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Assessment of the Larvicidal Potency of *Bacillus thuringiensis* Against the Moth of *Spoladea recurvalis* on Amaranths Plant

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Abstract
The control of Spoladeare curvalis, a major pest of amaranth plant, has been through the use of synthetic pesticides which have been reported to have detrimental effects. This study was aimed at assessing the larvicidal potency of B. thuringiensis against the larvae of Spoladea recurvalis on amaranth plant. The assessment of the larvicidal potency of B. thuringiensis isolates from cow range land and refuse dump sites in Zaria metropolis habouringCry1 gene (CR2, CR4, RD1) was carried out by spraying spore crystal mixture of B. thuringiensis isolates on amaranth plants infested with Spoladea recurvalis larvae. The mortality of S. recurvalis larvae, under a screen house were assessed after 24, 48 and 72hours of exposure to the spore crystal mixture of B. thuringiensis isolates. Spore crystal mixtures of B. thuringiensis isolates were effective in causing larvae mortality with more than 60% mean mortality rate of S. recurvalis larvae after 72hours of exposure. The mortality rate of Spoladea rescurvalis increased with increase in exposure period. Spore crystal mixture of Bacillus thuringiensis isolates should be considered as an alternative to synthetic pesticides for the control of Spoladea recurvalis larvae on amaranth plant.
Keywords: B. thuringiensis; spore; larvae; amaranth; larvicidal.

INTRODUCTION

Amaranthus spp are herbaceous plant, produced and consumed mainly for its nutritional and medicinal purpose. It is popularly known as African spinach, Efo and Ele-Efo (Ogwu, 2020). One of the limiting factors affecting the production of Amaranth spp is the damage cause by lepidoptera pests with *Spoladeare curvalis* being the most reported pest (Muralikrishna *et al.*, 2019).

The control of *Spoladeare curvalis* has been through the use of synthetic pesticides which is fraught with detrimental consequence such as environmental pollution prospective effect on non-target insect. The ready consumable nature amaranth without much processing warrants the search for a safer and environmentally friendly control agent such as Biocontrol Agents (Ezeh *et al.*, 2015).

B. thuringiensis is a Gram- positive entomopathogenic bacterium widely used as biological control agent against various economical important pest (Pujiastuti *et al.*, 2018). It has been reported that *B. thuringiensis* can be present in several different habitats such as soil, stored product dust, insect cadavers, grains, agricultural soils, olive tree-related habitats, different plant and aquatic environments (Arora *et al* 2007). Numerous *B. thuringiensis* strains have been isolated that shows activity against lepidopteran, dipteran or coleopteran insects. Thus *B. thuringiensis* could serve as an alternative pesticide for the control of *Spoladea recurvalis* on amaranth plant.

MATERIALS AND METHODS

Generation of Spore Crystal Mixtures

Single colonies of *B. thuringiensis* isolated from cow range land and refuse dump site harboring*Cry*1 gene was inoculated into 10mL Tranvers (T3) sporulation medium and incubated at 28ºC for 5 days on a rotatory shaker at 250rpm. After 5 days Spore crystal mixtures were harvested by centrifugation at 4000xg for 25 minutes. The pellets were resuspended in 5 mL distilled water and centrifuged again (4000xg for 10 minutes). This process was repeated twice. Then, the pellets of the spore crystal mixtures were resuspended in 5 mL distilled water and kept at -4˚C for the next phase of the study (Sahin *et al.*, 2018)

Nurturing of Amaranths Plants

Seeds of Amaranthus spp were obtained from Institute of Agricultural Research (IAR), Ahmadu Bello University Zaria.

Seedlings of the Amaranths were initially raised in nursery beds, in a screen house of department of Microbiology, Ahmadu Bello University Zaria, using a mixture of loamy soil and composed manure. After two weeks, seedlings were transplanted into portable plastic pots each containing 4 kg of the manure amended soil at the rate of one seedling per pot. The potted plants were placed in the screen house to prevent them from attack by herbivore and other insect pests. Watering was done regularly to prevent drying (Legwaila *et al.*, 2015).

