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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (8): 1142-1145, 2005
ISSN 1028-8880

The Impact of *Nosema apis Z.* Infestation of Honey Bee (*Apis mellifera* L.) Colonies after Using Different Treatment Methods and their Effects on the Population Levels of Workers and Honey Production on Consecutive Years

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Abstract: The aim of this study was to determine the natural occurance of *Nosema apis* in honey bee colonies and evaluated of *N. apis* presence in colonies after medical treatment with fumagillin and thymol in consecutive 3 years period. For this purpose, 208 honey bee colonies randomly selected for detection of *N. apis* infection from Aegean ecotype of *Apis mellifera anatolica,* 1 years old queen in April, 2002. The colony development performances and honey yields were evaluated through the years from 2002 to 2004. Infested colonies were classified in 3 groups as tried to be equalized in Nosema infestation level; Fumidil-B, thymol application and control (only sugar syrup feeding). The effects of using period of Fumidil-B, contains fumagillin and thymol on *N. apis* infected honeybee colonies were researched by determining winter losses, adult bees population, brood and honey production in consecutive years. Also, control group were impacted for same parameters. The adult bee worker population and brood surfaces of treated in both groups (Fumidil-B and thymol) were increased significantly ($p<0.05$) by years from 2002 to 2004. Honey production of thymol group was exhibited significant ($p<0.05$) increase by years, consequently. Significant decrease ($p<0.05$) was recorded in control group for winter mortality, brood production, adult bee worker population and honey yield than the other treatment groups. The study was conducted that, observations of bee hives and regular treatment of infested colonies supported healthy and more productive honey bees. Leaving colonies un-medicated caused severe problems in colony production. Thymol could be suggested to beekeeper as a qualified *Nosemiasis* prevent agent; cheap, practical and non-toxic in hives for organic honey production. Moreover, with using thymol, residue-free bee products could be handled economically.

Key words: *Nosema apis, Apis mellifera,* honeybee disease, fumidil-B, thymol

INTRODUCTION

Nosema disease is the most widespread of adult bee diseases[1,2] and occurs worldwide wherever bee colonies exist[3,4]. Nosema disease is caused by the spores of a microsporidian parasite *Nosema apis* that infect the epithelial cells of adult honeybee ventriculus[5]. Hence, microscopic examination of the ventriculus of adult bees is essential for the diagnosis of *Nosema apis*[6,7]. The incidence is highest in spring[8].

Nosema infested workers have a reduced life-span[9]: it is shorter by 22-44% than that of a healthy worker[10]. Spores of *N. apis* are ingested by adult honeybees during activities such as hive cleaning. Epithelial gut cells are multiplied by germination of spores. The infected gut cells rupture, releasing new spores that may re-infect the other honeybees[11]. All the Nosema-induced problems affect nectar collection by foragers which results in depressed honey yields[12-14].

Nosema infested queens have degenerated ovaries, their egg-laying capacity is seriously impaired[15]. Increased losses of bee colonies and their depopulation during the winter in temperate climates seem to be due to their infestation with Nosema[14,16,17].

Nosema apis can reduce of infestation of bee colonies[18] by: (I) Improving management practices of bee colonies, proper ventilation (ii) Fumigating combs and equipment with 80% acetic acid, (iii) Applying chemotherapy: the antibiotic Fumagilin obtained from *Aspergillus fumigatus* and produced by Abbott as Fumidil-B™ is the most effective compound to control *Nosema apis* in bee colonies. Fumidil-B is effective against nosema but it is expensive and is heat unstable[8].

Recently, suppressive effects of thymol have been demonstrated against *N. apis* spores. Honeybees are tolerant to the use of thymol[19]. The action of thymol as a miticide is not clear, but it is known to be most effective in

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the absence of honeybee brood[20]. Thymol is not detectable by taste in honey at concentrations less than 1.1 mg kg^{-1}[21]. Evidence suggests that thymol suppresses nosema disease in honeybees were fed sugar syrup containing 0.44 mM thymol as a preservative. During 20 years period, no incidence of *N. apis* was recorded in honeybee colonies[22]. It is also cheap, practical and guaranteed healthy for organic bee products.

The aim of this study was to impact of Nosema disease of honeybee colonies in natural occurred on the population levels of workers, evaluated results from applying different chemoteraphic agents and un-medication on colony performances in 3 years consecutive period.

MATERIALS AND METHODS

The study was conducted in 208 honey bee colonies in 2002 as the same age (1 year old) queen and approximately in same population level. *Nosema apis* was detected of 162 from 208 colonies at various level determined by only foragers that carry pollen loads on their hind legs. Degree of *Nosema apis* infestation-bee samples were collected from honey bee colonies and examine in accordance with haemocytometer methods by Cantwell[23]. Hives were classified in three groups tried to be equal consider of the level of infestation.

