Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **43** Issue **01 Year 2023** Page **30:43***

Field Application of Ethanolic and Aqueous Chili Extracts to Control Diamondback Moth (*Plutella Xylostella L.)* In Cabbage (*Brassica Oleracea Var. Capitata L.)*

Tony Fukatine[1], Wendy Wanio[2], Rudolph Tarue[3], Peter Navus[4], Kari Iamba[5]

[1] *Department of Agriculture, PNG University of Natural Resources and Environment, Private Mail Bag, Vudal, East New Britain, Papua New Guinea. Email: tonyfukatine@gmail.com*

[2]*Lab Technician, Department of Agriculture, PNG University of Natural Resources and Environment, Private Mail Bag, Vudal, East New Britain, Papua New Guinea. Email: waniow.43@gmail.com*

[3]*Lab Technician, Department of Agriculture, PNG University of Natural Resources and Environment, Private Mail Bag, Vudal, East New Britain, Papua New Guinea. Email: rtarue@gmail.com*

[4] *HoD of Department of Agriculture, PNG University of Natural Resources and Environment, Private Mail Bag, Vudal, East New Britain, Papua New Guinea. Email: prnavus@gmail.com*

[5] *Lecturer, Department of Agriculture, PNG University of Natural Resources and Environment, Private Mail Bag, Vudal, East New Britain, Papua New Guinea. Email: kari.iamba@gmail.com*

**Corresponding author's E-mail: kari.iamba@gmail.com*

Article History

Received: 18 June 2022
Revised: 05 March 2023
Accepted:08 March 2023

Abstract

The Diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae) is an economic oligophagous pest that feeds on the members of the Brassicaceae family. Cabbage as one of the brassicas is mostly targeted by the larvae of P. xylostella in tropical countries including Papua New Guinea (PNG). Attempts to control this pest continue to be a challenge for farmers since it is now resistant to commonly used synthetic insecticides. Chili (Capsicum frutescens L.) in particular is an insecticidal plant that has been proven to reduce the impact of pests including P. xylostella. Previous studies have focused on the extraction of chili phytochemicals with ethanol as an extraction solvent (tincture). The current study experimentally tested the potential of hot water chili extract. Tincture chili extract was relatively more effective than hot water extract against P. xylostella. The current study further confirms that the absence of chili extracts in dry conditions enhances the recruitment of the pest, P. xylostella. Furthermore, the tincture extract was also capable of withstanding rainy days due to its adhesive phytochemicals.

Keywords: ***Plutella Xylostella, Tincture, Hot Water, Rainfall, Relative Humidity***

1. Introduction

Cabbage, *Brassica oleracea* var. *capitata* L. (Brassicaceae) is a popular leafy vegetable grown globally. Originating from Western Europe, it is now very popular in tropical countries (FAO, 2000).

Cabbage is a highly favored vegetable within the Vudal area of East New Britain Province in Papua New Guinea and is grown all year round on a continuous cycle mostly for the local markets and consumption. The major constraint to cabbage production is insect pests. Among other pests, the Diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae) is the most notorious pest attacking cabbage from seedling to mature stage (Iamba & Malapa, 2020; Iamba & Waiviro, 2021; Iamba & Yoba, 2019). *P. xylostella* is an economic and cosmopolitan pest that is globally distributed (Zhang et al., 2016). Other pests that also affect the yield and market value of cabbage include the cabbage webworm, *Hellula undalis* F. (Lepidoptera: Crambidae); cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae); cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae); flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae); whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae); and the variegated grasshopper, *Zonocerus variegatus* (L.) (Orthoptera: Pyrgomorphidae) (Baidoo, Mochiah, & Apusiga, 2012; Fening et al., 2011; Mochiah et al., 2011). The cabbage plant is succulent and has a rich nutritional value therefore it attracts many herbivorous pests (Baidoo et al., 2012). The market value and quality of cabbage heads are greatly reduced since the larvae of DBM create feeding holes in the leaves, damage the growing buds, and tunnel into mature heads.

Control using synthetic insecticides has been unsuccessful due to the build-up of resistance in DBM over time (Iamba, Malapa, & Yoba, 2021; Xia et al., 2013). The negative impacts of synthetic insecticides on human health, the lack of expertise and equipment for safe handling among smallholder farmers thus suggest alternative approaches to pest management (Amoabeng et al., 2013; Coulibaly, Cherry, Nouhoheflin, Aitchedji, & Al-Hassan, 2007; Ntow, Gijzen, Kelderman, & Drechsel, 2006). Pesticide poisoning of farmers each year amounts to three million globally with 20,000 deaths relating to the indiscriminate use of agrochemicals (Darko & Akoto, 2008; Dinham, 2003). Coupled with the expensiveness of synthetic insecticides and concerns about environmental pollution, botanical insecticides have gained prominence (Mwine, Van Damme, Gerard, & Charles, 2011). Plant extracts or plant-derived products have received attention for their ability to reduce agricultural pests and diseases (Bajpai, Pande, Tewari, & Prakash, 2005; Devi & Pamila, 2000; Facknath, 2006; Ssekyewa, Mwine, Kalanzi, & Kudamba, 2008). And they were pursued as they are environmentally friendly having no negative impact on natural enemies (Ayalew & Ogol, 2006; Charleston, Kfir, Dicke, & Vet, 2006; Dadang & Prijono, 2009). Organic cabbage farming has been deemed as nature-friendly and preferred to inorganic farming which involves high chemical inputs (Baidoo et al., 2012). Plant extracts play an essential role in the plant protection program of an organic farming system.

