Neem as a cost-effective and potent biopesticide against the diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) and the cabbage webworm *Hellula undalis* F. (Lepidoptera: Crambidae)

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Abstract

Cabbage is an important cash crop to the resource-poor farmers in sub-Saharan Africa and offers a good source of vitamins and minerals. The diamondback moth (DBM), Plutella xylostella L. and the cabbage webworm, Hellula undalis F. are major pests causing significant losses to brassica crops worldwide. During the major and minor seasons of 2015, an experiment was carried out at the University of Ghana Soil and Irrigation Research Centre (SIREC), Kpong to determine the effect of some pesticides (synthetic insecticides-chlorpyrifos and lambda-cyhalothrin, botanicals - hot pepper fruit extract, aqueous neem seed extract, local insecticidal soap - 'alata samina' and water as control) in controlling the diamondback moth and the cabbage webworm on cabbage. Cabbage seedlings were transplanted onto 3m x 3m plots, and plots were labelled by randomly assigning treatments to them. The experiment was laid out in a complete randomised block design, consisting of six treatments in three replications. Treatments were applied weekly, two weeks after transplanting and data on the population of the diamondback moth and the cabbage webworm, multiple head formation were collected weekly. At the end of each season the resulting yield was assessed for marketability, and cost benefit analysis carried out to determine the cost: benefit ratio. The results revealed that the highest population of the diamondback moth was recorded in the plots sprayed with chlorpyrifos and lambda-cyhalothrin, with neem recording the least number of diamondback moth and cabbage webworm populations. The highest marketable yield of 13.82t/ha and 28.36t/ha was recorded for the neem sprayed plots with a cost: benefit ratio of 1:48.6 and 1:137.1 for both seasons, respectively, followed by pepper extract (3.92t/ha, 1:10.5) for the major season and 'alata samina' (8.86t/ha, 1:36.4) for the minor season. The aqueous neem seed extract can be used by resource-poor farmers in Ghana as the most cost-effective biopesticide against the diamondback moth and the webworm on cabbage.

Introduction

Cabbage (Brassica oleracea var. capitata) cultivation is an important source livelihood for small-scale farmers in sub-Saharan Africa due to its growing popularity for home consumption and the food industry (Abbey and Manso 2004; Owusu-Boateng and Amuzu, 2013; Fening et al., 2013; Forchibe et al., 2017). It offers nutritional benefits to its consumers due to its high nutritive value. However, owing to its susceptibility to insect pests in the field, severe losses of up to 100% have been recorded, especially for the diamondback moth (dbm), Plutella xylostella L (Chellaiah and Srinivasan, 1986; Lingappa et al., 2004; Fening et al., 2013). These pests attack the cabbage plant at different

growth stages and cause severe damage to the head of the crop as well as the wrapper leaves which results in significant decrease in its marketability (Eigenbrode et al., 1990; Baidoo and Adam, 2012; Fening et al., 2017). Consequently, the global cost of control of DBM was estimated in 2012 to be between US\$ 4 and 5 billion (Zalucki et al., 2012). Similarly, Sivapragasm (1994) reported that Hellula undalis F. (Lepidoptera: Crambidae) is responsible for 41% of the total mortality of plants during the pre-heading stage and multiple head formation (Fening et al., 2013, 2014). This pest has no economic threshold level to initiate insecticide application and makes it an important pest of cabbage (Sivapragasm et al., 2001). Therefore, a single larva boring into the growing shoot of a cabbage plant during pre-heading can result in the production of multiple unmarketable heads or the death of the plants.

Intensive and widespread use of synthetic insecticides has been and continues to be the main control strategy for these pests, especially against DBM (Barros et al., 1993; Ntow et al., 2006; Cheng et al., 2008). Synthetic insecticides have been ineffective, largely due to the development of resistance to most of the insecticides in use today, coupled with the negative impact on the environment and food safety concerns (Obeng-Ofori et al., 2002; Odhiambo et al., 2010; Fening, 2013). Thus, there is the need for alternative and sustainable pest management interventions against these pests. This study explored other user-friendly and cost-effective insecticides of plant origin for managing these two devastating pests of cabbage.

Materials and Methods

Study site

The study was carried out at the University of Ghana, Soil and Irrigation Research Centre, Kpong (N6°8'5.5464", E0°4'55.452"), in the Eastern region of the Coastal Savanna agro-ecological zone of Ghana. It has an annual rainfall between 700 and 1100 mm, an average annual temperature of 28 °C and a relative humidity of between 59 - 93 %. The main soil type is the vertisols (black clay soil). Experiments were undertaken between May and August, and September and December 2015 in the major and minor rainy seasons, respectively.

