Anales de Biología 48: 19-25, 2026
http://dx.doi.org/10.6018/analesbio.48.03
ISSN/eISSN 1138-3399/1989-2128

ARTICLE

Alternative material for monitoring varroa mites' infestation in honeybees' colonies

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Received: 25 June 2025
Accepted: 25 May 2026
Published on-line: 31 July 2026

Resumen

Material alternativo para el seguimiento de la infestación por varroa en colmenas de abejas melíferas

La varroa (Mesostigmata: Varroidae) es ectoparásita de las abejas. En diferentes regiones se emplean diferentes enfoques para su vigilancia. El estudio se centró en materiales alternativos para monitorear la infestación de varroa en colonias de abejas. Se comparó la eficacia del método de espolvoreado con cenizas de madera con métodos de lavado con etanol al 70% y espolvoreado con azúcar glas. El orden de eficacia de los tres métodos fue: etanol al 70% (38%) > cenizas de madera (33%) > azúcar glas (29%). No se observaron diferencias significativas en la población media de ácaros recuperados mediante los tres métodos. Si bien el etanol al 70% muestra un mejor porcentaje (38%), el método de las cenizas de madera (33%) podría ser una alternativa a los métodos de lavado con etanol al 70% o azúcar glas (29%).

Palabras clave: Material alternativo; Ceniza de madera; Etanol 70%; Azúcar glas; Varroa; Abeja.

Abstract

Varroa mites (Mesostigmata: Varroidae) are ectoparasites of honeybees. Different approaches are engaged in varroa mites' surveillance in different regions. The study focused on alternative material for monitoring varroa mites' infestation in honeybees' colonies. The efficacy of wood ashes dusting method was compared with 70% ethanol wash and icing sugar dusting methods. The order of efficacies of recovery of varroa from the external bodies of honeybees by the three methods is 70% ethanol (38%) > wood ashes (33%) > icing sugar (29%). There was no significant difference in the mean population of varroa mites recovered by the three methods. Although from this study, 70% ethanol shows better percentage (38%), but wood ashes method (33%) could be an alternative to either 70% ethanol wash or icing sugar methods (29%).

Key words: Alternative material; Wood ashes; 70% ethanol; Icing sugar; Varroa mites; Honeybees.

Ashes are white powdery byproducts of burned dry wood which are likely to contain good sources of potassium, calcium and other necessary nutrients. The ashes exhibited bio-insecticidal properties due to their ability to dehydrate insects and damage the exoskeleton (Wahedi *et al.* 2017). Although the efficacy was reported to depend on the concentration and environmental conditions (Miha *et al.* 2025). Despite this, the wood ashes are suspected to be useful for monitoring and surveillance of varroa infestation of honeybee colonies. Varroa mites (Mesostigmata: Varroidae) are ectoparasites of honeybees (Hymenoptera: Apidae) such as *Apis cerana* Fabricius 1793, *Apis mellifera* L. 1758, *Apis dorsata* Fabricius 1793, *Apis florea* Fabricius 1787 and *Apis andreniformis* Smith, 1858. The feeding behavior of varroa mites weakens the honeybees and eventually destroys honeybees' colonies overtime. Varroa mites attach to the external body of individual honeybees for survival by feeding on the fat tissue of the host (Ramsey *et al.* 2019, Han *et al.* 2024) . This causes disease called varroasis which tends to reduce the honeybees' population and affect the productivity of managed honeybees' colonies. This challenge impacts negatively on beekeepers' investment. High and significant parasitic load of varroa mites' infestation were reported to lead to the death of individual honeybees probably resulting to colony loss as observed globally (Roth *et al.* 2020). Huge losses of colonies of honeybees have impacted greatly on the economy of honey producing countries, beekeepers and allied businesses. Colony losses due to varroa mites have impacted pollination services, honey related industries, and honey bee conservation negatively.

