LARVICIDAL EFFECT OF METHANOL EXTRACTS OF *ZANTHOXYLUM XANTHOXYLOIDES* (LAM) AGAINST THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: PLUTELLIDAE) ON CABBAGE

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ABSTRACT
The larvicidal effect of methanol extracts of candlewood, Zanthoxylum xanthozyloides (Lam) against diamond back moth (DBM) larvae Plutella xylostella (L.) (Lepidoptera: Plutellidae) was investigated in the laboratory. Three concentrations of 20%, 15% and 10% for both leaf and stem bark of Z. xanthozyloides and a control were applied against the DBM larva. Results from the study showed that the extract yielded 90% and 70% larval mortality at concentration of 20% within 72 hrs of exposure to stem bark and leaf extracts, respectively. At 10%, 15% and 20% concentration, percentage repellencies for leaf extract were 30%, 60% and 70% whereas the bark extract gave 30%, 60% and 80% percentage repellencies respectively after 24 hrs of treatment. Decrease in larval damage of 33.3% and 41.7% were recorded for cabbage leaves treated with 20% of stem bark and leaf, respectively whereas the control yielded 100% damage. This study has revealed the bio-insecticidal potential of Z. xanthoxyloides in the management of P. xylostella.

INTRODUCTION
The diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) is one of the most destructive and problematic pest of cabbage in Ghana and world at large. Its economic importance is highly reflected globally as almost US$ 4 billion and US$ 5 billion is estimated annually for its control (Zalucki *et al*., 2012; Wei *et al*., 2013). Due to their damage, cabbage growers rely on calendar spray of high dosage of synthetic insecticides. Although synthetic insecticides are valued for their effectiveness and convenience yet the diamond back moth has developed resistance to almost all groups of insecticides including new ones (Nisin *et al*., 2000: Shelton *et al*., 2000: Sarfaz and Keddie, 2005).

Plants derived products and botanicals have been given priority recently to reduce the use of synthetic insecticides and losses caused by agricultural pests and diseases (Devi and Uupta, 2000; Tewary *et al*., 2005; Facknath, 2006; Ssekyewa e*t al*., 2008). One of such plants which have been given much attention in stored food protection is the candlewood *Zanthoxylum xanthoxyloides.*

The leaf, bark and root of *Z. xanthoxyloides* have been effective in protecting grains from storage product pests such as *Callosobruchus. maculatus*, *Sitophilus zeamais* and *Prostephanus truncates* (Udo, 2000; Owusu *et al.,* 2007) due to its antibacterial, antiviral and antivenin properties (Kassim *et al*., 2009).

The plant contains phenolic compounds, secondary metabolites and alkaloids of which benzo-

phenanthridines are the most common. The phenylpropaniods compounds coumarins in *Zanthoxylum* are very useful and have exhibited antibacterial, antiviral, anti-tumor and anticoagulant activities (Mabry and Ulubelen, 1980). Most of the *Zanthoxylum* species contains lignans specifically diarylbutirolactones and 2,6-diaryl-3, 7-dioxabicyclo [3.3.0] octanes which are responsible for the biological activities including the insecticidal and inhibitory effects on certain enzymes (Adesina, 2005). This study evaluated the effect of methanol extract of the leaves and stem bark of *Z. xanthoxyloides* against the diamond back moth on cabbage plant.

MATERIALS AND METHODS

The study was conducted at the Entomology laboratory in the Crop Science Department of the University of Ghana, Legon.

Insects collection and culture

The DBM larvae and pupae were collected from farmers' farm in Djorwulu (Accra, Ghana) using fine camel hair brush into petri dishes lined with tissue paper. Collected larvae and pupae were placed separately in plastic containers and larvae were provided with fresh cabbage leaves until adult emerged. Adult that emerged were allowed to mate at random and provided with cabbage leaves for oviposition. Eggs laid on leaves were transferred into plastic containers lined with filter paper and covered until they hatched. The 1st stage larvae that emerged fed on fresh tender leaves. Insects colonies were established under controlled laboratory conditions of 27 ± 2.0°C, 65-70% relative humidity and photoperiod of 12h:12h (L:D) and were fed on insecticide-free cabbage. Early 2nd stage of F_2 larvae from the population were used for the assays.

Plant collection and extraction

The leaves and stem bark of *Z. xanthoxyloides* were collected from University of Ghana botanical garden, Legon (Accra, Ghana). The plant parts were air-dried, pounded, milled and sieved with sieve of mesh size of 710 u, to obtain fine powder for the extraction. Methanol (70%) was used for the extraction.

One hundred grammes (100g) of the plants powder was added to 250 mL of methanol and kept in the dark for three days. The mixtures were filtered and the extracts concentrated using the rotary evaporator. 3 ml of the extracts was solubilized in 0.2 ml of acetone and 9.8 ml of distilled water was added to obtain stock concentration of 30%. Three concentrations 20%, 15% and 10% from the stock solution were prepared by serial dilution and used for the bioassays.

