HOW CAN WE QUALIFY THE REPELLENT EFFECT OF ESSENTIAL OIL ON INSECTS?

ANNE BUREL[1], LISE ROY[2], JUAN MANUEL GARCIA[1], AGATHE LABALETTE[1], PIERRE CHICOTEAU[1]

[1]NOR FEED SAS, 3 rue AMEDEO AVOGADRO, 49 070 BEAUCOUZE[2]CEFE, Equipe Interactions Biotiques, 1919 route de Mende, 34293 Montpellier
Contato: anne.burel@norfeed.net

Resumo: Arthropods have an economic and sanitary impact on feed and farm animals' production. The use of chemical insecticide against storage pests is restricted due to the residual toxicity and resistance appearance, especially when the level of production in the country is high. The loss of raw material caused by storage pests is estimated at 10% in stored products on a world scale. Moreover, using efficient insecticides in the presence of animals on the farm can sometimes be difficult.
Initially, a natural in-feed ingredient composed of essential oils such as lemongrass (Cymbopogon nardus), clove (Eugenia caryophyllus), oregano (Oreganum vulgare) and citrus extract, was developed to be a repellent against red-mites, a poultry ectoparasite. In order to develop a new application, this study evaluated the sensitivity of some insects (Tenebrio molitor, Alphitobius diasperinus and Sarcopha sp.) to this natural in-feed repellent
Palavras Chave: solução natural, ectoparasita do alimentaçao animais, oleos essenciales, moscas, tenebrio

HOW CAN WE QUALIFY THE REPELLENT EFFECT OF ESSENTIAL OIL ON INSECTS?

Abstract: Arthropods have an economic and sanitary impact on feed and farm animals' production. The use of chemical insecticide against storage pests is restricted due to the residual toxicity and resistance appearance, especially when the level of production in the country is high. The loss of raw material caused by storage pests is estimated at 10% in stored products on a world scale. Moreover, using efficient insecticides in the presence of animals on the farm can sometimes be difficult.
Initially, a natural in-feed ingredient composed of essential oils such as lemongrass (Cymbopogon nardus), clove (Eugenia caryophyllus), oregano (Oreganum vulgare) and citrus extract, was developed to be a repellent against red-mites, a poultry ectoparasite. In order to develop a new application, this study evaluated the sensitivity of some insects (Tenebrio molitor, Alphitobius diasperinus and Sarcopha sp.) to this natural in-feed repellent
Keywords: Natural feed additive, animal feed, ectoparasite, essentials oils, flies, darkling beetle

Introdução: The control of pests is a major issue in the agricultural sector (Klys et al. 2017). The litter beetle Alphitobius diaperinus is the most common worldwide insect pest found in poultry farms, being a possible vector in the transmission of Salmonella (Leffer et al. 2010). Arthropods such as flies can transfer diseases in poultry production.
Raw materials can also be impacted by storage pests. The coleopteran Tenebrio molitor is a pest in stored products (Martinez et al. 2017).
The repulsive impact of a natural repellent against red-mite composed of citronella (Cymbopogon nardus), clove (Eugenia caryophyllus), oregano (Oreganum vulgare) and citrus extract, via the feed, was demonstrated several times on red-mites (El Adouzi et al. 2017). The present study was set up to evaluate the potential repulsive effect of this natural product on other species than red-mites, for new applications, especially on feed pests, using electroantennography (EAG).

Material e Métodos: The capacity of insects to detect aromatic molecules was measured by EAG in a laboratory. This method, described by SYNTECH, 2004, is based on the discovery by Schneider (1957) who recorded small voltage fluctuations between the tip and base to determine pheromones perceived by insects. The amplitude of the tension measured is caused by the depolarisation of olfactory neurones situated in antennas of tested insects.
The antenna of alive coleopter and flies was separated from the head and placed in an electroantennography to obtain a connection between two glass electrodes with Ringer liquid included. The electric signal was registered and visualised by an oscilloscope linked to a computer.
A continuous air stream was oriented on the antenna. The test consisted in injecting punctually into this continuous air stream a determined quantity of volatile compounds to test ("puff"). The aim being the observation of eventual electrical responses and to register the associated amplitude.
Controls were made on each antenna, one with an injection of pure air (T-), another with a mix of 40 molecules generally used in a laboratory to obtain stimuli in major arthropod (T+). The antenna was maintained under a pure air stream for a minimum of 30 seconds between 2 puffs to allow olfactive receptors to recover. One antenna from 5 different insects was tested for each species. Two tenebrio T. molitor and A. diaperinus and a Sarcopha sp. type of fly were tested with this protocol.

