Management of *Helicoverpa armigera* (Hübner) with botanical extract (*Balanites aegyptiaca*) and endophytic entomopathogenic fungus *Metarhizium brunneum* (6c1) reared on okra plant

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Summary

Leaves chewing insects are widely recognized as herbivores which can influence agricultural productivity. Cotton bollworms are one of the most important crop pests worldwide. The larva is mainly responsible for causing damage; it is a polyphagous in nature and attacks more than 182 plant species. Moreover, well-managed pests like the cotton bollworm (*Helicoverpa armigera*) developed resistances against often used insecticides. Hence, concerns about the widespread of the environmental negative impacts of chemical insecticides coupled with the great damages in many areas that expected to be caused by the bollworm have led to focus on environmentally sound and sustainable alternative strategies for pest control. The development of new biopesticide against these and other pests is therefore of high importance, to overcome disadvantages of chemically synthetic plant protection products, which can be highly efficient but with indeterminate environmental effects.

Overall hypotheses of the thesis were that *Metarhizium brunneum* (Mycoinsecticide) and extracts of *Balanites aegyptiaca* L (Botanical insecticide) are effective in controlling cotton bollworm *Helicoverpa armigera* insect pest.

- Chapter 1 General introduction focus on literature review.

- Chapter 2 An *Metarhizium brunneum* strain 6c1 was evaluated for its capability to colonize okra plant with toothpick inoculation method. The studies reflected the ability of Mb-6c1 to colonize the whole plant tissue. Different parameters such as pupae success, area consumed, weight gain and faeces dry weight bioassay were tested to evaluate whether the colonization of Mb- 6c1 could affect the performance of bollworm by means of toothpick. The results revealed that *M brunneum* (6c1) showed a positive effect on the survival of cotton bollworm larvae conducted in the bioassay.

- Chapter 3 and 4 of this focus on *B aegyptiaca* as a botanical insecticide

- Chapter 3 A review on Desert Date *Balanites aegyptiaca* L. & Del.: general uses and future prospective as botanical insecticide.

- Chapter 4 Deleterious effects of oil and water extracts (2% (v: v): 5% (v: v) oil extract and 5% (w/v): 10% (w/v) water extract) of seed kernel of *B aegyptiaca* Del on the 2nd

instar larvae *H armigera* were studied. All results showed that larval survival was significantly influenced by treatments. Oil and water extracts have properties of larvicidal against *H armigera* which could potentially be used as an attractive alternative for pest management.

In order to clearly and widely identify the active ingredients which caused the deleterious effects and the modes of action, this study recommends that the bioassays should be applied in a field study and modes of action should be identified and dose-response curves assessed additionally, efficacy of different parts (leaves and roots) of *B aegyptiaca* against *H armigera* should be screened as well.

To the blessed memory of my father and mother

My my beloved wife, Bothina and son, Ibrahim

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Chapter One

General Introduction

Cotton bollworm (*Helicoverpa armigera* Hübner 1808; Lepidoptera: Noctuidae) considered to be one of the important pests for many crops worldwide (Lammers & Macleod, 2007). The larva is mainly responsible for causing damage; it is a polyphagous in nature and attacks more than 182 plant species, including different important cash crops, (cotton, sunflower, sorghum, maize), vegetables, fruit crops and tree species (Uddin et al., 2009). The annual yield loss is estimated to about US$ 2 billion reported in tropical and subtropical area. Furthermore, US$ 500 million worth pesticides are applied to control bollworm (Kass et al., 2018). Moreover, the bollworms cause about 10 to 60% of okra (*Abelmoschus* esculentus L) fruit infestations, however, under optimal environmental conditions the level of damage can boost 30 to 80% yield loss (Javed et al., 2019). Hence, concerns about the widespread of the environmental negative impacts of chemical insecticides coupled with the great damages in many areas that expected to be caused by the bollworm have led to a focus on environmentally sound and sustainable alternative strategies for pest control. Thus, a worldwide resurgence of interest in the use of predators, parasitoids and/or entomopathogenic microorganisms have intensively been studied for biological control of *Helicoverpa armigera* species (Gonzalez et al., 2016) and a significant advance in development and manufacturing of these agents in the future is expected with recent biotechnological discoveries (Hatting, 2012).

Plants are commonly colonized by a wide range of endophytic microorganisms, such as bacteria and fungi. Endophytes are microorganisms present in plant tissues without causing any apparent symptoms (Jalgaonwala et al., 2011). Fungal endophytes are widespread and quite diverse in nature (Arnold, 2007). Entomopathogenic fungi have been widely used for the control agricultural, for example *Beauveria bassiana* (Balsamo) Vullemin (Ascomycota: Hypocreales) is the best-studied endophytic fungal entomopathogen (Posada et al., 2010) and it is available as a commercial mycoinsecticide for control of the Colorado potato beetle (Faria & Wraight, 2007). Inoculation methods tested to establish *B. bassiana* as an endophyte include soil drenches and immersions (Tefera & Vidal, 2009), seed coatings (Jalgaonwala et al., 2011), radical dressings (Posada & Vega, 2005), root and rhizome immersions (Akello et al., 2009), stem injections (Posada et al., 2007) and foliar sprays (Quesada et al., 2006). Using these methods, researchers have introduced *B. bassiana* into the different types of crops. Also the insect-pathogenic fungus,

Metarhizium robertsii is a common inhabitant of soils worldwide that has been studied and used as an insect pathogen for biocontrol (Lomer et al., 2001). Several species of entomopathogenic fungi have been playing multiple roles in nature ranging from antagonists of plant pathogens to rhizosphere associates, endophytes, and possibly even plant-growth (Vega, 2008). Vega et al., (2012) reported that when used as mycoinsecticides, entomopathogenic fungi have demonstrable potential for the management of insect pests. *Metarhizium anisopliae* are producing pathogens implicated in toxicity of a number of insects, the action of cytotoxins is proposed by hyphae penetration causing symptoms such as partial or general paralysis and decrease irritability in mycoses insects that are consistent with the action of neuromuscular toxins (Sandhu et al., 2012).

The selection of fungal pathogens for controlling *Helicoverpa armigera* is necessary because the selection of isolates which combine the best possible characteristics for diminishing the target insects under glasshouse conditions is vital. Hence, the important characteristics of the isolate to be considered are (i) good mass production, such as high sporulation on artificial media, (ii) high virulence against target organisms, and (iii) the ability to withstand the pest environment (Hussain et al., 2014).

Botanical insecticides are made from substances extracted from plants or plant-derived secondary metabolites and used for insect control (Hikal et al., 2017). Essential oils extracted from redolent plants have been increasing considerably as insecticides due to their popularity in organic farming. These products are characterized with their repellent, insecticidal, antifeedants, growth inhibitors, oviposition inhibitors, ovicides, and growth-reducing effects on a variety of insects (Regnault-Roger et al., 2012 ; Suthisu et al., 2011)

Different components of *Balanites aegyptiaca* have several bioactive substances (Al Ashaal et al., 2010) which possess distinguished characteristics and important phytochemical components for instance, alkaloids, glucosides, flavonoids, phenolics, saponins, tannins, terpenoids, steroids, lipids, proteins, carbohydrates, organic acids, furanocoumarins, Diosgenin, N-trans-feruloyltyramine, N-cis-feruloyltyramine, trigonelline, balanitol and cardiac glycosides (Amadou, Le, & Shi, 2012 ; Koubala et al., 2013). Additionally, important phytochemical components (secondary metabolites) are useful for the management of various diseases and have potential insecticidal effects against medical, veterinary and agricultural pests (Pan et al., 2016)

According to Tesfaye (2015), the tree is a multipurpose with considerable economic possibilities for its enormous biological active components, all parts of the plant used as food or has effects as an antidote for the cure of infections or pests control, also he recommended that the ranch of tree acting as shelterbelt and soil reformer in arid regions and in saline soil and will be helpful in conservation of biodiversity. Hence, the main objective of the current study is to address the virulent of *Metarhizium brunneum* (Mb-6c1) and essential oils extracted from *Balanites aegyptiaca* on the management of cotton bollworm *Helicoverpa armigera* (Hübner).

The aims of the study were:

- To investigate the efficacy of strain 6c1 of the *Metarhizium brunneum* on *Helicoverpa armigera* (Hübner).
- To examine the ability of *Metarhizium brunneum* Mb-6c1 to occupy the tissue of okra plant.
- To determine the potential endophytic strain 6c1 of the entomopathogenic fungi as a bio-control agent against insect pests by confirming that the fungus still acts as a true insect pathogen after being introduced into plants.
- To propose essential oils extracted from *Balanites aegyptiaca* seed water extracts and seed crude oil in water emulsions as well. To evaluate the antifeedant and repellent of extracts from *B aegyptiaca* against 2nd instar larvae *H armigera*.
- To determine the possible significant implications of the produced bio-pesticides.

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Chapter Two

Efficacy of endophytic (*Metarhizium brunneum*) strain 6c1 in the control of old-world Bollworm (*Helicoverpa armigera*) in okra

2.1 Abstract

Metarhizium brunneum strain 6c1 was evaluated for its capability to colonize okra *Abelmoschus esculentus* (L.) with toothpick inoculation method. The results reflected the ability of Mb-6c1 to colonize the whole plant tissue. Different parameters such as pupae success, area consumed, weight gain and faeces dry weight bioassay were conducted to test whether the colonization of Mb-6c1 could affect the performance of bollworm *Helicoverpa armigera* by means of toothpick. The results showed that the inoculation had a significant pupae success, in particular, a gradual reduction of number of pupations were 80%, 73%, 20%, 13% for the control, Tween800.01% (v: v), (1×10^7conidia/ml) and (1×10^5conidia/ml), respectively. Consumed leaf area was significantly influenced by the treatment ($p < 0.05$) with a mean of 63.13 ±11.90 cm^2 and 54.65 ± 10.32 cm^2 for the high conc and low conc treatments, respectively. Nevertheless, Mb-6c1 increased the root dry weight of okra plants and acts as plant growth regulation. Mb-6c1 was crucial for the dietary uptake and growth of *H armigera*, and consequently could be a mycoinsecticide target for the development of pest management and environmentally friendly.

Keywords; *Metarhizium brunneum*, *Helicoverpa armigera*, *Abelmoschus esculentus*, Conidia, colonization

2.2 Introduction

Cotton bollworm *Helicoverpa armigera* (Hübner) cause about 10 to 60% okra *Abelmoschus esculentus* (L.) fruit infestations, however, under optimal environmental conditions the level of damage can boost 30 to 80% yield loss (Javed et al., 2019). The larva is mainly responsible for causing damage: it is a polyphagous in nature and attacks more than 182 plant species, including different important and cash crops, (cotton, sunflower, sorghum, maize), vegetables, fruit crops and tree species (Uddin et al., 2009). Hence, concerns about the widespread of the environmental negative impacts of chemical insecticides coupled with the great damages in many areas that expected to be caused by the bollworm have led to a focus on environmentally sound and sustainable alternative strategies for pest control. Thus, a worldwide resurgence of interest in the use of predators, parasitoids and/or entomopathogenic microorganisms have intensively been studied for biological control of *Helicoverpa armigera* species (Gonzalez et al., 2016) and a significant advance in development and manufacturing of these agents in the future is expected with recent biotechnological discoveries (Hatting, 2012).

Metarhizium is a genus of fungi that belongs to the family of Clavicipitaceae (class: Pyrenomycetes-sphaeroides, phylum: Ascomycota) and is spread worldwide (Roberts & St. Leger, 2004). Metarhizium spores attack insects and cause green muscardine disease. When the fungus comes into contact with the body of an insect host, they germinate and the mycelium penetrates the cuticle, while the fungus toxin ingestion by insect develops inside the body and ultimately kills the target after a few days (Freimoser et al., 2003). Currently, the fungus is used as a biological control agent against various insect species (Shah & Pell, 2003). In addition, species from the genus Metarhizium are used to manage and prevent infestations of various species of pest insects, including locusts, grasshoppers *Melanoplus femurrubrum* (Hunt & Charnley, 2011; Wang et at., 2011), malaria mosquitoes *Aedes aegypti* (Garza-Hernández et al., 2013), tobacco hornworm *Manduca sexta* (St. Leger et al.,1996), mealworm beetle *Tenebrio molitor* (Oliveira & Rangel, 2018), diamondback moth *plutella xylostella* (Batta, 2013), Wireworms *melanotus communis* (Kabaluk et at., 2005), termite *Coptotermes formosanus* (Wright, Raina, & Lax, 2009), Cockroach, *Supella longipalpa* (Sharififard et at., 2016),Chagas vector *Meccus pallidipennis* (Flores-Villegas et al., 2016) and House Fly, *Musca domestica* (Sharififard et al., 2011).

Joan Webber, (1981) suggested that, a protected elm tree against the beetle (*Physocnemum brevilineum*) was giving by endophytic fungus *Phomopsis oblonga*. The ability of fungi to occupy healthy tissue of the plant symptomless is very important to be considered. Many researchers worked on fungal entomopathogens as endophytes, in preparation for subsequent assessment of endophyte biological control. *Beauveria bassiana* have been tested to establish an endophyte (Brownbridge et al., 2012). However, *B. bassiana*, has the ability to exist as an endophyte in corn as a horizontally transmitted endophyte; *B. bassiana* infects hosts via germination of dry conidia following hydration on the leaf surface (Bing & Lewis, 1992; Wagner & Lewis, 2000) which may lead to pioneer methods for crop protection. Also the insect-pathogenic fungus, *Metarhizium robertsii* is a common inhabitant of soils worldwide that has been studied and used as an insect pathogen for biocontrol (Lomer et al., 2001). Several species of entomopathogenic fungi have been playing multiple roles in nature ranging from antagonists of plant pathogens to rhizosphere associates, endophytes, and possibly even plant-growth (Vega, 2008). Vega et al., (2012) reported that when used as mycoinsecticides, entomopathogenic fungi have demonstrable potential for the management of insect pests.

Metarhizium anisopliae are producing pathogens implicated in toxicity of a number of insects, the action of cytotoxins is proposed by hyphae penetration causing symptoms such as partial or general paralysis and decreasing irritability in mycosed insects that are consistent with the action of neuromuscular toxins (Sandhu et al., 2012).

The selection of fungal pathogens for controlling *Helicoverpa armigera* is necessary because the selection of isolates which combine the best possible characteristics for diminishing the target insects under glasshouse conditions is vital.

