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Note

Effects of the Fungus, *Beauveria bassiana*, on the Larval Development of the Greater Wax Moth, *Galleria mellonella*, (Lepidoptera: Pyralidae) under Laboratory Conditions

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Abstract Wax moths can cause many damages to honey bee colonies. These moths can destroy the combs, impair the development of bee colonies, and cause bee absconding. Few control strategies for these moths inside beehives are available. The fungus, *Beauveria bassiana*, is effective for the control of *Varroa* mites, and may be also useful for the control of wax moths. This study aimed to investigate the effects of three treatments (1, 3, and 6 g per larva) of this fungus on the development of the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) under laboratory conditions. Notably, the use of 1 g per larva of this fungus showed very few effects on larval development, and 80% of the larvae completed the development normally. The use of 3 g per larva caused low death rate (20%), and 70% of the test larvae pupated but failed to emerge as adults. Only 6 g per larva showed rapid deleterious effects and completely impaired the development of the test larvae (100% death rate). According to this study, the use of this fungus to control wax moths is not likely to be very effective especially high amount of *B. bassiana* is required to cause rapid death to larvae (the harmful stage of wax moths to bee colonies).

Keywords Honey bee, Wax moths, Fungi, *Galleria mellonella*

INTRODUCTION

During the recent years, the number of honey bee, *Apis mellifera* L. colonies has declined due to a colony collapse disorder phenomena (Oldroyd, 2007), which happened as a result of many factors that can harm the bees (vanEngelsdorp *et al*., 2009; Neumann and Carreck, 2010). Chemicals, in general, are not recommended to be used inside beehives because they can contaminate beeswax and bee products, besides harming the bees (Pettis *et al*., 2004; Bogdanov, 2006). Therefore, biological control agents are perfect and most recommended alternative treatments. Honey bee colonies are affected by many parasites and pests, and the major parasite is *Varroa* mite (*Varroa destructor*, Acari: Varroidae (Abou-Shaara, 2014). The use of *Beauveria bassiana* to control *Varroa* mite is considered among the best biological control options (Abou-Shaara and Staron, 2019), and has shown reasonable efficacy (Shaw *et al*., 2002; Meikle *et al*., 2008; Hamiduzzaman *et al*., 2012; Ahmed and Abd-Elhady, 2013; Sewify *et al*., 2015). Moreover, this fungus has no harmful effects on honey bees (Meikle *et al*., 2007; Meikle *et al*., 2008; Ahmed and Abd-Elhady, 2013), which encourages its application in beehives.

But the use of this fungus in beehives can contaminate the beeswax and may have further consequences on the wax moths. These moths are another pest to honey bees and can cause various damages to the colonies (Ellis *et al*., 2013; Kwadha *et al*., 2017), and feed mainly on beeswax. Few options are available to control these moths inside the beehives including non-chemical traps (Abou-Shaara, 2017). The host range of this fungus includes the greater wax moth (*Galleria mel-*

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lonella, Lepidoptera: Pyralidae), and previous studies have focused on the effects of this fungus on cuticle and enzymatic activities of wax moths (Samšiňáková *et al*., 1971; Dubovskiy *et al*., 2011; Hussein *et al*., 2012). The potential use of this fungus as a control method to these moths has not been considered widely. Only wax moth larvae cause damages to bee colonies. Therefore, this fungus can be considered as a potential control agent only if caused rapid death to larvae and prevent further development into pupae or adults. This point has not been sufficiently studied. Therefore, this study aimed to investigate the rapid effects of three treatments (1, 3, and 6 g per larva) of this fungus on the development of wax moth larvae under laboratory conditions.

MATERIALS AND METHODS

Greater wax moths were reared under laboratory conditions (temperature range from 25 to 30°C, and under complete darkness) on beeswax sections utilizing methods described by Ellis *et al*. (2013). The entomopathogenic fungi, *Beauveria bassiana*, were obtained from the Biological Control Unit of the Egyptian Association for Sustainable Agriculture. Forty Petri dishes (diameter 8.7 cm) were used in the study and in each dish 10 g of wheat flour mixed with 5 g of honey, 5 g beeswax and 2 g of bee pollens were placed. The spores (conidiospores) were placed in the Petri dishes mixed with the previous materials at three amounts (treatments) 1, 3 and 6 g.

