ECRONICON OPEN ACCESS

EC CLINICAL AND MEDICAL CASE REPORTS
Research Article

Virulence of Indigenous Nematode-Bacteria Complex from Kashere, Nigeria against *Phyllophaga* Spp., *Liriomyza* Spp., B*usseola fusca* and *Locusta migratoria*

Aliyu HU*, Wante SP, Labaran HS and Kela SL

Department of Biological Sciences, Faculty of Science, Federal University of Kashere, Gombe State, Nigeria

***Corresponding Author:** Aliyu HU, Department of Biological Sciences, Faculty of Science, Federal University of Kashere, Gombe State, Nigeria.

Received: September 15, 2022; **Published:** December 30, 2022

Abstract

The pathogenicity and the life cycle of the isolate of *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) associated with *Photorhabdus* spp. was studied against the larvae of *Phyllophaga* spp, *Liriomyza* spp., *Busseola fusca* and adult stage of *Locusta migratoria.* The study was done under laboratory conditions and this is the first record of such study in Nigeria. The efficacy of ranged from 60 - 100% and the lethal concentration of nematode-bacteria complex required to kill 50 - 99% of the pest populations ranged from 34 IJ/host to 509 IJ/host for adult of *Locusta migratoria* which is the most susceptible of the hosts, 89 IJ/larva to 141 IJ/larva for *Liriomyza* spp., 99 IJ/larva to 894/larva for *Busseola fusca* and 105 IJ/larva to 4073 IJ/larva for *Phyllophaga* spp. being the least susceptible host. The reproductive rate of the nematodes-bacteria complexes ranged from 1,462 infective juveniles per host for adult *Locusta migratoria*, 2,286 infective juveniles per larva for *Busseola fusca,* 1,576 infective juveniles per larva for *Liriomyza* spp. and 2,297 infective juveniles per larva for *Phyllophaga* spp. From the evidence obtained from this study therefore, the indigenous isolate of nematodes-bacteria complexes from kashere, Nigeria produced up to 100% mortality in all the hosts tested. This isolate also reproduced successfully in all the hosts hence it can be employed for the potential biological control and management of these pests in Kashere and Nigeria.

Keywords: *Heterorhabditis bacteriophora; Biological Control; Nigeria; Mortality; Reproductive Rate*

Introduction

Nigeria's quest for diversification of its revenue base which is largely dependent on the oil sector has led the Federal Government to shift to agriculture. Although the country is blessed with vast arable land for crop production, a major problem bedeviling the sector is the problem of insect pests. Most of the attempts made in the management and control of these insect pests is largely dependent on the use of chemical pesticides. Of these chemicals, synthetic pesticides abound in different brands for the control and management of these pests, though a few of plants and their secondary metabolites have also been in use [5]. The problems posed by synthetic chemical pesticides have been documented. These include their effects on animals and human health as well as their impact on the environment which affect the ecosystem. Some of these chemical pesticides accumulate in humans and as per the report of [15], can interfere with most of the biological processes and pathways especially certain proteins involved in cell growth and body repair because they can induce DNA mutations in human cells. On the ecological aspect, these pesticides affect the ecosystem killing the beneficial flora and fauna especially

insects as most of them are not host specific. This will further affect biodiversity especially of rare species. Another disadvantage is associated with the problem of resistance. Some of these insect pests develop resistance toward the synthetic chemical pesticides due to either genetic modification of the affected pests, use of sub-lethal doses or a times even when high concentrations of the chemical are used, the insects rapidly continue to grow causing damages to the crops as reported separately by [10,14]. The best alternative of controlling and reducing pests' infestation or attack of agricultural crops especially those due to insects or arthropods have to be found.

Nematodes-bacteria complexes are made up of nematodes that harbor bacteria in their gut. The nematodes belong to genera *Steinernema* and *Heterorhabditis* and their associated bacterial partners belong to the genera *Xenorhabdus* for *Steinernema* and *Photorhabdus* for *Heterorhabditis* respectively [13]. The nematodes and the bacteria together form an entomopathogenic complex and they have emerged as potential alternatives to chemical pesticides against several economically important insect pests of crops [4,7]. The nematodes-bacteria complexes are found free living in the soil. Upon finding a susceptible host, they enter the host's body through natural openings like spiracles, mouth and anus, and release the specific symbiotic bacteria which is responsible for the killing of the host through septicemia and toxemia usually within 24 - 72 hrs [7]. After one or two generations of the nematodes-bacteria complexes within the insect host and as a result of limiting nutrients, infective juveniles exit the cadavers to find new hosts in the environment [9]. Many species of the nematodes-bacteria complexes of both genera have been used in the biological control of a variety of pests with varying degrees of success [1,20,21,24]. The use of indigenous isolates is especially better suited for the inundative release against local pests for an effective management and control regime as observed by [6]. This is because indigenous isolates are better adapted to local environmental conditions as observed by [11,17]. The introduction of exotic nematodes-bacteria species may result in negative impact on native non-target pests as observed by [14].