The aims of the study were:

- To investigate the efficacy of strain 6c1 of the *Metarhizium brunneum* on *Helicoverpa armigera*
- To examine the ability of *Metarhizium brunneum* (6c1) to occupy the tissue of okra plant.
- To explore the problematic of pesticides resistance (*Helicoverpa armigera*)

2.3 Materials and methods

Experiments were carried out under greenhouse conditions and quarantine cabinet at the Department of Entomology, Faculty of Agriculture - Georg-August-University, Gottingen.

2.3.1 Insect rearing

2.3.1.1 Artificial diet

Cotton bollworm eggs were obtained from a laboratory strain (Bayer Crop Science Monheim, Germany) and incubated in a chamber at 22 °C; 60 % RH; 16:8 LD. Larvae started to hatch three days later, reared on an agar based artificial diet modified from Wakil et al., (2011) (Fig 2.1). The diet recipe completed to 1000 ml, heated on magnetic stirrer until boiling point. Then the followings were added: 2 g Methyl-4-hydrobenzoate, 4 ml ETOH 96 %, 15 g Agar-Agar, 20 g wheat sprouts, 125g Bean Flour, 2 g Wessons Salt Mixture, 4 ml sunflower Oil. The mixture was then stirred well for 5 minutes and let to cool to 70 °C. Thereafter 8 g Vitaminmix, 6 g Vitamin C, 10 g Yeast Extract and 2 g Streptomycin Sulfate. The diet was immediately poured into petri-dishes (5 cm in diameter), allowed to cool and dry. Then one neonate larva was placed on it.

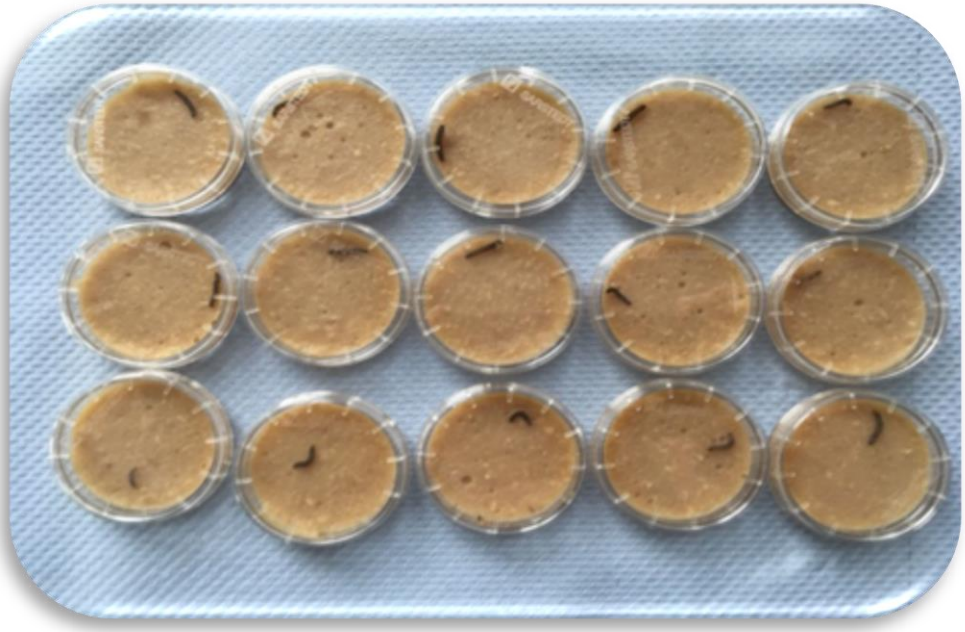

Figure 2.1. Rearing petri-dishes (5 cm in diameter) with artificial diet

2.3.1.2 Okra fruit diet

The newly larvae from eggs brought from Bayer (Bayer Crop Science Monheim, Germany) hatched were transferred separately and placed into rearing containers petri-dishes (9 cm in diameter). Each larva was supplied with okra fruit blocks. These pieces of fruit were maintained as fresh as possible by keeping them on moist filer paper. The food was renewed daily and faeces discarded, the petri-dishes were kept clean from faeces and food remains. Successive rearing experiments were conducted under climate chamber at 22 °C; 60 % RH; 18:6 LD (Fig 2.2**a**). When the larvae pupated; the date of pupation was recorded and adult emergence was watched closely to record the pupation period. The newly emerged adult's moths were sexed. The male and female were transferred into oviposition cage (Fig 2.2**b**). The cages were simply constructed from a cylinder with its top covered with muslin cloth and its lower end fitted on pot with water. The surface of water pot was separated from the contents of upper chamber by filter paper (125 mm diameter). Two small specimen glasses fitted with sponger soaked in a 7% honey solution, applied as food for adult moths, were tied on the walls of the cylinder and small fresh and tender branch of okra plant were placed inside the cage for egg deposition. The branch stalk dipped in water and such branches were renewed daily and examined for egg-laying.

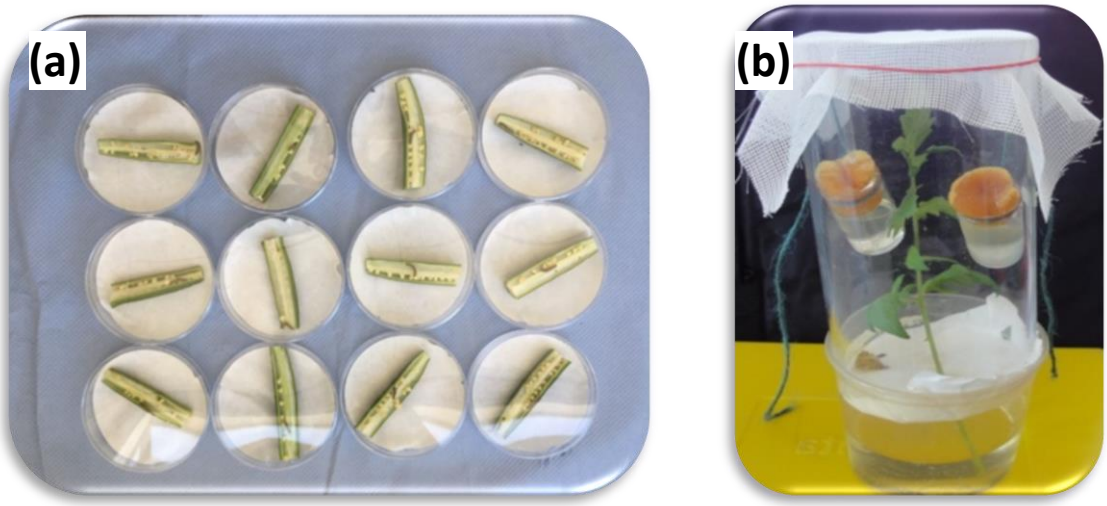

Figure 2.2 (**a**) Rearing petri-dishes (9 cm diameter) with okra block placed on moist filter paper (**b**) oviposition cage

2.3.2 Plant materials

Seed of okra *Abelmoschus esculentus* (L.) obtained from Agricultural Research Corporation – Sudan. Seedlings were grown in a greenhouse chamber (23 ± 3°C; 60 % RH; 18:6 LD). Two-week old plants were individually transplanted into plastic pots (15 cm diameter) with a mixture of non-sterile sand and soil. Plants were irrigated regularly and fertilized once a week.

2.3.3 Seed test

2.3.3.1 Surface sterilization of okra seeds

Okra seeds were surface sterilized in 70% ethanol for 90 min, then 3 min in 5.25% Sodium hypochlorite for, and finally again 90 min in 70% ethanol. Thereafter, seeds were washed in sterile water three times, then air dried on a filter paper in biosafety cabinet. For sterilization tests, randomly selected seeds were cultured on potato dextrose agar (PDA) and incubated at 25°C for 7 days.

2.3.3.2 Okra plant inoculation

Spore suspension of *Metarhizium brunneum* (6c1) was plated on autoclaved potato dextrose agar (PDA) and sub-cultured. Spores were washed from PDA by a few drops of Tween80. Then the removed material was transferred in sterile water (200 ml). The spore suspensions were stirred with magnetic stirrer for 10 min and the spore concentration was adjusted at about 1×10^7 conidia/ml and 1×10^5 conidia/ml. Plants were inoculated using the toothpick method, toothpicks were autoclaved five times in distilled water, then autoclaved after cooling, toothpicks were rinsed in distilled water, and autoclaved again according to (Scandiani et al., 2011). Okra plants were divided into four groups of 15 plants each. The toothpicks were inserted in high and low concentration of spore suspension *Metarhizium brunneum* (6c1), Tween 80 0.01% (v: v) and non-contaminated toothpicks for the control. Each treatment was injected on okra plants for one week.

2.3.3.3 Cross section of okra plant

The cross section was conducted to ensure endophytic strain of the *Metarhizium brunneum* (6c1) inside the plant tissue. For cross section test; randomly selected inoculated stem from each treatment was cut and then prepared on the slide and examined under light microscope.

2.3.3.4 Effect of *Metarhizium brunneum* (6c1) on *Helicoverpa armigera*

On each young plant bioassays were conducted where only unfolded new leaves were used. Consecutively, one larva per plant was transferred with fine brush onto the first growth stage 24 BBCH scale. The respective areas were covered with clip cages.

2.3.4 Measured variables

2.3.4.1 Pupae success

Pupae success was assessed at the end of larval stage and successfully entering the pupal stage. The number was recorded and expressed as percent pupal success. The date of pupation was recorded and the adult emerged was watched closely to record the pupation period.

2.3.4.2 Consumed leaf area

The setup consists of three translucent PVC sheets, black paper, a black square of precisely 100 mm^2 within a red frame and a 100 mm^2 [mm^2 paper] square. For each larva the total consumed leaf area [cm^2] was used as an indirect fitness parameter and photometrically assessed through particle analysis with Fiji Image J v1.52g. (Rueden et al., 2017; Schindelin et al., 2012).

2.3.4.3 Weight gain

Larvae were obtained from eggs laid by the same female reared on okra fruit, newly larvae were transferred separately and placed in rearing containers, petri-dishes (9 cm in diameter) and supplied with okra fruit blocks. When reached the second instar; larval weight was individually measured immediately before introduced into the clip-cages and again fully grown in order to calculate the weight gain (fresh fully grown instar weight – fresh second instar weight). The larvae were daily checked for molting and survival.

2.3.4.4 Number of pods

The pods were manually removed from harvested plants and number of pods per-plant was recorded.

2.3.4.5 Dry weight of below-ground

Plants were cut at soil line above soil level and the above-ground plant parts, leaves and stems, were collected, the below-ground parts were carefully collected and thoroughly washed many times with water to remove soil particles. Then roots were detached from sand particles and organic debris by flotation. Then the root was separated from the top (cut at soil line). The collected materials were placed in towel paper for 10 min then transferred into a paper bag. The effect of treatment on okra was investigated by measuring the dry weight of shoot and root compared to the control. Above and below-ground parts of the plants were oven dried at 60° C for one week till constant weight was achieved.

2.3.5 Statistical analysis

Data were statistically analyzed with SigmaPlot version (SigmaPlot 14.0). Analysis of variance (ANOVA) was used to test for treatment effect if so, the differences were separated used Bonferroni test at a probability of 0.05, unless otherwise stated.

2.4 Results

2.4.1 Seeds sterilization

No contamination was observed with sterilized seeds, cultured on PDA and incubated at 25°C for 7 days (Fig. 2.3). This showed the efficacy of the tested seed sterilization procedures for its ability to germinate and eliminate contamination.

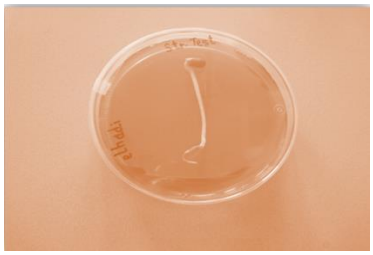

Figure 2.3 Seed sterilization

2.4.2 Cross section of okra plant

The cross section of okra stem was specifically conducted to test the growth the mycelium of endophytic strain of the entomopathogenic fungi *Metarhizium brunneum* inside the plant. The result revealed the distinctive colonization compared to the control (Fig. 2.4).

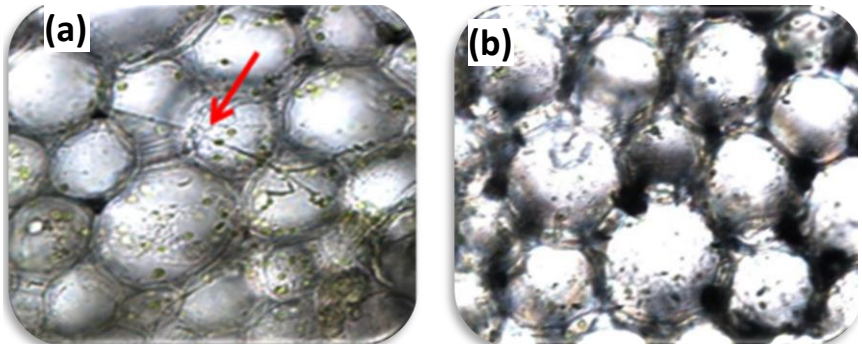

Figure 2.4 Entomopathogenic fungi on endophytic occupation of okra plants (**a**) red arrow shows the endophytic hyphae of *Metarhizium brunneum*, (**b**) control

2.4.3 Total life cycle

Total development duration (life cycle) of *Helicoverpa armigera* reared on artificial diet ranged from 43 to 46 days, average 43.70 ± 1.57days while for okra fruit it ranged from 43 to 48 days, average 45.6 ± 2.10 days (Fig 2.5)

