Entomology

The incidence of nosema *spp.* on honey bees (*Apis mellifera*) colonies in Pakistan

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Abstract

Honeybees (*Apis mellifera L.*) are important for the production of many valuable products such as honey, royal jelly, pollen, propolis, beeswax, bee venom, and play an important role in the pollination of different crops. Several parasites and pathogens, including *Nosema* spp. affect honeybee colonies. Nosemosis is produced by a microsporidian now considered a fungus that causes adult honey bee disorder. It infects the honeybee population and honey production. In this study the detection of Nosemosis (*Nosema* spp.) in *A. mellifera* and their affect on honeybee colonies. The samples of worker bees were collected from colonies of five different apiaries located at Honeybee Research Institute, National Agriculture Research Center Islamabad (HBRI NARC) in July and August of 2019. The result showed that out of 80 honeybee colonies, 20 colonies were found infected with Nosema spore. The infestation percentage of Nosema in honeybee colonies was found higher in July as compared to August. The higher infestation of Nosema in July might be due to higher relative humidity compared to August.

Keywords: *Apis mellifera*, nosema spp, apiaries, colonies, humidity

Introduction

Honeybees play one of the most important roles in the pollination of different plants (Ahmad. 1987, Hung *et al*. 2018) [1, 19]. Honeybees not only produce honey but also produce many other valuable products such as pollen, royal jelly, propolis, and bee venom, which play an important role in medicine (Viuda-Martos *et al*. 2017) [33]. Because of high honeybee colony mortality in many parts of the world, today's beekeepers confront tremendous challenges in maintaining healthy colonies capable of agricultural pollination. Because humanity's reliance on pollinator-dependent crops has expanded dramatically in the previous half-century, the death of lots of colonies has gotten attention (Aizen and Harder. 2009) [2]. Several parasites and pathogens, including *Nosema* spp, affect honeybee colonies. Nosemosis is produced by a microsporidian now considered a fungus that causes adult honey bee disorder. It infects the honeybee population and honey production.

Nosema apis was first detected in honey bees was in 1909 by German scientist zander (Zander. 1909) [37]. *N. apis* microspores are eaten by bees through food or water, by food change with other bees or worker bees performing duties to cleaning up dirty combs (Fries *et al*. 1992) [12]. *N. apis* is a microsporidian parasite that is considered to be the only etiological agent of European honeybees (*A. mellifera*), which can reduce worker longevity and result in significant winter colony losses (Higes *et al*. 2006) [17].

In 1993, *N. apis* was detected in European honeybees *A. mellifera* in Sweden (Fries. 1993) [10]. New species of *Nosema* called *N. ceranae* was first detected in Asian honey bees *A. cerana* (Fries *et al*. 1996) [11]. Researchers discovered a new microsporidian, *Nosema neumanni* in some Ugandan colonies. There are distinct variances between the two on basis of molecular and ultrastructural properties of *Nosema* spp. (Chemurot *et al*. 2017) [6]. In Taiwan, the infection of *N. ceranae* was the first detected in honey bees colonies *A. mellifera* in 2005 (Huang *et al*. 2005) [18]. The infection of *N. ceranae* in honey bees colonies *A. mellifera* was stated from Europe (Higes *et al*. 2006) [17]. the detection of *Nosema* spp. in 1995-2007 in the United States (Chen *et al*. 2008) [7]. In 2005-2006 *Nosema* spp. was detected from honey bees colonies in Turkey (Whitaker *et al*. 2011) [34]. *N. ceranae* was a poisonous pathogen of *A. mellifera* causing colony collapse of honey bees (Paxton. 2010) [26]. In South America, *N. ceranae* has recently been detected in native bumblebees (Plischuk *et al*. 2009) [28].

