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HYGIENIC BEHAVIOURAL MECHANISM OF RESISTANCE TO DISEASES AND PARASITES IN WEST AFRICAN HONEY BEE COLONIES APIS MELLIFERA ADANSONII (HYMENOPTERA: APIDAE)

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A B S T R A C T

Diseases, parasites and pests are problems of honey bee colonies in all parts of Nigeria with no protracted effort by beekeepers at improving colony hygiene for their domesticated bee colonies to curb the menace. Hygienic behavior is a genetic trait which has been a key assessment of general resistance of honey bees to diseases and parasites. Worker bees in healthy colonies detect, uncap and remove infected brood to reduce the spread of diseases. Forty two colonies of honey bees were selected in Ogun and Osun States and pathological tests were conducted to ascertain the presence and level of infection of the colonies with some diseases pathogens and *Varroa* mites that impact on the brood by killing it. Colonies were grouped into two categories: healthy colonies (HC) when they are free of parasites and brood diseases and infected colonies (IC) when they show the presence of parasites and brood diseases. Hygienic test was carried out using pin to kill some brood in both HC and IC, the numbers of dead broods uncapped and removed within 24 and 48hours were noted. Parasitic mite infestations were detected in 54.9 ± 5.91% (Mean ± S.D) of the colonies, 66.34 ± 6.16% (Mean ± S.D) of the colonies were having chalkbrood infections and there were no records of American and European foulbrood diseases. . Pin-killed brood assay revealed removal of dead brood within 24 and 48 hours, there is no significant difference observed between the rate of uncapping and removal of the dead brood in both IC and HC (t-critical = 1.782, and t-calc = 0.85, df = 12 and p = 0.05) The mean of dead brood uncapped and removed by the worker honey bees in IC is significantly greater than in the HC (t-critical = -1.782, and t-calc = 25.75df = 12 and P = 0.05). In *Apis mellifera adansonii*, uncapping and removal of dead brood in hygienic colonies is a mechanism of resistance and in infected colonies in addition to resistance, it reduces the spread of diseases and parasitic infections.

Keywords: Colony hygienic behaviour, Economic threshold level, Diseases, Parasites and Pests.

INTRODUCTION

The research work investigates if *Apis mellifera adansonii* like any other related species has developed hygienic behavioural mechanism to resist brood related diseases and parasitic *Varroa* mites. In the study, pin-killed brood bioassay test (Spivak and Reuter, 1998) was carried out on selected colonies managed by local beekeepers who have little or no knowledge about application of chemical treatments of honey bee diseases and parasitic infections. Diseases, parasites and pests are the major problems of beekeeping in Nigeria (Malaka and Fasasi, 2002, JAICAF, 2009, Oyerinde and Ande, 2009). 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 (Akinwande *et al.,* 2012a, 2013). Hygienic behaviour is a key assessment of general resistance of bees to diseases and pests. Hygienic bees easily detect, uncap and remove diseased or parasite-infested brood: brood infested with *Varroa*-mite (Peng *et al.,* 1987a; 1987b), brood infested with American foulbrood (Rothenbuhler, 1964a; 1964b.) and chalkbrood (Gilliam *et al*., 1983; 1988). It is widely

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known that hygienic worker honey bees remove dead or infected larvae and pupae from their cells to reduce the spread of diseases within a colony (Spivak and Gilliam, 1993, Spivak and Downey, 1998 and Spivak and Reuter, 1998). The development of chemotherapy in the management of honey bee diseases and parasites has many negative effects such as: loss of natural immunity, increase susceptibility to agrochemical toxicity and synergistic effects of diseases and chemicals as well as in- and out-of-hive insecticides (Johnson *et al*., 2009a, 2009b, 2010). Hygienic behaviour is a genetical trait and only 10% of commercial honey bees in United States are hygienic (Spivak and Gilliam, 1993, Spivak and Downey, 1998), although, this would have increased with a lot of effort in the last 10 years geared towards increasing the hygienic stocks of domesticated honey bees. To determine the hygienic behaviour of a colony, freeze-killed or pin-killed brood assay test or liquid nitrogen tests is required (Spivak and Downey, 1998, Spivak and Reuter, 1998). A hygienic colony would uncap and remove over 95% of the dead brood within 48 hours while a non-hygienic colony would spend some days to completely remove the dead brood (Spivak and Gilliam, 1993, Spivak and Reuter, 1998, 2001). The speed with which a colony removes dead brood is correlated with its ability to remove diseased and parasite-infested brood.

