Research Article

Biocidal Efficacy of *Heterorhabditis bacteriophora* against the African Armyworm (*Spodoptera exempta*) Towards Pest Control

Mohammed Akinlabi Rufai[1]*, Abideen Akinkunmi Wahab[2], Kamil Ayo Fasasi[1], Quadri Olusegun Adeshina[1] and Mary Tolulope Awotidebe[1]

[1]Parasitology Unit, Department of Zoology, Faculty of Basic and Applied Sciences, College of Science and Engineering, P.M.B. 4494, Osun State University, Osogbo, Nigeria; [2]Department of Microbiology, Osun State University, Osogbo, Nigeria.

Abstract | Studies on the biocidal activities of *Heterorhabditis bacteriophora* on African armyworm (*Spodoptera exempta*) was carried out in vivo. The *H. bacteriophora* was obtained from Osun state University farmland sites, Osogbo, Osun State while armyworm used in this study was obtained from the pot of a tomato plant in the botanical garden of Osun State University. Extraction of *H. bacteriophora* was done using decantation and centrifugation method of nematodes extraction. The five different concentrations of *H. bacteriophora* were made in distilled water (µg/l); one sample not treated with nematode was served as control. Biocidal efficacy was achieved by collecting soil samples, sterilized and kept into experimental plastic plates labelled as A, B, C, D, and E. This was further divided by weight into five equal portions labelled as A1-A5, B1-B5, C1-C5, D1-D5, and E1-E5, respectively. The observation was done after every twelve hours for five iterations, and the death rate was recorded. The death rates of armyworms in each soil sample after 72 hours were noticed to achieve biocidal efficacy. Maximum mortality (100%) was observed in highest concentration of nematodes (50%) after maximum time exposure (72 h); sample treated with 0% concentration had no mortality. The result shows that the death rate of the armyworm is proportional to the concentration of the *H. bacteriophora* and duration of application. Statistical analysis conducted using analysis of variance indicated that there was no significant difference in the overall biocidal activities of *H. bacteriophora* at 5% confidence limit ($F>0.05$). Statistical test conducted on the data also supports the reliability of the data with a correlation coefficient of $0.85<R2<1$.

Received | March 09, 2021; **Accepted** | June 02, 2021; **Published** | June 13, 2021
***Correspondence** | Mohammed Akinlabi Rufai, Parasitology Unit, Department of Zoology, Faculty of Basic and Applied Sciences, College of Science and Engineering, P.M.B. 4494, Osun State University, Osogbo, Nigeria; **Email:** akinlabi.rufai@uniosun.edu.ng
Citation | Rufai, M.A., Wahab, A.A., Fasasi, K.A., Adeshina, Q.O. and Awotidebe, M.T., 2021. Biocidal efficacy of *Heterorhabditis bacteriophora* against the African armyworm (*Spodoptera exempta*) towards pest control. *Pakistan Journal of Nematology*, 39(1): 41-45.
DOI | https://dx.doi.org/10.17582/journal.pjn/2021/39.1.41.45
Keywords | *Heterorhabditis bacteriophora*, *Spodoptera exempta*, Biocidal, Pest control

Introduction

The African armyworm is a migratory moth, the larvae (caterpillars) of which are important pests of pastures and cereal crops, predominantly in Africa south of the Sahara, Yemen, and certain countries of the Pacific region. Normally, only small numbers of this pest occur, usually on pastures. However, periodically the populations increase dramatically and mass migration of moths occur, covering many thousands of square kilometers and traversing international boundaries. They travel from field to field in great numbers, hence the name "armyworm" Grzywacz, 2006. The major upsurges occur in seasons of sporadic rainstorms and long sunny periods throughout the outbreak period.

Two entomopathogenic nematode (EPNs) genera

Links Researchers

viz., *Heterorhabditis* and *Steinernema* were reported as effective biological control agents against major insect pests (Ehlers and Shapiro-Ilan, 2005). Each of these nematodes contains a symbiotic bacterium, viz., *Photorhabdus* specific for *Heterorhabditis* and *Xenorhabdus* for *Steinernema* (Sáenz-Aponte *et al.*, 2014).