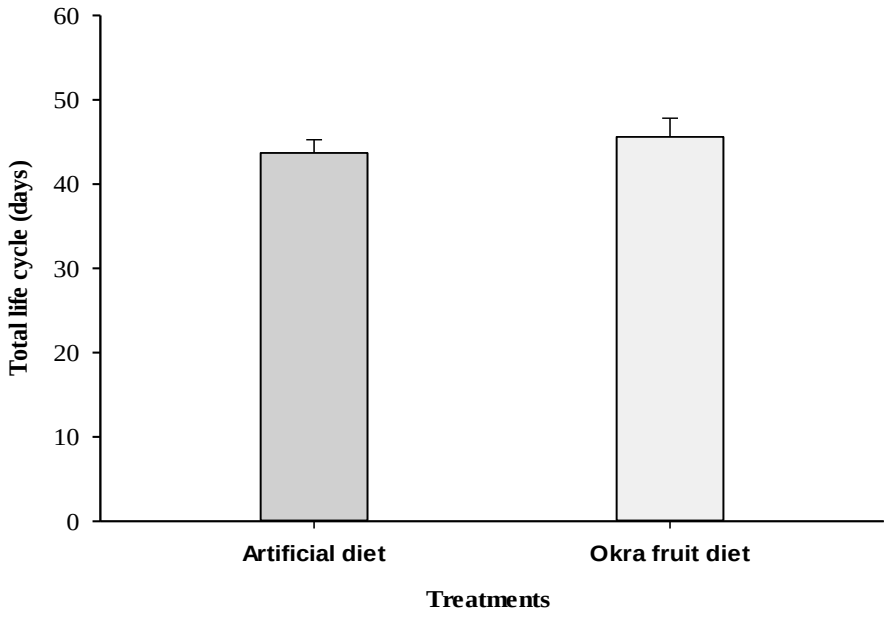

Figure 2.5 Total life cycle of *Helicoverpa armigera* reared on artificial diet and okra fruit under climate chamber at (22 °C; 60 % RH; 18:6 L)

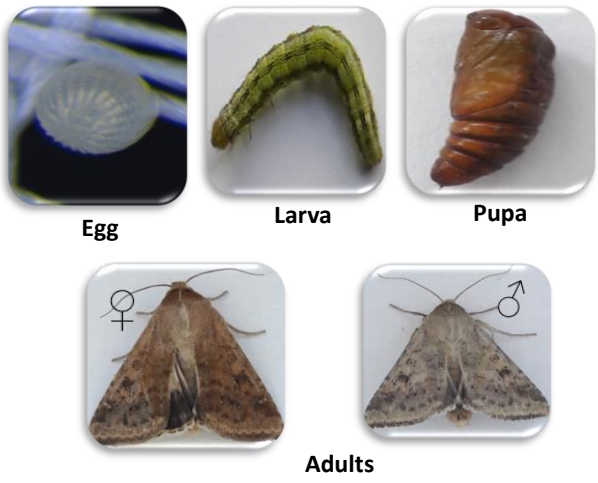

Figure 2.6 Life cycle of *Helicoverpa armigera* reared on okra

2.4.4 Pupae success

The study showed significant differences ($P < 0.05$) in the virulence of strain. After 12 days' post-treatment, in the control, the endophyte treatments reduced pupae success, however, the high conc and low conc of spoor suspension showed a gradual reduction of the number of pupations, toothpick inoculation, thus, okra plant showed high efficiency of suppression. The resulted values were 80%, 73%, 20%, 13% for the negative control, Tween80 0.01%, high conc and low conc, respectively (Fig2.7).

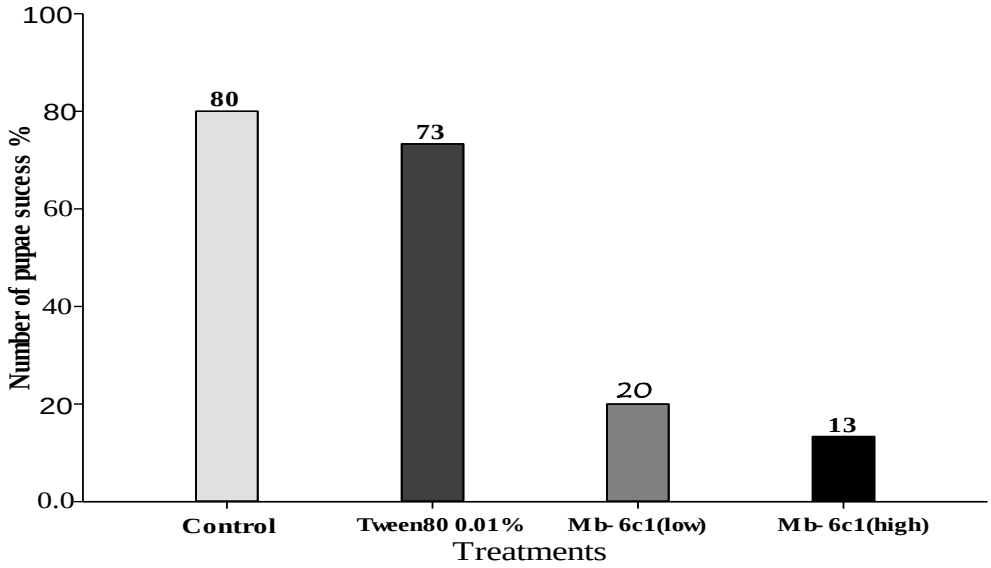

Figure 2.7 Percentage pupae success of *Helicoverpa armigera* on each treatment Mb-6c1 okra plant with *Metarhizium brunneum* low conc and high conc toothpick inoculation, okra plant, Tween80 0.01%with tooth pick inoculation, control clean okra plant.

Figure 2.8 Mortality symptom of *Helicoverpa armigera* in high conc and low conc of spore suspension

2.4.5 Consumed leaf area

Consumed leaf area (Fig 2.9) was significantly influenced by treatment ($p < 0.05$). The mean total consumed leaf area of negative control treatment was 91.79 ± 17.64 cm^2 and 75.01 ± 9.59 cm^2 for Tween 80 0.1%. Both were significantly higher than the high conc and low conc where the leaf area was 63.13 ± 11.90 cm^2 and 54.65 ± 10.32 cm^2, respectively.

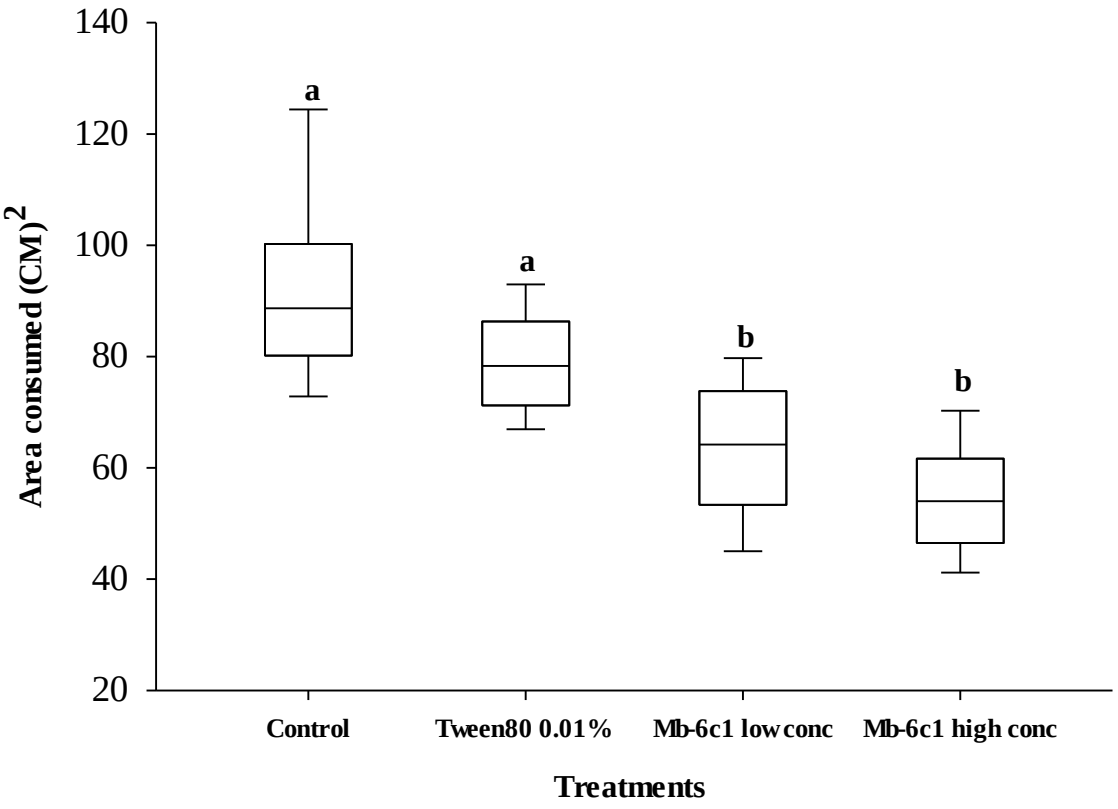

Figure 2.9 Consumed leaf area [cm^2] by *Helicoverpa armigera* larvae on okra plants for each treatment (*Metarhizium brunneum* Mb-6c1 low conc and high conc, Tween80 0.01%, negative control). Error bars represent ± SE. Same letters are not significantly different using least significant difference (LSD).

2.4.5.1 Consumed leaf area of *Helicoverpa armigera* larvae for each treatment per day

The daily consumed leaf area is resulted in the following values 19.742 cm^2, 12.156 cm^2, 8.689 cm^2, 5.266 cm^2 for the control, Tween80 0.01%, high conc and low conc of spore suspension, respectively. (Fig 2.10)

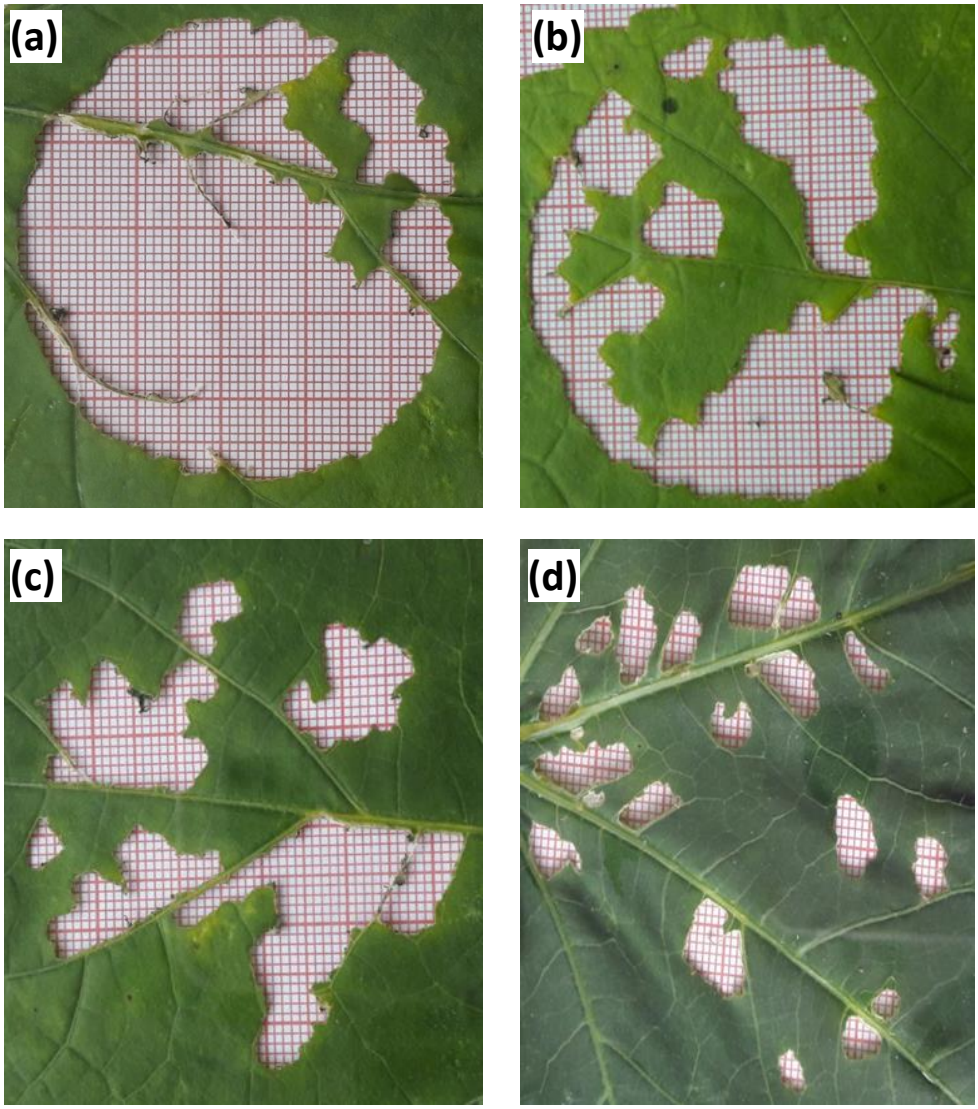

Figure 2.10 The pattern of damage on different treatments. (**a**) Control area consumed per day (19.742 cm^2), (**b**) Tween80 0.01% area consumed per day (12.126 cm^2) (**c**) Low conc area consumed per day (8.689 cm^2) and (**d**) High conc area consumed per day (5.266 cm^2)

2.4.6 Weight gain

Weight gained by *Helicoverpa armigera* larvae was significantly different between treatments ($p < 0.05$) and the higher value (0.13 ± 0.04) was recorded in the control compared to values reported in the high conc and low conc of Mb-6c1 (0.05 ± 0.04, 0.07 ± 0.06), however, no significant differences were detected between control and Tween80 0.01% (Fig 2.11)

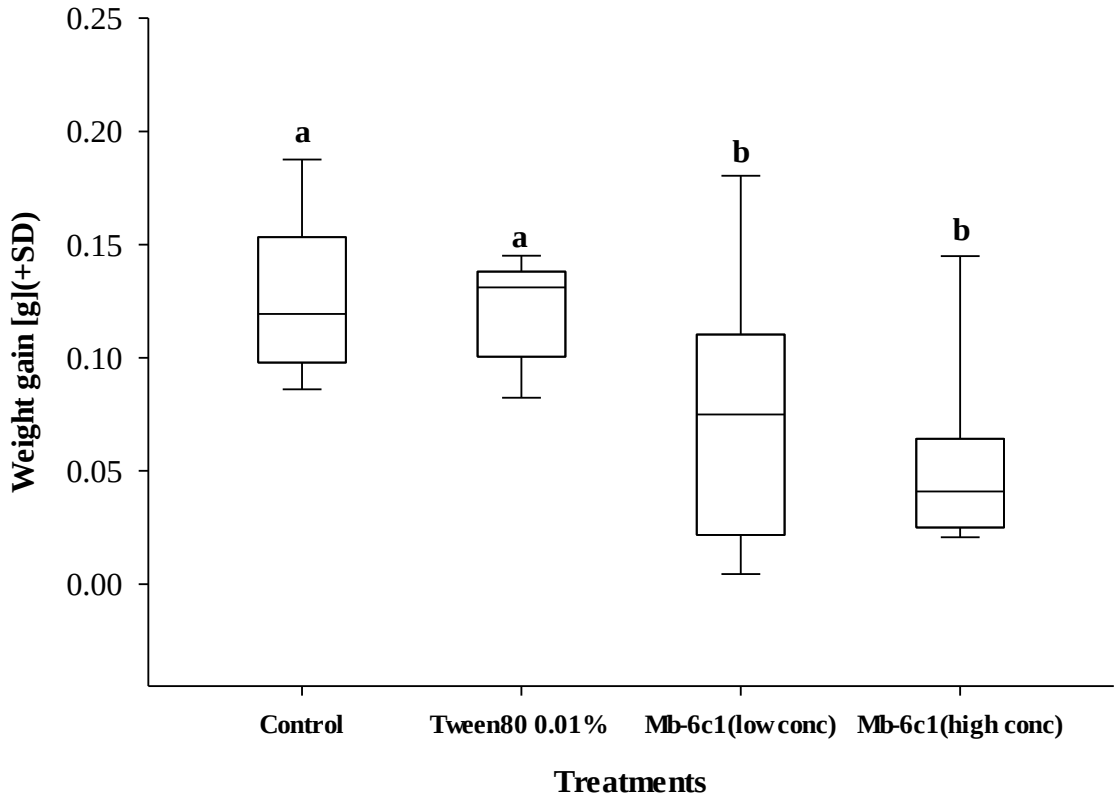

Figure 2.11 Effect of treatments (Mb-6c1 low conc and high conc, Tween80 0.01%, negative control) on weight gained by larva *Helicoverpa armigera*. Vertical bars with the same letter(s) are not significantly different ($P < 0.05$) using least significant difference.

