Evaluation of Non-Chemical Traps for Management of Wax Moth Populations within Honey Bee Colonies[1]

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ABSTRACT Wax moth larvae (Lepidoptera: Pyralidae) are serious pests on wax combs outside and inside of beehives. Management of wax moths is particularly difficult inside hive bodies housing weak colonies. In this study, five traps were designed and evaluated for their efficacy to attract mature larvae inside beehives. These traps were: (1) mesh envelope trap (MET), (2) cup trap (CT), (3) corrugated sheet (CS), (4) wooden sheet trap (WST), and (5) frame trap (FT). The traps were designed to take advantage of the behavior of mature larvae seeking protected locations for pupation. The numbers of larvae fluctuated during the study with a major peak at the end of June. MET attracted the greatest numbers of larvae, followed by CS, CT and FT, whereas WST did not attract any larvae. The internal traps, particularly MET, may reduce the damage from wax moth larvae by reducing existing moth populations inside the beehives, and therefore, prevent additional infestation.

KEY WORDS Honey bees, *Apis mellifera*, wax moths, traps, control

Diseases and pests of honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), can greatly hinder the advancement and reduce the economic benefits of beekeeping worldwide. The lesser wax moth (LWM), *Achoria grisella* (F.), and the greater wax moth (GWM), *Galleria mellonella* (L.) (both Lepidoptera: Pyralidae), are cosmopolitan in distribution and pest status. Wax moth larvae damage wax combs, wooden frames and beehive boxes. Wax moths can prevent new adult bees from uncapping their brood cells (known as galleriasis), and cause bald brood (Ellis et al. 2013). High infestations could lead to colonies absconding (Tsegaye et al. 2014). Although wax moths can invade both weak and strong honey bee colonies, weaker colonies are more severely impacted (Nielsen & Brister 1977).

Methods to protect stored combs from wax moths include the use of heat treatments (Charrière & Imdorf 1999), ozone treatment (James 2011), freezing method and climate manipulation (Ellis et al. 2013), and chemicals such as paradichlorbenzene crystals (Burges 1978). Methods to control adult wax moths outside the beehives include male sterilization with gamma rays (Jafari et al. 2010), sex pheromone traps (Sangramsinh et al. 2014), and light traps (especially with red light) (Mabrouk & Mahbob 2015). Maintaining strong colonies, supplementary bee feeding, and the use of tobacco leaf smoke have been suggested to reduce the wax moths within the beehives (Tsegaye et al. 2014). Ethanolic extracts of medicinal plants had been shown to have potential in killing wax moth larvae in

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in vitro tests (Zaitoun 2007). Surendra et al. (2010) found that extract of neem seed (*Azadirachta indica* A. Juss; Meliaceae) caused mortality of 85% to 94% in larvae. The efficacy of *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Clavicipitaceae) (El-Sinary & Rizk 2007), *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (Ellis & Hayes 2009), and entomopathogenic nematodes (Noosidum et al. 2010) have been evaluated against larvae. The cost-effectiveness and efficacy of the above within-hive management methods require additional investigations, particularly within weak colonies.

Using non-chemical methods and safe materials within honey bee colonies is very important for reducing exposure of bees and their products from contamination. Preventing wax moth larvae from feeding on the wax combs within the colonies is not a simple task due to their size and feeding habits. Eggs are approximately 0.44 × 0.36 mm (Arbogast et al. 1980). First-instar larvae range from 1 to 3 mm, and begin to feed on combs immediately after hatching (Ellis et al. 2013). It is possible to trap mature larvae by providing them with a suitable pupation site. This study aimed to develop traps of mature larvae that could help reduce existing moth populations, and therefore, reduce repeating infestations. The traps were made of simple, inexpensive materials. These traps do not prevent adult moth infestation, but could remove existing larvae. The trap with the highest trapping efficiency from this study will be recommended for use against wax moth infestations in weak honey bee colonies.

