Nig. J. Biotech. Vol. 37(1): 129-137 (June 2020)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v37i1.14

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Potentials of Indigenous *Bacillus thuringiensis* Isolates from the soil in controlling *Fusarium* wilt of Cucumber *cause by Fusarium oxysporum* f.sp *cucumerinum*

***[1]Akintokun, P. O., [2]Okuwa, A. O., [2]Oloyede, A. R., [2]Adebajo, S.O. and [2]Akintokun A. K.**

[1]Department of Plant Physiology and Crop Production, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.
[2]Microbiology Department, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Abstract

Cucumber (*Cucumis sativus L.*) production is generally low in Nigeria due to continuous soil nutrient limitation and diseases. However, the persistence in the use of agrochemicals for cucumber production in Nigeria is associated with high cost and deleterious effects on man, animal and the environment. This study was conducted to investigate the potentials of indigenous *Bacillus thuringiensis* (Bt), a spore-forming bacterium known for its insecticidal properties in controlling Fusarium wilt of cucumber. *Bacillus thuringiensis* strains were isolated from soil samples collected from different farm sites in Abeokuta, Nigeria, and identified phenotypically and molecularly. The *in-vitro* antagonistic activity of *B. thuringiensis* strains on *F. oxysporum* f.sp. *cucumerinum* was evaluated by dual culture method, followed by pot experiment in the screen house. 16S rRNA gene sequencing was performed on the antagonistic *B. thuringiensis* to confirm Bt species. The results of the *in-vitro* antagonistic activity revealed that most indigenous *B. thuringiensis* strains showed significant growth inhibition of *Fusarium oxysporium* f. sp*. cucumerinum*. Similarly, application of *B. thuringiensis* A and C isolates significantly suppressed the incidence of Fusarium wilt of cucumber in the screen house when compared to the control. The 16S rRNA gene sequencing technique identified the isolates A and C as *Bacillus thuringiensis* strain LTS-209 and *Bacillus thuringiensis* strain VITSJ-01, respectively. Hence, indigenous *B. thuringiensis* A and C isolates should be incorporated into cucumber cultivation for controlling Fusarium wilt disease of cucumber.

Keywords: Cucumber, *Bacillus thuringiensis,* Fusarium wilt, 16S rRNA gene
***Corresponding author:** akinpius97@yahoo.com Tel: +2348037215443

Introduction

Modern agriculture heavily depends on the application of agrochemicals for fertilizing the soil and for disease control. However, the over-use of these chemical compounds poses potential risks to human health and the environment, and can also lead to the chemical

resistance in the disease-causing agents (Akintokun and Taiwo, 2016). These effects had led to a total ban or restricted use of most chemical pesticides. Therefore, the need for biocontrol agents as an alternative to reduce synthetic chemicals usage is now being encouraged (Wen-Kun *et al*., 2014).

Fusarium oxysporum f. sp. cucumerinum is an economically important wilting pathogen of cucumber which can cause significant yield loss (Gamal, 2010). Biological control of Fusarium wilt by beneficial microorganisms are complex but most studies conducted previously have focused on using non-pathogenic fusarium spp. or other antagonists and their antagonistic activities could be through mechanisms such as competition for nutrients, competition for infection sites on roots and production of antibiotics (Gamal, 2010).

The ability of members of the Gram-positive genus *Bacillus* to form spores is advantageous for controlling a variety of soil-borne phytopathogenic fungi (Koumoutsi *et al*., 2007; Arguelles-Arias *et al.,* 2009; Chowdhury, 2013) and some are commercially marketed as biopesticides, biofertilizers and soil amendments because of their easy colonization, good competition and broad antimicrobial spectrum (Cazorla *et al*., 2007; Baysala *et al*., 2008; Chung *et al*., 2008; Choudhary and Johri, 2009). *Bacillus thuringiensis* (BT) is an ubiquitous, Gram-positive, spore-forming bacterium that produces parasporal crystals during the stationary phase of its growth cycle. The crystals comprise one or more crystal proteins (encoded by *cry* or *cyt* genes) that show specific toxicity against several orders of insects, including Lepidoptera, Diptera, Coleoptera, Hymenoptera, Homoptera, Orthoptera, and Mallophaga, as well as nematodes, mites, and protozoa (Weixing *et al*., 2012). *Bacillus thuringiensis* was initially characterized as an insect pathogen and its insecticidal activity was ascribed largely or completely to the parasporal protein crystals. Apart from crystal proteins, *Bt* strains are able to produce exoenzymes, such as proteases and *α*-amylases (Smitha, 2010) as well as *β*-exotoxins which are able to inhibit the growth of phytopathogenic fungi (Guo *et al*., 2008).

