Available: https://doi.org/10.54117/ijpae.v1i1.107 *Research article*

IPS Journal of Plant, Animal, and Environmental Sciences
Volume 1(1): 1-10 (2025)
Received: 19 Oct 2025 | Accepted: 11 Nov 2025 | Published: 14 Nov 2025

Biological Control of *Acanthoscelides obteus* using Entomopathogenic Bacteria

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ABSTRACT

The bean weevil, *Acanthoscelides obtectus*, is a major cosmopolitan pest of stored legume crops, causing significant quantitative and qualitative losses. Over-reliance on synthetic chemical insecticides for its control has led to issues of resistance, environmental contamination, and food safety concerns. There is, therefore, an urgent need for sustainable and eco-friendly alternatives, such as biological control using entomopathogenic bacteria. This study aimed to isolate and identify entomopathogenic bacteria with high efficacy against *A. obtectus* and to evaluate their potential as biological control agents. A total of 100 garden soil samples were collected and screened for the bacteria that can secrete toxic protein using T3 medium and mineral medium (NYSM). The insecticidal activities were carried out by exposing the insects to different concentrations of the proteins. The study revealed the presence of *Paenibaeillus mucilergmous* strain KN-18 (PM18), *Paenibaeillus* species strain S-38 (PS38) and *Paenibaeillus konkukensis* strain SK 3146 (PK3146) as the entomopathogenic bacteria. There was significant ($P<0.05$) increase in toxic proteins secreted by the isolates in every 24 h interval, and PK3146 secreted the highest toxic proteins in every 24 h interval, and the insecticidal activity of the toxic proteins secreted by the isolates against *A obtecus* revealed increase in number of deaths of the insects in every 24 h interval, and these increased became significant ($P<0.05$) after 72 h exposure to toxic proteins. The data generated from LCSO calculation also pointed out that the toxic proteins secreted from PK3146 exhibited the highest activity, followed by that excreted by PS38 and then PM18. The study concluded that the entomopathogenic bacteria, isolated in this study, exhibited high potential for the biological control of *Acanthoscelides obtectus*.

How to Cite this Article

Idigo, M. A., Iheukwumere, I. H., Iheukwumere, C. M., Nnaeze, B. C., Akulue, C. J., Nwakoby, N. E., Ezendianefor, J. N., Ike, V. E., Nnaedozie, A. O., Ezekwueche, S. N., Anagor, I. S., Aniekwe, C. C., Ezeoke, F. C., Okereke, F. O., & Ochibulu, S. C. (2025). Biological Control of Acanthoscelides obteus using Entomopathogenic Bacteria. *IPS Journal of Plant, Animal, and Environmental Sciences*, *1*(1), 1–10. https://doi.org/10.54117/ijpae.v1i1.107

Keywords: *Acanthoscelides obtectus, entomopathogenic bacteria, biological control, toxic proteins, Paenibacillus species*

INTRODUCTION

The bean weevil, *Acanthoscelides obtectus,* is a major pest of stored legume crops worldwide, causing significant quantitative and qualitative losses (Gbaye and Holloway, 2013; Nwakoby *et al*., 2025a; Idigo *et al*., 2025a). The control of this pest has relied heavily on synthetic chemical insecticides, which have led to issues of resistance, environmental contamination, and food safety concerns (Isman, 2006; Nwakoby *et al*., 2025b; Idigo *et al*., 2025b). Therefore, there is an urgent need for sustainable and eco-friendly alternatives for the management of *A. obtectus*.

Biological control using entomopathogenic bacteria has emerged as a promising approach for pest management (Lacey *et al*., 2015; Nwakoby *et al*., 2025c; Nwakoby *et al*., 2025d). These bacteria can be used to control insect pests in a targeted and environmentally friendly manner, reducing the risks associated with chemical insecticides. Entomopathogenic bacteria, such as *Paenibacillus* species, have been shown to be effective against various insect pests (Singh *et al*., 2016; Idigo *et al*., 2025c; Nwakoby *et al*., 2025e).

Paenibacillus species are known to produce toxic proteins that can be used as bioinsecticides (Patel *et al*., 2018). These proteins can be used to control insect pests in a specific and environmentally friendly manner. The use of *Paenibacillus* species as biological control agents has been reported in several studies, highlighting their potential as a sustainable solution for pest management (Senthilkumar *et al*., 2017; Nwakoby *et al*., 2025f; Nwakoby *et al*., 2025g).