Objective of the Study

The objective of this study therefore is to evaluate the efficacy of an indigenous isolate of *H. bacteriophora* associated with *Photorhabdus* spp. form Kashere, Nigeria against 4 important pests of crops in the area. The pathogenicity of the isolate against these important pests and their reproductive capacity were investigated under laboratory conditions.

Materials and Methods

Source of nematodes-bacteria complexes

The nematodes-bacteria complexes were isolated from soil samples collected from Kashere, Nigeria. The isolates have been characterized in previous studies and identified as *H. bacteriophora*. The associated bacteria was also identified as *Photorhabdus* spp. the isolate was grown on 5th instar larvae of the greater wax moth, *Galleria mellonella* as described by [23]. The fresh nematodes-bacteria complexes that emerged from the *G. mellonella* larval cadavers were collected using modified White traps [8] and used for pathogenicity studies.

Sources of insect hosts

Larvae of *Phyllophaga* spp, *Liriomyza* spp., *Busseola fusca* and adult stage of *Locusta migratoria* were obtained from farms situated in the study area. All hosts were maintained in the laboratory for 4 days to ensure that they are healthy before using them in the test.

Pathogenicity bioassay

The pathogenicity bioassays were conducted in Petri dishes lined with moistened double layer Whatman No. 1 filter paper, according to the methods of [8]. Aliquots of nematodes-bacteria complexes containing 0, 25, 50, 75 and 100 IJs/ml were added to the Petri dishes and allowed to stand for 30 minutes. 5 each of the hosts were placed in each of the Petri dish. The dishes were sealed with masking tape and kept in the dark to facilitate infection of the hosts by the nematodes-bacteria complexes. The experiments were done in three replicates

and the entire experiment was repeated on another date to minimize error. The control plates received only distilled water as treatment. Host mortality was checked during every 24h for up to 6 - 10 days depending on the hosts. Death caused by infection with nematodes-bacteria complexes was confirmed due to a change in colour of the cadavers which is evident due to the presence of symbiotic bacteria.

Reproductive rate of nematodes-bacteria complexes

The number of nematodes-bacteria complexes that emerged per cadaver of each host was counted per concentration for 10 days. This was achieved by removing infected cadavers and placing them on individual modified White traps [23] after rinsing them in distilled water. Nematodes-bacteria complexes that emerged from the cadavers were then collected and counted under the microscope. The nematodes-bacteria complexes emerging from the cadavers were counted daily for 10 days.

Statistical analysis

All experimental data were analyzed statistically and results are presented as mean ± standard error of mean. The significance of the difference in all bioassay experiments was determined by one way analysis of variance (ANOVA) and Tukey's test of significance. P values ≤ 0.05 were accepted as statistically not significant. LC_{50} and LC_{99} values were calculated using Probit analysis with SPSS version 2000 software.

Results

The effectiveness of indigenous isolate of *Heterorhabditis bacteriophora* from kashere, Nigeria, was evaluated against four important pests of agricultural crops. The isolate was evaluated for its virulence based on different concentrations and exposure time. Their ability to reproduce and emerge from the host cadavers was also investigated.

Data presented in table 1-4 shows the rate of mortality of the different hosts post exposure to the nematodes-bacteria complexes. In table 1, mortality of adult grasshoppers (*Locusta migratoria*) began 24 hrs post exposure to the nematodes-bacteria complexes for all the different concentrations reaching 100% at day 4 for 25 IJs. For 50, 75 and 100 IJs/ml, 100% mortality was achieved on day 3. For stem borers (*Busseola fusca*) mortality did not start until day 4 for the concentration of 25 IJs/ml. For the other concentrations mortality started on day 2. For all the concentrations however, 100% mortality was recorded on day 6 as shown in table 2. For larvae of vegetable leaf miners (*Liriomyza* spp.), a different trend was observed with 25, 50 and 75 IJs/ml. Mortality was recorded on day 1 for all concentrations except for 100 IJs/ml (Table 3). Data presented in table 4 shows mortality of white grubs (*Phyllophaga* spp.) exposed to nematodes-bacteria complexes. 25 IJs/ml and 50IJs/ml did not produce any mortality until after day 2 when it produced 20% mortality. Only 60% of the larvae died for 25IJs/ml. 75 IJs/ml of the nematodes-bacteria complexes however, produced 40% death of the Scarabaeid larvae after day 1, reaching 100% after day 4. 100 IJs/ml produced only 20% death of the Scarabaeid larvae after day 1 and reached 100% on day 4.