Land preparation, transplanting and application of treatments

The land was cleared of weeds, ploughed, harrowed and ridged. Certified seeds of

hybrid white cabbage (B. oleracea var. capitata) (cv. oxylus) were sown on nursery beds. The young seedlings were protected from pest attack with mosquito-proof netting (1.2 mm ×1. 2 mm of mesh size) (Forchibe et al., 2017). Standard agronomic practices such as weeding, and irrigation were carried out throughout the growing period as needed. Plants were fertilised using NPK 15-15-15 (5g/plant) and Ammonium sulphate (3g/plant) 2 and 6 weeks, respectively after transplanting. The experimental design was a randomized complete block with six treatments and three replicates. Cabbage seedlings were transplanted 30 days after germination. The planting distance was 0.5 m x 0.5 m and each plot measured 3 m x 3 m. Each plot was made up of six rows, with five plants per row, making 30 plants per plot. Inter-plot alley was 2m to prevent drift between adjacent plots. Treatments used were neem seed (Azadirachta indica) extract (50 g/L of water), pepper fruit (Capsicum frutescens) extract (20 g/L of water), local insecticidal soap solution ('alata samina'; bought from a local market) (7 g/L of water) and two commonly used synthetic insecticides Conpyrifos® (chlorpyrifos, 2 ml/L of water), and Lambda M® (lambdacyhalothrin, 2 ml/L of water) and tap water as control. Dosages used for the biopesticides were as recommended by previous research (Obeng-Ofori and Sackey, 2003; Biswas, 2013; Fening et al., 2013, 2014).

Treatment preparation and application

Ripe fruits of *Capsicum frutescens*, were obtained from a local market and 60g was obtained using an electronic balance. The weighed fruits were homogenised using an electric blender and allowed overnight in 3L of water. 150g of dry neem seeds were also weighed and pounded in a wooden mortar using a wooden pestle. The homogenate was

left overnight, and later sieved through a fine linen material (12-16 µm diameter mesh). The mixtures were further diluted with the required volume of water for spraying. 21g of 'alata samina' was also weighed and dissolved in 3L of water.

Treatments were applied using a 15 L capacity knapsack sprayer. For each treatment different knapsack sprayers were used to avoid contamination. Applications commenced 14 days after transplanting of seedlings for both the major and minor seasons and continued weekly thereafter until cabbage heads were fully mature, about 14 days to harvesting. The duration of treatment application was 7 weeks and the number of applications was 7 times per season.

Data collection

Ten cabbage plants from the inner rows were sampled weekly and examined thoroughly for the presence at *P. xylostella* and *H. undalis* larvae. Multiple heads were counted per plot and the percentage calculated by dividing the number of plants with multiple heads by the total number of plants and multiplied by 100%. That is:

 $\frac{\textit{Number of plants with multiple heads}}{\textit{Total number of plants per plot}} \times 100 = \textit{Percent multiple head formation}$

Identification of insects

Samples of larvae of diamondback moth and the cabbage webworm were reared in the laboratory to the adult life stage to allow for identification. All insects collected were identified by comparison with labelled reference specimen of the insect museum of the Department of Animal Biology and Conservation Science, University of Ghana.

Cost: benefit ratio analysis

At the end of the trial, cabbage heads of the inner rows were harvested and weighed for yield assessment (tonnes/hectare). Yields per plot were weighed and expressed as total yield (t/ha). Cabbage heads were classified into marketable and unmarketable heads, and their percentages calculated from the total yield per plot. The classification into marketable and unmarketable yield was based on the degree of damage on cabbage heads. Only marketable yield (expressed as actual marketable yield) was sold at the prevailing market prices. The Ghanaian currency, Cedi (¢) was converted to US\$ using the exchange rate at the time of study (1 Gh ¢ equals 3.82\$ and 3.95\$ for the major and minor seasons, respectively). The cost of cabbage per head was expensive during the minor seasons due to its scarcity during this period (mostly attributed to low rains and very few farmers engaging in production). Cost of labour was constant for all treatments and was not included in the total cost of production. Cost and benefit analysis were carried out for both seasons, using the costs of production and marketable yield, computed following the formulae by Amoabeng et al. (2014). All expenses and income were converted to per hectare basis.

Data analysis

Weekly count data were square root transformed and subjected to repeated measures of ANOVA. Percentage data were arcsine square root transformed prior to ANOVA. Mean cabbage head weight was also analysed using ANOVA. Significant differences in means were separated using SNK test ($P \le 0.05$). All analyses were carried out in GENSTAT version 12.0.

