SCIENTIA PLENA
www.scientiaplena.org.br
VOL. 18, NUM. 7 2022
doi: 10.14808/sci.plena.2022.070206

Occurrence of pollinating beetles on oil palm inflorescences treated with insecticides injection

Ocorrência de besouros polinizadores em inflorescências de dendezeiro tratado com injeção de inseticidas

J. I. L. Moura[1]; R. R. da C. Pereira*[2]; M. E. Caliman[3]; C. E. Pereira[2]

[1]*Estação Experimental Lemos Maia, Comissão Executiva do Plano da Lavoura Cacaueira, 45690-000, Una-BA, Brasil*
[2]*Centro de Formação em Ciências Agroflorestais, Universidade Federal do Sul da Bahia, 45604-811, Ilhéus-BA, Brasil*
[3]*Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, 45662-900, Ilhéus-BA, Brasil*

**rosaneilheus@gmail.com*
(Recebido em 01 de abril de 2022; aceito em 29 de junho de 2022)

The most important oil palm pollinators are *Elaeidobius subvittatus* and *Elaeidobius kamerunicus* (Coleoptera: Curculionidae). Thus, techniques for integrated pest management that preserve these beneficial organisms should be studied. Therefore, this research aimed to verify the effect of abamectin or imidacloprid injections on oil palm pollinating beetle incidence. The study was carried out at the Lemos Maia Experimental Station in the municipality of Una, Bahia state, using 12-year-old oil palm plants. Imidacloprid or abamectin was applied via injection and the emergence of *E. kamerunicus* and *E. subvttatus* adults in male inflorescences was verified. It was used a completely randomized design. The number of individuals of the species *E. kamerunicus*, regardless of the treatment applied, was higher than *E. subvittatus*. Furthermore, the number of *E. kamerunicus* and *E. subvittatus* that emerged of plants treated with abamectin or imidacloprid injections did not differ significantly from the control treatment, without insecticide. Thus, it is concluded that the application of abamectin or imidacloprid via injection in oil palm plants does not affect the number of individuals of *E. kamerunicus* and *E. subvittatus* emerged from male flowers.
Keywords: *Elaeidobius kamerunicus*, *Elaeidobius subvittatus*, *Elaeis guineensis*.

Os mais importantes polinizadores do dendezeiro são *Elaeidobius subvittatus* e *Elaeidobius kamerunicus* (Coleoptera: Curculionidae). Assim, deve-se buscar metodologias para o manejo integrado de pragas que preservem esses organismos benéficos. Por isso, nessa pesquisa objetivou-se verificar em dendezeiro o efeito de injeções de abamectina ou de imidacloprido sobre a incidência de besouros polinizadores. O estudo foi desenvolvido na Estação Experimental Lemos Maia, Ceplac em Una, no estado da Bahia utilizando-se dendezeiros com 12 anos. Aplicou-se imidacloprido ou abamectina via injeção e verificou-se a emergência de adultos em inflorescências masculinas. Foi utilizado o delineamento experimental inteiramente casualizados. O número de indivíduos da espécie *E. kamerunicus*, independentemente do tratamento aplicado, foi superior ao de *E. subvittatus*. Além disso, o número *E. kamerunicus* e de *E. subvttatus* que emergiram de plantas que receberam injeções de abamectina ou de imidacloprido não diferiram significativamente da testemunha, sem tratamento inseticida. Assim, conclui-se que a aplicação de abamectina ou de imidacloprido via injeção em dendezeiros não afeta o número de indivíduos *de E. kamerunicus* e *E. subvttatus* que emergem das flores masculinas.
Palavras-chave: *Elaeidobius kamerunicus*, *Elaeidobius subvittatus*, *Elaeis guineenses*.

1. INTRODUCTION

Oil palm (*Elaeis guineensis* Jacquin.) is a monoecious plant, so the male and female flowers are produced on the same plant but in inflorescences separated. This characteristic increase cross-pollination potential because female and male flowers are rarely simultaneously receptive on the same plant at the same time [1]. Thus, the cultivation of this plant depends on the action of pollinating insects for an adequate fruit production.

