Journal of King Saud University – Science 35 (2023) 102641

HOSTED BY

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

The aptness of entompathogenic bacteria against sawtoothed grain beetle (*Oryzaephilus surinamensis* [L.]) (Coleoptera: Silvanidae) in dates under laboratory conditions

Waleed Saleh Alwaneen

Advanced Agricultural & Food Technologies Institute, King Abdulaziz City for Science and Technology (KACST), Riyadh, Saudi Arabia

ARTICLE INFO

Article history:
Received 22 December 2022
Revised 5 February 2023
Accepted 8 March 2023
Available online 16 March 2023

Keywords:
Stored product insects
Microbial control
Entomopathogens
Date fruits
Saudi Arabia

ABSTRACT

Objective: The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L). is known as one of the most devastating insect pests in stored date fruits, infrequently found in newly harvested date fruits and raisins but developed a high plenty in dry fruits particularly after long storage. Indiscriminate use of pesticides and fumigants to control insects have resulted in insecticide resistance, environmental hazards, residual toxicity, and pest resurgence so the control trends have been changed and particularly biological control agents like entomopathogenic bacteria *Photorhabdus temperata* and *Xenorhabdus nematophila* being used to manage sawtoothed grain beetle.

Methods: During present study, the pathogenicity of *P. temperata* and *X. nematophila* was evaluated against this crucial pest of stored grains. The bacterial concentrations, 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 cells/ml of both bacteria were prepared and used for the experiments. The prepared solutions were applied directly to the date fruits infested with sawtoothed grain beetles and data were recorded.

Results: Significant results were observed for both bacterial treatments as the highest concentration of 10^8 cells/ml showed to be very effective against the beetle in terms of fecundity, adult emergence, and mortality. In terms of fecundity, 8.66 to 4.33 mean number of eggs were produced at bacterial *P. temperata* concentrations of 1×10^4 and 1×10^8 cells/ml, respectively. Similarly, the F_1 adult emergence for *P. temperata* was recorded 15.33 and 9 adults at concentrations of 1×10^4 and 1×10^8 cells/ml, respectively. Results showed a similar trend for the *X. nematophila* species.

Conclusions: These results indicate that utilizing bacteria as biological control agents can be advantageous for reducing insect populations in storage facilities and preventing the loss of grains and date fruits that have been stored in storage.

1. Introduction

Date fruits contain a high rich protein and good source of rapid energy because of high amount of carbohydrates which is 70–80% (Ghnimi et al., 2017). The sawtoothed grain beetle is a cosmopolitan pest found globally, it is responsible for sever losses, polyphagous feeding in nature and have broad range of stored goods, as well as cereal products, dried fruits, oil seeds and stored grains (Hashem et al., 2012). The sawtoothed grain beetle, infest date fruits which has low moisture content, with the calyx removed or those broken or with mechanical damage. The mechanical damages or the broken skin of date fruits facilitate the insects to deposit eggs and also help the juveniles to eat and bore inside the fruit flesh. Tunnels are made on the outer fruit skin and flesh by feeding of adults and larvae. During the high infestation, sawtoothed grain beetle consume all fruit contents leaving the skin or *exo*-carp integral. The beetle causes severe damage such as weight decrease, reducing the quality and quantity of the date fruits. Stored dry and semi dry date fruits face a basic issue of insect damage (Al-Dosary 2009). In stored products the date

Abbreviations: F_1, First generation; IPM, integrated pest management; EPNs, entomopathogenic nematodes; Tc's, toxins complexes; g, gram; ANOVA, One-way analysis of variance.

E-mail address: waleed478@hotmail.com,

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2023.102641

varieties are most infected by sawtoothed grain beetle and 40 to 75% losses have been reported (Mallah et al., 2016).

