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Hygienic behavioral response of *Apis mellifera* Ligustica against pin-killed and infectious brood

Joni Kumar and Pramod Mall

Abstract
The present investigations were carried out in apiary, Department of Entomology, G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand) India. Evaluation of colonies for the hygienic behaviour was done by using "pin killed" methodology. Twenty colonies were selected for this study. These colonies were divided into two categories: healthy colonies (HC) and European foul brood infected colonies (IC). Three frames of brood were taken out from every colony and killed by entomological pin. The pin killed brood cells were marked for the purpose of identification and the observations were recorded at 24 h and 48 h. The pin killed brood assay revealed the removal if killed cells within 24 h and 48 h. The mean removal of killed cells by worker bees in IC is significantly higher than in HC. Our study provides a clue of the importance of this behaviour in removal of pest and diseases infected brood from colony.

Keywords: Honey bee, *Apis mellifera,* hygienic behaviour, pin-killed method

1. Introduction
The brood rearing is an essential activity of bee colonies depending upon the availability of pollen, nectar and climatic factors prevalent in the locality. A strong colony has an advantage not only to have higher honey yield but also that such colonies are better adapted to fight the enemies and overcome adverse conditions. The brood rearing activities may be affected by the environmental factors like wind, temperature, relative humidity, rain etc. Hygienic behaviour in *Apis mellifera* consists of the removal of dead larvae from colony by worker**.** The removal of dead larvae/ any strange material from colony by worker is known as hygienic behaviour. A Pin-killed brood bioassay test [16] was carried out on selected colonies managed by local beekeepers that have little or no knowledge about application of chemical treatments for honey bee diseases and parasitic infections. There is little awareness among the local beekeepers on hive management practices that can best be employed to reduce the scourge of colony diseases and parasitic infections. However, the poor attention paid by beekeepers to management of colony diseases, parasites and pests has become an advantage; research investigations reveal that the bees have been dependent on self-defense or natural resistance [1, 2]. Hygienic behaviour is a key assessment of general resistance of bees to diseases and pests. Hygienic bees can easily detect, uncap and remove diseased or parasite-infested brood: brood infested with *Varroa*-mite [10, 11], brood infested with American foulbrood [12, 13] and chalk brood [5, 6]. It is widely accepted that hygienic behavior of bees play important role in removal of dead and infected broods from colony [15-16]. As part of our effort to better understand the details of this behavior, we examined whether grouped dead brood affected by bee hygienic behavior.

2. Materials and Methods
The present experiment was conducted in the apiary, Department of Entomology, G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand, India). Pantnagar is situated at the foot-hills of Himalayas at an altitude of 243.83 meters above the mean sea level (MSL), 290 N latitude and 79.30 E longitude. The climate is humid sub-tropical with maximum temperature ranging from 32 ºC to 45 ºC during summer and minimum ranging from 00 to 9 ºC during winter. The monsoon occurs from 3rd week of June and continues till the middle of September with average annual rainfall measuring 1400 mm. All colonies were marked for ease of collection of data and diagnosis. Brood combs in all the colonies were examined for presence of pests and diseases. Ropiness and non-ropy diagnostic tests were used to confirm the presence of American and European foulbrood diseases respectively.

Colonies that were found infected with any disease or infested with pests were grouped together as infected colonies (IC) while those free from any infections and infestations were grouped together as healthy colonies (HC). Fifteen healthy colonies (HC) and 5 infectious colonies (IC) were randomly selected for colony hygienic test (Table 2). To observe the hygienic behaviour "pin killed method" was used with slight modification in the method developed by [8] and further modified by [7] has been used. In this method, the broods were damaged or killed with an insect pin, which is used to pierce the sealed brood cells through the center of the cell cap, penetrating the body of the pupa. Three frames containing capped brood cells were taken out from each colony and damaged by insect pin. The pin killed brood cells were marked for the purpose of identification and the observations were recorded at 24 h and 48 h. These observation was repeated every fifteen days interval.

3. Results and Discussion

All the colonies were kept in same geographical area and there is no site related difference observed during study. All the bees are of same species. During honey flow season all colonies were shifted from Pantnagar (UK) to Pilibhit (U.P).

Of the twenty colonies, fifteen were found free from infestation of pest and diseases, whereas 5 colonies were infectious. In infectious colony, the mites population was recorded from 18-38 (number of mite) per colony. European foul brood infection was also found in mite infested colonies, where 80 to 130 brood cells were infested. There were no records of American foul brood and other diseases of honey bee. Each colony have both solid and scattered brood pattern possessing many empty cells. Response to disease diagnosis was same in both brood patterns.