The most important palm trees pollinators are found in the Curculionidae family as genera *Phyllotrox*, *Derelominus*, *Derelomus*, *Meredolus*, *Notolomus*, *Nodoncnemus*, *Derelomorphus*, *Prosoestus* and *Elaeidobius* [2]. The genus *Elaeidobius* includes the species *E. kamerunicus*, *E. subvittatus*, *E. singularis* and *E. plagiatus*. These species have a short biological cycle and visit only flowers of the genus *Elaeis* [3].

In southern Bahia, *E. subvittatus* and *E. kamerunicus* (Coleoptera: Curculionidae) are the most important oil palm pollinators. *E. kamerunicus* is larger than *E. subvittatus* and, consequently, transports a larger amount of pollen grains and can be more efficient in pollination. Thus, *E. kamerunicus* introduced in Malaysia from Africa allowing the suspension of assisted pollination and increasing oil production [4]. Later, *E. kamerunicus* was introduced into commercial oil palm plantations in Latin America. Embrapa introduced this species in the Amazonas and Pará states, Brazil. In 1994 the Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC), with the support of Embrapa, introduced *E. kamerunicus* in southern Bahia with the aim of improving the fertilization rate of oil palm fruits. Ten years after the introduction of these pollinators, the fertilization rate increase to 79% from 60% in plantations at the Estação experimental Lemos Maia (ESMAI), and the populations of *E. kamerunicus* beating those of *E. subvittatus* in municipalities where oil palm plants subspontaneous occur frequently [5].

These pollinators as adults feed on pollen and lay their eggs in male oil palm inflorescences. The larvae develop by consuming decaying floral parts and the pupae remain in the spikelets of the male flowers, where adults emerge [6, 7]. After emerging, adults are attracted by the fragrances of male and female inflorescences that have the same odor due to the exhaled odorant 4-allylanisole, also known as Estragole [8].

In the management of oil palm pests with phytosanitary products, it is important to know the effects of pesticides on non-target organisms, such as natural enemies and pollinators. This information is crucial for control tactics decision-making and preventing reduction in beneficial insect populations.

Endotherapy is a phytopharmaceutical tactic that consists of injecting or infusing phytosanitary product into the tree or palm tree trunk and translocated through vascular tissues to reach higher parts of the canopy, not reached by conventional sprays. The advantage of pesticide injection is its full use when incorporated into the vascular system [9-11] and distribution throughout the tree [12]. Biological control agents such as fungi, bacteria and resistance inducers can be used in endotherapy [13, 14]. It is considered an environmentally safe method, due to the non-exposure of non-target organisms, soil, water, air, wildlife, in addition to not be exposed to negative effects from weather conditions such as rain and solar radiation [15].

Tests with pesticide injections in palm trees have been used for over 40 years in the African, Asian and American continents [16]. Experiments conducted by Chihaoui-Meridja et al. (2020) [17], in Tunisia, with canary island date palm (*Phoenix canariensis* Chabau), aiming to control *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), the most invasive and destructive pest of arecaceous in the world [18].

According to Chihaoui-Meridja et al. (2020) [17], several control tactics have been adopted, including biological control with nematodes, enteropathogenic fungi and mass collection with pheromone. But, according to these authors, spraying with pesticides are the only way to preventively control adults that mate and oviposit in the plant leaf crown region. However, depending on the pesticide residual persistence, reapplications may be necessary. In turn, spraying affects non-target organisms and in the case of palm trees for landscape use, as canary island date palm trees, it is difficult to adopt such control method because are public areas where people pass through.

The experiments by Chihaoui-Meridja et al. (2020) [17] with injections of thiamethoxam and emamectin benzoate showed that thiamethoxam caused high mortality in adults in pupae and in larvae of *R. ferrugineus*. However, thiamethoxam had preventive rather than curative action. In turn, emamectin benzoate showed efficiency in mortality of pupae, larvae, and adults in the preventive and curative treatment.