Pesticides have been used as protectants all over the world because of their efficient pest control ability but on the other hand, the detrimental effects may include toxicity of stored food commodities causing sanitary and phytosanitary issues as well as life threatening effects on non-targeted organisms (Phillips and Throne 2010). For the sake of human health and safety, environment friendly the implementation of less expensive control measures has become the need due to the lack of awareness of pesticides cost, their detrimental effects on human health and development of genetic resistance in insect species against these chemicals (Aggarwal et al., 2016). Many countries are scrutinizing the traditional fumigation procedures as they are posing threats which include ozone depletion, potential risk of carcinogenic methyl Bromide and phosphine. Alternative strategies must focus on the efficacy against limited target specific species which are biodegradable into non-toxic products and recommended to be use in integrated pest management programs as well as eco-friendly measures development are in discussion. Plant-based chemicals, insect growth regulators and the insect pathogens like entomopathogenic bacteria, fungi, viruses and protozoan are being assessed as better alternatives to chemical based prepared insect control programs. Biological control has great attention over past few decades, as alternative to chemical insecticides or as a constituent of integrated pest management (IPM) (Subramanyam and Hagstrum 2012). These constitute effective alternatives to chemicals without producing adverse effects on the environment.

Entomopathogenic bacteria, *Photorhabdus temperata* and *Xenorhabdus nematophila* are gram negative, motile rod-shaped bacteria which belongs to family Enterobacteriaceae and form symbiosis with entomopathogenic nematodes (EPNs) *Steinernema* and *Heterorhabdus* (Akhurst and Boemare 1988; Akhurst et al., 1996). Due to this symbiosis, nematodes provide shelter to bacteria in their guts where they seek protection from soil stressors as well as antagonists such as telluric bacterial consortia and bacteria inside insect guts that is why the isolation from soil samples is not possible without their nematode host. This pathogenic symbiosis is able to parasitize as well as to kill the larval stages of host from orders Diptera, Hymenoptera, Lepidoptera, Orthoptera, Coleoptera and Isoptera (Boemare 2002; Belien 2018). The toxins complexes (Tc's) and *Photorhabdus* insect-related toxins show oral activity against insect species despite of the fact that oral infection is not important to the biology of *Photorhabdus or Xenorhabdus*. Gram-positive and gram-negative bacteria produced toxins which have high molecular weight and multi subunit with insecticidal properties (Waterfield et al., 2001). Bacteria *Photorhabdus or Xenorhabdus* encodes the toxins producing genes which co-exist with entomopathogenic nematodes. Strains of bacteria have been discovered with loci that encodes for toxins, some of which are associated to insect while others are not. The objective of this research is assessment of entomopathogenic bacterial toxicity towards sawtoothed grain beetle management and determining mortality of sawtoothed grain beetle in date fruits due to bacteria.

2. Materials and methods

2.1. Insects rearing

Oryzaephilus surinamensis adults were reared on date fruits and its healthy cultures were maintained in plastic jars of 1–2 kg sizes, covered with the muslin cloth; tightened with the elastic rubber band. These jars were placed in a SANYO incubator, which was set at 27 ± 2 °C and 70 ± 5 percent relative humidity in the laboratory. Adult of sawtoothed grain beetles were identified as per the males have spine like structure on femur of *meta*-leg, which is absent in females.

2.2. Bacterial culture maintenance

Entomopathogenic bacteria namely *Photorhabdus temperata* and *Xenorhabdus nematophila* were obtained from Korean Agriculture Collection. At first the culture on supplement agar plates was streaked over at 25 °C for two to four days. Obtained culture was filtered by streaking individual colony on nutrient agar. Refined bacterial culture was replicated in nutrient broth for 24 h. The serial dilution plate count method was used to draw a dilution curvature between the optical thickness and cells/ml in order to calculate and adjust the colony shaping units per unit volume. Distinctive concentrations of bacteria were prepared to apply against sawtoothed grain beetle.