Materials and Methods

Apiary and colonies. The study was conducted in a commercial apiary located at Damanhour, El-Behera, Egypt. Twenty-four beehives were prepared; each consisted of a standard 10-frame Langstroth box containing five frames covered with bees (2 food and 3 brood frames) (Figure 1). The extra space within the hives allowed the colonies to be more exposed to natural infestation by wax moths. All colonies were headed by young (<one year old) Carniolan × Egyptian hybrid queens. All the colonies were treated equally in regard to feeding.

Traps for wax moth larvae within the colonies. Traps were designed to have cracks, beeswax and/or wooden part to take advantage of the behaviors of mature larvae of utilizing cracks and wooden surfaces for pupation. These traps are: 1) cup trap (CT), 2) mesh envelope trap (MET), 3) wooden sheet trap (WST), 4) frame trap (FT), and 5) corrugated sheet (CS) (Figure 2). The CT trap consisted of a plastic jar (6.5 cm diameter and 10.5 cm length) filled with layers of tissue papers saturated with melted beeswax, and small holes along the walls of the jar to allow entry of larvae. The CT was designed to provide a safe place for pupation away from honey bees, and beeswax was used to attract mature larvae. The MET trap consists of wire mesh (11 pores/cm^2) envelope (20 × 20 cm), and a piece of wavy corrugated cardboard sheet covered with melted beeswax as an insert. The wire mesh envelope was designed to restrict access for honey bee workers, but allow access for mature larvae. The WST is a normal frame with a wooden sheet fixed inside in place of beeswax foundation. The FT trap consists of a normal frame of beeswax comb, and is covered with wire mesh from one side to prevent the access of worker bees. The CS trap consists of corrugated cardboard sheet (15 cm L × 40 cm W) with wavy surface. All traps were placed between the hive wall

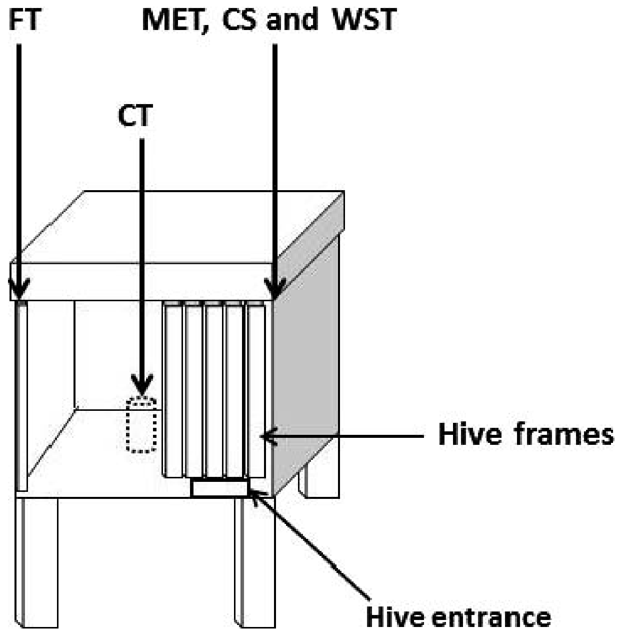

Fig. 1. Placement of traps within beehive. CT: cup trap; MET: mesh envelope trap; FT: frame trap; WST: wooden sheet trap; and CS: corrugated sheet.

and the first comb, except for CT and FT, which were placed after the last frame (Figure 1). It was not possible to place CT and FT in the same location as the other traps, because they can hinder the normal behavior of honey bees within the colonies. The five trap types were assigned randomly to the hives, with each design replicated four times. Four control hives (no traps) were also prepared.

Numbers of wax moths within colonies. The numbers of larvae and pupae in each trap, or on the beehive walls, frames and bottoms, were counted weekly from June until the end of October 2015. The larvae or pupae were removed during counting to avoid any overlap among readings. Because eggs and first-instar larvae are small and difficult to see, only second-instar or older larvae were counted. The mean numbers of larvae and/or pupae detected in each hive were calculated for each week, and plotted against sampling weeks to detect population trend.

