

Evaluation of Certain Botanical Preparations against African Bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: noctuidae) and Non Target Organisms in Chickpea, *Cicer arietinum* L

Nigussie Lulie and Nagappan Raja*

Department of Biology, Faculty of Natural and Computational Sciences, Post Box-196, University of Gondar, Gondar, Ethiopia

Abstract

Aqueous extract of individual and mixed form of *Azadirachta indica* A. Juss seeds kernel and leaves of *Milletia ferruginea*, Hochst and *Croton macrostachyus* Hochst was tested against African bollworm, *Helicoverpa armigera* Hubner. Antifeedant activity of selected plant extract was tested at 1%, 2.5%, 5% and 10% concentration against 4th instar larvae of *H. armigera* in the laboratory and 5% concentration was tested under field condition. All the tested plant extract showed 100% protection at 5% and 10% concentration. Among the various botanical treatment Neem Seed Kernel Extract (NSKE) and NSKE+BLE (Birbira Leaves Extract) was effective at 2.5% concentration with minimum pod damage. In the field observation among the botanicals, reduction of larval population in the treatment of NSKE, BLE and NSKE+BLE was statistically not significant ($p>0.05$; LSD). The lowest percentage pod damage (0.45%) was observed in Diazinon 60% EC treated plot followed by NSKE treated plot (3.90%) after second spray.

The highest mean yield was obtained from NSKE treated plot (781 g) followed by Diazinon 60% EC treated plot (719.33 g), NSKE+BLE (656.67 g) and BLE treated plot (653.33 g). Five days after second treatment there was a significant difference in the mean number of ants between NSKE+BLE, control and other treatments. The highest mean number of spiders was observed in control plot (3.6) and lowest (0.3) was in plot treated with Diazinon 60% EC. The reduction of lady bird beetle population among the botanicals treated plots was statistically not significant ($p>0.05$; LSD). The mean number of wasp population was the highest in control plot (3.3) and there was no wasp observed in Diazinon 60% EC treated plot. In conclusion, even though Diazinon 60% EC was found to be effective by considering the interaction of beneficial in the field botanical preparations are much better particularly NSKE and also suitable to spray under rain fed condition to protect the crop by small farming communities.

Keywords: Botanicals; *Helicoverpa armigera*; *Azadirachta indica*; *Milletia ferruginea*; *Croton macrostachyus*; Non-target organisms; Aqueous extract

Introduction

Chickpea is an important source of protein, carbohydrate, fibres, oil, calcium, phosphorus, magnesium, iron, zinc, β -carotene, unsaturated fatty acids. In addition, improves soil fertility by fixing atmospheric nitrogen in to soil [1,2]. Ethiopia is a largest producer of chickpea in Africa accounting for about 46% of production during 1994-2006 [3] and seventh largest producer in worldwide [4]. One of the constraints to reduce yield loss in chickpea is African bollworm, *Helicoverpa armigera* which causes 80% pod damage in early sown chickpea [5]; 21 to 36% pod damage in central highlands [6] and 100% pod damage in some localities of Yilmana Densa and Achefer areas in Gojjam in the 1990s [7]. Globally botanical pest management is gaining appreciation because of multiple mode of action such as antifeedant which inhibit normal development of insects, repellent, antijuvenile hormone activity, oviposition/ hatching deterrence, antifertility or growth disrupters and chemosterilants [8]. According to Purohit and Vyas [9] about 2121 plant species are reported to use in pest management programs. In Ethiopia, even though with rich floral diversity, about 30 plant species are recorded and most of them are used traditionally for the management of storage pests [10].

Azadirachta indica (Neem) and their products are considered as effective botanical pesticides due to controlling wide variety of insect pest including *H. armigera* [11]. Tebkew et al. [12] reported that crude neem extracts prepared from neem seeds collected from Melka Woreda has significantly reduced the percentage pod damage; similarly pod

damage on treated chickpea plot was lower than untreated plots [13]. The predatory lady bird beetle (*Mallada signatus*) pupation was delayed when they fed with neem product treated larvae of *H. armigera* thereby increasing the individual predatory activity [14,15]. In East Africa, predatory ants and anthocorids are most important natural enemies of *H. armigera* on corn, sorghum and sunflower [16]. Among the predators, ants kill up to 90% of *H. armigera* pupae in the soil [17]. Some other natural enemies such as wasps feed on egg and larvae; ants feed on egg, larvae and pupae; preying mantids feed on egg; spiders feed on egg, larva and adults of *H. armigera* [18]. However, indiscriminate use of chemical pesticides and their continuous application create intolerable environment to natural enemies and also to prevent re-entry in treated areas [16,19]. The neem products and their utilization are increasing world wide but in Ethiopia their use was not well explored in farming communities.

*Corresponding author: Nagappan Raja, Department of Biology, Faculty of Natural and Computational Sciences, Post Box-196, University of Gondar, Gondar, Ethiopia, E-mail: nagappanraja@yahoo.com

Received January 04, 2013; Accepted January 27, 2013; Published February 02, 2013

Citation: Lulie N, Raja N (2012) Evaluation of Certain Botanical Preparations against African Bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: noctuidae) and Non Target Organisms in Chickpea, *Cicer arietinum* L. J Biofertil Biopestici 3:130. doi:10.4172/2155-6202.1000130

Copyright: © 2012 Lulie N, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Milletia ferruginea (Birbira) belongs to the family Leguminaceae and subfamily Papilionoideae is a multipurpose tree widely distributed in Ethiopia [20,21]. The seeds and roots containing the toxic principle rotenone was widely used as insecticides and piscicides (to kill fishes) [22,23]. The crude extracts from the seed was toxic to stored grain pest *Sitophilus zeamais* [24]; aqueous and organic solvent extracts of seeds have killing effects on three species of aphids by contact [25] and aqueous seed extracts proved to show 100% mortality on termites under laboratory conditions [26].