2.3. Bacterial pathogenicity bioassay

In each jar, 50 g of date fruits were placed. The jars were secured with muslin cloth, fixed with elastic band and were placed in an incubator at temperature and humidity mentioned earlier. Following different concentrations, 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 cells/ml of both bacteria were prepared and used for the experiments. In the control treatment distilled water was used. There were three replicates for each treatment, each jar representing one replicate, and there were five pairs of the young beetles in each jar. The effectiveness of entomopathogenic bacteria against sawtoothed grain beetle were studied according to the following parameters.

2.4. Entomopathogenic bacteria effect on sawtoothed grain beetle fecundity

Eggs were counted on the third day following the bacterial post treatment. Three date fruits were chosen at random from each jar the number of eggs inside date fruits were counted. Average number of eggs were determined influence of different treatment on egg laying capacity (fecundity). Because the sawtoothed grain beetle's eggs are so tiny and delicate, therefore, only three date fruits from each jar were opened, and the eggs were counted in order to prevent any damage that would have an impact on the outcomes, such as adult emergence.

2.5. Sawtoothed grain beetle adult emergence (F_1) and mortality%

After four weeks of the bioassay, all the jars were opened, emergence of adults (F_1) and the mortality of former adults employed for the bioassay and the new adults were counted. The number of adults' (F_1) were calculated in individual jar to check the repression of *O. surinamensis* appearance by using diverse concentrations of entomopathogenic bacteria. In a similar manner, the mortality was assessed at 7, 14, and 21 days following treatment. At each observation, the dead adults were removed from the jars, the number of dead and live adults were counted, and the mortality percentage was computed.

2.6. Statistical analysis

The data were collected, tabulated and analyzed. One-way analysis of variance (ANOVA) was done using bacterial concentrations as the main factor and eggs laid, adult mortality and new adult emergence as the response factor. The SAS 9.2 was used to analyze the data.

3. Results

3.1. Number of eggs laid by sawtoothed grain beetle in date fruits treated with Photorhabdus temperata

According to the Table 1, all used concentrations of *P. temperata* Entomopathogenic Bacteria viz., 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 cells/ml significantly reduced sawtoothed grain beetle fecundity as compared to the control. The least number of eggs were counted in the date fruits treated with the bacterial concentrations of 1×10^7 and 1×10^8 cells/ml in which the values were 5.00 and 4.00 respectively. However, the concentration 1×10^5, 1×10^6, 1×10^7 and 1×10^8 cells/ml were statistically similar to each other while 1×10^4 cells/ml concentration was dissimilar statistically from the all other used concentration. All bacterial concentrations proved pathogenic against sawtoothed grain beetle fecundity biological trait and showed good results than the control. Moreover, the results showed that the number of eggs laid by sawtoothed grain beetles got decreased with the increase in the entomopathogenic bacterial concentrations.

3.2. Sawtoothed grain beetle adult (F1) emerged from date fruits treated with Photorhabdus temperata

All the bacterial concentrations of *P. temperata* 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 cells/ml were better statistically in the F_1 adult emergence (Table 2). The minimum number of 9.00 F_1 adults were emerged in the concentration of 1×10^8 cells/ml while 1×10^4 bacterial concentration showed maximum number of the 15.00 emerged adults. Moreover, the concentration 1×10^5, 1×10^6 and 1×10^7 cells/ml were showed statistically different results with one another. All bacterial concentrations indicated better F_1 adult's emergence inhibiting rate in stored date fruits as compared to the control.

3.3. Sawtoothed grain beetle adult (F1) emerged from date fruits treated with X. nematophila

The Table 3, shows that highest numbers of sawtoothed grain beetle eggs 7.00 were observed when the date fruits were treated with the bacterial concentration of 1×10^4 cells/ml. Similarly, the lowest number of the eggs was shown by the bacterial concentration 1×10^7 and 1×10^8 cells/ml. All concentration gives a better result as compared to the control. The concentration 1×10^4, 1×10^5 and 1×10^6 were not statistically dissimilar while the 1×10^7 and 1×10^8 were similar among themselves and significantly different from the all other concentrations. Along with increase in the bacterial concentrations the number of eggs laid was reduced.

