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DOI: https://doi.org/10.64388/IREV9I7-1713503

Studies on the Distribution of Bacillus Thuringiensis Isolated from Arable Soil in Keffi and Its Toxicity Against Mosquito Larvae

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Abstract- Plant pests and diseases affect 20%–40% of food production globally. Inadequate use of chemical pesticides to control pests has increased selection pressure, resulting in insect resistance and affects soil fertility. To resolve these issues, a new form of pest control is desperately needed. Most of the micro-organisms are capable of surviving in almost all sorts of environments. In the present scenario, development of diseases, resistanct varieties, and chemical pesticide resistance are a few major hurdles for insect pest management. Over the past two decades, biopesticides provide 1% of total plant protection worldwide; about 175 biopesticide compounds and 700 biopesticide products have already existed in the market globally. Entomotoxic microorganisms are becoming very effective as biocontrol agents of different pest and used as alternative to chemical pesticides. This study is aimed at distribution of Bacillus thuringiensis isolated from arable soil in Keffi and its toxicity against Mosquito larvae. Soil samples were collected using a clean spoon and inserted into sterile polythene bags from 30 different arable lands in Keffi. Isolation and identification of the bacteria was carried out using standard microbiological techniques. Light microscopy showed the presence of parasporal bodies produced by the isolates. Molecular identification was carried out using 16sRNA polymerase chain reaction method. Mosquito larvae were collected by allowing water containers filled with water to remain in an open space thereby facilitating laying of eggs by mosquitoes. The mean Standard deviation of the bacteria ranges from 1.2 × 10 2 cfu/g to 11.2 ×103 cfu/g. The overall occurrence of Bacillus thuringiensis in this study was 40.0%. The highest isolates were isolated from arable soil collected from Nasarawa road while the lowest with occurrence of 17.0% was isolated from arable soil from Nasarawa State University (main campus). Arable soil where maize was grown had the highest number of isolates.The highest mean Standard deviation of the bacterial load was from Bacillus thuringiensis isolated from Nasarawa road and the lowest mean standard deviation was from Nasarawa state university (Main campus). Crystal proteins detected from Bacillus thuringiensis isolates were Cry 1, 2, 3, 4, 7, 9 and 11. Cry 1, Cry 2 and Cry 11 were the most abundantly detected crystal protein genes among the isolates. The detection of these cry genes was carried out using a PCR technique. All the 12 Bacillus thuringiensis isolates in the study were toxic to the larvae of mosquitoes and therefore could be adopted as a form of biopesticide and consequently used in controlling mosquito breeding and other pests of economic importance.

I. INTRODUCTION

Insect pests have adverse and damaging impacts on agricultural production and market access. Up to 28% of the world food production is damaged by insects, either in the field or during storage (Pimentel, 2005). Current pest control strategies rely greatly on chemical insecticides, which lead to numerous harmful effects such as pesticide residues, development of insect resistance, and destruction of natural balance with beneficial insects. Such undesirable side effects prompted scientists to search for alternative, environmentally friendly control agents (Abo-bakr *et al*., 2020). However, there are alternatives such as biopesticides based on *Bacillus thuringiensis* (Bt) type microorganisms, with endotoxin protein crystals which are lethal to many pests of Lepidoptera, Coleoptera and Diptera. Bt-based biopesticides occupy 97% of the world's biopesticide market (Melo *et al*., 2016; Osman et al., 2015; Rodríguez *et al*., 2019; Sayed and Behle, 2017). *Bacillus thuringiensis* produces protein crystals made up of delta endotoxin, which bind to the receptors of the larvae's epithelial cells (Elleuch *et al*., 2016).

The entomopathogenic bacterium, *Bacillus thuringiensis* (Bt) is a rod-shaped, positive-gram, and spore-forming bacterium well-known for its insecticidal properties associated with its ability to produce crystal inclusions during sporulation. These inclusions are proteins encoded by Cry genes and have shown to be toxic to a variety of insects and other

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DOI: https://doi.org/10.64388/IREV9I7-1713503

groups such as nematodes and protozoa (Sauka *et al*., 2010).