- Control group (no medication applied but get only sugar solutions, 52 hives)
- Fumidil-B treated group (55 hives)
- Thymol treated group (55 hives)

The infestation level was classified as low (0.1 to 5.0 million spores per bee), medium (5.0 to 10.0 million spores per bee) and heavy (over 10.1 million spores per bee). When the spores were diagnosed more than 2 million in a bee, Fumidil-B and thymol were applied in treatment groups in spring and autumn. For this purpose, Fumidil-B (Abbott-USA) was dissolved 1 g Fumidil/colony/week in 150 mL 30% sugar syrup and given once a week to the bees for 4 consecutive weeks for 3 years period on infected colonies.

The sugar syrup concentration of 0.44 mM crystallized thymol is (equal to 66 micrograms thymol per mL of syrup, 0.000001 g) prepared and added in sugar syrup. of thymol medicated group with the same incidence of Fumidil-B application[8,22]. The control group was fed only with sugar syrup and no-medication was applied.

The effect of medication with Fumidil-B and thymol on the recovery of the infested colonies were made on the growth of adult bee population, brood surfaces and honey yield. Over wintering of treatment groups was also recorded. Adult bee population was determined by counting frames. Brood surfaces were determined by Puchta methods. Honey yields were obtained by weighing colonies beginning and at the end of nectar flow. The results were submitted to statistical analysis with the Duncan test, accepting $p<0.05$ as significant.

RESULTS

During the first year of the study, 162 of the 208 hives were infested by *N. apis*. The experimental design conducted with occurred 3 treatment groups with consider of equal in *N. apis* infestation level (low, medium and heavy) in each treatment. The average of nosema infection was 4.98 million in Fumidil-B group whereas 5.06 million in Thymol and 5.14 million in control groups, respectively (Fig. 1). *Nosema apis s*pores were sharply decreased in thymol groups in the 2004 spring. Even significant ($p<0.05$) decrease was recorded of *N. apis* spores Fumidil-B groups from 2002 to 2003, spores was nearly stable in 2004 of this group. The control group was the highest level of mean in *Nosema apis* spores which was showed slightly increase by years. Thymol group had the least *N. apis* spores and this number could be eliminated for incidence of *N. apis* occurance. So, less than 2 million spores in a bee could be accepted as healthy in colony condition whereas we were obtained this progress in 2004 by thymol group.

In next year, 2003 spring, winter losses were stated in all treatment groups. In 2003, fifteen hives were failed with over wintering in Fumidil-B group where as twenty-one hives were failed in control groups. Only 2 hives were lost in thymol groups in same year. Next year, eight hives from Fumidil-B group and 2 hives from thymol group were

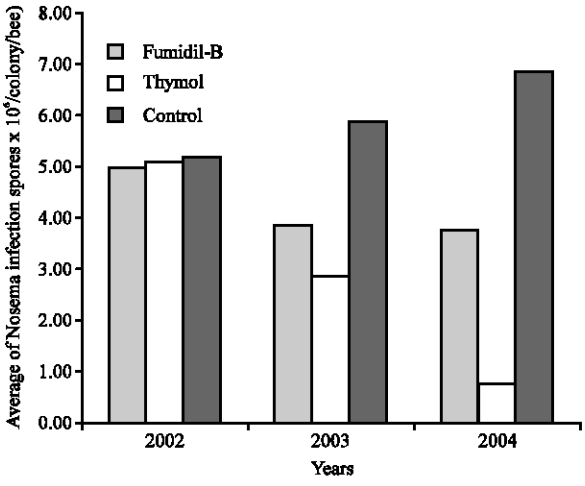

Fig. 1: Nosema infestation of honeybee colonies

Table 1: Means of adult worker bee population, brood surfaces and honey production of medicated (Fumidil-B and thymol) and control colonies from 2002 to 2004

	2002			2003			2004		
	F	T	C	F	T	C	F	T	C
Means of adult worker bee population (frames)	6.04±0.58^a	5.98±0.12^{ab}	5.78±0.79^{ab}	6.26±0.71^a	6.87±1.02^a	5.27±0.97^b	6.82±0.53^a	7.02±0.46^a	4.85±0.75^b
Means of brood Surfaces (cm^2/colony)	1992.52±428.7^{ab}	2243.45±156.7^a	2014.4±215.7^{ab}	2416.04±24.6^a	2845.41±142.5^a	1942.78±178.9^b	2838.13±216.8^a	2956.01±154.7^a	1902.75±106.1^b
Means of honey production (hive/kg/year)	18.42±1.12^a	18.75±0.48^a	17.96±1.17^b	20.01±2.04^a	22.75±0.75^a	16.78±2.12^b	21.15±1.97^a	23.45±0.79^a	15.79±1.45^b

^{a,b} Means within rows with no common superscript differ significantly ($p< 0.05$), F: Fumidil-B group, T: Thymol group, C: Control group

failed with over-wintering whereas 16 hives were failed in control group. The differences for winter losses in groups were important. The least winter loss was obtained from thymol group.