However, several studies have been conducted on the insecticidal activity of *Bacillus thuringiensis*, less attention has been paid to exploiting its antifungal activity for biological control of *Fusarium oxysporum f. sp. cucumerinum.* The present study was therefore undertaken to evaluate the ability of indigenous *Bacillus thuringiensis* isolated from cultivated and uncultivated soils in suppressing *Fusarium* wilt disease of cucumber.

Materials and Methods

Collection of samples

Soil samples were collected aseptically from three farm sites (Isolu, Federal University of Agriculture Abeokuta (FUNAAB) and Osiele) in Abeokuta, Nigeria where there was no previous record of application of *Bacillus thuringiensis*- based pesticides. The soil samples were taken 2 cm to 5 cm below the surface, after scraping the surface with sterile spatula. Soil samples were then transported immediately to the laboratory on ice box and stored in sterile plastic bags at 4°C until their processing.

The phytopathogenic *Fusarium oxysporum f.sp cucumerinum* was collected from the Department of Crop Protection, Federal University of Agriculture, Abeokuta, Abeokuta and confirmed at the laboratory of Department of Microbiology.

Isolation of indigenous Bacillus thuringiensis

Bacillus thuringiensis were isolated from the soil samples using the selective sodium acetate heat treatment method as described by Rathinam *et al*. (2007). Briefly, 10.0 g of each soil sample was suspended in 90 ml sterile distilled water and vortexed for 1 minute. The suspension was heated in a water bath at 80ºC for 30 minutes. One milliliter (1.0 ml) of each suspension was added to 10 ml of nutrient broth buffered with 0.25 M Sodium acetate, pH 6.8. The inoculated broth was incubated at 30ºC for 4 hours, followed by heating at 80ºC for 3 min. Suspensions were serially diluted to 10^{-6} and 1.0ml of 10^{-6} dilution was inoculated on nutrient agar plates using pour plate method. The sample dilution and agar medium were thoroughly mixed and allowed to solidify. The plates were incubated at 30ºC for 24 hrs. The number of colonies on the surfaces of

the agar plates were counted, recorded and expressed as Colony forming units per gram (CFU/g) of soil.
Pure bacterial isolates were obtained by sub-culturing on nutrient agar plates, incubated at 30°C. All pure cultures of bacterial isolates were maintained on nutrient agar slants and stored at 4°C for further studies.

Phenotypic characterization of Bacillus thuringiensis isolates
The *Bt*-like isolates were characterized by observing their colony morphologies (shape, size, elevation, surface, margin, pigmentation), endospore staining, parasporal body staining, Gram staining, motility and subjecting them to a series of biochemical tests. The identification of the isolates was then done using the Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

In vitro antagonistic assay of indigenous Bacillus thuringiensis isolates against Fusarium oxysporum f.sp cucumerinum
The indigenous *Bt* isolates were screened against *Fusarium oxysporum* f. sp *cucumerinum* (causing Fusarium wilt of cucumber) using the dual culture method as described by Sharma *et al.* (2012). Fresh mycelium of *F. oxysporum* f. sp. *cucumerinum* was inoculated at the centre of each potato dextrose agar (PDA) plate. On the opposite of the fungus, about 3.00cm from the fungus, a loop full of each *Bt* isolate was streaked. The *Bt* isolate was replaced with sterile distilled water in control treatments. The plates were then incubated at room temperature (25 ± 2 °C) for 3 days. The barrier between the fungus and the *Bacillus thuringiensis* strains indicated the antagonistic interaction between them.
The percentage inhibition of growth over control was calculated using the formula:
Growth inhibition = $1 - (a / b) \times 100\%$

a = radial growth of fungus interacting with antagonistic bacteria; b = radial growth of the fungus only in the control plate.