Croton has economic importance due to its essential oil content and various biologically active substances such as terpenoid, flavonoids and alkaloids [27-31]. Several species of Croton are reported to have wide range of insect controlling properties. According to Kubo et al. [32] diterpenes from *C. cajucara* inhibit the growth of *Heliothis virescens*. The dichloromethane and ethyl acetate fraction of *C. urucurana* causes 65% mortality in the larvae of *Anagsta kuehniella* due to the action of phenolic compounds catechin and galocatechin [33]. The adult mortality of *Dystercus maurus* was significantly higher in *C. urucurana* treated insects [34]. Even though, biopotential of Croton species are explored well in worldwide but there is no much work in Ethiopia. Therefore, present study was aimed to evaluate aqueous extract of *Azadirachta indica* seeds kernel, leaves of *Milletia ferruginea* and *Croton macrostachyus* against African bollworm *Helicoverpa armigera* and some non-target organisms interaction in chickpea field.

Materials and Methods

Plant materials collection and extraction

Mature and healthy *Azadirachta indica* seeds were collected from Metema area; located at 175 km west of Gondar town, Ethiopia. The pulp of the seed was removed initially and shade dried; later seed coat was removed from the dried seeds and the kernel was powdered by using mortar and pestle. Sixteen hours before spraying, botanical extract was prepared from the powder by following the procedure of Jain and Bhargava [35]. According to this procedure to prepare 5% concentration, 5 kg of neem seed kernel powder was dissolved in 100 liters of water and used to spray on one hectare of crop land. Since the experimental plot size in the present study for one treatment was 21.6 m², for this reason 10.8 g of neem seed kernel powder was soaked in 21.6 ml of water and stirred periodically to mix the contents well. After 16 h, contents were filtered by shema cloth and the volume was made up to 216 ml by adding freshwater. Finally, 30 ml of detergent soap solution was added as an emulsifier and sprayed. Leaves of *Milletia ferruginea* and *Croton macrostachyus* were collected in and around Gondar town, shade dried and powdered separately by using mortar and pestle. Three days before spray schedule, powdered leaves were soaked in water and stirred well periodically to facilitate thorough mixing. According to the procedure of Dodia et al. [36], 5% leaf extract was prepared and 30 ml of detergent soap was added as an emulsifier and sprayed.

Antifeedant activity

Antifeedant activity of the plant extract was tested at 1%, 2.5%, 5% and 10% concentration against IVth instar larvae of *H. armigera* in the laboratory by following the procedure of Jain and Bhargava [35] and Dodia et al. [36]. Healthy immature chickpea pods were collected from field and sprayed with respective concentration of plant extract individually. In a clean glass container 5 sprayed pods were kept and single healthy 4th instar larva of *H. armigera* was added to avoid cannibalism. The chickpea pods treated with soap solution was considered as untreated control and field recommended dose (1 L/

hectare) of Diazinon 60% EC was considered as positive control. The experiment was replicated three times and number of pod damaged by the larva of *H. armigera* was recorded up to 48 h and percentage of pod damage was calculated.

Field spraying of botanical preparations

Field study was conducted at Maksegnit, Gondar Zuria Woreda of North Gondar administrative zone in Amhara regional state, Ethiopia. The study area was located at an altitude ranging from 1912 -2848 masl, latitude 12°11' 24" N – 12°39' 40" N and longitude 37°24' 48" E -37°36' 00" E. The mean annual rain fall in the study area was 992.5mm and annual temperature was ranged from 13.5°C to 28.5°C. The study area was characterized by wet season from June to September and dry season from October to May. The botanical treatment was arranged in Randomized Complete Block Design (RCBD) with three replications [37]. The study plot (3 m×2.4 m) was divided into three blocks and each block further divided in to eight subplots. There was 1.5 m space between blocks and 1m space between subplots to facilitate easy movement for spraying and data collection and also to avoid mixing of botanical spray from one plot to another. Local desi variety of chickpea seeds were sown (<5 cm depth) in eight rows in each plot in early September 2011. The distance between the rows was 30cm and between the plants was 10 cm. Among the eight plots in each block six was treated with botanical preparations, one with chemical pesticide (Diazinon 60% EC) and one was untreated control. The details of the treatments given in the field was as follows; 5% Neem Seed Kernel Extract (NSKE); 5% Croton Leaves Extract (CLE); 5% Birbira Leaves Extract (BLE); 2.5% NSKE+2.5% BLE; 2.5% CLE+2.5% BLE; 2.5% NSKE+2.5% CLE; positive control Diazinon 60% EC and untreated Control. The field spraying was done in the evening time after sun shed to increase the residual action of botanicals since they degrade rapidly by UV light. A total of two round sprays were undertaken before harvesting.