There are different sampling methods for monitoring varroa mites' infestation in honeybees' colonies. These include ether roll method, icing sugar wash method, 70% ethanol wash, soap washing methods, sticky-boards and stick roll (De Jong *et al.* 1982, Calderone 1999, Ostiguy and Sammataro 2000, Dukku *et al.* 2020, Ontario Ministry of Agriculture, Food and Rural Affairs 2021). For better monitoring and effective control of varroa mites' infestation, beekeepers estimate varroa mites' density to determine level of infestation periodically in their colonies by random sampling and dislodging of mites from approximately 300 adult honeybees taken from each brood nest frames in the colonies (Lee *et al.* 2010). This was done by either using 70% ethanol wash, icing sugar wash, ether roll and stick roll to determine the population density of varroa mites' level in each colony in their apiaries (Branco *et al.* 2006, Lee *et al.* 2010). This is to enable beekeepers to take timely inform decisions on control measures and mitigations against varroa mites' infestation of the colonies to prevent colony loss (Guzman *et al.* 2010).

In Nigeria, despite reported cases of varroa mites' infestation of honeybees' colonies (Akinwande *et al.* 2013, Dukku & Vincent 2017), most beekeepers probably lack access to the materials needed for dislodging varroa mites from the external bodies of honeybees sampled from honeybees' colonies in their apiaries. In addition, the cost implication of assessing any of these materials (icing sugar wash, 70% ethanol wash, ether roll, sticky-boards and stick roll) is unbearable for the beekeepers to determine varroa loads in their colonies to take informed decisions with respect to its control measures. Hence, the study on alternative material for monitoring varroa mites' infestation loads in honeybees' colonies using wood ashes.

Materials and Methods

Study sites

The study was conducted between December 2017 and February 2018 at the Honeybees' Research Farm Centre, Department of Animal and Environmental Biology, Faculty of Basic and Applied Sciences, College of Science, Engineering and Technology, Osun State University, Osogbo Main Campus, Osun State, Nigeria. Before honeybees' samples collection, the population of honeybees in each of the five colonies (7º45'45''N; 4º 36'11''E) was estimated using the methods of Farrar (1937) and Fasasi (2008). Each colony population was within the range of 32,255 and 45,682 honeybees [with 22 top bars per Kenya Top-bar Hive (KTBH)] with the brood nest mostly located at the Centre of the KTBHs. Adult worker honeybees were sampled from each of the five colonies (Kenya Top bar hives - KTBH) in the Honeybee Research Farm centre.

Collection of honeybees' samples

Honeybees sampling was done from the brood nests every two weeks for six weeks between December 2017 and February 2018 at about

16.00– 18.00 (to prevent drifting or swarming of honeybees after colony disturbance). Above three hundred adult worker honeybees were collected from the honeybee brood combs into a 450 ml screw-cap vials. Three replicates of samples (>300 adult worker honeybees per sample) were taken each from the five colonies of honeybees per sampling session throughout the study period (Dietemann *et al.* 2013).

Varroa mites' dislodgement Methods

Tested method: Wood ashes of 230µm size dusting method

The whitish powdery ashes produced after dried logs of wood are ignited and cooled and referred to as wood ashes (Ofuya 1986, Oguntade & Adekunle 2010, Jaganathan *et al.*, 2018). The wood ashes are collected from ignition points (local indigenous kitchens where logs of wood are used regularly) in local communities where fire-woods are the only source of energy for cooking. These powdery ashes were collected and stored in airtight glass jar (2L) at room temperature for the laboratory bioassay. Whenever the ashes absorbed moisture, it is oven dried to eliminate the moisture content by 100% for use and reuse.