In vivo antagonistic assay of indigenous *Bacillus thuringiensis* isolates against *Fusarium oxysporum f.sp cucumerinum*

A beneficial bacterial-phytopathogen fungi interaction study was conducted using the method described by Baniasadi *et al.* (2009). The experiment was conducted in the screen house at Federal University of Agriculture, Abeokuta.

Seeds of cucumber (susceptible to infection by *Fusarium oxysporum* f.sp *cucumerinum*) were surface-sterilized with 5% Sodium hypochlorite solution for 2 minutes, washed three times in sterile distilled water and then air- dried at room temperature (25±2°C). Four milliliter (4.0 ml) of *Fusarium oxysporum* f. sp *cucumerinum* inoculum was mixed with 50.0 ml sterile distilled water and directly inoculated on the sterile soil, after which planting bags were immediately covered with black polyethylene bags for 48 hours. Five *Bt*-treated cucumber seeds and control seeds were planted per planting bag, and immediately covered with soil. All plants were kept in the experimental screen house under normal light and temperature conditions, and watered with sterile water at regular intervals. The seedlings were observed after 8 weeks to determine the disease incidence (DI). The percentage of cucumber seedlings in a bag that showed visible signs of infection was also calculated (Michel *et al.*, 1997). Disease severity was also recorded by visual observation of the disease symptoms with reference to the untreated infected controls. Disease index data were obtained and recorded using the scale of 0 to 4 (Soonthompoct *et al.,* 2001). Symptom severity was graded into five disease classes as follows: 0 (No disease or wilt i.e. apparently healthy seedlings), 1 (one to two leaves infected), 2 (three to five leaves infected or traces of stem rot), 3 (all leaves infected/stunted growth/stem rot) and 4 (damping off/ wilting/ seedling death). The disease reduction percentage (DRP) was also calculated as:
DRP = 100 x [1 - (DI of treatment / DI of controls)].

Molecular identification of the Bacillus thuringiensis isolates

Molecular characterization was performed on two isolates of *B. thuringiensis* that displayed outstanding antagonistic activity against *Fusarium oxysporum* f. sp *cucumerinum* using 16S rRNA gene sequencing method. The bacterial genomic DNA was extracted from the *Bt* isolates using PureLink Genomic DNA extraction kit (Invitrogen Life Technologies, USA), followed by amplification of 16S rRNA gene in 25.0 µl reaction mixture using 27F (5'-AGAGTTTGATCMTGGCTCAG – 3') and 1492R (5' – ACCTTGTTACGACTT – 3') primers (Blackwood *et al*., 2005). The amplified fragments were resolved by electrophoresis on a 1% agarose gel. The PCR amplicons were purified and directly sequenced by using the ABI 3730 Genetic Analyzer at STAB VIDA Technologies, Portugal. Sequence assembly and alignment were carried out using CLC 6.8.4 bio software. To identify the isolates, the gene sequences were compared with the GenBank sequences at the National Centre for Biotechnology Information (NCBI) database using BLASTn search tool.

Statistical analysis of data

Data were analyzed using the statistical package for social sciences (SPSS) version 16.0 for Windows (SPSS, Chicago IL, U.S.A). Means were separated using Tu key-Kramer HSD test at α = 0.05.

Results and Discussion

Isolation of indigenous Bacillus thuringiensis from soil samples

Table 1 shows total *Bacillus* counts of soil samples from three farm sites in Abeokuta. The results showed that there were significant differences in total *Bacillus* counts of cultivated and uncultivated soil samples from Isolu and FUNAAB farm sites, but there were no significant differences in the counts between Osiele and Isolu soil samples. Cultivated and uncultivated soil samples from FUNAAB farm sites recorded the highest and lowest counts respectively (Table 1). In general, soil samples from uncultivated farm sites had lower *Bacillus* counts than soil samples from the cultivated farm sites. These results are in agreement with Meihiar *et al*. (2014) who reported high counts of *Bacillus thuringiensis* from cultivated areas compared to uncultivated natural areas and interior arid areas. Also, Ralte *et al*. (2016) reported high counts of *Bacillus* species from agricultural land compared to non-agricultural land. This may be as a result of high organic and moisture contents as reported by Obeidat *et al*. (2004) and Meihiar *et al*. (2014) that soil with high organic matter and moisture content are very rich in *Bt* due to high levels of nutrients and insect activity.