H. bacteriophora are ubiquitous and are extraordinarily lethal pathogens to many important soil insect pests, yet they are safe for plants, vertebrate animals and humans (Gaugler, 1998). *Heterorhabditis* gains entry by abrading the intersegmental membranes of the insect using a dorsal tooth. Once inside the host insect, the nematode invades the hemocoel and releases the symbiotic bacteria that are held in the nematode's gut. The bacteria cause a septicemia, killing the host within 24-48 hours after penetration (Hall and Menn, 1999). The IJs then feed on the rapidly multiplying bacterial cells, degrade host tissues and mature into adults. One or more adult reproductive generations may develop within the host cadaver, depending on available nutrient resources (Poinar, 1990).

Physical observations and scientific investigations have shown that using pesticides to control African armyworm are environmentally damaging and expensive. Biocontrol technology is aimed at replacing environmentally damaging and expensive synthetic chemical pesticides. The use of beneficial or entomoparasitic nematodes (EPNs) as a biocontrol measure for insect pests provide several advantages: (i) more effective than chemical agents; (ii) persist within soil; (iii) sustainability; and (iv) safe for user and environment (Arthurs *et al.*, 2004; Ehlers and Shapiro-Ilan, 2005). Several attempts have been made by researchers to develop improved biocontrol technology, but there are wide gaps concerning efficiency in different locations. This work aims to bridge these gaps in knowledge through the use of indigenous strains of entomopathogenic nematodes.

This study therefore assesses the potency of indigenous *H. bacteriophora* as biocontrol of army worm invasion. Furthermore, no life-threatening risks have been associated with the use of beneficial nematodes.

Materials and Methods

Extraction of EPN (H. bacteriophora) from soil

EPNs species used in this study were isolated from non-polluted soil collected from Osun State University vegetable farmland (7°76'19.4"N and 4°60' 3.18"E). Extraction of nematodes, identification and their mass production were done as described by Flanders *et al.* (1996). Species were identified based mainly on morphometrics of the IJ, male, and hermaphroditic female under light microscopy according to procedures described by Uribe-Lorio *et al.* (2007); Elena *et al.* (2013).

Isolation inoculation and culturing of the bacterial symbionts

Isolation of the bacterial symbionts was done by "hanging drop" method as described by Poinar and Thomas (1966). Dead armyworms are washed with distilled water, dissected on petri-dish (one per armyworm), their hemolymph were picked up and were inoculated on nutrient agar medium for bacterial growth. Then plates were incubated at 35°C in the dark for 48-96 hours.

Sub-culturing

Sub-culturing is done to get a pure culture of the isolate of interest. From the initial plate containing the mixed culture, colonies with different morphological characteristics were picked separately on sterile nutrient agar plates and MacConkey agar plates. The plates were then incubated at 35°C for 48 hours.

Identification of the symbiotic bacterial isolates

Isolated cultures were characterized by microbiological and biochemical tests for the identification of symbiotic bacteria. Colony morphology was done for preliminary identification of the bacterium when the isolates were grown on MacConkey agar and Nutrient agar. The symbiotic bacterial isolates were studied for cell morphology under microscope (Singh *et al.*, 2011) and Gram reaction staining was done using 24 hours old cultures by the standard procedure (Dix *et al.*, 1992). One bacteria strain, designated 0813-124, was isolated from IJs, the first to be isolated from this species. The bacterial culture produced bioluminescence which was detected by the naked eye after adaptation to the dark for approximately 5 min. The colonies absorbed dye from both MacConkey agar plates and NBTA plates. The result of Biology identification system only supported the identification of the bacteria strain as *Photorhabdus* sp.

Source of Galleria mellonela used in the insect baits

The adult greater wax moth (*Galleria mellonela* L.)

were purchased from Forest Research Institute Jericho, Ibadan, Oyo state and were mass reproduced to larvae under laboratory condition as described by (Shapiro-Ilan and Gauglerm, 2002; Rufai *et al*., 2020).

