Mortality and Recovery Potential of Fall Armyworm Larvae (Spodoptera Frugiperda J.E. Smith) after Exposure to Bacillus thuringiensis Isolate

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ABSTRACT

The Fall Armyworm (FAW) (*Spodoptera fragiperda* (J. E. Smith) is a major insect pest of maize crop in America and its outbreaks was recognized in 2017 in Ethiopia to threatened by maize crops. Biological control is an effective and eco-friendly approach for the management of insect pest and also one of the most important alternative control measures providing environmentally safe and sustainable plant protection. Therefore, this study was carried out to evaluate the mortality and recovery potential of FAW larvae under *in vitro* conditions after exposure to *Bacillus thuringiensis* isolates at Ambo Agricultural Research Center during March to May in the year of 2020. Based on the toxicity screening test, totally seven isolates of *Bacillus thuringiensis* were used. Among treatments, three isolates (Bt19.1= 90.61%, Bt 3.1= 68.11% and Bt 92= 62.23%) with higher toxicity, one isolate, Bt 25= 54.39% with medium toxicity and three isolates (Bt 27= 49.12%, Bt 45= 47.16% and Bt 10.2= 27.04%) with lower toxicity were identified as compared with

standard check =75.13% 72 h after exposure. There was no larval mortality more than 24.56% at 24 h exposure to all *Bt* isolates. The bioassay results indicated that four *Bt* isolates were highly toxic against third instars larvae of FAW and caused more than 50% mortality. Among seven isolates of *Bt,* only one isolate of *Bt19.1* induced mortality above 90% against third instars larvae of FAW. *B. thuringiensis sub-species kurstaki* commercial produced induced 75.13% mortality against third instars larvae of FAW. The recovery potential of FAW bioassay, in the different evaluation days, highest death and lowest survival for all treatments was recorded on the fifteen days after the larvae were placed in the *Bt19.1* (83.33% and 16.67%, respectively) treated diet when comparing with negative control (36% death and64% live). The percentages of adult emergences from the highest to lowest were: 76.00%, 37.04%, 13.04%, 10.53% and 6.67% with the untreated control (-), *Bt25, Bt92, Bt3.1 and Bt kurstaki* isolate, respectively. But no adult is emerged from the treatment *Bt19.1* isolate. Commercially produced *Bt sub spp kurstaki* (6.67%) was resulted in lowest adult emergence next to *Bt19.1* isolate. Highest adult emergence was recorded in the treatments with negative control (76%) followed by *Bt25* (37.04%) isolate**.** There was a dramatic effect on the weight and length of pupa in the *Bt* isolates treatments, *Bt19.1, Bt3.1, Bt92 and Bt sub spp kurstaki* in which significant weight differences in comparison with control. The highest mean weight and length were registered on the negative control= 0.47 and 1.80 followed by *Bt25* =0.30 and 1.3. There were significant weight differences between the *Bt19.1 =0.14, Bt92=0.18 and Bt3.1* isolate *=0.16* and also *Bt sub spp kurstaki =0.12,* Further studies are required to validate under field conditions and molecular species level identification of the isolates should be studied.

Keywords: Fall armyworm, Bacillus thuringiensis, Toxicity, Mortality, Recovery potential.

1. INTRODUCTION

Maize is the primary importance to Ethiopian agribusiness, and it is grown widely in the national territory; the crop has a variety of socioeconomic interests across the country. But its production is constrained by various pests. The recently introduced alien invasive fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), has become a number one maize pest in Ethiopia. Since its first introduction to Ethiopia in 2017, FAW has spread to all maize growing areas across the country and infestations have been severe. In 2017 a total of 458 Districts were affected by the pest and out of the 2.1 million ha planted in 2017, more than 650,000 ha (22.23%) were infested by FAW and about 2 million farm families were affected (Girma, 2018). Although the insect is known to attack many crops, maize is the one which has suffered the highest damage. Estimated damage of up to 100% on maize and other crops has been reported by smallholder farmers due to FAW (Girma *et al*., 2019). Currently, FAW control in Ethiopia predominantly depends on the use of chemical insecticides that may lead to resistant population, environmental contamination, public health problems and incurs high production cost. Consequently, several alternative methods have been studied to replace use of synthetic insecticides among which biological control occupy an important position.

