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CONTROLLING AMERICAN FOULBROOD IN HONEYBEES BY SHOOK SWARM METHOD

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ABSTRACT: American Foulbrood (AFB), caused by the bacterium *Paenibacillus larvae* is a serious disease of honey bees worldwide that inflicts considerable economic losses to beekeepers. All work was undertaken in a commercial apiary that showed visual signs of AFB infection. Twenty four hives positive for AFB were identified from the apiary (4 colonies in each replicate). Half of the colonies were treated with shook swarm method while other half were left without any treatment. The treated colonies showed significant difference from untreated colonies (t test, P< 0.005) and moribund colonies got recovered with increased bee population while the control colonies collapsed. It is therefore concluded that shook swarm method should be incorporated for prevention as well as curing of AFB.

Key Words: Apis mellifera; Paenibacillus Larvae; Shook Swarm Method; American Foul Brood; Population; Prevention; Curing; Pakistan.

INTRODUCTION

Most of the diseases that affect honey bees are little more than a nuisance, but some are serious and a few are lethal not only to the individual bees but to the whole colony (Fries and Camazine, 2001). To diminish the impact of disease in honey bees is of interest not just because of the well-being of the bees and the value of the honey they produce for the beekeepers, but the value of pollination which is estimated to exceed the value of the products from beehives manifold (Delaplane and Mayer, 2000).

Prior to the arrival of the parasitic mite, *Varroa destructor*, the most important diseases of honey bees worldwide were the bacterial brood diseases European Foulbrood (EFB) and American Foulbrood (AFB). AFB is still the most deleterious bee disease throughout the world (Shimanuki, 1997), caused by the bacterium *Paenibacillus larvae*, that inflicts considerable economic losses to beekeepers (Genersch et al., 2006). This disease is considered to be especially severe because it can kill entire colonies, and once becomes established in a region its eradication is very difficult (Matheson and Reid, 1992). Early diagnosis is therefore important for preventing its spread. The spores are extremely infective and one dead larva may contain billions of spores (Hansen and Brodsgaard, 1999).

Contaminated hive material or products can cause outbreaks many years after the original disease was treated. Because AFB is very contagious and lethal at colony level, it is very important to have reliable methods to detect outbreaks before they spread and become more difficult to control. Reliable detection methods are also of great importance for studies of pathogen transmission within and between colonies. Methods exist for the identification of hives with sub clinical infection that require the inspection of either honey samples (Alippi, 1995; Nordstrom and Fries, 1995) or bee samples (Hornitzky and Karlovskis, 1989). The examination of honey for viable spores may be of value in tracing disease outbreaks (Hornitzky and Clark, 1991). Some companies that market queens and package bees improved for hygienic behaviour (HB) periodically check for AFB and routinely analyse honey samples as part of their efforts to prevent the disease. How-

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ever, it is difficult to predict the relationship between the number of spores present in honey and the appearance of the disease (Hansen and Brodsgaard, 1999).

According to Goodwin et al. (1993) three possible states of infection that exist are contamination; *P. larvae* are present in the hive but cause no ill effects sub clinical infection; *P. larvae* adversely affects at least the larvae, although no disease is apparent to observers, and clinical infection; *P. larvae* adversely affects the larvae and visible signs of AFB are present.

The bacterium is ingested by the larvae during feeding by adult bees, then resides in the larval gut, and competes with it for food (Bailey and Ball, 1991). If food is in short supply, the brood food will be consumed by the *P. larvae*, causing larval death by starvation and visible signs of AFB in the colony (Bailey, 1983). However, if there is plenty of food, the larva will develop normally and pupate, excreting the bacteria during development. This will leave potentially infective bacteria in the cell after the bees have emerged.

In recent years, an alternative form of treatment known as the shook swarm + oxytetracycline (OTC) method has been used for AFB affected colonies which aims to eliminate this potentially infective reservoir of bacteria. This old technique used before the advent of antibiotics involves the transfer of adult bees from the diseased colonies into a new hive box with new foundation (Morse and Shimauki, 1990). None of the brood comb is removed to the new colony, but this is all destroyed. The new colony is fed with sugar syrup containing dose of OTC which is thought to limit carry over of bacteria on adult bees. The feeding of sugar stimulates the colony to draw out the foundation in order for the queen to re-establish itself. Removal of the potentially infective material should reduce the possibility of further foul brood recurrence.