Another experiment with injections was conducted by Mashal and Obeidat (2019) [19] in date palm (*P. dactylifera* L) plantations in Jordan to control *R. ferrugineus*. These authors used two formulations (microemulsions) of emamectin benzoate (Revive® 4%, 40 g L^{-1} and Revive® 9.5%, 95 g L^{-1}) specially formulated for palm trees. To inject the two formulations into palm trees, they used a device named TMI (tree micro injection; Syngenta Plant Protection) that infused the compounds into vascular tissues at pressure of 2 bar. After 12 months, the average number of death individuals of *R. ferrugineus* in plants that received injections of Revive® 4% and 9.5% did not differ from each other but was higher than control treatment.

Although, endotherapy is a selective tactic to non-target organisms and the environment in general, there are no reports in the literature about the effect on pollinators in palm trees inflorescences. In oil palm the situation is more complicated because adults of *E. kamerunicus* and *E. subvittatus* feed on pollen and larvae breed inside the male flowers. In this case, the hypothesis that pesticide residues can affect these pollinates must be considered. Thus, this study aimed to evaluate the effect of abamectin and imidacloprid injections in oil palm on the pollinator incidence.

2. MATERIALS AND METHODS

The study was carried out at the Estação Experimental Lemos Maia (ESMAI), Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC), in the municipality of Una, Bahia state, Brazil (15º17'34" S, 39º04'30" W) in oil palm (*Elaeis guineensis* Jacquin.) plantations of Tenera variety in two areas (A and B), 1.5 km apart. Area A has 8 hectares and area B has 2.5 hectares with oil palms plants.

All plants were 12 years old and, in both areas, did not receive top-dressing. However, mowing, and leaf pruning were carried out.

In each area, 40 oil palm trees were randomly marked, 20 were used with insecticide treatment (injections) and 20 as control (without injections). Thus, in area "A" the plants received treatment with 40 ml of concentrated abamectin injection (0.72 g a.i. plant^{-1}) and in area "B", 20 ml of concentrated imidacloprid injection (4 g a.i. plant^{-1}). To apply the products to the interior of the oil palm stem, a hole 16 mm in diameter and 25 cm in depth was made with a drill attached to a chainsaw. The holes were approximately 40 cm height and inclined in relation to the stem axis to prevent pesticide runoff. The pesticides were injected into the hole with a syringe and after the hole was covered with a wooden plug to prevent entry of water.

After insecticides application, weekly field inspections were carried out to identify the male inflorescences and pollinator's oviposition site in the beginning of the anthesis. Three spikelets were collected (top, middle and base of male inflorescence sampled) by repetition. Each repetition consisted of one inflorescence per plant. These sample collections occurred 30 days after the injections for oil palms plants treated with abamectin and 60 days for those treated with imidacloprid because in studies carried out with injections in coconut palms (*Cocos nucifera* L.) by Moura et al. (2020) [20] was found that ascension of imidacloprid to the crown is slow, probably due to its high viscosity.

The spikelets were placed individually in identified polyethylene cups, covered with organza, and taken to the ESMAI laboratory. As the beetles emerged from the spikelets they were killed, identified, sorted by species, and counted. For this study, the species *Elaeidobius subvittatus* and *E. kamerunicus* were evaluated. An analogous procedure was adopted for control oil palms plants (without pesticide injections). Data were presented as total number of *E. subvittatus* and *E. kamerunicus* individuals emerged per repetition.

A completely randomized design with 2 treatments and 20 replications was used. Data were transformed by $\sqrt{(x + 0.5)}$ and then submitted to analysis of variance (ANOVA) comparing means by the F test ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

The number of individuals of the species *E. kamerunicus* in male inflorescences of oil palm, regardless of the treatment applied, was higher that *E. subvittatus* (Figure 1). This was expected because after the introduction of *E. kamerunicus* in southern Bahia, this species became dominant [5]. In the work developed by Moura et al. (2008) [5] it was found that the number of *E. kamerunicus* emerged from male spikelets was almost 15 times greater than *E. subvittatus*. These results corroborate those obtained by Chinchilla and Richardson (1991) [21] and Mariau and Genty (1988) [22] in studies carried out in Costa Rica, Honduras and Venezuela, respectively. This is probably due to the dispute for space for oviposition. Females of *E. kamerunicus* oviposit for a longer period and start before *E. subvittatus* that being at the end of the anthesis period and those, earlier, in the newly opened inflorescences, dominating the space [21].