Ten dishes were used per treatment and ten dishes were left as the control without fungi. In each dish, one larva of the greater wax moth (from the rearing stock) at age of 22 days (~3rd instar) was placed with a total of ten larvae per treatment. The experiment was done from October until the end of December 2019. The larvae were checked daily to observe their survival rate. The time at which larvae were died or pupated was recorded, as well as the time at which the adult emerged successfully. The length of pupa and adult were measured to check the variations between emerged adults.

RESULTS AND DISCUSSION

The numbers of dead and survived wax moths while exposed to different treatments of *B. bassiana* spores are presented in Table 1. All larvae in the control group completed their development and emerged as adults with a total period (calculated from 1 day old larva until emergence) of 59 to 82 days with mean (72.1 ± 2.69 days). The period of pupal stage until emergence ranged from 16 to 39 days with mean (29.1 ± 2.69 days). The normal development time of the greater wax moths ranges from 31 to 134 days based on environmental conditions (reviewed in Ellis *et al*., 2013). This indicates that the recorded developmental time is within the normal range of the development of the greater wax moths; therefore the used diet had no harmful effects on the development of the larvae. Treatments with 1 g of fungi per larva impacted the development of two larvae only. These two larvae were able to pupate after 80 days but failed to emerge, while the other eight larvae were able to complete their development normally after 62 to 84 days with mean (73.12 ± 2.79 days), and the pupal period was 19 to 41 days with mean (31.12 ± 3.10 days). Only one larva was able to complete the development normally after a period of 63 days (Pupal period was 20 days) when 3 g of fungi per larva were used. Two larvae died after 78 and 85 days, and seven larvae pupated after 70 to 75 days but failed to emerge. The length of pupa and adult moth were 2.1 to 2.2 cm and 1.4 to 1.5 cm, respectively for the control and the previous two groups with few differences than the reported typical lengths (1.2~2 cm for pupae and about 1.5 cm for adults) of the greater wax moth (Ellis *et al*., 2013). Thus, the emerged adult moths from treated groups were normal as the control group. No larvae were able to complete the de-

Table 1. Numbers of dead larvae, pupae and emerged adult wax moths while exposed to different amounts of *Beauveria bassiana* spores

Treatments	Total number of larvae	Number of dead larvae	Number of dead pupae	Number of emerged adults
1 g	10	0	2	8
3 g	10	2	7	1
6 g	10	10	0	0
Control	10	0	0	10

Fig. 1. Dead larva by the spores of the fungus, *Beauveria bassiana*.

velopment when 6 g of fungi per larva were used (Fig. 1). The ten larvae failed to pupate and died as larvae after 39 to 83 days with mean (59 ± 5.98 days).

The fungus was able to kill and impair the development of greater wax moth. In general, this result is corresponding with previous studies using laboratory experiments on larvae exposed to this fungus (Gupta *et al*., 1994; El-Sinary and Rizk, 2007). However, the argument that this fungus can cause high mortality to the 4th instar larvae (El-Sinary and Rizk, 2007) is not supported by the obtained results of this study. According to the present study, the high amount of fungi (3 and 6 g per larva) can hinder the development of the larvae than the low amount (1 g per larva). Notably, the effects are slow and appeared as early as 39 days and as late as 85 days. Wax moth larvae are expected to expose to a lower amount than 1 g and for a short period only during the field application of this fungus, especially the larvae tend to dig burrows inside the wax combs. Therefore, it is not anticipated that this fungus can cause any harmful effects on wax moth larvae under beehive conditions. Especially eight larvae were able to complete their development in a similar way to the control group when 1 g of the fungi was used, while larvae were in continual exposure to the fungus. Also, it is impossible to artificially contaminate wax combs with high amount of spores under field conditions.

CONCLUSION

Larvae are the harmful stage of wax moths to bee colonies. The majority of test larvae were able to complete their development to adults in case of using 1 g of *B. bassiana* per larva, and up to pupae in case of using 3 g per larva as showed in the present study. Only, 6 g per larva showed the ability to prevent the development of larvae to pupae or adults. It is not possible to expose wax moth larvae to such high amount of fungi per larva under hive conditions especially they feed inside the wax comb. Thus, this fungus is not appropriate as a potential method for wax moth control inside beehives, and research efforts should focus on other control options including the development of internal traps for wax moth larvae.

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