Estimation of pods damage

The number of chickpea pods damaged by the larvae of bollworm was counted before and after each spray schedule on predetermined and tagged five chick pea plants/plot. After the completion of botanical treatment, total number of damaged and undamaged pods was counted and percentage of pod damage was calculated by using the following formula.

$$\% \text{ pod damage} = \frac{\text{Total number of pods damaged}}{\text{Total number of pods}} \times 100$$

Estimation of non-target organisms

The number of non-target organism such as ants, wasps, lady bird beetles and spiders were counted and recorded on each plot. Data were taken for three times before treatment, after first and second treatment. Mean number of non-target organisms per plot was calculated.

Estimation of African bollworm larvae

The number of bollworm larvae was counted by visual observation on ten chickpea plant/plot and recorded. The data was collected before treatment, after first and second treatments. Mean number of *H. armigera* larvae per 10 plants was calculated.

Estimation of yield and yield loss

Chickpea crops were harvested from each plot separately, thrashed and weighed by using balancer. Mean yield weight was calculated for each treated plots and projected yield for hectare was calculated. The

percentage yield loss was calculated by using the following formula suggested by Judenko [38] and Walker [39].

$$\% \text{ Yield loss} = \frac{\text{MCYTP} - \text{MCYUP}}{\text{MCYUP}} \times 100$$

Whereas MCYTP=Mean Chickpea Yield of Treated Plot

MCYUP=Mean Chickpea Yield of Untreated Plot

Statistical analysis of data

The data collected from antifeedant activity of plant extracts in the laboratory and field trial was subjected to ANOVA followed by Least Significant Difference (LSD) test to separate individual mean significant difference at 5% level ($p < 0.05$) by using to SPSS version 16.

Results

Antifeedant activity of the plant extracts against IVth instar larvae of *H. armigera*

Antifeedant activity of tested plant extracts results demonstrates that at 1% concentration minimum pod damage of 13.33% was observed in NSKE and NSKE+BLE which was statistically not significant ($p > 0.05$; LSD). However, compared to other treatments and untreated control the result was statistically significant. At 2.5% concentration, NSKE+BLE combination showed minimum pod damage (3.33%) compared to other treatments. Among the different botanicals treatment except CLE, remaining results were statistically not significant ($p > 0.05$; LSD). The chickpea pods treated at 5%, 10% concentration of the plant extracts and positive control Diazinon 60% EC showed 100% protection against *H. armigera* larval infestation. All the control groups were observed with 100% pod damage (Table 1).

Effect of botanical extracts on *H. armigera* larvae in the field

Pre and post spray count for mean number of *H. armigera* larvae was recorded and presented in table 2. The distribution of larval population in the plot was not uniform before treatment. The plot assigned for different botanical treatment results showed significant difference within the plot ($p < 0.05$; LSD). The minimum number of larvae (9.0) was observed in plot allotted for the treatment of NSKE+BLE. Five days after first treatment with botanical application larval population was reduced significantly at 5% level ($p < 0.05$) compared to control. Among the various botanical treatment, reduction of larval population in the treatment of NSKE, BLE and NSKE+BLE was statistically not significant ($p > 0.05$; LSD). Five days after the second application mean number of *H. armigera* larvae was further reduced in each treatment.

Treatments	Concentration tested			
	1%	2.5%	5%	10%
NSKE	13.33 ± 5.77 ^c	10.0 ± 10.0 ^c	0.0 ± 0.00	0.0 ± 0.00
CLE	36.6 ± 5.77 ^b	30.0 ± 10.00 ^b	0.0 ± 0.00	0.0 ± 0.00
BLE	36.6 ± 5.77 ^b	13.3 ± 5.77 ^c	0.0 ± 0.00	0.0 ± 0.00
NSKE + BLE	13.3 ± 5.77 ^c	3.33 ± 5.77 ^c	0.0 ± 0.00	0.0 ± 0.00
CLE + BLE	36.6 ± 5.77 ^b	13.3 ± 5.77 ^c	0.0 ± 0.00	0.0 ± 0.00
NSKE + CLE	36.6 ± 5.77 ^b	13.3 ± 5.77 ^c	0.0 ± 0.00	0.0 ± 0.00
Diazinon 60% EC	0.0 ± 0.00 ^d	0.0 ± 0.00 ^c	0.0 ± 0.00	0.0 ± 0.00
Control	100.0 ± 0.00 ^a	100.0 ± 0.00 ^a	100.0 ± 0.00	100.0 ± 0.00

Values are mean percentage pod damage ± standard deviation of three replications. Within the column similar alphabets are statistically not significant by LSD ($p > 0.05$).

Table 1: Mean percentage of pod damaged by 4th instar larva of *H. armigera* exposed to different concentration of aqueous extracts.

