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Bio-insecticidal effects of plant extracts and oil emulsions of Ricinus communis L. (Malpighiales: Euphorbiaceae) on the diamondback, Plutella xylostella L. (Lepidoptera: Plutellidae) under laboratory and semi-field conditions

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ABSTRACT

Objective: The objective of this study is to evaluate the potential of using *Ricinus communis* leaf, root, seed kernel crude extracts and oil emulsion to control the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae).

Methodology and results: The effect of different treatments of *R. Communis* plant extract (20%) and oil emulsion (5% and 10%) and their persistence (0, 3 and 5 days after application; DAA) on mortality and oviposition behaviour of *P. xylostella* were tested in laboratory and field cage experiments. In general, *R. communis* products have strong larvicidal effect on *P. xylostella*, with 100% mortality recorded on 3rd instar larvae treated with 10% oil emulsion in both ingestion and contact toxicity tests. Aqueous extracts were significantly less toxic with the highest mortality rates (67.49 ± 1.98% and 70.86 ± 0.85%) recorded with seed kernel extract and the lowest with the root extract (53.98 ± 1.21% and 54.87 ± 1.88%), in topical toxicity and ingestion toxicity experiments, respectively. The adult emergence was significantly affected with the lowest emergence rate recorded in 5% oil emulsion, 57.72 ± 72% and 49.98 ± 0.98% in topical toxicity and ingestion toxicity tests, respectively. No significant different was noted between Dursban and aqueous extract treatments. Among emerged adults from larvae treated with oil and aqueous extracts, a 44–79% abnormal development as wings and legs deformation were observed. The sex ratio was skewed in favour of males among F1 progeny from Durban and *R. communis* treated insects and in favour of females in controls. In field-cage experiments, treated plants had strong larvicidal and oviposition deterrent index on *P. xylostella*. The oviposition deterrence index was highest with castor bean oil at a concentration of 10%. Diamondback females clearly discriminated between plants sprayed *with R. communis* products and those with water. Treating diamondback-infested cabbage plants with plant extracts and oil emulsions resulted in more than 59% mortality 7 DAA. Experiments on the residual effects revealed a significant decrease in larval mortality with time between the botanical application and insect release.

Conclusion and application of findings: In view of the low oviposition rates, oviposition deterrent, immature mortality, and the relatively low persistence of the toxic ricin oil, it can be expected that the use of R. communis product be suitable for *P. xylostella* population density reduction in the field.

Key words: botanicals, *Ricinus communis*, integrated pest management, Oviposition, *Plutella xylostella*.

INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is regarded as the most destructive insect pest of Brassicaceae crops that requires US$1.0 billion globally in estimated annual management costs (Talekar and Shelton 1993) in addition to the crop losses. The larvae are voracious defoliators with a potential to destroy the entire crop if left uncontrolled (Verkerk & Wright, 1996). Currently, available control option against *P. xylostella* relies on the application of chemical insecticides (pyrethrenoids and organophosphates) on the basis of spray schedule (Castelo Branco & Gatehouse, 2001). However, synthetic pesticides, while valued for effectiveness and convenience control of *P. xylostella*, pose certain problems, including phytotoxicity and toxicity to non-target organisms, environmental degradation and health hazards to farmers and insect resistance. *Plutella xylostella* is known to develop multiple- and cross-resistance to nearly all groups of pesticides applied in the field including new chemistries such as spinosyns, avermectins, neonicotinoids, pyrazoles and oxadiazines (Sarfraz & Keddie 2005; Sarfraz et al. 2005).

So far attempts for biological control of *P. xylostella* as alternative to chemicals have mainly concentrated on the use of parasitoids, predators and pathogens. Parasitoids are the key group of natural enemies of DBM and over 130 species including hymenopteran, have been widely reported to attack various life stages of DBM (for more detail see Sarfraz et al., 2005). In periurban areas of Benin, *Anomma nigricans* (Illiger) (Hymenoptera: Formicidae) have been reported as an important regulator of *P. xylostella* (Goudegnon et al. 2004). Moreover, several pathogens including fungi (Furlong, 2004), bacteria (Braun et al., 2004) and viruses (Cherry et al., 2004). Although increased efforts are made worldwide to develop integrated pest management programs, principally based on manipulation of natural enemies to control agricultural pests, these are often not affordable to African peasant farmers. Therefore, the development of alternative control methods for *P. xylostella* based on botanical pesticides, which are locally available, is of paramount importance to sustain the successful use of biological control against DBM in Africa. This group of pesticides is easily biodegradable and their use in crop protection is a sustainable alternative to synthetics (Immaraju, 1998; Juan & Sans, 2000; Carpinella et al*.,* 2002; Roy et al., 2005; Isman, 2006; Asogwa et al., 2010). Botanicals have been successful against a number of agricultural pests in Africa (e.g., Barbouche et al., 2001; Kétho et al., 2002; Lee et al., 2002; Bruce et al., 2004; Sanda et al., 2006; Ogendo et al., 2008, Agboka et al., 2009). In Togo, Dreyer (1987) demonstrated good efficacy of crude extract of neem seed against the larva of *P. xylostella*. However, the difficulty in collecting neem seed and the unavailability of locally made and ready-to-use botanical based pesticides did not allow the practical application of these alternatives. Apart from neem products, other extracts derived from indigenous plants including *Cymbopogon schoenanthus* L., *Ocimum basilicum* Basil (Labiae), *Hyptis suaveolens* L. (Lamiaceae) and the fish bean *Tephrosia vogelii* Hook F. (Leguminosae) recently gained attention with regard to their insect pest control potential (Kétho et al., 2002; Sanda et al., 2006; Agboka et al., 2009). In Togo only few studies have been conducted on insecticidal activities of plant extracts although many plants are known to possess potential for insect pest management. Kétho et al. (2002) have demonstrated the insecticidal activity of the *C. schoenanthus* against *Callosobruchus maculatus* L. (Coleoptera: Pteromalidae). Sanda et al*.* (2006), reported high toxicity of *Cymbopogon schoenanthus* L. in controlling *P. xylostella* under field conditions. Other botanical insecticide include *Ocimum basilicum* Basil (Labiae) and the physic nut *Jatropha curcas* L. (Euphorbiaceae). In order to offer more choice in pesticide plants to the user, there is need to assess other indigenous botanical pesticide, especially those with a presumably better potential of extension. Such plants, in addition to their inherent pesticidal effectiveness must be rustic, perennial and easily cultivated. One of these candidates may be the castor bean plant, *Ricinus communis L*. (Malpighiales:

