Indian Journal of Entomology Online published Ref. No. e23109 DoI. No.: 10.55446/IJE.2023.1109

POTENTIAL OF FUMAGILLIN AGAINST *NOSEMA* SP., A MICROSPORIDIAN ENDOPARASITE OF HONEY BEE *APIS MELLIFERA* L.

Archana Shukla[1]*, Ashwani Kumar[2] and Sanket Mahajan[1]

[1]Department of Biological Sciences, Faculty of Science, [2]Department of Entomology,
Naini Agricultural Institute,Sam Higginbottom University of Agriculture,
Technology and Sciences, Prayagraj 211007, Uttar Pradesh, India
*Email: archushukla001@gmail.com (corresponding author): ORCID ID 0000-0003-0288-0251

ABSTRACT

***Nosema apis* and *Nosema ceranae* are two kinds of microsporidian parasites that cause the recently identified illness known as nosemosis in European honey bees (*Apis mellifera*). 10 samples of adult honey bees from each infected colony of a single apiary were collected, located in the village of Handia, district of Prayagraj, India, from September 2022 to January 2023 to investigate the frequency of intestinal infections and the effectiveness of fumagillin antibiotic against nosemosis in *A. mellifera* with the use of monocular LED optical microscopy and a haemocytometer, the mean and median spore count of each composite sample was calculated. It was observed that 5% of the infected gut samples contained Nosema spores. The results show that 10% of colonies tested positive for Nosema, and fumagillin is effective against nosemosis.**

Key words: *Apis millifera, Nosema ceranae, Nosema apis*, Protozoa, fumagillin, endoparasite, microsporodian nosemosis, European honey bee, Prayagraj, intestinal infections, nosemosis

Honey bees have long been associated with vigour, assiduity, and collaboration in our environments. Several opponents of bee health pose a threat to honey bee survival as well as the livelihoods of people who profit from their labour. Exposure to chemicals, inadequate fodder, mite parasites, and diseases have led to a high annual death rate in colonies. The fungal infection nosemosis caused by spore forming endoparasite *Nosema ceranae* and *Nosema apis* is one of the possibilities involved in bee decline and is extremely common in both healthy and declining colonies. A 'new Nosema' typically connected with the Asian honey bee *Apis cerana*, in colonies of the European honey bee *Apis mellifera* first identified in Europe in 2006 has been known, *Nosema ceranae* is a species of Nosema that infects *A. mellifera* naturally (Higes et al., 2006). Since then, several reports on the prevalence of *N. ceranae* in *A. mellifera* and other host species (Klee et al., 2007; Chen et al*.,* 2008). *Nosema* sp., spores can be seen under a microscope with relative ease, and some skilled technicians may be able to distinguish between *N. apis and N. ceranae* spores by eye. However, species identification is difficult because both species can cause isolated or mixed infections in *A. cerana* and *A. mellifera* (Chen et al., 2009; Forsgren and Fries 2010; Milbrath et al., 2015). Beekeepers in India have previously reported dramatically rising colony losses, (Grupe and Quandt, 2020). Viruses (Foster et al., 2007) and nosemosis are the primary pathogens responsible for these increases in colony mortality (Martín et al., 2007). In the winter and fall of 2021 and 2022, Indian beekeepers suffered huge losses, and they have since observed an increase in overwintering mortality. In a study involving 100 colonies in the Prayagraj district, the prevalence of *Nosema* spores infection was observed. Effectiveness of a well-known antifungal drug fumagillin, used to treat *Nosema* infections was also evaluated to determine whether *Nosema* can be linked to these losses.

MATERIALS AND METHODS

During September 21, 2022 to January 26, 2023, samples of *A. mellifera* were taken using sterile forceps in collection jars filled with 70% ethanol from a single apiary situated in the village of Handia in the district of Prayagraj. When requested to gather samples of 10 adult bees from all infected hives, beekeepers consented to give the research samples. Bees at the hive entrance or on frames far from the brood nest were considered to be older bees. The Stevanovic et al. (2011) approach was used to demonstrate that there was no *Nosema* infection in the colonies. The feeding solution was prepared in sucrose syrup (50%w/v) with fumagillin concentration of 26.4 mg/ℓ (Heever et al., 2014) which was then stored in amber vials and utilized for 5 days as described by Raymann et al. (2017). From the ten selected colonies (infected), 10 adult worker bees were removed, and put in net bags, and then maintained overnight in an incubator (35^{o}C 67% RH). The next morning, five worker bees

were selected at random and placed in cages. There were 50 bees in each cage. A solution of 50% (w/v) sugar was given to all the groups. The infected controls received nothing and other groups of bees were fed fumagillin treatments from 22 September 2022 to 24 January 2023; total of three doses were given at an interval of 30 days as part of their diet. Gathered bee samples were then preserved in 70% ethanol at ambient temperature. Five worker bees were randomly chosen from each sample and viewed under a microscope to check for *Nosema* spores. The bees' abdomens were removed and crushed using a mortar and pestle in 1 ml of distilled water. To estimate the number of spores, extracts were diluted 1:10 in water and placed onto a Bright-line haemocytometer to quantify the spores/ bee as described by Cantwell (1970) and OIE (2018).