MATERIAL AND METHODS

Field survey was carried out between June and September for two years using 42 colonies belonging to some beekeepers in different parts of Osun and Ogun States in Nigeria. The colonies were labeled for ease of diagnosis and data collection. . The survey examined the colonies for the presence of *Varroa* mite (*Varroa Jacobson*), and brood diseases that damage the brood and impact on their development; it also sought the presence of hygienic behaviour in the bees. Diseases have been suspected to be problematic to beekeepers in Nigeria but impact has not been felt because many believed that *Apis mellifera adansonii* have developed many resistance mechanisms (Malaka and Fasasi, 2002, JAICAF, 2009, Oyerinde and Ande, 2009 and Akinwande *et al*., 2013).

The method used to diagnose the presence of *Varroa* mite is "alcohol wash". A wide mouth canning jar with a lid on one side and a size 8 mesh screen on the other side was used to collect bee samples, using a cup with a volume capacity to hold 280 – 300 bees, from a frame of brood in the colony brood nests. Alcohol was added to the bee samples and shaken gently to dislodge mites from the bees. The alcohol and the mites dislodged from the bees were emptied into a container. Brood combs in all the colonies were examined for signs of diseases. Features that were examined in the brood combs are discolorations, appearance of capping and presence of punctures, foul odour and dead brood. Confirmation tests for brood diseases were conducted using routine examination table/chart (USDA, 2010) (Table 1).

Table .1 Routine diagnostic tests for confirmation of foulbrood diseases and *Varroa* mites infestations

Pathogen/ pest examined	How it was Examined	Limit of examination	Acceptable limit of examination	Why it was examined?
Varroa mite	[1]Alcohol wash	Sampling of 75% of the colonies in the apiaries and 280 -300 bees for diagnostic test	Survey at least 50% of the colonies in the apiary Sample 300 bees for test	It affects the brood and adults
Chalkbrrod	[2]Observation of whitish or blackish chalky brood	Search for white or black mummies at hive entrances and brood cells	Presence of mummies and microscopic examination for fungal pathogens	It affects the brood
American foulbrood	Roppiness test	Examination of brood roppiness	Roppiness of brood and microscopic examination for bacteria pathogens	It affects the brood
European foulbrood	Non-ropy test	Examination of brood roppiness	Non-roppiness of brood and microscopic examination for bacteria pathogens	It affects the brood

Ropiness and non-ropy diagnostic tests were used to confirm the presence of American and European foulbrood diseases respectively. The presence of chalkbrood disease was diagnosed and confirmed by observing the presence of whitish or blacky chalky pre-pupal and pupal brood filling in the brood comb cells or forming litters at the hive entrances. Colonies that were found infected with any disease or infested with parasitic mites were grouped together as infected colonies (IC) while those that were free from any infections and infestations were grouped together as healthy colonies (HC). Fourteen colonies that consisted of 4 healthy colonies (HC) and 10 infectious colonies (IC) were randomly selected for colony hygienic test (Table 2).

The hygienic test was carried out according to the procedure used by Spivak and Reuter, 1998 and Spivak and Downey, 1998. In each colony, a comb section of sealed brood containing approximately 100 brood cells sampled with an open end rim of a tin on each side was used for the test. A long thin entomological pin was inserted to kill all the broods within the marked regions on both sides of the comb. The frame with the pin-killed brood was returned to the colony where it was removed and this exercise was repeated for other colonies. The number of dead brood uncapped and removed were counted in two time points 24 hours and 48 hours in all the colonies and comparisons were made between IC and HC. Recapping of the brood cells was not observed. The rate of uncapping and removal of dead brood was taken as a percentage of dead brood effectively uncap and removed in 48hours, Inferential statistics of two tailed *t*-test at a confidence interval of 95% were used to analyse the significant difference between the means and rate of dead brood uncapped and removed in HC and IC in 24hours and 48 hour time points.