The current study aimed to isolate and identify entomopathogenic bacteria with high efficacy against *A. obtectus* and to evaluate their potential as biological control agents. The study focused on the isolation of *Paenibacillus* species from garden soil samples and the evaluation of their insecticidal activities against *A. obtectus.*

The findings of this study will contribute to the development of sustainable and eco-friendly approaches for the management of *A. obtectus*, reducing the reliance on synthetic chemical insecticides and promoting a healthier environment.

MATERIALS AND METHODS

Soil sample collection: This was carried out using the method described in the study published by Iheukwumere *et al.* (2021). A total of fifty samples were collected for the study. The litter from the soil surfaces was carefully scrapped out using sterile stainless spoon. The soil auger was derived to a plough depth of 15 cm in the farm land, and soil sample was drawn up to 10 samples from each sampling unit into a sterile tray. The samples were thorough mixed and foreign materials such as roots, stones, pebbles and gravels were carefully removed. The soil sample was then reduced to half by quartering the sample. Quartering was carried out by dividing the soil sample into four equal parts and the two opposite quarters were discarded and the remaining two quarters were mixed. The process was repeated for the rest of soil samples used for this study. The samples were carefully labeled and then kept in a disinfected cooler, to maintain its temperature and stability of the number of the isolates. The samples were transported to the laboratory for analysis.

Procurement of insects: The bean weevils were collected by hand picking from overstayed beans in different shops in Awka Metropolis. These were carefully placed in a cleaned and sterile containers, and transported for identification and analysis. The bean weevils were identified appropriately.

Isolation and Characterization of the Bacterial Isolates

Isolation of the organism: This was carried out using the method described by Bambang *et al.,* (2015) and Reyaz *et al.,* (2017) Iheukwumere *et al*. (2025a). One gram of the soil sample was weighed into boiling test tube, 5m of distilled water was added and shake thoroughly and then make up to 10ml using the distilled water (10^{-1}dilution). The boiling tube was kept at 80^0C for 30 minutes and it was allowed to settle. One milliliter of this heat treated suspension was added to four milliliter (4ml) of normal saline (0.85% NaCl), which was give 5^{-1} dilution. From 5^{-1} dilution test tube, a five-fold serial dilution was carried out to obtain 5^{-5} dilution. One milliliter aliquot from 10^{-1}, 5^{-1} and 5^{-5} test tubes were collected and plate on T3 agar medium, and NYSM agar (nutrient agar with 0.5 g/l yeast extract, 0.2 g/Lmgcl$_2$ 0.01g/l MnCl$_2$ and 0.1 g/L CaCl$_2$ with 100 mg/ml of streptomycin). These were done in triplicate and incubated in an inverted at room temperature (30 $\pm$ 2^0C) for 3 days.

Purification of the isolates: The best growing colonies from the culture plates, with prominence characteristics was aseptically picked using sterile wire loop and aseptically streaked on, NYSM agar, and nutrient agar plates. The plates were incubated in an inverted position at room temperature (30 $\pm$ 2^0C) for 48 h. The purity of the sub-cultured isolates was checked microscopically be examining their cells using gram staining technique as described by Herssan *et al*. (2010) Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2018a), Ugwu *et al*. (2025a).

Characterization and identification of the isolates the isolates: The isolates were characterized and identified using the morphological, biochemical and molecular characteristics (Herssan *et al.,* 2010, Patit *et al.,* 2014, Bambang *et al.,* 2015).

Characterization and Identification of the Isolates

The isolates were sub cultured on nutrient agar (Biotech), incubated in inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions (Cheesbrough, 2010, Iheukwumere *et al*., 2017a; Ugwu *et al*., 2025b), biochemical reactions (Cheesbrough, 2010) and molecular characterization (Iheukwumere *et al*., 2018, Iheukwumere *et al*., 2018b; Ike *et al*., 2025a). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates.

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al*. (2025c), Iheukwumere *et al*. (2025d) Dim *et al*. (2025a).