2.4.7 Feces dry weight

The dry weight of feces produced by the larvae *Helicoverpa armigera* was significantly different between treatments ($p < 0.05$) and the higher value (0.12 ± 0.02) was recorded in the control compared to values reported in the high conc and low conc of Mb-6c1 (0.06 ± 0.0, 0.08 ± 0.02), however, no significant differences were detected between control and Tween80 0.01% (0.11 ± 0.01) (Fig 2.12)

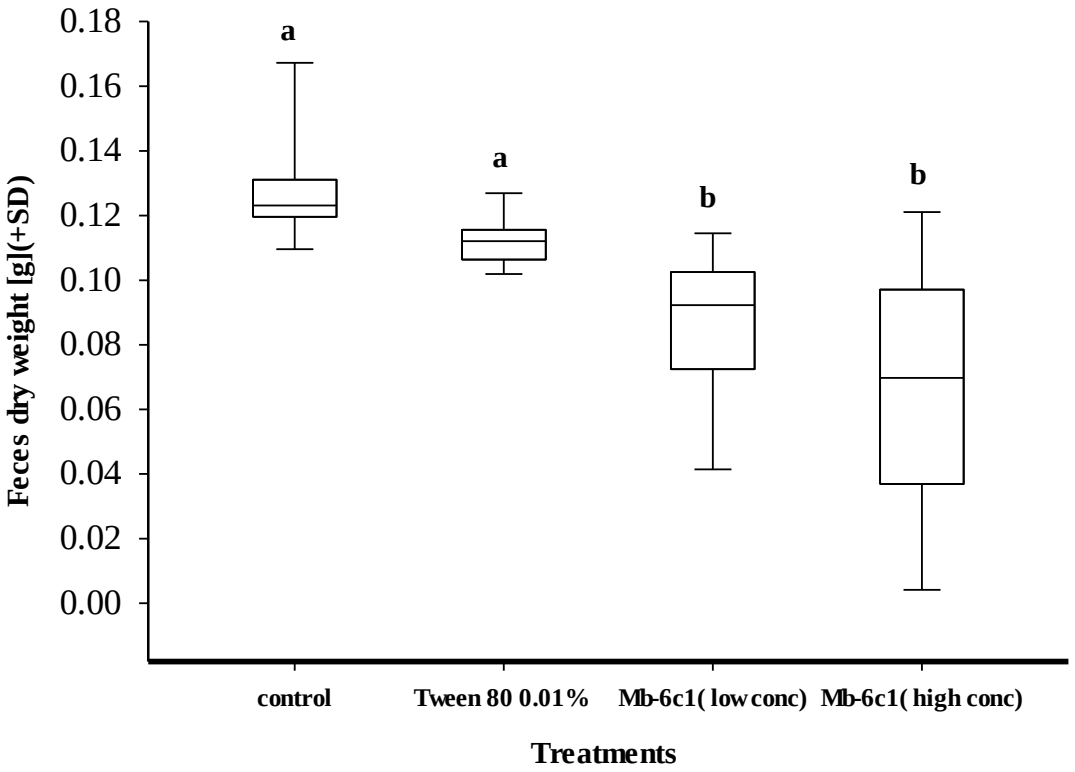

Figure 2.12 Effect of treatments (Mb-6c1 low conc and high conc, Tween80 0.01%, negative control) on dry weight of feces produced by the larvae *Helicoverpa armigera*. Vertical bars with the same letter(s) are not significantly different ($P < 0.05$) using least significant difference.

2.4.8 Number of pods

Pods harvested from each treatment were 2.66 ± 1.29, 2.72 ± 1.27, 2.93 ± 1.43, and 3.0 ± 1.30 for control, Twin80 0.01%, high conc and low conc, respectively (Fig 2.13)

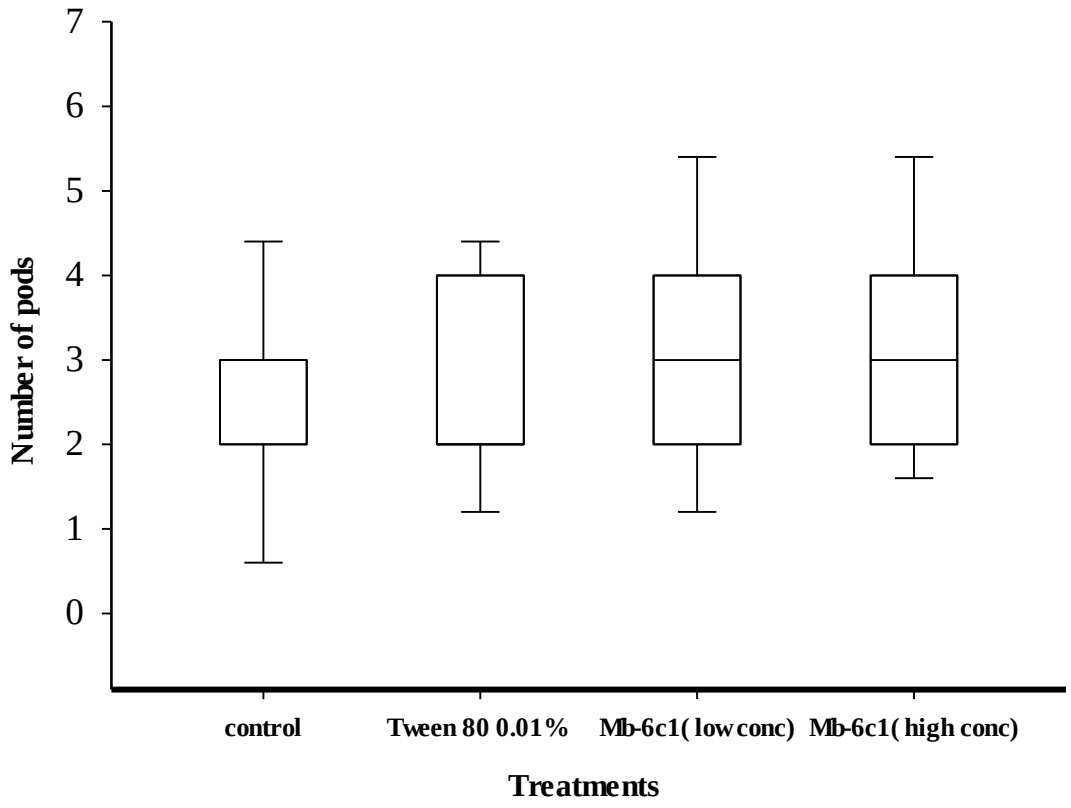

Figure 2.13 Number of pods per plant produced for each treatment (*Metarhizium brunneum* Mb-6c1 low conc and high conc, Tween80 0.1% negative control, toothpick inoculation)

2.4.9 Dry weight of below-ground plant biomass

Fig (2.14) shows the results of the below-ground dry matter of okra plant obtained with high conc and low conc of Mb-6c1 that was significantly ($P < 0.05$) different compared to the control and Tween80 0.01%, however, no significant differences were observed between the control and Tween80 and the same result was reported between the low conc and high conc.

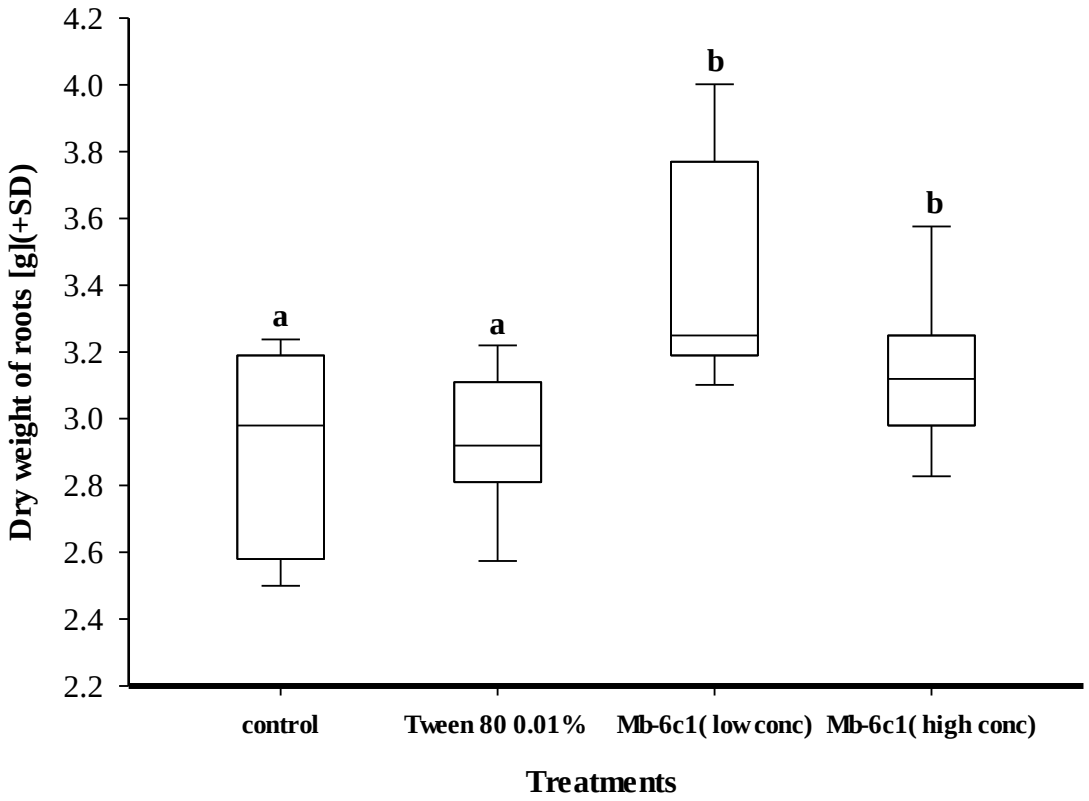

Figure 2.14 Dry weight of roots okra plants for each treatment (*Metarhizium brunneum* Mb-6c1 low conc and high conc Tween80 0.01%, negative control, toothpick inoculation)

2.5 Discussion

The possibility for utilizing a fungal delivering system through endophytic phenomena could be beneficial in managing the population insect pests with such leaves chewing insects as *Helicoverpa armigera*. Our result showed that the toothpick inoculation with a strain of the entomopathogenic fungi *Metarhizium brunneum* (6c1) was able to endophytically-colonize okra plants. After 2 weeks, the cross section of the okra plant showed colonization of Mb-6c1. Previous reported that the ideal inoculation methods include among others seed coating and immersions, root inoculation, stem injection and flower sprays which result in systemic colonization of plants by fungal entomopathogen (Tefera & Vidal, 2009; Biswas et al., 2012; Brownbridg et al., 2012). Thus, our results are in accordance with those obtained by (Gurulingappa et at., (2010) who studied entomopathogenic as endophytic fungi in family Malvaceae. Similar results were reported in other crops, for example. coffee (Posada et at., 2007), corn (Wagner and Lewis, 2000), cassava (Greenfield et al., 2016) and potato (Jones, 1994). Moreover, the mechanism of Metarhizium species to colonize plant tissue are described by many researchers (Behie, Jones, & Bidochka, 2015 ; Akello & Sikora, 2012).

The diet plays an important role in the development of larvae *H armigera*, this is evident from finding of the modified diet that showed better growth and shorter developing period compared to okra fruit block diet. Our result are in line with Wu & Gong, (1997) who reported an average larvae duration 11,7 days for larvae fed with an artificial diet while 15 days for larvae fed on cotton leaves. However, the nutritive value of diets greatly influences the healthiness of growth and development duration of the larvae.

Entomopathogenic fungi can be effective not only when in direct contact with the target host, but thorough endophytic colonization of the effective spore or produces toxic substance acquired by larvae *H armigera*. Our experiments showed that Mb-6c1 is virulent against larvae, on another hand the intoxication of the larvae can have a significant effect on the developing stages.