Euphorbiaceae), a wild growing plant in all ecological areas in Togo and other parts of the world (Weiss, 2000). Castor bean plant contains ricin toxin, one of the most toxic and easily produced plant toxins worldwide (Thomas et al., 1980; Bojean, 1991; Ogunniyi, 2006). However, to the best of our knowledge, very few studies have been conducted to investigate the pesticidal activity of *R. communis*. The very limited data on toxicity in target insects comprise mainly information on aqueous extract of castor bean products rather than on its oil. Aunty et al. (2006) demonstrated high larvicidal activity of aqueous extracts from leaves of *R. communis* against four mosquito species, *Culex pipiens* (L.), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) and *Anopheles maculipennis* (Meigen). No data on the effects of *R. communis* on agricultural pests is available. Therefore, the present work aimed at assessing the efficacy of aqueous extracts and oil emulsion of castor bean plant in controlling *P. xylostella.*

MATERIALS AND METHODS

Plant materials*:* The cabbage variety KK CROSS, of Japanese origin, widespread in Togo, was purchased from the local suppliers and used in all experiments. Different parts of castor bean plant (leaf, root and seed kernel) used were harvested from plants growing on the area of the Université de Lomé campus.

Insect culture : A laboratory culture of *P. xylostella* originating from specimens collected on field-grown cabbage plants at the market gardening site near the "Port Autonome de Lomé", was established at the "Station d'Expérimentations Agronomiques de l'Ecole Supérieure d'Agronomie de l'Université de Lomé" (06°17'N, 001°21'E) following the protocol developed by Ibrahimi et al. (2009). The insects were continuously reared in cages for three generations on cabbage variety KK CROSS plant. Cabbage plants at eight to ten-leaf stage were placed inside oviposition cages containing 8-10 pairs of DBM. The oviposition cage consisted of transparent cubic Plexiglas's container (30 × 30 × 30 cm), with a fine nylon mesh installed on the top-side. A small cotton-wool wick soaked in 10% honey solution was placed in each oviposition cage as a source of carbohydrate for adults. In other to synchronize insect rearing, adult DBM were kipped on the plants for 12 hr. Early 3rd instar larvae were used in all experiments; early 2nd instars larvae were found to be too fragile mostly dying during handling.

Preparation of aqueous extracts of castor bean leafs, root and seed kernel: Extraction of castor bean leaf, root and seed kernel was carried out as simple as possible for easy adoption by the peasants growers. Hence, crude aqueous extracts of each plant organ at 20% were prepared by soaking 200g pounded fresh-plant material, respectively, in 1l of tape water, and left for 12 hr and then filtered through muslin cloth. A solution of emulsifier was added to each preparation before both laboratory and field trials.

Extraction and formulation of castor bean seed kernel oil: Ripe castor bean seed were sun-dried before oven drying at 40°C for 72 hr. Grains obtained after manual decortications, were ground using a pestle and mortar. The toxic glucoprotein, ricin, is known to remain in the seed cake and is not transferred to the oil fraction when extracted by cold pressing of the seeds (Cosmetic Ingredient Review Expert Panel, 2007). Therefore, oil extraction was done using ethanol as solvent. Castor bean seed powder obtained was moistened and the paste is modelled in sticks of 150g each, and oil extracted with 90% ethanol in a soxhlet extractor. The excess solvent was removed under reduced pressure; and the oil was placed in desiccators to remove any remaining water and stored at 4 ± 2°C. The amount of oil obtained from 150 g paste of castor bean seed was 26.75 ml weighing 24.6 g. Castor bean oil was thereafter formulated with a water and emulsifier solution (soap without any detergent) to prepare the different concentrations of oil emulsion for both laboratory and field-cage trials.