	Mortality per day of adult grasshoppers						
Conc (IJs/ml)	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Control	0	0	0	0	0	0	0
25	20	40	80	100	100	100	100
50	20	60	100	100	100	100	100
75	40	80	100	100	100	100	100
100	40	80	100	100	100	100	100

Table 1: *Percentage mortality of adult grasshoppers (Locusta migratoria) exposed to nematode-bacteria complex.*

Key: Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes); ml: Milliliter of Distilled Water.

	Mortality per day of stem borers					
Con (IJs/ml)	**1**	**2**	**3**	**4**	**5**	**6**
Control	0	0	0	0	0	0
25	0	0	0	20	60	100
50	0	40	40	60	80	100
75	0	40	60	60	80	100
100	0	40	60	60	80	100

Table 2: *Percentage mortality of larvae of stem borers (Busseola fusca) exposed to nematode-bacteria complex.*

Key: Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes); ml: Per Milliliter of Distilled Water.

	Mortality per day of larvae of vegetable leaf miners (*Liriomyza* spp.)						
Conc (IJs/ml)	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Control	0	0	0	0	0	0	0
25	40	80	100	100	100	100	100
50	60	100	100	100	100	100	100
75	60	100	100	100	100	100	100
100	0	0	20	40	80	100	100

Table 3: *Percentage mortality of larvae of larvae of vegetable leaf miners exposed to nematode-bacteria complex.*

Key: Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes); ml: Per Milliliter of Distilled Water.

	Mortality per day of larvae of Scarabaeid						
Concentration/days	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Control	0	0	0	0	0	0	0
25	0	20	40	40	60	60	60
50	0	20	60	80	100	100	100
75	40	60	80	100	100	100	100
100	20	40	60	100	100	100	100

Table 4: *Percentage mortality of larvae of Scarabaeid (Phyllophaga spp.) exposed to nematode-bacteria complex.*

Key: Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes); ml: Per Milliliter of Distilled Water.

The results were subjected to statistical analysis using one way analysis of variance and Tukey's test of significance at $P \leq 0.05$. Result presented in table 5-8 showed that there was no significant differences between the concentrations in their ability to kill the different hosts. From table 9, results of Probit analysis of nematodes-bacteria complexes against the 4 hosts showed that grass hoppers were most susceptible to the nematodes-bacteria complexes requiring about 34 IJs/ml to kill 50% of its population and 509 IJs/ml to kill 99%, followed by vegetable leaf miners that required about 89 IJs/ml to kill 50% of their population and 14 IJs/ml for 99%. This was followed by the stem borers and they required about 99 IJs/ml to kill 50% of their population and 894 IJs/ml for 99%. The Scarabaeid larvae being the least susceptible requiring a higher concentration of about 105 IJs/ml of the nematodes-bacteria complexes to kill 50% of its population and 4073 for 99%.

	Mortality per day					
Conc (IJs/ml)	**1**	**2**	**3**	**4**	**5**	**6**
25	0.6667 ± 0.5774[a]	1.6667 ± 0.5774[a]	1.6667 ± 0.5774[a]	1.0000 ± 1.0000[a]	0.0000 ± 0.0000	0.0000 ± 0.0000
50	1.3333 ± 0.5774[a]	1.6667 ± 0.5774[a]	1.3333 ± 0.5774[a]	0.6667 ± 1.1547[a]	0.0000 ± 0.0000	0.0000 ± 0.0000
75	2.0000 ± 1.0000[a]	2.0000 ± 0.0000[a]	1.0000 ± 1.0000[a]	0.0000 ± 0.0000[a]	0.0000 ± 0.0000	0.0000 ± 0.0000
100	2.3333 ± 0.9003[a]	1.6667 ± 0.5774[a]	1.0000 ± 1.0000[a]	0.0000 ± 0.0000[a]	0.0000 ± 0.0000	0.0000 ± 0.0000

Table 5: *Mean mortality of grasshoppers exposed to different concentrations of nematode-bacteria complex.*

Means with the same letters are not significantly different at P ≤ 0.05 using Tukey's test.

Key: Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes); ml: Milliliter of Distilled Water.

	Mortality per day					
Conc (IJs/ml)	**1**	**2**	**3**	**4**	**5**	**6**
25	0.0000 ± 0.0000[a]	0.0000 ± 0.0000[a]	0.6667 ± 1.5774[a]	1.0000 ± 0.0000[a]	1.0000 ± 1.0000	0.0000 ± 0.0000
50	0.0000 ± 0.0000[a]	1.3333 ± 1.1547[a]	1.0000 ± 0.0000[a]	1.0000 ± 0.0000[a]	0.3333 ± 0.5774	0.0000 ± 0.0000
75	0.0000 ± 0.0000[a]	1.6667 ± 0.5774[a]	1.0000 ± 1.0000[a]	0.3333 ± 0.5774[a]	0.6667 ± 1.1547	0.0000 ± 0.0000
100	0.0000 ± 0.0000[a]	1.3333 ± 0.5774[a]	1.0000 ± 1.0000[a]	0.6667 ± 1.1547[a]	0.3333 ± 0.5774	0.0000 ± 0.0000

Table 6: *Mean mortality of larvae of stem borers exposed to different concentrations of nematode-bacteria complex.*

Means with the same letters are not significantly different at P ≤ 0.05 using Tukey's test.