To each of the honeybees' samples in the 450 ml screw-cap vials, 100 g of powdery wood ashes was added. The honeybees and the ashes in the 450 ml screw-cap vial were mixed thoroughly to coat all the external bodies of the honeybees including rolling side-ways horizontally and vigorously between 5-10 minutes to dislodge the varroa mites from the bodies of the adult worker honeybees. Thereafter, the 450 ml screw-cap vial was turned upside-down and the powdered wood ashes from the vial were discharged onto plain white paper cardboard (30cmx30cm). The shaking was repeated for another 5 minutes to ensure all mites were dislodged from the bodies of the honeybees. Collected varroa mites were rinsed with water to remove the ashes and placed on absorbent paper to dry up the water on them. Thereafter, the varroa mites were counted and recorded accordingly. As a final check, all the adult worker honeybees were examined individually for any remaining varroa mites.

70 % ethanol wash method

To each of the honeybees' samples (300) in the 450 ml screw-cap vial, 100 ml of 70% ethanol was added to cover all the honeybees. The 450 ml screw-cap vial containing the honeybees' samples was vigorously shook for 5-10 minutes to dislodge the varroa mites from the external bodies of the adult worker honeybees. Thereafter, the mixture of the dead honeybees, varroa mites and the 70% ethanol were poured into 2 litres plastic mesh retaining the honeybees but allowing the passage of varroa mites and 70% ethanol which in turn pass through a 2 litres plastic sieve made with plain white cloth retaining the varroa mites but allowed the passage of 70% ethanol only into a receiving container for re-use. The process was repeated to ensure all mites are dislodged and the varroa mites were counted and recorded according to the method of De Jong *et al.* (1982), Fries *et al.* (1991) and Lee *et al.* (2010). As a final check all the worker honeybees were examined individually for any remaining varroa mites.

Icing sugar dusting method

One hundred grams (100 g) of powdered icing sugar were added to each of the honeybees' samples (300) in the 450 ml screw-cap vials. The honeybees and powdered icing sugar in the 450 ml screw-cap vial were mixed thoroughly to coat all the external bodies of the adult honeybees including rolling side-ways horizontally and vigorously for 5-10 minutes to dislodge the varroa mites from the bodies of the adult worker honeybees. Thereafter, the 450 ml screw-cap vial was turned upside-down and the powdered icing sugar from the screw cap vial shook onto plain white paper cardboard (30cmx30cm). The shaking was repeated for another 5 minutes to ensure all mites were dislodged from the honeybees based on the method of Macedo *et al.* (2002). As a final check all the adult worker honeybees were examined individually for any remaining varroa mites. Collected varroa mites were rinsed with water to remove the icing sugar and placed on absorbent paper to dry up the water on them. Thereafter, the varroa mites were counted and recorded accordingly.

Calculation of varroa mites' infestation level of adult worker honeybees

The number of worker bees in each sample were counted and the infestation level calculated with the following formula (Dietemman *et al.*, 2013):

$$ILBV = (VPN1 + BNS)/100$$

where:

ILBV: Infestation level of bees with varroa mites.

VPN1: Varroa parasites number found in a sample after first wash.

BNS: Adult worker honeybees' number in a sample.

Efficiency of the extraction of varroa mites from adult worker honeybees

The efficiency of the extraction method was assessed as follows (Dietemann *et al.* 2013):

EM= EVM/(VPN1+ EVM)

VPN1: Varroa parasites number found in a sample after first wash.

EVM: Additional or extra washes (with same solvent) until no more mites are found.

VPN1 + EVM: Total number of mites in the sample after first and repeated wash

Statistical analysis

Data collected from all the three methods were compared and subjected to one-way Anova using excel sheet.

Results

Table 1 shows the mean±SD of the numbers of sampled honeybees and varroa mites recovered using the three comparative methods of extracting varroa mites from the external bodies of adult worker honeybees from this study. Mean±SD of honeybees' samples for each of the three methods ranged between 317±0.56 and 364.33±1.96 for each colony, while numbers of varroa mites per 100 honeybees according to each varroa mites' monitoring method, were 91 (wood ashes dusting), 104 (70% ethanol wash) and 78 (icing sugar dusting) (Table 2). One-way Analysis of Variance of the three compared methods of varroa mites' dislodgement from the external bodies of honeybees ($F_{2,12,0.05}$ = 0.3037, p= 0.7436, $F_{critical}$ = 3.8853) show that there was no statistically significant difference in the mean mites' load sampled (Table 3), while the mean±SD of varroa mites extracted from the honeybee samples from each of the colonies using the three methods were between 142.00±0.93 and 629.67±0.43 (Table 1). The study shows that the wood dusting method is effective comparatively (Tables 1 & 2). However, the order of effectiveness of the three varroa mites' dislodging methods is 70% ethanol wash (38%) > wood ashes dusting (33%) > icing sugar dusting (Fig. 1).