Laboratory tests

Topical and ingestion toxicity of castor bean plant extracts and oil emulsion to third instar Plutella xylostella larvae: In topical toxicity experiment, 3rd instar larvae were topically treated with castor bean leaf, root and seed kernel aqueous extracts at a concentration of 20%, with oil emulsions at concentrations of 5 and 10% or with the synthetic pesticide Dursban (5% active ingredient, prepared by diluting 5g of product in 10ml tape water), widely used insecticide for diamondback control in the experimental zone, by applying the solution on the anterior pronotum of the larvae using a micro-litter syringe. Hundred and

five (105) 3rd instar *P. xylostella* larvae were inoculated per treatment in group of 15 individuals. A further 105 3rd instar larvae were topically treated with emulsified water, 1% ethanol and untreated check, referred to as control 1, control 2 and control 3, respectively. Insects were thereafter transferred to clean plastic Petri dishes (150 x 20 mm^2) containing untreated 9 cm diameter cabbage leaf disc and incubated in seven groups of 15 for 7 days at 25 ± 1°C, 75 ± 5 % r.h. .

In ingestion toxicity test, discs (9 cm in diameter) cut from cabbage leaves were immersed individually in the different concentrations of oil or aqueous extracts for one minute. The discs were left to dry at ambient temperature for 5 min, and transferred to clean plastic Petri dishes (150 x 20 mm^2). Test insects were inoculated in group of 15 individuals by feeding on treated leaf disc for 24 h. The experiment set up is similar as in topical toxicity test and larvae fed with cabbage leaf discs treated with emulsified water, 1% ethanol in water and untreated check served as controls.

In both topical application and ingestion tests, untreated leaf discs were used to feed the insects and changed every 24 hours. Following this methodology, *P. xylostella* larvae could be kept on fresh leaves throughout the whole experimental period of seven days. The experiment was repeated three times and larval mortality was assessed at 24-h intervals. To assure independence of values, each group of 15 insects per treatment was assessed only once, thus group 1 was assessed at 24 h only, group 2 at 48 h and so on to group 7 at 7 days. Insects that survived and developed to pupae were followed until adult emergence and percent emergence, moth deformities, sex-ratio of adult moths and longevity were determined for each treatment.

Field-cage experiments

Experiment 1: Direct treatment of diamondback moth infested cabbage plant in cage experiments

The experiment was conducted to evaluate the efficacy of castor bean plant extracts and oil in controlling *P. xylostella* under semi-field conditions. Experiments were carried out at the experimental station of the "Station d'Expérimentations Agronomiques de l'Ecole Supérieure d'Agronomie de l'Université de Lomé" located in the southern guinea Savannah region of Togo. The tests were carried out in the field in wooden insect cages (50 x 50 x 90 cm) with four mesh sides (the two lateral and the front and the top side), and the remaining two sides made of wood. Five plastic pots (11 x 7.5 x 8.5 cm), with one cabbage plant at eight-leaf stage, were used per cage. Inside each cage, 60 to 90 *P. xylostella* larvae were released onto plants and the cages were maintained at ambient conditions. Treatments consisted of applying per plant 10 ml of aqueous extract of *R. communis* root, leaf, and seed kernel at a concentration of 20%, oil emulsion of *R. communis* at concentrations of 5 and 10% and of the synthetic pesticide Dursban, twenty-four hours after releasing the insects. Plants were sprayed with a hand sprayer covering both sides of the leaves. Emulsified water, 1% ethanol in water and untreated check served as control. Treatments were replicated three times and mortality was scored daily for seven days. Insects that died immediately after spraying were not included in the analyses.

Experiment 2: Residual activity of the botanical treatments

The residual activity of the *R. Communis* extracts and oil on DMB larvae was evaluated over time. Healthy cabbage plants in the field at eight-leaf stage were sprayed with castor bean aqueous extracts, oil emulsion, Dursban, at their respective concentrations and control solutions. Treated plants, all sprayed at the same time, were transferred to the insect cages immediately (0 h) or three (72 h) and five days thereafter. Inside each cage, 60 to 90 larvae were released onto plants and held at ambient conditions. Each treatment consisting of one cage with 5 cabbage plants was replicated three times. The 3 levels of control as mentioned above were considered and insects were monitored daily for 7 days and mortality was recorded.

Experiment 3: Effect of different treatments on the oviposition behaviour of P. xylostella.

No-choice experiment: In no-choice experiment, cabbage plants, produced singly in pots, were treated at ten-leaf stage as described in the previous sections with oil emulsion at 10%, aqueous extracts at 20% and Dursban 5%. Once treated, plants were transferred immediately to oviposition cages. Each treatment consisted of three cages with one potted cabbage plant each, and control 1, control 2 and control 3 as described above were used for comparison. Approximately 1 h after spraying the plants, one 1-day old adult *P. xylostella* pair was released inside the cage for oviposition.

Choice experiment: The choice experiment was conducted to determine if females DBM could successfully discriminate between plants treated with *R. communis* products and untreated ones. In the no-choice tests, the DBM did not discriminate between

untreated plants and plants treated with emulsifier water and with 1% ethanol (for details see Results). Hence females *P. xylostella* were given the choice between cabbage plants treated with *R. communis* aqueous extracts, oils emulsion or Dursban at the same concentration as in no-choice experiment and untreated control. Each combination (treated vs. untreated) was repeated three times. To reduce bias between treated and untreated plants inside each cage, the pots were placed in the diagonally opposed angles of each cage. Two newly emerged pairs of *P. xylostella* were released in the centre of each cage and provided with a 10% sucrose solution as food source.

In both no-choice and choice experiments, plants were gently shaken four days after releasing the moths, to remove surviving moths and eggs deposited on the cabbage were tagged and counted under a binocular microscope. The number of eggs laid per plant as well as oviposition deterrence index (ODI) (choice experiment) for the aqueous extracts, oils and Dursban was calculated using the formula: [(C - T)/(C + T)] * 100 (Akhtar and Isman, 2003), with C being the number of eggs laid on the untreated control plants and T the number of eggs laid on the treated plants.