Table 1
The number of eggs (Mean ± SE) laid by *Oryzaephilus surinamensis* female in stored dates treated with *Photorhabdus temperate* counted on the third day following the bacterial post treatment.

Bacterium *sp*	Concentration	Number of eggs (Mean ± SE)
Photorhabdus temperata	1×10^4	8.66 ± 1.5 cd
	1×10^5	7.00 ± 1.00 bc
	1×10^6	6.33 ± 1.52 ab
	1×10^7	5.33 ± 1.52 ab
	1×10^8	4.33 ± 0.57 a
	Control (distilled water)	10 ± 1.00 d

Means followed by the same letters are not significant, at ($\alpha = 0.05$).

Table 2
The number of F_1 adults (Mean ± SE) of *Oryzaephilus surinamensis* emerged in stored dates treated with *Photorhabdus temperate* counted on the fourth week following the bacterial post treatment.

Bacterium *sp*	Concentration	Number of adults (Mean ± SE)
Photorhabdus temperata	1×10^4	15.33 ± 1.52c
	1×10^5	13.00 ± 2.00 bc
	1×10^6	12.66 ± 1.52 bc
	1×10^7	11.33 ± 0.50 ab
	1×10^8	9.00 ± 1.00 a
	Control (distilled water)	30.00 ± 2.00 d

Means followed by the same letters are not significant, at ($\alpha = 0.05$).

Table 3
The number of eggs (Mean ± SE) laid by *Oryzaephilus surinamensis* female in stored dates treated *Xenorhabdus nematophila* counted on the third day following the bacterial post treatment.

Bacterium sp	Concentration	Number of eggs (Mean ± SE)
Xenorhabdus nematophila	1×10^4	7.00 ± 1.00c
	1×10^5	6.66 ± 0.57c
	1×10^6	6.00 ± 1.00 bc
	1×10^7	4.66 ± 0.57 ab
	1×10^8	4.00 ± 1.00 a
	Control (distilled water)	9.66 ± 0.57 d

Means followed by the same letters are not significant, at ($\alpha = 0.05$).

3.4. Number of adult F1 sawtoothed grain beetle emerged from date fruits treated with X. nematophila

The bacteria *X. nematophila*, showed significant inhibition of sawtoothed adult emergence. According to the Table 4, most effective concentrations which results in minimum number of new adults produced was observed to the 1×10^8 cells/ml while the least effective concentration was 1×10^4 cells/ml producing the greatest number of new emerged adults of sawtoothed grain beetle. However, all concentrations showed a smaller number of the adults as compared to the control. It was observed that when the higher concentration was used then a smaller number of sawtoothed grain beetle adults emerged.

3.5. Sawtoothed grain beetle adult mortality treated with X. nematophila

Adult mortality was calculated in percentage by counting the dead and live adults in each jar at various time interval of the post treatment. The bacterial content and exposure time had a clear correlation with the mortality. The maximum adult mortality with 1×10^8 *X. nematophila* cells/ml concentrations after the seven days

Table 4
The number of F_1 adults (Mean ± SE) of *Oryzaephilus surinamensis* emerged in stored dates treated with *Xenorhabdus nematophila* counted on the fourth week following the bacterial post treatment.

Bacterium *sp*	Concentration	Number of adults (Mean ± SE)
Xenorhabdus nematophila	1×10^4	20.66 ± 0.57 d
	1×10^5	18.00 ± 1.00c
	1×10^6	16.33 ± 0.57 bc
	1×10^7	15.00 ± 1.00 bc
	1×10^8	12.00 ± 1.00 a
	Control (distilled water)	30.33 ± 2.00 e

Means followed by the same letters are not significant, at ($\alpha = 0.05$).

Table 5
Mortality (%) of *Oryzaephilus surinamensis* adults at various time intervals in stored dates treated with *Xenorhabdus nematophila*.