Occurrence of Nosema in honeybees from different countries of Asia region report of *N. ceranae* and *N. apis* in China, Japan, Taiwan study in 1992, 2007-2008 host is *A. mellifera* and *A. ceranae* (Chen *et al*. 2009) [8]. In 2009 *N. ceranae* was reported in colonies of *A. mellifera* in Japan (Yoshiyama and Kimura. 2011) [36]. *N. ceranae* report in *A. mellifera* colonies in different Asian countries i.e. Jordan, Saudi Arabia, Turkey (Haddad. 2014, Ansari *et al*. 2017, Muz *et al*. 2010) [15, 3, 23]. In Thailand and Vietnam the detection of *N. ceranae* in both hosts of honeybees (*A. mellifera*, *A. ceranae*) in the year of 2008-2009, 2006 (Klee *et al*. 2007, Chaimanee *et al*. 2010) [20, 5]. *Nosema* spp. reported in different countries of Northern Europe, *N. ceranae* and *N. apis* detect in European honeybees *A. mellifera* in Denmark, Finland, Sweden, and Lithuania (Klee *et al*. 2007, Paxton *et al*. 2007, PAŞCA *et al*. 2019) [20, 27, 25].

Nosema disease decreases worker longevity to 22-44%, which directly decreases honey production and indirectly affects crop pollination (Fries *et al*. 2003) [13]. Infected worker bees negative effect on the orientation of flight that reduced the duration and distance covered during orientation (Wolf *et al*. 2016) [35]. Spores of *N. apis* were observed in the hemolymph

of larvae of honey bees (Gilliam and Shimanuki. 1967) [14]. Up to 180 million spores can be found in the guts of highly infected bees (Cantwell. 1970) [4].

Due to infection of *N. apis* and *N. ceranae*, immature spores were observed in epithelial cells of the midgut (Higes *et al*. 2006) [17]. Spore of *N. apis* and *N. ceranae* was also observed on worker mouthparts and in drones' semen, due to the food exchanging the horizontal transmission of spores to queen (Smith. 2012) [30]. *N. ceranae* was negative impact on the body of drone (Retschnig *et al*. 2014) [29]. The drone was sensitive to the infection caused by *N. ceranae* (Traver and Fell. 2011) [32]. Through the exchange of food from infected workers nurse bees transfer spores of *N. ceranae* to queen bees (Higes *et al*. 2009) [16]. The infection affects the queen's reproductive health as *N. apis* infects the terminal oocytes of the queen (Liu. 1992) [21].

According to the best of our knowledge, the systematic work on incidence of Nosema on honey bees missing in Pakistan. However, it has been reported from India (Kashmir), Iran, China, Turkey, and many other countries. The beekeepers in Pakistan often do report symptoms similar to Nosema and severe colonies losses. So, there is a dire need for a reference study to verify the Nosema in the *A. mellifera* colonies. This is a huge research gap, due to which thousands of beekeepers of the country may be being the loss of millions of rupees in losses of the bees population and honey production. This study to verify the Nosema in their colonies is essential.

Materials and Methods

Randomly Collected worker bee samples from different colonies in five various apiaries placed at NARC and vicinity. All the sampled bees were older foraging bees. About 10-15 bees from each colony were taken in a zip bag.

Bees were tested fresh, frozen (0°F), and for later test stored in alcohol. All samples taken in a zip were put in the freezer for killing the bees. Removed abdomen from bees and put into a mortar. Using a pestle, grind up the guts in mortar. Ground guts with the water make use of pestle. Coverslip was used cover the sample on hemocytometer. Use of pipette for taking a sample, the first drop of the sample tested and then takes more sample drops from grind solution for the confirmed accuracy for a bitter result; both sides of the hemocytometer are filled. First filled one side, after clearing out the pipette, again take a drop of the sample and then fill the other side of a hemocytometer. Put a drop of sample in the triangular slot. Both sides of hemocytometer to be sure it filled conformed. Let to keep it for 90 seconds to settle down all the spores for easy tested.

Hemocytometer placed under microscope lens (1000X) to find the spore. Nosema spores which were oval elliptical shaped with a dark outline, looked like a boiled egg. count. Rinsed all equipment washed and dried up early for the next test of the sample and put in storage. Wash only with a silky fabric cloth, not a paper towel to avoid scraping.

Results and Discussion

A lab test of honeybee (*Apis mellifera)* worker bees analyzed for the infection with *Nosema spp* depicted the results that out of 80 honeybee colonies, 20 colonies were found infected with *Nosema spore*. The twenty infected colonies are from five different apiaries as shown in table 1. The infestation percentage of Nosema in honeybee colonies was found higher as compared to August. The percentage infestation in various apiaries was 12.5, 11.1, 50, 5, and 50 in apiaries 1,2,3,4, and 5 respectively. Due to the moon soon rainy season and humid conditions prevailed in the area and favorable environmental conditions created the pandemic situation of Nosemosis.