To calculate the percentage efficacy of each trap type, the number of larvae and pupae collected by a trap was divided by the total number of larvae and pupae inside each hive (including those in the trap), and multiplied by 100.

Statistical analysis. Means and standard deviations (SD) were calculated for the numbers of larvae and pupae and the percentages efficacy of traps. One-way analysis of variance was used to detect significant differences in the total numbers of larvae and pupae among sampling weeks, and in the mean numbers, total numbers and percentage efficacy among trap types, at the threshold of 0.05 (PROC GLM, SAS Institute 2004). Means were separated using Duncan's multiple range test (DMRT). Larval and pupal abundance was log-transformed, and percentages efficacy were arcsine-transformed, before statistical analysis.

Fig. 2. Traps evaluated in the study. CT: cup trap; MET: mesh envelope trap; FT: frame trap; WST: wooden sheet trap; and CS: corrugated sheet.

Results

The mean numbers of larvae and pupae fluctuated over time (Figure 3). The population peaked at Week 4 (the end of June) with a mean ($\pm$SD) of 2.6 $\pm$ 7.5 larvae and pupae, followed by Week 6, 10 and 20 with means of 1.3 $\pm$ 2.3, 1.0 $\pm$ 1.4 and 1.0 $\pm$ 1.4 larvae and pupae, respectively ($F = 3.27$, df = 19, 460 $P =$ 0.0001). The SD was higher than the mean because sampling at many dates did not recover any larva or pupa (i.e., count = 0).

The numbers of larvae and pupae collected outside of the traps (pooled over the study period) were not significantly different among the trap types ($F = 0.32$, df = 4, 395, $P = 0.8650$) (Table 1). The number of larvae and pupae collected inside

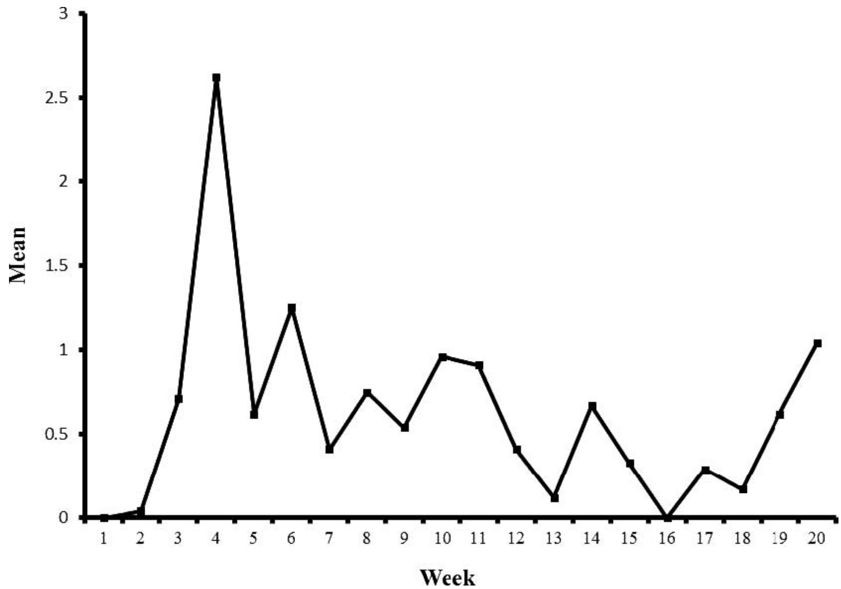

Fig. 3. Weekly fluctuations in the mean numbers of larvae and pupae within hives over the study period. Means from all colonies were pooled.

MET was significantly higher than those collected by other trap types ($F = 15.06$, df = 4, 395, $P = 0.0001$). WST failed to capture any larvae. Based on percentage of efficacy, the most effective design was MET, followed by CS, CT and FT ($F = 27.22$, df = 4, 155, $P = 0.001$).

