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GREATER WAXMOTH (Galleria mellonella L.)– AN IMPORTANT INSECT PEST OF HONEYBEES

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Abstract: Honey bees are highly important insects in the world which are known for their honey, wax, and other hive products and pollination of many crops around which human nutrition revolves. The decline in the bee population globally has been attributed to many factors interactive with each other. The greater wax moth in most honeybee colonies, *Galleria mellonella* Linnaeus, is a ubiquitous insect. In the midrib of the honeybee comb, the greater wax moth larvae build tunnels through the surface of both the pollen bee brood and honey unsealed cells. Crawling larvae leave underneath web masses that induce galleries and subsequent colonies to be expelled. In especially tropical and subtropical zones, larvae are drastic and are considered to be one of the factors contributing to the significant reduction of both wild and protected honeybee colonies. In bee colonies, the insect was historically considered a problem, but most experiments have relied on the pest as a framework for in vivo toxicology and pathogenicity experiments. It's quite effectively widespread, significant concern has been raised for further research findings to determine time-integrated management approaches. Furthermore, our understanding of this pest is very less. This review gives an overview of the latest understanding of the importance of resource loss to biology and the possibilities for management.

***Index Terms* - Biology, *Galleria mellonella*, Honeybee colonies, Infestation**

INTRODUCTION

In Hymenoptera, the super family Apoidea is considered to be the most significant group of insect pollinators, with an estimated 25,000 species described belonging to 250 genera and 13 families. Throughout India, approximately 1000 dominant bee species are found as pollinators **(**Grimaldi and Engel, 2005). Honey bees are an important part of the natural environment and influence the forest as well as the agricultural environment. Nearly one-third of the human diet comes from insect-pollinated plants and 80% of pollinated bees (Anonymous, 2014). The decline in the bee population globally has been attributed to many factors interacting with each other. Among these, trading of honey bees and hive products internationally (non-native species) (Pirk *et al.*, 2015), loss of habitat and fractionation, injudicious use of pesticides (Meixner, 2010; Goulson *et al.*, 2015; Johnson *et al.*, 2015). Five species of honey bees are found all over India, namely *Apis florea, Apis cerana, Apis dorsata, Apis mellifera, and Trigona iridipennis*. However, only *A. cerana* and *A. Mellifera* are commonly reared in hives. One of the biggest challenges to improving beekeeping industries involves identifying and offering ways to mitigate the adverse effects of honey bee pests and diseases. Honey bees colonies are infested by various insect enemies like hive beetle, wasps, ants, termites, mites, birds, mammals, and wax moth, which cause considerable losses. These enemies weaken the colony, thereby decreasing honey production and pollination. Among the insect pests of honey bees, one of the most destructive and economically important wax pests in the world is the greater wax moth (*Galleria mellonella* L.) under the family Pyralidae of lepidopteran order (Chang and Hsieh, 1992). Wax is one of the most useful and important products of bee-keeping which has its usage, particularly in the pharmaceutical and cosmetics industries. Many nutrients like pollen and honey are found in wax which attracts various pests including mammals. The larval stage of *G. mellonella* (with its 7 instars), the only feeding stage with the longest life cycle of all developmental stages, builds its silk-lined feeding tunnel in the honeycomb and feeds on wax, pollen, and brood cocoons. Adults do not feed on wax combs (Charriere and Imdorf 1997). This causes extensive loss of honeycombs and ultimately honey bees absconding from the weakened colonies (Ayman and Atef, 2007). Yearly, they destroy a lot of combs; attack the wax foundation and can reduce stored combs and weaken young colonies to a pile of debris. If the colonies they attack are unable to repel them, wax moths inflict enormous damage in apiaries. The colony's susceptibility to attacking moth can be caused by many factors such as starvation, illness, loss of the queen, or worker bees' large-scale mortality due to pesticidal poisoning. Wax moths may also be implicated in the spread of contagious diseases; especially the foulbrood disease. These moths are responsible for heavy economic losses reaching up to 60 to 70 percent to beekeepers in developing countries including India (Paddock 1918; Kapil and Sihag, 1983 and Hanumanthaswamy *et al.*, 2008).

