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Technical note

Effectiveness of the smoke of fruits of *Guazuma ulmifolia* (Sterculiaceae) and vapors of Thymol for control of *Varroa destructor* infesting Africanized bees

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Abstract:

The mite *Varroa destructor* is a scourge in honey bee colonies worldwide. Conventional chemical-based control treatments can contaminate colony products and cause resistance in the parasite. Plant-source compounds are promising alternatives. The effectiveness of smoke from dried *Guazuma ulmifolia* fruit and vapors from thymol crystals was evaluated in control of *V. destructor* in colonies of Africanized bees (*Apis mellifera*) in Yucatan, Mexico. Three treatments were used during a three-week experimental period. In Group 1, colonies were administered five to eight puffs of smoke from dried *G. ulmifolia* fruits twice a week. In Group 2, they were administered 4-8 g of thymol crystals once a week. Group 3 was a control and received no treatment. Collections of 200 to 300 adult bees from each colony were done prior to treatment (day 0) and after treatment at 7, 14 and 21 d. These were processed to quantify colony infestation levels and treatment efficacy. Overall *V. destructor* infestation levels in adult bees decreased in all three groups after 21 d, with differences between treatments. Levels were lowest in Group 2, followed by Group 1 and the control. Efficacy at the end of the treatments was 41 % in Group 1 and 69% in Group 2. Compared to the control, application of thymol crystals provided the most effective alternative control method against

V. destructor. However, regular application of *G. ulmifolia* fruit smoke also reduced mite infestation levels, and this resource has the advantage of being locally available.

Key words: *Guazuma ulmifolia*, Thymol, Alternative control, *Varroa destructor*, Africanized bees, *Apis mellifera*, Yucatan.

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The *Varroa destructor* mite remains one of the principal health problems in beekeeping worldwide[1]. A serious threat, it negatively impacts the development, survival and productivity of *Apis mellifera* colonies intended for honey production[2,3] and crop pollination[4]. An ectoparasite affecting bee pupae and adults, *V. destructor* causes a reduction in the body weight of workers at emergence and shortens their lifespan[5]. Bee colonies with intense *V. destructor* infestations can also suffer from increases in viral diseases, mainly deformed wing virus. Transmitted by female *V. destructor* while feeding on bee pupae and adults, it causes declines in population and honey production in infested colonies; when the mite population grows exponentially the bee colony dies[2,6]. In Europe and the United States of America, *V. destructor* continues to destroy managed colonies and is considered to be one of the factors associated with bee colony collapse and mass mortality, a phenomenon known as colony collapse disorder[2,7]. Widespread loss of colonies is negatively impacting honey bee pollination services in various agricultural crops[8]. This same phenomenon occurs in Mexico. When *V. destructor*-infested colonies are not treated, infestation levels quickly increase, reducing honey production[9], and, in conjunction with other diseases, can cause colony collapse and mortality[10].

In an effort to control or eliminate *V. destructor* from honey bee colonies, beekeepers resort to different control methods, including application of approved and prepared pyrethroid-based chemicals[3]. However, some also use products such as homemade powders, ointments and wooden strips, which are unauthorized for use in bees and often include acaricides such as amitraz, bromopropylate and coumaphos. These can contaminate honey and other products from bee colonies[11], thus risking their rejection on the international market.

In response to this challenge, natural mite control alternatives are being developed. To date various products of plant origin have been tested, such as thymol obtained from *Thymus vulgaris* (Lamiaceae)[12,13], menthol from *Mentha arvensis* and *Mentha piperita* (Lamiaceae)[14,15], as well as formic acid and oxalic acid[12,16]. These have the advantages of acceptable efficacy in the presence of larva and pupae, easy application, lower risk of

contaminating honey, wax, pollen and other bee colony products, as well as a reduced likelihood of mites developing resistance to them[3], as occurs with commercial acaricides containing mainly pyrethroids[17].

