APIMONDIA SCANDINAVIA 2025
SWEDEN | DENMARK | NORWAY

PP-389 [Bee Biology]

In vivo evaluation of "progen" probiotic against *Nosemaceranae*

Naser Tajabadi[1], Mojtaba Moharami[2], Rasoul Bahraini[3], Somayeh Tazeh kam[4], Pegah Valizadeh[1], Shabnam Parichehreh[1], Hadi Tajabadi[5]. Nader Mashaykhi Sardoei[1], Ahad Nejad Mohammad Nameqi[1]

[1]Department of Honey Bee, Animal Science Research Institute of Iran, Agricultural Research Education and Extension Organization, Karaj, Iran
[2]Razi Vaccine and Serum Production Research Institute, Agricultural Research Education and Extension Organization, Karaj, Iran
[3]Department of Biological Sciences, University of Alberta, Edmonton, Canada
[4]Department of Animal Science, Faculty of Animal Science and Aquatic Science, Sari Agricultural Sciences and Natural Resources University. Sari, Iran
[5]Faculty of Basic Sciences, Central Tehran Branch, Islamic Azad University, Tehran, Iran

ABSTRACT

Nosema ceranae is a microsporidian fungus that infects the epithelial cells of the midgut of honey bees. The honey bee gut microbiota consists of a wide range of bacteria, including numerous Lactic Acid Bacteria. In this research, the effect of the specific honey bee probiotic "Progen" on *N. ceranae* spores and honey bee survival was tested. Briefly, *N. ceranae* spores were first extracted and confirmed by DNA extraction and PCR. Then, the frames containing covered pupa from colonies without *N. ceranae* infection and without previous treatment with antibiotics or Fumagillin were kept overnight in the incubator. The newborn bees were collected the next day and starved for 2 hours. Each bee was inoculated with approximately 50,000 spores. After feeding, groups of 40 bees were placed in wooden cages and kept in an incubator at a temperature of 30 degrees Celsius and a relative humidity of 65%. The negative control bees were fed separately with 5 microliters of sugar syrup without spores and the positive control with 5 microliters of sugar syrup with spores without probiotics. The treatments with three doses of 0.5, 1 and 1.5 grams per liter of probiotic "Progen", positive control treatment and negative control treatment in most of them were treated in a completely randomized design for 21 days. Daily mortality, number of spores, feed consumption and water consumption were recorded. The results showed that the highest percentage reduction of *N. ceranae* spores in each bee compared to the positive control is related to the treatment containing 1.5 grams of probiotic with an average of 2.99E±06 spores per bee, with 82.63%. The treatment containing 1.5 grams of probiotics with 15.5% deaths had the lowest amount of mortality during the experiment. The treatment containing 1.5 grams of probiotics had a significant difference with the positive control treatment in the factors of spore count, survival and mortality at the level of 5%.

MATERIAL AND METHODS

Nosemaceranae Spore Extraction

Inoculation of Honey bees with *Nosemaceranae* Spores

Incubation overnight of the pupa frame → Collected newly hatched bees → Inoculated each bee with approximately 50,000 spores

Incubated at 30°C and 60% relative humidity for 21 day

Feeding with probiotics and applying treatments

Probiotic doses of 0.5, 1 and 1.5 g/l syrup → Feeders changed every four days → Weighing the amount of water and syrup consumed → Removed daily dead bees and spores counted in dead bees → Transferred and maintained at -20°C → Spore counting using a hemocytometer → Statistical analysis

RESULTS

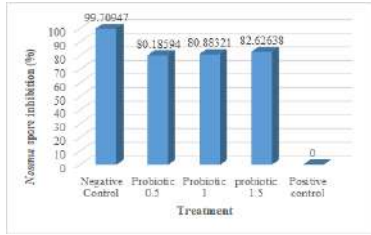

Figure 1: Effect of different doses of "Progen" probiotic on preventing the growth of *N. ceranae* spore.

