

RESEARCH ARTICLE

Effect of Some Honeybee Diseases on Seasonal Mortality of *Apis mellifera intermissa* in Algeria Apiaries

Noureddine Adjlane^{1,2} · Nizar Haddad³

Received: 19 October 2015 / Revised: 29 August 2016 / Accepted: 13 September 2016 / Published online: 22 September 2016
© Zoological Society, Kolkata, India 2016

Abstract With a view to identify the pathogens and to establish the role of these pathogens in regulation of the density of honey bee population occurring in the apiaries of the area concerned samples of honeybee were collected from the beekeepers in some parts of central Algeria. It is revealed that *Nosema* sp., *Varroa destructor*, *Paenibacillus larvae* are associated with the disease manifestation in honey bees. The presence of *Nosema* sp., *Varroa destructor*, *Paenibacillus larvae* was analyzed using standard OIE methods. Spores of *Paenibacillus larvae* were detected in 56.6 % in winter 52.32 % in spring, 29.33 % in autumn and 11.25 % in summer. *Nosema* infestation was recorded in 47.91 % bee individuals during spring. *Varroa* infestation rate was maximum 12.57 % in summer and lowest 3.44 % in spring. Analysis of data indicates that Bumerdes and Tipaza, diseases induced mortality exceeds 10 % in honeybee. There exists a significant correlation between *Nosema* disease and mortalities in honeybees. Seasons play significant role, irrespective of pathogens, in disease manifestation.

Keywords Honey bee · Diseases · Mortality · Apiary · Algeria

✉ Noureddine Adjlane
adjlanenoureddine@hotmail.com

Nizar Haddad
drnizarh@gmail.com

¹ Département de Biologie, Université M'hamed Bougara, Avenue de l'indépendance, 35 000 Bumerdes, Algeria

² Laboratoire de Biologie et de Physiologie Animale, ENS kouba, Vieux-Kouba, Algeria

³ Bee Research Department, National Center for Agriculture Research and Extension, P.O. Box 639, Baq'a 19381, Jordan

Introduction

Honey bees are social insects. They play a vital role in the sustainability of the ecosystems and biodiversity. It is estimated that one-third of the human diet depends directly or indirectly on the role of pollinators, of which honey bees represent a large proportion (Haddad et al. 2007; Shamout et al. 2014). Worldwide high mortality of honey bee colonies (*Apis mellifera*) is a serious threat to apiculture. Of the several mortality promoting factors (VanEngelsdorp et al. 2009), high load of parasites and pathogens, such as the ectoparasitic mite *Varroa destructor* and certain viruses (Dainat et al. 2012) are burning problems. Such problems have already been identified by a good number of workers (Guzman-Novoa et al. 2010; Adjlane et al. 2012; Copley and Jabaji 2012; Adjlane et al. 2013).

As apiculture is a good source of economy in Algeria and the beekeepers are facing problem in maintaining the bee colonies because of unusual mortality of the bees we aimed to investigate the problems with a view to protect the bee colonies from destruction.

Materials and Methods

Sampling

Honeybee samples were collected from 4 regions: Blida, Bumerdes, Algiers and Tipaza (center of Algeria) during 4 seasons 2013–2014. In each case, 100–150 honeybees were shaken from brood frames into plastic vial containing ethanol 90°. Each sample was numbered and transferred to the laboratory for further studies. Survey was conducted by selecting 20 beekeepers at random in each area to record the loss of colonies and the reasons for the same.

To determine the infestation of mite 300 workers were collected from the brood frames of each colony. We separated the mites from their host by placing the workers in jars containing 90 % ethanol by shaking for 3 min (Shimanuki and Knox 2000).

Method of Lindstrom et al. (2008) was applied to detect the bacteria *Paenibacillus larvae*. Catalase method (Haynes 1972) was applied for biochemical and microbiological confirmation. Also, the technique of Murray and Aronstein was applied for further confirmation in cases of positive samples.

Nosema were detected and counted separately for each sample according to the protocols provided by Office International des Epizooties (Oie 2005).

The spores were counted following the method of Cantwell (1970). Soesensen (2009) was followed to determine the degree of infestation of disease in bee colonies induced by *Nosema*.

The data obtained are analyzed with Statistica 8.0 software following the variance analysis process (ANOVA).