Statistical analysis: Median survival times (MST) were calculated using SPSS (SPSS, 1999). The efficacy of the different treatments was compared using the final mortalities (i.e. final cumulative mortalities). Differences in mortality rates, rate of pupation, adult emergence, sex ratio, adult deformities and adult longevity, eggs per plant and oviposition deterrent index were analyzed by analysis of variance (ANOVA) and means were separated using Student-Newman-Keuls test (PROC MIXED procedure, SAS institute, 1997) and the probability level was set at $\alpha = 0.05$. Percentage data and ratios were arcsine-transformed before analysis.

RESULTS

Laboratory tests

Topical and ingestion toxicity of castor bean plant extracts and oil emulsion to third instar larvae: In both topical and ingestion tests, aqueous extracts and oil emulsion of castor bean plant caused significantly higher P. xylostella mortalities than the control ($P < 0.0001$, Tables 1 and 2). Oil emulsion was the most active against P. xylostella larvae followed by the Dursban, both being significantly more active compared to the aqueous extracts. In both tests, no difference was noted among the 3 levels of control. Dead larvae were characterized by burned cuticle in R. communis treatment particularly in oil emulsion treatments. Among aqueous plant extracts, the seed kernel induced significantly higher mortality when compared to the roots and leave extracts (Tables 1 and 2). In oil treatment, large number of larvae died shortly after getting in contact with or ingesting the castor bean oil. The median survival time (MST) of P. xylostella was the lowest in oil emulsion treatments and the highest in the 3 controls (Tables 1 and 2).

Table 1: Mean *Plutella xylostella* 3rd instar mortality and Median Survival Time (MST) in topical application of aqueous extracts and oil of castor bean, *Ricinus communis*.

Treatments	Larval mortality (% ± SE)[a]	MST(days ± SE)[a]
Seed kernel extract (20%)	67.49 ± 1.98c	3.01 ± 0.09b
Roots extract (20%)	53.98 ± 2.41d	4.74 ± 0.57c
Leaf extract (20%)	58.98 ± 1.73d	3.86 ± 0.17b
Oil (5%)	82.19 ± 2.71b	1.05 ± 0.03a
Oil (10%)	100.00 ± 0.00a	1.02 ± 0.01a
Dursban (5%)	88.46 ± 2.97b	3.12 ± 0.77b
Control 1 (Emulsified water)	3.79 ± 1.07e	>7[d]
Control 2 (1% ethanol in water)	3.28 ± 0.85e	>7[d]
Control 3 (untreated check)	2.22 ± 0.95e	>7[d]
df	8,18	
F	1280	
P	< 0.0001	

[a]Means in a column followed by the same are not significantly different ($P < 0.05$; Student-Newman-Keuls test).

Table 2: Mean mortality of 3rd instar larvae of *Plutella xylostella* and Median Survival Time (MST) in ingestion toxicity tests of aqueous extracts and oil of castor bean.

Treatments	Larval mortality (% ± SE)[a]	MST(days ± SE)[a]
Seed kernel extract (20%)	70.86 ± 0.85c	3.16 ± 1.03b
Root extract (20%)	49.89 ± 3.02d	4.12 ± 0.87b
Leaf extract (20%)	51.87 ± 0.88d	3.78 ± 0.79b
Oil (5%)	89.58 ± 1.90b	1.04 ± 0.04a
Oil (10%)	100.00 ± 0.00a	1.01 ± 0.01[c]a
Dursban (5%)	88.46 ± 2.97b	3.07 ± 0.82b
Control 1 (Emulsified water)	0.00 ± 0.00e	>7[d]
Control 2 (1% ethanol in water)	0.00 ± 0.00e	>7[d]
Control 3 (untreated check)	0.00 ± 0.00e	>7[d]
df	8,18	
F	1975	
P	< 0.0001	

[a]Means in a column followed by the same letter are not significantly different ($P < 0.05$; Student-Newman-Keuls test).

Percent adult emergence, percentage of adults without deformities as well as longevity of adult *P. xylostella* were significantly affected by the previous treatments on larvae (Tables 3 and 4). While all adult moths that emerged in untreated, 1% ethanol and emulsified water present normal morphological traits, significantly higher proportion of adults emerging from *R. communis* treatments presented abnormal development as wings and legs deformation (Tables 3 and 4). In general, *P. xylostella* appears to be more sensitive to castor bean oil than to aqueous extracts. There was no difference between females and male longevity ($P > 0.05$), however when treated with castor bean products, longevity of both males and females adult *P. xylostella* was significantly reduced as compared to the controls (Tables 3 and 4). The sex ratio of emerging moths was male-biased for the *R. communis* products and Dursban and female biased in untreated, 1% ethanol and emulsified water controls (Tables 3 and 4), and did not vary in ingestion test (Table 4). In topical application test however the sex ratio was significant affected by treatments (Table 3).