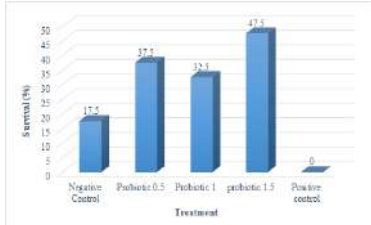

Figure 2: Effect of different doses of "Progen" probiotic on Survival of honey bee after infection with *N. ceranae*

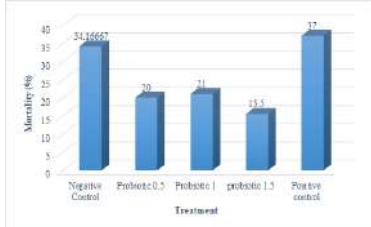

Figure 3: Effect of different doses of "Progen" probiotic on Mortality of honey bee after infection with *N. ceranae*

The results showed that the highest percentage reduction of *N. ceranae* spores in each bee compared to the positive control is related to the treatment containing 1.5 grams of probiotic with an average of 2.99E±06 spores per bee, with 82.63%. The treatment containing 1.5 grams of probiotics with 15.5% deaths had the lowest amount of mortality during the experiment.

CONCLUSION

In conclusion, the use of "Progen" probiotic at a dose of 1.5 g/L of sugar syrup significantly reduced *Nosema* spore count in *Nosema* spore-infected bees. These results showed that treatments consuming probiotics had a lower mortality rate than the negative control treatment. Therefore, it can be said that feeding with probiotics reduces the negative effects of stressors in honeybees.

ACKNOWLEDGMENTS

Animal Science Research Institute of Iran(ASRI),
Agricultural Research Education and Extension Organization, Karaj, Iran
and
Takgene Group, Tehran, Iran

Researcher information:

Naser Tajabadi

Honey Bee Department, Animal Science Research Institute of Iran (ASRI),
Agricultural Research Education and Extension Organization, Karaj, Iran
ntajabadi@yahoo.com
Office Phone: 0098-26-3425-6001
H.P. 0098-912-0939288

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[1]Department of Honey Bee, Animal Science Research Institute of Iran, Agricultural Research Education and Extension Organization, Karaj, Iran
[2]Razi Vaccine and Serum Production Research Institute, Agricultural Research Education and Extension Organization, Karaj, Iran
[3]Department of Biological Sciences, University of Alberta, Edmonton, Canada
[4]PhD. Student in Animal Genetics and Breeding, Department of Animal Science, Faculty of Animal Science and Aquatic Science, Sari Agricultural Sciences and Natural Resources University. Sari, Iran
[5]Faculty of Basic Sciences, Central Tehran Branch, Islamic Azad University, Tehran, Iran

Nosema ceranae is a microsporidian fungus that infects the epithelial cells of the midgut of honey bees. The honey bee gut microbiota consists of a wide range of bacteria, including numerous Lactic Acid Bacteria. In this research, the effect of the specific honey bee probiotic "Progen" on N. ceranae spores and honey bee survival was tested. Briefly, N. ceranae spores were first extracted and confirmed by DNA extraction and PCR. Then, the frames containing covered pupa from colonies without N. ceranae infection and without previous treatment with antibiotics or Fumagillin were kept overnight in the incubator. The newborn bees were collected the next day and starved for 2 hours. Each bee was inoculated with approximately 50,000 spores. After feeding, groups of 40 bees were placed in wooden cages and kept in an incubator at a temperature of 30 degrees Celsius and a relative humidity of 65%. The negative control bees were fed separately with 5 microliters of sugar syrup without spores and the positive control with 5 microliters of sugar syrup with spores without probiotics. The treatments with three doses of 0.5, 1 and 1.5 grams per liter of probiotic "Progen", positive control treatment and negative control treatment in most of them were treated in a completely randomized design for 21 days. Daily mortality, number of spores, feed consumption and water consumption were recorded. The results showed that the highest percentage reduction of N. ceranae spores in each bee compared to the positive control is related to the treatment containing 1.5 grams of probiotic with an average of 2.99E±06 spores per bee, with 82.63%. The treatment containing 1.5 grams of probiotics with 15.5% deaths had the lowest amount of mortality during the experiment. There was no significant difference in the consumption of sugar syrup and water between experimental treatments at the 5% level. The treatment containing 1.5 grams of probiotics had a significant difference with the positive control treatment in the factors of spore count, survival and mortality at the level of 5%.