Results

Effect of treatments on pests

The diamondback moth (DBM), *Plutella xylostella* infestation started in the first week of

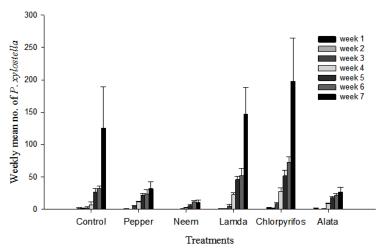


Fig 1. Effects of treatments on mean (± SE) weekly count of DBM larvae per cabbage plant in the major season, 2015, Kpong, Ghana

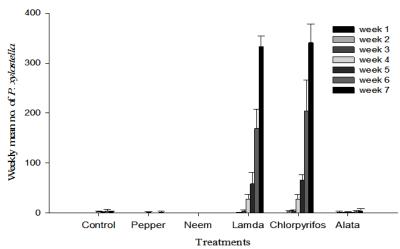


Fig 2. Effects of treatments on mean (± SE) weekly count of DBM per cabbage plant in the minor season, 2015, Kpong, Ghana

sampling and increased steadily for the major and minor seasons (Figs. 1 and 2). The control (tap water) and the synthetic insecticides (lambda cyhalothrin and chlorpyrifos)treated plots recorded the highest number of DBM larvae infested plants per plot for the major season, while for the minor season, the synthetic insecticide sprayed plots recorded the highest number of DBM larvae infested plants per plot. Neem-treated plots however, recorded the least number of DBM for both seasons, followed by 'alata samina' and pepper-treated plots. There was a significant difference among the treatments in controlling the DBM in both seasons ($F_{5,125} = 30.49$; P < 0.0010 and $F_{5,125} = 99.82$; P < 0.0010 for major

and minor seasons, respectively). The effect of each treatment on DBM population among the weeks of sampling for both seasons were also significantly different ($F_{6,\ 125}=83.45;\ P<0.0010$ and $F_{6,\ 125}=85.23;\ P<0.0010$). The interaction between the sampling weeks and treatments was also significant for the major and minor seasons ($F_{30,\ 125}=3.95;\ P=0.0070$ and $F_{30,\ 125}=29.41;\ P<0.0010$). A t- test showed that there was no significant difference in DBM counts between the major and minor seasons ($P=0.795,\ t=0.27$).

The population of the cabbage webworm, *Hellula undalis* was low throughout the sampling period for both seasons. Many treatment plots did not record any *H. undalis*

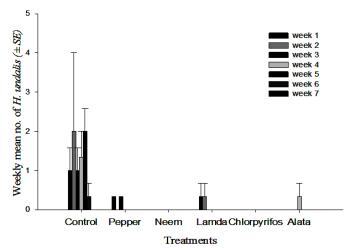


Fig 3: Effects of treatments on mean (± SE) weekly counts of *H. undalis* larvae per cabbage plant during the major season, 2015 in Kpong, Ghana

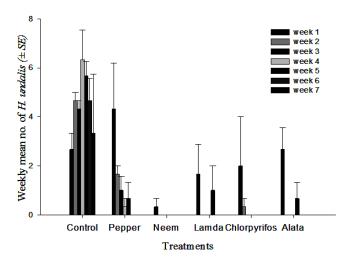


Fig 4: Effects of treatments on mean (± SE) weekly counts of *H.undalis* per cabbage plant during the minor season, 2015 in Kpong, Ghana

for the whole sampling period. Peaks were observed in the second and fifth week in the major season and fourth week in the minor season for the control plot (Figs. 3 and 4). There was a significant difference among the treatments in controlling *H. undalis* for both seasons ($F_{5,125} = 5.86$; P = 0.0090 and $F_{5,125} = 39.43$; P < 0.0010). The effect of each treatment on *H. undalis* population among the weeks of sampling was not significantly different for the major season ($F_{6,125} = 1.22$; P = 0.314) but significantly different for the minor season ($F_{6,125} = 6.90$; P = 0.0010). However, the interaction between the sampling weeks and treatment was not significant for both seasons

(F $_{30,125} = 0.99$; P = 0.476 and F $_{30,125} = 1.98$; P = 0.053). A t-test showed that there was no significant difference in *H. undalis* counts for both seasons (P = 0.243, t = -1.30).

Damage assessment using multiple heads

Damage due to multiple heads was not significant for the different treatments for the major season but was significant during the minor season, with highest numbers recorded in the control plots, followed by pepper and chlorpyrifos-treated plots. Neem-treated plots recorded the least number of multiple heads followed by 'alata samina'-treated plots during the minor rainy season (Table 1).