Bacillus-like species are gram-positive bacteria that are ubiquitous in soils. Many of Bacillus-like bacteria are demonstrated as beneficial microbes widely used in industry and agriculture. However, the knowledge related to their diversity and distribution patterns in soils is still rudimentary (Liu *et al*., 2019).

II. MATERIALS AND METHODS

Study Area:
The study was carried out in Keffi, Nigeria. Keffi is approximately 68 Km from Abuja, the Federal Capital Territory and 128 Km from Lafia, Capital of Nasarawa State. The area lies in latitude 85'N of the equator and longitude seven 8'E, it is situated on altitude of 850 M up sea level (Akwa *et al*., 2007). The mean yearly rainfall is ± 2,000 millimeters and is always heavier during the rainy months with its highest downpour around July through September (Yohanna *et al*., 2019).

Sample Collection:
Soil samples was collected by scraping off surface material with a sterile spatula and then obtaining a 10g sample 2 to 5 cm below the surface. Soil samples were taken from 30 different locations of arable farmlands in Keffi. All samples were placed in sterile plastic bags aseptically and stored at 4°C until processed.

Isolation of *Bacillus thuringiensis*

The acetate selection method modified by Ammouneh *et al*. (2010) was used. 1 gram of each soil samples will be suspended into a sterilized conical flask containing 10 ml nutrient broth medium and 0.25M sodium acetate, the mixtures will be shaken at 180-200 rpm for 4 h at 30 °C. Heat treatment will then be applied for 3 min at 80 °C to kill vegetative cells. The samples will then be plated on nutrient agar plates and allowed to grow by incubation at 30°C for 72 hours.

Gram staining and Biochemical characterization of isolates

Isolates were subjected to Gram staining and biochemical test including indole, Voges-proskauer, methylred, citrate, catalase, urease and starch hydrolysis as described by Chuku *et al*., (2016).

Determination of Densisty

Density of the Bacillus thuringiensis isolates were determined using the method described by Lubov *et al*., (2015).

Toxicity Test against Mosquito larvae

Toxicity test was carried out according to the method described by Thomas et al., (2014). Water containers were left to stand in an open space at ambient temperature of 30ºC for 7 days to facilitate laying of eggs by the mosquito. The containers were monitored daily to observe the emergence of larvae.

III. RESULTS AND DISCUSSION

Biochemical Characteristics

Twelve of gram-positive and spore forming bacilli were isolated from soil. Morphological, physiological and biochemical characteristics of isolates are shown in table 2 below. All isolates showed a positive reaction to Catalase, VP and starch hydrolysis. Biochemical data revealed a negative test to indole and oxidase tests. The isolates of Bt.8 and Bt.4 were negative to Citrate and endospore staining respectively.

Table 1: Morphological and Biochemical Characterization of Isolates

Isolates	Bt1	Bt2	Bt3	Bt4	Bt5	Bt6	Bt7	Bt8	Bt9	Bt10	Bt11	Bt12
Gram Reaction	+	+	+	+	+	+	+	+	+	+	+	
Morphology	R	R	R	R	R	R	R	R	R	R	R	R
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	_	+	+	+	+

Endospore staining	+	+	+	_	+	+	+	+	+	+	+	+
Methyl-red	_	_	_	_	_	_	_	_	_	_	_	_
Indole	_	_	_	_	_	_	_	_	_	_	_	_
Oxidase	_	_	_	_	_	_	_	_	_	_	_	_
Voges-proskauer	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+

Source: Laboratory work, 2024

Keys:

+: positive _ : negative R: Rods

Table 2: Frequency of Isolation of *Bacillus thuringiensis* Isolates

Sample site	No. of samples	Mean colony count (cfu/g)	No. of isolates	Percentages (%)
Angwan lambu	6	6.4×10^3	2	33
Nasarawa road	6	11.2×10^3	5	83
Kaduna road	6	8.1×10^2	3	50
NSUK campus	6	1.2×10^2	1	17
BCG	6	2.2×10^3	1	17
Total	30		12	40

