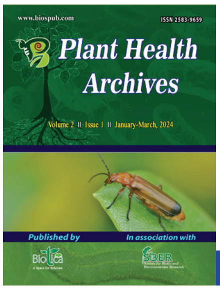

DOI: 10.54083/PHA/2.1.2024/13-17

Plant Health Arch., 2024, 2(1):13-17

Plant Health Archives

e-ISSN: 2583-9659

January-March, 2024

Article ID: PHA028

Research Article

Study on the Distribution of Olfactory Antennal Sensilla of *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) and their Response to Some Botanical Extracts

Suleiman, M.[*], Halliru, M., Sani, I., Yusuf, M.A. and Abdullahi, K.B.

Dept. of Biology, Umaru Musa Yar'adua University, Katsina (PMB 2218), Nigeria

Open Access

Corresponding Author

Suleiman, M.

✉: mohammed.suleiman@umyu.edu.ng

Conflict of interests: The author has declared that no conflict of interest exists.

How to cite this article?

Suleiman, M., Halliru, M., Sani, I., *et al*., 2024. Study on the Distribution of Olfactory Antennal Sensilla of *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) and their Response to Some Botanical Extracts. *Plant Health Archives* 2(1), 13-17. DOI: 10.54083/PHA/2.1.2024/13-17.

Abstract

The repellency potential of *Euphorbia balsamifera* Aiton, *Lawsonia inermis* L. and *Mitracarpus hirtus* (L.) DC against *Sitophilus zeamais* was assessed at 30±2 °C and 70±5% R.H. The botanicals were applied as chloroform extracts at the rate of 6.25, 12.50, 25.00, 50.00 and 100.00 mg ml^{-1} per 20 g sorghum grains. Percent repellency of the botanicals against *S. zeamais* was taken at 1 and 24 hour after exposure (HAE). Scanning electron microscopy (SEM) was conducted for examination and identification of olfactory antennal sensilla of the weevil. This was enhanced by the aforementioned repellency test with antennal distal flagellomere of the weevils excised. The SEM showed that sensilla chaetica (SC), sensilla trichoidea (ST) and sensilla basiconica (SB) were the types of antennal sensilla of *S. zeamais* identified. Results from repellency tests conducted revealed that ST and SB were the olfactory sensilla located on the last distal flagellomere of the weevils. It was also found that the botanicals had promising repellent activity against *S. zeamais* and might be used in the protection of stored sorghum grains.

Keywords: Botanicals, Odour detection, Olfactory sensilla, Repellency, *Sitophilus zeamais*

Introduction

Botanical repellents are preferred substances because they provide protection while having little effect on the environment and keep pest insects away from treated materials by stimulating their olfactory or other sensors (Divekar *et al*., 2022). Plant material's repellency has long been used by humans, who simply hang damaged plants in their homes, a technique that is still common in developing nations (Maia and Moore, 2011). Ngegba *et al*. (2022) noted that some plant species were identified to have repellent properties and found to be safe for pest control. Repellents are also reported to reduce pesticide deposit and guarantee bio-safety of food commodities, consumers and the environment. It is further elucidated that the use of plant extracts is less bio-hazardous (Sharma *et al*., 2023).

About 297 plant species were reported as repellents Govindarajan *et al.* (2011). Out of 230 plant species reviewed by Zoubiri and Baaliouamer (2014) for their potentiality of as source of insecticides, more than ten were found to show repellency potential against some insect pests. The leaves, stems, barks, seeds and oil of these plant species contain a variety of bioactive substances, including terpenoids, alkaloids, glycosides, phenols, tannins and flavonoids (Verma *et al.*, 2016).

The repellent action of some botanical products might be due to the presence of volatile substances and pungent smell that makes the insects to embark on reversible action, hence, moving away from source of the substances (Chaudhary *et al*., 2017).