Table 1. Means ± SD of wax moths (larvae and pupae) collected over the study period inside and outside of traps, and the efficacy of trap types.

Trap	Means of wax moths collected			Trap efficacy %
	Inside	Outside	Combined (inside + outside)	
Mesh envelope trap (MET)	0.93 ± 3.14a	0.33 ± 1.23	1.27 ± 4.30a	74.48 ± 36.95a
Corrugated sheet (CS)	0.32 ± 0.79b	0.35 ± 0.98	0.67 ± 1.42abc	50.72 ± 44.49b
Cup trap (CT)	0.16 ± 0.56bc	0.22 ± 0.71	0.38 ± 0.87bcd	44.73 ± 49.70b
Frame trap (FT)	0.03 ± 0.24c	0.32 ± 1.01	0.36 ± 1.03cd	16.67 ± 38.92c
Wooden sheet trap (WST)	0c	0.27 ± 1.06	0.27 ± 1.06d	0d
Control	—	—	0.77 ± 1.34ab	—

Means followed with the same letter within each column are not significantly different according to DMRT at $\alpha = 0.05$.

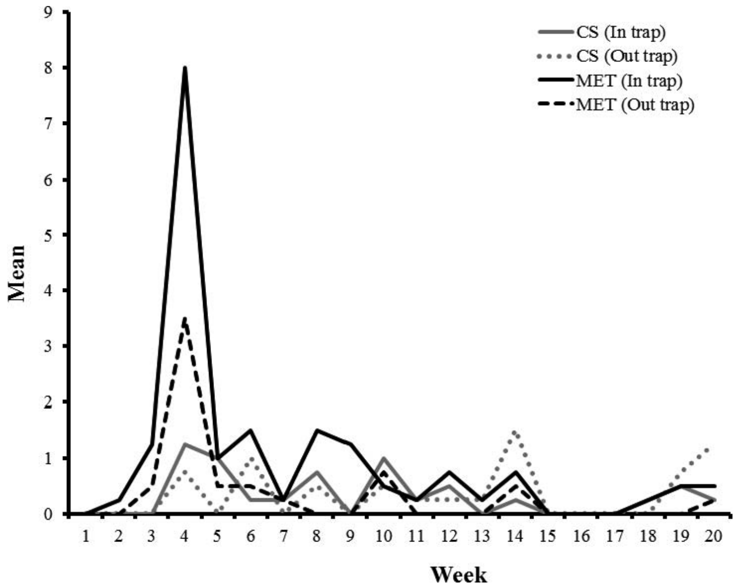

Fig. 4. Mean numbers of larvae and pupae observed inside and outside of corrugated sheet traps (CS) and mesh envelope traps (MET).

The MET consistently captured more larvae and pupae inside than outside the traps, except at Week 10 (Figure 4). The mean numbers of larvae and pupae captured inside CS were lower than those outside at Week 6, 13, 14, 19, and 20 (Figure 4). The mean numbers of larvae and pupae captured inside was lower than those outside at Week 6, 7, 11, 14, 15, 17, 19, and 20 for FT, and at Week 7, 9, 10, 15, 17, and 20 for CT (Figure 5).

Discussion

The numbers of larvae captured and observed within hives fluctuated greatly during the study, with a major peak at the end of June (Week 4 of the study). Mabrouk & Mahbob (2015) found wax moths to present throughout the year, but with greater variations in the numbers of adult wax moths inside light traps from April to June and from September to November in Egypt. This suggests that wax moth populations are not stable over time and greatly fluctuate.