Treatment	Before spraying	After first spray	After second spray	% reduction
NSKE	13.33 ± 2.51 ^{bc}	6.66 ± 2.08 ^c	1.33 ± 1.15 ^e	90.02
CLE	13.0 ± 4.00 ^a	10.66 ± 3.51 ^b	6.66 ± 2.51 ^b	48.77
BLE	10.14 ± 1.52 ^{cd}	7.14 ± 1.00 ^c	3.28 ± 2.31 ^d	67.65
NSKE + BLE	9.0 ± 4.00 ^d	4.33 ± 1.52 ^{cd}	3.33 ± 1.52 ^d	63.00
CLE + BLE	12.33 ± 7.02 ^{ab}	8.0 ± 4.58 ^c	4.33 ± 1.15 ^{cd}	64.88
NSKE + CLE	13.3 ± 6.02 ^a	7.0 ± 2.64 ^c	5.3 ± 2.08 ^{bc}	60.15
Diazinon 60% EC	10.6 ± 2.51 ^c	2.6 ± 2.51 ^d	0.0 ± 0.00 ^f	100
Control	10.1 ± 2.00 ^{cd}	17.3 ± 3.57 ^a	14.0 ± 2.64 ^a	-38.66

Values are mean ± standard deviation of three replications. Within the column similar alphabets are statistically not significant by LSD ($p > 0.05$).

Table 2: Mean number of *H. armigera* larvae recorded in experimental plot before and after spraying.

The minimum number of larva (1.33) was observed in NSKE treated plot followed by BLE (3.28) and NSKE+BLE (3.33). In positive control Diazinon 60% EC treated plot, there was no *H. armigera* larva. The percentage reduction of larval population was 100% in Diazinon 60% EC treated plot followed by NSKE (90.02%) when compared to before spraying. However, in control plot the larval population was increased compared to before spraying.

Chick pea pod damaged by the larvae of *H. armigera*

Mean number and the percentage of pods damaged by *H. armigera* before treatment, after first and second treatments were presented in table 3. Five days after first treatment, mean number of pods damaged by the larvae of *H. armigera* was significantly ($p < 0.05$) decreased in treatments compared to control. The lowest percentage pod damage (0.45%) was observed in Diazinon 60% EC treated plot followed by NSKE treated plot (3.90%) after second spray. There was no statistically significant pod damage between NSKE and BLE treatment. The highest percentage of pod damage was observed in untreated plot (22.22%) followed by CLE+BLE (14.52%), CLE (14.33%), NSKE+CLE (9.41%), NSKE+BLE (8.00%) and BLE (5.22%) treated plot. Among the six botanical treatments NSKE was proved to be more effective in reducing pod damage in the field.

Values are mean ± standard deviation of pods damaged in 5 plants/plot. Within the column similar alphabets are statistically not significant by LSD ($p > 0.05$).

Effect of botanicals on chickpea yield

The yield of processed chickpea at the end of cropping season from each treatment was recorded (Table 4). The highest mean yield was obtained from NSKE treated plot (781 g) followed by Diazinon 60% EC treated plot (719.33 g), NSKE + BLE (656.67 g) and BLE treated plot (653.33 g). Where as lowest mean chickpea yield was obtained in control plot (419.33 g) followed by CLE (522 g) and NSKE + CLE (525 g) and CLE + BLE treated plots (556.67 g). Overall yield was significantly higher in treated plots compared to untreated plot. From the result it is clear that highest yield was recorded from plots treated with NSKE and positive control Diazinon 60% EC. The yield obtained from NSKE treated plot and Diazinon 60% EC treated plot was statistically not significant ($p > 0.05$; LSD value 89.19). The overall percentage of yield loss was 46.31% and 41.70% if the field was not treated with NSKE and Diazinon 60% EC respectively.

Effect of botanicals and chemical pesticides on non target organisms

The mean number of non target organisms in the field before

Treatment	Before spraying	After first spray	After second spray	Total number of pods
NSKE	16.6 ± 3.57 ^c (9.18%)	8.8 ± 3.41 ^f (4.87%)	7.06 ± 2.8 ^d (3.9%)	180.67
CLE	22.3 ± 5.16 ^b (15.07%)	25.6 ± 6.25 ^b (17.3%)	21.2 ± 4.81 ^b (14.33%)	147.93
BLE	18.7 ± 2.93 ^c (11.36%)	12.1 ± 2.57 ^e (7.35%)	8.6 ± 2.92 ^d (5.22%)	164.6
NSKE + BLE	20.8 ± 3.91 ^b (13.21%)	16.8 ± 2.5 ^d (10.67%)	12.6 ± 2.91 ^c (8.00%)	157.47
CLE + BLE	28.8 ± 6.34 ^a (19.36%)	29.8 ± 5.78 ^a (20.03%)	21.6 ± 6.00 ^b (14.52%)	148.73
NSKE + CLE	28.8 ± 5.47 ^a (19.08%)	21.2 ± 4.93 ^c (14.05%)	14.2 ± 4.97 ^c (9.41%)	150.87
Diazinon 60% EC	25.9 ± 5.78 ^b (14.64%)	2.26 ± 2.08 ^a (1.27%)	0.8 ± 1.47 ^e (0.45%)	176.87
Control	20.5 ± 7.14 ^{bc} (13.13)	32.6 ± 9.75 ^a (20.88%)	34.7 ± 15.12 ^a (22.22%)	156.13

Table 3: Mean number of damaged pods recorded in control and experimental plot.