The patterns of okra leaves damage caused by intoxicated *H armigera* in Mb-c61 leaves were characteristically different. For instance, in the control our negative control the damaged area was regular in shape and the larvae tended to consume the whole leaf and even tried to damage the clip cage, the same pattern was observed for Tween80 0.01% with less severity. However, larvae in okra leaves treated with the low conc and high conc of Mb-6c1 tended to avoid the

endophytic entomopathogenic fungi which resulted in an inconsistent pattern of consumption. Our finding concurs with Russo et al., (2019) who reported that the significant reduction in consumption has been attributed to toxic substance by entomopathogenic fungi inside the host that lead to effect the survival and development of subsequent generation. Also, aphids test choice preferred to fed on plants that were not colonized with entomopathogenic fungi *Fusarium oxysporum* (Martinuz et al., 2012)

In our study, Mb-6c1 was found to cause significant reduction in the larval weight, thus, the results clearly indicate that both low conc and high conc showed significant pupal success (20%, 13%, respectively) and were not able to pupate when larvae are feeding on Mb-6c1 treated plants. However, Tween80 0.01% do not have an effect on larvae of *H armigera*. Our observation is supported by (Revathi et al., (2011) who reported that *M anisopliae* showed more than 70% mortality of *H armigera* in bioassay. Inculcation of plant with entomopathogen fungi showed higher levels of terpenoids which are contemplated secondary metabolites to act as plant defense against an herbivorous insect (Shrivastava et al., 2015)

Survival of fungal pathogens in an edaphic habitat is reported to enhance crop canopy (Álvarez-Loayza et al., 2011) and many of the pathogens can survive saprophytically in soils (Raaijmakers et al., 2009). *M. brunneum* used in this study enhanced the crop growth by means of proliferation of roots and increased vegetative growth which might have played an important role as bio fertilizer and could consequently manage pests. This finding is in agreement with the report of Mukherjee et al., (2013) who reported that the mechanisms by which Metarhizium boosts plant growth are likely to be multifactorial, similarly as reported for species of Trichoderma which stimulate plant-growth-promoting effects that include antibiosis, parasitism, induction of host plant resistance, and competition. However, *M. brunneum* could act as producer of plant growth-promoting auxins on roots and open new window in the industry of plant growth regulation from entomopathogenic fungi.

2.6 Conclusion

This study concluded that treatment of okra plant with Mb-6c1 resulted in the reduction of the consumed area, weight gain, dry weight of feces and pupae success and this could affect the performance of bollworm by means of toothpick inoculation. An endophytic application of Mb-

6c1 significantly altered *H armigera* larval behavior which larvae avoiding treated host plant and reducing their feeding activity. Further studies could evaluate the presence of fungal entomopathogenic into immature insect bodies.

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Chapter Three

A review on Desert Date *Balanites aegyptiaca* L. & Del.: general uses and future prospective as botanical insecticide

3.1 Abstract

The desert date *Balanites aegyptiaca* (L.) Del. (Zygophyllaceae) is an evergreen, woody perennial xerophytic and spinous flowering tree. The tree reaches approximately ten meters in height., widely distributed across dry lands of arid and semi-arid regions. It is traditionally used in urban and rural areas. Approximately, all parts of the plant are useful and have been used as a medication the treatment of a wide range of in Africa and Asia, thus, various parts are used in healing systems and other folkloric medicines for the treatment of wide range of diseases i.e., syphilis, jaundice, liver and spleen problems, epilepsy, yellow fever. Furthermore, it revealed insecticidal, antihelminthic, antifeedant, molluscicidal, larvicidal, and contraceptive activities. Researches have been carried out experiments using different techniques to evaluate the biological active components, in vitro and in vivo. To support most of these claims, this review presents the general uses and future prospective of desert date plant as a botanical insecticide.

Keywords; *Balanites aegyptiaca*, insecticide, antifeedant, antihelminthic, molluscicidal, larvicidal

3.2 Introduction

Balanites aegyptiaca (L.) Delile, known as 'desert date', is a drought-tolerant perennial tropical spiny shrub or tree up to 10 meter in height. it belongs to the family Balanitaceae. It is a multi-branched, evergreen tree distributed throughout the dryland areas of Africa and Asia, namely, in the Sudano-Sahelian region of Africa, the Middle East and South Asia (Hall, 1992; Hines & Eckman, 1993). It is a prevailing natural tree that grows wildly in open woodland or natural savannah sometimes cultivated for its fiber, oil and medicinal values (Zang et al., 2018). It is a multi-purposes tree as it is used for food, fodder, construction and furniture in almost all parts of Africa and South Asia (Fadel Elseed et al., 2002). Also, the whole plant or its proportions and components contain many beneficial materials and essential minerals for human and animal health.

Different parts of *Balanites aegyptiaca* have several bioactive substances (Al Ashaal et al., 2010) which possess distinguished characteristics and important phytochemical components; that include alkaloids, glucosides, flavonoids, phenolics, saponins, tannins, terpenoids, steroids, lipids, proteins, carbohydrates, organic acids, furanocoumarins, Diosgenin, N-trans-feruloyltyramine, N-cis-feruloyltyramine, trigonelline, balanitol and cardiac glycosides (Amadou, Le, & Shi, 2012; Koubala et al., 2013). Moreover, important phytochemical components (secondary metabolites) are useful for the treatment of a wide range of diseases and have potential insecticidal effects against medical, veterinary and agricultural pests. In Sudan *B aegyptiaca* jeopardized due to desertification, overgrazing, and very important factor is cutting to produce a charcoal. (Lazim, 2007) report that *B. aegeyptiaca* leaves and twigs are useful in fodder due to richness of protein in dry season. The potential of *B. aegyptiaca* remains unexplored therefore, the objective of this review is to point out the overall potential use and future prospective as botanical insecticide.

3.3 Botanical taxonomy

The desert date tree *Balanites aegyptiaca* belongs to the family Balanitaceae (Zygophyllaceae) which comprised of approximately 25 genera and 240 species and is predominant in tropical, subtropical and warm temperate regions. It is often found in the drier areas in Africa and Asia. It is a perennial spiny shrub or tree with flowering and fruiting occurring annually (Bhandari,

1990). It is appointed to the following botanical classification which was reported by many researchers (Chapagain & Wiesman, 2006) ; Poongkodi, 2015 ; NPDC, 2017)

Kingdom	Plantae - Plants
Subkingdom	Tracheobionta - Vascular plants
Super division	Spermatophyta - Seed plants
Division	Magnoliophyta - Flowering plants
Class	Magnoliopsida - Dicotyledons
Subclass	Rosidae
Order	Sapindales
Family	(Balanitaceae) Zygophyllaceae - Creosote-bush family
Genus	Balanites Delile – balanites
Species	*Balanites aegyptiaca* (L.) Delile - desert date
Synonyms	*Ximenia aegyptiaca* L. (excl. Balanites roxburghii Planch)
	Agialida senegalensis van Tiegh., Agialida barteri van Tiegh
	Balanites latifolia (van Tiegh.) Chiov (Dwivedi et al., 2009)

3.4 Ecology

Balanites aegyptiaca (L.) Delile is a branching thorny tree, a highly drought-tolerant evergreen plant (Figure 3.1**a**) and a dicotyledonous flowering species (Orwa et al., 2010). The plant can grow in different habitat and climatic conditions (Pandey, 2005). The tree survives in various kinds of soil but is mainly found on alluvial soils with deep sandy loam with free access to water such as valley floors, river banks or the foot of rocky slopes (Chothani & Vaghasiya, 2011). The tree also has good adaptive mechanisms to grow and thrive under combined water and salinity stresses (Maksoud & El Hadidi, 1988). The species is intolerant to shade, and thus, it prefers open woodland or natural savannahs for renewal (Berhaut, 1979; Arbonnier, 2000). The tree can be grown at highlands up to 2000 m altitude with a mean annual temperature of 20 to 30°C and mean annual rainfall of 250 to 400 mm (TWI, 1998; Schmidt; Lars & Joker, 2001).

3.5 Botanical Description

The plant can grow and reach up to 10 m in height (Rathore et al., 2005; Yadav, and Panghal, 2010). The leaves are alternate, bright green, leathery (Figure 3.1**b**), two foliate, petioles are 3–6 mm long, and leaflets are elliptic, obovate and have broadly pointed petioles up to 5 mm long (Chothani & Vaghasiya, 2011). The spines of the plant are simple, straight, stout yellow, rigid, green, alternate, supra-axillary, up to 5 cm long. The inflorescence is supra axillary clusters or rarely supra cemose. Chapagain & Wiesman (2006) reported that the flowers are small, bisexual, yellowish-white (Figure 3.1**b**), fragrant with 5-6 mm in diameter, in axillary clusters. The sepals are five in number (free), ovate and 3 mm long. The petals are five in number (two free), oblong-obovate, longer than the sepals. The stamens are ten in number, filaments glabrous, and anthers are dorsifixed. The ovary is ovoid, silky, five-celled and ovules are solitary in each cell, the style is short and conical. The fruit is date-like and an ovoid drupe found on a short thick stalk and is faintly five grooved, ranges from 2 to 5.6 cm in length and from 1.5 to 4 cm in diameter. Immature fruit is green and tomentose (Figure 3.1**c**). The ripe fruit is yellow or pale brown with a brittle coat (Figure 3.1**d**) and it contains four layers (Mohammed et al., 2002). According to Orwa et al., (2010) the outer skin, fleshy pulp, the woody shell within which the seed is found are referred to as epicarp (Figure 3.1**e**), mesocarp (Figure 3.1**f**), endocarp and the kernel of the nut (Figure 3.1**g**) respectively. The fruit is enclosed in a brown or reddish-brown sticky pulp, the pulp is bitter-sweet and edible. The seed (nut) is a hard stone with light brown, pendulous and ex-albuminous; 1.5 to 3 cm long (Figure 3.1**g**) (Kirtikar, K.R. and Basu, 1933).

Generally, flowering time occurs from October to April, whilst the fruiting takes place from December to July each year (Elghazali et al., 1994; Bein et al., 1996). A mature tree produces 100 - 150 kg of fruits annually. The plant starts flowering and fruiting at 5 to 7 years of age and continues fruits production for about two decades. Maximum fruit production is when the trees reach 15 to 25 years old. The trunk is grooved, short and often branching from near the base and its color ranges from dark brown to deeply grey, the bark is splintered longitudinally and ragged with yellowish-green color and patches where it is shedding (Chothani & Vaghasiya, 2011). In Sudan, *Balanites aegyptiaca* was reported as one of the longest-lived savannah trees living more than a hundred years (Bourliere, F. & Hadley, 1983; Orwa et al., 2010).

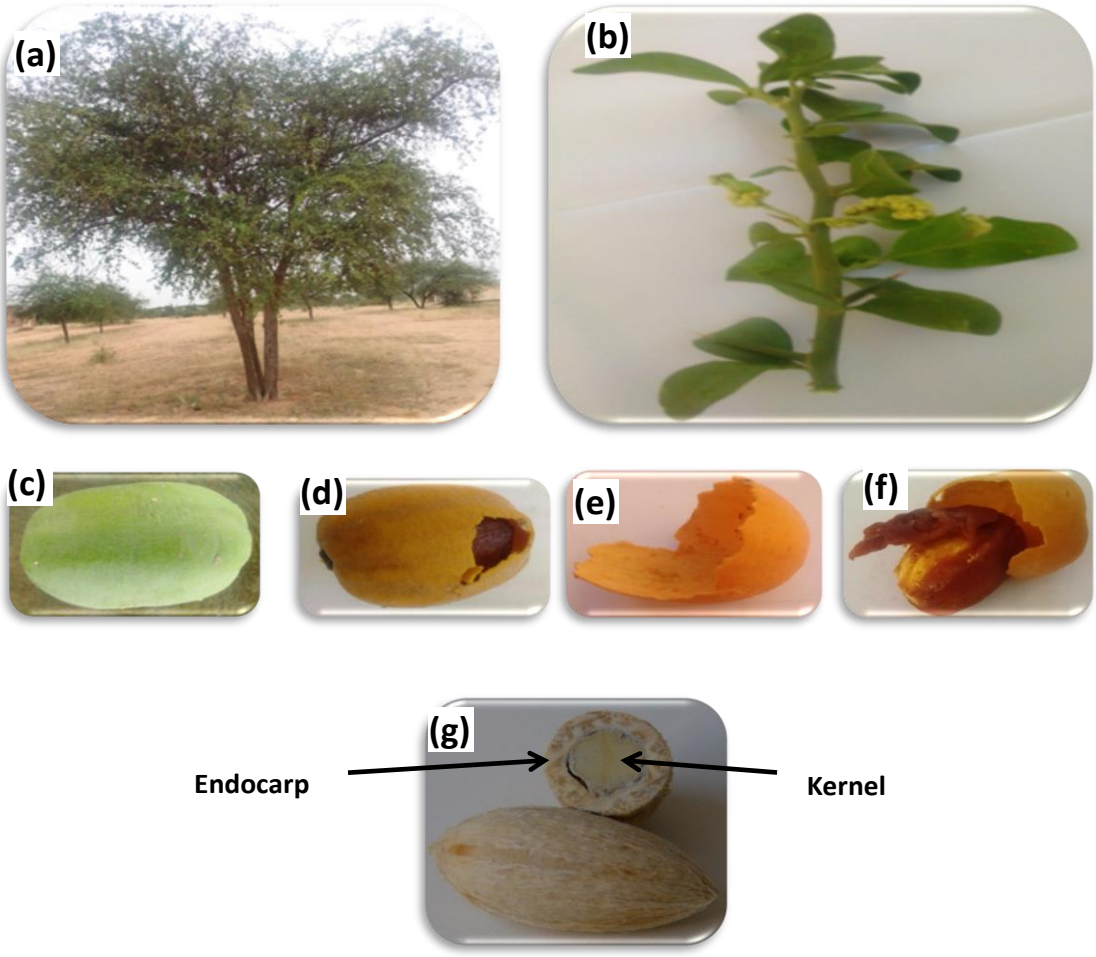

Figure 3.1 *Balanites aegyptica* (L.) Delile, (**a**) tree (**b**) Green leaves with yellow flowers (**c**) Immature fruit (**d**) Mature fruit (**e**) Epicarp (**f**) Mesocarp (**g**) Nut

3.6 General uses

The desert date; *Balanites aegyptiaca* is a multipurpose plant; almost every part of the plant is useful including roots, trunks, barks, leaves, flowers, seeds and fruits (Zang et al.,2017). The tree and its parts are used for food, fodder, shade, building and medicinal applications mostly in all parts of Africa and South Asia (Elfeel, A.A. & Warrag, 2011). However, Gad et al., (2006) explained that the most important part of the tree is its fruits. The fruit is known as the desert date (English) and lalob (Arabic). Many researchers clearly explained that the fleshy pulp of both unripe and ripe fruit is edible and could be eaten fresh or dried as it contains essential minerals.