Toxicity effect

This was done by adopting method described by Maa and Liao, (2000) and Botwe *et al*., (2012). A fixed volume of 0.1 uL of the various concentrations of each plant extracts was topically applied on 10 larvae of DBM which were then transferred into containers containing cabbage leaf disc. Each treatment had three replications with water alone used as control. The response measured was insect mortality. Insects were considered dead when they fail to respond to probing by blunt probes.

Repellency test and damageassessment

For repellency test, cabbage leaf discs of diameter 0.5cm±0.2 treated with various plant extracts and untreated leaf were placed opposite to each other in a petri dish lined with moistened filter paper. Ten larvae of the DBM were introduced and left for 24 hrs. The numbers of DBM found on the treated and untreated leaves were recorded. Percentage repellency was calculated using the formula;

Damage on treated leaves caused by DBM larvae was assessed by visual scoring on a scale of (0 – 4) where 0 is no damage and 4 is total damage to the leaves.

$$\% \text{ repellency} = \frac{\text{Total No. of Larvae - No. on treated leaves}}{\text{Total number of larvae}} \times 100$$

Data Analysis

Data on mortality, repellency and were analysed using Analysis of variance at 0.05 probability

level using Genstat Statistical Package 9.2 (9th Edition). Mortalities were corrected using Abbot's formula. Means were separated using Duncan Multiple Range test.

RESULTS

Developmental stages

Eggs laid took 4.5 days to hatch. The four larval developmental stages lasted for 12-15 days with the 1st stage at 2.5 days, 2nd stage at 3 days, 3rd stage at 3.5 and the 4th stage at 4 days. The period for pupation lasted for 6 days and adults that emerged lived for about 5-7 days.

Contact toxicity of *Z. xanthoxyloides* stem bark on *P. xylostella* larvae

Table 1 shows the result of contact toxicity of *Z. xanthoxyloides* stem bark on *P. xylostella* larvae. After 24 hrs of treatment, 53.3% mortality of larvae was observed at a concentration of 20% while 33.3%, 13.3% and 0.0% mortalities were recorded for 15%, 10% and the control, respectively. At 48 hrs, mortalities of 20.0%, 46.7% and 73.3% were recorded at 10%, 15% and 20% concentrations, respectively. By 72 hrs, 20% concentration gave 90.0% larvae mortality whereas 15% yielded 73.3 % and 10% also recorded 30.0% mortality. No mortality was recorded in the control. Mortality for each treatments were significantly different (Fpr. = 0.001: P <0.05).

Contact toxicity of *Z. xanthoxyloides* leaf on *P. xylostella* larvae

Result of contact toxicity of *Z. xanthoxyloides* leaf on *P. xylostella* larvae is shown in Table 2. At 24 hrs of exposure, larval mortalities for each concentration 20%, 15%, 10% and control were 33.3%, 26.7%, 6.7% 0.0%, respectively. Within 48 hrs, larval mortalities increased to 16.7%, 43.3% and 53.3% at 10%, 15% and 20% concentrations, respectively. Larval mortality increased by 72hrs of exposure at 20% recorded 70.0%, 15% had 53.3 % and 10% recorded 23.3% mortalities. There was no larval mortality in the control. Mortality for each treatments were different significantly (Fpr. = 0.001: P <0.05).

Repellency and damage effect on cabbage leaves treated with *Z. xanthoxyloides* stem bark extract

The repellency effect on *P. xylostella* and larval damage on cabbage leaves treated with *Z. xanthoxyloides* stem bark at 24 hrs is presented in Table 3. As repellency increased, larval damage on treated cabbage leaves also decreased. At 10%, 15% and 20% concentrations, percentage repellency and damage were 30.0% and 60.0%, 80.0% and 75.0%, 66.7% and 33.3%, respectively. For control, no insect was repelled and 100% damage was recorded.

Table 1: Number of dead larvae after 72 hours of exposure to *Z. xanthoxyloides* stem bark extract

	% Mortality (±S.E) hours after treatment		
Conc(%)	**24**	**48**	**72**
20	53.3 ± 0.3^{a}	73.3 ± 0.3^{a}	90.0 ± 0.0^{a}
15	33.3 ± 0.3^{b}	46.7 ± 0.3^{b}	73.3 ± 0.3^{b}
10	13.3 ± 0.3^{c}	20.0 ± 0.0^{c}	30.0 ± 0.3^{c}
Control	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}

Means marked with different letter within a column are significantly different (P<0.05)

Table 2: Number of dead larvae after 72 hours of exposure to *Z. xanthoxyloides* leaf extract

	% mortality (±S.E) hours after treatment		
Conc (%)	**24**	**48**	**72**
20	33.3 ± 0.3[a]	53.3 ± 0.3[a]	70.0 ± 0.0[a]
15	26.7 ± 0.3[a]	43.3 ± 0.3[b]	53.3 ± 0.3[b]
10	6.7 ± 0.3[b]	16.7 ± 0.3[c]	23.3 ± 0.3[c]
Control	0.0 ± 0.0[c]	0.0 ± 0.0[d]	0.0 ± 0.0[d]

Means marked with different letter within a column are significantly different (P<0.05)

Table 3: Percentage repellency and larval damage on cabbage leaves after 24 hours exposure to *Z. xanthoxyloides* stem bark extract

	Mean ± S.E	
Conc (%)	**% Repellency**	**% Damage**
20	80.0 ± 0.0[a]	33.3± 0.3[d]
15	60.0 ± 0.3[b]	66.7± 0.0[c]
10	30.0 ± 0.0[c]	75.0 ± 0.1[b]
Control	0.0± 0.0[d]	100 ± 0.0[a]

Means marked with different letter within a column are significantly different (P<0.05)

Table 4: Percentage repellency and larval damage on cabbage leaves after 24 hours exposure to *Z. xanthoxyloides* leaf extract.