Treatment	Yield/g/plot	Projected yield per/ ha in kg	% yield loss / hectare compared to control if not treated
NSKE	781 ± 16.28 ^a	1446.3	46.31%
CLE	522 ± 49.11 ^{cd}	966.67	19.67%
BLE	653.33 ± 15.27 ^b	1209.87	35.82%
NSKE + BLE	656.67 ± 70.23 ^b	1216.05	36.14%
CLE + BLE	556.67 ± 18.58 ^c	1030.87	24.59%
NSKE + CLE	525 ± 39.23 ^{cd}	972.22	20.13%
Diazinon 60% EC	719.33 ± 49.00 ^{ab}	1332.09	41.70%
Control	419.33 ± 95.44 ^d	776.54	-

Values are mean ± standard deviation of three replications. Similar alphabets within the column was statistically not significant by LSD (P>0.05).

Table 4: Chickpea yield per plot in grams.

spraying, after first and second spraying was recorded. When consider the population of ants before treatment there was a significant difference between the plots (p<0.05, LSD). Five days after first treatment there was no significant difference among the botanical treatments. However, there was a significant difference between botanical treatments and Diazinon 60% EC and untreated control. The highest number of ants population was recorded in control (5.33) and lowest was in Diazinon 60% EC treated plot (0). The mean number of ants in six botanical treatments ranged from 1.3 to 2.0 which was not statistically significant (p>0.05, LSD). Five days after second treatment there was a significant difference in the mean number of ants between NSKE+BLE, and control. There was no significant difference among NSKE, BLE, BLE, BLE+BLE and NSKE+BLE treated plots (Table 5).

The mean number of spider population in pre treatment count was statistically not significant (p>0.05; LSD) between the plots assigned for botanical treatment. Five days after first treatment there was a significant difference between positive control, control and other treatments. The highest mean number of spiders was observed in control plot (3.6) and lowest (0.3) was in plot treated with Diazinon 60% EC. Five days after second treatment, spider population was significantly (p<0.05; LSD) decreased in the treated plots compared control (Table 6).

In the case of lady bird beetles before treatment in all the plots there was no significant difference (p>0.05; LSD). Five days after first treatment among the botanicals treated plots the result was statistically not significant (p>0.05; LSD). However, compared to chemical pesticide treated plot and control plot result was statistically significant (p<0.05;

LSD). The highest mean number of lady bird beetles was observed in control plot (3.66) followed by NSKE treated plot (2.6) and the difference was statistically not significant at 5% level by LSD (Table 7).

When consider the wasp population, LSD test showed no significant difference before treatment. Five days after first treatment, in general mean number of wasps was decreased in the experimental plot compared to control plot. The highest mean number of wasp population was recorded in control plot (3.3) and there was no wasp observed in Diazinon 60% EC treated plot. Five days after second treatment, there was a slight decrease in the mean number of wasps compared to first treatment. The LSD test showed that wasp population in the plot treated with NSK, BLE and CLE was significantly increased compared to Diazinon 60% EC treated plot. Among the botanicals treatment, mean number of wasp population was statistically not significant (p>0.05; LSD) except NSKE + BLE treated plot (Table 8).

Discussion

Natural products in insect pest management programs are gaining recognition in recent years due to environmental pollution, pest resistance and resurgence caused by indiscriminate use of synthetic chemical pesticides. In Ethiopia, marginal farmers cannot afford the cost of chemical pesticides and moreover chemical pesticides are not advisable for crops that are mainly grown under rain fed condition particularly in highlands of Ethiopia. Therefore, an attempt was made to find out ecofriendly pest management strategies by utilizing locally available plant materials. In the laboratory findings higher percentage of pod damage was observed at lower concentration of botanicals than at higher concentration. Among the botanicals tested, NSKE was found to be most effective as compared to other botanical extracts even at lower concentration. The better results of NSKE may be due to antifeedant or repellent property and this is in line with the observation of Gilani [40] who has reported that neem plant extracts deter insects from feeding. Redferen et al. [41] also reported that neem compound azadirachtin

Treatments	Before treatment	After first treatment	After second treatment
NSKE	2.0 ± 1.00 ^a	1.6 ± 0.57 ^b	1.33 ± 1.15 ^{bc}
CLE	2.0 ± 1.00 ^a	2.0 ± 1.00 ^b	1.66 ± 1.15 ^{bc}
BLE	2.0 ± 1.00 ^a	1.3 ± 0.57 ^b	1.66 ± 0.57 ^{bc}
NSKE+BLE	1.6 ± 1.15 ^a	1.3 ± 0.57 ^b	0.6 ± 0.57 ^c
CLE + BLE	2.6 ± 0.57 ^a	2.0 ± 0.00 ^b	2.33 ± 0.57 ^b
NSKE +CLE	1.6 ± 0.57 ^a	1.3 ± 0.57 ^b	1.0 ± 1.00 ^{bc}
Diazinon 60% EC	2.0 ± 1.00 ^a	0.0 ± 0.00 ^c	0.0 ± 0.00 ^c
Control	2.33 ± 1.15 ^a	5.6 ± 0.57 ^a	5.33 ± 1.15 ^a

Values are mean ± standard deviation of three replications. Similar alphabets within the column was statistically not significant (p>0.05; LSD).

Table 5: Mean number of ants recorded in experimental and control plots.