Resultado e Discussão: Each species tested reacted positively to at least one of the volatile compounds from the natural in feed repellent. The experiment demonstrated a clear electrophysiological response to the volatile molecule cocktail emitted by this product ($p<0.05$; Kruskal-Wallis). On all species, the median amplitude of electric responses to the volatile molecule provided by NM, and T+ were significantly different compared to T- ($P<0.05$; Mann Whitney pair-wise test).
This test exposes the capacity of insects to detect volatile molecules from NM. While this method does not allow to determine whether the effect of the product is attractive or repellent for arthropods and insects, the repellent

effect of certain essential oils contained in this product formulation, such as lemongrass (C. nardus), clove (E. caryophyllus), oregano (O. vulgare), have been clearly demonstrated (Soon Il, et al. 2004; George, et al. 2009). Lemongrass is also known for its repellent effect against fleas and biting insects (E.Maggi et al. 2006). Moreover, the repulsive effect of this product has already been demonstrated on mites and flies (El Adouzi et al. 2017, Labalette 2015). Thus, further research is needed to confirm the potential repellent effect of this product on in-feed pests.

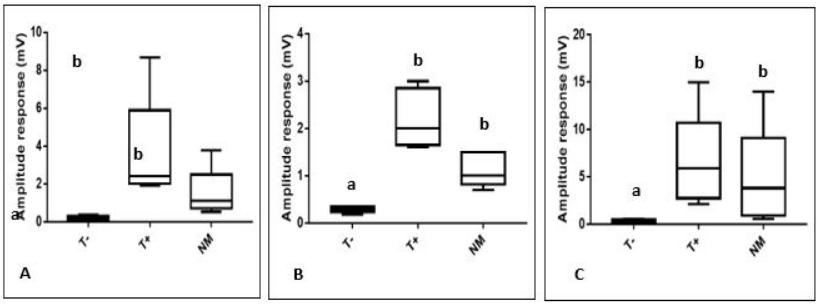

Figure 1: Boxplot of amplitude responses to different puffs A: *A. diaperinus*, B: *T. molitor*, C: *Sarcophaga sp.*. Values with different superscript within the same graph differ significantly ($p<0,05$, Mann-Witney pair-wise test). T-: Negative control, T+ positive control NM: Natural in feed repellent

Conclusão: This study showed a significant response to the olfactive compounds from this in-feed repellent on insects. While its repellent effect has been demonstrated on mites and flies (El Adouzi et al. 2017, Labalette 2015) these first results show a potential interest for a new application against storage pests. Further research is needed to confirm these promising observations.

Agradecimentos: A special thanks to the Centre of functional Ecology and the evolutive (CEFE) team for the realisation of the study.

Referências Bibliográficas: El Adouzi M., Arriaga A., Buatois B., Lapeyre B., Dormont L., Bonato O., Roy L., 2016, Response of the PRM *D. gallinae* to changing VOCs emmited by its host *Gallus gallus domesticus* fed with additive repellents. EURAAC, 8th symposium of the European Association of Acasiologist. Klys M.; Malejky N.; Nowak-Chmura M. 2017. The repellent effect of plants and their active substances against the beetle storage pests. Journal of Stored Products Research George D.; Smith T; Shiel R; SparaganoJ O.; Guy H. 2009. Mode of action and variability in efficacy of plant essential oils showing toxicity against the poultry red mite, D. gallinae; Veritanary pasithology Labalette A .2015. Ectoparasitas: solução natural Nor-Mite via ração; saúde animal AVES e OVOS, nº 241 - setembro/outubro 2015 Leffer; Kuttel J.; Martins L..; Pedroso A.; Astolfi-Ferreira C., Ferreira F.; Piantino Ferreira A. 2010. Vectorial competence of larvae and adults of A. diaperinus in the transmission of Salmonella Enteritidis in Poultry A.M. Vector borne and zoonotic disease Volume 10, Number 5 Magi E.; Jarvis T.; I. Miller 2006 Effects of Different Plant Products against Pig Mange Mites Martínez L.; Plata-Rueda A.; Colares H.; Campos J.; Dos Santos M.; Fernandes F.; Serrão J.; Zanuncio J. 2014. Toxic effects of two essential oils and their constituents on the mealworm beetle, T. molitor Soon-Il K.; Jee-Hwan Y.; Jun-hyung T.; Young-Joon A. 2004. Acaricidal activity of plant essential oils against D. gallinae Veterinary Parasitology 120 (2004) 297–304