Testing for biocidal efficacy of entomopathogenic nematodes against African armyworm (Spodoptera exempta)

Biocidal efficacy was determined in the laboratory; about 100 g of soil sample was weighed into five experimental plastic plates (coded Plate A, B, C, D and E), each of which was thoroughly mixed to achieve homogeneity of the soil sample, and then each sample was further divided by weight into five equal portions labelled A1-A5, B1-B5, C1-C5, D1-D5, and E1-E5, respectively. Biocidal efficacy was determined by placing a total of 10 armyworm per one aerated plastic containers containing the soil samples.10, 20, 30, 50 and 0 infective juveniles of EPNs species and its bacteria symbiont were added respectively to all the plates containing the soil sample and army worms using a dropping pippete. The nitrogen, sulfur and phosphorus in the soil were able to sustain the microbial growth. Plate E group were not treated with EPNs and served as the control. Plastic plates were labeled and placed at room temperature. The biocidal efficacy was achieved by monitoring the death rates of armyworms in each soil over 24 hours (Del Valle *et al*., 2013).

Statistical analysis

The data obtained from this research and the inferences thereof were subjected to Descriptive Statistics including mean, and standard deviation and Inferential Statistics including correlation, and test statistics using Microsoft Excel Software, and Statistical Package for Social Sciences (SPSS).

Results and Discussion

The study shows that *H. bacteriophora* and its bacteria symbiont, *Photorhabdus* had high biocidal effect on armyworm, *S. exempta*. The results indicate that the no. of death of armyworm, *S. exempta* recorded generally increased with the introduction of *H. bacteriophora* and its bacteria symbiont, *Photorhabdus* extract concentrates over time presented in Figures 1 and 2. The trend shows that at 0% of the concentrates, 0% of the nematodes were exterminated after 72 hours. The average life span of armyworm has been well established to be 9-14 days while adult female lives an average of 10 days. The fully matured armyworms were used in this study hence, their deaths would have occurred from natural causes at 0% concentration. The study found that 100% of the armyworms were exterminated after 72hours of application at 50µg/l *H. bacteriophora* extracts concentration. The death count of the armyworm occurred proportionally with time, and the death rate was observed to be greatest at 50% concentrate and lowest at 10% concentration.

The biocidal efficacy was achieved by monitoring the death rates of armyworms in each sample over 72 hours with various concentrations of *H. bacteriophora* and its bacteria symbiont-*Photorhabdus*. At the end of the period, the result showed that plate treated with no EPNs had no mortality. Mortality began at 12-13hrs time with 30 and 50µg/l concentration. The rate of mortality was the same in these concentrations till 18-19 hours with 3 mortalities each. At 21-22 hour, mortalities of 1, 2, 3 and 5 were observed in 10µg/l, 20µg/l, 30 µg/l and 50µg/l, respectively. At 72 hour, mortalities increased steadily in 30 µg/l and 50µg/l concentrations with 50µg/l having the highest mortalities (10) followed by 30 µg/l (5). The total mortality was highest in 50µg/l (10), only plate with highest no of EPNs and its bacteria symbiont (50) showed great deal of biocidal activities than plates treated with 10, 20 and 30 EPNs and its bacteria symbiont. The rate of biocidal activity was influenced by the populations of EPNs. This result showed that EPNs is highly active on armyworm, from this part of the world and it lends credence to several works in other parts of the world where *H. bacteriophora* (Ehlers and Shapiro-Ilan, 2005; Berry *et al*., 1997) and *Steinernema* spp. (Sáenz-Aponte *et al*., 2014) have been used extensively for field control of agricultural insect pests invasion.

The results corroborate the discussions from Figures 1 and 2 as the mean death rate of 6.4 armyworms per half day was recorded with a standard deviation of 0.49. Mathematical models of the death count of the nematodes with concentration and time as independent variable were generated in the form: $y=g(c)$, and $y=k(t)$, respectively which can be used to extrapolate the deaths of nematodes for any desired length of time in hours, and extract concentration. Where y represents the expected death count, t represents the independent variable of time, $t:t=12x:2\leq z\leq l$, and c represents the independent variable of concentration, $c:c=5x:0\leq x \leq 4$. These inference equations are supported by the corresponding coefficients of correlation which were

found to be very strong ($0.88 \leq R^2 \leq 1$).