Key: Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes); ml: Milliliter of Distilled Water.

	Mortality per day						
Conc (IJs/ml)	**1**	**2**	**3**	**4**	**5**	**6**	**7**
25	2.3333 ± 0.5774[b]	1.3333 ± 0.5774[a]	1.3333 ± 0.5774[a]	1.0000 ± 1.0000[a]	0.0000 ± 0.0000[a]	0.0000 ± 0.0000[a]	0.0000 ± 0.0000[a]
50	2.6667 ± 0.5774[b]	2.0000 ± 1.0000[a]	0.3333 ± 0.5774[a]	0.0000 ± 0.0000[a]	0.0000 ± 0.0000[a]	0.0000 ± 0.0000[a]	0.0000 ± 0.0000[a]
75	3.3333 ± 0.5774[b]	1.6667 ± 0.5774[a]	0.3333 ± 0.5774[a]	0.0000 ± 0.0000[a]	0.0000 ± 0.0000[a]	0.0000 ± 0.0000[a]	0.0000 ± 0.0000[a]
100	0.0000 ± 0.0000[a]	1.3333 ± 0.5774[a]	1.0000 ± 1.0000[a]	1.3333 ± 1.5275[a]	1.3333 ± 0.5774	1.0000 ± 1.0000[a]	0.3333 ± 0.5774

Table 7: *Mean mortality of larvae of vegetable leaf miners exposed to different concentrations of nematode-bacteria complex.*

Means with the same letters are not significantly different at P ≤ 0.05 using Tukey's test.

Key: Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes); ml: Milliliter of Distilled Water.

	Mortality per day						
Conc (IJs/ml)	**1**	**2**	**3**	**4**	**5**	**6**	**7**
25	0.3333 ± 0.5774[a]	0.3333 ± 0.5774[a]	1.0000 ± 0.0000[a]	0.6667 ± 1.1547[a]	0.6666 ± 0.5774[a]	0.3333 ± 0.5774[a]	0.0000 ± 0.0000
50	0.0000 ± 0.0000[a]	1.3333 ± 0.5774[ab]	1.3333 ± 0.5774[a]	1.0000 ± 0.0000[a]	1.0000 ± 0.0000[a]	0.0000 ± 0.0000[a]	0.0000 ± 0.0000
75	1.6667 ± 0.5774[b]	1.6667 ± 0.5774[b]	1.0000 ± 0.0000[a]	0.3333 ± 0.5774[a]	0.3333 ± 0.5774[a]	0.0000 ± 0.0000[a]	0.0000 ± 0.0000
100	0.6667 ± 0.5774[ab]	1.0000 ± 0.0000[ab]	1.3333 ± 0.5774[a]	1.3333 ± 1.5774[a]	0.6667 ± 1.1547	0.0000 ± 0.0000[a]	0.0000 ± 0.0000

Table 8: *Mean mortality of larvae of Scarabaeid exposed to different concentrations of nematode-bacteria complex.*

Means with the same letters are not significantly different at P ≤ 0.05 using Tukey's test.

Key: Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes); ml: Milliliter of Distilled Water.

Insect hosts	LC_{50}	LC_{99}	Chi square
SCB	105.241	4073.562	1.193
SB	99.344	894.388	1.378
GH	34.015	509.534	0.100
VLF	89.870	14.871	11.340

Table 9: *Probit analysis of nematodes-bacteria complexes at 48hrs post exposure of the hosts.*

Key: SB: Stem Borers (Busseola fusca); GH: Grass Hoppers (Locusta migratoria); VLF: Vegetable Leaf Miners (Liriomyza spp.); SCB: White Grubs (Gwazarma) (Scarabaeid spp.); LC_{50}: Lethal Concentration of Nematodes-Bacteria Complexes Required to Kill 50% of the Hosts; LC_{99}: Lethal Concentration of Nematodes-Bacteria Complexes Required to Kill 99% of the Hosts.

Reproductive rate of nematodes-bacteria complexes in hosts

Data presented on table 10-14 shows that yield of nematodes-bacteria complexes that emerged from all the cadavers reduced progressively from day 1 of emergence to day 10 for all the concentrations. The rate of reproduction in the hosts ranged from 1200.00 ± 164.00 for *Locusta migratoria* at concentration of 25 IJs/ml to 2498.67 ± 189.07 for *Phyllophaga* spp. at concentration of 75 IJs/ml.