DISTRIBUTION

Galleria mellonella (Pyralidae; Lepidoptera) is an insect ubiquitously distributed all over the world where honeybees are present. This pest causes major harm especially in the tropical and sub-tropical regions, in the honeybee colonies of Asian honeybee (*Apis cerana*). The greater wax moth was first recorded by Paddock in the year 1918 (Paddock, 1918). However, Williams (1997) and Shimanuki (1980) later characterized the pest as a ubiquitous insect in beekeeping. A recent case study in Kenya using novel scenario models has forecasted the future spread of honeybee pests, including the greater wax moth, in ecological areas that are widely considered unsuitable for pests, even though there are some regions currently free of pests (Makori *et al.*, 2017). The distribution pattern of the pest is also likely to change, with the changing climatic parameters.

BIOLOGY

Eggs: The size of the larger wax moth eggs varies with an overall length and width of 0.478 mm and 0.394 mm (Ellis *et al* 2013, Paddock, 1918). They are pearly white to light pink in color and have a rough texture due to wavy lines running diagonally at regular intervals. Throughout the developmental period, the egg changes from white to yellowish color. At approximately 4 days prior to hatching, the larva is visible as a dark ring within the egg. The completely developed larva is observable through the chorionic egg twelve hours before hatching (Paddock, 1918).An average of 300-600 eggs (maximum up to 1800) is laid by a single female in their two-week lifespan. Under controlled conditions, eggs can be seen deposited in clusters, pressed to the bottom edge of the paper strips given for egg-laying, however at certain periods, some eggs are also laid in small cracks of hives under nest conditions. (Venkatesh *et al* 2017).

Larvae: Upon hatching, wax moth larvae are approximately 1-3 mm in length and 0.12-0.15 mm in diameter (Smith 1965, Paddock 1918). Owing to the lack of sex-specific external morphological characters, sexing in males and females is not yet possible at the larval stage. The larva is polypodtype (Eruciform), with six legs on the thorax and a number of prolegs on the third to sixth abdominal segments. Immediately, the freshly hatched larvae begin to feed and spin the webbing. The larvae tunnel into the comb after hatching, covering their tunnels with a silky web as they reached the interior, where the larvae pass across a mass of webbing from comb to comb. They moult very slowly when resources are scarce (Vijayakumar *et al* 2019). The head capsule is yellowish and thinner than the prothoracic region (Paddock, 1918), which is more prominent. After hatching, the thoracic legs are well-formed; however, the abdominal legs are not noticeable until the larva is around 3 days old. During its growth, a greater wax moth larva molts 7 times. Most of the growth and size increment happens during the final 2 instars. Larval development lasts for almost 6-7 weeks at 29°-32°C and high humidity. A mature wax moth larva measures approximately 20 mm in length (Paddock 1918).

Pupa: Depending on the temperature, the developmental period of greater wax moths from larvae to pupae inside the cocoon varies from 3.75 days to 6.4 days (Paddock, 1918). Inside the cocoon, the newly formed pupa is white and becomes yellow after 24 hours. The pale colour of the pupa eventually becomes dark brown after 4 days towards its maturity (Hanumanthaswamy, 2008; Paddock, 1918). The size of pupae ranges from 5 mm to 7 mm in diameter and 12 mm to 20 mm in weight (Paddock, 1918). Female pupae are normally longer than males. The pupa is of obtect type, and all of its extremities are fused to the body by a hormone-induced during ecdysis. Pupation often takes place in spun cocoons covered with faecal pellets and frass, and provided with an opening which serves as an exit for the enclosing adult (Williams, 1997).On the eighth abdominal segment, the female pupa has a cloven sternum and a pair of small external rounded knobs on the ventral side of ninth abdominal segment, which are absent in male (Juhi *et al*., 2019).

Adult: The wax moth adult shows well defined sexual dimorphism. During late-night or night hours, eclosion of adult moths from the pupae takes place (Juhi *et al.*, 2019). Following two days of emergence, the perkiness of moths can be noticed. The female wax moth measures about 15-20 mm in body length, 31 mm in wingspan, and 169 mg in weight (Ellis *et al.,* 2013). The male is considerably smaller and less dark in colour compared to the females. The female moths have an almost straight distal forewing margin as opposed to the notched wing margin in male border (Smith, 1965; Shimanuki, 1981). In addition, the female has a forward-projected labial palp that gives the mouthparts a beak-like appearance, whereas it is deeply bent upwards and hooked inwards in males. The female antennae are 10-20% longer than those of the male (Paddock, 1918). Greater wax moths do not feed as adults and the longevity for females and males are 12 and 21 days, respectively (Paddock, 1918).GWM is a multivoltine insect, which undergoes between 4-6 generations annually.