Insect antennae have sensory organs called sensilla that are essential for identifying a range of cues that lead to the discovery of appropriate environments, necessary resources and potential mates (Ali *et al*., 2016). Previous investigation reported how antennal sensilla of various insect species were characterized based on their structure. However, despite its olfactory function, little is reported about *S. zeamais*'s

Article History

RECEIVED on 28th November 2023 RECEIVED in revised form 03rd March 2024 ACCEPTED in final form 10th March 2024

antennal sensilla. It is therefore against this background that this study was designed to conduct SEM of *S. zeamais* antenna for identifying olfactory sensilla, their distribution response to chloroform extracts of some botanicals.

Materials and Methods

Rearing of the Test Insects

One hundred mature *S. zeamais* were gathered from infected grain storage areas at Katsina Central Market and then placed into individual plastic containers (500 ml) with sterilized sorghum grains (250 g) as the main food source. The containers were covered with muslin cloth and placed in an incubator at 30±2 °C and 70±5% relative humidity for 14 days of oviposition (Suleiman *et al*., 2018). Emerged adult weevils were collected from the bottles for the subsequent experiments.

Preparation of Botanicals

From an uncultivated area, an adequate quantity of fresh foliage of *E. balsamifera*, *L. inermis* and *M. hirtus* were gathered. To get rid of any dust and other undesirable particles, the leaves were washed with distilled water. After that, they were shade-dried for 14 days at room temperature in Biology Laboratory 3 of Umaru Musa Yar'adua University, Katsina (UMYUK). The dried leaves were first mashed into a powder using a laboratory blender and then sieved through a laboratory sieve with an 80 micron mesh size.

In conical flasks, 100 g of each plant powder were dissolved in 400 milliliters of chloroform individually. After being firmly corked, the conical flask mouths were chilled for 48 hours. After the extract was separated using muslin cloth and filtered through Whatman No. 1 filter papers, it was vacuum-pumped. The filtrate was concentrated separately by evaporating excess solvents in a rotary evaporator that was set to rotate at a speed of 3 to 6 rpm for eight hours. The residual surplus solvents were evaporated by pouring the aliquot into crucibles and setting them on a water bath. Before being used in the lab tests, the resultant extracts were air-dried to eliminate any remaining solvent and refrigerated at 4 °C.

Scanning Electron Microscopy (SEM) Analysis of S. zeamais's Antennal Sensilla

An analysis of *S. zeamais*' antennal sensilla under an electron microscope was conducted in accordance to Ali *et al*. (2016) to ascertain whether the distribution pattern affects their ability to repel the test botanicals. Ten *S. zeamais* antennae were removed and cleansed for five seconds in an ultrasonic bath (250 W) to remove any last bits of grime. The procedures were done under a stereomicroscope with a 40X magnification. They were dehydrated twice in 100% ethanol for 15 minutes to remove any remaining water or lipid droplets. The dehydration process involved ethanol series treatments of 30, 45, 60, 75, 90 and 95% for 15 minutes each. The antenna preparations were palladium/gold sputter-coated (40:60) and mounted on a stub using double-sided sticky tape once the critical point dried. Next, the antennae were examined using a scanning electron microscope (Model: Phenom Pro X, Phenom-world BV, Netherlands).

Antennal Sensilla Distribution and Reaction to Repellent Actions

To ascertain if the antennal sensilla distribution influences the insect's reaction to volatile chemicals, four sets of ten weevils each that had been exposed to sorghum grains for three days beforehand were made. Chloroform leaf extracts of the test botanicals were given to the first group of weevils that still had their antennae attached. The fourth, fifth and sixth flagellomeres of the second set were removed. The following is how the identical insects were instantly exposed to the botanicals for the repellency test.