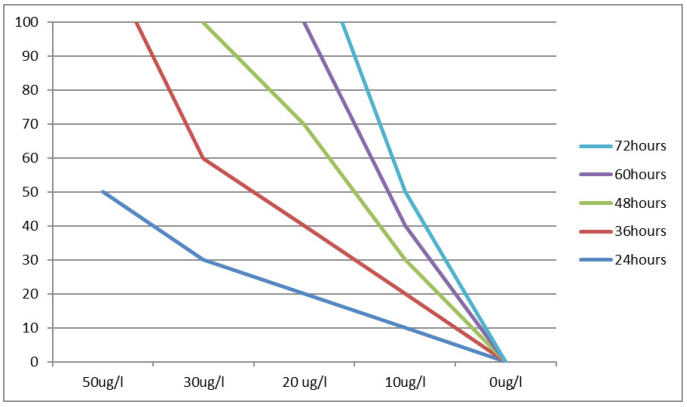

Figure 1: *Death rate of armyworm-Spodoptera exempta Relative to Concentration of Heterorhabditis bacteriophora and its bacteria symbiont-Photorhabdus Extract.*

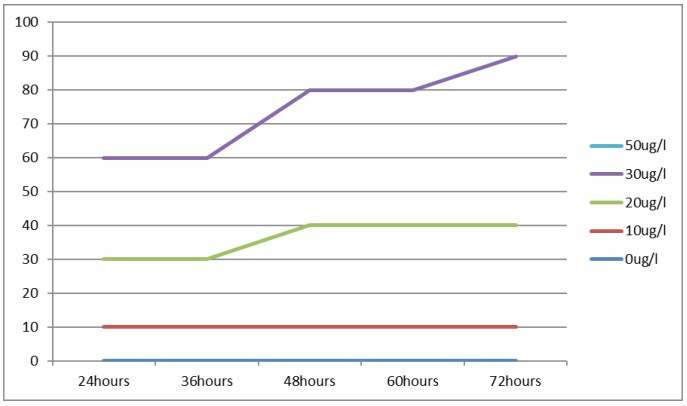

Figure 2: *Death rate of of armyworm-Spodoptera exempta relative to Time of Application of the Concentrations of Heterorhabditis bacteriophora and its bacteria symbiont- Photorhabdus.*

This study established that extracts of *H. bacteriophora* concentrates are detrimental to armyworm and the death rate of the armyworm is proportional to the concentration of the extract, and time of application. The study also revealed that the death rate of the armyworm is different for different concentrations, and for different length of time of application.

The study recommends that extracts of *H. bacteriophora* is efficient for the extermination of armyworm at 50% concentration when applied for 2 ½ days, 30% of the concentrates may also be used to exterminate this pest when applied for a period of 3 days. It is recommended that the local strain of *H. bacteriophora* with its bacteria symbiont be employed for the biocontrol of agricultural pests in the study area to reduce cost of using other control methods and for its ecofriendly feature. The study recommends that more research be done on the effect of *H. bacteriophora* on the geophysical properties of soil.

Acknowledgements

The authors appreciate the assistance of the technical staff of Zoology laboratory of Osun State University for their co-operation during this study. The authors would like to thank the Tertiary Education Trust Fund (TET fund) for financial support. The authors would like to extend their gratitude to the Osun State University for their logistic support and given them platform with which the fund was assessed.

Novelty Statement

The study may be exploited along with other suitable strategies in the bio- control of insect vectors of parasitic diseases and insect crop pests.

Author's Contribution

RMA conceived and designed the study, participated in data collection, data analysis and interpretation and also drafted the paper for publication. WAA, FKA and SOA participated in the study design and reviewed the paper. AQO, UJC participated in sample collection, laboratory work and data collection. All authors have read and approved the final manuscript.

Conflict of interests

The authors have declared no conflict of interest.

References

Arthurs, S., Heinz, K.M. and Prasifka, J.R., 2004. An analysis of using entomopathogenic nematode against above ground pests. Bull. Entomol. Res., 94: 297-306. https://doi.org/10.1079/BER2003309

Berry, R.E., Liu, J. and Groth, E., 1997. Efficacy and persistence of *Heterorhabditis marelatus* (Rhabditida: Heterorhabditidae) against root weevils (Coleoptera: Curculionidae) in strawberry. Environ. Entomol., 26: 465-470. https://doi.org/10.1093/ee/26.2.465

Del Valle, E.E., Dolinski, C., Barreto, E.L.S. and Souza, R.M., 2013. Time period between infection of *Heterorhabditis baujardi* LPP7 (Nematoda: Rhabditida) and soil application of *Galleria mellonella* (Lepidoptera: Pyralidae) cadavers on the emergence of infective juveniles. Nematol. Brasil., 37: 26-28.