SEASONAL ACTIVITY

Different workers, who have reported multiple overlapping generations of wax moth in a year, have performed seasonal occurrence studies. The wax moth populations are a persistent source of preserved and unused combs, improperly cleaned wax, and weak or poorly maintained colonies. Based on food supply, temperature, pest habitat, over one year, it is possible to create multiple generations. Successful wax moth's population are active from March to October (Garg and Kashyap 1998) However, its highest response was recorded between June to November (Ramachandaran and Mahadevan, 1951; Brar *et al.*, 1985; Gupta 1987). The highest infestation of this pest was noted during the dearth period in South India (Viraktamath, 1989). In preserved combs, it hibernates in larval (about 70 percent) and pupal stages (about 30 percent)

ECONOMIC SIGNIFICANCE OF GREATER WAX MOTH

Due to the destructive feeding habit of its larvae, the GWM is taken into the account the foremost significant pest of honeybee products (Williams, 1997). The larva relies on the skins of the pollen, honey, wax, cast-off honeybee pupa produces tunnels within the comb, and creates masses of webs on the frame. (Türker *et al.*,1993: Williams, 1997; Nielsen and Brister 1979; Shimanuki, 1980). Damage happens when the larvae form a silk-lined network through the Walls of hexagonal cells and over the surface of the comb. The tunnels and borings formed on the wax caps by the larvae chosen sites through which honey spills, making the parts unfit for consumption (Shimanuki, 1981). The silken threads comprise emerging bees that die of hunger, a process described as galleries (Williams, 1997). Furthermore, Infestation of apiaries by larvae of the greater wax moth also leads to the destruction of colonies, absconding, and reduction of the migratory bee swarms (Williams, 1997). The potential economic wax deficit attributed to *G. mellonella* in Iran, the infestation was around 38 percent. (Jafari *et al.,* 2010). Honey bees have typically evolved hygienic and hygiene behaviors against hive intruders, like social encapsulation (Ellis *et al.*, 2003; Neumann *et al.,* 2001). The GWM uses counter-attack strategies to avoid bee defenses, and as such, adults will still emerge from pupae encapsulated with a layer of propolis and fly out of the hive if honeybees strike violently (Nielsen and Brister, 1977). Additionally, as bees are less aggressive during the grooms, gravid females enter poorly secured colonies and deposit eggs masses in crevices and comb colonies with loosely packed pollen (Nielsen and Brister, 1977).

NATURE AND EXTENT OF THE DAMAGE

Most people still consider the greater wax moth as a valuable insect since its larvae are being used in several countries as fish bait; so that they are commercially raised to get their larvae. It does, however, trigger significant commercial losses annually, to the beekeepers. Typically the larvae remain in beehives which feed on wax and young bees, fill the hive tunnels with silk threads. The larvae are especially harmful to colonies that are old or unguarded, and to combs stored. However, most Asian honeybee colonies are vulnerable to infestation by wax moths (Adalakha and Sharma 1975; Brar *et al.*, 1985; Viraktamath 1989). Damage to several folds in *A. Mellifera* and *A. cerana* Colonies is increased during the dearth and rainy seasons. In *A. Dorsata*, its pattern of seasonal infestation varies from that of the domesticated bees. The wax moth population begins to grow in March, hitting its peak in August (99-100%) and then decreases till after February (Thakur, 1991). Moth destroys brood, cells, pollen, and honey areas at all stages. Wax moth larvae can start reducing the web and debris to a mass of combs. Extreme outbreak results in a reduction of brood rearing, foraging activity, and eventually colony exclusion from the nest. Weak colonies (53%) are much more vulnerable to infestation with wax moths especially in comparison with strong colonies (11%) (Thakur, 1991).Other workers (Newton, 1917; Kreb, 1982; Jyothi *et al.*, 1990).Infestation in abandoned combs recorded by one percent (Nielsen and Brister, 1979). The wax moth attraction was noticed to be more attractive towards strong colonies than to weak colonies.