Gram staining technique: A thin smear was made in a cleaned grease free microscopic slide (75mm×25mm), air dried heat heat-fixed (Iheukwumere *et al*., 2017b; Iheukwumere *et al*., 2025e; Dim *et al*., 2025b). The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with cleaned water. This was followed by decolourizing the slide content with 95%w/v

ethyl alcohol for 10seconds and then rinsed with cleaned water. The smear was then counter stained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al*. (2017c), Ike *et al*. (2025b), Iheukwumere *et al*. (2025f).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35 ± 2^0C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al*. (2017d), Iheukwumere *et al*. (2018c), Iheukwumere *et al*. (2025g).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Ike *et al*. (2025c) Egbe *et al*. (2025a), Dim *et al*. (2025c).

Indole test: The test was carried out as described by Cheesbrough (2010), Ekechukwu *et al*. (2025a), Egbe *et al*. (2025b), and Obianom *et al*. (2024). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37˚C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025h), Ike *et al*. (2025d), and Ekechukwu *et al*. (2025b). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al*. (2025e), Egbe *et al*. (2025c), and Obiefuna *et al*. (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production

Urease test: The test was carried out as described by Cheesbrough (2010),Iheukwumere *et al*. (2025i), Iheukwumere *et al*. (2025j), and Ekechukwu *et al*. (2025c). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. The presence pink/red colouration indicated positive urease test

Methyl red test: The test was carried out as described by Cheesbrough (2010). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2020), Iheukwumere *et al*. (2022b), Ekesiobi *et al*. (2025). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of α-naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2022c), Iheukwumere *et al*. (2024), and Iheukwumere *et al*. (2025k). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2022d), Iheukwumere *et al*. (2025l), and Obiefuna *et al*. (2025b). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c) Iheukwumere *et al*. (2023a), and Iheukwumere *et al*. (2023b). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al*., 2025m; Iheukwumere *et al*., 2022e; Chude *et al*., 2020)

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72°C for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al*., 2022f; Iheukwumere *et al*., 2025n; Ejike *et al*., 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with ≥95% similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al*., 2017; Iheukwumere *et al*., 2022g; Nwike *et al*., 2017).

Detection of crystalline inclusions: The culture smears was prepared, heat fixed and stained with Coomassie Brilliant Blue Stain (0.133% Coomassie Brilliant Blue G250 in 50% acetic acid). Then, the smear was washed softly in running tap and observed microscopically using x100 objective lens (Subbiah and Abidha, 2010).

Production of toxic proteins: a loopful of the test organism was inoculated into a 500 mL Evlemeyer flask containing 150 mL of feather meal medium that composed of 0.5 g/L NH_4Cl, 0.5 g/L NaCl, 0.3g/l K_2 HPO4, 0.4 g/L KH_2 PO_4, 0.1 g/L $MgCl_2$ $6H_2O$, 0.1 g/L yeast extract and 10 % chicken feather. This was maintained at pH 7.5, with manual shaking at (30 $\pm$ 2^0C) for 96-120 h. Samples were withdrawn at intervals to determine the toxins (protein mg/l) during crystal proteins production (Patil *et al.,* 2014).

Toxin (Protein mg/l) determination: Five milliliter (5 mL) of the culture medium was centrifuged at 10,000 rpm for 10 minutes and the resulting pellets were washed twice with normal saline (0.85% NaCl) and twice with distilled water. These pellets were then be suspended in 1ml of NaO (50mg/L, pH= 12.5) in order to solubilize protein crystals. After 2 h of incubation at 37°C, total proteins in the supernatant was measured after re-centrifuged at 10,000 rpm for about 10 minutes using Bradford method (Patil *et al.,* 2014).

Precipitation of the toxins: This was carried out using the method described by Fernando *et al.,* (2010). The supernatant that was generated from toxic protein production was subjected to ammonium tetraoxosulphate (vi)$(NH_4)_2SO_4$) precipitation using 80 % $(NH_4)_2$ SO_4 solution. The precipitate was obtained by filtering the solution using what man N01 filter paper. The crystals obtained on the filter paper were air dried.

Insecticidal Activities of the Toxic Proteins

Preparation of the toxic proteins: The toxins were prepared by dissolving 0.01 g, 0.02 g, 0.03 g, 0.04 g, 0.05 g, 0.06 g, 0.07 g, 0.08 g, 0.09 g and 0.10 g respectively of the toxic proteins in 100 mL of phosphate buffer saline (PBS) to form 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 ppm respectively.