Many factors lead to the variations in weekly counts of larvae and pupae. In the current study all colonies had approximately the same strength (i.e., five frames covered with bees), but moths exhibited their own preference to invade some colonies more than others. Although wax moths preferentially invade weak colonies, mated females appear to be more attracted to some colonies for unknown reasons (Nielsen & Brister 1977). Additionally, not all eggs hatch at the same time, and not all larvae reach full maturity at the same time, which could explain the weekly variations in larvae densities observed within colonies (regardless of

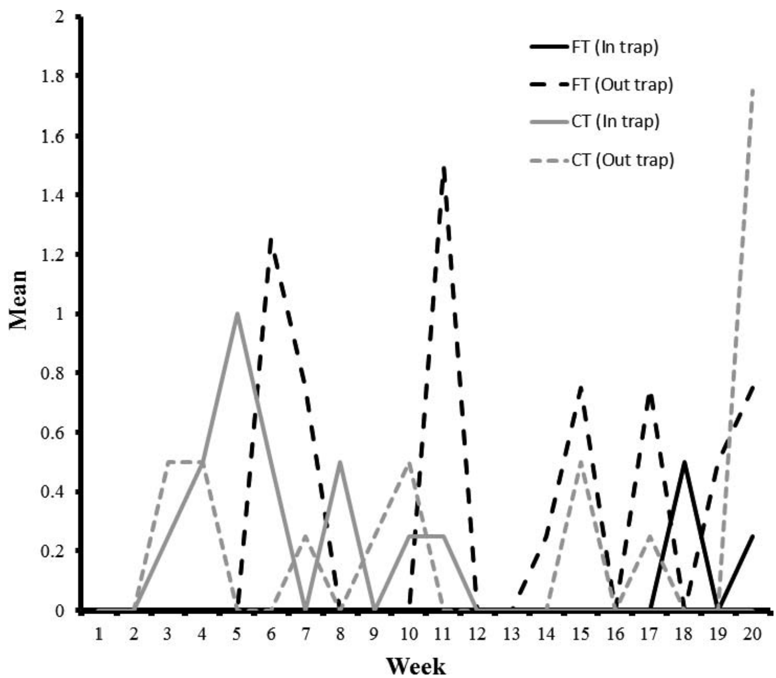

Fig. 5. Mean numbers of larvae and pupae observed inside and outside of frame traps (FT) and cup traps (CT).

the trap types). Moreover, honey bee workers have their own strategies to defend against wax moths, including biting larvae and removing them from colonies (personal observations), which may reduce the numbers of observed larvae within the hives.

In the present study, MET trapped more larvae than the other trap types. This trap contains pieces of corrugated sheet with a wavy surface, which reflects the suitability of this material for pupation. MET attracted more larvae than CS, likely due to a layer of beeswax deposited over the corrugated sheet. Mature larvae tend to excavate furrows in a suitable pupation location, most commonly wooden parts of the beehive (Ellis et al. 2013). FT, which contains beeswax comb covered with wire mesh on one side, attracted very few larvae. The low attraction may be explained by the placement of FT away from the combs and the hive entrance. Trap location may have a significant role in the number of attracted larvae, and mature larvae may select locations close to combs for pupation. WST did not attract any larvae, despite the behavior of larvae to dig burrows in wooden wall for pupation. Traps were placed near combs covered with bees, and the presence of bees may have discouraged mature larvae from utilizing this type of traps for pupation. Also, the wood type (semi-soft and smooth) used in the WST trap might have impacted the choice of wax moth larvae to pupate on it as the larvae may prefer rougher woods to slightly softer ones.

It is not clear why some larvae did not enter the traps while other larvae did. Based on the observations taken during the study, most of the detected larvae or pupae outside traps were found under combs directly, which means that mature larvae often pupate directly under combs without searching for any other place to pupate. However, most larvae that did search for a suitable pupation location were found in traps or on the hive wall close to combs (in cases where traps were placed away from the combs and hive walls).

This study indicates that it is possible to trap wax moth larvae within colonies using a MET, without any harmful impacts on honey bees. This type of trap is recommended for use in weak colonies and should be checked every week to remove any trapped larvae. Future development of internal traps for wax moths should consider both trap location (placement between wax comb and beehive wall) and trap material (to be wavy and covered with beeswax, and trap shape should be considered).

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