Source: Field and Laboratory work, 2024

Table 4.5: Toxicity of Bt isolates against Mosquito larvae

	After 4 hours					After 24 Hours				
Dilution Factor (ml)	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
	No. of live larvae					No. of live larvae				
Bt1	2	1	3	5	5	0	0	0	0	0
Bt2	2	1	3	4	5	0	0	1	0	0

Bt3	2	2	3	5	5	0	0	0	0	0
Bt4	3	2	2	5	5	0	0	0	1	0
Bt5	2	2	2	5	5	0	0	0	0	0
Bt6	1	3	3	5	5	0	0	1	0	0
Bt7	2	2	3	5	5	0	0	0	0	0
Bt8	3	2	3	5	5	0	0	0	0	0
Bt9	2	2	2	5	4	0	0	0	0	0
Bt10	3	3	3	5	5	0	0	0	0	0
Bt11	1	2	3	5	5	0	0	0	0	0
Bt12	2	2	3	5	5	0	0	0	0	0

Source: Laboratory work, 2024

IV. DISCUSSION

Current pest control strategies rely greatly on chemical insecticides, which lead to numerous harmful effects such as pesticide residues, development of insect resistance, and destruction of natural balance with beneficial insects. Such undesirable side effects prompted scientists to search for alternative, environmentally friendly control agents (Abo-bakr *et al*., 2020). Accordingly, the present work was proposed to isolate and characterize *Bt* isolates from arable soil and to test their effect against mosquito larvae.

Bt can be isolated from soil, leaves, dead larvae or water (Liang *et al.,* 2011 and Valicente *et al.,* 2010). The *Bt* strain produces crystal proteins that have been successfully used for controlling the mosquito population (Liang *et al.,* 2011).

It is well known that the characteristic shape of the mosquitocidal crystals is the spherical-shaped crystals. *Bt* produces this type of crystals (Charles and de Barjac, 1982), to which *Culex* larvae are more susceptible (Boisvert, 2005). In the present study, the highest mortality percentage recorded was 100%, obtained by the isolate Bt22.19, as compared to other strains in the untreated control.

The result of the toxicity test of the isolates on mosquito larvae which shows that the isolates had biopesticidal activity against the larvae is in agreement with the work of Adebayo and Uthman (2021) in their work carried out in Ilorin, Kwara state. It is also in further agreement with the findings of Aksoy *et al* (2015) in their wok carried out to test the toxicity of *Bacillus thuringiensis* isolated from soil on Culex larvae.

The detection of various crystal genes which serve as a virulent factor in the isolates shows the diversity of these crystals proteins among the bacteria. The occurrence of Cry 1, Cry 2 and Cry 11 genes in all 12 of the isolates is in agreement with the work of Adebayo and Uthman (2021).

Besides the Cry toxins, *Bacillus thuringiensis* contains other virulence factors such as alpha and beta exotoxins, hemolysins, enterotoxins, chitinases, and phospholipases (Rajendran *et al*., 2018). However, the concentration and activities of these substances vary, and the precise contribution of each is not discovered yet, hindering the determination of the real toxic spectrum of an isolate that produces more than one type of toxin (Praca *et al*., 2004).

V. CONCLUSION

The results obtained in this shows the efficiency of the *Bacillus thuringiensis* in controlling mosquito larvae. The use of *Bacillus thuringiensis* as a biocontrol agent

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DOI: https://doi.org/10.64388/IREV9I7-1713503

against mosquito larva is preferred as it is environmentally friendly unlike the regular pesticides used in killing mosquitoes in most communities. It is still necessary to search for more microbial toxins to control insects' orders which have the ability to develop resistance against selected insecticides. Screening of soil samples from different sources and habitats may be useful to obtain *Bacillus thuringiensis* strain with broader host ranges and novel crystal proteins.

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