Treatments	Before treatment	After first treatment	After second treatment
NSKE	2.6 ± 1.15 ^{ab}	2.0 ± 1.73 ^b	1.66 ± 2.08 ^b
CLE	1.6 ± 0.57 ^b	1.3 ± 0.57 ^b	1.0 ± 1.00 ^b
BLE	1.3 ± 0.57 ^b	1.3 ± 0.57 ^b	1.0 ± 0.00 ^b
NSKE+BLE	1.3 ± 0.57 ^b	1.0 ± 1.00 ^b	0.6 ± 0.57 ^b
CLE + BLE	2.0 ± 1.00 ^b	1.6 ± 0.57 ^b	1.33 ± 0.57 ^b
NSKE +CLE	2.0 ± 1.00 ^b	1.0 ± 0.00 ^b	0.66 ± 0.57 ^b
Diazinon 60% EC	2.33 ± 0.57 ^a	0.3 ± 0.57 ^c	0.33 ± 0.57 ^b
Control	2.66 ± 0.57 ^a	3.6 ± 0.57 ^a	3.33 ± 0.57 ^a

Values are mean ± standard deviation of three replications. Similar alphabets within the column was statistically not significant (p>0.05; LSD).

Table 6: Mean number of spiders recorded in experimental and control plots.

Treatments	Before treatment	After first treatment	After second treatment
NSKE	2.3 ± 1.15 ^a	2.6 ± 0.57 ^{ab}	2.33 ± 0.57 ^{ab}
CLE	2.0 ± 1.00 ^a	2.0 ± 1.00 ^b	2.0 ± 1.00 ^b
BLE	2.33 ± 0.57 ^a	2.0 ± 1.00 ^b	1.33 ± 1.15 ^b
NSKE+BLE	1.6 ± 0.57 ^a	1.3 ± 0.57 ^b	1.33 ± 0.57 ^b
CLE + BLE	2.33 ± 0.57 ^a	2.0 ± 1.00 ^b	2.0 ± 1.00 ^b
NSKE +CLE	1.6 ± 0.57 ^a	1.6 ± 0.57 ^b	1.33 ± 0.57 ^b
Diazinon 60% EC	2.3 ± 1.15 ^a	0.6 ± 0.57 ^c	1.0 ± 0.00 ^b
Control	2.0 ± 1.00 ^a	3.6 ± 0.57 ^a	3.66 ± 0.50 ^a

Values are mean ± standard deviation of three replications. Similar alphabets within the column was statistically not significant ($p > 0.05$; LSD).

Table 7: Mean number of predatory lady bird beetles recorded in experimental and control plots.

Treatments	Before treatment	After first treatment	After second treatment
NSKE	1.6 ± 0.57 ^a	1.33 ± 1.15 ^{bc}	1.0 ± 1.00 ^b
CLE	1.6 ± 0.57 ^a	1.3 ± 0.57 ^{bc}	1.0 ± 0.00 ^b
BLE	2.33 ± 0.57 ^a	1.6 ± 0.57 ^b	1.66 ± 0.57 ^b
NSKE+BLE	2.0 ± 0.00 ^a	1.6 ± 0.57 ^b	1.33 ± 1.15 ^{bc}
CLE + BLE	1.3 ± 0.57 ^a	1.0 ± 1.00 ^{bc}	0.66 ± 1.15 ^{bc}
NSKE +CLE	2.0 ± 1.00 ^a	1.3 ± 0.57 ^{bc}	0.33 ± 0.57 ^c
Diazinon 60% EC	1.6 ± 0.57 ^a	0.0 ± 0.00 ^c	0.0 ± 0.00 ^c
Control	1.66 ± 0.57 ^a	3.3 ± 0.57 ^a	3.0 ± 1.00 ^a

Values are mean ± standard deviation of three replications. Similar alphabets within the column was statistically not significant ($p > 0.05$; LSD).

Table 8: Mean number of wasps recorded in experimental and control plots.

has antifeedant effect on insects.

In the field study, reduction in mean number of *H. armigera* larval population was higher in positive control Diazinon 60%EC followed by NSKE. The second best botanical treatment was BLE. The reduction in mean number of *H. armigera* on chickpea plot treated with botanicals may be associated with individual or combined properties of either antifeedant or repellent or oviposition deterrent or antifertility. Among the botanicals, NSKE was found to be superior and it is agreed with the report of Rajput et al. [42]. They have reported that neem products are superior in reducing pest population due to the repellent activity against the larvae of different instars on the treated plots as a result lower the number of *H. armigera* compared to control plot and /or due to the antifeedant effect on the larvae of *H. armigera*. The different neem extracts (aqueous, ethanolic and hexane) have shown ovicidal properties against the eggs of *H. armigera* [43]. In addition to NSKE, BLE also showed about 48.77% reduction in the mean number of *H. armigera*. This is in accordance with the findings of Barbozasilva et al. [44]; they have reported that cis-dehydrocrotonin extracted from *Croton cajucar* bark inhibits the growth of *Heliothis virescens*. The percentage reduction of *H. armigera* in BLE treated plots may be due to the antifeedant and/or toxic effects. It is known that rotenone have been used as insecticides since 1848 when they were applied to plants to control leaf eating caterpillars. Bekele [24] also observed that crude extracts from the seeds of Birbira was toxic to *Sitophilus zeamais*. Ishaaya et al. [45] suggested that Birbira products have both contact and stomach poison to insects and kill insects slowly but causes them to stop feeding almost immediately.