Time interval	Concentration	Mortality (%)
Day 7	1×10^4	10.00
	1×10^5	15.00
	1×10^6	20.00
	1×10^7	25.00
	1×10^8	30.00
	Control (distilled water)	5.00
Day 14	1×10^4	15.00
	1×10^5	25.00
	1×10^6	40.00
	1×10^7	45.00
	1×10^8	60.00
	Control (distilled water)	5.0
Day 21	1×10^4	45.00
	1×10^5	65.00
	1×10^6	65.00
	1×10^7	65.00
	1×10^8	70.00
	Control (distilled water)	10.00

of post treatment was 30%. On the other hand, the same treatment resulted in a maximum mortality rate of up to 70% at day 21 of post treatment (Table 5).

4. Discussion

Date fruits are an extremely valuable fruit because they are only harvested once in a year with proper storage and insect prevention, may be eaten for several years. The usage of entomopathogenic organisms is very safe for people and other animals, including cattle that eat date fruits incorporated into their diets as an essential nutrient. The ability of entomopathogenic bacteria to inhibit the sawtoothed grain beetle from laying eggs has been proven by current data. *Xenorhabdus spp*., a symbiotic bacterium, is one of the well-known biocontrol agents used in pest management (Zhou et al., 2002; Gulcu et al., 2012). According to (Richards and Goodrich-Blair 2010), *X. nematophila* is an entomopathogenic bacterium with a wide host range and strong toxicity against insect pests; nonetheless, similar results were seen in our investigation. Similar findings were reported that *P. temperata and X. nematophila* were reported as deadly pathogens that produced proteins and secondary metabolites which prove toxic against a variety of insects (Dowling and Waterfield 2007; Sheets et al., 2011; Ng'ang'a 2015; Namsena et al., 2016). In particular, discussing *X. nematophila*'s pathogenicity against adults of various insects has demonstrated that Drosophila melanogaster and *Manduca sexta* adults quickly died after being infected with *X. nematophila* (Kim et al., 2017). These bacteria produce a number of compounds that are insecticidal and can be utilized to combat a variety of species, including insects (Abd-Elgawad 2022; Tomar et al., 2022; (Waterfield et al., 2001).

In present study the emergence of F1 adults was very low in the treated date fruits as compared with the saw-toothed grain beetle adults where distilled water was applies as control treatment. It was noticed during observation that adults were not active in the treated date fruits as compared to the date fruits where water was applied. When adults were not active it reflects the deterrent properties of the metabolites produces by the bacteria. This deterrent property of bacteria has affected the normal activities like feeding, copulation, and fecundity. The present data has shown the evidence of low fecundity and F1 adult emergence in the treated date fruits which have also been reported in other studies (Bode 2009; Kusakabe et al., 2022). Keeping continue, there are several natural products synthesized from the toxic produced by several species of *Photorhabdus* and *Xenorhabdus*, secretions and being used commercially for the management of several issues regarding human health and food storage and protection ((da Silva et al., 2020);Cimen et al., 2022; (Ünal et al., 2022)). All these findings are in favor of the present results outcomes.

5. Conclusions

Present study results showed that bacterial effectiveness was strongly correlated with exposure period; larger concentrations of *X. nematophila* were more effective than lower concentrations against *O. surinamensis*. An investigation revealed that the entomopathogenic bacteria *X. nematophila* was more aggressive against *O. surinamensis* when applied to the date fruits infested with it. The study's findings led to a successful and secure biological control procedure and will in the future direct an effective IPM program for this crucial pest.

Disclosure of funding

The authors declare that they don't have any particular funding for this study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Author would like to thanks the Advanced Agricultural & Food Technologies Institute, King Abdulaziz City for Science and Technology (KACST), Riyadh, Saudi Arabia.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2023.102641.