TABLE 1					
Mean (± SE) percentage multiple cabbage head counts at harvesting for treatments					
in the major and minor rainy seasons of 2015, Kpong, Ghana					

Treatment	% Multip	% Multiple heads			
Treatment	Major season	Minor season			
Control	1.67±0.882	$52.2 \pm 6.759a$			
Pepper	1.00 ± 1.00	36.7 ± 8.819 be			
Neem	0.00 ± 0.00	$4.4 \pm 1.111c$			
Lambda-Cyhalothrin	2.00 ± 0.58	$21.1 \pm 5.56d$			
'Alata Samina'	0.67 ± 0.67	14.40 ± 2.94 dc			
Chlorpyrifos	0.00 ± 0.00	27.8 ± 4.01 de			
F	1.13	22.49			
P	0.4070	< 0.0010			

Means with the same letter(s) are not significantly different (P < 0.05, SNK test) within columns

TABLE 2

Cost: benefit ratio evaluation for the major rainy season of managing pests with neem seed extract, pepper fruit extract, 'alata samina' and two synthetic insecticides (Lambda-cyhalothrin and Chlorpyrifos)

Treatment	Total cabbage Yield (t/ha)	Actual Marketable Yield (t/ha)	Cost Of Plant Protection (US\$/ha)	Income From Marketable Yield (US\$/ha)	Net Benefit (US\$/ha)	Benefit Over Unsprayed Treatment	Cost: Benefit Ratio
Neem	$17.80 \pm 2.61a$	13.82±1.35a	130	7235	7105	6314	1:48.6
Pepper	$12.37 \pm 1.30ab$	3.92±4.09b	110	2052	1942	1151	1:10.5
'Alata samina'	$14.57 \pm 2.75ab$	1.41±0.87c	80	738	658	-133	1: -1.7
Chlorpyrifos	$13.40 \pm 1.99ab$	3.57±4.81b	120	1869	1749	958	1:8
Lambda	$6.03 \pm 2.08b$	0±0d	120	0	-120	-911	1:-7.6
Control	$12.37 \pm 1.27ab$	1.51±1.84c	0	791	791	0	-

Means within columns with different letters differ significantly (P<0.05)

Cost: benefit ratio

From the cost: benefit analysis, the results indicated that neem seed extract had the highest cost: benefit ratio compared to all the other treatments for both seasons (Tables 2 and 3). This was followed by chlorpyrifos and pepper in the major season (Table 2) and 'alata

samina' and pepper in the minor season (Table 3). 'Alata samina' and lambda-cyhalothrin had negative cost: benefit ratios in the major season, while the conventional insecticides lambda-cyhalothrin and chlorpyrifos had a negative cost: benefit ratios (least) for the minor season (Tables 2 and 3).

TABLE 3

Cost: benefit ratio evaluation for the minor rainy season of managing pests with neem seed extract, pepper fruit extract, 'alata samina' and two synthetic insecticides (Lambda-cyhalothrin and Chlorpyrifos)

Treatment	Total cabbage Yield (t/ha)	Actual Marketable Yield (t/ha)	Cost Of Plant Protection (US\$/ha)	Income From Marketable Yield (US\$/ha)	Net Benefit (US\$/ha)	Benefit Over Unsprayed Treatment	Cost: Benefit Ratio
Neem	28.36±0.971a	28.36±0a	130	17949	17819	17818	1:137.1
Pepper	6.503±0.7364c	1.63±5.77b	110	4114	4004	4003	1:36.4
'Alata samina'	15.05±3.052b	8.86±6.54c	80	5608	5528	5527	1:69.1
Chlorpyrifos	10.02±3.702bc	0±0d	120	0	-120	-121	1:-1
Lambda	5.07±2.809c	0±0d	120	0	-120	-121	1:-1
Control	0.59±0.0664c	0.002±0.19d	0	1	1	0	-

Means within columns with different letters differ significantly (P<0.05)