Results and Discussion

Infestation rate of American foulbrood, *Nosema* and *Varroa* in the honeybees *A. m. intermissa* varied with the localities and the seasons (Figs. 1, 2, 3). It is evident that, on average 41.07, 47.91, and 8.8 % bee individuals were attacked by the diseases caused by the agents like bacteria *P. larvae*, protozoans *Nosema* and mites *Varroa* respectively. Results of ANOVA tests clearly indicate that there exists significant difference in the infestation rate between the season (for bacteria $p < 0.01$; for *Nosema* $p < 0.003$; for *Varroa* $p < 0.01$). However, results of ANOVA tests failed to establish the impact of localities on the infestation

rate of bacteria, *Nosema* and mites ($p > 0.05$) in the honeybee occurring in the apiaries of the localities concerned.

Hansen and Brodsgaard (1999) in their review stated that the bacteria multiply in the brood during spring. But, in the present study, it is evident that irrespective of localities infestation rate of *P. larvae* is almost equally high during winter and spring. However, Lindstrom et al. (2008) opined that the distance between the colonies plays significant role in spreading the bacterial spore. The problem became unmanageable with the increasing number of apiaries within a short distance. Because, short distance about 1 km or less (Lindstrom et al. 2008) ensures contamination from one bee colony to other. As, in Algeria, beekeepers are practicing migratory beekeeping device without taking any measure regarding infection of *P. larvae* the problem is of serious concern. Though practice of application of antibiotics in the apiaries is in progress. In Algeria the queen available in the market are not free from *P. larvae* infection. Perhaps, for this reason the American foulbrood disease is a serious threat to apiculture in Algeria.

The incidence of *Nosema* in honeybees is very much influenced by the environmental factors irrespective of geographical area of the globe (Mussen et al. 1975; Dyess and Wilson 1978; Moeller 1978; Ball and Bailey 1991; Fries 1993; Swart 2003; Barbancon and L'Hostis 2007; Martin-Hernandez et al. 2007; Soesensen 2009).

The influence of climatic factors is very much pronounced in Algeria also. Because we noted that, almost all the samples in our collection were contaminated with *Nosema* in winter, spring, summer and autumn but the infestation rate varied from 1 million spores in summer and autumn to 5 million spores in winter and spring per honeybee. This suggests that the spring and winter provide

Fig. 1 Contamination rate of American foulbrood in honeybees in the study area, in Algeria in different seasons during 2013–2014

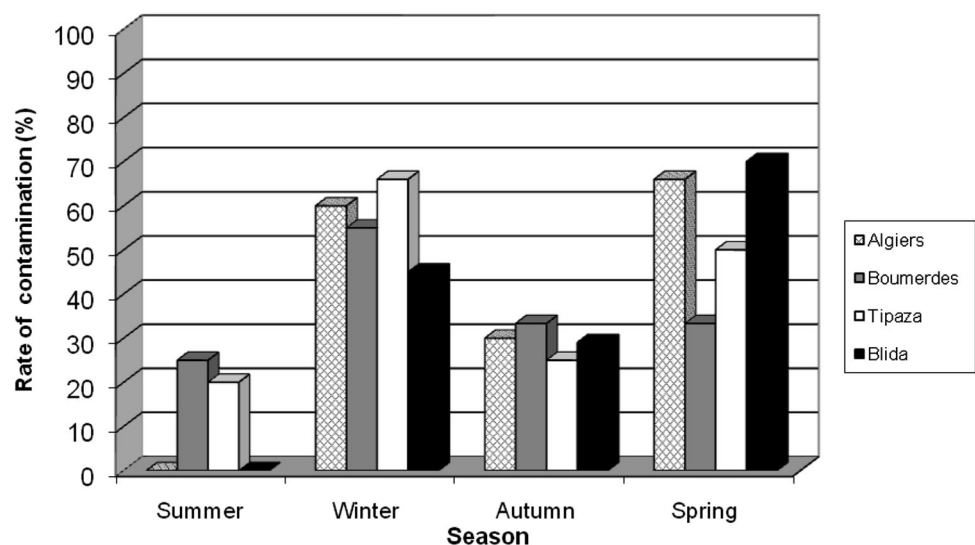


Fig. 2 *Nosema* contamination rate of the honeybees in the study areas in Algeria, in different seasons during 2013