	Yield per day									
Conc (IJs/ml)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
25	212.00 ± 16.00[a]	209.33 ± 30.29[a]	184.00 ± 10.58[a]	152.00 ± 22.27[a]	134.67 ± 56.05[a]	112.00 ± 30.19[a]	76.00 ± 4.00[a]	65.33 ± 18.04[a]	30.67 ± 8.33[a]	25.33 ± 16.16[a]
50	209.33 ± 26.63[a]	193.33 ± 30.55[a]	193.33 ± 30.02[a]	149.33 ± 26.63[a]	114.67 ± 6.11[a]	102.33 ± 27.23[a]	66.67 ± 22.03[a]	57.33 ± 19.73[a]	42.67 ± 15.14[a]	24.00 ± 16.00[a]
75	216.00 ± 22.27[a]	193.33 ± 16.65[a]	156.33 ± 4.00[a]	136.00 ± 22.27[a]	118.67 ± 48.05[a]	78.67 ± 34.48[a]	78.67 ± 37.17[a]	60.00 ± 20.78[a]	42.67 ± 15.14[a]	26.67 ± 9.24[a]
100	252.00 ± 48.66[a]	234.67 ± 49.36[a]	213.33 ± 36.29[a]	185.33 ± 28.94[a]	148.00 ± 28.84[a]	94.67 ± 32.58[a]	80.00 ± 34.18[a]	64.00 ± 13.85[a]	37.33 ± 8.33[a]	34.67 ± 2.31[a]

Table 10: *Reproductive rate of nematodes-bacteria complexes in Locusta migratoria*

Means with the same letters are not significantly different at P ≤ 0.05 using Tukey's test.

Key: Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes); ml: Per Milliliter of Distilled Water.

	Yield per day									
Conc (IJs/ml)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
25	312.00 ± 34.18^{a}	293.33 ± 44.24^{a}	270.67 ± 41.05^{a}	238.67 ± 61.10^{a}	212.00 ± 73.32^{a}	158.67 ± 42.77^{a}	96.00 ± 36.00^{a}	88.00 ± 40.00^{a}	64.00 ± 18.33^{a}	48.00 ± 24.33^{a}
50	330.67 ± 60.71^{a}	318.67 ± 40.07^{a}	309.33 ± 37.17^{a}	280.00 ± 64.37^{a}	246.67 ± 56.19^{a}	221.33 ± 69.55^{a}	160.00 ± 35.55^{ab}	150.67 ± 46.36^{a}	121.33 ± 64.66^{a}	65.33 ± 22.03^{a}
75	345.33 ± 80.93^{a}	333.33 ± 46.19^{a}	310.67 ± 57.18^{a}	288.00 ± 32.00^{a}	258.67 ± 65.03^{a}	228.00 ± 48.49^{a}	204.00 ± 62.48^{b}	132.00 ± 58.92^{a}	116.00 ± 70.31^{a}	65.33 ± 24.44^{a}
100	365.33 ± 103.18^{a}	338.67 ± 53.72^{a}	294.67 ± 20.13^{a}	297.33 ± 22.03^{a}	268.00 ± 41.76^{a}	224.00 ± 31.24^{a}	197.33 ± 8.33^{ab}	136.00 ± 18.33^{a}	98.67 ± 10.07^{a}	66.67 ± 36.30^{a}

Table 11: *Reproductive rate of nematodes-bacteria complexes in stem borers (Busseola fusca).*

Means with the same letters are not significantly different at P≤0.05 using Tukey's test.

Key: Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes); ml: Per Milliliter of Distilled Water.

	Yield per day									
Conc (IJs/ml)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
25	228.00 ± 24.98^{a}	218.67 ± 2.31^{ab}	196.00 ± 4.00^{a}	180.00 ± 13.87^{a}	180.00 ± 10.58^{a}	150.67 ± 22.03^{a}	120.00 ± 8.00^{a}	108.00 ± 43.27^{a}	96.00 ± 34.18^{a}	68.00 ± 24.98^{a}
50	229.33 ± 52.81^{a}	176.00 ± 48.66^{a}	168.00 ± 59.19^{a}	152.00 ± 45.43^{a}	138.67 ± 50.81^{a}	108.01 ± 38.16^{a}	92.00 ± 34.64^{a}	77.33 ± 15.14^{a}	64.00 ± 18.33^{a}	52.00 ± 13.86^{a}
75	202.67 ± 8.33^{a}	202.67 ± 8.33^{ab}	190.67 ± 10.07^{a}	181.33 ± 18.90^{a}	161.33 ± 10.07^{a}	154.67 ± 4.05^{a}	128.00 ± 14.42^{a}	105.33 ± 14.05^{a}	67.00 ± 20.08^{a}	69.67 ± 22.28^{a}
100	256.00 ± 22.27^{a}	250.67 ± 12.22^{b}	222.67 ± 22.03^{a}	190.67 ± 20.13^{a}	164.00 ± 4.00^{a}	149.33 ± 14.05^{a}	121.33 ± 15.14^{a}	100.00 ± 24.33^{a}	81.33 ± 23.44^{a}	60.00 ± 20.78^{a}

Table 12: *Reproductive rate of nematodes-bacteria complexes in vegetable leaf miners*

Means with the same letters are not significantly different at P≤0.05 using Tukey's test.

