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BRIEF REPORT

Potential use of *Apis mellifera* L. honey in the management of the cucurbit powdery mildew caused by *Podosphaera xanthii* (Castagne) under greenhouse conditions

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Abstract Powdery mildew by *Podosphaera xanthii* (Castagne) is a major disease of greenhouse cucurbitaceous crops worldwide. Honey by honeybees has been reported as an antimicrobial for diseases in humans, animals, and plants. The aim of this study was to assess *Apis mellifera* honey against *P. xanthii* in cucumber plants. During nine consecutive weeks, four different honey concentrations (2.0%, 2.5%, 3.0% and 3.5%), a chemical control (Azoxystrobin) and an untreated check (water) were evaluated. Except for honey at 2%, every concentration was significantly different from the untreated check. Honey concentrations at 3% and 3.5% were found to be the most effective, and their area under disease progress curve (AUDP) was statistically comparable to that of Azoxystrobin with 1048.3, 642.3 and 575.8 AUDP, representing 72.4%, 83.1% and 84.8% of efficiency compared to the untreated check, respectively. These results provide preliminary information on the potential use of honey in managing strategies of the disease under greenhouse conditions.

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PALABRAS CLAVE
Cultivos cucurbitáceos;
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Control biológico;
Programas de manejo integrado;
Condiciones de crecimiento controladas

El potencial de la miel de *Apis mellifera* L. para el manejo del mildiu polvoriento en cucurbitáceas causado por *Podosphaera xanthii* (Castagne) bajo condiciones de invernadero

Resumen El mildiu polvoriento causado por *Podosphaera xanthii* (Castagne) es una enfermedad importante en invernaderos de cucurbitáceas en todo el mundo. La miel de las abejas melíferas ha sido reportada como un antimicrobiano contra enfermedades de humanos, animales y plantas. El objetivo de este estudio fue evaluar el desempeño de la miel de *Apis mellifera* frente a *P. xanthii* en plantas de pepino. Durante 9 semanas consecutivas se evaluó el impacto de 4 concentraciones de miel (2%; 2,5%; 3% y 3,5%) comparado con el de un agente químico (zoxistrobina) y uno absoluto (agua). Exceptuando la miel al 2%, cada concentración fue significativamente diferente del control absoluto. Mieles al 3% y 3,5% fueron las más efectivas y el área bajo la curva del progreso de la enfermedad (ABCPE) fue de similar significancia estadística a la observada con azoxistrobina (valores de ABCPE: miel al 3%, 1.048,3; miel al 3,5%, 642,3; azoxistrobina, 575,8), lo que representa el 72,4%; el 83,1% y el 84,8% de eficiencia, respectivamente, en comparación con el testigo absoluto.

Powdery mildew caused by the fungus *Podosphaera xanthii* (Castagne) is one of the most relevant foliar diseases of Cucurbitaceae grown under greenhouse conditions worldwide, including cucumber, melon and squash[13,15]. The use of synthetic fungicides is currently the main practice to manage the disease; however, there are reports of a widespread and rapid resistance development in the fungus to these products[12]. Therefore, alternative strategies for the management of this pathogen are required.

The use of natural substances to reduce the disease severity has been reported[6]; however, most efforts have focused on bioproducts derived from plants, algae, and microorganism[10,15]. Honey from honeybees is a natural antimicrobial of human, animal[1] and plant pathogens, including fungus; nevertheless, a very limited number of reports have been published for pathogenic microorganisms associated with vegetables and these have focused on in vitro conditions[2]. So, the aim of this study is to evaluate the use of honey produced by the western honeybee *Apis mellifera* L. for cucurbit powdery mildew caused by *P. xanthii* under greenhouse conditions.