Insecticidal study: A total of fifty (50) *Acanthoscelides obtecus* (Bean weavils) were used for this study. The set-up was carried out using 1000 mL beaker (Pyrex) containing 50 bean weavils, the beakers were covered with perforated lids (1.0 mm hole) and 50 mL of the prepared toxic proteins was carefully sprayed into the beaker (Anonymous, 2023). Mortality rate was determined at 24, 48 and 72 h respectively. The number of death from each set-up was taken and recorded. The LCSO and LC90 were estimated using probit analysis (Fernando *et al.,* 2010).

Statistical Analysis

The data generated were expressed in percentage and Tables.The significance of the study was determined using Analysis of Variance (ANOVA) at 95% confidence level. Post-hoc analysis was carried out using Tukey's HSD (Honestly Significant Difference) test from IBM SSPS version 30.0.

RESULTS

The three isolates Q, R and S showed deviations in their colours on bacteriological medium, slight variations in their position of endospore, and also variations in utilization of xylose, Arabinose and mannitol. They showed similar characteristics in their surface appearances, Gram reaction, shape, spore formation, shape of spore, reaction to citrate, catalase, indole, casein, gelatin, urease, nitrate, methyl red, Voges Proskauer, oxidase, hydrogen sulphide production and utilization of glucose and trehalose (Table 1).

The absorbance ratio of the nucleic acids extracted from the isolates at 260nm and 280nm lied between 1.80 - 1.90, pointing that the nucleic acids were all DNA (Table 2). The molecular characteristics showed the presence of *Paenibaeillus mucilergmous* strain KN-18 (PM18), *paenibaeillus* species strain S-38 (PS38) and *Paenibaeillus konkukensis* strain SK 3146 (PK3146) (Table 3)

There was significant ($P<0.05$) increased in toxic proteins secreted by the isolates in every 24h interval, and PK3146 secreted the highest toxic proteins in every 24h interval (Table 4). The insecticidal activity of the toxic proteins secreted by the isolates against *Acanthoscelides obtecus* (Bean weavils) is shown in table 5. There was increase in number of deaths of the insects in every 24h interval, and these increases became significant ($P<0.05$) after 72h exposure to toxic proteins. Also, the number of deaths of *A. obtectus* increased with increase in concentration of the toxic proteins, of which the optimum concentration was detected at 600 ppm. The data generated from LCSO calculation also pointed out that the toxic proteins secreted from PK3146 exhibited the highest activity, followed by that excreted by PS38 and then PM18 (Table 6).

Table 1: characteristics of the bacterial isolates

Characteristics	Q	R	S
colour	Cream	Cream white	Milky
surface	rough	rough	Slight rough
Cream reaction	positive	positive	Positive
shape	rods	rods	Rods
Spore formation	Yes	Yes	Yes
Shape of spore	Oval	Oval	Oval
Positon of spore	Central	Terminal	Central
Catalase	-	-	-
Citrate	-	-	-
Indole	-	-	-
Casein	-	-	-
Gelatin	-	-	-
Glucose	+	+	+
Tetrahalose	+	+	+
Xylose	-	+/-	-
Arabinose	-	-	-
Mannitol	-	+/-	-

Urease, H_2S, nitrate reduction, MR, VP, Oxidase (negative)

Table 2: Nature of the nucleic acids extracted from the isolates

Isolates	Conc (µg/ml)	280nm	260nm	260/280
Q	146.34	1.7720	3.2610	1.84
R	109.22	1.6140	2.9370	1.82
S	127.14	1.7130	3.1180	1.82

Table 3: Molecular Characteristics

Parameters	Q	R	S
Max score	1810	1552	1552
Total score	1810	1552	1552
Query cover (%)	100	100	100
E-value	0.0	0.0	0.0
Identity (%)	100	100	100
Accession Number	CP035456.1	CP187970.1	CP027059.1
Description	*Paenibacillus mucilaginous* strain KN-18 chromosome genome (PM18)	*Paenibacillus* species strain S-38 chromosome complete genome (PS38)	*Paenibacillus konkukensis* strain SK3146 chromosome (PK3146)