Method of Rejitha *et al.* (2014), modified by Suleiman *et al*. (2018) was applied to investigate the repellent effect of chloroform extracts of the test botanicals. For each treatment, a device consisting of three 100 ml plastic bottles joined by 2 clear plastic tubes measuring 150 mm in length and 10 mm wide at an angle of 180° was created. A, B and C were the labels on the three plastic bottles, with B denoting the middle chamber. In bottle A of each apparatus, twenty grams (0.02 kg) of sorghum grains were combined with one milliliter (1 ml) of chloroform leaf extracts of *E. balsamifera* at a range of concentrations (6.25, 12.50, 25.00, 50.00 and 100.00 mg ml^{-1}, respectively). As a control, an additional 0.02 kg of sorghum grains devoid of extract were added to C. In bottle B, ten (10) adult *S. zeamais* from the study's rearing period were added. The setups for *M. hirtus* and *L. inermis* were the same. This experiment was replicated three times.

At one and 24 hours following exposure, the quantity of weevils migrating from bottle B to bottle A or C was counted (HAE).

Repellency (%) was determined as given by Sakuma and Funkami (1985) below.

$$PR = \left[1 - \frac{NT}{NT+NC}\right] \times 100$$

Where,

PR = Percentage Repellency;

NT = Number of weevils in the botanical-treated bottle; and

NC = Number of weevils in the control bottle.

Comparable trials were carried out with the other two groups of weevils. Only the sixth flagellomere was removed from the fourth set of insects, whereas the fifth and sixth flagellomeres of the third group were removed. There were three duplicates of each trial.

Data Analysis

The analysis of the gathered data was done with GraphPad Prism (version 7.03). They were initially determined to be non parametric using the Shapiro-Wilk normalcy test. Consequently, after each exposure period, the degree of significance in the percent repellencies amidst the plants at varied doses against the weevils was tested using Kruskal Wallis statistics. At the 5% level of significance, the Dunn's multiple comparisons test was utilized to distinguish between substantially different means.

Results and Discussion

Types of S. zeamais Antennal Sensilla and their Distribution

Sensilla chaetica (SC), Sensilla trichoidea (ST) and Sensilla basiconica (SB) are three distinct species of sensilla found throughout the antennal segments of the weevil, including the scape, pedicel and flagellum, according to scanning electron microscopy analysis of the antenna (Figure 1). This finding corroborates Fouda *et al*. (2016) who noted that *S. oryzae* and *S. granarius* have comparable sensilla kinds. Additionally, certain insect pests of stored goods, including *T. granarium*, *T. variabile* and *T. castaneum*, were shown to have these kinds of antennal sensilla (Wei *et al*., 2015; Ali *et al*., 2016). The types of sensilla are briefly explained here.

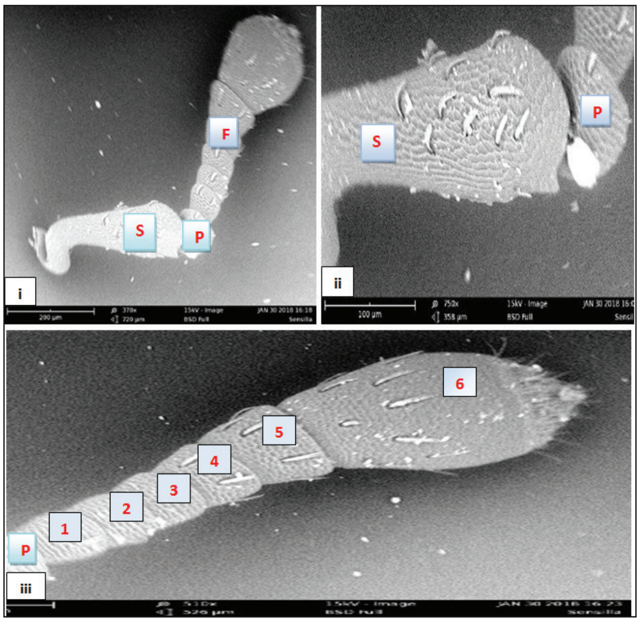

Figure 1: *S. zeamais* antenna scanning electron micrographs of the whole antenna (370x); scape and pedicel segments (1150x); and flagellum segment (510x). S stands for scape, P for pedicel, F for flagellum and 1 to 6 for the number of flagellomere

Sensilla Chaetica (SC)

The distribution of these was seen over all antennal segments, with a greater concentration closer to the surface, particularly on the scape as opposed to the flagellomeres [Figure 2 (i-iii)]. They have spherical collar-like sockets at the base and a cuticular surface. Every SC had a thorn-like form, curving and a blunt tip.