A variety of plant extracts originating from different plant families have been studied for their efficacy to reduce pest pressure. Most of these plants containing insecticidal properties can be sourced locally and thus are readily available to local farmers (Iamba & Masu, 2020; Iamba & Waiviro, 2021). Some plants that have been found to possess insecticidal properties are *Azadirachta indica* A. Juss. (Sapindales: Meliaceae), *Cassia sophera* L. (Fabales: Fabaceae), *Cymbopogon schoenanthus* L. (Poales: Poaceae), *Ocimum americanum* L. (Lamiales: Lamiaceae), *Securidaca longepedunculata* Fres. (Polygalales: Polygalaceae), *Synedrella nodiflora* Gaertn. (Asterales: Asteraceae), *Chromolaena odorata* L. (Asterales: Asteraceae), *Capsicum frutescens* L. (Solanales: Solanaceae), *Allium sativum* L. (Asparagales: Amaryllidaceae) and *Carica papaya* L. (Brassicales: Caricaceae) (Amoabeng et al., 2013; Belmain & Stevenson, 2001; Fening et al., 2011; Obeng-Ofori & Ankrah, 2002; Owusu, 2000). Extracts derived from leaves, flowers, fruits, and seeds of insecticidal plants have shown ovipositional deterrence, feeding reduction, and larval mortality of *P. xylostella* on treated cabbage leaves (Abbasipour, Mahmoudvand, Rastegar, & Basij, 2010; Charleston, Kfir, Dicke, & Vet, 2005; Chen, Chang, Hou, & Cheng, 1996; Shin-Foon & Yu-Tong, 1993). Plant extract from chili, *Capsicum frutescens*, has been proven to reduce the activity of *P. xylostella* in cabbage (Iamba & Malapa, 2020; Iamba, Malapa, et al., 2021; Iamba & Waiviro, 2021; Iamba & Yoba, 2019). Homemade extracts of hot pepper and garlic produced positive results as emamectin benzoate (ATTACK®) while proving more better than the insecticide lambda-cyhalothrin (BOSSMATE®) by reducing the impact of *P. xylostella* larvae and *B. brassicae* (Fening et al., 2013).

This study investigates and discusses chili contents that were extracted via ethanol and hot water. Homebase chili extraction is done simply by grounding and diluting with water (Fening et al., 2013; Mwine et al., 2011). Simple home-based aqueous extraction should be cheap, easy to adopt by farmers, and effective in controlling *P. xylostella*. Whereas ethanolic extraction involves the use of ethanol as an extractionl vent for extracting chili phytochemicals (Iamba & Malapa, 2020; Iamba & Waiviro, 2021; Iamba & Yoba, 2019). Ethanol is a common chemical solvent for phytoextraction while hot water is a simple home-based method. It was hypothesized that ethanolic chili extracts would be more concentrated and hence effective than hot water. Although separate studies of ethanolic and hot water chili extracts were conducted in the past, an experimental comparison was required. This study thus quantifies the effectiveness of these two chili extracts on *P. xylostella* abundance and other associated damages to cabbage plants.

Materials And Methods

Study site

The study was carried out at the campus of PNG University of Natural Resources & Environment (PNG UNRE). PNG UNRE is in Vudal and is one of the six government universities in Papua New Guinea that supports the sustainable management of Agricultural resources as well as that of Fisheries, Forestry, Climate Change, and Animal Sciences. The experimental site is situated at 4°21' 01.90" S and 152°00' 33.44" E at an approximate altitude of 51m above sea level (Iamba & Waiviro, 2021; Iamba & Yaubi, 2021). This research site referred to as the 'academic crops section' is where most field experiments are done on tropical vegetables and crops. Cabbage in particular is cultivated mostly from August to October annually as it coincides with the schedule of student research projects.

The soil type is classified as sandy loam, well-drained, fertile, calcareous and relatively alkaline (Iamba & Yoba, 2019; Malagat & Iamba, 2021a, 2021b). Sandy loam soil has been proven successful in raising seedlings since it possesses good binding properties (Howcroft, 2002). Soil sterilization is not necessary for sandy loam soils except when there is a pathogenic infection. Since Vudal is within the tropics, it receives a great deal of rainfall that is experienced all year round even in the driest month. The humidity is considered high with an average rainfall of 2780 mm per year and an intervening mild dry season (Malagat & Iamba, 2021a). The average relative humidity in a day ranges from 77-79% with a mean temperature of 27-29 °C (Iamba, Yoba, et al., 2021). These meteorological conditions are suitable and conducive for plant cultivation (Howcroft, 2002).

Nursery

Before the study, a nursery was established by sowing cabbage seeds in nursery trays at the nursery house. The K-K cross cabbage variety (*Brassica oleracea* var. *capitata*) was used in this study. K-K Cross is an improved medium-sized hybrid variety that is popular in tropical and sub-tropical countries because of its high heat tolerance and early maturity (58 days after transplant) (Iamba & Waiviro, 2021). Raw nursery soil was placed in a semi-cut galvanized bin and sterilized by heating over an open intense fire while stirring every 5 minutes. Specific nursery trays of dimension 30x40cm were filled with sterilized soil and carefully leveled.