Field-cage experiments:

Experiment 1: Direct treatment of diamondback moth infested cabbage plant in cage experiment: The highest mortality of DBM was obtained in plants treated with oil emulsion followed by Dursban (Table 5). No significant differences in mortality were found between the root and leaf extracts, both being significantly less toxic compared to seed kernel extract (Table 5). The majority of dead insects were recorded during the first 4 days after the applications, i.e., 76.0 ± 1.2%, 61 ± 2.1%, 55 ± 1.5) and 53 ± 1.7) for Dursban, seed kernel, leaf and root aqueous extracts, respectively. The median survival time (MST) of *P. xylostella* was significantly higher in the 3 control treatments than in aqueous extract and Dursban treatments. Lower MST values were registered in Dursban treatment compared to aqueous extract treatments (Table 5)

Experiment 2: Residual activity of the botanical treatments

Except when *P. xylostella* larvae were released immediately after spraying, no further mortality was recorded in the 3 levels control treatments (i.e., untreated check, emulsifier solution and 1% ethanol in water treatments) (Table 6). In all treatments, dead insects were found in most cases attached to the leaf surface. As in laboratory tests, insects that died on plant treated with *R. communis* products showed clearly burned cuticle. Significant effect of both treatments and time from application to insect release was observed ($F = 1986$; df = 8; $P < 0.0001$ and $F = 4093$; df = 2; $P < 0.0001$, respectively) with significant interactions between the treatments and days after application ($F = 347.773$; df = 16; $P < 0.0001$). *P. xylostella* mortality was always significantly higher in *R. communis* products treatments than in the control treatments ($P < 0.0001$). For aqueous extract treatments on 3- and 5-day-old spray residues, except for seed kernel extract, similar mortality rates were recorded, which were significantly lower than those on fresh residues. Significant decrease in larval mortality was noted over time for oil emulsion, seed kernel extract and Dursban treatments (Table 6).

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Table 5: Effect of different castor bean extracts and oil on DBM infesting cabbage plants in field cage experiments.

Treatments[b]	Mortality (% ± SE)[a]	MST[c] (days ± SE)
Seed kernel extract (20%)	67.15 ± 0.35c	2.61 ± 0.01c
Root extract (20%)	60.92 ± 3.41d	3.46 ± 0.08d
Leaf extract (20%)	58.76 ± 3.41d	3.50 ± 0.24d
Oil (5%)	97.92 ± 1.41a	1.02 ± 0.02b
Oil (10%)	100.00 ± 0.00a	0.73 ± 0.01a
Dursban (5%)	82.85 ± 3.41b	2.90 ± 0.08c
Control 1 (Emulsified water)	5.45 ± 0.37e	>7[d]
Control 2 (1% ethanol in water)	2.22 ± 0.17e	>7[d]
Control 3 (untreated check)	3.45 ± 0.37e	>7[d]
df	8,18	
F	16,029	
P	0.004	

[a]Seven days cumulative mortality; [b]means in the same columns followed by the same letter are not significantly different ($P < 0.05$; Student-Newman-Keuls test); [c]median survival time; [d] MST exceeded the observational period.

Table 6: Mortality (% ± SE) of 3rd instar of DMB on cabbage plants at different time intervals after treatment with different castor bean extracts and oil in field-cage experiment.

	Mortality (% ± SE)[a]		
		Days after application	
Treatments	0	3	5
Seed kernel extract (20%)	51.72 ± 3.20Ba	23.41 ± 1.72Cb	14.11 ± 1.47Bc
Root extract (20%)	48.44± 3.1Ba	19.94 ± 0.48Db	14.07 ± 0.78Bb
Leaf extract (20%)	42.44± 3.1Ba	17.94 ± 0.48Db	12.07 ± 0.78Bb
Oil (5%)	95.92 ± 1.31Aa	47.92 ± 2.22Bb	9.92 ± 2.17Cb
Oil (10%)	100.00 ± 0.00Aa	51.41 ± 1.72Bb	12.14 ± 1.47Bb
Dursban (5%)	75.21± 1.09Aa	65.56 ± 2.24Ab	29.15 ± 1.15Ac
Control 1 (Emulsified water)	6.87 ± 1.17Ca	0.00 ± 0.00Eb	0.00 ± 0.00Db
Control 2 (1% ethanol in water)	5.09 ± 0.92 Ca	0.00 ± 0.00Eb	0.00 ± 0.00Db
Control 3 (untreated check)	3.48 ± 1.04Ca	0.00 ± 0.00Eb	0.00 ± 0.00Db
df	8,18	8,18	8,18
F	1069	996.718	206.763
P	< 0.0001	< 0.0001	< 0.0001

[a]Means within columns followed by the same upper case and within rows followed by the same lower case letter are not significantly different ($P < 0.05$; Student-Newman-Keuls test).

Experiment 3: Effect of different treatments on the oviposition behaviour of P. xylostella.

No-choice experiment: Diamondback females laid significantly less eggs on plants treated with castor bean products, than on those in the 3 control treatments (Figure 1), with the lowest number of eggs recorded on plant sprayed with castor bean oil (i.e., 14.75 ± 0.61) (df = 7, F = 855.739 and P <0.0001). Similar number of eggs was recorded on plants treated with Dursban (75.75 ± 7.86), root extract (76.63 ± 2.38) and leaf extract (79.50 ± 0.50) a(Figure 1). No significant differences were found between the control treatments (Figure 1).

Choice experiment: Diamondback females clearly discriminated between plants sprayed *with R. communis* products and untreated ones (Figure 2). Castor bean plant extracts and oil emulsion treatments significantly reduced oviposition on the plants offered. More eggs were laid by *P. xylostella* on plants treated with water than those treated with *R. communis* products and Dursban (df = 4, F = 109.371, $P < 0.0001$; Figure 2).