Total varroa mites across all the five colonies for each method were 4,735, 5,409 and 4,160 for wood ashes dusting, 70% ethanol wash and icing sugar dusting respectively (Table 2). The general infestation level of honeybees (number of varroa mites per honeybee) for each method were 0.91 (wood ashes dusting), 1.04 (70% ethanol wash) and 0.78 (icing sugar dusting).

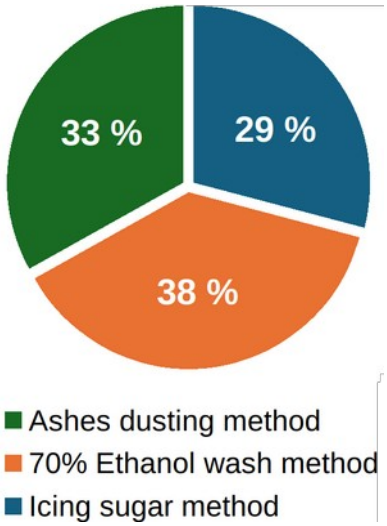

Figura 1. Porcentaje (%) del total de ácaros varroa recuperados por cada uno de los tres métodos.

Figure 1. Percentage (%) of total varroa mites recovered by each of the three methods.

	Wood ashes dusting		70% ethanol wash		Icing sugar dusting	
Experimental Colonies	Honeybees sampled	Varroa mites collected	Honeybees sampled	Varroa mites collected	Honeybees sampled	Varroa mites collected
OSBHK (1)	359.33±1.56	569.33±2.09	334.00±2.10	629.67±0.43	364.33±1.96	491.33±2.71
OSBHW (2)	317.00±0.56	427.33±2.92	354.67±1.35	467.33±1.34	351.67±1.70	336.00±0.81
OSBHX (3)	357.67±1.98	245.67± 0.59	351.67±0.74	280.33±1.40	356.33±2.47	166.00±2.10
OSBHY (4)	350.00±2.23	190.67±1.89	347.00±0.80	266.00±1.17	341.33±1.33	166.00±2.43
OSBHZ (5)	344.00±1.18	142.00±0.93	344.67±2.63	159.67±0,45	349.67±1.25	227.33±1.74

Tabla 1. Media±DE de las abejas muestreadas y los ácaros *Varroa* recolectados para la comparación de los tres métodos de extracción de ácaros *Varroa* de los cuerpos de las abejas.

Table 1. Mean±SD of honeybees sampled and *Varroa* mites collected for comparison of the three methods of extracting *Varroa* mites from the bodies of honeybees.

	Wood ashes dusting	70% ethanol wash	Icing sugar dusting
Sum of *Varroa* mites across all the five colonies	4735	5409	4160

Sum of sampled bees	5184	5208	5290
Mean±SD of *Varroa* mites	947.0±15.54	1081.8±15.24	1058.0±13.36
Infestation level: No. of varroa mites/100 honeybees.	91	104	78

Tabla 2. Comparativa: Media ± DE de ejemplares estudiados y nivel de infestación de ácaros varroa para cada método de monitoreo.

Table 2. Comparative: Mean±SD of studied specimens and infestation level of varroa mites per each method of monitoring.