References

Abd-Elgawad, M.M., 2022. *Xenorhabdus spp*.: An overview of the useful facets of mutualistic bacteria of entomopathogenic nematodes. Life 12 (9), 1360.
Aggarwal, N., Thind, S., Sharma, S., 2016. Role of secondary metabolites of actinomycetes in crop protection. Plant Growth Promoting Actinobacteria, Springer, pp. 99-121.
Akhurst, R., Boemare, N., 1988. A numerical taxonomic study of the genus *Xenorhabdus* (Enterobacteriaceae) and proposed elevation of the subspecies of *X. nematophilus* to species. Microbiology. 134 (7), 1835–1845. https://doi.org/10.1099/00221287-134-7-1835.
Akhurst, R.J., Mourant, R.G., Baud, L., Boemare, N., 1996. Phenotypic and DNA relatedness between nematode symbionts and clinical strains of the genus *Photorhabdus* (Enterobacteriaceae). IJSM. 46 (4), 1034–1041. https://doi.org/10.1099/00207713-46-4-1034.
Al-Dosary, N., 2009. Role of the Saw-toothed grain beetle (*Oryzaephilus surinamensis* L.) (Coleoptera: Silvanidae) in date palm fruit decay at different temperatures. *Basra.* J Vet. Res. 8 (2), 1–14.
Belien, T., 2018. Entomopathogenic nematodes as biocontrol agents of insect pests in orchards. CAB Reviews. 13 (058), 1–11. https://doi.org/10.1079/pavsnnr201813058.
Bode, H.B., 2009. Entomopathogenic bacteria as a source of secondary metabolites. Curr. Opin. Chem. Biol. 13 (2), 224–230. https://doi.org/10.1016/j.cbpa.2009.02.037.
Boemare, N., 2002. Biology, taxonomy and systematics of *Photorhabdus* and *Xenorhabdus*. J. Entomol. Nematol. 35–56. https://doi.org/10.1079/9780851995670.0035.
Cimen, H., Touray, M., Gulsen, S.H., Selcuk, H., 2022. Natural products from *Photorhabdus* and *Xenorhabdus*: Mechanisms and impacts. Appl. Microbiol. Biotechnol. 1–13. https://doi.org/10.1007/s00253-022-12023-9.

da Silva, W.J., Pilz-Júnior, H.L., Heermann, R., da Silva, O.S., 2020. The great potential of entomopathogenic bacteria *Xenorhabdus* and *Photorhabdus* for mosquito control: A review. Parasites Vectors 13 (1), 1–14. https://doi.org/10.1186/s13071-020-04236-6.

Dowling, A., Waterfield, N.R., 2007. Insecticidal toxins from Photorhabdus bacteria and their potential use in agriculture. Toxicon. 49 (4), 436–451. https://doi.org/10.1016/J.TOXICON.2006.11.019.

Ghnimi, S., Umer, S., Karim, A., Afaf, K., 2017. Date fruit (*Phoenix dactylifera* L.): An underutilized food seeking industrial valorization. NFS J. 6, 1–10. https://doi.org/10.1016/J.NFS.2016.12.001.

Gulcu, B., Hazir, S., Kaya, H.K., 2012. Scavenger deterrent factor (SDF) from symbiotic bacteria of entomopathogenic nematodes. J. Invertebr. Pathol. 110 (3), 326–333. https://doi.org/10.1016/j.jip.2012.03.014.

Hashem, M.Y., Ahmed, S.S., El-Mohandes, M.A., Gharib, M.A., 2012. Susceptibility of different life stages of saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) to modified atmospheres enriched with carbon dioxide. J Stored Prod Res. 48, 46–51. https://doi.org/10.1016/J.JSPR.2011.09.002.

Kim, I.H., Aryal, S.K., Aghai, D.T., Casanova-Torres, Á.M., Hillman, K., Kozuch, M.P., Mans, E.J., Mauer, T.J., Jean-Claude, O., Ensign, J.C., 2017. The insect pathogenic bacterium *Xenorhabdus innexi* has attenuated virulence in multiple insect model hosts yet encodes a potent mosquitocidal toxin. BMC Genomics 18 (1), 1–25. https://doi.org/10.1186/s12864-017-4311-4.