Table 1: Total counts of the Bacillus spp. isolated from the different farm soil samples

Farm sites	**Total *Bacillus* counts ($\times 10^6$ cfu/g± S.E)**	
	Cultivated soil	**Uncultivated soil**
Osiele	11.60 ± 1.20^{b}	8.00 ± 0.57^{ab}
Isolu	11.00 ± 1.52^{b}	10.00 ± 1.520^{a}
FUNAAB	13.66 ± 0.88^{a}	6.33 ± 0.88^{b}

Note: Results are presented as mean values ± standard error of the mean from three replicates. Means with different letters along the same column are significantly different at α = 0.05.

From the study, a total of eight isolates were putatively identified as *Bacillus thuringiensis* based on endospore staining, parasporal body staining, morphological and biochemical

characterization. The morphological and biochemical characteristics of the isolates are shown in Table 2. All the *Bt* isolates were able to ferment glucose, lactose, mannitol and sucrose sugars, but were unable to ferment maltose sugar.

Table 2: Morphological and biochemical characteristics of the *Bacillus thuringiensis* isolated from the different farm soil samples

Characteristics	***Bacillus thuringiensis* isolates**							
	***Bt* A**	***Bt* B**	***Bt* C**	***Bt* D**	***Bt* E**	***Bt* F**	***Bt* G**	***Bt* H**
Gram reaction	+	+	+	+	+	+	+	+
Morphology	rods	rods	rods	Rods	rods	Rods	rods	rods
Endospore staining	+	+	+	+	+	+	+	+
Parasporal body staining	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+
Maltose	–	–	–	–	–	–	–	–
Indole	–	–	–	–	–	–	–	–
Methyl red	–	–	–	–	–	–	–	–
Voges Proskauer	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+
Hydrogen sulphide	–	–	–	–	–	–	–	+
Sucrose	+	+	+	+	+	+	+	+
Urea	–	–	–	–	–	–	–	–
Oxidase	–	–	–	–	–	–	–	–
Catalase	+	+	+	+	+	+	+	+

Note: (−): negative reaction (+): positive reaction

In vitro antagonistic assay of Bt isolates against Fusarium oxysporum f.sp cucumerinum

All the eight *Bacillus thuringiensis* isolates were able to inhibit the growth of *Fusarium oxysporum f.sp cucumerinum in-vitro.* They all displayed varying fungicidal activities against the phytopathogen. *Bacillus thuringiensis* C had the highest percentage of inhibition (92%) while *Bacillus thuringiensis* G had the least percentage inhibition (0%) (Figure 1). This is in concordance with Batista-Junior *et al.* (2002) who reported that *Bacillus thuringiensis kurstaki* HD1 strains inhibited the growth of *Fusarium solani, Fusarium oxysporum* and *Colletotrichum sp* phytopathogens. The inhibiting effect of *Bacillus thuringiensis* strains on phytopathogenic fungi can be associated with enzyme production that can act against the fungal cell wall (Figueiredo *et al.*, 2010).

Percentage disease incidence and reduction of Phytopathogenic Fusarium oxysporum f.sp cucumerinum growth by Bacillus thuringiensis isolates under screen house.