Approximately 300 seeds were sown in nursery beds while experimental field plots were constructed. Cabbage seedlings were nurtured and maintained for four (4) weeks in the nursery house before transplanting to ensure acclimatization. The acclimatization process was done to boost their physiological development so they can better adapt to field environmental conditions. Close monitoring of pests and diseases was a vital part of the nursery management as well as daily watering of nursery beds. Seedlings that are raised in a nursery have a higher chance of survival than direct-sown seeds (Iamba & Waiviro, 2021).

Chili extraction using Ethanol

Hot pepper or bird-eye chili pods were bought from the market and collected locally. The stalks of ripened chili were removed and dried in the sun for 2 weeks to reduce the moisture content. Then they were grounded in an electric blender to acquire fine powdered material. About 2.5±0.5 kg of the grounded material was obtained using an electronic balance and placed in a clean desiccator. About 1000ml of 75% ethanol (C_2H_6O) was added to the desiccator and covered tightly. The desiccator prevented the evaporation of ethanol and protected the hygroscopic contents to react with air-water humidity. The contents were left on a designated lab bench for 7 days (1 week) for the ethanol to degrade the cell walls and release phytochemical constituents. After 1 week, the resultant mixture was filtered using filter paper and the filtrate was collected in a 500ml beaker. The beaker was left open in a well-ventilated room to allow for the evaporation of excess ethanol. Then the mixture was stored in a separate 500ml container that had a poly seal ('P') lid for preventing further evaporation. As suggested by Fening et al. (2013), a few drops of natural oil and soap were added to the mixture to enhance its adhesiveness when applied to the leaf. Before spraying, the filtrate was mixed with rain water at a concentration of 10ml of ethanolic chili L^{-1} *v/v* and applied using the conventional method of spraying (Iamba & Malapa, 2020).

Chili extraction using Hot water

Chili pods for hot water extraction were bought from the market and collected locally. The stalks of ripened chili were removed and dried in the sun for 2 weeks to reduce the moisture content. Then they were grounded in an electric blender to acquire fine powdered material. About 2.5±0.5 kg of the grounded material was obtained using an electronic balance and placed in a steel pot. About 2000ml of rainwater was added to the pot and covered tightly with a lid. The pot was placed on a stove burner and boiled for approximately 30 minutes. Boiling time was restricted to 30 minutes to avoid denaturing phytochemicals. The boiled mixture was allowed to cool down for 12 hours and then filtered using filter paper. The filtrate was collected in a 1000ml beaker and transferred to 1L storage containers. The storage container had a poly seal ('P') lid for preventing any vaporization of filtrate. A few drops of natural oil and soap were added to the mixture to enhance its adhesiveness when applied to a leaf. Before spraying, the filtrate was mixed with rain water at a concentration of 20ml hot waterer chili L^{-1} *v/v* and applied using the conventional method of spraying (Fening et al., 2013).

Experimental design

The study was carried out over a three (3) year period from 2019 – 2021. A study on ethanolic chili extract was done in 2019 while a study on hot water chili extract took place in 2020. A combination of both ethanolic and hot water extracts was done in 2021. Regardless of the treatments and study year, all experiments underwent field preparation including site clearance and plot constructions which occurred three (3) weeks before the nursery phase to allow for soil solarization. Bushes and weeds growing on the experimental site were cut, dried, and burnt. Then the soil was plowed followed by the building of proper drainages for excessive water and to avoid any waterlog conditions. The plowed field was divided into experimental plots following a Complete Randomized Block design (CRBD). The plot size, plant spacing, and plant density followed standard recommendations for cabbage cultivation (Iamba & Yaubi, 2021). Each plot had a dimension of 2x3m ($6m^2$) with a spacing of 40cm between plants and 50cm between rows (Iamba & Waiviro, 2021). So, the plots had a plant density of 30 plants per plot [($6m^2$ / (0.4 x 0.5)].

As aforementioned, in the year 2019, ethanolic chili treatment (T1= ethanolic chili extract) was used and compared with a control (T2= control). The control plot received no extract treatment and was only sprayed with water. Each treatment was replicated three times giving a total of six (6) experimental plots in 2019. In the year 2020, hot water chili treatment (T3= hot water chili extract) was used and compared with a control (T2= control). Since each treatment was also replicated three times, it also had six (6) experimental plots in 2020. Finally, for the year 2021, all three treatments were combined. The trial included three treatments: T1 = ethanolic extract, T2 = hot water chili extract and T3 = control. Each treatment was replicated 4 times giving a total of 12 experimental plots.

Sampling parameters

For all experiments, data were collected three days after spraying and three weeks after transplanting. Three plants were randomly selected per plot and measurements on three (3) parameters were recorded in a field datasheet. The three parameters measured were (1) abundance, (2) defoliation (%), and (3) leaf area index (LAI). So in 2019, a total of 54 data points were collected per sampling time (6 plots x 3 plants per plot x 3 variables per plant). The year 2020 also had a total of 54 data points while the year 2021 had 180 data points per sampling time. These response variables demonstrated the efficacy of the two chili treatments on *P. xylostella*. The procedure and protocol used were adopted from (Iamba, Malapa, et al., 2021). The abundance was the number of *P. xylostella* larvae counted during each sampling time. Larvae were observed with less disturbance in the event of opening cabbage leaves for counting. BioLeaf Foliar Analysis application (App) in an android phone was used to measure the leaf area index (LAI) and defoliation (%) (Iamba & Homband, 2020). The non-destructive approach was engaged when measuring these two parameters. A sample of the attached leaf was placed against white cardboard, then a clear shot of 13-megapixel resolution was taken with the phone camera. Both the defoliation and LAI were scanned and processed in the App. Data about the three parameters were collected twice per week for a total period of 5 weeks. Rainfall (mm) and Relative humidity (%) data were collected from the university weather station at Vudal and a weekly (5 days) average was computed and used for analysis. Both rainfall and relative humidity were accorded as important factors therefore they were also included as abiotic parameters.