The seed kernel produces high finesse of oil, the plant contains 30-60%, which is edible and used as cooking oil for over thousand years (Vonmaydell, 1986; Obidah et al., 2009). Pulpy fruit contains sixty to seventy percent of carbohydrates, crude protein, steroidal saponins, vitamin A and C (Dawidar & Fayez, 1969). The kernel is also rich in protein and mineral contents (Okia et al., 2011). The fruit is outstanding banana, guava, mango, and papaya in protein content and quality , it is similar to sesame and groundnuts oils (Abualfutuh, 1983). The leaf of *Balanites aegyptiaca* (L.) Del. is one of the preferable fodder among goats (Elseed et al., 2002), and it plays an important role as range plant in the desert during severe drought. In addition the living tree is used as trap for insect (Gour & Kant, 2012), wind barriers and for agroforestry purposes (Guinand, Y. & Lemessa, 2001; Tesfaye, 2015) . According to Zang et al., (2018) the bark produces fibres and natural gums that are used as glue. Furthermore, Orwa et al., (2010), pointed out that the branches are used as a fence for keeping livestock and the seeds are used to make beads and jewelry. The wood serves as a good source of firewood, charcoal and timber for indoor furniture and tool handles. For medicinal applications approximately all the parts of *Balanites aegyptiaca* are traditionally used in several traditional medicines worldwide. This plant has got enormous importance as being used in the treatment of wide range of illness and disorders, including among others jaundice, intestinal worm infection, skin boils, leucoderma, wounds, malaria, syphilis, epilepsy, dysentery, constipation, diarrhea, hemorrhoid, stomach aches, asthma and fever (Yadav, J. P. & Panghal, 2010). Furthermore, the plant bark is used for the cure of leprosy, epilepsy, yellow fever, jaundice, syphilis and also acts as a fumigant for healing circumcision wounds (Sarker et al., 2000 ; Hamid, O, Wahab M, 2001; Bukar et al., 2004). The boiled root, root bark and root molding of the tree are used as chowder against stomach pain, anthrax, in cure of diarrhea, haemorrhoid and acts as an antidote for snake biting. Moreover, many researchers reported that the seed, fruit and fruit kernel are used as ointments, to cure cough, colic pain, oral hypoglycemic cure, hypocholesterolemic, diuretic, a mild laxative, purgative, mouth ulcer cure, anticancer, antioxidant, anti-inflammatory, antidiabetic antiparasitic and schistosomicide (Ojo et al., 2006; Motaal et al., 2012).

3.7 *Balanites aegyptiaca* as botanical insecticide

According to Tesfaye, (2015) the tree is a multipurpose with considerable economic possibilities for its enormous biological active components. All parts of the plant are used as food or have effects as antidote for cure of infections or pests' control. Also, he recommended that the ranch of tree acting as shelterbelt and soil reformer in arid regions and in saline soil and will be helpful in conservation of biodiversity. Most of the studies of *Balanites aegyptiaca* (L.) Del, that used different plant parts as botanical insecticide, have shown promising results of botanical and insecticidal activities against pest. In his study Mageed (1990) evaluated *Balanites aegyptiaca* as a Mosquito Larvicide. He found that water extracts of fruit kernel were very effective as larvicides against mosquitoes (*Aedes aegypti*, *Aedes arabiensis* and *Culex quinquefasciatus*). Also, significant effect of phytochemical (saponin extract, fruit mesocarp fraction and shoot extract) against larvae by causing larval mortality and dramatically reducing the number of mosquitoes was recorded (Gour & Kant, 2012).

Different extracts from several parts of *Balanites aegyptiaca* were shown to raise antifeedants and molluscicidal activities against various snail pests (Plants & Lymnaea, 2010). Seeds of desert date *Balanites aegyptiaca* (L.) Del. contain many phytochemicals which act as secondary metabolites and have different biocidal effects against store pests such as khapra beetle *Trogoderma granarium* (Elamin & Satti, 2013). A study which tested the effect of leaf powder of *Balanites aegyptiaca* as bio insecticide on Bean weevil *Callosobruchus maculatus* revealed high insecticidal activity increasing mortality rate and repellency (Sani, 2016). Furthermore, Bruchidae et al., (2013) reported that *Balanites aegyptiaca* acetone leaf extract has potential activity, insecticidal effect and repellency properties against cowpea bruchid *Callosobruchus maculatus* F., larvae and pupae. Moreover, hexane extracts from kernel and seed applied against khapra beetle (*Trogoderma granarium*) and red flour beetle (*Castaneum tribolium*) recorded complete mortalities, high insecticidal and superior effects (Mohamed, N. M. 2003). Recently, Wakawa, Sambo, & Yusuf, (2019) tested leaves extract as anaesthetic on a beneficial organism (fish) and showed mortality at 3% concentration.

3.8. Conclusions

Extensive studies carried out using different in vitro and in vivo techniques revealed that most parts and components of *Balanites aegyptiaca* (L.) Del. act as a potential natural pesticide and an alternative to synthetic chemical pesticides. It has been experimentally proved that *B. aegyptiaca* possesses effective components against pests. Similarly, the desert date *Balanites aegyptiaca* (L.) Del., tree can play a leading role and has promising functions to be an attractive and safe alternative in near future against manufactured chemical insecticides, especially in the management of pests of warehouses, houses and fields, in accordance with the global revolution against the widespread of chemical pesticides. Consequently, more extensive studies are needed to provide information on the active principle phytochemical components of this pioneer tree, especially on their nontoxic and environmentally friendly effects.

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Chapter Four

Potential applications of botanical extracts of the *Balanites aegyptiaca* against *Helicoverpa armigera* reared on okra

4.1 Abstract

The effect of oil and water extract on the 2nd instar larvae of *Helicoverpa armigera* reared on okra *Abelmoschus esculentus* were studied to evaluate the potential application of extracts of *Balanites aegyptiaca* as botanical pesticide. The efficacy of the oil and water extracts against the *H armigera* was tested with (2%,5% (v: v) oil extract and 5%,10% (w/v) water extract) of seed kernel of *B aegyptiaca* Del. All treatments significantly influenced the larval survival of the *H armigera*. The 2% oil extract did not significantly differ from the 5% (0.469 ± 0.516 and 0.333 ± 0.488, respectively), however the number of survivals was higher in the negative control (0.869 ± 0.352) and Tween80 0.01% (v: v) (0.800 ± 0.414) while for water extract it was higher in the control (0.867 ± 0.352) than in the 10% (w/v) water extract (0.333 ± 0.488). Furthermore, no significant difference was detected between the control and 5% (w/v) water extract. Oil and water extracts have properties of larvicidal against *H armigera* which could potentially be used as an attractive alternative for pest management.

Keywords; *Balanites aegyptiaca*, *Helicoverpa armigera*, botanical insecticide, larvicidal, *Abelmoschus esculentus*

4.2 Introduction

Cotton bollworm (*Helicoverpa armigera* Hübner 1808; Lepidoptera: Noctuidae) which is a polyphagous in nature is one of the important pests for many crops worldwide (Lammers & Macleod, 2007). The larva is mainly responsible for causing crop damage and it attacks more than 182 plant species, including different important and cash crops, (cotton, sunflower, sorghum, maize), vegetables, fruit crops and tree species (Uddin el al., 2009). The annual yield loss by the cotton bollworm is estimated at about US$ 2 billion in tropical and subtropical regions where approximately US$ 500 million worth pesticides are applied to control it (Kass el al., 2018). The bollworms cause about 10 to 60% okra fruit yield loss and could even reach80% loss under optimal environmental condition (Javed et al., 2019).

Concerns about the widespread of the environmental negative impacts of chemical insecticides coupled with the great damages in many areas that are expected to be caused by the bollworm have led to focus on environmentally sound and sustainable alternative strategies for pest control. Thus, a worldwide resurgence of interest in the use of predators, parasitoids and/or entomopathogenic microorganisms have intensively been studied for biological control of *Helicoverpa armigera* species (Gonzalez et al., 2016), and a significant advance in development and manufacturing of these agents in the future is expected with recent biotechnological discoveries (Hatting, 2012).

Plants or plant materials cantina diverse botanical insecticides that have been widely studied and utilized by researchers. The history of application of botanical insecticides for the protection of crops and stored products from insects has been reported back to 400. b. C in Roma (Dayan et al., 2009), in ancient China (Liu et al., 2007) and different parts of developed countries. Botanical insecticides were extensively used and have been touted as attractive alternative to synthetic chemical products for pest management (Dwijendra Singh, 2014). Prior to the development and commercial success of the synthetic insecticides, people widely used different modified parts of some aromatic plants as repellents against insects (Isman, 2006 ; Benelli et al., 2015)

The desert date tree (*Balanites aegyptiaca*) belongs to the family Balanitaceae (Zygophyllaceae) which comprise of approximately 25 genera and 240 species that are predominant in tropical, subtropical and warm temperate regions. It is often found in the drier areas in Africa and Asia. It

is a perennial spiny shrub or tree with flowering and fruiting occurring annually (Bhandari, 1990). *Balanites aegyptiaca* (L.) Delile is a branching thorny tree, highly drought-tolerant evergreen plant and a dicotyledonous flowering species (Orwa et al., 2010). Many researchers clearly explained that the fleshy pulp of both unripe and ripe fruit is edible and could be eaten fresh or dried as it contains essential minerals. The seed kernel produces finest oil, which is edible and used as cooking oil for over thousand years (Vonmaydell, 1986; Obidah et al., 2009). (Orwa et al., 2010), pointed out that the branches are used as a fence for keeping livestock and the seeds are used to make beads and jewelry. The wood serves as a good source of firewood, charcoal and timber for indoor furniture and tool handles. For medicinal applications; approximately, all the parts of *Balanites aegyptiaca* are traditionally used in several traditional medicines worldwide. This plant has got enormous importance and is being used in the treatment of wide range of illness and disorders, including among others jaundice, intestinal worm infection, skin boils, leucoderma, wounds, malaria, syphilis, epilepsy, dysentery, constipation, diarrhea, hemorrhoid, stomach aches, asthma and fever (Yadav, J. P. & Panghal, 2010).

According to Tesfaye, (2015), the tree is multipurpose with considerable economic possibilities for its enormous biological active components. All parts of the plant are used as food or have effects as antidote for cure of infections or pests' control. Most studies of *Balanites aegyptiaca* (L.) Del, that used different plant parts as botanical insecticide has shown promising results of botanical and insecticidal activities against pest. In his study on evaluation of *Balanites aegyptiaca* as a Mosquito Larvicide Mageed (1990) found that, water extracts of fruit kernel were very effective as larvicides against mosquitoes (*Aedes aegypti*, *Aedes arabiensis* and *Culex quinquefasciatus*). Also, significant effect of phytochemical (saponin extract, fruit mesocarp fraction and shoot extract against larvae by causing most lethal, larval mortality and significantly reducing the number of mosquitoes was recorded (Gour & Kant, 2012). Bruchidae et al., (2013) reported that *Balanites aegyptiaca* acetone leaf extract has potential activity, insecticidal effect and repellency properties against cowpea bruchid *Callosobruchus maculatus* F., larvae and pupae. Moreover, hexane extracts from kernel and seed applied against khapra beetle (*Trogoderma granarium*) and red flour beetle (*Castaneum tribolium*) recorded complete mortalities, high insecticidal and superior effects (Mohamed, N. M. 2003). Recently, Wakawa and Yusuf, (2019) tested leaves extract as anaesthetic on a beneficial organism (fish) and showed mortality at 3% concentration.

The aims of the study were:

- To propose essential oils extracted from *Balanites aegyptiaca* seed water extracts and seed crude oil in water emulsions as well. To evaluate the antifeedant and repellent of extracts from B *aegyptiaca* against 2nd instar larvae *H armigera*.
- To determine the possible significant implications of the produced bio-pesticides.

4.3 Materials and methods

Experiments were carried out under greenhouse conditions and quarantine cabinet at the Department of Entomology, Faculty of Agriculture - Georg-August-University, Gottingen.

4.3.1 Plant material and pest

4.3.1.1 Okra fruit diet

Eggs of *Helicoverpa armigera* were brought from Bayer (Bayer Crop Science Monheim, Germany). Upon hatching, the newly larvae were then transferred separately and placed in rearing containers, petri-dishes (9cm in diameter). Each larva was supplied with okra fruit blocks. The pieces of fruits were maintained as fresh as possible by keeping them on moist filter paper (Fig 4.1**a**). The food was renewed daily and the larvae were kept in climate chamber at 22 °C; 60 % RH; 18:6 LD. The petri-dishes were kept clean from faeces and food remains. When the larvae pupated, the date of pupation was recorded and adult emergence was watched closely to record the pupation period. The newly emerged adult moths were sexed, during which males and females were transferred into oviposition cage (Fig4.1**b**). The cages were simply constructed from a cylinder with its top covered with muslin cloth and its lower end fitted on pot fitted with water, the surface of water pot was separated from the contents of upper chamber by filter paper (125 mm diameter) sheet. Two small specimen glasses were fitted with sponger soaked into a 7% honey solution and applied as food for adult moths and were tied on the walls of the cylinder, small fresh and tender branches of okra plant were placed inside the cage for deposition of the eggs. The branch stalk dipped in water and such branches renewed daily and examined for egg-laying date.

Figure 4.1 (**a**) Rearing petri-dishes (9 cm diameter) with okra block placed on moist filter paper (**b**) oviposition cage

4.3.2 Seed test: Surface sterilization of okra seeds

Okra seeds were surface-sterilized with 70% ethanol for 90 min, then with 5.25% Sodium hypochlorite for 3 min and finally again with 70% ethanol for 90 min. Thereafter, seeds were washed with sterile water three times, then air dried on a filter paper in bicarbonate. For sterilization test, randomly selected seeds were cultured on potato dextrose agar (PDA) and incubated at 25°C for 7 days.

4.3.3 Plant materials

Seeds of okra *Abelmoschus esculentus* (L.) obtained from Agricultural Research Corporation – Sudan. Seedlings were grown in a greenhouse chamber (23 ± 3°C). Two-week-old plants were individually transplanted into plastic pots (15 cm diameter) with a mixture of non-sterile sand and soil. Plants were irrigated regularly and fertilized once a week.

4.3.4 Host plant

After surface sterilization, okra seeds were grown in pots with soil (Neundorfer Einheitswert) and one-third sand, and placed in a greenhouse until BBCH22, watered on demanded and

fertilized with Hakaphos Blau (15/10/15 NPK) and micronutrients (Compo Expert Münster, Germany) according to manufacture instruction on a weekly basis.