Biological control is an effective and eco-friendly approach for management of insect pest and also one of the most important alternative control measures of providing environmentally safe and sustainable plant protection. The success of biological control will depend on understanding the adaptation and establishment of applied biological control agents in agricultural ecosystems (Nester *et al*., 2002). Microbial pathogens and arthropod bio-control agents have been successfully used in agricultural systems.

They are safe for non-target vertebrates and for the environment and production costs have been significantly reduced in recent times as they are mass produced in liquid media (Mahmoud, 2017).

Fall armyworm is susceptible to at least 16 species of entomopathogens including viruses, fungi, protozoa, nematodes and bacteria. Among the pathogens, *Bacillus thuringiensis*, *Metarhizium anisopliae* and *Beauveria bassiana* are caused significant level of mortality in FAW population and help to reduce leaf defoliation in crops (Molina-Ochoa *et al.,* 2003). Although several species of bacteria have been used as microbial control agents of a variety of insects, but only *Bacillus thuringiensis* (*Bt*) has been used for practical pest control. The common soil bacterium *Bacillus thuringiensis* (*Bt*) produces crystals containing proteins that are toxic to certain insects, but are harmless to most other organisms including people, wildlife and most beneficial insects (Schnepf 1998). *Bt* toxins have been shown to be completely safe to users and the environment. The extensive laboratory studies including mammalian toxicity studies, coupled with no reported cases of human or animal disease after more than 15 years of widespread use demonstrate that the tested isolates are not toxic or pathogenic (Rechcigl *et al.* 2000).*Bacillus thuringiensis* is a gram-positive, rod-shaped, motile, facultative anaerobic, spore-forming bacterium, widely used as bio-control agent against insect pests (Fernando *et al*., 2010). It is an active component in many commercial bio-pesticides (Rosas-Garcia, 2009; Sanahuja *et al*., 2011; Sanchis, 2011) and the effectiveness of this species is due to its ability to produce protein crystals during sporulation that contain insecticidal toxins known as delta - endotoxins (Cry and Cyt) (Bravo *et al*., 2011). The *Bt* has been produced Para sporal crystalline inclusion bodies constituted by highly specific insecticidal toxins which are proteinaceous by nature. These toxins are mainly active against Lepidopteran species and also showed toxicity against Dipteran and Coleopteran species (Vidyarthi *et al*., 2002; Martin *et al*., 2010).

The use of *Bt* product is gradually being increased as the alternative to chemical pesticides, as organic pesticides cause too many ill effects to human beings when they consume insecticides treated products (Kandibane *et al*., 2010). So far more than 50,000 *Bt* strains have been isolated from different environments (Sadder *et al*., 2006). It has been reported that *Bt* can be present in several different habitats such as soil, stored product dust, insect cadavers, grains, agricultural soils, olive tree related habitats, different plants and aquatic environments (Iriarte *et al*., 2000; Xavier *et al*., 2007). The mode of action of these endotoxins can be summarized in the following steps: 1) after Cry protein ingestion, it is activated in the mid gut by proteases under high pH conditions, 2) activated protein binds to specific receptors in the insect mid gut and 3) the toxin forms pores in the mid gut membrane, releasing mid gut contents into the body cavity and eventually killing the insect (Bravo *et al*., 2007; Likit ivatanavong *et al*., 2011; Pardo López *et al*., 2012; Vachon *et al*., 2012). However, empirical information on this approach is still scanty in Africa in general and Ethiopia in particular. Therefore, this study was carried out to evaluate the mortality and recovery potential of FAW larvae under *in vitro* conditions after exposure to *Bacillus thuringiensis* isolates

2. MATERIALS AND METHODS

The investigation was carried out at the Department of Entomology, Ambo Agricultural Research Center during March to May in the year of 2020.