Assessment of the Larvicidal Potency of Spore Crystal Mixture of *B.thuringiensis*

Crude spore crystal mixtures of *B. thuringiensis* isolates (100ml) generated, were used to spray the larvae using a small hand-held trigger sprayer that produces a fine spray of a relatively narrow range of droplet sizes to determine the potency of the mixture. The spray was applied on each plant infested with 10 larvae each. Each seedling was sprayed separately. Each treatment was applied to a single seedling in duplicates. Each pot was labeled, indicating the treatment and date of application. The second instar larvae were used because the first instar larvae (leaf miners) are not susceptible to a pesticide with a stomach poison mode of action such as *B. thuringiensis* (Legwaila *et al.*, 2015). The larvae were counted before treatment. The larvae mortality was assessed at 24, 48, and 72 hours after treatment. Any larvae that did not show signs of life after prodding with a needle was considered as dead and recorded (Legwaila *et al.*, 2015).

Data Analysis

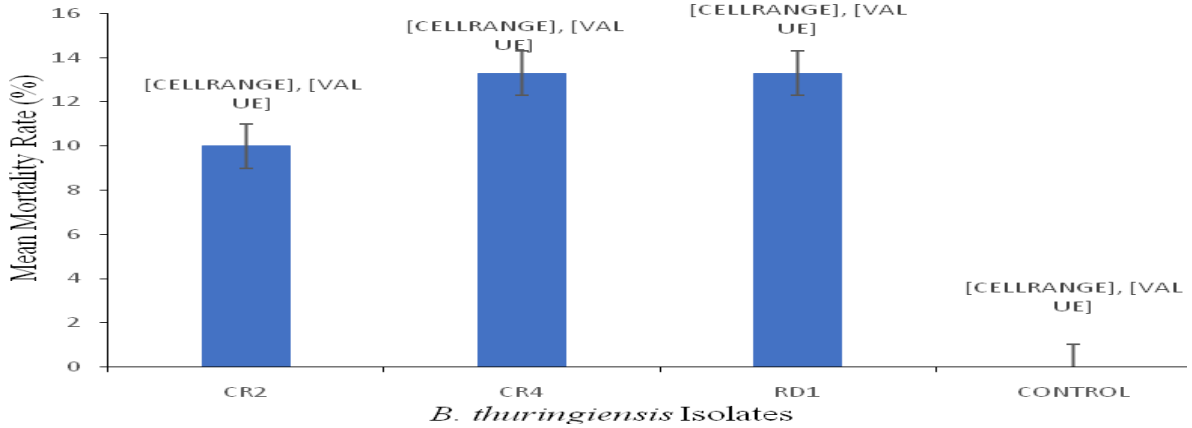
Data obtained from the study were analysed using the SAS JMP Pro 14 statistical package, one-way analysis of variance (ANOVA) was used to compare the differences between the mean toxicity of the crystal spore mixture derived from each isolate. Averages were separated using Tukey's Honestly significant difference test where significant effects were found. Results were then presented in charts (Statistics User's Manual, 2002).

RESULTS

Larvicidal Activity of Spore Crystal Mixtures Derived from *B. thuringiensis* Isolates