The control seedlings exposed to *Fusarium oxysporum f.sp cucumerinum* but not inoculated with any *Bacillus thuringiensis* strains expressed disease symptoms like leaf wilting, leaf curl, stem rot, leaf blight, root rot and were not able to survive till the fourth week of planting. In contrast, seedlings inoculated with *Bacillus*

thuringiensis strains showed outstanding antifungal effects. The percentage disease incidence for the fungus was highest in the control and *Bacillus thuringiensis* isolate G (100%) while the least (6%) was recorded in *Bacillus thuringiensis* C. The percentage disease reduction was highest in *Bacillus thuringiensis* isolate C (95%) and lowest was recorded in control and *Bacillus thuringiensis* isolate G (0%) as shown in Figure 2. This is similar with the report of Ahmed *et al*. (2006) who reported that under screen house conditions, *Bacillus* species isolates were effective in reducing disease incidence and disease severity of cucumber wilt caused by *Fusarium oxysporum f.sp cucumerinum* and increased the percentage of healthy plants compared to the control.

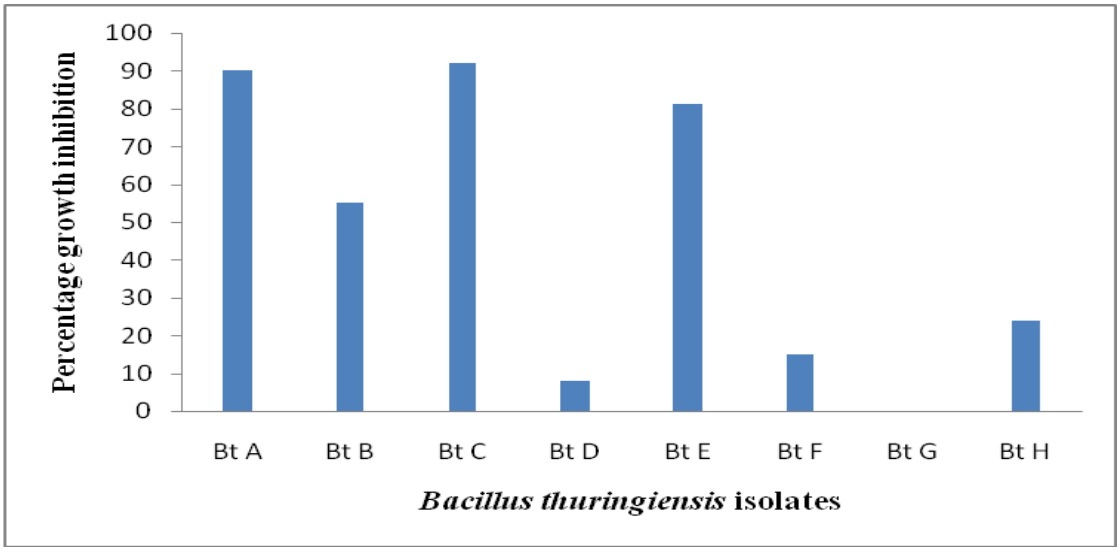

Fig. 1: *In vitro* Antagonistic activity of *Bacillus thuringiensis* against *Fusarium oxysporum* f.sp *cucumerinum*