Dix, I., Burnell, A.M., Griffin, C.T., Joyce, S.A.,

Nugent, M.J. and Downes, M.J., 1992. The identification of biological species in the genus *Heterorhabditis* (Nematoda: Heterorhabditidae) by cross-breeding second generation amphimictic adults. Parasitology, 104: 509-518. https://doi.org/10.1017/S0031182000063770

Ehlers, R.U. and Shapiro-Ilan, D.I., 2005. Mass production of entomopathogenic nematodes. In: Grewal, P., Ehlers, R-U. and Shapiro-Ilan, D. (Eds.) nematode as biological control agent. CABI Publishing, Wallingford, UK, pp. 65-79. https://doi.org/10.1079/9780851990170.0065

Elena, I., Nadezhda, S. and Sergei, S., 2013. Morphological and molecular characterization of *Steinernema costaricense* Uribe-Lorio, Mora and Stock, 2007 (Panagrolaimorpha: Steinernematidae) isolate from Bush Augusta State Park, Missouri, USA July 2013. Syst. Parasitol., 85: 219-234. https://doi.org/10.1007/s11230-013-9424-9

Flanders, K.L., Miller, J.M. and Shields, E.J., 1996. *In vivo* production of *Heterorhabditis bacteriophora* 'Oswego' (Rhabditida: Heterorhabditidae), a potential biological control agent for soil inhabiting insects in temperate regions. J. Econ. Entomol., 89: 373-380. https://doi.org/10.1093/jee/89.2.373

Gaugler, R., 1998. *Rhabditida*: *Steinernematidae* and *Heterorhabditidae*. In: Weeden, C.R., Shelton, A.M. and Hoffmann, M.P. (Eds.). Biological control: A guide to natural enemies in North America. Cornell University, London, UK.

Grzywacz, D., 2006. Novel controls for armyworm in East Africa adopted, demonstrated, disseminated - national/regional organizations. DFID Crop Protection Programme, Final Technical Report, R8408, pp. 9.

Hall, F.R. and Menn, J.J., 1999. Biopesticides used and delivery. Humana Press Inc. New Jersey. pp. 271-299.

Poinar, G.O. Jr. and Thomas, G.M., 1966. Significance of *Achromobacter nematophilus* Poinar and Thomas (Achronobacteriaceae: Eubacteriales) in the development of the nematode, DD-136 (Neoaplectana: Steinernematidae). Parasitology, 56: 385-390. https://doi.org/10.1017/S0031182000070980

Poinar, G.O.Jr., 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae. In: Gaugler, R. and Kaya, H.K. (Eds.). Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, FL. pp. 23-61.

Rufai, A.M., Adenigba, A.P. and Olaniran, O.D., 2020. Production of greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae) under laboratory conditions for mass production of entomopathogenic nematodes (EPNs). Katsina J. Sci., 7: 176-182

Sáenz-Aponte, A., Pulido, O.F. and Jaramillo, C., 2014. *Photorhabdus luminescens* SL0708 (Enterobacteriales: Enterobacteriaceae). Afr. J. Microbiol. Res., 8: 3123-3130. https://doi.org/10.5897/AJMR2014.6977

Shapiro-Ilan, D.I. and Gaugler, R., 2002. Production technology for entomopathogenic nematodes and their bacterial symbionts. J. Ind. Microbiol. Biotechnol., 28: 137-146. https://doi.org/10.1038/sj/jim/7000230

Singh, S., Moreau, E., Inman, F. and Holmes, L.D., 2011. Characterization of *Photorhabdus luminescens* growth for the rearing of the bacterial nematode *Heterorhabditis bacteriophora*. Indian J. Microbiol., 52: 325-331. https://doi.org/10.1007/s12088-011-0238-7

Uribe, L., Mora, M. and Stock, S.P., 2007. *Steinernema costaricense* n. sp. and *S. puntauvense* n. sp. (Rhabditida: Steinernematidae), two new entomopathogenic nematodes from Costa Rica. Syst. Parasitol., 68: 167-182. https://doi.org/10.1007/s11230-007-9098-2