Data analysis

The data generated from this study were not normally distributed as confirmed by the Shapiro-Wilk test ($p< 0.05$). Therefore the skewness was corrected using the Generalized Linear Model (GLM) (Iamba, 2022). Three distribution or exponential families of GLM known as Gaussian, Poisson, and Gamma distributions were fitted to the data. According to the analysis of deviance and Akaike information criterion (AIC), Gamma distribution fitted the data well. The Analysis of Deviance involving the residual deviance is a good test to determine the Goodness of Fit of a model [6, 8, 9] while AIC estimates the quality of models in the class of both linear and Generalized Linear models (GLM) (Iamba 2022). Using the analysis of deviance in the full model, $\gamma = x_1 + x_2 + x_3 + x_4$; where γ = abundance, x_1 = treatment, x_2 = year, x_3= rainfall, and x_4 = relative humidity, the Gamma model had low residual deviance (G^2*=172.7*) and low AIC (*AIC=1078.9*). The other two exponential families had higher G^2 and *AIC* values and therefore were considered not suitable. The computation for Gamma was performed in RStudio (version 4.0.3) with a log link.

All graphs about abundance, LAI, and defoliation were constructed with the ggplot2 package. Analysis of variance (ANOVA) was performed to obtain the significance level of a response variable subjected to the effect of several independent variables. The Tukey HSD test was

used to separate the treatment means and group them according to the probability of mean differences at the alpha level (*p = 0.05)*. The interaction plots were iteratively computed with cowplot and ggplot2 packages in RStudio. Faceted correlation plots and categorical ranges of abiotic variables (i.e. rainfall) were done using the flexplot function in RStudio. Chi-square test χ^2 with ANOVA (*p-value*) and degrees of freedom (*df*) was used as a test of independence between categorical variables. Pearson correlation was used to show the relationship between two continuous variables while linear regression tested the effect of independent variables on the response variable subjected to different factors.

2. Result And Discussion

Results

A total of 1246 *P. xylostella* larvae were sampled over six weeks in 2021 in cabbage plots. Abundance was relatively low from week 1 to week 3 then gradually increased in week 4 reaching its peak in week 6 (Fig. 1). There was a significant relationship between treatments and weeks on DBM abundance (χ2= 68.96, df=10, p< 0.001) (Fig. 1). Both treatments and weeks influenced the population dynamics of DBM (df=3, deviance=1872.5, p< 0.001). Abundance did not differ significantly between control (3.89 mean ±1.16 SE, p> 0.05) hot water extract (3.45 mean ±1.24 SE, p> 0.05) while being significant for tincture extract (2.11 mean ±0.44 SE, p< 0.05). The mean abundance was lower in tincture extract than in the other two treatments (Table 1). The abundance was significant for all sampling weeks: week 1 (0.19 ± 0.05, p< 0.05), week 2 (0.03 ± 0.02, p< 0.05), week 3 (0.83 ± 0.34, p< 0.05), week 4 (2.22 ± 0.83, p< 0.05), week 5 (7.04 ± 1.49, p< 0.05) and week 6 (13.97 ± 4.96, p< 0.05). Week 6 had the highest mean abundance since the cabbage plants were

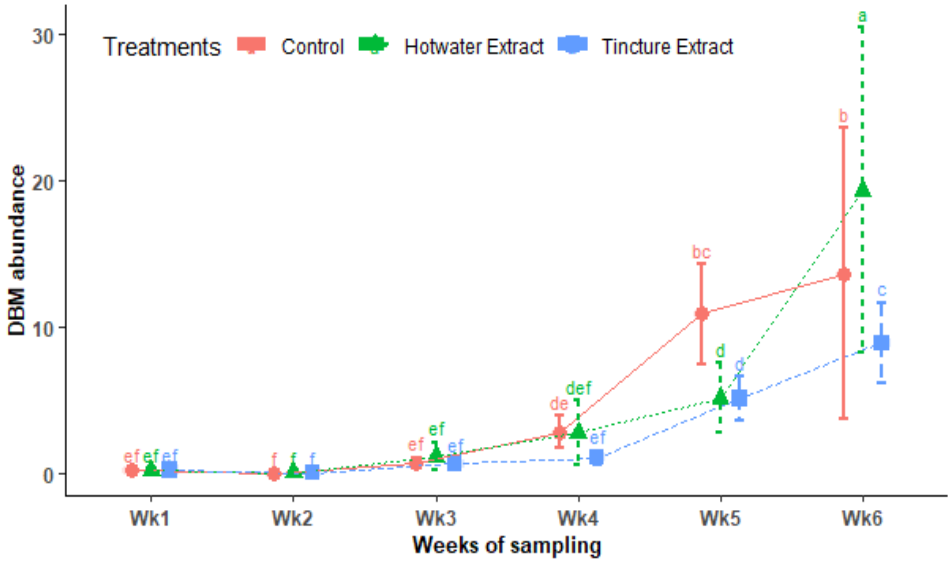

Figure 1. Graphical representation of the model, *glm(abundance~ weeks+treatments),* where weeks and treatments are categorical variables and abundance as the response variable. The points represent the meanwhile the vertical bars are standard error of the means. Treatments having the same letter are not statistically significant at α=0.05.