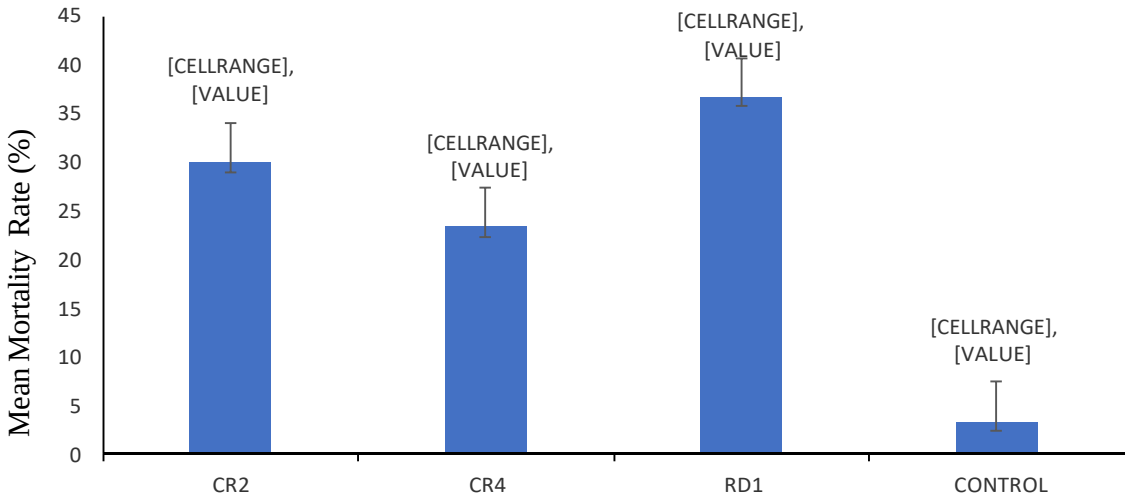
After 24h following the folia application of the spore/crystal mixture derived from the three B. thuringiensis isolates, the mean mortality rate ranged from 10% to 13.3% (Figure 1), with the mean mortality rate of the three *B. thuringiensis* isolates (CR2, CR4 and RD1) being significantly different from the control at P<0.05.

Exposing the larvae to the spore/crystal mixtures for another 24h (i.e. at 48h) period, further indicated significant increase in larval mortality due to action of the *B. thuringiensis* spore crystal mixture compared to the untreated control. Increase in larval mortality of 10, 20 and 22% were recorded among larvae treated with the product derived from isolates CR4, CR2 and RD1 respectively. However, ANOVA test showed that there were no significant differences in the mean larvae mortality rate among larvae treated with the three products 48h after exposure with a P <0.05 (Figure 2). Similar observations were made when the exposure period was extended to 72h with significantly higher larval mortality recorded among larvae treated with the products compared to the control (Figure 3). Nevertheless, 43, 33 and 43% increase in larval mortality were observed among the larvae treated with products derived from isolates CR4, CR2 and RD1 respectively 72h after exposure (Figure 4).



p = 0.0118 df = 11 f = 7.1667

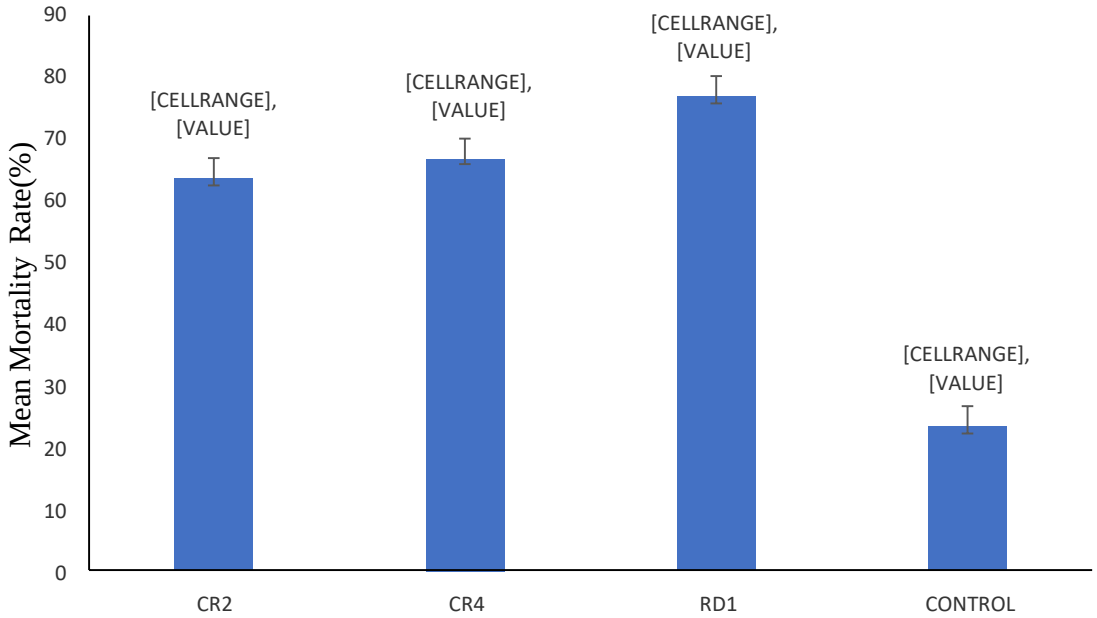
Figure 1: Mortality of *Spoladea recurvalis* larvae 24 hours after exposure to spore/crystal mixtures (mean ± SE). Bars with different letter are significantly different (p < 0.05).