MANAGEMENT TACTICS

A strong hive is the best defense against wax moth here; we concentrate on the strategies of past and present management and further highlight their benefits and limitations that should serve as a basis of managing for Wax moth's successful control.

1. CULTURAL METHODS:

The GWM are most efficiently managed through the maintenance of proper sanitation. This includes maintaining the colony healthy and covering cracks and crevices with appropriate sources of food (particularly in places invaded by Asian honeybees because they are weak proponents). In addition, beekeepers must reduce the application of pesticide usage to periodically replace combs and kill infested combs exhibiting galleries signs. In addition, beekeepers need to have a suitable storage facility for hive materials that are vulnerable to pest attacks and to protect colonies from diseases and pests. Such approaches are quick to introduce and do not have any harmful effects on all honeybee populations and non-target species. The cultural activities, however, the cultural practices are routine and only operate well in beekeeping operations on a small scale. Management methods like strengthening honeybee colonies via feeding, reducing and adding unoccupied frame, reducing super are the best practices for reducing wax moth in a number of infected combs and strongly increment of pollen, nectar, brood, bee population and honey yields, Significantly low wax moth infestation level and high honey yield were recorded. It is also interesting to note that giving fed in dearth periods is a pre-dominant wax moth control method (Gela *et al.*, 2013). Leave the infested combs empty in the heat for a few minutes. During the initial stages of infestation, destroy the tunnels to kill wax moth larvae. During the advanced stage of infestation. The whole comb must be destroyed to check the wax moth population. Do not leave the empty combs in open for a long period at the apiary site. (Omkar, 2017).

2. PHYSICAL METHODS:

These techniques are advantageous since the growth and development of GWM are dependent on environmental factors such as temperature. However, the techniques are only applicable in the absence of living honeybee stages (Charles, 2017). The disruption of the GWM growth period can be achieved by exposing the GWM resistance spectrum of beekeeping equipment and bee combs to temperatures beyond (heating technique) or even below (freezing technique) (Ritter *et al.*,2006). These approaches are useful when GWM's growth and development are preferable. It relies on environmental variables, such as temperature (Gulati *et al.,* 2004). The techniques are, furthermore, only valid in the absence of living honeybee stages and in small-scale apiculture, as increased costs will be needed for large-scale agriculture (Charles *et al.*, 2017). Providing artificial Cold -70C for 4-5 hrs or -120C for 2 hrs is another effective way of killing all stages (Gulati and Kaushik, 2004). Infested combs can also be exposed to cold rooms or refrigerator equipment such as home freezers set at −7 °C to −15 °C for 2–4.5 hrs (Charles, 2017). The most rapid pest control is obtained by deep freezing at -l7°C, the usual temperature of a butcher's freezer room. Longer exposures are effective at temperatures above, but near, 0°C, such as those in cold-rooms and domestic refrigerators (H.D. Burgers, 1978). Hot water at 60ºC for 4-5 to kill all larvae of *G.mellonella* (Gulati and Kaushik, 2004). Exposed to higher temperatures of approximately 45–80 °C for a period of 1–4 h in large scale farming. The combs are kept in hot water for 3-5 hrs for small-scale farming. It must be observed, similarly, a certain heating sags and distorts wax. (Charles, 2017). 80 minutes at 46°C or 40 minutes at 49°C (Charrière and Elmendorf, 1997). Cold frames and portions of the hive at -7 ° C will destroy all wax moth stages within 4-5 hours. -150 C for 2 hours or –12°C for 3 hours or –7°C for 4.5 hours effective and kills all stages (Charrière and Elmendorf 1997).