matured by then. Overall, the tincture extract was effective in lowering the population of DBM throughout the growth weeks. Hot water extract also showed some promising results in week 4 and week 5 but was not effective as control and tincture extract in week 6 (Fig. 1). There was no significant relationship between treatments and weeks for plant height (χ^2= 5.88, df=10, p= 0.83) (Fig. 2). Treatments did not have any significant effect on plant height (df= 2, deviance= 2.99, p=0.22) but weeks had a significant effect (df= 5, deviance= 282.83, p< 0.001). Plant height did not differ significantly between control (14.32 mean ±0.39 SE, p> 0.05) and hot water extract (14.09 mean

±0.34 SE, p> 0.05) while being significant for tincture extract (14.88 mean ±0.36 SE, p< 0.05). The mean plant height was higher in the tincture extract than in the other two treatments (Table 1. The plant height was significant for all sampling weeks: week 1 (9.81 ± 0.23, p< 0.05), week 2 (11.66 ± 0.28, p< 0.05), week 3 (14.05 ± 0.23, p< 0.05), week 4 (17.47 ± 0.36, p< 0.05), week 5 (16.81 ± 0.42, p< 0.05) and week 6 (19.18 ± 0.56, p< 0.05). Week 6 had the highest mean plant height since the cabbage plants reached maturity by then. Overall, tincture extract was effective in boosting plant height, especially in week 5 and week 6. Hot water extract was not as effective as the control treatment in boosting plant height (Fig. 2).

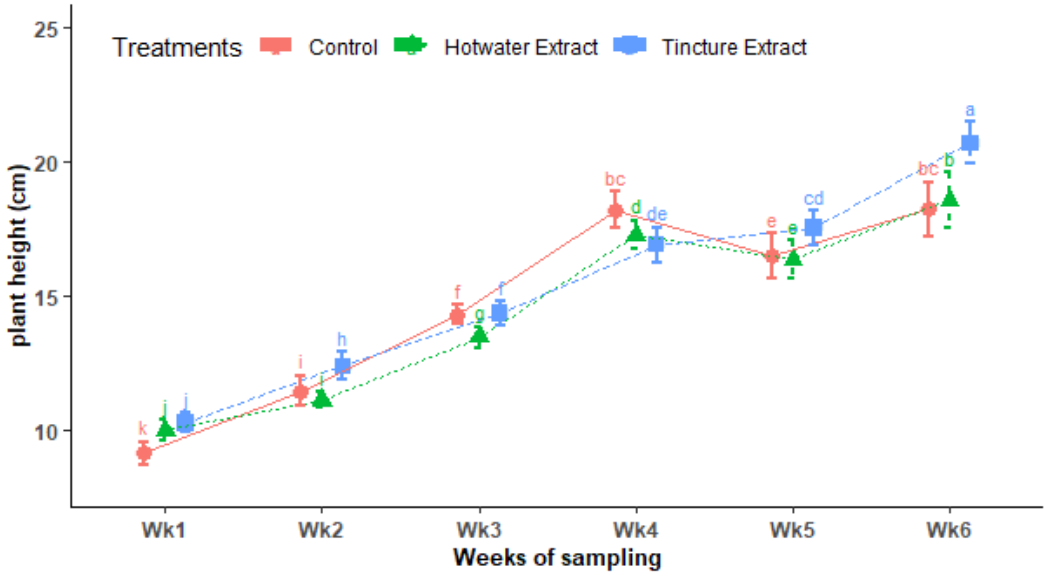

Figure 2. Graphical representation of the model, *glm(height~ weeks+treatments),* where weeks and treatments are categorical variables and plant height is the response variable. The points represent the meanwhile vertical bars as are standard error of the means. Treatments having the same letter are not statistically significant at α=0.05.

There was no significant relationship between treatments and weeks for head width (χ^2= 6.62, df=10, p= 0.76) (Fig. 3). However, each categorical variable, treatments (df= 2, deviance= 17.52, p< 0.001) and weeks (df= 5, deviance= 280.02, p< 0.001) had a significant effect on head width. Head width differed significantly for control (19.16 mean ±0.56 SE, p< 0.05), hot water extract (17.09 mean ±0.48 SE, p< 0.05) and tincture extract (17.51 mean ±0.57 SE, p< 0.05). The mean head width was lower in hot water and tincture extract than in the control treatment (Table 1). The head width was significant for all sampling weeks: week 1 (15.15 ± 0.36, p< 0.05), week 2 (18.31 ± 0.48, p< 0.05), week 3 (24.96 ± 0.92, p< 0.05), week 4 (17.74 ± 0.50, p< 0.05), week 5 (15.82 ± 0.65, p< 0.05) and week 6 (13.13 ± 0.41, p< 0.05). The mean head width reached the highest peak in week 3 then gradually declined towards week 6. Overall, cabbages sprayed with tincture and hot water extract had a reduced head width than the control treatment (Fig. 3).