The percentage of removed and dead brood observation from HC and IC were recorded at 24 and 48 hours (Table 1 and 2). The percentage of dead brood that were removed and uncapped after 24 hours in HC range from minimum 35.33 per cent to a maximum of 38.09 per cent with 37.57±1.10% Mean ±S.D (Table 1) while in IC, the percentage of dead brood that were removed and uncapped after 24 hours range from minimum 38.06 per cent to a maximum 43.15 per cent with 41.21±1.77 per cent (Mean ±S.D) (Table 2). The mean value of removed and uncapped brood of IC is significantly higher than HC in 24 hours (t-calculated= 4.32, t-critical=1.73, df =18 and p=0.05) (Table 3). Similarly, after 48 hours, the range of dead brood removed and uncapped in HC was from minimum 88.71per cent to a maximum of 92.08 per cent with 90.39±0.99 per cent (Table 1) while in IC, the range from minimum 96.96 percent to a maximum of 100.0 percent with 98.28±1.11 per cent (Table 2). The mean of dead brood removed by the worker bees in IC is significantly greater than in the HC (t-calculated= 14.12, t-critical=1.73, df =18 and p=0.05) (Table 4).

Worker bees start hygienic behavior by puncturing the capped brood cell, making small holes in it, followed by uncapping of the brood cell and removal of the brood. However, the brood can be partially or totally removed. Rarely, punctured cell was followed by capped cell. The brood pattern of all colonies revealed that the bees actively removed the dead or infected broods. This brood pattern could be used to assess the performance of colony, queen bee health and its egg laying capacity. Queen relays in removed cells, which hatch out at different interval to form solid or compact appearance of broods. [12, 18, 16] claim that spotty brood patterns are created due to hygienic behaviour of the bees to naturally resist Chalk brood and American foulbrood. The Observation of both HC and IC performing uncapping and removal of dead brood supports this ascertains. Although, in IC, uncapped and removed dead brood is higher and spent significantly the same time in uncapping and removing of diseased brood than HC. [3, 10] found that healthy colonies are more efficient in detecting, uncapping and removing diseased or dead brood as compared to the non-healthy colonies and the overall colony efficiency shows that the healthy colonies removed more diseased brood than the non-healthy colonies within the same time frame. [19, 4] claim that bees in non-healthy colonies observed recapping cells that were previously uncapped by other worker bees. This claim supports our results where some infected colonies were found with compact or solid brood patterns.

Our results show that bee can develop hygienic behavior in response to the attack of diseases and pests in absence of any external treatment of colony. Further study is required at molecular level to identify the genes responsible for hygienic behaviour to better understand how bees behave overtime.

Table 1: Number of dead brood removed and uncapped at 24 hrs and 48 hrs in healthy colony (HC)

Colony No.	Colony status	Percent of dead brood removed and uncapped		Percent of dead brood not removed	
		24 hrs	48 hrs	24 hrs	48 hrs
1.	HC	38.09%	89.50%	61.91%	10.5%
2.	HC	39.85%	90.47%	60.15%	9.53%
3.	HC	35.33%	88.71%	64.67%	11.29%
4.	HC	38.09%	90.12%	61.91%	9.88%
5.	HC	37.61%	91.65%	62.39%	8.35%
6.	HC	38.09%	91.00%	61.91%	9.00%
7.	HC	36.85%	88.85%	63.15%	11.85%
8.	HC	38.09%	91.95%	61.91%	8.05%
9.	HC	38.09%	92.08%	61.91%	7.92%
10.	HC	38.09%	90.45%	61.91%	9.55%
11.	HC	36.85%	89.35%	63.15%	10.65%
12.	HC	38.09%	90.55%	61.91%	9.45%
13.	HC	37.61%	90.47%	62.39%	9.53%
14.	HC	35.33%	89.95%	64.67%	10.05%
15.	HC	37.61%	90.76%	62.39%	9.24%
Mean +/-S.D		37.57+/-1.10%	90.39+/-0.99%		

Table 2: Number of dead brood removed and uncapped at 24 hrs and 48 hrs in infected colony (IC)

Colony No.	Colony status	No. of dead brood removed and uncapped		No. of dead brood not removed	
		24 hrs	48 hrs	24 hrs	48 hrs
1.	IC	40.65%	97.36	59.35%	2.64%
2.	IC	41.85%	98.00%	58.15%	2.00%
3.	IC	43.15%	100.00%	56.85%	0.00%
4.	IC	42.38%	98.15%	57.62%	1.85%
5.	IC	38.06%	96.96%	61.94%	3.04%
Mean +/-S.D		41.21+/-1.77%	98.28+/-1.11%		

Table 3: Paired T-test for difference between mean of removed and uncapped dead brood in HC and IC in 24 hrs (at 95% confidence level)

Colony status	Mean of dead brood removed	Standard deviation	Number of colonies	Standard error	df	t-calc	t-critical
HC	37.57	1.10	15	0.841	18	4.32	1.73
IC	41.21	1.77	5				

t-cal > t-critical

Table 4: Paired T-test for difference between mean of removed and uncapped dead brood in HC and IC in 48 hrs (at 95% confidence level)

Colony status	Mean of dead brood removed	Standard deviation	Number of colonies	Standard error	df	t-calc	t-critical
HC	90.39	0.99	15	0.558	18	14.12	1.73
IC	98.28	1.11	5				

t-cal > t-critical

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