Of the methods available today, adult bee sampling has been shown to reflect the current disease status of the colony most correctly (Nordström et al., 2002). However, the method needs further evaluation at different levels to determine its usefulness and limitations both for screening purposes and epidemiological studies.

It has been stated that one of the main factors that increase the virulence of a disease is the route of transmission between hosts (Lipsitch et al., 1996). Horizontal transmission refers to pathogen transmission between individuals within generation which is equivalent to transmission between colonies in honey bee system while vertical transmission means pathogen transmission between individuals of different generations. AFB has been thought to be mainly horizontally transmitted, level virulence (Fries and Camazine, 2001). However, little is known about AFB modes of transmission under natural conditions. Several authors have reported honey bee lines to be tolerant to AFB (Spivak and Gilliam, 1998) but fail to note the *P. larvae* spore loads of the examined hives.

This investigation aims to determine the effect of shaking technique or shook swarm method in a commercial apiary of *Apis mellifera* for controlling AFB.

MATERIALS AND METHODS

The field area of Haripur was selected for the experimental purpose. To take part in this trial 24 *Apis mellifera* colonies infected with same level of infection (as determined by the larval mortality and Foulbrood stretch test, in which a small dry stick is inserted into diseased larvae and removed slowly. The remains of larvae will be of a light coffee colour and will stretch with the consistency of glue into a fine thread) and of same size were selected (Table 1). Half of the colonies were used as control i.e., not subjected to any treatment while the other half of the colonies following a preliminary 48h stay in a cool place (starvation phase: the bees are grooming each other) without food were subjected to shaking technique i.e., bees were shook from their old combs in hives on 4” strip of un drawn wax foundation with the help of funnel and kept for two days. During this

Table 1. Status of honeybee colonies before start of experiment

T_1 (later subjected to shook swarm method)			T_2(Control)		
Bee frames	Brood frames	Honeycombs	Bee frames	Brood frames	Honeycombs
4.0	1.5	1.5	4.0	2.0	2.0
3.5	2.0	2.0	1.5	1.5	1.5
4.0	2.5	1.5	5.0	2.5	2.5
3.5	2.0	1.0	3.0	1.0	2.0
3.5	2.0	2.0	5.0	2.5	2.0
5.0	2.5	2.5	3.0	1.5	0.5
4.0	2.0	1.5	4.0	2.0	1.0
3.5	1.5	2.0	3.5	1.5	0.5
4.0	2.0	2.0	3.5	1.5	2.0
3.5	1.5	0.5	4.5	2.0	1.5
0.5	1.5	1.0	5.0	2.0	2.0
4.0	2.5	0.5	4.0	2.5	2.5
Mean 3.5	1.95	1.5	3.8	1.87	1.6

time the bees started building comb and all the pathogenic spores were excreted in the environment through mouth and with faeces. After that old combs with brood were removed and heavy wooden parts from the infected hive were burnt and the bees were transferred to new clean hives with new foundation sheets. Treatments were carried out at the same time i.e. April, 2006 within the apiary during early evening to lessen the chance of the shaken bees for drifting or being robbed in the vicinity. Plastic sheets were spread on the ground while treating bees which were later burnt out to avoid spread of infection in open area.

Up to the end of the active season the observation i.e. foraging behaviour, brood rearing, health of colony, increase in population etc were made on all the colonies.

RESULTS AND DISCUSSION

American foulbrood is the most severe disease (Matheson, 1996) that without proper treatment results not only in death of affected bee colonies but also in death of entire apiaries. Lately the disease is becoming a problem in the world. The shook swarm technique has been used for the control of foulbrood in several countries, including France, Denmark and Australia (Hornitzky and White, 2001). The traditional methods of control through killing and burning of affected bee families those were in use until several years ago and the treatments of the other bee families with antibiotics and sulfonamides were a real hazard with regard to the accumulation of drug residues in honeybee products. That is why the use of antibiotics and sulfonamides in most European countries is prohibited by the law (Law on Apiculture, 2003). It is therefore very important to develop and implement the alternative methods for the control of AFB that exclude the use of antibiotics.