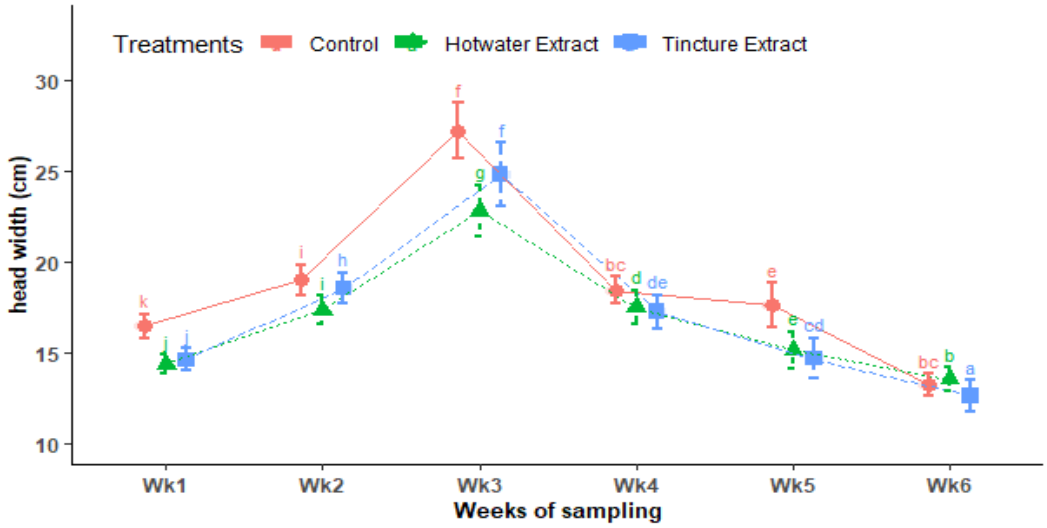

Figure 3. Graphical representation of the model, *glm(width~ weeks+treatments),* where weeks and treatments are categorical variables and head width is the response variable. The points represent the meanwhile the vertical bars are standard error of the means. Treatments having the same letter are not statistically significant at α=0.05.

There was a significant interaction between treatments and weeks for DBM abundance (Table 1). Treatments such as tincture extract were able to lower the population of DBM on a temporal scale. Hot extract and control treatment did not perform well in terms of controlling DBM larvae. No interaction was detected between treatments and weeks for plant height (Table 1). It can be presumed that plant height was influenced by time (weeks) and not treatments. Nil interaction was also reported between treatments and weeks for head width. However, treatments and weeks had a solo significant effect on head width. The head width of tincture and hot water extra was generally lower than that of the control treatment. Since *Brassica oleracea* var. *capitata* becomes round as it forms the head, we prefer a head that is well compacted or dense. The width of well-compacted heads would surely be lesser than uncompacted heads. In the case of market value, compacted heads are preferred over loose and puffy heads.

Table 1. The effects of treatments and weeks on (a) pest abundance, (b) plant height (cm), (c) head width (cm), and their interaction were obtained by taking the ANOVA of respective GLM models ($\hat{Y}= \beta_0 + \beta_1X_1 + \beta_2X_2$). Where $\hat{Y}$ = *dependent variable*, β_0= *intercept*, β_1=*coefficient*, X=*independent variable.* Treatments and weeks were used as independent variables while dependent variables included abundance, plant height, and head width.

(a) Abundance	***df***	**Deviance**	**Resid. *df***	**Resid. deviance**	***p***
Null (intercept)			395	4948.6	
Treatments	2	76.48	393	4872.1	< 0.001
Weeks	5	1948.99	388	2923.1	< 0.001
Treatments x Weeks	10	71.53	378	2851.6	< 0.001
(b) Plant height (cm)	***df***	**Deviance**	**Resid. *df***	**Resid. deviance**	***p***
Null (intercept)			395	475.62	
Treatments	2	2.995	393	472.63	0.2237
Weeks	5	282.826	388	189.80	< 0.001
Treatments x Weeks	10	5.903	378	183.90	0.8233
(b) Head width (cm)	***df***	**Deviance**	**Resid. *df***	**Resid. deviance**	***p***
Null (intercept)			395	806.88	
Treatments	2	17.516	393	789.37	< 0.001
Weeks	5	280.022	388	509.34	< 0.001
Treatments x Weeks	10	6.620	378	502.72	0.76

As abiotic factors affect the durability and longevity of botanical extracts, analysis was done on rainfall and relative humidity. In this study, both rainfall (mm) and relative humidity (%) affected the population of DBM. There was a significant association between rainfall and treatments in terms of abundance (χ^2= 66.24, df=4, p< 0.001). The effect of rainfall and treatments on DBM abundance was significant (df=4, deviance= 71.08, p< 0.001). Extreme conditions include high rainfall and high RH as it may limit the effectiveness of botanical extracts to control pests. There was no significant difference between hot extract (0.77±0.34, p> 0.05) and tincture extract (1.22±0.31, p> 0.05) under low rainfall while the control's abundance was significant (2.44±0.64, p< 0.05) (Fig. 4). Tincture extract still maintains low abundance under moderate (2.27±0.86, p< 0.05) and high rainfall (4.00±1.49, p< 0.05). Based on these results, tincture extract (TE) was deemed more effective against DBM than hot water control (Fig. 4). Control treatment had the highest population of DBM under high rainfall mainly due to the absence of plant extracts (8.46±3.48, p< 0.05). Hot water extract succumbed to high rainfall therefore DBM abundance is significantly high (7.29±3.14, p< 0.05). DBM population increased during high rainfall since heavy downpour is capable of washing off phytochemicals thus creating a free space for pests' recruitment.