Beekeepers in rural communities of the state of Yucatan, Mexico, have reported the use of various plant-based products to control *V. destructor* infestations with acceptable results in some regions of the state. Recent data provided by rural beekeepers to the Yucatan State Ministry of Rural Development (Secretaria de Desarrollo Rural del Gobierno del Estado de Yucatán; SEDER-Yucatan) indicate that they have been controlling parasitosis in bee colonies by using the dried fruit of the West Indian elm tree *Guazuma ulmifolia* (*pixoy* in Mayan language; Sterculiaceae) as fuel in the bee smoker. Application of the smoke of this fruit has been reported to be sufficient to control *V. destructor* infestations in bee colonies without the use of other commercial products or control methods. They report that the dry fruit must be collected directly from the tree, that the smoke does not irritate the bees or beekeeper, leaves no scent in honeycombs and does not affect queen bee egg production. However, this information has not been verified under controlled conditions with experimental colonies following research protocols. This is needed to confirm the reported results and, if effective, develop application methods that would allow its use as an alternative mite control technique. The present study objective was to assess the efficacy of dried *G. ulmifolia* fruit when used as fuel in bee smokers as an alternative for controlling the mite *V. destructor* in colonies of Africanized bees (*Apis mellifera*) under conditions simulating those prevalent in rural apiaries in Yucatan, and compare its performance to that of thymol crystals from the *T. vulgaris* plant, also widely used as an alternative mite control measure.

The study was done in the experimental apiary of the Faculty of Veterinary Medicine and Zootechny of the Autonomous University of Yucatan (FMVZ- UADY), where bee colonies are managed following practices similar to those in the state's honey producing regions. The installations are located in Xmatkuil, Yucatan, 15.5 km south of the city of Merida, Yucatan (20°52' N; 89°36' W). Climate in the area is warm sub-humid with summer rains (Aw0). Average annual rainfall is 985 mm, average annual temperature is 26.8°C and average annual relative humidity is 78 %[18]. The most important floral resources in this region in terms of nectar and pollen for bee colonies are goldeneye or *tajonal* (*Viguiera dentata*), which blooms from January to February, and bastard logwood or *ts'iits'ilche'* (*Gymnopodium floribundum*), which blooms from February to May[19]. Under these conditions, bee colonies normally have brood throughout the year, with peaks between February and May[20].

The colonies in the experimental apiary are kept in Langstroth-type hives. For the present study the colonies were housed in a single box (brood chamber only) or two boxes (brood chamber and one honey super), distributed similarly among treatments. All the colonies had naturally mated Africanized queens, were heavily populated with adult bees occupying at least eight of the ten honeycombs present in the brood chamber, and contained a similar

number of honeycombs containing brood in different developmental stages (eggs, larvae and pupae), honey and pollen. They were also naturally infested with the *V. destructor* mite, with no treatment or control methods applied for at least six years prior to data collection.

Before implementing the treatments, a preliminary diagnosis was made of each hive to measure *V. destructor* infestation levels in adult bees. This was done to ensure that the experimental groups had similar infestation levels at the beginning of the evaluations.

Evaluation of smoke from *G. ulmifolia* fruit and thymol crystals as natural alternative products for control of *V. destructor* was done over a three-week period. The hives were divided into three experimental groups.

Group 1 (G1): This group consisted of twelve colonies (ten colonies with a brood chamber and one honey super, and two with only a brood chamber). These colonies were administered smoke from the burning of dried *G. ulmifolia* fruit. Approximately 220 g of dried *G. ulmifolia* fruit were placed in a bee smoker, and the smoke applied at the colony entrance and the hives opened to apply smoke between the combs of the brood chamber and honey super (in the case of double colonies). Five to eight puffs were applied to each colony twice a week over the three-week experimental period, the number of puffs varying depending on bee defensive response and hive size. The hives were then closed. This application procedure is similar to that used in routine examinations of colonies.

Group 2 (G2): This group consisted of ten colonies (nine colonies with a brood chamber and honey super, and one colony with only a brood chamber). Thymol crystals (96.8% purity) were placed in each hive at seven-day intervals[16]. In the brood chamber hives only 4 g crystal were used, while in the double hives 8 g were used. For application, the crystals were placed in disposable plastic lids (250 ml) covered with a wire mesh to prevent the bees from removing the crystals from the colony, which would reduce effectiveness. The lids with the crystals were inserted into the hive entrance using a piece of wire, which allowed for easy insertion and removal.