	Mean ± S.E	
Conc (%)	**% Repellency**	**% Larval Damage**
20	70.0 ± 0.0[a]	41.7± 0.3[d]
15	60.0 ± 0.3[b]	66.7± 0.0[c]
10	30.0 ± 0.0[c]	84.0 ± 0.1[b]
Control	0.0 ± 0.0[d]	100± 0.0[a]

Means marked with different letter within a column are significantly different (P<0.05)

Repellency and damage effect on cabbage leaves treated with *Z. xanthoxyloides* leaves extract

Table 4 is the repellency effect on *P. xylostella* and larval damage on cabbage leaves treated with *Z. xanthoxyloides* leaves extract. The level of repellency and larval damage recorded was significantly dependent on concentration. There was 0.0% repellency and 100% damage for untreated cabbage leaves. Repellency at 10%, 15% and 20% concentrations were 30.0%, 60.0% and 70.0% while larval damage on treated cabbage leaves were 84.0%, 66.7% and 41.7%, respectively.

DISCUSSION

The result from the study indicates that methanol extract of the leaves and stem bark of *Z. xanthoxyloides* plant possess insecticidal potential (Adesina, 2005) against the larvae of *P. xylostella*. Survival of *P. xylostella* larvae was reduced when they were exposed to the various concentration of leaf and bark extracts of *Z. xanthoxyloides*. This confirms the fact that most plant based pesticides exhibit larvicidal effects (Kétho *et al.*, 2002; Sanda *et al*., 2006; Ogendo *et al*., 2008; Agboka *et al*., 2009).

Larval mortality increased with time and concentration for both the leaf and stem bark of *Z. xanthxyloides*. Similar studies revealed that methanol extracts of the root and leaves of *Z. xanthoxyloides* at 2 g/ml induced 100% mortality in *P. truncatus* after 72 hrs of exposure (Eziah *et al*., 2013). Subsequently, the toxic effects of *Z. xanthoxyloides* have also been demonstrated on storage pests (Udo *et al*., 2004, Owusu *et al*., 2007).

The effectiveness of *Z. xanthoxyloides* on *P. xylostella* larvae may be attributed to a high level of bioactive compounds and some secondary metabolites presents in *Zanthoxylum* plants. These metabolites include alkaloids, aromatic amides, lignans, aliphatic ketones, mono and sesquiterpenes sterols, phenolypropanoids, coumarins and carbohydrate residues (Marr and Tang 1992; Adesina, 2005). Further Studies have also shown that the leaf contains essential oil monoterpene hydrocarbons (98.2%) whereas the stem and root bark of *Z. xanthoxyloides* contain benzophenanthridine, furoquinoline and aporphine alkaloids. Among these, secondary metabolites, zanthoxylol, a sterol and phenolic compound present in the extracts of *Zanthoxylum* species is responsible for the insecticidal activity (Wongo, 1998).

The different plant part extracts exhibited different percentage reductions on the target pest at different time intervals. It was observed that the bark of *Z. xanthoxyloides* was more effective than the leaf as it shows a high level of insecticidal activities against *P. xylostella* larva. This may be attributed to the fact that the bark contains more of the secondary metabolites which are responsible for the insecticidal properties than the leaf. Therefore the performance of a plant extract to a target insect depends solely on the part of the plant from which the extract is obtained.

The performances of the plant species in repelling the insects indicate the presence of chemical compounds that stimulate or cause the insects to avoid or make oriented movement away from the stimulus source (Dethier *et al*., 1960). The presence of alkanoids and flavonoids and other metabolites in the stem bark and leaves extracts of *Z. xanthoxyloides* (Adesina, 1986) is the possible cause for the repellency observed. The higher level of repellency exhibited by *Zanthoxylum* leaf and stem bark implies high amounts of chemical compounds in the extract which caused the larvae to avoid the treated cabbage leaves. This confirms Owusu *et al*. (2007) finding who reported that methanol extracts of *Z. xanthoxyloides* was an effective repellent to stored product beetles. Repellent properties of *Z. xanthoxyloides* have also been documented on *Musca domestica* (Bisseleua *et al*., 2008).

In conclusion, the potential of *Z. xanthoxyloides* has good prospects in the control of the larval stage of *P. xylostella.* However, methanol extract of the plant has to be tested on egg stage of *P. xylostella*. Moreover additional studies are

required to ascertain the effect of root extract of *Z. xanthoxyloides* on the insect.

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