The ODI was significantly higher in *R. communis* treated plants compared to the control (Figure 2), indicating complete oviposition deterrent effects by castor bean plant oil and extracts at the tested concentrations. The highest oviposition deterrent effects of castor bean plant were noted with oil emulsion followed by the seed kernel extract. No difference was found between root, leave extracts and Dursban treatments (Figure 2).

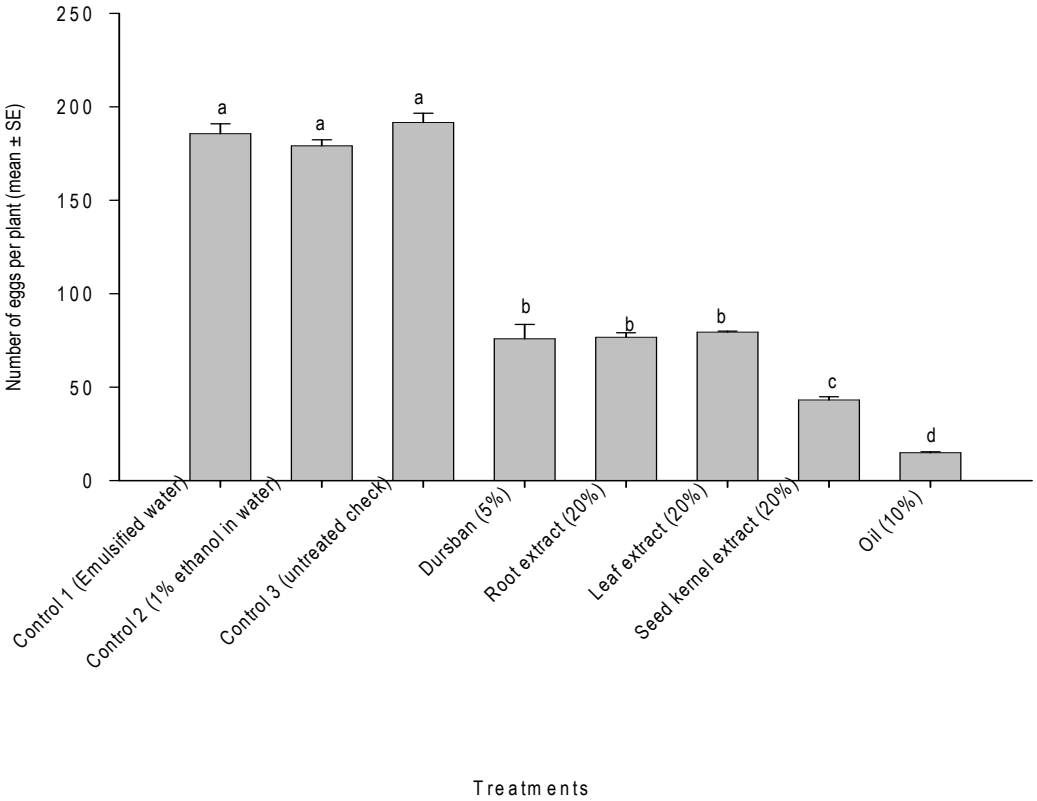

Figure 1: Mean (± S.E.) number of eggs laid by *Plutella xylostella* females per plant in no-choice experiment in field cage experiments. Different letters above bars indicate significant differences between means at $P = 0.05$ (Student–Newman and Keuls test).

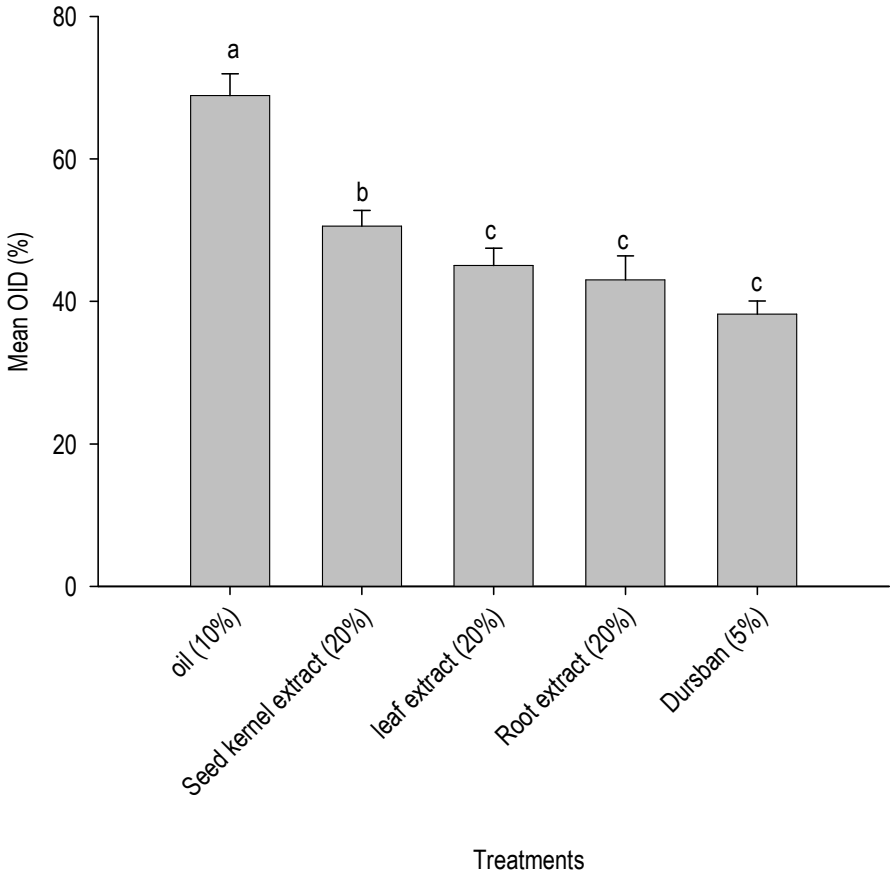

Figure 2: Oviposition deterrence index (ODI) of different extracts and oil of castor bean.
Different letters above bars indicate significant differences between means at $P = 0.05$ (Student-Newman-Keuls test).