RESULTS

The apiaries are located within the same geographical location. The bees are of the same species and there is no site related differences observed in the colony diseases and parasitic infestation found, the brood patterns and hygienic behavior**.**

Colony health status: In all the apiaries sampled for colony brood diseases and *Varroa* mite infestations, six (14.29%) were healthy colonies (HC) and 36 (85.71%) were infectious colonies (IC). In the IC, *Varroa* mite infestations were detected in 54.9 ± 5.91% (Mean ± S.D) of the colonies (Fig. 1), chalkbrood infections were prevalent in 66.34 ± 6.16% (Mean ± S.D) of the colonies, 33.4 ± 4.47% (Mean ± S.D) colonies have both *Varroa* mites and chalkbrood infections while there were no records of American and European foulbrood diseases (Fig. 1).

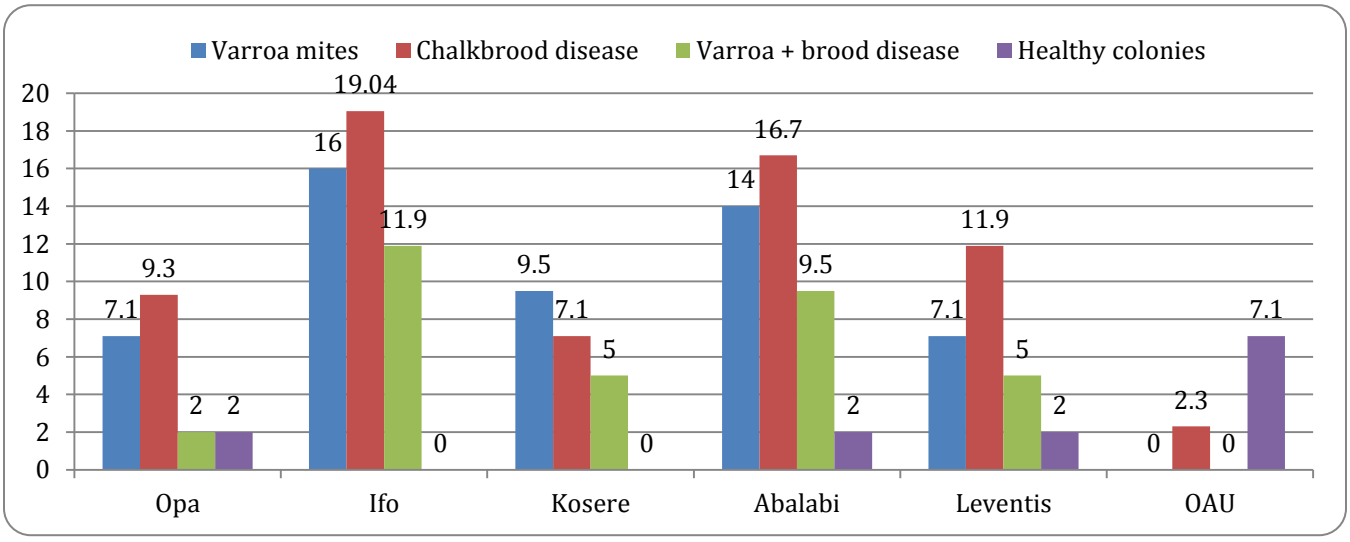

Figure 1. Mean percentage distribution of *Varroa* mites infestations and diseases in the apiaries.

Patterns of brood: The brood patterns observed in each of the colonies were mixtures of compact and scattered brood patterns Patterns of brood in 9 (21.43%) colonies have mostly solid or compact brood patterns with few empty brood cells, while the remaining 33 (78.57%) colonies have brood combs with scattered or spotty brood patterns possessing many empty brood cells. It was observed that compact brood patterns do not have direct association with either the HC or IC with either Varroa mites or chalkbrood only and or with both and

similarly, spotty brood patterns do not relate to neither group. Response to disease diagnosis tests in both colonies with compact and spotty or scattered brood patterns are the same.