Key: Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes); ml: Per Milliliter of Distilled Water.

	Yield per day									
Conc (IJs/ml)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
25	314.67 ± 47.38^{a}	328.00 ± 31.24^{a}	285.33 ± 28.09^{a}	152.00 ± 22.27^{a}	134.67 ± 56.05^{a}	112.00 ± 30.19^{a}	76.00 ± 4.00^{a}	65.33 ± 18.04^{a}	30.67 ± 8.33^{a}	25.33 ± 16.16^{a}
50	345.33 ± 25.40^{a}	336.00 ± 24.00^{a}	193.33 ± 30.02^{a}	149.33 ± 26.63^{a}	114.67 ± 6.11^{a}	102.33 ± 27.23^{a}	66.67 ± 22.03^{a}	57.33 ± 19.73^{a}	42.67 ± 15.14^{a}	24.00 ± 16.00^{a}
75	400.00 ± 42.14^{a}	373.33 ± 16.65^{a}	156.33 ± 4.00^{a}	136.00 ± 22.27^{a}	118.67 ± 48.05^{a}	78.67 ± 34.48^{a}	78.67 ± 37.17^{a}	60.00 ± 20.78^{a}	42.67 ± 15.14^{a}	26.67 ± 9.24^{a}
100	396.00 ± 4.00^{a}	321.33 ± 28.38^{a}	213.33 ± 36.29^{a}	185.33 ± 28.94^{a}	148.00 ± 28.84^{a}	94.67 ± 32.58^{a}	80.00 ± 34.18^{a}	64.00 ± 13.85^{a}	37.33 ± 8.33^{a}	34.67 ± 2.31^{a}

Table 13: *Reproductive rate of nematodes-bacteria complexes in larvae of scarabaeids.*

Means with the same letters are not significantly different at P≤0.05 using Tukey's test.

Key: Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes); ml: Per Milliliter of Distilled Water.

	Yield per host			
Conc (IJs/ml)	**SB**	**GH**	**VLF**	**SCB**
25	1781.33 ± 408.10[a]	1200.00 ± 164.00[a]	1545.33 ± 137.19[a]	2085.33 ± 78.21[a]
50	2204.00 ± 462.19[a]	1153.33 ± 188.99[a]	1257.33 ± 362.09[a]	2388.00 ± 189.78[a]
75	2281.33 ± 441.55[a]	1157.33 ± 175.56[a]	1463.33 ± 40.41[a]	2498.67 ± 189.07[a]
100	2286.67 ± 166.73[a]	1462.67 ± 101.69[a]	1596.00 ± 28.00[a]	2297.33 ± 227.48[a]

Table 14: *Reproductive rate per host at different concentrations of nematodes-bacteria complexes.*

Means in the column followed by similar letters are not significantly different at P ≤ 0.05 using Tukey test of significance.

Key: SB: Stem Borers (Busseola fusca); GH: Grass Hoppers (Locusta migratoria); VLF: Vegetable Leaf Miners (Liriomyza spp.); SCB: White Grubs (Gwazarma) (Scarabaeid spp.); Conc: Concentrations; IJs: Infective Juveniles (Nematodes-Bacteria Complexes).

