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Natural Products can Efficiently Control the Greater Wax Moth (Lepidoptera: Pyralidae), but are Harmless to Honey Bees

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Abstract

Honey bees (*Apis mellifera* L.) have great global socioeconomic and environmental importance. However, the greater wax moth (*Galleria mellonella* L.) is a pest that causes serious worldwide damage to honey bee colonies. Good beekeeping practices and physical, chemical, or natural methods can be used to control wax moths. The use of natural products is a more sustainable option because of their lower toxicity to the environment and the colony. Therefore, we evaluated the efficiency of four natural products for greater wax moth control: neem oil (*Azadirachta indica*), eucalyptus oil (*Eucalyptus* spp.), tobacco extract (*Nicotiana tabacum*), and malagueta pepper extract (*Capsicum frutescens*). We also evaluated their effects on adult bees and on the population growth of colonies. The 4th instar wax moths and adult bees were subjected to *in vitro* bioassays of different concentrations of the products. The results allowed us to establish a concentration for each product that was safe for the bees and effectively controlled the moth. Then, we sprayed them on bee colonies to evaluate their effects on population growth. The neem and eucalyptus oils caused wax moth mortality at low concentrations, but did not affect colony population growth. However, they did have a toxic effect on adult bees. The tobacco and pepper extracts efficiently controlled the moth, but did not cause adult bee mortality or interfered with the population growth of the colonies. Therefore, the tobacco and pepper extracts could efficientlycontrol the greater wax moth, without damaging honey bees.

Introduction

The honey bee (*Apis mellifera* L.) (Apidae: Apini) is an eusocial bee species that is socioeconomically and environmentally important across the globe. Apiculture produces highly valuable commercial products and is responsible for the pollination of more than 70% of the main crops grown across the globe (Klein et al., 2006). Honey bee colonies are affected by several pests, such as the greater wax moth (*Galleria mellonella* L.) (Lepidoptera: Pyralidae), which can cause significant losses (Charrière & Imdorf, 1999; Kwadha et al., 2017). Moth caterpillars, especially between the 4th and 5th instars, can indirectly affect the survival of bees through the destruction of their wax combs, construction of galleries, and the consumption of stored food (Kwadha et al., 2017). A deterioration in the wax combs can cause the colony to abscond and can reduce potential reutilization of the hive (Fletcher, 1975).

The use of a sanitary management through proper practice by the beekeeper can considerably reduce the incidence of the pest, and is an efficient way of keeping the moth population under control. There are also physical and chemical methods that can be used to control *G. mellonella* (Büyükgüzel, 2009). However, physical and chemical methods are very expensive and may have toxic effects on the bees. Furthermore, these methods may contaminate the bee products (Bollhalder 1999; Rortais et al., 2017). Therefore, the use of natural products is a reasonable alternative for wax moth

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control (Farghaly et al., 2017) because they generally have a low environmental contamination risk and there are few, if any, harmful residues in the bee products (Schmutterer, 1990; El-Wakeil, 2013).

Several oils and plant extracts have been shown to efficiently control wax moths (El-Wakeil, 2013). Neem oil (*Azadirachta indica*) has been reported to be an important insecticide that is thought to be toxic tomore than 400 insect species due to the presence of a secondary metabolite, azadirachtin, which inhibits larval development (Senthil-Nathan, 2015). Eucalyptus oil (*Eucalyptus* spp.) has eucaliptol as its main constituent and can also effectively control several crop pests, including *Alphitobius diaperinus* (Pinto Júnior et al., 2010) and *Spodoptera frugiperda* (Souza et al., 2010). The malagueta pepper (*Capsicum frutescens*) has several natural amides in its fruit composition that have insect repellent properties (Maliszewska & Tegowska 2012). Among them, capsaicin acts as a neurotoxin that causes insect paralysis and death (Iorizzi, 2000). Tobacco extract (*Nicotiana tabacum)* also has neurotoxic effects on some insect genera (Taillebois et al., 2018).

The use of natural products for wax moth control can be a more sustainable and safer option than chemical control. Therefore, the aimsof this research were to evaluate the effects of neem oil, eucalyptus oil, malagueta pepper extract, and tobacco extract on greater wax moths; their toxicity to adult bees; and their effect on colony population growth. The results showed that at some concentrations, the natural products did not cause harmful effects to the bee colonies or to the adult bees and could efficiently control wax moths.