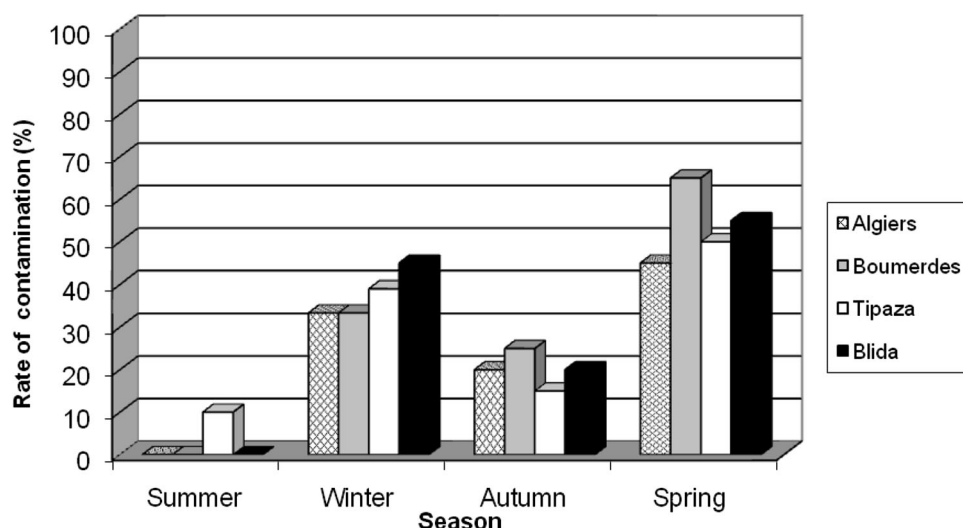
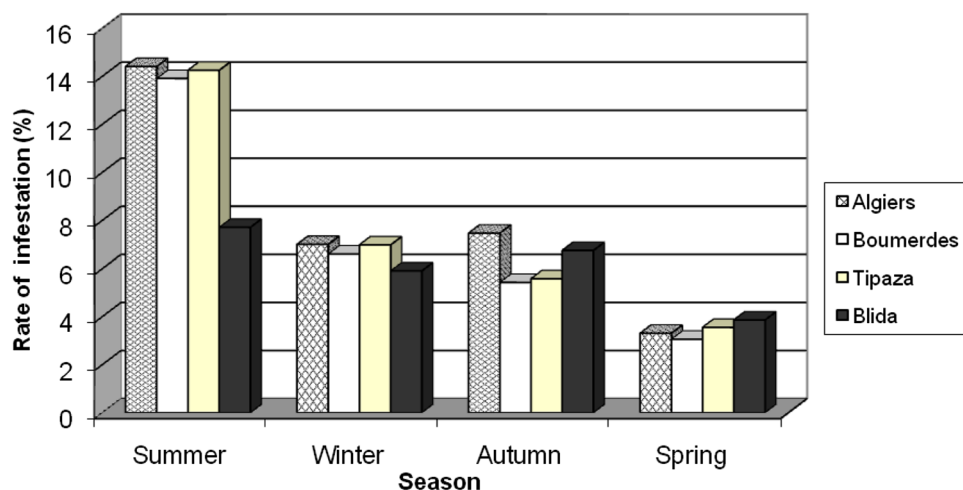


Fig. 3 *Varroa* infestation rate in honeybees in the study areas, during 2013–2014 in Algeria



congenial atmosphere to spread the disease caused by *Nosema*. According to Mussen et al. (1975) 10 million spores per bee stage of infestation seems to be critical to get rid of severity of the disease and thereby ensuring mortality of the infected bee. As this state of infection mostly coincides with the winter season mass mortality including death of the queen in a honeybee colony is inevitable.

Like *P. larvae* and *Nosema*, *Varroa* infestation in honeybees is also influenced by the seasons. But in this case, higher infestation rate is coincided with the summer while in spring the rate of infestation noted is minimum. It is evident that the climate of the apiary area regulates the growth of mite population (Kraus and Page 1995; Branco et al. 1999; Dietemann et al. 2013). According to Vandame (1996) in Mexico occurrence of phoretic mites in maximum number coincides with the availability of higher number of broods of the honeybee during spring. The same author also speculated that the *Varroa* females are adapted to use the broods to achieve the breeding success in course

of phoreses. In the Mediterranean climate of California the density of *Varroa* infestation colonies increases 286 times in a year (Kraus and Page 1995). Branco et al. (1999) opined that the rapid growth of the parasite population is not due to the higher reproduction rate of the mites but because of, availability of host (brood) almost round the year. However, in temperate climates, varroa reproduces when there is no brood. It seems that such adaptations of varroa are reflected in the mortality rates of honeybees occurring in Boumerdes, Tipaza, Algiers and Blida.