3. BOTANICALS:

Neem, Pongamia, Tulasi, (Surendra *et al.* 2010) Eucalyptus, Annona, Clerodendron plant extracts works effectively in control of wax moth larvae. A Plant extract, Muristeron -A from kernels of ipomoea shows the fatal and molting effect on grown-up *G.mellonella* larva. (Bolchi, 1979).*Azadirachtin* disrupted the molting process in larva and pupa and molts of last instar larvae and also induced mortality (Malczeuska *et al*, 1988). The essential oil of *Origanum majorana* was highly fatal to *G. mellonella* (Mohamed et al.2014). Neem oil, cedar oil, clove oil, peppermint oil, Karang oil, and neem seed kernel extract highly effects mortality greater wax moth (*Galleria mellonella*) in storage conditions. (Kalpana *et al.,* 2017). Some studies show peppermint ethanolic extract, Chinese propolis, cinnamon, clove, and Egyptian ethanolic extracts show mortality against *G.mellonella* 4^{th} instar larvae at higher concentrations (Asmaa *et al*. 2017). Negramina oil (*Siparunaguianensis*Aubl.) was equally effective in suppressing the greater and lesser wax moth population without affecting the bees (Ferreira *et al.,* 2017). Leaf extracts of *H. sativum*, *Raphanus sativus*, *Linumu sitatissimum* , *Cucurbita moschata*, and Vicia sativa, Husk extracts of P. psyllium are said to be highly effective in the control of wax moth larvae (Lalita *et al.* 2018). Mint, Chinese propolis, cinnamon at different concentrations works against the late larval instar of wax moth (Sanad and Mohanny 2015). Tobacco leaf extract, neem oil, castor oil, and groundnut oil were moderately effective in checking egg-laying by *G. mellonella*in live colonies. (Shylesha,1987). That feeding the *Galleria mellonella*larvae in artificial diets contains 0.5, 1, 2, and 4% NeemAzal-T/S caused 100% mortality. The larvae could not molt to the next instar, while 0.25% was the lowest value of the larval mortality. The results also indicated that rearing the *G. mellonella* on the different ages of beeswax combs with 4% NeemAzal-T/S showed that all the tested wax moth larvae died during the first week of treatment. Treatment of beeswax with NeemAzal-T/S at different dosages resulted in retarded growth and death of larvae and pupae of *G. mellonella* and that the mortality was concentration-dependent. The mortality increased from 63% at 20 ppm via 69% at 40 ppm to 100% at 80 ppm NeemAzal-T/S. (Elbehery *et al*., 2016).

4. BIO CONTROL:

With the development of new techniques in the field of biotechnology, there was the potential of making a breakthrough using *Bacillus thuringiensis* (Ritter *et al.,* 2006). Furthermore, a strain of GWM that had decreased the expression of genes involved in the binding of Cry protein was identified, resulting in *Bt*. Inefficient towards wax moths (Dubovskiy *et al.,* 2016). The bacterium *Bacillus thuringiensis* attacks moth larvae without harming the bee or spoiling honey for human consumption. *Bt*. isolates are effective in preventing infestation of *Galleria mellonella* on stored honeycombs and showed protection range varied from 89.5 – 44.3 percent (Taredahalli *et al.,* 2013). It was observed that early instar larvae of greater wax moth were potentially vulnerable to commercial V-Bt and induced higher rates of mortality.(Vijayakumar *et al.,* 2019). The *Bacillus thuringiensis* bacterium were found in 1911 and has already been widely used for many years for plant protection. For its operation against the Wax Moth, the bacterial strain of product B-401 was used. Spores containing a toxin are formed by the bacterium. The poison is liberated and destroys the intestinal walls as the larvae eat the spores. Adult Wax Moths doesn't feed on this item and are thus not threatened by it. *Bacillus thuringiensis* is a bacterium that is safe to vertebrates (man, livestock) and bees and left no wax or honey traces. (Charrière and Elmendorf, 1997). *Apanteles galleria,* a larval parasite found effective against wax moth larvae. (Gulati and Kaushik, 2004) Some hymenopteran parasitoids specifically *Apanteles galleria* Wilkinson (Braconidae) attack the caterpillars of *G. mellonella* (Ahmad *et al.* 2014), *Apameles galleria* started parasitizing only from second instar larvae of *G.mellonella*. During various larval instars, the percentage of accumulated parasitization showed that the third instar larvae of waxmoth. The most favoured for parasitization was (88.89 followed by the fourth instar larvae in which 68.89% parasitization was recorded) (Chhuneja and Yadav, 2012). *Galleria mellonella* was highly prone to entomopathogenic fungus, *Beauveria bassiana* against third instar larvae. The efficacy of the fungus was high at all the tested concentrations under lab conditions for 11 days. Mean mortality was slightly different between concentrations ($F= 35.75$, $P < 0.05$) and significantly higher compared to control in all doses. The fungus has a strong capacity to handle wax moth populations and serves as a potential biological control agent against *G. mellonella* (Adam *et al.,* 2017). Extreme freezing environment, EPN (isolates 69, 79, 99, 102, or D) recorded that 800 IJs / dish (containing 10 larvae) induced larval mortality, excluding isolate 69 at 10 ° C. As a control treatment, *Steinernema Cubana* with a contagious nature at high temperatures was used. D isolates, mortality at 10 ° C hit 100 percent and at 4 ° C the wax moth larvae's highest kill was 81.7 percent from isolate 999 percent (Mráček *et al.,* 1997). The red imported fire ants (RIFA) *Solenopsis Invicta* and *Solenopsis germinate* feed on immature stages of but when evaluated as a biological control agent of the moth in stored super combs, *S. Invicta* could only be effective in combination with promoted light and ventilation conditions (Charles,2017). It is also clear that it remains a long shot to use either entomopathogens or transgenics to express their toxins.