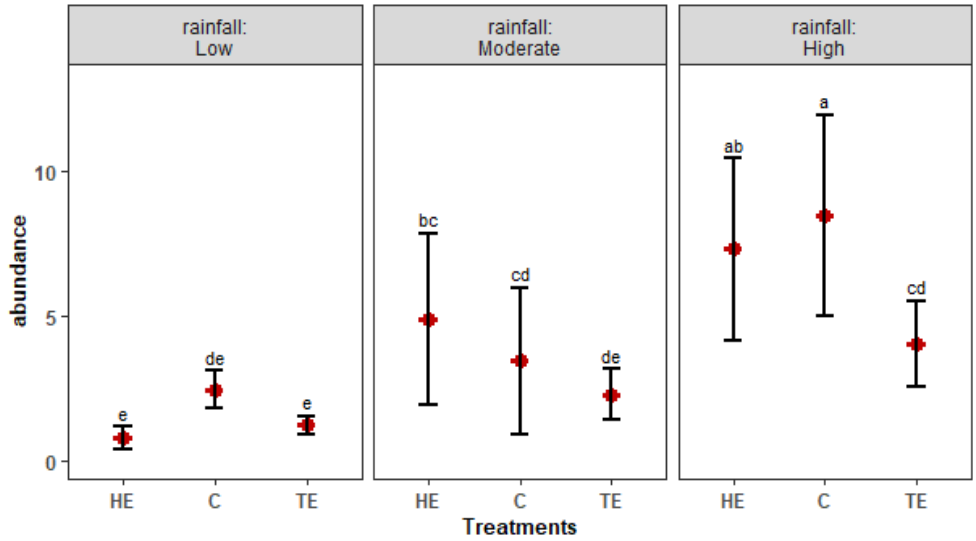

Figure 4. The rainfall was grouped into 3 categories; low (0 - 1mm), moderate (1 - 6.2mm), and high (6.2 - 21.5mm) by using the *flexplot()* function in R. The points represent the meanwhile the vertical bars are standard error of the means. Treatments having the same letter are not statistically significant at α=0.05. (USGS U.S. Department of the Interior – rates of rainfall)

There was a significant association between RH and treatments in terms of abundance (χ^2= 51.39, df=4, p< 0.001). The effect of treatment and RH on DBM abundance was significant (df=4, deviance= 52.81, p< 0.001). RH is also an important abiotic factor that can influence the population dynamics of pests i.e., DBM. There was no significant difference between hot water extract (0.77±0.46, p> 0.05) and tincture extract (0.89±0.32, p> 0.05) under low RH while control's abundance was significant (2.19±0.72, p< 0.05) (Fig. 5). Tincture extract was able to maintain low abundance under moderate (2.37±0.71, p< 0.05) and high rainfall (3.88±1.50, p< 0.05). Based on these results, tincture extract (TE) was again deemed more effective against DBM than hot water and control (Fig. 5). Control treatment had the highest pest abundance due to the absence of plant extracts. Generally, as RH increases, the population and activity of DBM are expected to increase as well. Hot water extract succumbed to RH and was not able to control DBM effectively at extreme RHs.

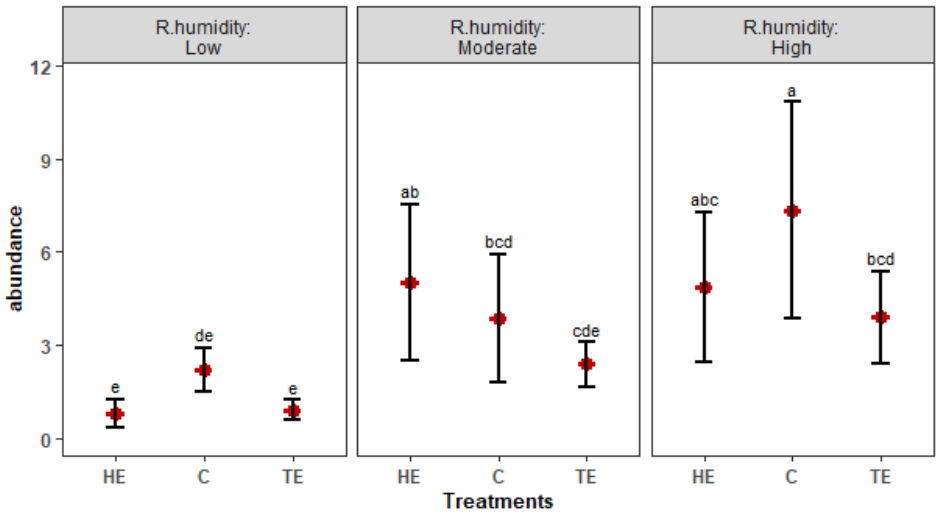

Figure 5. The relative humidity (RH) was grouped into 3 categories; low (63 – 66%), moderate (66 – 89%), and high (89 – 100%) by using the *flexplot()* function in R. The points represent the meanwhile the vertical bars are standard error of the means. Treatments having the same letter are not statistically significant at α=0.05.

Discussion

The study was aimed at providing improved protection to cabbage plants by testing chili extracted through ethanol and hot water against the Diamondback moth *(Plutella xylostella* L.*)*. In general, chili extract was recommended as a better alternative to control cabbage pests than synthetic insecticides mainly due to its effectiveness and compatibility with natural enemies (Antonious, Meyer, Rogers, & Hu, 2007; Iamba & Yoba, 2019; Zehnder, Simonne, Briggs, Bannon, & Ruff, 1997). When compared to garlic, chili was more effective in suppressing the abundance of *P. xylostella* (Baidoo & Mochiah, 2016). Diamondback moth (DBM) continues to be a destructive pest of cabbage, especially in the Vudal area and other tropical regions (Iamba & Malapa, 2020; Iamba, Malapa, et al., 2021; Iamba & Waiviro, 2021; Zhang et al., 2016).