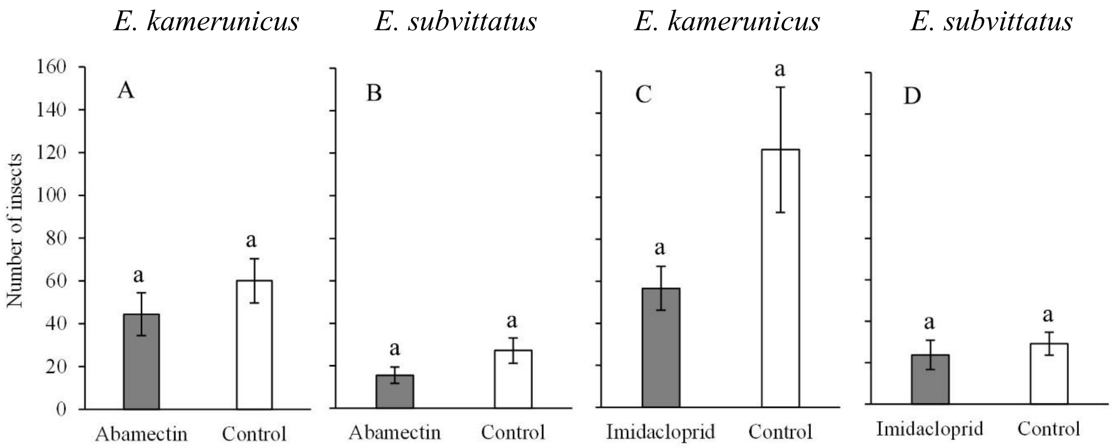

Figure 1. Number of individuals of Elaeidobius kamerunicus (A, C) and Elaeidobius subvittatus (B, D) in male inflorescences of oil palms plants treated with abamectin and imidacloprid injection. Means followed by the same letter does not differ significantly from each other by the F test ($p \leq 0.05$). Bars at the top of columns indicate the standard error of the mean.

There was no significant difference between treatments (Figure 1), i.e., the number of pollinators of the species *E. kamerunicus* and *E. subvittatus* that emerged from the male flowers of plants that received injections of abamectin or imidacloprid did not differ statistically from the control treatment, without insecticide.

Both imidacloprid and abamectin act in the transmission of insect nerve impulses in an irreversible way. Imidacloprid is a neonicotinoid and binds to nicotinic acetylcholine receptors in the postsynaptic membrane [23-25]. Different to acetylcholine, imidacloprid is not immediately hydrolyzed by acetylcholinesterase. In this way, nerve impulses are continuously transmitted leading to nervous system hyperexcitation and insect death [26].

Abamectin belongs to the group of avermectins and acts as an agonist to GABA (gamma-aminobutyric acid) which is an inhibitory neurotransmitter. It causes an excessive increase in membrane permeability to the Chlorine ion, mimicking the calming effect of GABA, super-inhibits the central nervous system and causes death by paralysis [27].

Imidacloprid applied via spray is effective in controlling *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae) [23] and *Strategus aloeus* L. (Coleoptera: Scarabaeidae) [28], important oil palm pests. However, the use of this insecticide in spraying plants may be impact on non-target organisms such as pollinators and natural enemies [29-33] and, due to its ability to translocate in the plant, it has the potential to contaminate nectar and pollen [34].

Pereira et al. (2020) [35] observing the behavior of *Apis melifera* (Hymenoptera: Apidae) that ingested and were exposed to imidacloprid verified that this neonicotinoid caused mortality in 100% of bees. Furthermore, in similar conditions Yang et al. (2008) [36] observed behavioral changes in individuals of this species. Setyawan et al. (2020) [37] studied the effects of imidacloprid and other phytosanitary products on the mortality and fecundity of *E. kamerunicus* and verified that, under laboratory conditions, this neonicotinoid applied via direct spray, killed 40% of the insects and negatively affected the fecundity of this pollinator.