Table 4: Toxic protein content from the broth culture

Time (h)	PM18 (mg/L)	PS38 (mg/L)	PK3146 (mg/L)
24	8.82 ± 0.14	9.02 ± 0.07	11.28 ± 0.11
48	13.67 ± 0.21	13.88 ± 0.11	16.34 ± 0.17
72	16.07 ± 0.04	16.27 ± 0.22	18.56 ± 0.12
96	16.17 ± 0.14	16.29 ± 0.17	19.72 ± 0.07

Table 5: Insecticidal activities of the toxic proteins from the isolates

Conc (ppm)	Number of Death Recorded											
	PM18				PS38				PK3146			
	24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h
100	13	22	26	29	16	27	31	42	23	33	41	48
200	19	27	31	33	21	31	34	44	27	37	47	50
300	22	29	34	35	25	35	37	47	29	41	48	50
400	24	33	37	39	29	39	43	50	34	47	50	50
500	27	38	42	50	31	41	47	50	40	50	50	50
600	27	38	43	50	32	41	48	50	40	50	50	50
700	28	39	43	50	32	41	48	50	40	50	50	50
800	28	39	44	50	32	41	48	50	40	50	50	50

N= 50

Table 6: Lethal doses (LC50)

Time (h)	PM18 (ppm)	PS38 (ppm)	PK3146 (ppm)
24	450	300	140
48	190	70	50
72	80	60	30
96	50	40	10

DISCUSSION

The bacterial isolates Q, R, and S exhibited similar characteristics in terms of their surface appearances, Gram reaction, shape, spore formation, and biochemical reactions. However, they showed deviations in their colors on bacteriological medium, position of endospore, and utilization of certain sugars. These findings are consistent with previous studies that have reported variations in the characteristics of Paenibacillus species (Grady *et al.,* 2016; Nwakoby *et al*., 2025i; Idigo *et al*., 2025d; Nwakoby *et al*., 2025j).

The molecular characteristics of the isolates revealed the presence of *Paenibacillus mucilergmous* strain KN-18 (PM18), *Paenibacillus* species strain S-38 (PS38), and *Paenibacillus konkukensis* strain SK 3146 (PK3146). These findings are in line with previous studies that have reported the use of molecular techniques for the identification of *Paenibacillus* species (Senthilkumar *et al*., 2017; Nwakoby *et al*., 2025l; Nwakoby *et al*., 2025m).

The study showed that the isolates produced toxic proteins that were effective against *Acanthoscelides obtectus* (Bean weavils). The toxic proteins secreted by the isolates caused significant mortality in the insects, with PK3146 exhibiting the highest activity. These findings are consistent with previous studies that have reported the insecticidal activity of *Paenibacillus* species against various insect pests (Patel *et al*., 2018; Idigo *et al*., 2025e).

The insecticidal activity of the toxic proteins increased with increase in concentration and exposure time. The optimum concentration of the toxic proteins was detected at 600 ppm, which is consistent with previous studies that have reported the dose-dependent activity of insecticidal proteins (Singh *et al*., 2016; Nwakoby *et al*., 2025n; Nwakoby *et al*., 2025o).

The study concluded that the *Paenibacillus* species isolated in this study, particularly PK3146, PS38, and PM18, exhibited high potential for the biological control of *Acanthoscelides obtectus*. These findings are in line with previous studies that have reported the potential of *Paenibacillus* species as biological control agents (Lacey e*t al.,* 2015; Nwakoby *et al*., 2025p).

CONCLUSION

This study demonstrated the potential of entomopathogenic bacteria, particularly *Paenibacillus mucilergmous* strain KN-18, *Paenibacillus* species strain S-38, and *Paenibacillus konkukensis* strain SK 3146, as biological control agents against *Acanthoscelides obtectus.* The toxic proteins secreted by these bacteria exhibited significant insecticidal activity, highlighting their potential as sustainable and eco-friendly alternatives to synthetic chemical insecticides for pest management.

Acknowledgments

We are grateful to all our study participants who join the study voluntarily. We are grateful to ZAHARM Analytical and Research Laboratory, Amawbia, Awka Anambra State, Nigeria for providing enabling environment, resources and techniques for this study. We really salute their wonderful efforts.

Conflict of interests: The authors declare that they have no conflict of interests.

Funding: This research did not receive specific grant from any funding agencies.

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