Discussion

The findings of this study revealed that neem seed extract effectively controlled DBM and H. undalis. Azadirachtin which is the most abundant active ingredient in neem, is known to induce a physiological effect on insects by interfering with the synthesis and release of ecdysteroids which disrupts larval moulting in hemi- and holometabolous insects (Mordue and Blackwell, 1993; Anibal and Condor, 2007). The effective control of these insects can be attributed to the diverse modes of action of azadirachtin present in neem. Neem is also known to act as a deterrent when sprayed to plants and alters some properties of the cabbage plant such as the leave colour which is known to be an attracting factor to DBM and other insect pests (Sarfraz et al., 2006). Gaby (1988) indicated that botanicals like neem are very important in altering the attractive properties of cruciferous plants to DBM. This finding concurs with studies done by Lidet et al. (2009); Sow et al. (2013); Begna and Damtew (2015), who recorded similar effects on DBM when neem was used and Prasannakumr et al. (2014) who recorded effective control of H. undalis when cabbage plants were treated with neem. The effect of pepper fruit extract on DBM and H. undalis as observed in this study, can be attributed to the presence of capsaicinoid elements (Antonious et al., 2006; Fening et al., 2013), alkaloids, saponins and flavonoids (Habimana and Hakizayezu, 2014). A similar study by Shazia et al. (2006), reported that pepper effectively reduced the incidence and severity of the P. xylostella leading to high number and percentage of marketable heads, compared to the control. The insecticidal soap, 'alata samina' on the other hand also had a significant effect on the insect pests than conventional

pesticides. 'Alata samina' is a local soap made from potash and its insecticidal property is attributed to the presence of potassium salts of several fatty acids which is believed to disrupt pest's cellular membrane, once it touches the insect (especially the larval forms, since they are soft bodied) leading to the insect cells leaking their contents causing dehydration and death (Osborne and Henley, 1982).

On the other hand, synthetic insecticidestreated plots, recorded a constant build-up of DBM populations throughout the two sampling seasons, but induced significant control of H. undalis to below damaging levels. Hill and Foster (2000), suggested that, synthetic insecticides will remain essential for the management of DBM larvae since it feeds on the marketable portions of the crop. However, the insecticides used in this study; lambda-cyhalothrin and chlorpyrifos did not control DBM, leading to severe damage of crops. The DBM has been reported to have developed resistance to several groups of insecticides, making it difficult to be controlled in the field (Branco and Gatehouse, 1997; Odhiambo, 2005; Eziah et al., 2009; Odhiambo et al., 2010). Therefore, high numbers recorded in the lambda-cyhalothrin and chlorpyrifos-treated plots is possibly due to resistance as was reported by Eziah et al. (2009) and Odhiambo et al. (2010). Moreover, DBM was reported to have acquired cross resistance to commonly used pyrethroids and multiple resistance to pyrethroids and organophosphates (Odhiambo et al., 2010). It is generally known that continuous usage of insecticides usually results in the emergence of insecticide resistance populations in insect pests. The results obtained from this study would be the case as studies by Horna et al. (2008) and Afari-Sefa et al. (2015) showed that these insecticides are among the frequently

used insecticides on cabbage in Ghana. A survey by Amoabeng et al. (2016) recorded high usage of lambda-cyhalothrin among cabbage farmers despite its ineffectiveness as recorded in this study.

The cost benefit ratio obtained from the assessment was totally dependent on the price of the commodity during the study period. Fluctuations in the US\$ exchange rate also played a major role in the total income and benefits obtained over control. The study indicated that neem seed extract was the most cost-effective biopesticide against the two major insect pests of cabbage as was also reported by Ezena et al. (2015). Additionally, toxicity trials for neem products particularly neem oil and aqueous extracts have shown low toxicity to mammals (Boeke et al., 2004). It is therefore recommended for use in small scale cabbage production, especially in organic farming systems. Nevertheless, farmers need to be educated on the economic benefits of neem-based pesticides as well as their positive impacts on humans and the environment to increase its adoption for use in the control of insect pests. There is also the need for government interventions and policies to promote local production and adoption in Ghana. Additionally, due to the short shelf life, photosensitivity and slow killing rates of biopesticides such as neem, (Campos et al., 2016), appropriate extraction and storage methods needs to be employed to standardize and maintain its composition and potency.

Conclusion

The results of this study offer an environmentally friendly, cost-effective and safe biopesticide to control DBM and *H. undalis* on brassica crops, especially for resource-poor smallholder

farmers in Ghana for enhanced livelihoods. Using neem as a plant-based insecticide will ensure the production of safe and quality vegetables, thus minimising the risk of insecticide residues accumulating in harvested produce. In addition to the negative effects of conventional pesticides on the consumer and the environment, most of them are no more effective in controlling DBM and H. undalis on cabbage, as they are likely to have developed resistance to most of these insecticides in use today. In contrast, neem as a botanical insecticide exhibits several modes of action (e.g. anti-feedant, causes abnormal and delayed moults, growth regulator, increased mortality, sterility effect, etc.), thus making it difficult for the DBM and *H. undalis* to develop resistance against it.

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