2.1. Insect Rearing

FAW larvae liberated in the entomology greenhouse trials originated from a culture collected from unsprayed maize farm from West Hararghe Zone, Oromia region and maintained on natural diet of maize grown on pots and covered by cages, for 10 generations. Typical daily temperatures range from 20°C minimum to 38°C maximum during the growing season, with a mean monthly relative humidity in excess of 70%. Insect development was supervised every other day, when pupae turned from orange-red to dark red,

they were placed in groups of 40 inside collapsible insect cages to provide room for emerging adults to fly and mate, a sponge with a solution of 10% sugar in a Petri dish was provided as food source. Egg masses laid on leaves of maize, cages side and maize stem were collected and placed in a plastic container with moist paper towels. Neonate larvae were transferred to bioassay trays with 1 ml of artificial diet. Third instar larvae were used in the bioassays.

2.2. Evaluation of *Bacillus thuringiensis* (Bt) Isolates Against FAW

The larvae of FAW were obtained from FAW larvae established in the Entomology greenhouse trial of Ambo Agricultural Research Center. Seven local isolates of *Bacillus thuringiensis* spp. (Bt19.1, Bt10.2, Bt25, Bt45, Bt92, and Bt3.1and Bt27) were obtained from Ambo Agricultural Research Center while the commercial *Bacillus thuringiensis sub-species kurstaki*was obtained from Sineria Company in Nairobi, Kenya (Table 1).

Table 1: Details of *Bacillus thuringiensis* (*Bt)* isolates used in this experiment against FAW larvae

Isolate code	Cell/ml	No of Inoculated larvae per treatment	Area of collection	Host	Year of collection
Bt19.1	10^6	20	West wollega	Soil	2019
Bt10.2	10^6	20	West wollega	Soil	2019
Bt25	10^6	20	West wollega	Soil	2019
Bt45	10^6	20	West wollega	Soil	2019
Bt92	10^6	20	West wollega	Soil	2019
Bt3.1	10^6	20	West wollega	Soil	2019
Bt27	10^6	20	West wollega	Soil	2019
Bt sub-species kurstaki	20g/20L water	20	Commercial	-	

2.3. Insect Toxicity Assay

Laboratory bioassays were conducted at Ambo Agricultural Research Center in Pathology laboratory. Mortality data were generated by a maize leaf dip bioassay on FAW third instars larvae. Standard leaf-dip bioassays were conducted, using sterilized glass Petri dishes (9 cm). Test solutions of *Bt* isolate were made in double distilled autoclaved water. Fresh unsprayed leaves were cut, washed thoroughly with distilled water, and shade-dried at room temperature (28 °C). The larvae were transferred to the maize shoots and leaves cut into pieces and weighed to 20 g of diet previously sprayed by *Bt* isolate suspension. This suspension was obtained with *B. thuringiensis* isolates scrapped from the plates, so the concentration of spores and crystals were very high and adjusted. Maize leaf (5cm length) were dipped for 10 sec in a 10^6 cell $\cdot$ ml^{-1} concentration of various *Bt* isolates, allowed to air dry, placed in plastic Petri dishes and inoculated with 20 larvae per Petri dish. Maize leaf (5cm length) dipped into sterile distilled water was also included negative control (untreated leaf).The treatments were laid out in a Complete Randomized Design (CRD) with three replications.Larval mortality was assessed after 24, 48 and 72hours of exposure of the third instars larvae to the treatments.Larvae that failed to respond to moderate prodding were considered as dead. The number of dead larvae in each replicate was converted into proportions of the total number of larvae introduced and expressed as a percentage. Mortality data were corrected for natural mortality using Abbott's correction formula (Abbott, 1925):

$CM\ (\%) = \frac{(\%T - \%C)}{(100 - \%C)} \times 100$; Where, **CM** is the corrected mortality, **T** is the mortality in treated leaf and **C** is the mortality in untreated leafas standard check.