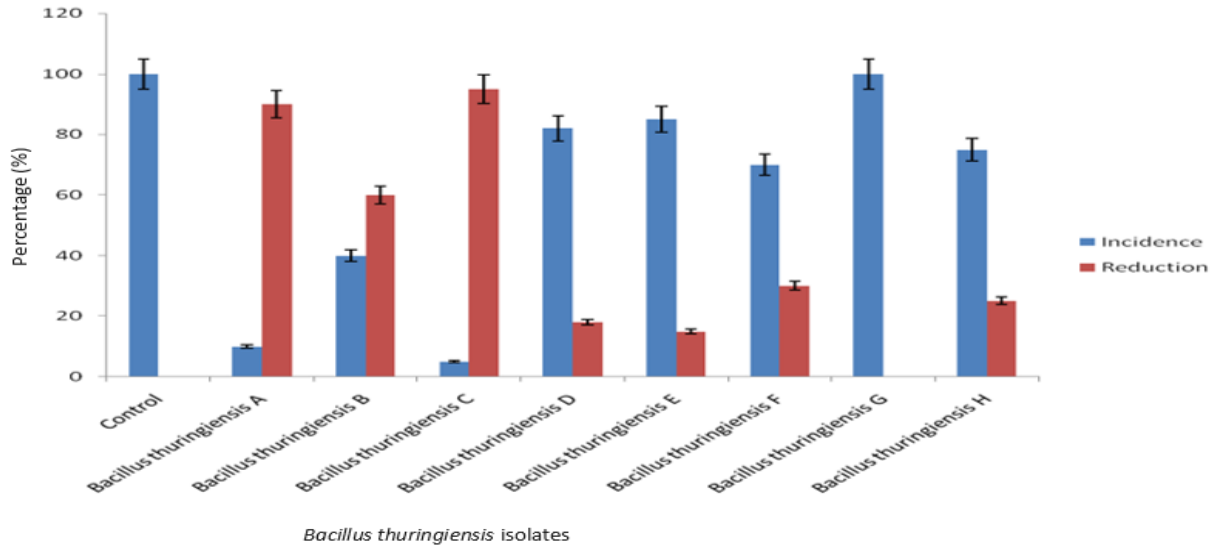

Fig. 2: Percentage disease incidence and growth reduction of phytopathogenic *Fusarium oxysporum f. sp cucumerinum* by *Bacillus thuringiensis* isolates under green house.

Molecular identification of the Bt isolates

The gel electrophoresis of the PCR products indicated that the 16S rRNA gene of the two strains were located at 1,000 base pairs as shown in Plate 1. Sequencing of the PCR products followed by BLASTn searches at GenBank of the NCBI library revealed that *Bt* isolate A and *Bt* isolate C showed 97% and 99% similarity to *Bacillus thuringiensis* strain LTS-209 and *Bacillus thuringiensis* strain VITSJ-01 respectively. The results of the molecular characterization were consistent with the morphological and biochemical characteristics of the antagonistic *Bt* isolates. The results obtained in this study is in agreement with Upasana and Ronald (2015) who reported that 16S rRNA genes of some *Bacillus thuringiensis* strains isolated from finished spiced food were located at 1,000 base pairs.

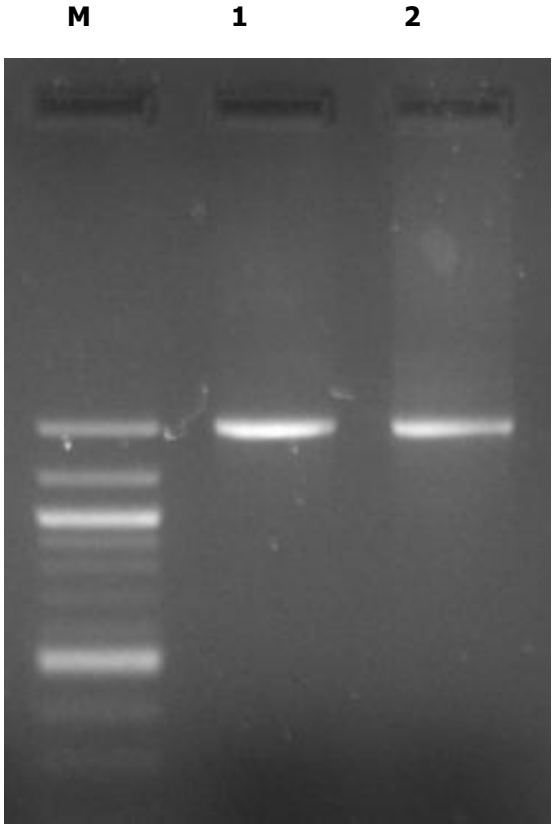

Plate 1: Agarose gel electrophoresis showing amplified 16S rRNA genes of the *Bacillus thuringiensis* isolates.

M: Molecular ladder – 100bp

Bt isolate A: *Bacillus thuringiensis* strain LTS-209

Bt isolate C: *Bacillus thuringiensis* strain VITSJ-01

Conclusion and Recommendation

This study confirmed that *Bacillus thuringiensis* strains LTS-209 and VITSJ-01 are highly effective against the *Fusarium oxysporum* f. sp *cucumerinum*. The implication of this is that the potent danger that may occur through application of pesticides to the environment is averted, hence, the stability of ecosystem is guaranteed in

the use of the above strains. However, continuous study with these strains is now needed on the field trials, under different local environmental conditions in order to enhance the sustainability of the crops in Nigeria by making use of the bacteria as bio-fungicides.