B. thuringiensis Isolates

p = 0.0022df = 11 f = 12.4444

Figure 2: Mortality of *Spoladeare curvalis* larvae 48 hours after exposure to spore/crystal mixtures (mean ± SE). Bars with different letter are significantly different (p < 0.05).



B.thuringiensis Isolates

p = 1.63 x 10^{-5} df = 11 f = 49.5833

Figure 3; Mortality of *Spoladea recurvalis* larvae 72h after exposure to spore crystal/mixtures (mean ± SE). Bars with different letter are significantly different (p < 0.05).

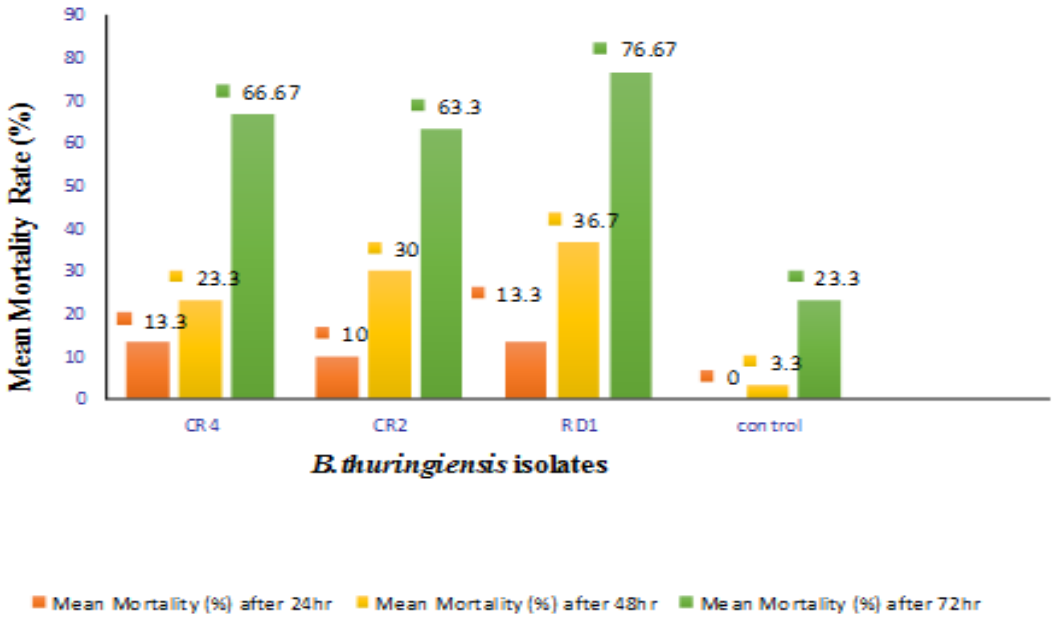


Figure 4.4. Effect of Period of Exposure of Spore Crystal Mixture of *B. thuringiensis* on the Mortality Rate of *Spoladeare curvalis*