2.4. Bioassays of Recovery Potential of FAW Larvae

The recovery bioassays were conducted with only four isolates (*Bt19.1, Bt25, Bt92, and Bt3.1)* that caused above 50% mortality in the toxicity assay along with standard check and untreated control. One or two concentrations that caused above 50% mortality in the diet above were used. For the recovery potential of the FAW larvae, the highest dose included in the diet overlay bioassay (10^6cell· ml^{-1}) was used. Control treatments consisted of diet plus sterile distilled water and commercial *Bt* product of *B. thuringiensis sub-species kurstaki*. FAW third instars larvae were exposed to the *Bt* isolates suspension as mentioned above. After three days of exposure, only those larvae moved when prodded with a small paint brush were transferred to fresh diet. Controls were also transferred to fresh diet after the three days period. Three replications were conducted per treatment for each population. Recovery and development were evaluated every three days, the parameters recorded for each insect was: 1) survival (live/dead), 2) weight (g), 3) life stage, and 4) adult emergences.

2.5. Data Analysis

All the data recorded were subjected to one-way and two-way analysis of variance (ANOVA) by using the PROC GLM procedure in SAS version 9.4 (SAS institute, 2019) and difference among means were compared by the Tukey's highest significant different (HSD) tests at 5% level of significance. Larval mortality data was corrected according to Abbott's procedure (Abbott 1925) before further statistical analyses.

3. RESULTS AND DISCUSSIONS

3.1. Insect Toxicity Assay

The result of toxicity assay showed that there were significant differences among treatments in larval mortality after each three-exposure time (Table 2).

The highest mortality was recorded 72 h after exposure while the lowest were recorded 24 h after exposure. The maximum mortality observed 24 and 72 hours after exposure was 24.56 and 90.61 %, respectively. After 24 h of exposure of the larvae to the treatments, most treatments showed lower larval mortality among each other and the control; however, Bt19.1 isolate caused the highest larval mortality (>20%) followed by standard Bt isolate (19.30%) and Bt25 isolate. Standard Bt isolate, *Bt sub-species kurstaki*, gave 19.30 per cent larval mortality followed by Bt25 isolate (15.79%). This is in line with the mode of action of *Bt* strains as their proteins upon ingestion by the target insect pests take about 48 to 72 h to be converted into toxic protein crystals which further bind with mid gut epithelial cells and cause mortality (septicemia) symptoms (Bravo *et al*. 2017).

Table 2: Effects of different *Bacillus thuringiensis* isolates on mean percent mortality of fall armyworm third instars larvae.

Treatments	Corrected larval mortality (%)		
	24 h	48 h	72 h
Bt19.1	24.56^{a}	85.67^{a}	90.61^{a}
Bt10.2	1.75^{d}	5.36^{ef}	27.04^{e}
Bt25	15.79^{b}	44.64^{c}	54.39^{cd}
Bt45	7.02^{cd}	19.49^{def}	47.16^{d}
Bt92	12.28^{bc}	31.68^{dc}	62.23^{bcd}
Bt3.1	12.28^{bc}	49.90^{bc}	68.11^{bc}
Bt27	7.02^{cd}	21.15^{de}	49.12^{d}
Bt sub-species kurstaki (+ control)	19.30^{ab}	69.88^{ab}	75.13^{ab}
Control (-)	0.00^{d}	0.00^{f}	0.00^{f}
LSD	7.57	20.86	18.12
CV%	39.74	33.40	20.06

Means followed by the same letter within a column are not significantly different at 5% probability level.

Forty-eight hours after exposure, the isolates showed a pattern similar to that of 24 h, with significant differences among the isolates for killing larvae (Table 2). The highest mortality (>80%) was caused by Bt19.1 isolate followed by standard Bt isolate (69.88%), Bt3.1 isolate (49.90%) and Bt25 isolate (44.64%).The bioassay results after 72 h of exposure indicated that four *Bt* isolates i.e *Bt19.1, Bt3.1, Bt92 and Bt25*were highly toxic against third instars larvae of FAW which caused more than 50% mortality. Among seven isolates of *Bt,* only one isolate of Bt19.1 induced mortality above 90% against third instars larvae of FAW (Table 2). The percentage of mortality was: 90.61, 68.11, 62.23, 54.39, 49.12, 47.16 and 27.04 with isolates of, *Bt27, Bt45 and Bt10.2,* respectively. Bioassay results showed that the commercial *B. thuringiensis sub-species kurstaki* l induced 75.13% mortality against third instars larvae of FAW.