Destruction rate of honeybee colonies varied with the diseases and the localities considered for survey (Fig. 4). However, the results of correlation tests revealed that the correlations between the rate of loss in respect to foulbrood disease ($R = 0.21$, $p > 0.05$) and that of *Varroa* induced disease ($R = 0.009$; $p > 0.05$) are not statistically significant while the mortality rate of bees due to *Nosema* induced disease has significant correlation ($R = 0.63$; $p < 0.05$) from statistical view point. Studies suggest that the destruction rates of bee colonies in Europe and United

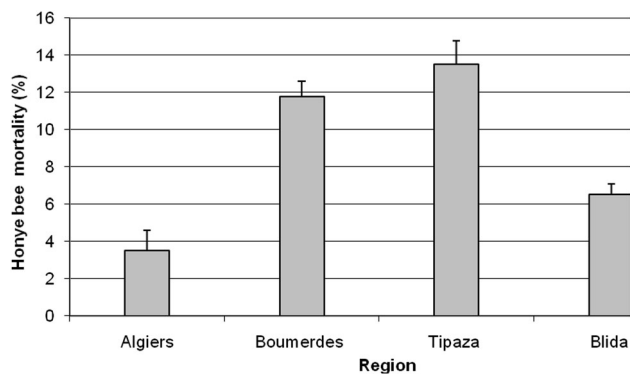


Fig. 4 Mortality rate in honeybees due to the attack of diseases in different localities of Algeria during 2013–2014

States are cumulative effects of *Nosema* and *Varroa* diseases (Ribière et al. 2006; Cox-Foster et al. 2007; Higes et al. 2008).

In view of the above factors it is concluded that attempts should be made by the experts to detect the initiation of bee-disease in the apiaries at the early state with a view to prevent the spread of the same in other colonies. Therefore, necessary steps in this regard, should be taken by the Government through the employment of vigilant experts of the field concerned.

