Thiamethoxam (Neonicotinoïd) and Spinosad (Bioinsecticide) Affect Hypopharyngeal Glands and Survival of *Apis mellifera intermissa* (Hymenoptera: Apidae)

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1 Introduction

To avoid contaminating non target animals and pollution during pesticide treatments of crops, neonicotinoïds were developed during the ninetees. These neurotoxics target insect nicotinic acetylcholine receptors (nAChRs) making them harmless to mammals. However, they proved to be very toxic toward honeybees. Thiamethoxam is one of the most commercialized neonicotinoïd. It converts to clothianidin, a highly remnant neonicotinoïd. Thiamethoxam impairs olfaction, orientation, locomotion, queens' fitness, survival and indirectly, harms the immune system of honeybees (Brandt et al. 2016). It also alters hypopharyngeal glands of honeybees, therefore threatening the supply of the brood and the queen (Renzi et al. 2016).

Spinosad was developed to replace synthetic insecticides. Despite the low environmental and toxicological risk of this natural derivate of a soil bacteria, when liquid, it is highly toxic to honeybees especially by ingestion (Mayes et al. 2003). The primary mode of action of spinosad is to act on nAChRs. It also acts on GABA and chlorine channel receptors.

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Hypopharyngeal glands which secrete royal jelly to feed larvae and queen of honeybees play a key role in colony development. Any disturbance in their functioning can impair colony fitness and survival. Many insecticides among which neonicotinoïds impair hypopharyngeal glands structure and functioning (Hatjina et al. 2013). Our study aimed to assess and compare the effects of acute ingestion of LC_{50} and chronic ingestion of $LC_{50}/5$ of thiamethoxam and spinosad on hypopharyngeal glands development and survival of native honeybee race *Apis mellifera intermissa.* We used acini diameter and protein content of the head as parameters to assess physiological status of hypopharyngeal glands.

2 Materials and Methods

The oral toxicity at 24 h of thiamethoxam and spinosad (LC_{50} 24 h) were determined on newly emerged honeybees (*Apis mellifera intermissa* from non treated apiaries in Annaba, Algeria). Afterwards, five groups of newly emerged honeybees were set up. The first and second group were exposed to continuous source of thiamethexam and spinosad respectively through sugar syrup and pollen pastry at a concentration of $LC_{50}/5$. The two other groups were exposed respectively to thiamethexam and spinosad, 24 h only, at a concentration of LC_{50}. The last one served as control. After 6, 9 and 14 days, two pools of honeybees were collected from each group and their heads were removed. For the first pool, the heads were dissected and the hypopharyngeal glands (HPGs) collected. The diameter measurement of an average of 20 acini per HPG was performed in order to assess the HPG development (Hatjina et al. 2013). For the second one, the heads were put in 1 ml Phosphate buffer

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saline. Afterwards, proteins were extracted following Fortini et al. (2009) and quantified following Hartfelder et al. (2013). Protein content of the head served as a second parameter to assess HPG development (Renzi et al. 2016). In parallel, five other groups of newly emerged honeybees were set up and exposed continuously to thiamethoxam and spinosad respectively at $LC_{50}/5$ for the first two groups and at $LC_{50}/2$ for the two others against control group. Each 24 h, the number of dead bees was recorded during 60 days. For both experiments, sugar syrup and pollen pastry were changed twice a week.

3 Results and Discussion

The LC_{50} of thiamethexam and spinosad was 0.31 and 24 ng/µl respectively. This corresponds to LD_{50} of 2.48 and 192 ng/bee respectively.

Acute exposure to thiamethoxam decreases significantly acini diameters only at 14 days when spinosad leads to significant decrease at all ages (Fig. 1a). No significant difference is exhibited between both insecticides at any age. Chronic exposure to thiamethoxam decreases acini diameters at all ages (Fig. 1b). For spinosad, a significant decrease is noticed at 9 and 14 days. Similarily, no significant difference is exhibited between both insecticides at any age. In addition, no significant difference of acini diameters is observed between both acute and chronic treatment neither after thiamethoxam nor after spinosad exposure.

The decrease of acini diameter after insecticide ingestion is accompanied by the deterioration of acini which appear with irregular shapes.

Protein content of the head is less sensitive to chronic treatment and especially to acute one with both insecticides. Indeed, protein content of the head decreases significantly only at 9 days after acute exposure to thiamethoxam and spinosad (Fig. 1c). However, after chronic exposure, we noticed a significant decrease at 6 and 9 days for both insecticides (Fig. 1d).

Both thiamethoxam and spinosad decrease honeybee survival for both concentrations. However, only spinosad at $LC_{50}/2$ exhibits significant survival decrease compared to control.