Kusakabe, A., Wang, C., Xu, Y.-M., Molnár, I., Stock, S.P., 2022. Selective Toxicity of Secondary Metabolites from the Entomopathogenic Bacterium *Photorhabdus luminescens* sonorensis against Selected Plant Parasitic Nematodes of the Tylenchina Suborder. Microbiol. Spectr. 10 (1), e02577–e12521. https://doi.org/10.1128/spectrum.02577-21.

Mallah, N.A., Sahito, H.A., Kousar, T., Kubar, W.A., Jatoi, F.A., Shah, Z.H., 2016. Susceptibility of different varieties of stored date palm fruits infested by saw tooth grain beetle, *Oryzaephilus surinamensis* (L., 1758) under laboratory conditions. J. Entomol. Zool. Stud. 4, 438–443.

Namsena, P., Bussaman, P., Rattanasena, P., 2016. Bioformulation of Xenorhabdus stockiae PB09 for controlling mushroom mite, Luciaphorus perniciosus Rack. BIOB 3 (1), 1–7. https://doi.org/10.1186/s40643-016-0097-5.

Ng'ang'a, P., 2015. In Silico and Molecular Analysis of Xpta Protein Toxin Genes of *Xenorhabdus* Sp. and Efficacy of the Bacterium on *Sitophilus Zeamais* and *Prostephanus Truncatus*. University of Nairobi.

Phillips, T.W., Throne, J.E., 2010. Biorational approaches to managing stored-product insects. Annu. Rev. Entomol. 55, 375–397. https://doi.org/10.1146/annurev.ento.54.110807.090451.

Richards, G.R., Goodrich-Blair, H., 2010. Examination of *Xenorhabdus nematophila* lipases in pathogenic and mutualistic host interactions reveals a role for xlpA in nematode progeny production. Appl. Environ. Microbiol. 76 (1), 221–229. https://doi.org/10.1128/AEM.01715-09.

Sheets, J.J., Hey, T.D., Fencil, K.J., Burton, S.L., Ni, W., Lang, A.E., Benz, R., Aktories, K., 2011. Insecticidal toxin complex proteins from *Xenorhabdus nematophilus*: structure and pore formation. J. Biol. Chem. 286 (26), 22742–22749.

Subramanyam, B., Hagstrum, D.W., 2012. Alternatives to pesticides in stored-product IPM. Springer Science & Business Media.

Tomar, P., Thakur, N., Yadav, A.N., 2022. Endosymbiotic microbes from entomopathogenic nematode (EPNs) and their applications as biocontrol agents for agro-environmental sustainability. Egypt J. Biol. Pest Control. 32 (1), 1–19. https://doi.org/10.1186/s41938-022-00579-7.

Ünal, M., Yüksel, E., Canhilal, R., 2022. Biocontrol potential of cell suspensions and cell-free supernatants of different Xenorhabdus and Photorhabdus bacteria against the different larval instars of *Agrotis ipsilon* (Hufnagel)(Lepidoptera: Noctuidae). Experimental Parasitol. 242,. https://doi.org/10.1016/j.exppara.2022.108394 108394.

Waterfield, N.R., Bowen, D.J., Fetherston, J.D., Perry, R.D., 2001. The tc genes of *Photorhabdus*: a growing family. Trends Microbiol. 9 (4), 185–191. https://doi.org/10.1016/S0966-842X(01)01978-3.

Zhou, X., Kaya, H.K., Heungens, K., Goodrich-Blair, H., 2002. Response of ants to a deterrent factor (s) produced by the symbiotic bacteria of entomopathogenic nematodes. Appl. Environ. Microbiol. 68 (12), 6202–6209. https://doi.org/10.1128/AEM.68.12.6202-6209.2002.