In general, there were significant differences in larval mortality between treated and untreated control. According to the results of toxicity tests against FAW larvae *Bt*19.1isolates (90.61%) obtained the toxicity is higher than *Bt* sub-species kurstaki (75.13%). Three isolates (Bt19.1= 90.61%, Bt3.1= 68.11% and Bt92= 62.23%) higher toxicity, one isolate (Bt25= 54.39%) had medium toxicity and three isolates (Bt27= 49.12%, Bt45= 47.16% and Bt10.2= 27.04%) had lower toxicity when comparing with commercial Bt sub-species kurstaki (+ control) after 72h of exposure. The first three isolates (Bt19.1= 90.61%, Bt3.1= 68.11% and Bt92= 62.23%) had best results for further studies for FAW larvae to control. Estela *et al*. (2004) and Makhlouf *et al*. (2016) demonstrated the same trend of larval mortality on *Helicoverpa armigera*, i.e., 72 and 85%, respectively, at 120 h of exposure to many soil isolates of *Bt*. The current investigation revealed that four *Bt* isolates, namely *Bt19.1, Bt3.1, Bt92 and Bt25* proved very effective against third instars larvae of FAW. Thus, the results of these mortality studies of *Bt* isolates on FAW clearly indicated the difference in insecticidal activity among them.

The results are in close agreement with that of Justin *et al.* (1989) reported that the variation in insecticidal activity among the various *Bt* sub-species. The toxicity of *Bt* mainly depends upon the delta endotoxin. The delta endotoxins produced by different strains have different spectra of insecticidal activity. The present findings agree with the results obtained by Adams *et al*. (1996) who reported that the different strains are known to vary in their toxicity and different isolates of the same strain may show variable levels of toxicity against same species of insect pest. Pokharkar *et al*. (2002) reported that toxicity of *Bt* isolates persisted at least for 120 h on cabbage leaves.

3.2. Recovery Potential of FAW Larvae

The results of an experiment conducted to determine the recovery potential of FAW larvae after exposure to different *Bt* isolates with respect to survival, adult emergence, weight and life stages are presented as follows:

3.2.1. Survival

Table 3 showed the average survival percentages of FAW at different developmental time (days). The maximum survival (98%) was recorded on the third day from un-treated diet (negative control) followed by*Bt*25 (85.19%) and the minimum survival (16.67%) was recorded in the *Bt*19.1treated diet. The same trend was observed after 12 and 15 days. In general, the highest mortality and lowest survival was recorded form diet treated with the *Bt19.1* (83.33% & 16.67%, respectively) while lowest mortality and highest survival were recorded from diet treated with negative control (8% mortality& 92% survival).

Table 3: Mean Fall armyworm survival percentages throughout developmental stage after exposure to different *Bt* isolates.

Code	% Mortality	Inoculated population after treatment	Life stage after 3 days	Survival		Life stage after 12 days	Survival		Life stage after 15 days	Survival	
				Live	dead		Live	Dead		Live	Dead
Bt19.1	90.61^{a}	6^{f}	larvae	1^{f} (16.67)	5^{bc} (83.33)	Larvae 1(16.67)	1^{e} (16.67)	0^{e} (83.33)	pupa	1^{e} (16.67)	0(83.33)
Bt25	54.39^{c}	27^{b}	larvae	23^{b} (85.19)	4^{c} (14.81)	Larvae 7(25.93) pupa 12(44.44)	19^{b} (70.37)	4^{d} (29.63)	pupa	19^{b} (70.37)	0(29.63)
Bt92	62.23^{bc}	23^{c}	larvae	12^{d} (52.17)	11^{a} (47.83)	larvae 6(26.09) pupa 3(13.04)	9^{c} (39.13)	12^{a} (52.17)	pupa	9^{c} (39.13)	0(52.17)
Bt3.1	68.11^{bc}	19^{d}	larvae	14^{c} (73.68)	5b^{c} (26.32)	larvae 7(36.84) pupa 1(5.26)	8^{c} (42.11)	6^{c} (57.89)	pupa	8^{c} (42.11)	0(57.89)
Btkurstaki	75.13^{ab}	15^{e}	larvae	9^{e} (60)	6^{b} (40)	pupa 2(13.33) larvae 3(20)	5^{d} (33.33)	4^{d} (66.67)	pupa	5^{d} (33.33)	0(66.67)
Control (-)	0.00^{d}	50^{a}	larvae	49^{a} (98)	1^{d} (2)	Pupa46(92)	46^{a} (92)	9^{b} (8)	pupa	46^{a} (92)	0(8)
LSD	15.96	1.62	-	1.45	1.45	-	1.45	1.45	-	1.45	-
CV	15.36	3.91	-	4.54	15.30	-	5.57	13.99	-	5.57	-