5. CHEMICAL METHODS:

Sulphur, Acetic acid, Ethylene bromide, Calcium cyanide, Methyl bromide, Phosphine, Para dichlorobenzene (PDB) Naphthalene and Carbon dioxide are fumigants which were historically using and have been extensively studied towards wax moth. At present, for use as a fumigant, only carbon dioxide is recommended. Fumigants were applied under airtight conditions in preserved combs. All fumigants targets and destroy all life stages of the moth, except PDB, which does not destroy eggs Acetic acid vapors causes ovicidal and adulticidal effect on wax moth and suggested that longer exposure of the larva in cocoon was essential to cause its mortality, Based on of these observations an immediate treatment of the combs after removing them from the colonies is suggested so that hatching of eggs did not take place. (Charrière and lmdorf 1997) .Use of few chemicals and fumigant insecticides such as sulphur dioxide, acetic acid, formic acid, Para dichlorobenzene (PDCB), methyl bromide or phosphine is detrimental to the bee colonies and were being used to monitor the wax moth on the beeswax combs in storage conditions (Whitcomb, 1967; Calderon, 2000). An important step to preserve the combs from greater wax moths is the fumigation of stored combs. To prevent honeybee combs from wax moth infestation, Naphthalene balls can be used. Sulphur fumes produced by burning sulphur over live charcoal (@28.35gm/0.16m^3 space) could also be used to disinfect new combs, sulphur fumigation (@25.30gm / lit m3) recognised to be the safest and easiest to use. Phostoxin pellet fumigation applied in an air-tight space is regulated by GWM cellophane sheets (Mahmood *et al*.1994).The only suitable chemical methods employ poisonous gases. Ethylene oxide (mixed with an inert gas), ethylene dibromide, and phosphine are safer than the highly poisonous methyl bromide, which is very effective. Such hazardous materials should be applied only by properly trained personnel (Burgers, 1978).More specifically, they are toxic to bee populations and non-target insects and in many regions where beekeeping is practised, and they currently face significant challenge.

TABLE.1. Treatments that can be used for the effective control for *Galleria melonella*

BOTANICALEXTRACTS, VOLATILE AND ESSENTIAL OILS	MICROBIALS	CHEMICALS
Azadirachta indica	*Bacillus thuringiensis*	Sulphur fumigation
Ocimum basilicum	*Beauveriabassiana*	Acetic acid
Vernonia amygdalina	*Pseduomonasputida*	Formic acid
Annona squamosa		Eugenol
Cymbopogon citratus		Para dichlorobenzene
Origanum majorana		Methyl salicylate
Cymbopogon proximus		Naphthalene
Pongamia pinnata		
Ocimum sanctum		
Pepper mint oil		
Blue gum oil		
Basil oil		

CONCLUSION

The greater wax moth continues to be a global challenge to bee health and the beekeeping industry. The bee-keepers should strive to take control and manage the damages caused by GWM through IPM. An essential aspect of the IPM program must be botanical, bio control and cultural practices mainly in small-scale apiculture operational activities and in circumstances where the population growth rate of the pest is high. If the emphasis moves to the production of new methods, temporal and spatial differences need to be taken into consideration as they are likely to promote the efficacy of these methods in various regions of beekeeping. In particular, the successful introduction of IPM involves cooperative actions, in particular by beekeepers in close vicinity, since in the case of repellent items; the risk of practicing on an apiary having a detrimental effect on a neighboring apiary cannot be excluded.

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