DISCUSSION

The results of the present study showed that castor bean plant extracts and oil could be toxic to larvae of *P. xylostella* through contact and ingestion. Plant based pesticides have been found to exhibit larvicidal effects (e.g., Kétho et al., 2002; Sanda et al., 2006; Ogendo et al., 2008; Agboka et al., 2009). Under field conditions, Sanda et al. (2006), find that *Cymbopogon schoenanthus* L. could significantly reduced larval population of *P. xylostella*. Torres et al. (2001) report 100% mortality against *P. xylostella* larvae with 10% aqueous solution of the wood bark of *A. pyrifolium* in Brazil. Using aqueous extracts from leave of *R. communis,* Aouinty et al. (2006) have reported high larvicidal activity against 2nd and 4th instar larvae of four mosquito species, *Culex pipiens* (L.), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) and *Anopheles maculipennis* (Meigen). The high mortality rate of *P. xylostella* larvae in both laboratory and cage experiments could be due to toxic effect of the plant. Castor bean oil and pure compounds of *R. communis* have been reported to exhibit high toxic effects in target animals (Olsnes, 2004; Bigalke & Rummel, 2005; Kumar et al., 2007; He et al., 2007). The toxicity of the plant is ascribed to the presence of ricin, a water-soluble glycoprotein concentrated in the seed endosperm but present in lesser concentrations in other parts of the plant and reputed to be one of the most poisonous of the naturally occurring compounds (Darby et al., 2001; Frederiksson et al., 2005; Kozlov et al., 2006; El-Nikhely et al., 2007). This fact explained the comparatively high effects recorded in oil and seed kernel extract compared to the leave and root extracts. According to Tokarnia et al., 2002, whilst the seeds are the primary source of toxin, the rest of the plant may also be considered to be slightly toxic. The mechanism of toxicity has been elucidated in great detail. Following uptake into cells by endocytosis, ricin causes acute cell death by inactivation of ribosomal RNA, inhibiting

protein synthesis (e.g., Roberts & Smith, 2004; Utskarpen et al., 2006; Parikh et al., 2008). Although the high toxicity of ricin could explain the high larval mortality recorded in this study, the burning effect of the *R. communis* products could be an important factor in the host death particularly in contact toxicity tests.

In the present work, a large number of adult died during emergence, exhibiting abnormal development. Morphological alterations are common in insects treated with botanical based insecticides and have been attributed to the reduction in the concentration of ecdysone (a steroidal prohormone of the major insect moulting hormone 20-hydroxyecdysone) (Schmutterer, 1990; Mordue-Luntz and Nisbet, 2000) or to its delayed release into the circulatory fluid (Jacobson et al., 1983; Marco et al., 1990).

The results from the cage study corroborated previous laboratory data on the high toxicity of botanical oil emulsion to *P. xylostella* larvae, thus confirming the high potential of *R. communis* components for controlling the diamondback. Our results showed that direct treatment of diamondback-infested bean plants can be very effective for control of *P. xylostella*. The majority of diamondback larval mortality is probably a result of direct contact of the applied toxin on the larva. However, the significant decline in larval mortality in the residual study indicated a strong decline in activity of the toxin applied to leaf surfaces. Increasing time after application resulted in significantly decreasing diamondback larval mortalities, showing a probable decrease in toxin activity under field conditions. Ricin is known to quickly disappear when exposed to UV radiation and high temperatures (Martinez-Herrera et al., 2006). A number of factors including high temperature for detoxifying castor seed meal (e.g., 100°C for 30 min; 120°C for 25 min) have been investigated, and have been reviewed by Anandan et al. (2004). In addition to the toxic effect on larvae, castor bean products acted as ovipositional deterrent as it was shown in the ovipositional test. *P. xylostella* adult tended not to oviposit on cabbage plants treated with the oil and, to certain extend, on plant treated with aqueous extracts, indicating a repellent effect. Ovipositional deterrent effects of botanical pesticides on many crop pests including Lepidopteran, Homopteran and Dipteran species (Singh & Singh, 1998; Bruce et al., 2004; Showler et al., 2004; Greenberg et al., 2005; Hossain & Poehling, 2006; Seljåsen & Meadow, 2006; Adebowale and Adedire, 2006; Boateng & Kusi, 2008; Agboka et al., 2009) have been reported. According to Udayagiri and Mason (1995), chemical cues play a major role in host selection. Similar results have been observed by Bruce et al. (2004), who found that application of neem oil at 0.075 ml per maize plant leads to a reduction in the number of eggs laid by *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Eldana saccharina* Walker (Lepidoptera: Pyralidae) of 88 and 49%, respectively, compared with the control. Oviposition deterrence was also observed in *Mussidia nigrivenella* (Lepidoptera: Pyralidae) by Agboka et al. (2009) who report significantly higher oviposition deterrence index with neem oil at 2.5 and 5%, *Jatropha curcas* at 5% and *Hyptis suaveolens* at 20%. Lowery and Isman (1993) suggest that this deterrence resulted from a variety of compounds working in concert with another, producing different behavioural responses, which vary in magnitude between species.