Materials and Methods

Experimental Site

The experiment was conducted at a site located at the coordinates 22°50’ S and 48°25’ W, with an average altitude of 720 m.

Preparation of the Natural Products

The natural products were neem oil, eucalyptus oil, tobacco extract, and malagueta pepper extract. These products were chosen based on their availability and low cost so that they would be accessible to beekeepers. The neem and eucalyptus oils were commercial brands (*Codipa*® – 98% azadirachtin and *BioEssência* – 100% oil). The tobacco extract was obtained by chopping 500 g of pure tobacco into pieces and diluting them in 2 Lof boiling water. After 24 h, the solution was agitated and filtered. Then, 200 mL of ethyl alcohol was added and the volume was made up to 2 L. The malagueta pepper extract was prepared by macerating 100 g of pepper in 2 Lof water and leaving the mixture to settle for 24 h. Then, the mixture was agitated and filtered to obtain the extract (Guerra, 1985; Santos et al., 1988).

Effect of the Natural Products on *Galleria mellonella* Larvae

The 4th instar *Galleria mellonella* larvae were deprived of food for 24 h before the trials. Then, five larvae were placed in Petri dishes with filter paper containing1.5 mL of one of the natural products. The products were diluted with distilled water in order to obtain the following concentrations: 0.0%, 0.5%, 1.0%, 2.5%, 5.0%, 10.0%, 20.0%, 40.0%, 80.0%, or 100.0%. The Petri dishes were kept atroom temperature in the dark. The larval mortality was observed every 15 minutes for 4 h, which was16 assessments in total. Each treatment was replicated five times.

Effects of the Natural Products onAdult Bees

The lethal effect of the natural products on adult bees was evaluated by collecting 10 individuals from the central frame of a colony and immediately taking them to the laboratory. Then, they were anesthetized with CO_2 and placedin plastic containers with filter paper. The filterpaper contained 2.0 mL of one of the natural products. Each natural product had the following concentrations: 0.0%, 0.5%, 1.0%, 2.5%, 5.0%, 10.0%, 20.0%, 40.0%, 80.0%, or 100.0%. Therefore, 400 bees were used in each replicate and there were five replicates. The container also had a feeder that provided the bees with sugar syrup (1:1) *ad libitum*. Mortality was evaluated every 15 minutes over 4 h, what resulted in 16 counts in total.

Effects of the Natural Products on Colony Population Development

In vitro bioassays and adult bee and caterpillar analyses were used to establish a concentration for each product that was not lethal tothe bees but effectively controlled the moths. A total of 20 standardized colonies were used to evaluate any possible sub-lethal effects of the products on colony population growth. Each colony had two central brood frames that were sprayed with 2 mL of one of the products or distilled water as the control. Therefore, each product plus the control were applied tofour colonies. The concentration of each product was 0.5% neem oil, 0.5% eucalyptus oil, 0.5% tobacco extract, and 10% malagueta pepper extract. The brood frames contained an open brood and a capped brood area that were evaluated by a modified methodology proposed by Al-Tikrity et al. (1971). Evaluation was done on a weekly basis for 35 days, i.e., six times in total.

Statistical Analysis

All Statistical analysis was performed in R. Measures of central tendency were used to compare the data. The data were normal and homocedastic. Therefore, ANOVA and Tukey’s test were used to analyze any differences between the averages. The differences were considered statistically significant when $P<0.05$ (Zar 1996).

Results

Wax Moth Mortality

The neem oil, eucalyptus oil, and tobacco extract led to wax moth mortality at all concentrations ($P<0.05$). However, the pepper extract only caused mortality at concentrations above 10% ($P<0.05$; Fig 1).