References

Ahmed, G.A., Sagitov, A.O. and Mahdy, A.M.M. (2006). Biological control of greenhouses cucumber wilt caused by *Fusarium oxysporum f. sp. cucumerinum* of *Bacillus* spp. Plant Protection and Quarantine. 4: 92-94.

Akintokun, A.K. and Taiwo, M.O. (2016). Biocontrol potential of individual specie of Rhizobacteria and their consortium against phytopathogenic *Fusarium oxysporum* and *Rhizoctonia solani*. *International Journal of Scientific Research in Environmental Science* 4 (7): 0219-0227.

Arguelles-Arias, A., Ongena, M., Halimi, B., Lara, Y., Brans, A., Joris, B., Fickers, P. (2009). *Bacillus amyloliquefaciens* GA1 as a source of potential biotics and other secondary metabolites for biocontrol of plant pathogens. *Microbial Cell Fact*. 8: 63–68.

Baniasadi, F., Shahidi, S., Bonjar, G. H., Baghizadeh, A., Nik, A. K., Jorjandi, M., Aghighi, S. and Farokhi, P. R. (2009). Biological control of *Sclerotinia sclerotiorum*, causal agent of sunflower head and stem rot disease, by use of soil borne Actinomycetes isolates. *Journal of Agricultural Biological Science* 4:146–151.

Batista-junior, C.B., Albino, U.B., Martines, A.M., Saridakis, D.P., Matsumoto, L.S., Avanzi, M.A. and Andrade, G. (2002). Efeito fungistático de *Bacillus thuringiensis* e de outras bactérias sobre alguns fungos fitopatogênicos. *Pesq. Agropec. Bras* 37: 1189-1194.

Baysala, O., Çalışkanc, M. and Yeşilovaa, O. (2008). An inhibitory effect of a new *Bacillus subtilis* strain (EU07) against *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Physiol. Mol. Plant Path.* 73: 25–32.

Blackwood, C.B., Oaks, A., Buyer, J.S. (2005). Phylum and class-specific PCR primers for general microbial community analysis. *Applied and Environmental Microbiology* 71:6193-6198.

Cazorla, F.M., Romero, D., Pérez-García, A., Lugtenberg, B.J.J., de Vicente, A. and Bloemberg, G. (2007). Isolation and characterization of antagonistic *Bacillus subtilis* strains from the avocado rhizoplane displaying biocontrol activity. *Journal Applied Microbiology*. 103:1950-1959.

Choudhary, D.K. and Johri, B.N. (2009). Interactions of *Bacillus* spp. and plants with special reference to induced systemic resistance (ISR). *Microbiol. Res.* 164: 493-513.

Chowdhury, S.P., Dietel, K., Rändler, M., Schmid, M., Junge, H., Boriss, R., Hartmann, A. and Grosch, R. (2013). Effects of *Bacillus amyoliquefaciens* FZB42 on lettuce growth and health under pathogen pressure and its impact on the rhizosphere bacterial community. *Microbiol. Res.*16 8: 688-776

Chung, S., Kong, H., Buyer, J.S., Lakshman, D.K., Lydon, J., Kim, S. and Roberts, D.P. (2008). Isolation and partial characterization of *Bacillus subtilis* ME488 for suppression of soilborne pathogens of cucumber and pepper. *Applied Microbiology Biotechnology* 80: 115-123.

Figueiredo, M.V.B., Seldin, L., Araujo, F.F. and Mariano,R.L.M.(2010). Plant growth promoting rhizobacteria: Fundamentals and applications. In: D.K. Maheshwari, (ed.): Plant Growth and Health Promoting Bacteria. Springer- Verlag, Berlin, Heidelberg, pp. 21-42.

Gamal, A. (2010). Controlling of *Fusarium* Wilt of Cucumber by Antagonistic Bacteria. *Journal of Life Sciences.* 4(7): 1934-7391.