In all apiaries, about 25% of colonies were found infected with Nosema spores. However, in the present study, the infection with Nosema was observed in July and August in summer. Microscopic examination of the midgut content and raw matter showed the occurrence of a large number of microsporidian spores. Spores shaped are oval to elliptical. A midgut infected with Nosema appears white, swollen, and without rings or constriction. The climate of Islamabad (NARC) and apparent humidity during summer seem to favor *Nosema* spreads. About 25% of the 80 investigated colonies are infected with *Nosema spp*. The high infection could be related to the prevalence of high humidity.

Table 1: Occurrence of Nosema spp in two months (July and August) in Colonies from Islamabad region

Apiary	Total colony tested for Nosema	Infected colonies (July)	Infected colonies (August)	Total infected Colonies	Percentage %
A 1	24	02	01	03	12.5%
A 2	09	01	00	01	11.1%
A 3	20	06	04	10	50%
A 4	20	00	01	01	5%
A .5	10	03	02	05	50%

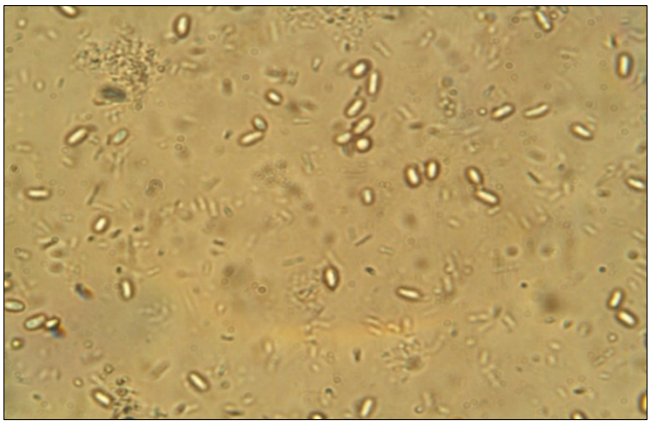

Fig 1: Nosema spore in honey bees

Adult bees samples are infected colonies No 12 out of 40 in July 2019 and 08 out of 40 in August 2019. In weak colonies, about 40% were infected in July, about 24% in August. as shown in table 2. In strong colonies, about 20% were infected in July, and 13.3% in August. as shown in table 3.

Table 2: Nosema infection in weak colonies of honey bees

Pathogen	Month	Sampling No.	% Positive
Nosema spore	July	20	40%
Nosema spore	August	25	24%

Table 3: Nosema infection in strong colonies of honey bees

Pathogen	Month	Sampling No.	% Positive
Nosema spore	July	20	20%
Nosema spore	August	15	13.3%

Nosema infection generally gets its high ranking in humid seasons of the year (Martín-Hernández *et al*. 2007) [22]. In the Mediterranean Sea regions, In summer the humidity is high; therefore, during this period the Nosemosis infection is very high in the ranking (Özkırım and Keskin. 2001) [24]. Northwestern Iran such as the Aras Baran region has a mountainous climate and atmospheric humidity in spring, which creates conducive conditions for enough occurrence of Nosemosis in honey bee apiaries, and the infection rate has been reported to be high in spring (23.9%) and the lowest in summer and fall (TOPÇU and ARSLAN. 2004) [31]. In spring the high infection rate was observed as compared to the other two seasons and low infection rate in summer and there is no infection rate in autumn. In spring infestation rate is high due to high humidity (Dar and Ahmad. 2013) [9].

Conclusion

Considering the highest level of infection in summer, the highest parasitic infection of bees in Islamabad (NARC) such as Nosema spp. which is the most commonly, observed among bees in this region, can lead to a decrease in the population of the colonies. Consequently, these vulnerable colonies would demonstrate a high rate of Nosemosis in summer (with suitable humidity and temperature for the spread of Nosemosis). Generally, the dissemination of Nosemosis in bee colonies of Islamabad was high in summer and low in other periods of the year. It is suggested that the epidemiological studies on Nosema infection be conducted continuously according to the climatic and the geographical conditions of Islamabad and the statistical concern regarding the infection in honeybee colonies be presented based on the season.

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