The impairment of HPG due to chronic exposure to thiamethoxam demonstrated by our study confirms previous work (Renzi et al. 2016). The acute exposure to high thiamethoxam concentration has the same impact on HPG as chronic one to a lower concentration. The bioinsecticide spinosad also has negative effects on HPG and exhibits the same impairment induced by thiamethoxam. In addition, both acute and chronic exposure to spinosad are harmful to HPG. Also, our study partially confirms that protein content

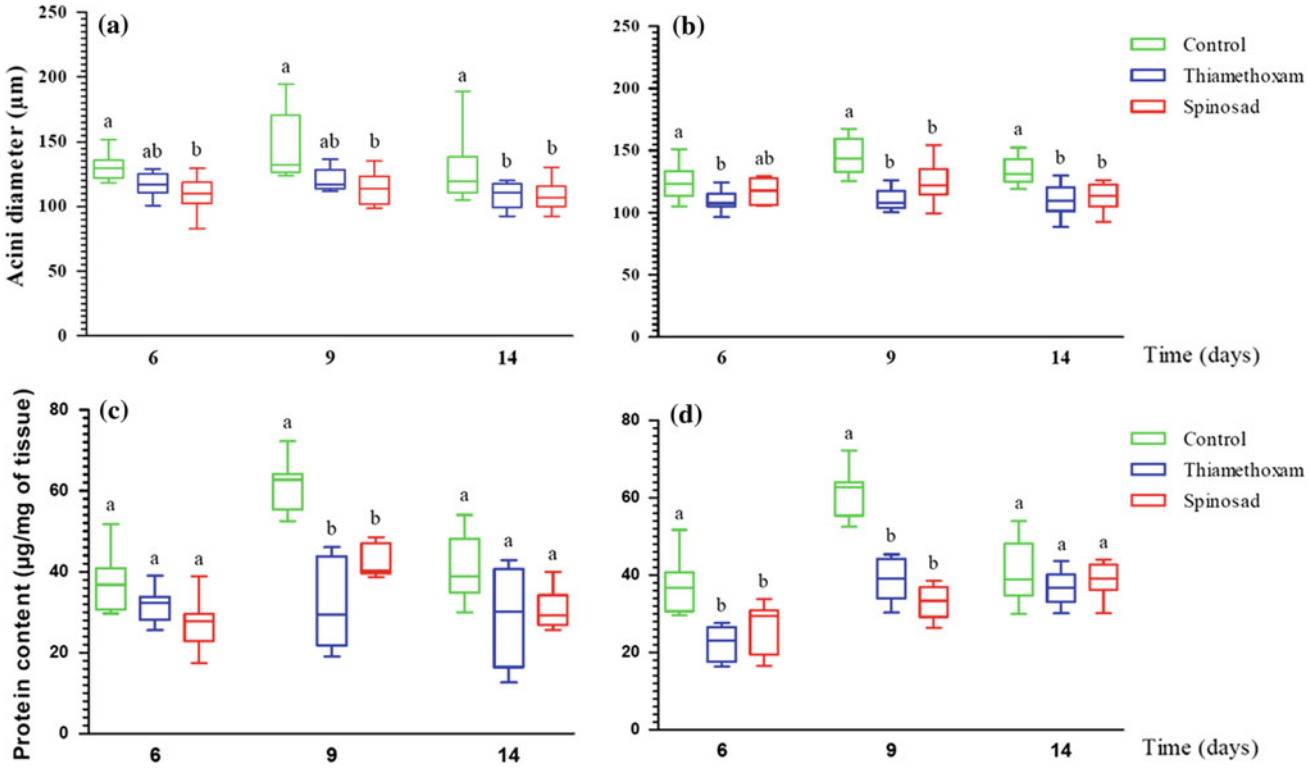

Fig. 1 Thiamethoxam and spinosad effect on acini diameter and protein content of the head. (**a**): Effect of acute treatment on acini diameter; (**b**): Effect of chronic treatment on acini diameter; (**c**): Effect of acute treatment on protein content of the head; (**d**): Effect of chronic treatment on protein content of the head

of the head evolves following the acini diameter but with some gaps, which was already mentioned by Renzi et al. (2016).

The absence of a significant decrease of survival after thiamethoxam exposure observed during our experiment complies with Köhler et al. (2012) results when honeybees were treated with nicotine. Also, our results reveal that spinosad has a detrimental effect on survival when a high concentration is ingested.

4 Conclusion

Acute (LC_{50}) and chronic ($LC_{50}/5$) exposures to thiamethoxam and spinosad impair hypopharyngeal glands by reducing acini diameters and protein content of the head of native honeybee *Apis mellifera intermissa*. However, these two parameters are not affected in exactly the same way. We conclude that even if it is a bioinsecticide, spinosad harm hypopharyngeal glands like thiamethoxam. In addition, spinosad shortens honeybee survival more than thiamethoxam, probably due to the multiple targets of this insecticide in the nervous system. Therefore, our study proves that both thiamethoxam and spinosad are harmful to honeybees.

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