The overall pod damage was significantly lower in treated plots as compared with the control. Among the treatments, the lowest pod damage was observed in Diazinon 60% EC and NSKE treated plots. The second best botanical treatment with higher protection was BLE. The biopotency of NSKE and BLE may be due to antifeedant, repellency

and oviposition deterrence properties of these extracts, consequently lowering the number of *H. armigera* larvae from the treated plots. This is in line with the findings of Sehgal and Ujagir [46]; they have reported that using neem seed extracts in chickpea field, the pod damage was lowered compared to untreated plots. Sadawarte and Sarode [47] also indicated similar results that neem was effective in reducing pod damage at 5 or 6% concentration. Jeyakumar and Gupta [48] reported that neem product was superior in anti-oviposition activity on *H. armigera*. The overall insect populations and pod damage was lower in treated plots than untreated plots resulting in higher yields. Chickpea yield was significantly higher in plot treated with NSKE and positive control (Diazinon 60% EC). Sadawarte and Sarode [47] also suggested that NSKE can be used in place of the highly toxic insecticides because of its safety to beneficial insects and lowest cost.

During the pod formation stage of chickpea plants, spiders, ants, lady bird beetles and wasps were observed in all plots due to availability of prey. These non target organisms are generalized predator and also natural enemies of *H. armigera*; they can feed either in egg or larva or pupa or adult moth. After treatment, mean number of each non target organism was reduced in treated plots. The highest reduction was observed in Diazinon 60% EC treated plots. It is known that chemical pesticides are not safer to non-target organism due to its contact toxicity. The reduction in the number of natural enemies in botanicals treated plots may be due to less availability of prey. Therefore, it is possible that the natural enemies may migrate to the control plot or nearby plots in search for their prey. In general, botanical extracts, particularly NSKE proved to have significant reduction in insect population thereby chickpea yield was increased. The cost of commercially available Diazinon 60% EC was 200 Ethiopian Birr (\$10.899). However, if the farmers prepare botanical pesticides by themselves the cost of input becomes nil since the materials are locally available. Therefore, these widely and freely available eco-friendly botanical biopesticides are suitable for resource poor farming community to protect their chickpea crop particularly under rain fed condition against African bollworm *H. armigera*.

Acknowledgements

First author thank the University of Gondar for providing financial assistance under teaching and learning program to conduct post graduate research.

References

- Muehlbauer FJ, Abebe T (1997) *Cicer arietinum* L. New Crop Fact Sheet.
- Guar PM, Tripathi S, Gowda CLL, Ranga Rao GV, Sharma HC, et al. (2010) Chickpea seed production manual. International Crops Institute for Semi arid tropics.
- EARI (2006) (Ethiopian Agricultural Research Institute) Crop protection department progress report for the period 1994-2006.
- Jones R, Audi P, Shiferaw B, Gwata E (2006) Production and marketing of kabuli or chickpea seeds in Ethiopia; experience from Ada district. International Crops Research Institute for the Semi-Arid Tropics, Kenya.
- ICRISAT (1991) Growing chickpea in Eastern Africa. India.
- Gelatu B, Million E (1996) Chickpea in Ethiopia. In: Adaptation of chickpea in the West Asia and North African region. 1-6.
- Melaku W, Melkamu A, Birhane A, Fentahun M (1998) Research on insect pests and disease of field crops in North Western Ethiopia. 12-14.
- Metcalfe RL, Metcalfe ER (1992) Plant kairomones in insects ecology and control. 1st edn. Chapman and Hall, UK.
- Purohit SS, Vyas SP (2004) Medicinal plant cultivation: A scientific approach. 165-168.