Discussion of Results

The effectiveness of the isolate was tested against four important insect pests of agricultural products in Kashere. The isolate was very effective as it killed all the hosts and the infective juveniles recycled successfully in the hosts used as they were able to kill, reproduce within the hosts and successfully emerged from the hosts. The ability of the nematodes-bacteria complexes to kill the various hosts varied with *Locusta migratoria* being the most susceptible hosts since it took only 1 day for the nematodes-bacteria complexes to kill 20% for 25 and 50 IJs/ml and achieving 100% mortality on day 4. 75 and 100 IJs/ml killed 40% of the hosts on day 1 and achieved 100% on day 3. Probit analysis revealed that 34 IJs/ml of the nematodes-bacteria complexes were required to kill 50% of the population of *Locusta migratoria* which is the least observed. This was followed closely by *Liriomyza* spp. in terms of susceptibility to the nematodes-bacteria complexes. However, in *Liriomyza* spp. there was a delay in mortality for 100 IJs/ml due to competition of the nematodes-bacteria complexes for space and nutrients. This competition may be due to the small size of the hosts. Results of Probit analysis showed that 89 IJs/ml of the nematodes-bacteria complexes were required to kill 50% of *Liriomyza* spp. and only 14 IJs/ml were required to kill 99% of *Liriomyza* spp. This result further confirms the competition between the nematodes-bacteria complexes within the host. Larvae of *Busseola fusca* were less susceptible as they required 4 days to start experiencing the effect of the nematodes-bacteria complexes. For 25 IJs/ml, 20% of larvae of *Busseola fusca* were killed and 100% mortality was achieved on day 6. For 50, 75 and 100 lJs/ml 40% of the larvae of *Busseola fusca* were killed after day 2 with 100% mortality achieved on day 6. Larvae of *Phyllophaga* spp. were the least susceptible to the nematodes-bacteria complexes. They required longer exposure time for the nematodes-bacteria complexes to kill Scarabaeids (*Phyllophaga* spp.) than the other hosts. Probit analysis also revealed that the larvae of *Phyllophaga* spp. required the most number of nematodes-bacteria complexes of 105 IJs/ml to produce 50% mortality and 4073 IJs/ml to produce 99% mortality. However, the ability of the isolates to kill, reproduce in all the hosts and also to emerge from the hosts is consistent with the observations of Peters, 1996 who observed that *Heterorhabditis bacteriophora* were regarded as generalist and that this characteristics reflects their wide global distribution.

The nematodes-bacteria complexes were observed to have effectively recycled in 70 - 100% of the tested pests. According to [2] a good control potential of nematodes-bacteria complexes is above 70% effectiveness. Effectiveness of the nematodes-bacteria complexes also depend on their ability to recycle successfully in a susceptible host and emerge after a successful reproductive cycle from the carcass and to seek for new hosts in the environment as observed by [1,7]. The nematodes-bacteria complexes isolated in this study killed the entire hosts and reproduced successfully in them. They also emerged from the hosts. The larvae of *Phyllophaga* spp. produced the highest number of nematodes-bacteria complexes of 2498.67 ± 189.07 per larva at 75 IJs/ml. This was followed closely by larvae of *Busseola fusca* which produced 2286.67 ± 166.73 nematodes-bacteria complexes per larva at 100 IJs/ml. *Liriomyza* spp. produced 1596.00 ± 28.00 nematodes-bacteria complexes per larva at 100 IJs/ml and the least was produced by *Locusta migratoria* which produced 1462.67 ± 101.69 nematodes-bacteria complexes at 100 IJs/ml. The observations made in this study are consistent with those of [1] who observed that the

pathogenicity of nematodes-bacteria complexes against a specific insect host differ. Inoculums size of the nematodes-bacteria complexes also determines host mortality and the number of progeny produced as reported by [1,6,12]. Results obtained from this investigation are consistent with findings of other authors in South Africa who isolated indigenous nematodes-bacteria complexes and evaluated them against indigenous pests with various degrees of efficacy [18,19,22].

High effectiveness for entomopathogenic nematode-bacteria complex was recorded as 70% and above as observed by [3]. However, according to [16], effectiveness in the laboratory may not translate to effectiveness on the field, hence it is important for more research to be conducted so as to further evaluate the efficacy of this isolate on the field for the effective control of the tested pests in the study area.

Conclusion

The key to achieving sustainable agriculture and food security in Nigeria is ensuring that crops stay healthy and protected from damage by pests and diseases. One of the ways of achieving this important goal is through the use of biological pesticides based on nematode-bacteria complex as alternative sources of pest management and control in the country.

From the evidence obtained in this investigation all the hosts tested with the isolated nematodes-bacteria complexes was susceptible producing up to 100% mortality. More so, the nematodes-bacteria complexes isolated were able to reproduce in all the hosts and emerge to seek new hosts making them cost effective. Considering these attributes, it could be suggested that these nematodes-bacteria complexes have the potential for the effective control of these important insect pests of agricultural produce in Kashere namely *Locusta migratoria*, larvae of *Phyllophaga* spp, larvae of *Liriomyza* spp. and larvae of *Busseola fusca*. Therefore, these Nigerian isolates of entomopathogenic nematodes-bacteria complexes meet the requirements of a good candidate for the effective biological control of these important pests of crops in Kashere. Therefore, the isolate should be adopted for biological management and control of pests in Nigerian agriculture.