4.3.5 Seed powder

fruits of *Balanites aegyptiaca* obtained from Agricultural Research Corporation –Sudan. The dry fruits of desert date were crushed lightly to remove epicars, the mesocarps were then dissolved in water for two days, cleaned and dried to obtain the nuts. The completely dried nuts were grounded as follows; before milling, the mill was cleaned with pressured air as well as 70 % Ethanol. Then the nuts were put into a zip-lock plastic bag and cracked by hitting with stones and hammer. The husks and kernels were manually separated, milled (Retsch Haan, Germany, Type SK1; sieve No.2 with round 2 mm diameter perforation) and sieved through a metal tea sieve (1 mm mesh width). The powdered materials were then stored in darkness (wrapped in tin foil and stored in a box) at 10 °C until further analysis. (Fig 4.2)

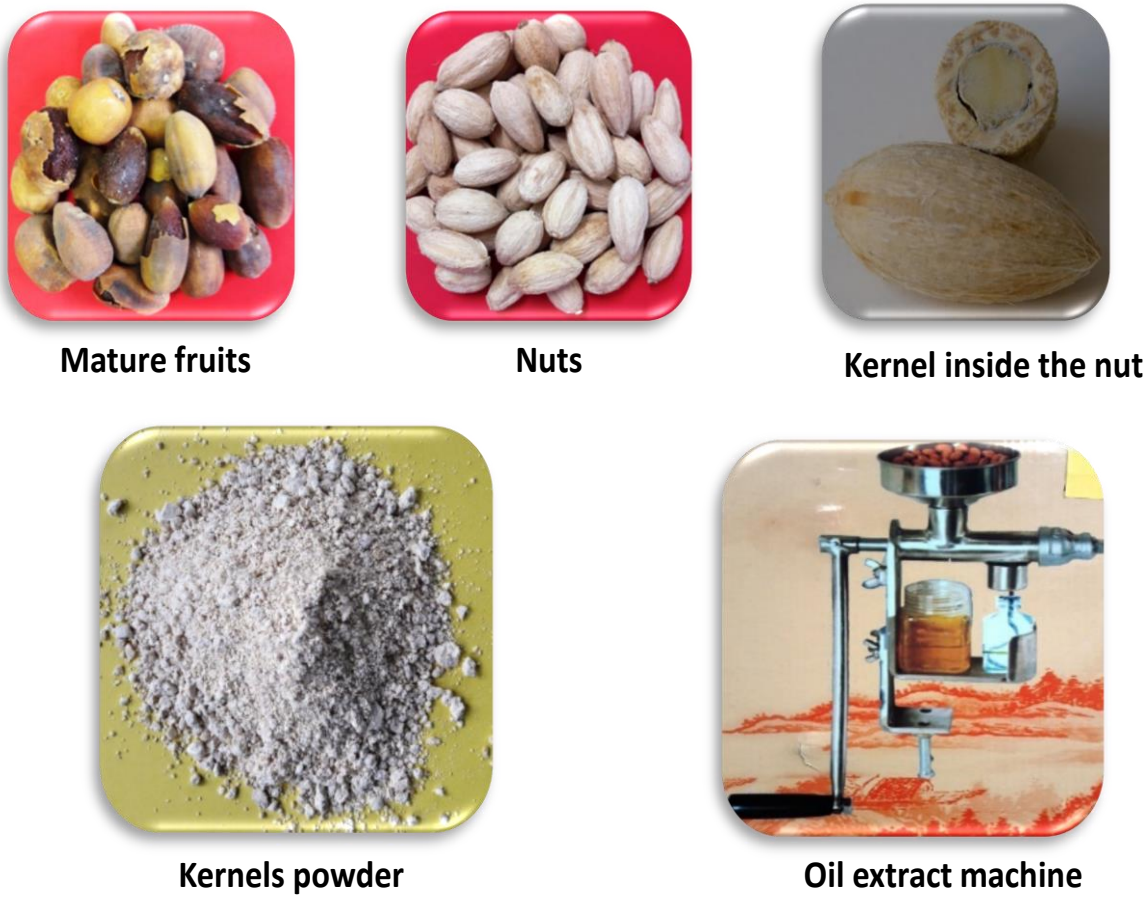

Figure 4.2 Process of oil and water extracts *Balanites aegyptiaca*

4.3.6 Crude oil extract

Kernels were cut with a kitchen knife into smaller sized chunks and pressed with a hand oil press. Then the extracted crude oil was filtered through single layered gaze (mesh dimensions: 0.2 mm length 0.2 mm width). Crude oil and Tween80 were measured and transferred with Eppendorf pipets, H_2o dest. The mixture was measured to the desired treatment amounts with a measuring cylinder and added to the respective spraying bottles (for spraying application) or glass bottles for the behavior assay. The filtered crude oil in its desired treatment concentrations and 0.01 % Tween80 (v/v) in the respective concentrations was added with Eppendorf pipets. The compounds were stirred with a magnetic stirrer for 20 min at 1000 rpm. The resulting emulsions were stored in their respective bottles in darkness (wrapped in tinfoil and stored in a box) at 10 °C until further usage.

4.3.7 Warm water extract

Nut's powder was thoroughly mixed with a teaspoon. It was then weighed with a fine scale into the desired amounts for treatment concentrations and put into wide neck glass Erlenmeyer flasks. The flask contents were stirred with a magnetic stirrer for 20 min at 500 rpm and then placed onto a shaker over night at 250 rpm. On the following day, the suspensions were sieved trough double layered gaze fabric (mesh dimensions: 0.2 mm length, 0.2 mm width) and the filtrates poured into fine muzzle hand spraying bottles for application (Bürkle turn´n´spray, 1.2 ml push-1; ± 0.1 ml, Bürkle Bad Bellingen, Germany). The bottles were stored in darkness (wrapped in tinfoil and stored in a box) at 10 °C until further usage. Calculate the amount of seed material = X needed

$$X= (\text{Concentration } [\%]/100) * \text{Suspension Amount}[ml]$$

4.3.8 Measured variables

4.3.8.1 Eggs hatch rate

Eggs boated from male and female were reared on okra fruit. Eggs harvested from upper side muslin cloth ((Fig 4.1**b**) oviposition cage) were carefully placed with a camel fine brush individually in sterile petri-dish (9cm) with filter paper (ten eggs into petri-dish). Five different treatments namely; control, tap water, Tween80 0.001% (v/v), 2% (v/v) and 5%(v/v) oil extracts of *Balanites aegyptiaca* sprayed with spray flask spray flask (2.8oz, 200ml) for efficacy test against hatched eggs in a climatic chamber under controlled condition (22 °C; 60 RH; 18:6 LD),

and each treatment was replicated 20 times. The rate of hatched eggs in each treatment was recorded.

4.3.8.2 Repellent activity of water and oil extracts (*Balanites aegyptiaca*)

The repellent biomasses of water and oil extract against the larvae of *Helicoverpa armigera* were tested in three treatments, namely; control, 5% (v/v) oil extract and 10% (w/v) water extract. Okra leaves were placed on moist filter paper in Petri-dishes, sprayed with the three above mentioned treatments, and allowed to dry under shade. The released ten 2nd instar larvae were kept on climate chamber at 22 °C; 60 RH; 18:6 LD. Repellent activities were then observed after 2 days.

4.3.8.3 Larvae survival

The efficacy of oil and water extracts of *Balanites aegyptiaca* was tested by spraying the host plant, okra, with 2% (v/v) and 5%(v/v) oil extract and water extract 5%(w/v) and 10%(w/v). Before a leaf was sprayed, the bottle was shaked to ensure the homogeneity of botanical pesticide. The negative control and Tween control (0.001%(v/v) treated areas were allowed to dry for approx. 20 min then introduced with one 2nd instar larva for each treatment and the respective areas were covered with clip cages. After caged leaf areas were consumed by the larvae, treatment applications were repeated on new leaves and the larvae with their clip cages were transferred. This process was repeated until larvae either dead or pupated, then the host plants were placed in a growing chamber at 22 °C; 60 RH; 18:6 LD. Each treatment was replicated 15 times.

4.3.8.4 Pupae weight

At the end of larval stage and the start of the pupal stage, the weight of each insect was determined on sensitive balance.

4.3.8.5 Plant dry biomass

The above-ground plant parts (leaves and stems) were collected. Plants were cut at soil surface and dried in an oven at 60° C for one week till constant weight was achieved. The efficacy of the treatment compared to the control was tested.

4.3.9 Statistical analysis

Data were statistically analyzed with SigmaPlot version (SigmaPlot 14.0). Analysis of variance (ANOVA) was used to test for treatment effect if so, the differences were separated used Bonferroni test at a probability of 0.05, unless otherwise stated.

4.4 Results

4.4.1 Repellent activity of water and oil extract

The 2nd larvae instar of *Helicoverpa armigera* avoided okra leave treated with 5% (v/v) oil and 10% (w/v) water extracts compared to the control which indicates the potential efficacy of extracted materials as depicted in (Fig 4.3)

Figure 4.3 Repellent activities of water and oil seed of *Balanites aegyptiaca* at 10 %, (w/v) 5% (v/v) after 48 h.

4.4.2 Eggs hatched rate

The total number of hatched eggs was significantly ($P < 0.05$) different between treatments. The recorded number of hatched eggs was significantly higher in the control (0.950 ± 0.224) than in oil extract 2% (0.450 ± 0.510) and 5% (0.350 ± 0.489). However, no significant difference was detected among the control, tap water (0.850 ± 0.366) and Tween (0.700 ± 0.470) treatments (Fig 4.4).

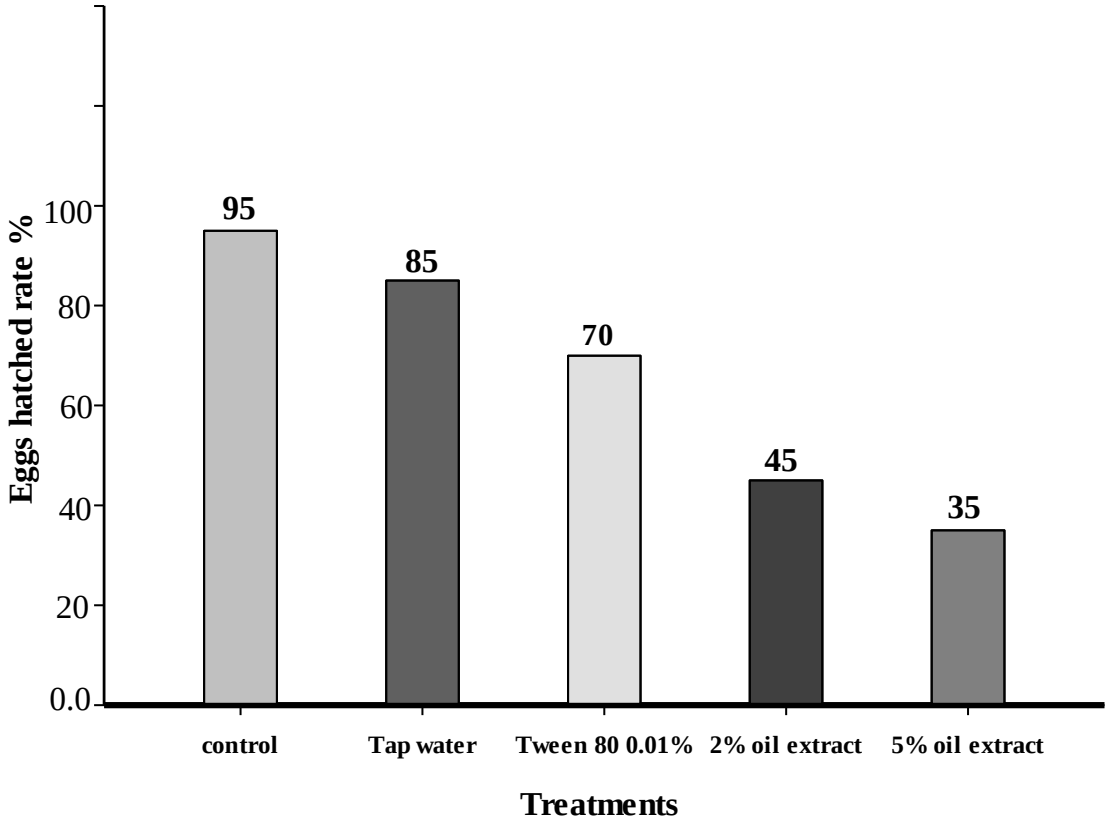

Figure 4.4 Percentage Eggs hatched rate of *Helicoverpa armigera* for each treatment (2% (v/v) and 5% (v/v) oil extract, Tween 80 0.01% (v/v), Tap water, Negative control)

4.4.3 Effect of crude oil on *Helicoverpa armigera* 2nd instar

Survival was significantly ($P < 0.004$) affected by treatment. (Fig 4.5) shows that no significant differences were observed between 2% and 5% oil extract treatment (0.469 ± 0.516 and 0.333 ± 0.488, respectively) however, the number of survivals was higher in the negative control (0.869 ± 0.352) and Tween (0.800 ± 0.414), thus, no significant differences were recorded for the later treatments.