All antennal segments had SC; however, they were mostly focused on the scape. It has been revealed that SC can carry out touch chemoreception and mechanoreception (Fouda *et al*., 2016). It is also explained that these sensilla most likely pick up on changes in the antennal positions (Namikawa and Amornsak, 2016). This might have been the cause of SC's greater focus on the scape as opposed to the flagellum. The presence of SC as mechanoreceptors on the flagellum, in especially the distal flagellomeres of maize weevil, might be associated to their participation in host assessment during the behaviour of antennal drumming, as suggested by Namikawa and Amornsak (2016).

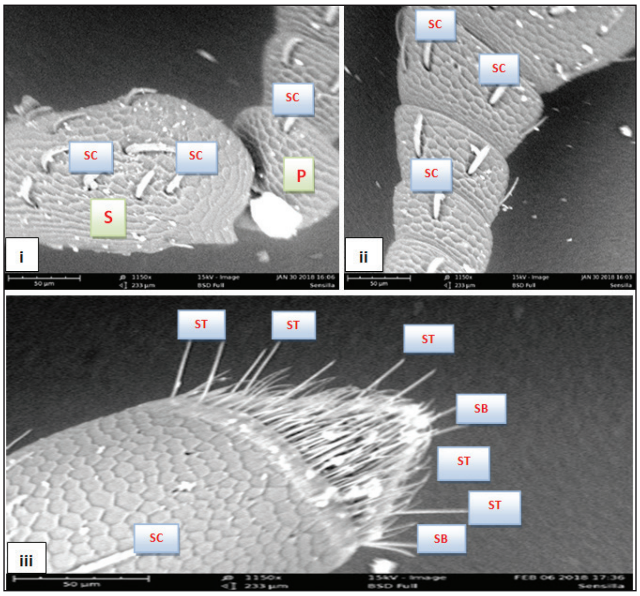

Figure 2: *S. zeamais* antenna scanning electron micrographs displaying three distinct sensilla types (1150x): (i) scape and pedicel; (ii) flagellum; and (iii) the sixth flagellomere [the letters stand for Sensilla chaetica (SC), Sensilla trichoidea (ST), Sensilla basiconica (SB) and scape (S)]

Sensilla Trichoidea (ST)

These were located [Figure 2(iii)] at the tip of the final (6th) distal flagellomere. On the scape, pedicel and first through fifth flagellomeres, no ST was observed [Figure 2(i) and (ii)]. ST had a smooth, hair-like, long, slender and flexible surface. ST was almost straight or slightly curled, with a prickly tip.

Sensilla Basiconica (SB)

Only the final distal flagellomere had this kind of sensilla. The smooth surface of SB was distinguished by a blunt, rounded, or narrow tip [Figure 2 (iii)]. On the antenna tip, there were less SB than ST.

On the weevil's distal (6th) flagellomere, ST and SB were discovered to be concentrated. The distribution of ST and SB is consistent with earlier observations (Ali *et al*., 2016; Fouda *et al*., 2016) where it was noted that *S. oryzae*, *S. granarius* and *T. castaneum*'s tip of their last antennomere had these sensilla present. Also, Li *et al*. (2013) revealed that *Quadrastichus erythrinae* Kim had ST on the tip of its distal flagellomere.

