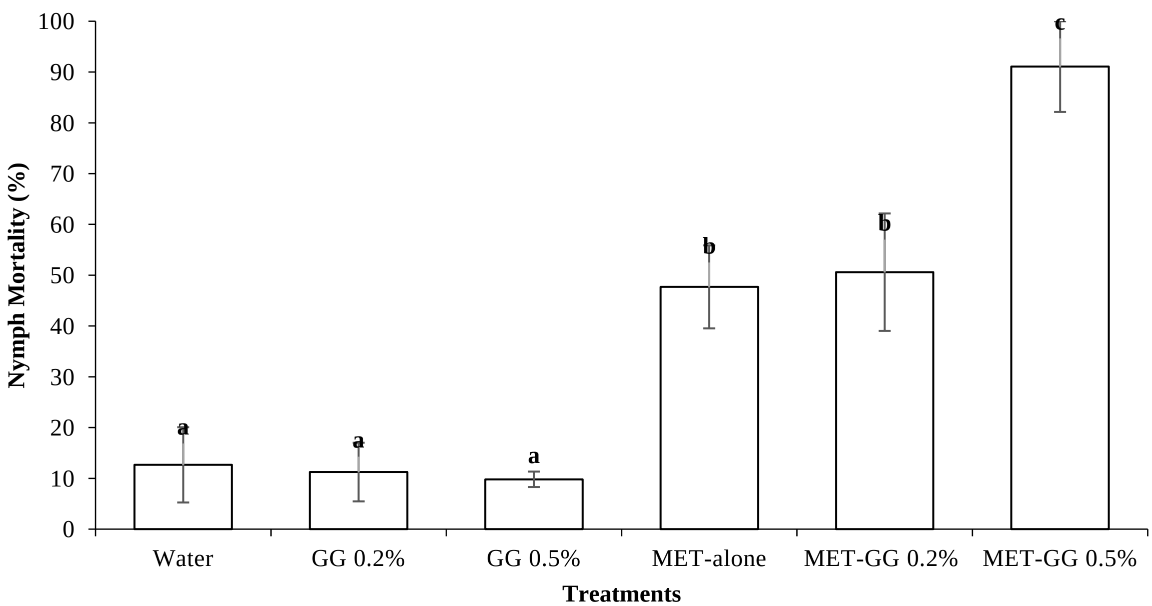

Fig. 4. Effects of GG as carrier matrix on the entomotoxic activity of MET against *Bemisia tabaci* nymphs. MET: *Metarhizium anisopliae*; GG: *G. ulmifolia* gum. Different letters indicate significant differences among treatments, bars on each column represent the standard error (Pairwise contrast Tukey, P≤0.05).

4. DISCUSSION

Natural gums are promising material with biotechnological applications. They are non-toxic, stable, easily available, and associated with less regulatory issues as compared to their synthetic counterpart (Avachat *et al*., 2011). In the present work, we evaluated the hydration properties of GG and its potential as carrier agent for an entomopathogenic fungus. The GG yield was relatively low (67.1 g/kg) in comparison to seed gum from other plant species, like *D. regia* (400 g/kg) and *O. basilicum* (200 g/kg) (Hosseini *et al.*, 2010; Pacheco-Aguirre *et al*., 2010). Nevertheless, the gum yield was similar to that of *S. japonica* seed (92.2-33.3 g/kg) and *L. leucocephala* seed (75.2 g/kg) (Cerqueira *et al.*, 2009; El Kader *et al*., 2008). The analytical data of GG shows specific characteristics. For example, the total carbohydrates were lower than that reported for *D. regia* seed gum (95.31 %) (Pacheco-Aguirre *et al.*, 2010), but slightly higher than that reported for *O. basilicum* seed gum (79.63 %) (Hosseini *et al.*, 2010) and *Acacia senegal* (L.) wild seed gum (84.10 %) (El-Kheir *et al*., 2008). In addition, the ash content was higher than values reported by commercial gums (El-Kheir *et al*., 2008; Elkhalifa & Hassan, 2010), which indicates that GG had a similar purity to that of non-traditional gums.

The increase of conductivity when increase GG concentration suggests the presence of ionic charges in its chemical structure (Nedjhioui *et al*., 2009). This condition was observed in a good W_{DIS} (79.6 %) of GG at room temperature, as the presence of ionic charges cause electrostatic repulsive interactions between themselves facilitating the formation of hydrogen bonds with water (Choi *et al*., 2003). This behavior has been also observed in GG indicating a thermal stability of their polymeric structure, which favors the potential application of GG as a carrier for biological control agents that must be protected from high temperatures (Basfar *et al*., 2003). In the case of carriers, the swelling ability has great influence on the improvement of dissolution rate of poorly water-soluble biocides (Viral *et al*., 2010). Likewise, the swelling ability is used also in the retardation of release of active substances from polymeric matrix (Kadajji & Betageri, 2011).

The viscosity is also an important characteristic of polymeric matrix to gain fundamental understanding of the processability of the material (Sadiku *et al*., 2011). In this case, GG had a non-Newtonian behavior indicating a disentanglement process and an increase of average end-to-end distance of polymer chains due to shearing, where the viscosity and shear stress increased with the increase of GG concentration, probably due to the formation of a polymeric network (Aalaie *et al*., 2007). The decrease in viscosity of both GG solutions when temperature increase indicates the thermo-dependent behavior of the solutions. Understanding the rheological behavior of polymeric melts is helpful in understanding the structure property in these materials for the formation of microbial agent carriers. We might think that the GG gum is favorable for delivering the biocide or decrease the biocide entrapment (Razavi *et al*., 2013).

Apart of the hydration properties of GG, the potential of this gum as carrier for microbial control agent MET conidia to enhance their entomotoxic activity was also evaluated. In this sense, we used two concentrations (0.2 % and 0.5 %) of GG in the conidial suspension of MET that were sprayed on *B. tabaci* nymphs under greenhouse conditions. The use of the GG matrix at 0.2 % did not enhance the activity of MET. The insecticidal activity of this treatment was not significantly different to that of MET-alone (lower than 50 %). This rate of mortality was similar to those observed in other works, where *M. anisopliae* was applied against *B. tabaci* nymphs. For example, Ruiz-Sánchez *et al*., (2011), Flores *et al*., (2012) and García *et al*., (2013) found that rates of mortality of *B. tabaci* nymphs ranged from 40 to 50 % using bare spores of *M. anisopliae*. The increase of GG from 0.2 % to 0.5 % increased significantly the nymphal mortality (e.g 91.1 ± 8.9 % against 50.6%). This effect could clearly be attributed to an increase in

polymer concentration as well as swelling properties of the gum, which could lead to better adhesion of MET to the cuticle of *B. tabaci* nymphs. We underlie the advantage of using GG as carrier agents of MET, since they are relatively cheap, non-toxic and eco-friendly due to their natural origin (Nazarzadeh Zare *et al*., 2019).
The purity of gum from *G. ulmifolia* seed was similar to non-traditional gums. The GG showed good water dispersion and offered thermal stability under high temperatures. The GG when added to the MET spore suspension improved its effectiveness against *B. tabaci* nymphs. The GG showed good capacity of interaction with water. The typical characteristic of GG includes ionic charge gum, high solubility and stable SI. The GG showed an adequate viscosity and thermal stability at low concentrations. When evaluated as carrier and protecting agent for the biological control fungus MET, there was an increase in mortality of *B. tabaci* nymphs when increasing the GG concentrations in the spore suspension, which suggests a major retention of MET in GG. More studies of chemical and structural composition of the gum should be carried out prior to use it as new source of polymer to improve the activity of entomopathogenic fungi.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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