Group 3 (G3): Containing twelve colonies (ten double hives and two with just a brood chamber), this group was a control, receiving no anti-mite treatment during the experimental period.

Collections of adult bees (200-300 bees per collection) were taken from each colony to quantify the effectiveness of the *G. ulmifolia* fruit smoke (G1) and thymol crystals (G2) treatments. Collection was done prior to treatment (day 0) and after application of each treatment at 7, 14 and 21 d. Samples of the adult bees and the mites infesting them were stored in vials containing 80% alcohol, and marked with the collection date, colony number and treatment group.

In the laboratory, the bee samples were placed in plastic containers and 250 ml 80% ethyl alcohol added until the bees were completely covered. These were then mechanically agitated at 180 rpm for 30 min and the alcohol filtered through white gauze to collect any mites. All the mites collected from each adult bee samplewere counted. This methodology successfully removes all mites from the bee body, allowing quantification of infestation level (%) and that for all adult bees (% IAB) in each group, using the formula[21]:

% IAB (No. mites / No. bees) x 100

At the end of the experimental period (21 d), efficacy of the *G. ulmifolia* fruit smoke (G1) and thymol crystals (G2) treatments was calculated based on mite infestation levels in adult bees using the formula[22]:

E = 1- (A x D / B x C) x 100

Where: E= treatment efficacy; A= mite infestation level in control group (G3) before treatment application (d 0); B= mite infestation level in control group (G3) after treatment completion (d 21); C= mite infestation level in treatment group (G1 or G2) before application (d 0); D= mite infestation level in treatment group (G1 or G2) after each treatment (d 7, 14 and 21).

Post-treatment *V. destructor* infestation levels in all three groups were compared with a one-way ANOVA and Tukey multiple comparison test (95% confidence level). Analyses were done with the Statgraphics Centurion ver. XV program[23], and those results expressed as percentages (% infestation) were arcsine transformed (angular transformation)[24].

Before the treatments were begun (d 0), *V. destructor* infestation in adult bees did not differ between the groups (F= 0.00; g.l. 2,31; *P*=0.99): 13.5 ±5.8 % for G1, 13.3 ±3.2 % for G2 and 13.4 ±3.9 % for G3 (Table 1). This indicates that infestation level distribution was similar in the experimental colonies of the three groups.

Table 1: *Varroa destructor* infestation levels in adult bees (%) in the three experimental groups at d 0, and after treatment application on d 7, 14 and 21

	Varroa destructor infestation levels in adult bees (%)		
	Group 1 (*G. ulmifolia*)	Group 2 (*T. vulgaris*)	Group 3 (Control)
Day 0	13.5 ± 5.8 [a]	13.3 ± 3.2 [a]	13.4 ± 3.9 [a]
Day 7	8.8 ± 2.8 [a]	10.9 ± 3.8 [a]	12.7 ± 5.5 [a]
Day 14	7.7 ± 2.9 [a, b]	6.4 ± 3.3 [a]	12.0 ± 6.3 [b]
Day 21	5.2 ± 2.4 [a]	2.8 ± 1.4 [b]	8.8 ± 3.8 [c]

[a,b] Different letter superscripts in the same row indicate significant difference (P<0.05).

Seven days after the first application of *G. ulmifolia* fruit smoke (G1) infestation levels had dropped to 8.8 %, while in the thymol crystals treatment (G2) they had dropped to 10.9 %, and in the control (G3) to 12.7%. No significant differences occurred between the three treatments at this time (F= 2.57; g.l. 2,31; *P*=0.09). However, after the final application at 21 d infestation levels in G1 had decreased to 5.2 %, those in G2 to 2.8 % and in G3 to 8.8 % (Table 1). The three groups differed from each other (F = 13.73; g.l. 2,31; *P*=0.0001), with G2 exhibiting the greatest reduction, followed by G1 and G3.