DISCUSSION

The larvicidal effect of the spore crystal mixture of *B. thuringiensis* isolates in 24 to 72hr may be attributed to the presence of *Cry* 1 gene in the *B. Thuringiensis*isolates also, the presence of spore enhances the activity of the crystals. This report is similar to the findings of Aswal and Bisht (2012) who also reported that *B. thuringiensis* Kurtakiis effective against the larvae of *Spoladea recurvalis* after evaluating the bio-efficacy of new generation insecticides, botanical and microbial insecticides. Effectiveness of *B.thuringiensis* against other Lepidoptera pest has also been reported by researchers such as Pujiastuti *et al*. (2018),who reported *B. thuringiensis* isolated from soil to be effective as biocontrol agent against *Sposoptera litura*, and Gebremariam *et al* (2021) who found out that Ethiopian isolates of*B. Thuringiensis* has toxicity effect on the larvae *of Galleria mellonella*.

B. thuringiensis has also been reported to be effective against other orders of insect such as dipteran and Hemiptera. Torres- Quintero*et al* 2016 found *B. thuringiensis* to be effective in causing mortality of *Myzuspersicae*. Cabra and Hernandez- Fernandez (2019) Reported that *B. Thuringiensis* is toxic to*Bemisiatabaci (White fly) (Hemiptera)*, Riskuwa-Shehu *et al*. (2019) reported *B. thuringiensis*to be toxic against Mosquito larvae (Diptera)

Other bio-pesticides such as entomopathogenic fungi and neem leaf has also been reported to be effective against larvae of *Spoladeare curvalis* (Aderolu *et al*., 2013 and Opisa *et al*., 2018).

The mortality rate of more than 60 % after 72hours of exposure recorded in this research (Figure 3), indicates that the Spore crystal mixture derived from the *B. thuringiensis* isolates were highly effective in causing larvae mortality of *Spoladeare curvalis*. The high mortality rate of the larvae may be as a result of *B. thuringiensis* proteinaceous protoxins contained in the spore and crystalline parasporal body which is dissolve in the alkaline midgut of insects, reacting with proteolytic enzymes, acting as toxins(Talaei-Hassanloui *et al*., 2014), Also, it has been reported that the second instar larvae (L2) of *S. recurvalis* are more susceptible to biopesticides. The result obtained in this research is in line with several other researchers, Aswal and Bisht (2012) reported an 85% reduction in larval population of *S. recurvalis* when treated with *B. thuringiensis*. Similar results were obtained with *B. thuringiensis* against *S. recurvalis* by Leena *et al* (2005).

More than 60% mortality rate of larvae of other insect pest caused by *B. thuringiensis* has been reported by other researchers such as Lobo *et al*. (2018), who reported 100% mortality rate of *Aedes aegypti* 3rd instar larvae caused by *B. thuringiensis* found in the soil of Cerrado region of Brazil and Santos *et al*. (2009) reported 70% mortality of *S. eridania* larvae.

The increase in the mortality recorded after each period of exposure to each of the spore/crystal mixture of *B. thuringiensis* recorded (Figure 4) may be due to the fact the increase time allows the toxins to accumulate, and the toxin's (δ-endotoxin) effect increase increases as exposure period increase. This is similar to a report by El Husseini (2019) who recorded an increase on the mortality rate of the second instar larvae of *S. recurvalis* fed on diet mixed with different concentration of *B. thuringiensis Kurstaki*. Similar to this report also is a report by Majeed *et al* (2018) who evaluated the toxicity of indigenous *B. thuringiensis* isolates *Helicoverpa armigera* larvae using in vitro bioassay method and revealed that larvae mortality was significantly affected by period of exposure. Toxicity studies for indigenous *B. thuringiesis* isolates from Malang City, East Java on *Aedes aegypti* larvae carried out by Gama *et al.*, (2013) revealed that 72h exposure time, was more effective than 24h.

CONCLUSION

Application of sporecrystal mixtures derived from three *B. thuringiensis* isolates which harbour *Cry1* gene was toxic to *Spoladea recurvalis* larvae under screen house conditions with as high as 76.67% mean mortality rate of *S. recurvalis* larvae after 72h of exposure.

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