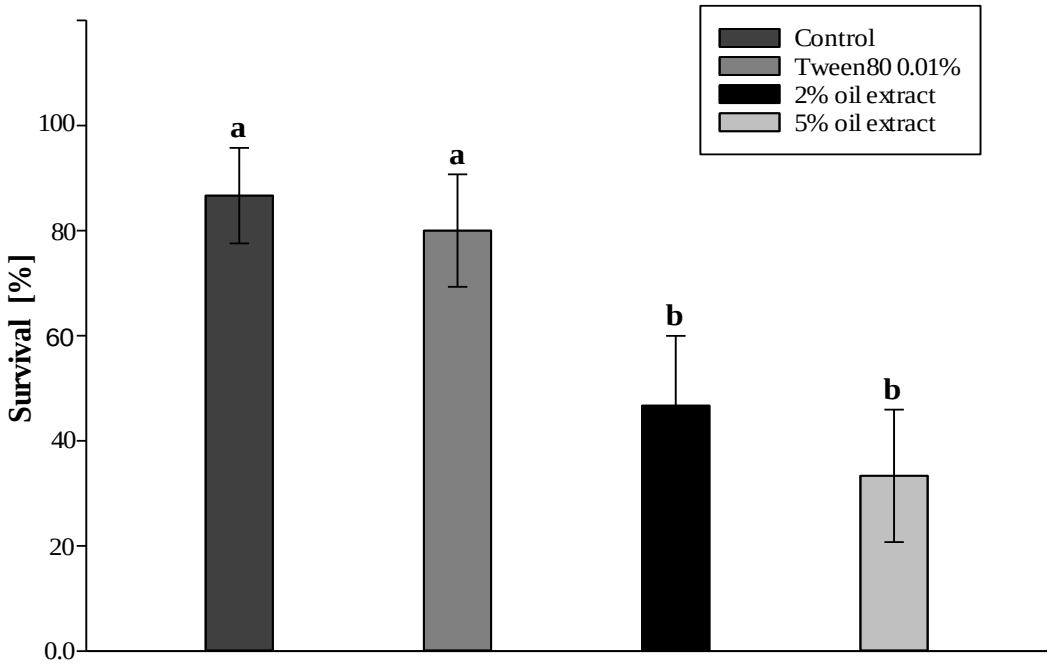

Figure 4.5 Effect of treatments (2% (v/v) and 5% (v/v) oil extracts, Tween 80 0.01% (v/v), Negative control) on larva survival. Same letters are not significantly different using the least significant difference (LSD)

4.4.4 Effect of warm water extracts on *Helicoverpa armigera* 2nd instar

Number of survivals differed significantly between treatments ($P < 0.012$). The number was 0.867 ± 0.352, 0.333 ± 0.488 and 0.53 ± 0.51 for the control, 10% (w/v) water extract and 5% (w/v) water extract, respectively. No difference was detected between the control and 5% (w/v) water extract (Fig. 4.6)

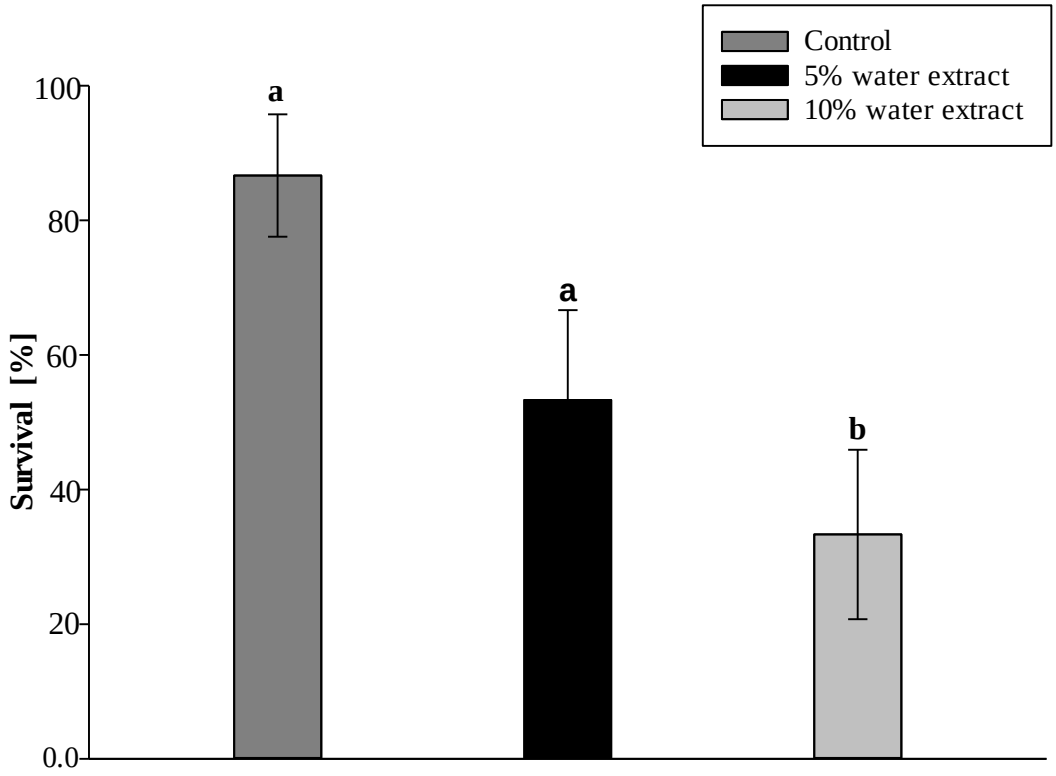

Figure 4.6 Effect of treatments (Negative control, 5% (w/v) and 10% (w/v) water extracts) on larval survival. Same letters are not significantly different using the least significant difference (LSD)

4.4.5 Pupae weight

Pupae weight was significantly (P < 0. 001) influenced by treatments. The pupae weight was higher in the negative control (0.357 ± 0.102) and Tween control (0.292 ± 0.152) than in the 2% and 5 % oil extract (0.171 ± 0.145 and 0.141 ± 0.137, respectively) (Fig. 4.7)

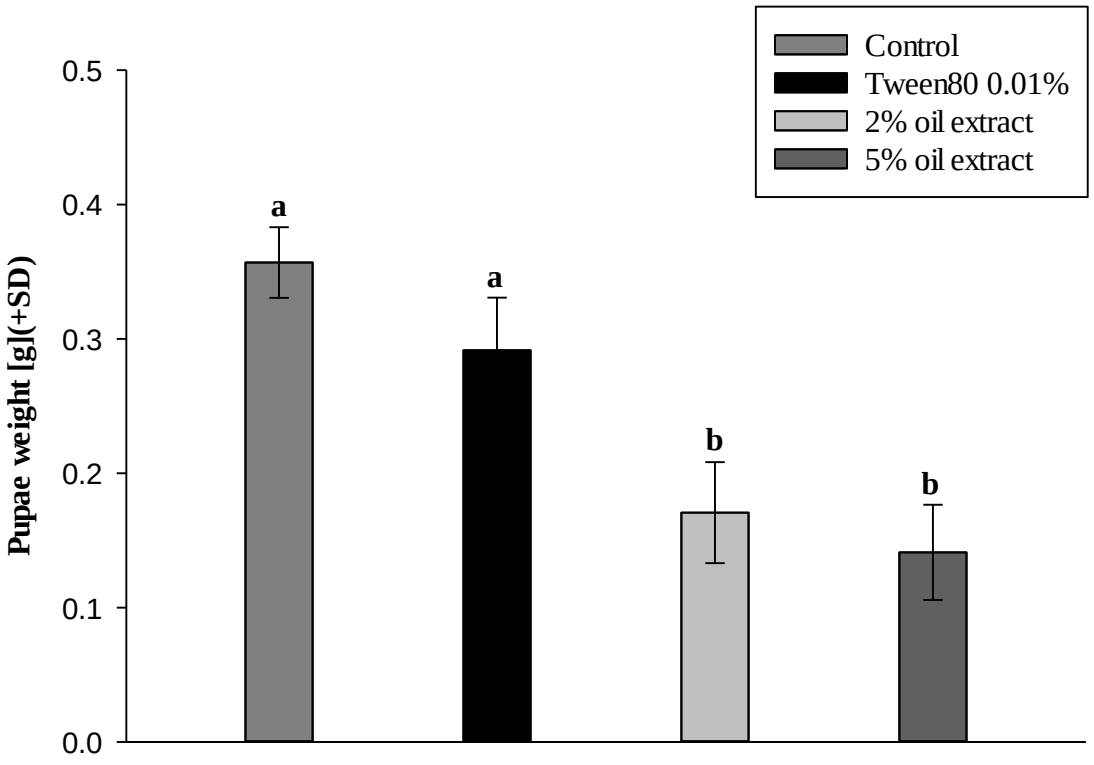

Figure 4.6 Effect of treatments (2% (v/v) and 5% (v/v) oil extracts, Tween 80 0.01% (v/v), Negative control) on Pupae weight. Same letters are not significantly different using the least significant difference (LSD)

4.4.6 Plant biomass for oil extract

Table 1 shows above ground dry biomass of the host plant harvested at 25 days. 5% (v/v) and 2% (v/v) oil extract *Balanites aegyptiaca* was significantly ($P < 0.001$) higher than the negative control and Tween80 0.01% (v/v).

Table 1 Average above ground host plant biomass [g] (±SD) assessed at 25 days. Sorted by treatments. Asterisks (*) indicate significant differences between treatments

Treatments	N	Mean [g](+SD)
5% (v/v) oil extract	15	5.34 ± 0.91*
2% (v/v) oil extract	15	5.18 ± 1.23*
Tween800.01% (v/v)	15	3.34 ± 0.75
Control	15	3.090 ± 0.56

4.4.7 Plant biomass for water extract

Table 2 shows above ground dry biomass of the host plant harvested at 20 days. The results showed that 10% (w/v) water extract was significantly ($P < 0.05$) higher than 5% (w/v) water extract and control.

Table2 Average above ground host plant biomass [g] (±SD) assessed at 20 days. Sorted by treatments. Asterisks (*) indicate significant differences between treatments.

Treatments	N	Mean [g](+SD)
10%(w/v)*Balanites aegyptiaca* water extract	15	5.30+0.90*
5% (w/v) *Balanites aegyptiaca* water extract	15	4.57+1.11
Control	15	3.74+.0.83

4.5 Discussion

The total of *Helicoverpa armigera* eggs hatched differed significantly between treatments ($P < 0.05$) and was significantly higher in the control (0.950 ± 0.224) than in oil extract 2 % (0.450 ± 0.510) and 5 % (0.350 ± 0.489). However, no significant differences were detected between the control, tap water (0.850 ± 0.366) and Tween (0.700 ± 0.470) treatments. The results of oil extract on eggs hatching rate are consistent with many studies that explained that the oil extract of *Balanites aegyptiaca* depressed egg metabolic rate and led to decreased, rapid effect on eggshell by clogging pores which led to increase in water loss and a reduced viability of the eggs. This effect on the development of eggshells itself probably plays a role in controlling the diffusion of gasses that is consistent with Woods et al. (2005) who mentions in his experiment the wax and crystalline chorionic as candidate controlling oxygen and water traffic across the eggshell of *Manduca sexta*. Management over eggshell action may play several important roles in life cycle of *H armigera*, moreover eggs of *Tribolium confusum* exposed to acetone vapors have achieved excellent mortality (Tunç et al., 1997) also Zrubek & Woods (2006) pointed out that hypoxia depressed egg metabolic rates. The eggshell is consists of a waxy layer play the penetrated rather by of oil-based products then by water-based formulations (Campbell et al., 2016).

In all *Balanites aegyptiaca* with exception of the 5% water extract, survival 2nd instar *Helicoverpa armigera* was lower than in the negative control and Tween oil extract which could be due to the toxic compound in oil and water extract. The higher saponins and tannins content of *Balanites aegyptiaca* could be the reason for decreased survival (Amos et al., 2001). Moreover, it concluded that saponin present in *Balanites aegyptiaca*, *Ilex apocea*, (Brem et al., 2002) *Solanum laxum*, (Soulé et al., 2000) *Balanites roxburghii*, (Simandi & Publishers, 1991) *Medicago truncatula* (Lei et al., 2019) interfere with the insects feeding behavior or has property of entomotoxicity.

Un survival of 2nd instar was due to the repellent effect of the phytochemical towards the larvae which reduce feeding by ingestion of the toxic substance which includes alkaloids, glucosides, flavonoids, phenolics, saponins, tannins, terpenoids, furanocoumarins, Diosgenin, N-trans-feruloyltyramine, N-cis-feruloyltyramine, trigonelline, balanitol and cardiac glycosides leading to decreased survival, This effect has also been observed by Gour & Kant, (2012). Many

researchers reported alkaloids have strong antifeedant properties agonist pests (Brem et al., 2002:Mao & Henderson, 2007:Sani, 2014:Gabriel Paulraj et al., 2014).

(Shao, 2017) tested the oil extracted from *Rosmarinus officinalis* on *Tribolium confusum* and recorded 86.22% mortality rate, also Hamzavi & Moharramipour, (2017) in their experiment observed 100% death rate on same pest treatment for the oil extracted from *Eucalyptus camaldulensis*. Moreover, Mageed (1990) on evaluation of *Balanites aegyptiaca* as a Mosquito larvicide found that water extracts of fruit kernel were very effective as larvicides against mosquitoes (*Aedes aegypti*, *Aedes arabiensis* and *Culex quinquefasciatus*). Also different extracts from several parts of *Balanites aegyptiaca* were shown to raise antifeedants and molluscicidal activities against various snail pests (Plants & Lymnaea, 2010). Seeds of desert date *Balanites aegyptiaca* (L.) Del. contains many phytochemicals which act as secondary metabolites and have different biocidal effects against store pests such as khapra beetle *Trogoderma granarium* (Elamin & Satti, 2013).

The results clearly indicate that both water and oil extracts of *Balanites aegyptiaca* showed survival and pupae weight reduction over control of *H armigera*. Hence, it was concluded that hydrophilic compounds were present in desert date that involved in reducing survival of bollworm larvae. Moreover, it was concluded that there are some secondary metabolites present in *Balanites aegyptiaca,* Datura, (Jawalkar et al., 2016) *Pyrethrum,* (Ojiako, Dialoke et al., 2015) Rotenone, (Zubairi, Othman et al., 2016) *Carvone*, (Koul et al., 2008) Jatropha, (Bashir & El Shafie, 2013) and Neem (Campos et al., 2016) that are capable of inhabiting the larval of herbivores.

Plant biomass was significantly influenced by treatments depending on the area consumed by larvae *Helicoverpa armigera*. For example, in the negative control and tween control the pest has the ability to consume the whole area around the clip cages which covers the treated area while for the oil and water extract of *Balanites aegyptiaca* it has less capability to damage the clip cages area due to phytochemical compounds acting as antifeedant and repellent substances. This agrees with Berenbaum, (2010) who mentioned that phytochemical such as furanocoumarins act as skip of a side against herbivores. Moreover, desert date has plenty of tannin compounds which have negative influence on the insect in addition to that other studies showed that saponins also have potent narcotic activity (Amos et al., 2001)

4.6 Conclusions

The result illustrated that the extracts of *Balanites aegyptiaca* exhibited a wide spectrum of biopesticide activities, our results proved the repellent and antifeedant effect of oil and water extracts of desert date. This assessment also showed that oil and water extracts had an age-dependent toxicity against *H armigera* larvae 2nd instar. Generally, our result demonstrates the efficacy of *B aegyptiaca* against *H armigera* and also pointed out the pathways in which the influence of oil and water extracts may be recognized as a means of managing bollworm. The reduction in the number of larvae could effectively reduce the old-world bollworm populations in subsequent generations. More research is needed to extensively study what is pointed out by our experiment which includes:

- localize the fraction which contains the active compounds
- The question here was whether the observed repellence of the compound responsible could be confirmed.

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D E C L A R A T I O N S

1. I, hereby, declare that this Ph.D. dissertation has not been presented to any other examining body either in its present or a similar form. Furthermore, I also affirm that I have not applied for a Ph.D. at any other higher school of education.

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2. I, hereby, solemnly declare that this dissertation was undertaken independently and without any unauthorised aid.

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