However, even in this work, when compared to the other insecticide treatments studied, imidacloprid had the lowest mortality rate for *E. kamerunicus*, indicating its potential for use in oil palm plantations using techniques capable of reducing direct exposure from the pollinating insect to the phytosanitary product, such as injections in the plants (Figure 1).

No residue of imidacloprid was detected in nectar or pollen when injected into apple trees during spring, but 0.39 ng g^{-1} in pollen was detected when injected in late spring, but still below the maximum residue limit established by the environmental agency of the United States for bees [38].

Ahmed et al. (2010) [39] applied imidacloprid, via injection and soil, in date palm and verified efficient control of green pit scale insect (*Palmapsis phoenicis* Ramachandra Rao Hemiptera: Asterolecaniidae), and residue of this insecticide in the plant was not observed. Dembílio et al. (2015) [18] applied imidacloprid and abamectin via injection in *Phoenix canariensis* and verified the efficiency of these pesticides in the control of *Rhynchophorus ferrugineus*.

Research on the pesticides application via injection indicates that this tactic is efficient in the phytosanitary management of crops. Thus, endotherapy is an alternative that can help reduce the negative impacts of pesticides on beneficial organisms because reduce the contact probability of the pesticide with these non-target organisms.

Imidacloprid and abamectin injections in oil palm in southern Bahia probably does not change the dynamics of these pollinators because the short biological cycle, frequent emission of male inflorescences with subsequent emergence of thousands of pollinators and constant supply of these pollinators through oil palm trees subspontaneous present in phytogeographic landscape of southern Bahia [5]. In Brazil, more studies are needed, such as more appropriate formulations. Finally, the determination of lethal concentration (LC50) for larvae and adults of *E. kamerunicus* and *E. subvittatus* and residues of imidacloprid and abamectin present in male flowers and pollen are important analyses to complement this study.

4. CONCLUSION

The application of abamectin or imidacloprid via injection in oil palm does not affect the number of pollinators of the species *E. kamerunicus* and *E. subvittatus* that emerge from the male flowers of the plants.

5. REFERENCES

1. Conceição HEO, Muller AA. Botânica e morfologia do dendezeiro. In: Viégas IJM, Muller AA, editors. A Cultura do dendezeiro na Amazônia brasileira. Belém (PA): Embrapa Amazônia Oriental; 2000. p. 31-44.
2. Henderson A. A review of pollination studies in the Palmae. Bot Rev. 1986 Jul;5(3):221-59. doi: 10.1007/BF02860996
3. Mariau D, Houssou M, Lecoustre R, Ndigui B. Insectes pollinisateurs du palmier et taux de nouasion en Afrique de l'ouest. Oleagineux. 1991;1:43-51.
4. Syed RA, Law IH, Corley RHV. Insect pollination of oil palm: introduction, establishment and pollinating efficiency of *Elaeidobius kamerunicus* in Malaysia. Planter. 1982;58(681):547-61.
5. Moura JIL, Cividanes FJ, Santos Filho LP, Valle RR. Polinização do dendezeiro por besouros no sul da Bahia. Pesq Agropec Bras. 2008 Mar;43(3):289-94. doi: 10.1590/S0100-204X2008000300001
6. Cunha RNV, Lopes R, Dantas JCR, Rocha RNC. Procedimentos para produção de sementes comerciais de dendezeiro na Embrapa Amazônia Ocidental [Internet]. Manaus (AM): Embrapa Amazônia Ocidental; 2007 [cited 2022 Mar 30]. (Documentos, 54). Available from: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/46497/1/Doc-54.pdf
7. Tuo Y, Koua HK, Hala N. Biology of *Elaeidobius kamerunicus* and *Elaeidobius plagiatus* (Coleoptera: Curculionidae) main pollinators of oil palm in West Africa. Eur J Sci Res. 2011;49(3):426-32.
8. Hussein MY, Lajis NH, Kinson A, Teo CB. Laboratory and field evaluation on the attractancy of *Elaeidobius kamerunicus* Faust to 4-allylanisole. Porim Bulletin. 1989;18:20-6.
9. Aćimović SG, Cregg BM, Sudin GW, Wise JC. Comparison of drill-and needle-based tree injection technologies in healing of trunk ports on apple trees. Urban For Urban Green. 2016 Sep;19(1):151-7. doi: 10.1016/j.ufug.2016.07.003
10. Costonis AC. Tree injection: Perspective macro-injection/micro-injection. J Arboric. 1981 Oct;7(10):275-8.