Efficacy during the first week was 32 % in G1 and 14 % in G2, but in the second week had increased to 36 % in G1 and 47 % in G2 (Table 2). Total efficacy after the three applications (21 d) of each treatment was 41 % in G1 and 69 % in G2. Compared to Group 3 (Control), Group 1, treated with *G. ulmifolia* fruit smoke, exhibited a significant reduction in infestation levels at the end of the 21-d experimental period, with 41 % efficacy. However, Group 2, treated with thymol crystals, experienced an even greater reduction in infestation (lower than in Groups 1 and 3), with an overall efficacy of 69 %.

Table 2: Estimated efficacy (%) of application of *G. ulmifolia* fruit smoke and thymol (*T. vulgaris*) crystals in control of *V. destructor* infestation after application of each treatment

	Treatment efficacy (%)		
Treatment	Day 7	Day 14	Day 21 (final)
G. ulmifolia (G1)	32	36	41
T. vulgaris (G2)	14	47	69

Results for Group 1 suggest that frequent application of *G. ulmifolia* fruit smoke may contribute to lowering *V. destructor* infestation levels in honey bee colonies when routinely used as fuel to generate smoke during colony management. This treatment's average efficacy (41 %) exceeded that of other commercial organic products such as Hive-Clean® (made with organic acids (formic, citrus and oxalic), propolis extract, essential oils and sugar syrup). When tested under tropical conditions and with Africanized bees[25], Hive-Clean® has exhibited relatively low efficacy (16.7 %) compared to its rather high efficacy (91.6 %) in a temperate climate (Poland) and with European bees[26]. In addition, application of *G. ulmifolia* fruit smoke had no apparent negative effect on mortality in adult bees or offspring, nor did it repel adult bees.

Use of thymol crystals in Group 2 resulted in 69 % efficacy after 21 d, the most effective among the three test groups. This is similar to a previous study of the efficacy of thymol, thymol in gel[13], and formic acid crystals[16], in which the essential oil was found to be an effective alternative method for control of *V. destructor* in Africanized bee (*Apis mellifera*) colonies in the environmental conditions of Yucatan.

Group 3, the control, also exhibited a reduction in *V. destructor* infestation levels (13.4 to 8.8 %) over the experimental period, although much less than those in the G1 and G2 treatments. Natural decreases in *V. destructor* infestation levels in adult bees in the absence of control measures may result from a population dynamic of mites in bee colonies known as the parasite "dilution effect". In this phenomenon bee population size increases when the availability of food is grater during flowering seasons, thus increasing the number of bees in the colony, diluting the mite population amid a greater number of individuals in the colony and lowering the infestation level in adult bees[27].

Controlling *V. destructor* mites in honey bee colonies using plant-origin compounds is preferable to application of conventional pyrethroid-based acaricides or the use of home-made wooden strips and ointments incorporating chemicals such as coumaphos since the latter can leave residues in the honey[28] and may generate resistance in mites[29].

Essential oils extracted from different plants have been evaluated as potential insecticides for control of certain parasites[30]. The present study is the first report of use of *G. ulmifolia* fruit smoke as a mite control method in honey bee colonies, although alcohol extracts of *G. ulmifolia* leaves are known to be toxic to *Aedes aegypti* mosquito larvae, causing 35% mortality[31]. Phytochemical compounds in *G. ulmifolia* have also been reported to have potential activity in the control of various insects and mites affecting domestic turkeys (*Meleagris gallopavo*)[32]. The present results apparently support first-hand accounts from beekeepers in Yucatan that continual use of dried *G. ulmifolia* fruit as fuel in bee smokers provides sufficient control of *V. destructor* in colonies of Africanized bees. Indeed, they claim they use no other method to control this parasite.

When used during routine management of Africanized honey bee colonies in the state of Yucatan, Mexico, the smoke of dried *G. ulmifolia* fruit proved an effective alternative method for control of the mite *V. destructor*. Thymol crystals were even more effective at controlling this parasite. However, *G. ulmifolia* fruits have the advantages of being readily available in the study region, and the smoke from them does not irritate bees or beekeepers, leaves no scent in honeycombs and has no effect on queen bee egg production.

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