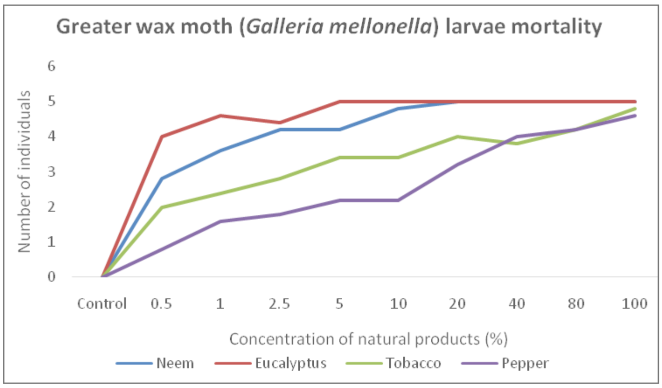

Fig 1. Greater wax moth (*Galleria mellonella*) mortality by different natural products atvariousconcentrations (%).

Adult Bee Mortality and Population Development

The effects of the products on adult bee mortality were observed and the results showed that eucalyptus and neem oil concentrations above 5% affected bee survival compared to the lower concentrations. However, only the pepper extract at 100% concentration and tobacco extract concentrations above 80% affected bee survival ($P>0.05$; Fig 2).

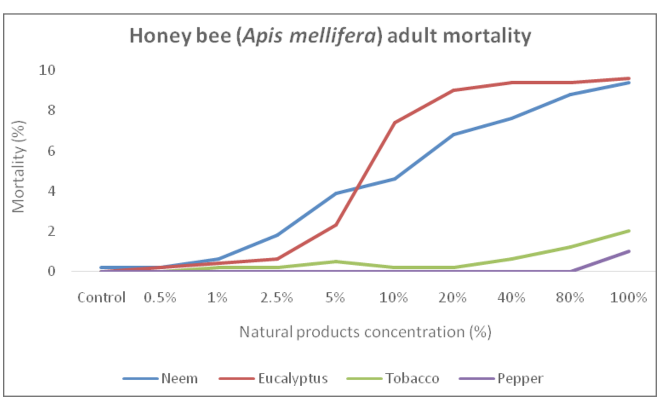

Fig 2. Adult honey beemortality for the different natural products atvarious concentrations (%).

Furthermore, applying the plant extracts to the colonies did not affect the open and closed breeding areas compared to the control ($P>0.05$; Fig 3).

Discussion

All four natural products efficiently controlled the greater wax moth. However, the oils and the tobacco extract caused wax moth mortality at relatively low concentrations

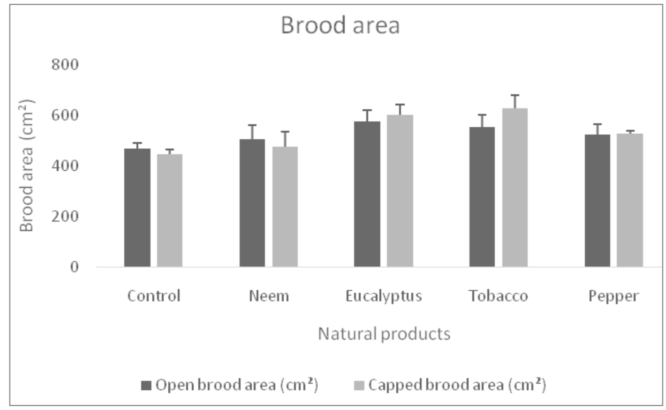

Fig 3. Population growth of *A. mellifera* colonies after using the different natural products.

(0.5%), whereas the pepper extract caused pest mortality only at concentrations above 10%. Negative effects were observed in adult bees when the two oil concentrations were higher than 5% (46% and 76% mortality, respectively, for the neem oil and the eucalyptus oil). In contrast, the tobacco and pepper extracts only caused bee mortality when used in concentrations above 80% and 100%, respectively. Furthermore, the mortality rate was smaller than that caused by the oils (10–20% mortality). However, none of the extracts harmed colony population growth.

The neem and eucalyptus oils, and the tobacco and pepper extracts efficiently controlled the greater wax moth. Both the oils and the tobacco extract caused wax moth mortality at relatively low concentrations (0.5%), whereas the pepper extract only caused pest mortality at concentrations above 10%.