Hygienic response: The percentages of brood cells uncapped and removed in HC and IC at 24 and 48 hours observation time were recorded (Table 2a and b). Percentages of dead brood that were uncapped and removed after 24 hours in HC range from a minimum of 35 percent to a maximum of 37.50 percent with 36.6 ± 1.14% (Mean ± S.D) (Table 2a) while in the IC, the percentages of dead brood that were uncapped and removed after 24hours range from a minimum of 37.50 percent to a maximum of 43.0 percent with 39.5 +/- 1.74% (Mean +/- S.D) (Table 2b). The mean of dead brood uncapped and removed by the worker honey bees in IC is significantly higher than in HC in 24 hours (*t*-crirtical = 1.782 and *t*-calculated = 3.66, df = 12 and *P* = 0.05) (Table 3). Similarly, in a time point of 48 hours, the range of dead brood uncapped and removed in HC is from a minimum of 89.9 percent to a maximum of 90.80 percent with 90.47 +/-0.43% (Mean +/- S.D) (Table 2a) while in the IC, the percentages of dead brood that were uncapped and removed range from a minimum of 96.93 percent to a maximum of 99.0 percent with 97.5 +/- 0.86% (Mean +/- S.D) (Table 2b). The mean of dead brood uncapped and removed by the worker honey bees in IC is significantly greater than in the HC (*t*-critical = -1.782, and *t*-calc = 25.75 df = 12 and P = 0.05) (Table 4). In all the colonies, the rate of uncapping and removal range from1.85, to 2.08. Mean rate of uncapping and removal of dead brood in IC is 2.02+/-0.04 and in HC is 1.88+/-0.03 (Table 2). There is no significant difference observed between the rate of uncapping and removal of the dead brood in both IC and HC (*t*-critical = 1.782, and *t*-calc = 0.85, df = 12 and P = 0.05) (Table 5).

Table 2a. Number of dead brood uncapped and removed after 24 hours and 48 hours time point intervals in selected hygienic colonies (HC).

Colony	No. of pin killed brood	Colony Status	No. of dead brood uncapped and removed		No. of dead brood not removed		Rate of removal
			24hrs	48hrs	24hrs	48hrs	
OAU	99	HC	37 (37.50%)	89 (89.90%)	62	10	1.85
Opa	101	HC	38 (37.30%)	92 (91.00%)	62	9	1.92
Abalabi	103	HC	36 (35.00%)	93 (90.20%)	67	10	1.88
Levents	98	HC	36 (36.60%)	89 (90.80%)	51	9	1.85
Mean +/-S.D			36.6 +/-1.14%	90.47+/- 0.43%			1.88+/-0.03

Table 2b. Number of dead brood uncapped and removed after 24 hours and 48 hours time point intervals in selected infected colonies (IC) colonies.

Colony	No. of pin Killed brood	Colony Status	Number of dead brood removed		No. of dead brood not removed		Rate of removal
			24hrs	48hrs	24hrs	48hrs	
Opa	100	IC	39 (39.00%)	98 (98.00%)	61	2	2.04
Opa	99	IC	39 (39.39%)	95 (97.95%)	60	4	1.98
Abalabi	101	IC	39 (38.61%)	99 (98.01%)	62	2	2.06
Abalabi	98	IC	40 (40.01%)	95(96.93%)	58	3	1.98
Leventis	100	IC	38 (38.00%)	99(99.00%)	52	1	2.06
Leventis	100	IC	40 (40.00%)	97(97.00%)	60	3	2.02
Leventis	97	IC	39 (42.26%)	94 (97.00%)	57	3	1.96
Ifo	103	IC	41 (39.80%0	100(97.08%)	62	3	2.08
Ifo	100	IC	43(43.00%)	98(98.00%)	57	2	2.04
Ifo	99	IC	37 (37.50%)	97(97.97%)	62	2	2.02
Mean +/- S.D			39.5+/- 1.74%	97.5+/- 0.86%			2.02+/-0.04

Table 3. Two tailed *t*-test of difference between Means of dead brood uncapped and removed in HC and IC in 24 hours (*Confidence Interval* = 95%).

Colony status	Mean of dead brood removed	Standard deviation	n	*df*	Standard error	*t*-calc	*t*-critical
HC	36.6	1.14	4	12	0.793	3.66	1.782
IC	39.5	1.74	10				

t cal > *t* critical

Table 4. Two tailed *t*-test of difference between Means of dead brood uncapped and removed in HC and IC in 48 hours (*Confidence Interval* = 95%)

Colony status	Means of dead brood removed	Standard deviation	n	*df*	Standard error	*t*-calc	*t*-critical
HC	90.47	0.03	4	12	0.273	25.75	1.782
IC	97.50	0.86	10				

t cal > *t* critical

Table 5. Two tailed *t*-test of difference between rate of uncapping and removal of dead brood in HC and IC in 48 hours (*Confidence Interval* = 95%).