The current study consisted of two experiments carried out independently and simultaneously in different cucumber (*Cucumis sativus* L.) greenhouses at the Experimental Station of the Faculty of Agronomy of the Autonomous University of Sinaloa (Culiacan, Sinaloa, Mexico; 24°37′26″ N, 107°26′31″ W) during November 2020 to April 2021. Two young plants with two true leaves were transplanted into pots (105 cm length, 15 cm height and 16 cm width) containing 7 kg of dried coconut stem (30% dust and 70% fiber) (Tracomex®). Plants were artificially inoculated with *P. xanthii* by gently dropping conidia from infected cucumber leaves. Then, to increase the humidity level, the inoculated plants were covered with transparent polyethylene for 24 h.

The honey used was obtained from a local *A. mellifera* colony and four different concentrations were evaluated (2.0%, 2.5%, 3.0% and 3.5% v/v with tap water). The commercial fungicide Amistar® (Syngenta) (Azoxystrobin 2 g/l) was used as chemical control, while the plant treated with water was used as untreated check. A 2 l hand sprayer (Truper®) was used for the treatment applications, which consisted in covering the entire surface of the leaves. The applications started 24 h after the inoculation and continued weekly until the leaf surface of the untreated plants was almost completely diseased (covered by powdery mildew), totaling nine evaluations.

The disease severity was recorded weekly before each application using half of the total leaves chosen randomly and it was determined based on the percentage of leaf area covered by *P. xanthii* following the subsequent visual scale of Horsfall and Barrett[8] with modifications: 1 = 0–10% infected leaf surface (ils); 2 = 11–20% ils; 3 = 21–30% ils; 4 = 31–40% ils; 5 = 41–50% ils; 6 = 51–60% ils; 7 = 61–70% ils; 8 = 71–80% ils; 9 = 81–90% ils; 10 = 91–100% ils. The severity of the powdery mildew was assessed, using the following formula[4]: disease severity index (DSI) (%) = [Σ (class frequency × score of rating class)/(total number of observations) × (maximal disease index)] × 100. On the other hand, the mean area under the disease progress curve (AUDPC) was calculated using the formula proposed by Pandy et al.[11], AUDPC = D [1/2(Y1 + Yk) + (Y2 + Y3 + . . . + Yk − 1)], where D = time interval; Y1 = first DSI; Yk = last DSI; and Y2, Y3, Yk − 1 = intermediate DSI. Finally, treatment efficacy (TE) was calculated after the last evaluation using the AUDPC values according to the following formula reported by Elagamey et al.[6], TE (%) = [(Control − treatment)/Control] × 100.

Each experiment was conducted as a completely randomized design with ten plants per treatment considering each plant as a technical replicate. A combination of data from both experiments was performed since no significant difference was observed among assays ($p \leq 0.05$). The combined data (20 plants) were subjected to an analysis of variance and comparison of means using Tukey's test ($p \leq 0.05$) with SPSS version 26 software.

Figure 1 Infected cucumber leaves with powdery mildew (*Podosphaera xanthii*), untreated check (left) and honey at 3.5% (right) after nine weekly sprays.

The results in Table 1 showed that honeys at 3.0 and 3.5% were statically similar to the chemical control based on AUDPC values, with 1048.3, 642.3, 575.8, respectively, representing 72.4, 83.1 and 84.8% of efficiency compared to the untreated check (Fig. 1). Honey at 2.5% had an intermediate level compared to the chemical control and untreated check with 1653.8 AUDPC and 56.5% efficiency. Only honey at 2.0% was similar to the untreated check with 3540.3 and 3799.3 AUDPC, respectively, which represents only 6% of efficiency. These results indicate that honey had an antibiotic effect on *P. xanthii* that changes at different concentrations. Honey is mainly composed of carbohydrates (60–85%) and water (12–23%) plus other compounds in small amounts, such as organic acids, minerals, vitamins, enzymes, proteins, amino acids, phenols, and flavonoids[3]. The osmotic pressure produced by these sugar concentrations, along with the activity of the phenols and flavonoids, are reported as the key antimicrobial factors[5,9]; therefore, the increase of honey results in a higher concentration of these compounds.