Means with the same letter are not significantly different at 5% probability level. Figures in parenthesis indicate means total percentage of dead and live values up to the given days.

On the last evaluation (day 15), the percentages of survival were: 92, 70.37, 42.11, 39.13, 33.33 and 16.67 from the highest to lowest with the treatments and negative control, *Bt25, Bt3.1, Bt92, Bt sub-species kurstaki* (+ control), and *Bt19.1*. Table 21 showed that survival of the FAW population after treatment with *Bt* isolates and commercial *Bt sub-species kurstaki* were decreased from three days after transferred to non-treated diet, on the last evaluation day (day 23).

In general, mortality in this population high throughout all the treatments with an average death percentage of 83.33% of the *Bt*19.1,66.67% *Bt kurstaki*, 57.89% *Bt*3.1,52.17% *Bt* 92, and 29.63% of*Bt*25 when comparing with negative control (8%), respectively at 15th day. The medium percentiles of FAW were recovered: 70.37% of *Bt*25, 42.11% of *Bt*3.1, 39.13%, of *Bt*92, 33.33% of *Bt kurstaki*, and 16.67% of *Bt*19.1 when comparing with negative control (92%) at 15th day. Previous work has proven that fall armyworm has the potential to recover from *Bt* protein damage when exposed as second or third instars (Hardke *et al*. 2011, Binning *et al*. 2014).

3.2.2. Adult Emergence

The impact of all treatments against *FAW* in terms of adult emergence was significant ($P \leq 0.05$) (Table 4).The percentages of adult emergences from the highest to lowest were: 76.00%, 37.04%, 13.04%, 10.53% and 6.67% with the untreated control (-), *Bt25, Bt92, Bt3.1 and Bt kurstaki* isolate, respectively. But no adult is emerged from the treatment *Bt19.1* isolate. Commercially produced *Bt sub spp kurstaki* (6.67%) was resulted in lowest adult emergence next to *Bt19.1* isolate. Highest adult emergence was recorded in the treatments with negative control (76%) followed by *Bt25* (37.04%) isolate**.**

Initial adult emergences on the 21stday were zero except in the negative control and *Bt25*isolate, with percentage emergence of 10% and 3.70%, respectively. Percentage adult emergence had increased from 10% and 3.7% in both control and *Bt25* isolate treatment to 76% and 37.04%, respectively on 27th date. On the last day (27th), the highest adult emergence was recorded in *Bt25 i*solate (37.04%) followed by *Bt92* (13.04%) and*Bt3.1* (10.53%) isolates.

Table 4: Fall armyworm cumulative adult emergence and weight after exposure to different *Bt* isolates

Code	% Mortality	Inoculated population after treatment	The last Live pupa after treatment	Average weight of pupa (g)	Average length of pupa (cm)	% of Initial adult emergence (21day)	% of Total adult emergence (27day)	% of Non-adult emergence
Bt19.1	90.61^{a}	6^{f}	1^{e} (16.67)	0.14^{e}	1^{d}	0^{c}	0.00^{f}	100.00^{a}
Bt25	54.39^{c}	27^{b}	19^{b} (70.37)	0.30^{b}	1.3^{b}	1^{b} (3.70)	37.04^{b}	67.96^{e}
Bt92	62.23^{bc}	23^{c}	9^{c} (39.13)	0.18^{c}	1.25^{b}	0^{c}	13.04^{c}	86.96^{d}
Bt3.1	68.11^{bc}	19^{d}	8^{c} (42.11)	0.16^{d}	1.12^{cd}	0^{c}	10.53^{d}	89.47^{c}
Bt kurstaki	75.13^{ab}	15^{e}	5^{d} (33.33)	0.12^{f}	1.2^{bc}	0^{c}	6.67^{e}	93.33^{b}
Control (-)	0.00^{d}	50^{a}	46^{a} (92)	0.47^{a}	1.80^{a}	5^{a} (10)	76.00^{a}	24.00^{f}
LSD	15.96	1.62	1.45	0.02	0.11	0.73	1.70	1.92
CV	15.36	3.91	5.57	4.73	4.96	40.69	4.01	1.40