Alternative methods of control are essentially related to the development and implementation in the practice of methods for early diagnosis of the disease via detection of *P. larvae* spores in the bee honey and bees wax (Hansen and Rasmussen, 1986; Ritter, 2003).

A widely used alternative method is the artificial swarm method, used in several modifications- with restrain of bees in a dark premise, without restrain, with interchanging the places of combs in the hive (Ritter, 2003) while our method was simply transfer of honeybees by reducing the spores in the mouth of bees by starving them and the shifting the bees to new clean hives with new foundation sheets.

SPSS statistical programme version 14 analyzed the data. Comparisons between means were made using the least significant difference (LSD) at 0.05 probabilities. The selected colonies were com-

pared and it was found that all the colonies were same in bees (t test, $P > 0.05$), brood frame (t test, $P > 0.05$) and honey combs (t test, $P > 0.05$). After applying the shook swarm technique the results from our experiment clearly demonstrate that swarms decrease their spore load significantly as at the end of experiment the colonies treated with swarm method were still alive. The treated and untreated colonies were compared and significant results were obtained for bees (t test, $P < 0.005$), brood (t test, $P < 0.005$) and honey combs (t test, $P < 0.005$) respectively. None of the treated colonies showed any symptoms of AFB and thus all the colonies that survived eventually decreased their spores load to undetectable levels (Table 2). The study shows that artificial swarming is an efficient treatment for AFB. The results from artificial swarming are congruent with the study of vertical transmission of *P. larvae* spores in natural swarms. The fact that no colony or swarm showed any clinical disease post shaking and that the decrease rate was similar to all colonies, shows that there is some mechanism that aid the bees to reduce the spore's load they carry before they have any brood. It is probably the same mechanism that reduces spore loads in natural swarms, but the nature of this mechanism needs further study.

In Denmark, the shaking method is successfully used (Hansen and Brodsgaard, 2003).

The method involves the transfer of adult bees in non-infected combs on frames with mounted wax foundation or strips and burning the brood combs from clinically ill families. Transferred bees consume the contaminated honey while building the new combs. The results showed that the shaking method reduced considerably the number of *P. larvae* spores to safe levels, and according to some German investigators, a complete decontamination could be achieved (Oehring, 1998).

Although shaking AFB hives is an efficient control method, there are also good arguments to continue stamping out the diseased colonies where this method is used. In Sweden, this technique has dramatically diminished the rate of diseased colonies since applied in 1974 (Anon, 2005). Data from New Zealand also show that stamping out of diseased colonies has decreased the number of colonies that become infected each year (Goodwin and Van Eaton, 1999). In Denmark, where shaking of AFB diseased colonies is allowed the prevalence of AFB is higher than in Sweden (Hansen, 1992).

It is an economic loss to the individual bee keeper to burn the infected colonies, but there is also a substantial cost to shaking in manual labour and investments in

Table 2. Status of honeybee colonies after applying shook swarm technique to half of colonies at the end of experiment for three replications

T_1 (subjected to shook swarm method)			T_2(Control)		
Bee frames	Brood frames	Honeycombs	Bee frames	Brood frames	Honeycombs
6.5	2.0	10.0	2.0	0.5	0.0
7.5	3.0	8.0	4.5	1.5	2.0
8.5	3.5	11.0	2.0	0.5	0.0
5.5	2.0	7.0	0.0	0.0	0.0
5.5	2.5	4.0	1.5	0.5	0.0
6.5	2.0	6.5	2.0	0.0	0.0
8.0	3.0	8.0	3.5	0.5	1.0
4.5	1.5	4.5	0.0	0.0	0.0
5.5	2.5	10.0	3.5	0.5	1.0
4.5	1.5	6.0	0.0	0.0	0.0
6.5	2.0	7.0	2.0	0.5	0.0
7.0	2.5	8.0	2.0	0.5	0.0
Mean 6.3	2.3	7.5	1.9	0.4	0.3

clean equipments. Unless queen excluders are used, colonies may abscond (Hornitzky and White, 2001) and queen losses do occur in the process of shaking. As a result of these procedures the bee colonies did not show any signs of mortality at the end of the experiment suggesting that this method could be successfully used for the control of AFB in Pakistan.

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