Colony status	Rate of removal of dead brood	Standard deviation	n	*df*	Standard error	*t*-calc	*t*-critical
HC	1.88	0.33	4	12	0.165	0.85	1.782
IC	2.02	0.04	10				

t cal < *t* critical

DISCUSSIONS

The patterns of broods found in all colonies revealed the bees are actively removing dead and or infected brood or preventing infections by the removal. Asthe pattern of brood could be used to judge the colony and queen bee health and queen egg laying capacity. Bees remove the dead larvae leaving the spotty brood appearance and the queen relays in those cells, which hatch out at different times to form a solid or compact brood appearance,. Rothenbuhler, 1964a; Taber, 1982; Spivak and Reuter, 1998; 2001 claim that spotty brood patterns are created due to hygienic behaviour of the bees to naturally resist Chalkbrood and American foulbrood. Observation of both IC and HC performing uncapping and removal of dead brood supports this ascertions. The IC, although uncapped and removed more dead brood, spent significantly the same time uncapping and removing diseased brood compared to HC. The rate of uncapping and removal is not significantly different. The results contradicted those of Palacio *et al*., (2010); Arathi *et al*., (2000) that healthy colonies are more efficient in detecting, uncapping and removing diseased brood compared to the non-healthy colonies and the overall colony level efficiency showed healthy colonies removed more diseased brood than the non-healthy colonies within the same time frame. Palacio *et al.,* (2010) claim that bees in healthy colonies detected diseased brood (chalkbrood mummies) more rapidly than bees in non-healthy colonies. Spivak and Gilliam (1993); Boecking and Drescher (1994); Boecking (1999), Thakur *et al.* (1997); Gramacho and Spivak (2003); Arathi and Spivak (2001); Arathi *et al.* (2006) all claim that bees in non-healthy colonies were observed recapping cells that were previously uncapped by other worker bees. This claim agreed with our observation where some infected colonies were found with compact or solid brood patterns.

Adult honey bees *Apis mellifera adansonii* like other species of bees express hygienic behaviour by uncapping and removing dead or infected brood or mummified brood due to chalkbrood infection. According to these authors: Park *et al*. (1937); Woodrow and Host (1942); Rothenbuhler (1964a, 1964b); Gilliam *et al*. (1983); Milne (1983); Taber (1982); Spivak and Reuter (1998); Palacio *et al*. (2000, 2010); Spivak and Reuter, (2001) the behaviour is a mechanism of resistance to brood diseases.

CONCLUSION

The mixtures of spotty and solid brood patterns found among the healthy and infected colonies eliminate the queen as a potential source for weak laying. Where there is persistence of spotty brood patterns it could weaken

the colony populations and increase their susceptibility to diseases and parasitic infections which can lead to overall colony collapse. Diseases and parasitic infections are undoubtedly the economic issues affecting beekeeping activities. Many of the colonies suffer diseases like chalkbrood and dreaded parasitic *Varroa* mite infestations. These have been passed unnoticed by the beekeepers and remain untreated posing increased stress on the domesticated honey bee colonies for many years. Inspite of poor hive and colony health management and lack of chemotherapy, the stress might have created additional opportunity for the bee population to evolve a strong mechanism to resist diseases and parasitic infection or to persist when infected. The implication of this research study is that *Apis mellifera adansonii* colonies like other species develop colony behaviour to resist brood related diseases when there is no chemical treatment. In addition to colony resistance mechanisms, there might be individual bee resistance mechanism employed to reduce parasitic mite infection and diseases, effectiveness of one cannot be ascertained. For non-brood related diseases, colony resistance may be entirely different. Therefore, with brood and non-brood related diseases and parasitic infections together in a colony, brood uncapping and removal may not provide enough natural protection. Again, this is a cross-sectional study; the report obtained is not an expression of how the colonies will behave overtime, Colony hygienic resistance reduces as the status of infections reduces from high to low overtime. It is not recommended to treat diseases and *Varroa* mites with chemotherapy because of negative factors that follows the use of chemical treatments.. However, regular inspection of colony to remove agents of diseases and parasitic infections and good colony management methods may be applied to complement the bees natural mechanism of resistance.

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