Guo, S., Liu, M., Peng, D., Ji, S., Wang, P., Yu, Z. and Sun, M. (2008). "New Strategy for Isolating Novel Nematicidal Crystal Protein Genes from *Bacillus thuringiensis* Strain YBT-1518. *Applied Environmental Microbiology* 74 (22): 6997-7001.

Holt, J.G., Krieg, N.R., Sneath, p. H. A., Stanley, J.T., Williams, S. T. 1994. Bergey's Manual of Determinative Bacteriology 9th Edn. Baltimore, M. D. Williams and Wilkins (Ed.)

Koumoutsi, A., Chen, X.H., Vater, J., Borriss, R., Deg, U. and Yoz, E. (2007). positively regulate the synthesis of bacillomycin D by *Bacillus amyloliquefaciens* strain FZB42. *Applied Environmental Microbiology*. 73: 6953-6964.

Meihiar, M., Ahmad, M. and Kebebo, E. (2014). Isolation and characterization of different *Bacillus thuringiensis* strains from Syria and their toxicity to the Mediteranean flour moth *Ephestia kuehniella Zeller (*Pyralidae*:* Lepidoptera). *Jordan J. Agric. Sci.* 8:196-207.

Obeidat, M., Hassawi, D. and Ghabeish, I. (2004). Characterization of *Bacillus thuringiensis* strains from Jordan and their toxicity to the Lepidoptera *Ephestia kuehniella* Zeller. *African J. Bacteriol*. 3: 622-626.

Michel, V.V., Wang, J.F., Midmore, D.Y. and Hartman, G.L. (1997). Effect of intercropping and soil amendment with urea and calcium oxide on the incidence of bacterial wilt of tomato and survival of soil-borne *Pseudomonas solanacearum* in Taiwan. *Journal of Plant Pathology.* 46:600-610.

Ralte, Z.O., Senthilkumar, N. and Gurusubramanian, G. (2016). Diversity and Toxicity of *Bacillus thuringiensis* from Shifting Cultivation (Jhum) Habitat. *Biocontrol Sci*. 21(2): 99-111.

Rathinam, R.X., Pandian, P.N., Gopalakrishnan, V.M. and Kunthala, J.A. (2007). Isolation of lepidopteran active native *Bacillus thuringiensis* strains through PCR panning. *Asian-Pacific Journal of Molecular Biology and Biotechnology* 15(2): 61-67.

Sharma, T., Navin, K. and Nishant, R. (2012). Isolation, screening and characterization of PGPR isolates from rhizosphere of rice plants in Kashipur region (Tarai region). *Journal Biotechnology International*. 5(3): 69-84.

Smitha, R.B. (2010). Dual Production of Endotoxin and Amylase from *Bacillus thuringiensis s*ubsp *kurstaki* by Fermentation and Efficacy Studies of Endotoxin against Eriophyid Mite, Ph.D. Thesis, University of Calicut, India. Pg203

Soonthompoct, P., Trevathan, L.E., Gonzalez, M.S. and Tomaso-Peterson, M.(2001). Fungal occurrence, disease incidence and severity, and yield of maize symptomatic for seedling disease in Mississippi. *Mycopathologia* 150:39-46.

Upasana, H. and Ronald, L. (2015). Spore Prevalence and Toxigenicity of *Bacillus cereus* and *Bacillus thuringiensis* Isolates from U.S Retail Spices. *Journal of food Protection* 78(3): 590-596.

Weixing, Y., Lei, Z.,Yingying, L., Neil, C., Donghai, P., Lifang, R.and Ming, S. (2012). Mining New Crystal Protein Genes from *Bacillus thuringiensis* on the Basis of Mixed Plasmid-Enriched Genome Sequencing and a Computational Pipeline. *Applied and Environmental Microbiology*.78 (14): 4795-4801.

Wen-Kun, H., Jian-Hua, S., Jiang-Kuan, C., Gang-Feng, W., Ling-An, K., Huan, P., Shu-Long, C. and De-Liang, P. (2014). Efficacy of Fungus *Syncephalastrum Racemosum* and Nematicide Avermectin against the Root-Knot Nematode *Meloidogyne incognita* on Cucumber. *Microbiol. Res.*.39(2):89-97.