	SS	Df	MS	*F*	p_{-value}	f_{-crit}
Between groups	156326.8	2	78163.4	0.3037	0.7436	3.8853
Within groups	3088159	12	257346.6			
Total	3244486	14				

Tabla 3. Análisis de varianza unidireccional de tres métodos comparados de desprendimiento

de ácaros varroa de los cuerpos externos de abejas recolectados de nidos de cría en colonias de abejas.

Table 3. One-way Analysis of Variance of three compared methods of varroa mites' dislodgement from the external bodies of honeybees collected from brood nests in honeybees' colonies.

Discussion

There are two methods of diagnosing honeybee colonies for the presence of varroa mites. One of the methods is to uncap honeybees' brood cells and remove the larvae or pupae for examination. Varroa mites generally have preference for brood cells to complete their life cycle and examining the brood cells for varroa mites is more productive during monitoring of varroa mites in apiaries (Ruttner and Ritter 1980). The second method which is easy and faster involves examination of the external bodies of adult worker honeybees by shaking them in specific solution or powdered materials which dislodged varroa mites from the external bodies of honeybees. Within a brief period, a large population of honeybees can be sampled by this second method. It facilitates the detection and estimation of varroa mites' infestation level in honeybee colonies in any affected apiary.

Varroa destructor Anderson & Trueman 2000 is mostly found attached to the intersegmental membranes of the abdominal sternites or tergites of adult honeybees and are difficult to see and dislodged without a few minutes' agitation or rigorous shaking in either liquid or powdered materials. Several types of powdered materials such as icing sugars, ether roll, sticky boards, Slovenian powdered sugar, baby powder, lyophilized royal jelly, corn starch, confectioners powdered sugar and granulated sugar (Fakhimzadeh *et al.* 2011, Ontario Ministry of Agriculture, Food and Rural Affairs 2021) are used for Varroa mites' monitoring or recovery materials apart from the use of 70% ethanol, detergent solutions (Stolbov & Vasikov 1976) and hot water (Grobov 1977), hexane or gasoline (Ruttner & Ritter 1980), 96% ethanol (De Jong & Goncalves 1981, De Jong *et al.* 1982). Gasoline was reported to be 100% effective for dislodging varroa mites from the external body of adult honeybees, but its fume is toxic and highly flammable (Ruttner and Ritter 1980). In Brazil, Goncalves *et al.* (1979) reported that ethanol was equally effective, but in most countries including Nigeria, it is costly due to present economic crunch and not readily available, but this study showed that there are possible and effective indigenous materials that can be used in alternative. The dry powdery wood ashes are readily and freely available within farm settlements and locations of the apiaries close to charcoal fire points operated by crop farmers.

For the detection of varroa mites, we assessed three methods of dislodging varroa mites from adult honeybees to compare their efficiency for extracting the mites. The three methods include the 70% ethanol wash, icing sugar and ashes dusting methods, respectively. The 70% ethanol wash method is most effective followed by wood ashes and icing sugar respectively and comparatively according to this study. However, powdery wood ashes (indigenous material) which ranked second is also effective and is commonly available locally where most beekeepers operate within locations of their apiaries, and it is relatively affordable at no cost.

Beekeepers are expected to sample varroa mites at least twice a year both in dry and wet seasons specifically. This will give an idea of the varroa mites' population in both seasons immediately before the treatment period. Beekeepers may also consider taking samples if the colonies appear weak and stressed. Beekeepers may also consider taking samples before and after treatment is applied to determine if the treatment was effective at lowering the population of mites in the colony. All these are possible based on the availability of the ashes at zero or reduced cost.

The three varroa mites' monitoring methods studied were efficient for extracting varroa mites

from the external body of honeybees when monitoring and determining varroa infestation level in honeybee colonies, but 70% ethanol method is most efficient comparatively. However, none of the three methods compared was distinctly superior to the other because the differences in recovery of varroa mites were not significant. Therefore, wood ashes dusting method can be used as an alternative to 70% ethanol wash and icing sugar methods in extracting varroa mites from the external body of honeybees to determine level of varroa mites' infestation in honeybee colonies.

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