The effect of treatments on adult bee population, brood production and honey yield were found significant ($p<0.05$). After treatment of Fumidil-B and thymol of infested colonies, adult worker bee populations and brood surfaces were increased during the period between end of April and mid July in 2002, 2003 and 2004, consequently (Table 1). The worker bee population was increased slightly in both of medicated groups whereas slight decrease was obtained in control group. The differences of adult bee population and brood production between medicated and control groups were important ($p<0.05$) in statistic. The adult bee population and brood production in thymol group was higher than the others (Table 1). Honey production of medicated bee colonies were also increased by years consequently whereas significant decrease ($p<0.05$) was found in control group (Table 1).

DISCUSSION

The research conducted that Fumidil-B is an effective treatment for *Nosema apis* in infested honey bee colonies. Even the Aegean Region have temperate climates than the other parts of Turkey, *Nosema apis* detected in most apiaries in the region. But, the results were proved that *N. apis* could give very harmful effect on colony performances in case of non-treated. *N. apis* could be the reason for the high winter losses in present study with the similar results of most researchers[14,16,17,19,24].

In present study, control group was exhibited regularly decrease in overwintering, colony developments and also honey yield. The Fumidil-B group was given better performance by years for adult bee population, brood production and honey yield than the control group. Shimanuki *et al.*[18] and Lensky[16] were also agreed with proper medication of *N. apis* infested colonies could increased the adult and brood production in the colony. Also, we expected to eliminate the negative residual effect of *N. apis* and for decrease the infestation level in honey bee colony by regularly application of Fumidil-B in consecutive 3 years. The methodology of present study was in similar concept with Furgala and Gouchnauer[25] and Jeffre and Allen[24] who reported the treatment of medication and survey of colonies should be repeated in second or more years (up to ten years) to reduce residual level of infection to an economically unimportant level. Otherwise, *N. apis* could be survive deterioration effect on honey bee colonies in years. That is why, we were repeated the treatments in medicated groups even the Nosema spores were in negligible level.

But the Fumidil-B group was exposed rear performance than thymol group in all characteristics. These results with similar with Fries *et al.*[14] and Fulton[8] who reported the Fumidil-B kills the active stages of *Nosema apis* but not the spores and it's effect diminish overtime. Also with the same results of Fries[5], present findings proved that even fumagillin is administered in consecutive periods, infection levels still be harmful not finished completely. Thymol group was exhibited the best results from any others. Similar results were obtained by Rice[22] that sugar syrup containing 0.44 mM thymol was effective in controlling nosema disease in over wintering honeybees. Honeybees condense the syrup by evaporating off excess moisture and so increase the concentration of thymol per volume of syrup. At this point, mature spores within digestive tract of bees and within the comb have been killed. So, similar with Fulton[8], present result were proved that with time, the level of infective *N.apis* spores in thymol group were declined to a level that does not cause nosema disease by consecutive years (Fig. 1).

Also, honey yield was increased by medication of infested colonies whereas non-treated group was decreased from 2002 to 2004. Similar results obtained by Furgala and Gouchnauer[25] and Pohorecka and Skubida[19] that *N. apis* depressed honey yields in the colony if hives were not medicated.

As a conclusion, the early detection of *N. apis* in honey bee colonies and observation of colonies regularly is very important for preventing apiaries. The bee colonies should be medicated whenever necessary, will enable us to stop using the preventive application of drugs to bee colonies. Proper dose and application of drugs are important for handled healthy and clean and organic bee products. So, thymol could be suggest to beekeepers as

cheap, practical and safe biochemical for against Nosema infection instead of Fumidil-B in temperate climates for providing residue-free bee products. Moreover, with using thymol, residue-free bee products could be handled economically.

ACKNOWLEDGMENTS

We wish to thank the Sinerji Agricultural Products Company for financial assistance, Prof. Yaacov Lensky for suggesting the idea about the research and local beekeepers allow us to work in their apiaries.

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