Bibliography

1. Arun KY and Lalramliana. "Efficacy of indigenous entomopathogenic nematodes from Meghalaya, India against the larvae of taro leaf beetle, *Aplosonyx chalybaeus* (Hope)". *Journal of Parasitology* 36 (2012): 149-154.
2. Georgis R., *et al*. "Successes and failures in the use of parasitic nematodes for pest control". *Biological Control* 38 (2006): 103-123.
3. Georgis R and Manweiler SA. "Entomopathogenic nematodes: a developing Biological control technology". In: Evans K. (edition.) Agricultural Zoology Reviews. Intercept, Andover, UK (1994): 63-94.
4. Georgis R. "Formulation and application technology". In: Gaugler R, Kaya HK, editors. Entomopathogenic nematodes in biological control. Boca Raton: CRC Press (1990): 173-191.
5. Giovanni B., *et al*. "Commentary: Making green pesticides greener? The potential of plant products for nanosynthesis and pest control". *Journal of Cluster Science* 28 (2017): 3-10.
6. Hominick WM and Reid AP. "Perspectives on entomopathogenic nematology". In: Gaugler, R. and Kaya, H. K, editions. Entomopathogenic nematodes in biological control. Boca Raton: CRC Press (1990): 327-345.
7. Kaya HK and Gaugler R. "Entomopathogenic nematodes". *Annual Review of Entomology* 38 (1993): 181-206.
8. Kaya HK and Stock SP. "Techniques in insect nematology". In Lacey L. A. (edition.) Manual of Techniques in Insect Pathology. San Diego, C.A: Academic Press (1997): 281-324.
9. Koppenhoffer AM. "Nematodes". In L. A. Lacey and H. K. Kaya eds. Field Manual of Techniques in Invertebrate Pathology: Application and evaluation of pathogens for control of insects and other invertebrate pests. Second edition. Dordredt: Springer (2007): 249-264.

10. Nyasami JO., *et al*. "Laboratory and field investigations using indigenous entomopathogenic nematodes for biological control of *Plutella xylostella* in Kenya". *International Journal of Pest Management* 54 (2008): 355-361.

11. Noosidum, A., *et al*. "Characterization of new entomopathogenic nematodes from Thailand: Foraging behaviour and virulence to the greater wax moth, *Galleria mellonella* L. (Lepidoptera:Pyralidae)". *Journal of Nematology* 42 (2010): 281-291.

12. Peters A and Ehlers RU. "Susceptibility of leatherjackets (*Tipula paludosa* and *Tipula oleracea*; Tipulidae; Nematocera) to the entomopathogenic nematode *Steinernema feltiae*". *Journal of Invertebrate Pathology* 63 (1994): 163-171.

13. Poinar GO. "Nematodes for biological control of insects". Raton, Florida; CRC Press (1979).

14. Schroer S and Ehlers RU. "Foliar application of the entomopathogenic nematode *Steinernema carpocapsae* for biological control of diamondback moth larvae (*Plutella xylostella*)". *Biological Control* 33 (2005): 81-86.

15. Segal D and Glazer I. "Genetics for improving biological control agents: the case of entomopathogenic nematodes". *Crop Protection* 19 (2000): 685-689.

16. Shapiro-Ilan DI., *et al*. "Efficacy of *Steinernema carpocapsae* for control of the lesser peach tree borer, *Synanthedon pictipes*: Improved aboveground suppression with a novel gel application". *Biological Control* 54 (2010): 23-28.

17. Shapiro-IIan DI and Gaugler R. "Production technology for Entomopathogenic nematodes and their bacterial symbionts". *Journal of Industrial Microbiology and Biotechnology* 28 (2002): 137-146.

18. Stokwe NF. "Entomopathogenic nematodes: Characterisation of a new species, long-term storage and control of obscure mealybug. *Pseudococcus viburni* (Hemiptera: Pseudococcidae) under laboratory conditions". Thesis, Stellenbosch University, Private Bag X1, Matieland (Stellenbosch), South Africa (2009).

19. Stokwe NF and Malan AP. "Potential control of mealybugs using Entomopathogenic nematodes". *South African Fruit Journal* 7 (2010): 38-42.

20. Tomalak M., *et al*. "Glasshouse applications". In: Grewal PS, Ehlers R-U, Shapiro-Ilan DI, editors. Nematodes as biocontrol agents. Oxon: CABI Publishing (2005): 147-166.

21. Valle EED., *et al*. "Dispersal of *Heterorhabditis Baujardi* LPP7 (Nematoda: Rhabditida) applied to the soil as infected host cadavers". *International Journal of Pest Management* 54 (2008): 115-122.

22. Van Niekerk S. "The use of entomopathogenic nematodes to control citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae) on citrus in South Africa". Thesis, Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, Matieland (Stellenbosch), South Africa (2012).

23. Woodring JL and Kaya HK. "Steinernematid and Heterorhabditid nematodes: A handbook of biology and techniques". Arkansas agricultural experiment station, Southern Coorperative Bulletin (1988): 331.

24. Williams EC and Walters KFA. "Foliar application of entomopathogenic nematode *Steinernema feltiae* against leaf miners on vegetables". *Biocontrol Science and Technology* 10 (1999): 61-70.

Volume 6 Issue 1 January 2023