RESULTS AND DISCUSSION

Nosema is affecting over 10% of bee colonies, and data shows a considerable drop in spore mean count after fumagillin administration ($p=0.42$, $df=2$, $f=0.74$). There is a need for a quick, accurate, and efficient assay to check for *Nosema* in samples submitted by beekeepers due to the high incidence of the disease in Prayagraj. A "loss of seasonality" is reportedly the characteristic of the particular type of nosemosis brought on by *Nosema* sp. According to reports, more colonies will have measurable infection levels in the spring, which means more individuals will be sick and show a greater spore load. Generally, early spring will bring out the pathological signs of nosemosis (creeping bees and dysentery inside the hive). Colonies will then decline before the season even begins (Fries, 2010). Moreover, counsel beekeepers to use Fumagillin-B to treat seriously diseased hives before the winter. Unfortunately, our knowledge of the *Nosema* infection cycle and the elements that could lead to a more severe outbreak within colonies is limited. It is vitally necessary to conduct further study on the yearly cycle and the variables that affect nosemosis severity.

ACKNOWLEDGEMENTS

Authors thank the Sam Higginbottom University of Agriculture Technology and Sciences Prayagraj, Uttar Pradesh, India for providing facilities. The authors acknowledge Mr Ajeet Kumar a bee keeper for providing honey bee samples.

FINANCIAL SUPPORT

The study received no funding.

AUTHOR CONTRIBUTION STATEMENT

AS wrote the paper. AK created the experimental procedure. SM translated the manuscript.

CONFLICT OF INTEREST

The researcher affirms that no financial or commercial ties that might be viewed as having a conflict of interest existed throughout the course of the research.

REFERENCES

Cantwell G E. 1970. Standard methods for counting *Nosema* spores. American Bee Journal 222-223.

Chen Y, Evans J D, Smith I B, Pettis J S. 2008. *Nosema ceranae* is a long-present and wide-spread microsporidian infection of the European honey bee (*Apis mellifera*) in the United States. Journal of Invertebrate Pathology 97(2): 186-188.

Cox-Foster D L, Conlan S, Holmes E C, Palacios G, Evans J D, Moran N A, Lipkin W I. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. Science 318(5848): 283-287.

Forsgren E, Fries I. 2010. Comparative virulence of *Nosema ceranae* and *Nosema apis* in individual European honey bees. Veterinary Parasitology 170(3-4): 212-217.

Fries I. 2010. *Nosema ceranae* in European honey bees (*Apis mellifera*). Journal of Invertebrate Pathology (103): 73-79.

Grupe A C, Quandt C A. 2020. A growing pandemic: A review of *Nosema* parasites in globally distributed domesticated and native bees. PLoS Pathogens 16(6): e1008580.

Higes M, Martin R, Meana A. 2006. *Nosema ceranae*, a new microsporidian parasite in honey bees in Europe. Journal Invertebrate Pathology (5): 92-93.

Klee J, Besana A M, Genersch E, Gisder S, Nanetti A, Tam D Q, Paxton R J. 2007. Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. Journal of Invertebrate Pathology 96(1): 1-10.

Martin-Hernandez R, Meana A, Prieto L, Salvador A M, Garrido-Bailon E, Higes M. 2007. Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. Applied and Environmental Microbiology 73(20): 6331-6338.

Milbrath M O, Van Tran T, Huang W F, Solter L F, Tarpy D R, Lawrence F, Huang Z Y. 2015. Comparative virulence and competition between *Nosema apis* and *Nosema ceranae* in honey bees (*Apis mellifera*). Journal of Invertebrate Pathology (125): 9-15.

OIE–Office International Des Epizooties. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 2.2.4. Nosemosis of honey bees. https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.02.04_NOSEMOSIS_FINAL.pdf2018.

Raymann, K, Shaffer Z, Moran N A. 2017. Antibiotic exposure perturbs the gut microbiota and elevates mortality in honey bees. PLoS Biology 15(3): e2001861.

Stevanovic J, Stanimirovic Z, Genersch E, Kovacevic S R, Ljubenkovic J, Radakovic M, Aleksic N. 2011. Dominance of *Nosema ceranae* in honey bees in the Balkan countries in the absence of symptoms of colony collapse disorder. Apidologie (42): 49-58.

Van den Heever J P, Thompson T S, Curtis J M, Ibrahim A, Pernal S F. 2014. Fumagillin: an overview of recent scientific advances and their significance for apiculture. Journal of Agricultural and Food Chemistry 62(13): 2728-2737.

(Manuscript Received: December, 2022; Revised: April, 2023;
Accepted: April, 2023; Online Published: April, 2023)
Online First in www.entosocindia.org and indianentomology.org Ref. No. 23109