11. Wise JC, Vanwoerkom AH, Acimovic SG, Sundin GW, Cregg BM, Vandervoort C. Trunk injection: a discriminating delivering system for horticulture crop IPM. Entomol Ornithol Herpetol. 2014;3(2):1-7. doi: 10.4172/2161-0983.1000126
12. Kobza M, Juhásová G, Adamcíková K, Onrusková E. Tree injection in the management of Horse-Chestnut leaf miner *Cameraria ohridella* (Lepidoptera: Gracillariidae). Gesunde Pflanzen. 2011 Jan;62:139-43. doi: 10.1007/s10343-011-0236-z
13. Aćimović SG, Zeng Q, McGhee GC, Sundin GW, Wise JC. Control of fire blight (*Erwinia amylovora*) on apple trees with trunk-injected plant resistance inducers and antibiotics and assessment of induction of pathogenesis-related protein genes. Front Plant Sci. 2015 Feb;6(16):1-10. doi: 10.3389/fpls.2015.00016
14. Bahadou SA, Ouijja A, Boukhari MA, Abdessalem T. Development of field strategies for fire blight control integrating biocontrol agents and plant defense activators in Morocco. J Plant Pathol. 2017 Oct;99(special issue):51-8. doi: 10.4454/jpp.v99i0.3909
15. Aćimović SG, Martin DKH, Turcotte RM, Meridith CL, Munck IA. Choosing an adequate pesticide delivery system for managing pathegens with difficult biologies: case studies on *Diplodia cortícola*, *Venturia inaequalis* and *Erwinia amylovora*. In: Topolovec-Pintaric S, editor. Plant diseases: Current threats and management trends. London (UK): IntechOpen; 2019. p. 1-36. doi: 10.5772/intechopen.87956
16. Ferry M, Gomez S. Assessment of risks and potential of injection techniques in integrated programs to eradicate the red palm weevil: review and new perspectives. Fruits. 2014 Apr;69(2):143-57. doi: 10.1051/fruits/2014005
17. Chihaoui-Meridja S, Harbi A, Abbes K, Chaabane H, La Pergola A, Chermiti B, Suma P. Systematicity, persistence and efficacy of selected insecticides used in endotherapy to control the red palm weevil *Rhynchophorus ferrugineus* (Olivier, 1790) on *Phoenix canariensis*. Phytoparasitica. 2020 Feb;48:75-85. doi: 10.1007/s12600-019-00776-5
18. Dembilio Ó, Riba JM, Gamón M, Jacas JA. Mobility and efficacy of abamectin and imidacloprid against *Rhynchophorus ferrugineus* in *Phoenix canariensis* by different application methods. Pest Manag Sci. 2015 Aug;71(8):1091-8. doi: 10.1002/ps.3889
19. Mashal MM, Obeidat BF. The efficacy assessment of emamectin benzoate using micro injection system to control red palm weevil. Heliyon. 2019 Jun;5(6):01833. doi: 10.1016/j.heliyon.2019.e01833
20. Moura JIL, Pereira RRC, Santos Filho LP, Bispo KC, Valle RR. Injeção de imidacloprido no controle do pulgão-preto-do-coqueiro. Entomol Commun. 2020 Nov;2:ec02029. doi: 10.37486/2675-1305.ec02029
21. Chinchilla CM, Richardson DL. Pollinating insects and the pollination of oil palms in Central America. ASD Oil Palm Papers. 1991;2:1-18.
22. Mariau D, Genty PH. Contribución de IRHO al studio de los insectos polinizadores de la palma de aceite en África, América del Sur e Indonesia. Palmas. 1988 Jan;9(1):33-8.
23. Martínez LC, Plata-Rueda A, Gonçalves WG, Freire AFPA, Zanuncio JC, Bozdogan H, et al. Toxicity and cytotoxicity of the insecticide imidacloprid in the midgut of the predatory bug, *Podisus nigrispinus*. Ecotoxicol Environ Saf. 2019 Jan;167:69-75. doi: 10.1016/j.ecoenv.2018.09.124
24. Matsuda K, Buckingham SD, Kleier D, Rauh JJ, Grauso M, Sattelle DB. Neonicotinoids: Insecticides acting on insect nicotinic acetylcholine receptors. Trends Pharmacol Sci. 2001 Nov;22(11):573-80. doi: 10.1016/S0165-6147(00)01820-4
25. Tan J, Salgado VL, Hollingworth RM. Neural actions of imidacloprid and their involvement in resistance in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Pest Manag Sci. 2008;64(1):37-47. doi: 10.1002/ps.1464
26. Gwaltney-Brant S. Insecticides and Molluscicides. In: Plumlee K, editor. Clinical Veterinary Toxicology. Saint Louis (US): Mosby Inc; 2004. p. 177-178.
27. Srivastava PK, Singh VP, Singh A, Tripathi DK, Singh S, Prasad SM, et al. Pesticides in crop production: Physiological and biochemical action. Hoboken (US): Wiley; 2020.
28. Martínez LC, Plata-Rueda A, Zanuncio JC, Serrao JE. Comparative toxicity of six insecticides on the rhinoceros beetle (Coleoptera: Scarabaeidae). Fla Entomol. 2014 Sep;97(3):1056-62. doi: 10.1653/024.097.0308
29. Iwasa T, Motoyama N, Ambrose JT, Roe RM. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. Crop Prot. 2004 May;23(5):371-8. doi: 10.1016/j.cropro.2003.08.018
30. Jervis MA, Kidd NAC, Fitton MG, Huddleston T, Dawah HA. Flower-visiting by hymenopteran parasitoids. J Nat Hist. 1993;27(1):67-105. doi: 10.1080/00222939300770051

31. Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One. 2012 Jan;7(1):e29268. doi: 10.1371/journal.pone.0029268
32. Whitehorn PR, O'Connor S, Wäckers FL, Goulson D. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science. 2012 Mar;336(6079):351-2. doi: 10.1126/science.1215025
33. Yang EC, Chang HC, Wu WY, Chen YW. Impaired olfactory associative behavior of honeybee workers due to contamination of imidacloprid in the larval stage. PLoS One. 2012 Nov;7(11):e49472. doi: 10.1371/journal.pone.0049472
34. James DG, Price TS. Fecundity in two spotted spider mite (Acari: Tetranychidae) is increased by direct and systemic exposure to imidacloprid. J Econ Entomol. 2002 Aug;95(4):729-32. doi: 10.1603/0022-0493-95.4.729
35. Pereira RC, Barbosa WF, Lima MA, Vieira Lima JO, Guedes RNC, Silva BKR, et al. Toxicity of botanical extracts and their main constituents on the bees *Partamona helleri* and *Apis mellifera*. Ecotoxicol. 2020 Mar;29:246-57. doi: 10.1007/s10646-020-02167-7
36. Yang EC, Chuang YC, Chen YL, Chang LH. Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). J Econ Entomol. 2008 Dec;101(6):1743-8. doi: 10.1603/0022-0493-101.6.1743
37. Setyawan YP, Naim M, Advento AD, Caliman JP. The effect of pesticide residue on mortality and fecundity of *Elaeidobius kamerunicus* (Coleoptera: Curculionidae). Earth Environ Sci. 2020;468:12020. doi: 10.1088/1755-1315/468/1/012020
38. Coslor C, Vandervoort C, Wise JC. Insecticide dose and seasonal timing of trunk injection in apples influence efficacy and residues in nectar and plant parts. Pest Manag Sci. 2018 Nov;75(5):1453-63. doi: 10.1002/ps.5268
39. Ahmed MA, Abdelbagi AO, Elshafie HA. Trunk injection with neonictoniods insecticides to control the green pit scale insect (*Palmapsis phoenicis* Ramachandra Rao) (Homoptera: Asterolecaniidae) infesting date palm in northern Sudan. Acta Hortic. 2010;882:937-55. doi: 10.17660/ActaHortic.2010.882.109