Previous studies have shown that neem oil is an efficient insecticide (Abreu, 1998; Souza & Vendramim, 2000; Vinuela, et al., 2000; Senthil-Nathan, 2015; Chaudhary, 2017). It shows selective insect control because different insect groups have different resistance levels towards its mode of action (Senthil-Nathan, 2015). One of its action mechanisms is to block the synthesis of growth hormones (ecdysteroids), which implies that metamorphosis is interrupted in immature insects (Isman, 2006). The higher susceptibility of the greater wax moth compared to the adult bees could be due to the fact that it belongs to the order Lepidoptera, which are more susceptible to azadirachtin (Zhong et al., 2017) and because it was at an immature growth phase (4th instar larvae) (Schumutterer, 1990; Moreira et al., 2008). Despite the higher susceptibility of the wax moth, neem oil also had negative effects on adult bee survival when used at concentrations above 5% and these effects were dose-dependent.

Eucalyptus oil has also been shown to be an important natural insecticide and can efficiently control several crop pests (Kim et al., 2003; Pinto Júnior et al., 2010; Souza et al., 2010; Fhati & Shakarami, 2014). The oil, even at low concentrations, led to significant wax moth mortality, but also caused adult bee mortality when applied at concentrations above 5%.

The greater susceptibility of the greater wax moth compared to bees may also be due the larvae having a less developed chitin layer compared to adult individuals, which limits larvae physical protection against eucalyptus oil (Chapman, 2013). In a similar way to neem oil, the eucalyptus oil led to significant mortality in bees when used at concentrations >5%, and its action was also dose dependent.

The lack of harmful effects on colony population growth after the application of neem and eucalyptus oils may have been due to reduced exposure to the product because bee larvae remain individually housed within the alveoli.

The 0.5% tobacco extract and the 10% pepper extract efficiently controlled the greater wax moth and did not cause bee mortality, even at high concentrations. They also did not influence colony population growth. The tobacco extract was only harmful to adult bees at doses >80% (safer levels compared to neem and eucalyptus oils). One of the main components of tobacco is nicotine, which is found in species of the genus *Nicotiana* (Isman, 2006). High doses of nicotine may affect larval survival and reduce the foraging activity of adult bees (Singaravelan et al., 2006) because the compound acts on the nervous system. It competes with acetylcholine in the synapses of the axons and promotes the generation of new nerve impulses that lead to spasmodic contraction, convulsions, and insect death (Taillebois et al., 2018). The alkaloid insecticides include nicotinoids and neonicotinoids, whose active ingredients are synthetic nicotine compounds. They are widely used to control several agricultural pests around the world (Blacquierre et al., 2012).

The pepper extract had significant effects on greater wax moth mortality when applied at concentrations equal to or greater than 10%. Although the mortality rates of the pest occurred at higher concentrations of this extract than the other products, its use did not cause toxicity to adult bees, even when applied in high concentrations, and it did not interfere in colony population growth. Among the compounds present in the fruits of the genus *Capsicum*, the phenols capsaicin and dihydrocapsaicin, as well as diterpenoids, flavonoids, phenolic compounds, and saponins, have lethal, anti-feed and repellent effects on invertebrates (Leete & Louden, 1968; Maliszewska & Tegowska, 2012). The capsaicin lethal dose for *Apis mellifera* bees is greater than 100 μg per individual (Flesar et al., 2010). Therefore, it is considered relatively non-toxic and is proving to be a safe compound that can be used in bee colonies. Despite the low toxicity of the extract towards bees, there was a significant effect on mortality and the consequent control of the wax moth. It is possible that *G. mellonella* has a greater susceptibility to capsaicin (Sarwar, 2015).

These results show that the use of tobacco and pepper extracts is an effective alternative for wax moth control because they show reduced toxicity to bees compared to neem and eucalyptus oils and have a greater margin of safety. The compounds were extracted using ethyl alcohol as a solvent and distilled water was usedto further dilutethe mother solution composition, which may also have resulted in a lower toxic effect on moth larvae and adult bees compared to neem and eucalyptus oils. However, the increased toxicity of the neem and eucalyptus products towards bees may have been due to fact that the products were oils.

The natural products based on neem, eucalyptus, tobacco, and pepper efficiently controlled the wax moth. However, the neem and eucalyptus oils have a lower margin of safety comparedto the pepper and tobacco extracts, and cause higher mortality among adult bees, even at low concentrations. Therefore, extracts of tobacco and pepper are safer alternatives when attempting to control the greater wax moth while maintaining bee survival.

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