In the current study, the healthy plants were weekly sprayed, starting 24 h post inoculation with *P. xanthii*. According to this methodology, on the treated plants, the fungus did not complete the infection process before the first application; therefore, the applications can be considered preventive. On the other hand, there are different factors that might influence the antimicrobial efficacy of honey, such as botanical origin, honey immaturity, high nectar flux and artificial feeding of bees[7], all of which should also be investigated. Additionally, the bee species can also have a major share in the honey antibiotic ability[1]. Therefore, further studies are yet needed to support the use of honey into crop management strategies. To the best of our knowledge, this is the first *in vivo* study designed to evaluate honey as a fungicide of plant pathogens.

Additionally, the use of honey has been investigated as a plant growth promoter with interesting results in different crops such as tomato (*Solanum lycopersicum* L.)[14], resulting in the improvement of multiple plants traits such as foliar area, height, and stem width.

In conclusion, this study indicates that *A. mellifera* honey applications can significantly reduce powdery mildew in cucurbits under greenhouse conditions, contributing to the reduction of chemical fungicides in disease management.

Table 1 Effect of the treatments on the powdery mildew (*Podosphaera xanthii*) disease severity index (DSI (%) ± SD), the area under disease progress curve (AUDPC) and efficiency (E%).

Treatment	DSI (%) Weeks after inoculation									AUDPC	E%
	1	2	3	4	5	6	7	8	9		
Untreated check	34.9 ± 17.3 a	43.7 ± 18.8 a	61.3 ± 25.7 a	68.9 ± 22.6 a	72.7 ± 22.9 a	79.6 ± 10.5 a	79.6 ± 10.4 a	82.1 ± 25.0 a	95.5 ± 5.1 a	3799.3 a	–
Chemical control	7.2 ± 2.5 b	8.2 ± 2.1 b	15.7 ± 13.2 c	9.1 ± 5.7 b	27.3 ± 19.6 b	4.5 ± 0.0 c	4.5 ± 0.0 d	4.8 ± 2.68 d	14.7 ± 5.2 b	575.8 c	84.8
Honey 2.0%	22.3 ± 8.5 ab	47.5 ± 24.6 a	39.9 ± 22.0 b	79.6 ± 10.4 a	51.3 ± 28.6 ab	79.6 ± 10.4 a	77.7 ± 8.5 a	37.7 ± 23.3 b	91.8 ± 6.1 a	3540.3 a	6.8
Honey 2.5%	10.9 ± 4.3 b	8.1 ± 4.0 b	9.5 ± 8.3 c	10 ± 5.1 b	36.8 ± 27.9 b	44.0 ± 24.9 b	44.0 ± 24.9 b	17.6 ± 12.68 c	26.1 ± 10.1 b	1653.8 b	56.5
Honey 3.0%	10.9 ± 4.5 b	8.1 ± 2.0 b	13.8 ± 6.4 c	8.2 ± 6.1 b	39.9 ± 22.0 b	4.5 ± 0.0 c	16.1 ± 5.9 c	23.3 ± 14.2 c	22.3 ± 8.5 b	1048.3 bc	72.4
Honey 3.5%	7.2 ± 2.5 b	9.1 ± 5.7 b	8.2 ± 6.1 c	9.4 ± 6.0 b	24.2 ± 12.75 b	7.3 ± 6.3 c	7.3 ± 6.26 d	16.7 ± 25.9 c	29.9 ± 10.4 b	642.3 c	83.1

Azoxystrobin 2 g/l (Amistar®, Syngenta) was used as chemical control. Means with different letters in the columns indicate significant differences according to Tukey's means test ($p \leq 0.05$).

CRediT authorship contribution statement

Conceptualization: JACD, MALB and NLL. Investigation and methodology: JACD, JAEU, NLL, VDP and MALB. Data curation and formal analysis: JACD, VDP, CALO and JAEU. Writing – original draft: JACD, WARA and JAEU. Writing – review & editing: JACD, CALO, WARA and JAEU.

Consent of publication

All authors declare consent of publication.

Ethical approval and consent to participate

All authors agree to participate in this research article.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

Data and materials used during the current study are available from the corresponding author on reasonable request.

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