In conclusion, the potential of *R. communis* in *P. xylostella* control has good prospects. However, oils including aqueous extracts of seeds have to be tested on non targets insects including parasitoids before being included in integrated pest management programs currently being developed. Moreover additional studies are required in other to develop appropriated formulation and application method of *R. communis* based pesticide.

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Table 3: Effects of topical application of castor bean extracts and oil on adult DBM emergence, deformities, sex ratio and longevity.

Treatments	% Emergence[1]	% Adults without deformities[1]	Sex ratio (males/females)	Adult longevity (days)[2]	
				Male	Female
Seed kernel extract (20%)	75.29 ± 2.41b	30.85 ± 1.09c	1.18 ± 0.08b	9.85 ± 0.98Ac	9.04 ± 1.01Ac
Root extract (20%)	79.84 ± 3.01b	53.49 ± 2.41b	1.04 ± 0.06c	11.08 ± 0.24Aa	10.82 ± 1.24Aa
Leaf extract (20%)	77.45 ± 1.20b	46.28 ± 1.98b	1.16 ± 0.13b	10.58 ± 1.08Ab	10.17 ± 0.98Ab
Oil (5%)	57.72 ± 1.57c	23.14 ± 2.78c	1.63 ± 0.15a	9.28 ± 0.86Ac	8.18 ± 1.04Ad
Oil (10%)	-[3]	-[3]	-[3]	-[3]	-[3]
Dursban (5%)	76.19 ± 1.26b	95.58 ± 2.45a	1.24 ± 0.09b	10.86 ± 0.98Ab	10.10 ± 0.85Ab
Control 1 (Emulsified water)	98,42 ± 1,08a	100.00 ± 0.00a	0.97 ± 0.05c	13.41 ± 0.95Aa	12.49 ± 1.06Aa
Control 2 (1% ethanol in water)	98.12 ± 1.58a	100.00 ± 0.00a	0.96. ± 0.41c	13.72 ± 0.83aA	12.72 ± 1.02Aa
Control 3 (untreated check)	98.87 ± 0.98a	100.00 ± 0.00a	0.98 ± 0.07c	13.25 ± 1.05Aa	12.48 ± 2.45Aa
df	7,16	7,16	7,16	7,16	7,16
F	206.31	1099	5.133	10.8	5.133
P	< 0.0001	< 0.0001	0.003	< 0.0001	0.003

Means followed by the same lower case letter within a column and means adult longevity followed by the same upper case letter within line are not significantly different, ($P < 0.05$; Student-Newman-Keuls test).

[1]Average of 90 pupae, 3 replications; [2]Average of 45 moths, 3 replications, [3]treated larvae died before pupation.

Table 4: Effects of ingestion of castor bean extracts and oil on adult DBM emergence, deformities, sex ratio and longevity.

Treatments	% Emergence[1]	% Adults without deformities[1]	Sex ratio (males/females)	Adult longevity (days)[2]	
				Male	Female
Seed kernel extract (20%)	77.35 ± 1.28b	35.18 ± 1.13c	1.62 ± 0.19a	10.02 ± 1.09Ac	9.58 ± 1.52Ad
Root extract (20%)	87.14 ± 1.75b	56.27 ± 1.74b	1.35 ± 0.75a	12.74 ± 1.11Aa	11.96 ± 0.98Ab
Leaf extract (20%)	82.05 ± 2.13b	49.09 ± 1.09b	1.27 ± 0.09a	11.01 ± 1.13Ab	10.95 ± 1.02Ac
Oil (5%)	49.98 ± 0.98c	20.76 ± 1.97d	1.73 ± 0.45a	9.11 ± 0.98Ac	9.02 ± 1.55Ad
Oil (10%)	-[3]	-[3]	-[3]	-[3]	-[3]
Dursban (5%)	80.85 ± 2.91b	91.58 ± 3.01a	1.09 ± 0.26a	11.01 ± 1.21Ab	10.85 ± 0.96Ac
Control 1 (Emulsified water)	98.39 ± 1.54a	100.00 ± 0.00a	0.97 ± 0.74a	12.98 ± 1.09Aa	12.14 ± 1.68Aa
Control 2 (1% ethanol in water)	98.41 ± 1.22a	100.00 ± 0.00a	0.96 ± 0.81a	12.79 ± 1.10Aa	12.36 ± 1.39a
Control 3 (untreated check)	97.49 ± 1.90a	100.00 ± 0.00a	0.98 ± 0.09a	13.86 ± 1.08Aa	12.63 ± 1.62Aa
df	7,16	7,16	7,16	7,16	7,16
F	238.992	1380	1.048	6.745	2.834
P	< 0.0001	< 0.0001	0.38	0.001	0.004

Means followed by the same lower case letter within a column and means adult longevity followed by the same upper case letter within linear not significantly different ($P < 0.05$; Student-Newman-Keuls test).

[1]Average of 90 pupae, 3 replications; [2]Average of 45 moths, 3 replications, [3]treated moth died before pupation.

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