Reaction of S. zeamais Antennal Sensilla to Repellent Activities of Plant Materials

Chloroform extracts of the test botanicals revealed differing repellent activities against the weevils with full antennomeres when applied at 6.25, 12.50, 25.00, 50.00 and 100.00 mg ml^{-1} within 1 and 24 HAE. Table 1 shows that *E. balsamifera* applied at 6.25 mg ml^{-1} repelled 17.78±1.11% to 30.55±2.78% *S. zeamais* from 1 to 24 hours of treatment. At 12.50 mg ml^{-1} it resulted in percentage repellency of 27.78±2.78% to 41.07±1.79%. The repellency was similarly increasing with increase in concentration resulting in highest activity ranging from 71.03±2.41 to 87.74±1.24 at 100.00 mg ml^{-1} of the botanical within 1 to 24 hours HAE.

Table 1 additionally demonstrates that *L. inermis'*s percent repellencies against *S. zeamais* within 1 and 24 HAE were 23.23±1.67 and 39.17±0.83, respectively, at a dosage of 6.25 mg ml^{-}1. At 12.50, 25.00, 50.00 and 100.00 mg ml^{-1} it resulted in 41.91±0.45 to 51.85±1.95%, 62.10±2.76 to 66.67±0.00%, 68.26±1.59 to 78.70±2.45% and 73.55±2.12 to 89.63±0.37%, respectively, within 1 to 24 HAE.

The same pattern was also observed in *M. hirtus* treated grains where the repellency ranged from 17.78±1.11 to 76.67±1.67% at 1 HAE and 32.78±4.34 to 85.51±1.21% at 24 HAE (Table 1).

Kruskal Wallis statistics revealed that there was significant difference ($p<0.05$) in percentage repellency among the botanicals at 25.00 and 50.00 mg ml^{-1} against *S. zeamais* within 1 HAE, but there is no discernible change ($p>0.05$) in treatments with 6.25, 12.50 and 100.00 mg ml^{-1}. In 1 and 24 HAE, weevils with removed flagellomeres showed no reactivity when grains or plants were present (Table 1).

Table 1: Repellent properties of chloroform leaf extracts of some botanicals administered at different doses to *S. zeamais* before and after the excision of distal flagellomeres

Botanicals	Concentration (mg ml^{-1})	Mean Repellency (% ± S.E.)			
		1 HAE		24 HAE	
		Before Excision	After Excision	Before Excision	After Excision
E. balsamifera	6.25	17.78±1.11^{a}	-	30.55±2.78^{a}	-
	12.50	27.78±2.78^{b}	-	41.07±1.79^{b}	-
	25.00	33.33±0.00^{b}	-	59.25±3.70^{c}	-
	50.00	45.77±2.17^{b}	-	71.73±4.70^{d}	-
	100.00	71.03±2.41^{d}	-	87.74±1.24^{e}	-
L. inermis	6.25	23.23±1.67^{a}	-	39.17±0.83^{a}	-
	12.50	41.91±0.45^{b}	-	51.85±1.95^{b}	-
	25.00	62.10±2.76^{c}	-	66.67±0.00^{c}	-
	50.00	68.26±1.59^{c}	-	78.70±2.45^{d}	-
	100.00	73.55±2.12^{d}	-	89.63±0.37^{e}	-
M. hirtus	6.25	17.78±1.11^{a}	-	32.78±4.34^{a}	-
	12.50	38.73±2.82^{b}	-	46.67±3.33^{b}	-
	25.00	43.67±4.23^{c}	-	54.23±2.17^{c}	-
	50.00	63.06±1.95^{c}	-	72.62±1.19^{d}	-
	100.00	76.67±1.67^{d}	-	85.51±1.21^{e}	-

[Note: HAE = hours after exposure; Means in the same column followed by different letter superscript are significantly different at $p<0.05$]

The olfactory function of ST and SB is indicated by their distribution pattern, as evidenced by the fact that the removal of the sixth distal flagellomere, which carried the two types of sensilla, prevented the weevils from reacting to the presence of the botanicals. When the antennae remained intact, however, the weevils exhibited strong repulsive actions. Ali *et al*. (2016) showed comparable outcomes when *T. castaneum*'s final three distal flagellomeres were excised and subjected to different volatile substances. The present study revealed the olfactory function of the ST and SB on the tip of *S. zeamais* antennae, which is in line with earlier findings that olfactory sensilla are present at the apex of *S. oryzae* antennae (Omar, 2012).