Means with the same letter are not significantly different at 5% probability level. Figures in parenthesis indicate means total percentage of dead and live values up to the given days.

3.2.3. Weight and Length of Pupa

A significant difference among the five isolates with regard to the weight and length of pupa was recorded (Table 4). There was a dramatic effect on the weight and length of pupa in the *Bt* isolates treatments, *Bt19.1, Bt3.1, Bt92 and Bt sub spp kurstaki*in which significant weight differences in comparison with control. The highest mean weight and length were registered on the negative control= 0.47 and 1.80 followed by *Bt25* =0.30 and 1.3. There were significant weight differences between the *Bt19.1 =0.14, Bt92=0.18 and Bt3.1* isolate *=0.16* and also *Bt sub spp kurstaki =0.12* (Table 26). Similarly, Capinera (2014) also reported the pupa constructs a loose cocoon, oval in shape and 20 to 30 mm in length, by tying together particles of soil with silk. All the pupa treatments length was below the previous range studied (Table 4).

4. CONCLUSIONS AND RECOMMENDATIONS

With growing threat of human health and degradation of environment due to excessive use of chemical insecticides for pest control; microbial based bio pesticides have emerged as safe and effective alternatives. The potential of entomopathogens as a resource material in agriculture and related areas is now a well-established fact. Bacterial based bio pesticides are effective in controlling various lepidopteron insect pests independently. These bio-pesticides which would be less toxic than conventional products could be used by the bottom level farmers. Although some bacterial isolates cannot completely fulfill the need of chemical pesticides it can be used in combination with other cultural practices as IPM. In conclusion, the present findings indicate that the third instar larvae were more susceptible at 3 days (72 h) post-treatment than at 24 h post-treatment. Among treatments, three isolates (Bt19.1= 90.61%, Bt 3.1= 68.11% and Bt 92= 62.23%) with higher toxicity, one isolate, Bt 25= 54.39% with medium toxicity and three isolates (Bt 27= 49.12%, Bt 45= 47.16% and Bt 10.2= 27.04%) with lower toxicity were identified as compared with standard check =75.13% 72 h after exposure.

The toxicity assay results indicated that four Bt isolates were highly toxic against third instars larvae of FAW and caused more than 50% mortality but one isolate, Bt19.1, induced mortality above 90%.

The result from recovery potential of FAW larvae showed that the highest survival rates were recorded on the third day after larval exposure in the untreated diet (negative control) = 92% and Bt25= 85.19%, while the lowest survival rate recorded in the Bt19.1=16.67%. In the different evaluation trial, the highest mortality and lowest survival were recorded on the fifteen days after larval exposure in the Bt19.1 when comparing with negative. The highest adult emergence (76.00%) was recorded from Bt25 isolate, whereas the lowest (6.67%) were recorded in standard check (Bt sub spp *kurstaki*). No adult was emerged from the treatment Bt19.1 isolate. The Bt isolates Bt19.1, Bt3.1, Bt92 and also Bt sub spp *kurstaki* were completely reduced pupa weight and length in comparison with control. Both Bt isolates which caused more than 50% mortality could be promising biological control agents against *S. fruguperda* as an alternative to synthetic insecticides in maize. Further studies, however, are required to validate its effectiveness under field conditions and additional recovery experiments with molecular level analysis should be designed to determine additional factors that may play a significant role in the potential for recovery after Bt exposure in fall armyworm.

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