10. Tesfahun G, Bayu W, Teafaye A (2000) Indigenous techniques of crop pest control in Wollo. Pest Manag J Ethiopia 6: 64-68.
11. Roopa PK, Gouda B, Mandihalli (2003) Ovipositional effects of neem extracts on different insects. Karnataka J Agri Sci 16: 251.
12. Tebkew D, Adane T, Asmare D (2002) Potentials for botanicals in controlling the African bollworm. In: proceedings of the national workshop on African bollworm management in Ethiopia: status and need. 106-114.
13. Gossa MW (2007) Effects of neem extracts on the feeding, survival, longevity and fecundity of African bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) on cotton.
14. http://www.nap.edu/openbook.php?record_id=1924&page=1
15. Ma DL, Gordh G, Zalucki MP (2000) Toxicity of biorational insecticides to *Helicoverpa* spp. (Lepidoptera: Noctuidae) and predators in cotton field. Inter J Pest Manag 46: 237-240.
16. Van den Berg H (1993) Natural control of *H. armigera* in smallholder crops in East Africa. PhD Thesis, Wageningen University, Wageningen, Netherlands.
17. Greathead DJ, Grling DJ (1985) Distribution and economic importance of *Heliothis* and their natural enemies and host plants in Southern and Eastern Africa. IOBC *Heliothis* Work Group, India.
18. Desta G, Ermias S, Ridwan M (2004) African bollworm management in Ethiopia status and needs. 24.
19. Emden HF Van (1989) Pest control. 2nd Edition, Cambridge University Press. 27-37.
20. Bekele J, Daniel G, Meried N, Emiru S (2002) Toxicity of Birbira (*Milletia ferruginea*) seed crude extract to some insect pests as compared to other botanical and synthetic insecticides. 11th NAPRECA Symposium Book of Proceedings, Antananarivo, Madagascar 88-96.
21. Karunamoorthi K, Bishaw D, Mulat T (2009) Toxic effects of traditional Ethiopian fish poisoning plant *Milletia ferruginea* (Hochst) seed extracts on aquatic macro invertebrates. Eur Rev Med Pharmacol Sci 13:179-185.
22. Holden MJ, Raitt DFS (1974) Manuals of Fisheries Science Part 2 - Methods of resource investigation and their application. Food and Agriculture Organization of the United Nations, Italy.
23. Bekele J (1998) Investigation of flavonoids from Birbira. 167.
24. Bekele J (2002) Evaluation of the toxicity potential of *Milletia ferruginea* (Hochst) Baker against *Sitophilus zeamais*. Inter J Pest Manag 48: 29-32.
25. Mulatu B (2007) Contact bioassay of an endemic plant to Ethiopia on three aphid species. Ethiopian J Biol Sci 1: 51-62.
26. Getahun D, Jembere B (2006) Evaluation of toxicity of crude extracts of some botanicals on different castes of macrotermes termites. Pest Management Journal of Ethiopia 10: 15-24.
27. Peres MT, Delle Monache F, Cruz AB, Pizzolatti MG, Yunes RA (1997) Chemical composition and antimicrobial activity of *Croton urucurana* Baillon (Euphorbiaceae). J Ethnopharmacol 56: 223-226.
28. Peres MT, Pizzolatti MG, Yunes RA, Monache MD (1998) Clerodane diterpenes of *Croton urucurana*. Phytochem 49: 171-174.
29. Suárez AI, Compagnone RS, Salazar-Bookaman MM, Tillett S, Delle Monache F, et al. (2003) Antinociceptive and anti-inflammatory effects of *Croton malambo* bark aqueous extract. J Ethnopharmacol 88: 11-14.
30. Anazetti MC, Melo PS, Durán N, Haun M (2004) Dehydrocrotonin and its derivative, dimethylamide-crotonin induce apoptosis with lipid peroxidation and activation of caspases-2, -6 and -9 in human leukemic cells HL60. Toxicology 203: 123-137.
31. Fischer H, Machen TE, Widdicombe JH, Carlson TJ, King SR, et al. (2004) A novel extract SB-300 from the stem bark latex of *Croton lechleri* inhibits CFTR-mediated chloride secretion in human colonic epithelial cells. J Ethnopharmacol 93: 351-357.
32. Kubo I, Asaka Y, Shibata K (1991) Insect growth inhibitory nor-diterpenes, cis-dehydrocrotonin and trans-dehydrocrotonin, from *Croton cajucara*. Phytochem 30: 2545-2546.
33. Silva LB, Silva W, Macedo MLR, Peres MTL (2009) Effects of *Croton urucurana* extracts and crude resin on *Anagasta kuehniella* (Lepidoptera: Pyralidae). Braz Arch Biol Technol 52: 653-664.
34. Silva LB, Xavier ZF, Silva CB, Faccenda O, Candido ACS, et al. (2012) Insecticidal effects of *Croton urucurana* extracts and crude resin on *Dysdercus maurus* (Hemiptera: Pyrrhocoridae). J Entomol 9: 98-106.
35. Jain PC, Bhargava MC (2007) Entomology novel approaches. 165-309.
36. Dodia DA, Patel IS, Patel GM (2010) Botanical pesticides for pest management.
37. Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research, (2nd edn). Wiley, USA.
38. Judenko E (1973) Analytical method for assessing yield losses caused by pests on cereal crops with and without pesticides. Tropical Pest Bulletin 31.
39. Walker PT (1997) The assessment of crop losses in cereals. Insect Sci Appl 4: 97-104.
40. Gilani G (2001) Neem the wonder tree. 3-7.
41. Redferen RE, Warthen JD, Liebel EL, Mills GD (1980) The antifeedant and growth disrupting effect of Azadirachtin on *Sopdoptera ferugiperda* and *Onceopeltus faseiatus*. 87-91.
42. Rajput AA, Sarwar M, Bux M, Tofique M (2003) Evaluation of synthetic and some plant origin insecticides against *Helicoverpa armigera* (Hubner) on chickpea. Pak J Biol Sci 6: 496-499.
43. Jhansi BR, Singh RP (1993) Biological effects of neem on insect pests. 12: 17-19.
44. Silva LB, Silva W, Macedo MLR, Peres MTL (2009) Effects of *Croton urucurana* extracts and crude resin on *Anagasta kaehniella*. Braz Arch Biol Technol 52.
45. Perry AS, Yamamoto I, Ishaaya I, Perry RY (1998) Insecticides in agriculture and environment: Retrospects and prospects (Applied agriculture). (1stedn) Springer, USA.
46. Sehgal VK, Ujagir R (1990) Effect of synthetic pyrethroids, neem extracts and other insecticides for the control of pod damage by *Helicoverpa armigera* (Hübner) on chickpea and pod damage-yield relationship at Pantnagar in northern India. Crop Protection 9: 29-32.
47. Sadawarte AK, Sarode SV (1997) Effect of neem seed extract, cow dung and cow urine alone and in combination against the pod borer complex on pigeon pea. International Chickpea and Pigeon pea 4: 37.
48. Jeyakumar P, Gupta GP (1999) Effect of neem seed kernel extract (NSKE) on *Helicoverpa armigera*. Pesticide Res J 11: 32-36.