Not only does it contribute to olfactory detection, as this study and other publications have shown (Fouda *et al*., 2016; Namikawa and Amornsak, 2016), Mechanoreceptive functions of ST have been identified in *S. oryzae*, *S. granarius* and *T. castaneum* (Ali *et al*., 2016; Fouda *et al*., 2016). Additionally, ST was mentioned as a crucial sensilla for sex pheromone perception (Fouda *et al*., 2016; Namikawa and Amornsak, 2016).

The antennal apex's olfactory function is further confirmed by the presence of SB there. Prior research has verified that SB on many insects' antennae participated in the sensing of odours (Ali *et al*., 2016; Fouda *et al*., 2016). Further, Li *et al*. (2013) stated that the sensilla wall's thickness and existence of pores within the wall are necessary for the olfactory functions of SB. The thick-walled SB can sense temperature and relative humidity in addition to being sensitive to carbon dioxide and odours (Miller, 1972).

Conclusion

Results showed that leaves of *E. balsamifera*, *L. inermis* and *M. hirtus* were repulsive against *S. zeamais* in stored sorghum. *E. balsamifera* was more repellent than the other botanicals. It was found that the chosen botanicals' repelling properties depended on their concentration, becoming more potent as the concentration of the botanicals rose. As exposure times shrank, so did the repellent properties.

Repellent activity of the botanicals indicates that they could be employed as part of integrated pest management techniques.

Comprehending the varieties and arrangement of antennal sensilla in *S. zeamais* yields baseline data that may be useful for subsequent investigations into the locations accountable for detecting odours. It has been demonstrated that the presence of ST and SB on the tip of the final distal flagellomere serves olfactory purposes, which accounts for the weevils' ability to respond to the repellency of the botanicals. Botanicals that can inhibit *S. zeamais*'s antennal sensilla so that they are unable to sense the presence of sorghum grains in storage need further research. The examination of *S. zeamais* antennae's intricate structures, or ultrastructures, necessitates the use of transmission electron microscopy (TEM). It is hereby recommended that more research be done on the active compounds that provide the repelling activity.

Acknowledgements

We acknowledge the sponsorship of this study by the Nigerian Tertiary Education Tax Fund (TETFUND) as part of its University Based Research (UBR) initiative.

References

Ali, S.A.I., Mory, M.D., Ali, S., Wang, M., 2016. Effects of the antennal sensilla distribution pattern on the behavioural responses of *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Florida Entomologist* 99(1), 52-59. DOI: https://doi.org/10.1653/024.099.0110.

Chaudhary, S., Kanwar, R.K., Sehgal, A., Cahill, D.M., Barrow, C.J., Sehgal, R., Kanwar, J.R., 2017. Progress on *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides. *Frontiers in Plant Science* 8, 610. DOI: https://doi.org/10.3389/fpls.2017.00610.

Divekar, P.A., Narayana, S., Divekar, B.A., Kumar, R., Gadratagi, B.G., Ray, A., Singh, A.K., Rani, V., Singh, V., Singh, A.K., Kumar, A., Singh, R.P., Meena, R.S., Behera, T.K., 2022. Plant secondary metabolites as defense tools against herbivores for sustainable crop protection. *International Journal of Molecular Sciences* 23(5), 2690. DOI: https://doi.org/10.3390/ijms23052690.

Fouda, M.A., Al-Dali, A.G., Ghannam, I.S., 2016. Ultrastructure of sensory receptors on the antennae and mouthparts of the adult, *Sitophilus oryzae* L. and *Sitophilus granarius* L. (Coleoptera: Curculionidae). *Journal of Nuclear Technology in Applied Science* 4(1), 25-33.

Govindarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K., Anandan, A., 2011. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine* 1(1), 43-48. DOI: https://doi.org/10.1016/S2221-1691(11)60066-X.

Li, J, Guo, Q., Han, S., Jiang, L., Liang, G., 2013. Types, morphologies and distribution of antennal sensilla of *Quadrastichus Erythrinae* (Hymenoptera: Eulophidae). *Florida Entomologist* 96(4), 1288-1297. DOI: https://doi.org/10.1653/024.096.0407.

Maia, M.F., Moore, S.J., 2011. Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria Journal* 10(Suppl. 1), S11. DOI: https://doi.org/10.1186/1475-2875-10-S1-S11.

Miller, M.C., 1972. Scanning electron microscope studies of the flagellar sense receptors of *Perdesmisa discus* and *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *Annals of the Entomological Society of America* 65(5), 1119-1124. DOI: https://doi.org/10.1093/aesa/65.5.1119.

Namikawa, S.K., Amornsak, W., 2016. Antennal sensilla morphology of *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae), a larval parasitoid of the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). *Agriculture and Natural Resources* 50(5), 374-379. DOI: https://doi.org/10.1016/j.anres.2015.12.003.

Ngegba, P.M., Cui, G., Khalid, M.Z., Zhong, G., 2022. Use of botanical pesticides in agriculture as an alternative to synthetic pesticides. *Agriculture* 12(5), 600. DOI: https://doi.org/10.3390/agriculture12050600.

Omar, Y.M.M., 2012. Morphological studies on some external and internal structures of rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), a major pest of the stored cereals in Egypt. *Journal of Plant Protection and Pathology*, *Mansoura University* 3(8), 843-863. DOI: https://doi.org/10.21608/JPPP.2012.84169.

Rejitha, T.P., Reshma, J.K., Mathew, A., 2014. Study of repellent activity of different plant powders against cockroach (*Periplanata americana*). *International Journal of Pure and Applied Bioscience* 2(6), 185-194.

Sakuma, M., Funkami, H., 1985. The Linear Track Olfactometer: An assay device for taxes of the German cockroach, *Blatella germanica* (Linn.) towards their aggregation pheromomne. *Applied Entomology and Zoology* 20(4), 387-402. DOI: https://doi.org/10.1303/aez.20.387.

Sharma, A., Dutta, P., Mahanta, M., Kumari, A, Yasin, A., 2023. Botanicals as a source of nanomaterial for pest and disease management. *Plant Health Archives* 1(3), 96-101. DOI: https://doi.org/10.54083/PHA/1.3.2023/96-101.

Suleiman, M., Rugumamu, C.P., Ibrahim, N.D., 2018. Repellency potential of some botanicals against the maize weevil, *Sitophilus zeamais* (Motschulsky, 1855) (Coleoptera: Curculionidae) in Stored Sorghum. *Polish Journal of Entomology* 87(1), 85-99. DOI: https://doi.org/10.2478/pjen-2018-0007.

Verma, S., Sharma, S., Malik, A., 2016. Termicidal and repellency efficacy of botanicals against *Odontotermes obesus*. *International Journal of Research in Biosciences* 5(2), 52-59.

Wei, C., Ren, B., Chen, X., Zhou, X., Wang, W., Wang, Z., 2015. Scanning electron microscope observations on the antennal sensilla of two stored grain pests *Trogoderma granarium* and *Trogoderma variabile* (Coleoptera: Dermestidae). *Florida Entomologists* 98(1), 140-148.

Zoubiri, S., Baaliouamer, A., 2014. Potentiality of plants as source of insecticide principles. *Journal of Saudi Chemical Society* 18(6), 925-938. DOI: https://doi.org/10.1016/j.jscs.2011.11.015.