References

- Adjlane, N., S.E. Doumandji, and N. Haddad. 2012. Situation de l'apiculture en Algérie: Facteurs menaçant la survie des colonies d'abeilles locales *Apis mellifera intermissa*. *Cahiers Agriculture* 21: 235–241. doi:10.1684/agr.2012.0566.
- Adjlane, N., K. Ameur Lain, N. Lecksir, N. Gharabi, and N. Haddad. 2013. Detection of *paenibacillus larvae* spores in honey samples from beekeepers of the central region of Algeria. *Journal of Microbiology, Biotechnology and Food Sciences* 3(1): 81–83.
- Ball, L., and B.V. Bailey. 1991. *Honey bee pathology*, 125. London: Academic Press.
- Barbançon, J.M., and M. L'Hostis. 2007. Pathologie. *Nosema qui est ? La Santé de l'Abeille* 3: 139–143.
- Branco, M.R., N.A.C. Kidd, and R.S. Pickard. 1999. Development of *Varroa jacobsoni* in colonies of *Apis mellifera iberica* and Mediterranean climate. *Apidologie* 30: 491–503.
- Cantwell, G.E. 1970. Standard methods for counting *Nosema* spores. *American Bee journal* 110: 222–223.
- Copley, T.-R., and S.H. Jabaji. 2012. Honeybee glands as possible infection reservoirs of *Nosema ceranae* and *Nosema apis* in naturally infected forager bees. *Journal of Applied Microbiology* 112(1): 1–7.
- Cox-Foster, D.L., S. Conlan, E.C. Holmes, G. Palacios, J.D. Evans, and N.A. Moran. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* 318: 283–287.
- Dainat, B., J.D. Evans, Y.P. Chen, L. Gauthier, and P. Neumann. 2012. Dead or alive: Deformed wing virus and *Varroa destructor* reduce the life span of winter honeybees. *Applied Environmental Microbiology* 78: 981–987. doi:10.1128/AEM.06537-11.
- Dietemann, V., F. Nazzi, S.J. Martin, D. Anderson, B. Locke, K.S. Delaplane, Q. Wauquiez, C. Tannahill, E. Frey, B. Ziegelmann, P. Rosenkranz, and J.D. Ellis. 2013. Standard methods for *Varroa* research. *Journal of Apicultural Research* 52: 1–49. doi:10.3896/IBRA.1.52.1.09.
- Dyess, E.G., and C.A. Wilson. 1978. A study of the seasonal variations of *Nosema apis* Zander of honey bee in Mississippi. *American Bee Journal* 118: 33–35.
- Fries, I. 1993. *Nosema apis*: A parasite in the honey bee colony. *Bee World* 74: 5–19.
- Guzman-Novoa, E., L. Eccles, Y. Calvete, J. McGowen, P.G. Kelly, and A. Corra-Benitez. 2010. *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie* 41: 443–450.
- Haynes, W.C. 1972. The catalase test, an aid in the identification of *Bacillus larvae*. *American Bee Journal* 112: 130–131.
- Haddad, N., A. Shammout, and A. Al-Nsour. 2007. The economic value of honeybees for crop pollinisation in Jordan. In 40th *Apimondia international apicultural congress*, Melbourne, p. 115.
- Hansen, H., and C.J. Brodsgaard. 1999. American foulbrood: A review of its biology, diagnosis and control. *Bee World* 80: 5–23.
- Higes, M., R. Matin-Hernandez, C. Botias, E. Garrido-Bailon, A.V. Gonzales-Porto, and L. Barrios. 2008. How natural infection by *Nosema ceranae* causes honeybee colony collapse. *Environmental Microbiology* 10: 2659–2669.
- Kraus, B., and R.E. Page. 1995. Population growth of *Varroa jacobsoni* Oud in Mediterranean climates of California. *Apidologie* 26: 149–157.
- Lindstrom, A., S. Korpela, and I. Fries. 2008. Horizontal transmission of *Paenibacillus larvae* spores between honey bee (*Apis mellifera*) colonies through robbing. *Apidologie* 39: 1–8.
- Martin-Hernandez, R., A. Meanna, L. Priero, M. Salvador, E. Garrido-Bailon, and H. Higes. 2007. Outcome of colonization of *Apis mellifera* by *Nosema ceranae*. *Applied and Environmental Microbiology* 73: 6331–6338.
- Moeller, F.E. 1978. *Nosema* disease—its control in honey bee colonies. *US Department of Agricultural Technical Bulletin* 1569: 22–32.
- Mussen, E.C., B. Furgala, and R.A. Hyser. 1975. Enzootic levels of *Nosema* disease in the continental United States. *American Bee Journal* 115: 48–50.
- Oie, A.H.S. 2005. *Manual of diagnostic tests and vaccines for terrestrial animals*, 1343. Paris: World Organisation Animal Health.
- Soesensen, P.E. 2009. Breeding *Nosema*—free colonies in Denmark. In *Proceedings Apimondia 41st congress*, Montpellier, p. 132.
- Shimanuki, H., and D.A. Knox. 2000. *Diagnosis of honey bee diseases*. Agriculture handbook no. AH690. US Department of Agriculture, Beltsville.
- Ribière, M., P. Lallemand, A.L. Iscache, F. Schurr, O. Cele, P. Blachard, V. Olivier, and J.P. Faucon. 2006. Infectious chronic bee paralysis virus (CBPV) excretion in honey bee (*Apis mellifera* L.) faeces: A way of spread. In *Proceeding second European conference*, ed. V. Vesley, and D. Titera, pp. 21–22. Apidology, EurBee, Prague.
- Shammout, S., N. Haddad, and O. Abuobaid. 2014. The monetary value of ecosystem services provided by insects (a case study for selected crops in Jordan). *Jordan Journal of Agricultural Sciences* 10: 02–07.
- Swart, D.J. 2003. *The occurrence of Nosema apis (Zander), Acarapis woodi (Rennie) and the cape problem bee in the summer rainfall region of South Africa*. Master of Science. and Euden Gradum Rhodes University, 50p.

- VanEngelsdorp, D., J.D. Evans, C. Saegerman, C. Mullin, E. Haubruge, B.K. Nguyen, M. Frazier, J. Frazier, D. Cox-Foster, Y. Chen, R. Underwood, D.R. Tarpy, and J.S. Pettis. 2009. Colony collapse disorder: A descriptive study. *PLoS One* 4(8): e6481.
- Vandame, R. 1996. Importance de l'hybridation de l'hôte dans la tolérance à un parasite. Cas de l'acarien parasite *Varroa jacobsoni* chez les races d'abeilles *Apis mellifera* européenne et africanisée au Mexique. Ph.D. dissertation, Université Claude Bernard Lyon 1, France.