Several authors have tested the effect of ethanolic and aqueous chili extracts either alone or concurrently to control pests (Amoabeng et al., 2013; Baidoo & Mochiah, 2016; Fening et al., 2013; Vasconcelos, Martins, de Oliveira, & Duarte, 2014). According to the results, DBM abundance was low on cabbage plants treated with ethanolic chili extract as confirmed by Vasconcelos et al. (2014). Chili extracted via ethanol was able to lower DBM abundance and minimize defoliation (Iamba & Yoba, 2019). Chili extract possesses antifeedant and repellent properties that have been proven effective against *P. xylostella* (Iorizzi, Lanzotti, Trematerra, & Zollo, 2000; Khan, Pickett, Berg, Wadhams, & Woodcock, 2000). Ethanolic extract was more effective in terms of pest mortality than water, hexane, and diethyl ether extracts (Mhazo, 2018). However, ethanolic chili extract was not so effective in increasing leaf area and reducing defoliation of cabbage foliage. Most active phytochemicals of plant extracts are prone to accelerated degradation under field conditions (Koch & Lawson, 1996). Botanicals extracted through ethanol can be affected by environmental factors such as rain, temperature, and humidity (Iamba & Masu, 2020).

However, in terms of leaf area, hot water chili extract significantly contributed to bigger cabbage leaves. A similar study found that aqueous chili (20g L^{-1} *w/v*) was more effective in controlling *P. xylostella* than the other treatments (Fening et al., 2013). It was assumed that hot water chili extract was able to reduce feeding by providing leaf surface protection. Chili contains capsaicinoids which is an active phytochemical group associated with caustic or spicy characteristics (Reifschneider, 2000). Other phytochemicals include diterpenoids, flavonoids, saponins, and phenolic compounds that act as antifeedant and parasite repellence (Iorizzi, Lanzotti, & Trematerra, 2000; Madhumathy, Aivazi, & Vijayan, 2007). Cabbages treated with botanical extracts including chili produced comparable yields

(Begna & Damtew, 2015). The absence of plant extract in control treatment triggers an increase in *P. xylostella* severity (Reuben, Yahya, Misangu, & Mulungu, 2006). Botanical extracts including chili extracted through the water were equivalent to or better than synthetic insecticides in controlling *P. xylostella* (Amoabeng et al., 2013). According to the results, the temporal pest abundance under hotwater extract was better or comparable with the ethanolic extract. For defoliation, both the hot water and ethanolic extracts reported low values. Simo et al. (2019) recommended aqueous extract rather than an ethanolic extract of pepper be used as a bio fungicide against the black pod disease (*Phdeytophthora megakarya*). Both aqueous and ethanolic extracts from chili pepper at a concentration of 50mg/L were toxic to two mosquito larvae, *Anopheles* and *Culex* (Ahmed, 2015).

Extreme environmental conditions can determine the effectiveness of plant extracts under field conditions. About 2 to 5 mm of rain is capable of washing off more than 50% of phytochemicals after 1 hour of application (Pick, Van Dyk, & De Beer, 1984). It has also been shown that compounds such as imazaquin which is present in herbicides can easily be lost to foliar wash-off (Reddy & Locke, 1996). An increase in relative humidity (RH) under both ethanolic and hot water extract did not have any significant effect on the abundance of *P. xylostella* larvae. Chili extract was reported to withstand rainy days due to its adhesive phytochemicals such as capsaicinoids, diterpenoids, flavonoids, saponins, and phenolics that can strive under extreme climatic conditions (Begna & Damtew, 2015; Iorizzi, Lanzotti, Trematerra, et al., 2000; Madhumathy et al., 2007). Leaf area increased significantly with increasing RH under control and ethanolic extract while non-significant under hot wate extract. Despite the adverse effect of environmental factors, chili extract can persist. And as RH increased, defoliation of ethanolic extract decreased significantly. In terms of rainfall (RF), pest abundance of ethanolic extract, hot water extract and control treatment generally decreased as RF increased. Abiotic conditions such as decreased rainfall were regarded as a major factor in regulating the population dynamics of *P. xylostella* (Harcourt, 1986).

Ecologically, hot and dry conditions are usually conducive for *P. xylostella* (Shelton 2001). The rainy season contributes to a decrease in the larval population of *P. xylostella* and creates an unfavorable conditions for the development of immature stages (Ahmad & Ansari, 2010). Rain can dislodge *P. xylostella* larvae from the foliage which then can easily be drowned in soil water (Talekar & Shelton, 1993). About 100 percent of the mortality of I– IV instar larvae of *P. xylostella* might be due to rain (Iga, 1985). While, Rain can also wash off the eggs of *P. xylostella*, and I instar feeding in the leaf axel can be easily drowned in the trapped water after a downpour (Annamalai, Itô, & Saito, 1988). The relations between leaf area, defoliation, and RF were not significant implying that RF is not a limiting factor to leaf area and defoliation. There was a significantly high number of pests under low RF while a significant increase was observed under moderate RH. A similar scenario was also observed for the leaf area under both RH and RF. Increased defoliation was observed under moderate RF while being high under low RH. A significant increase in the *P. xylostella* population was reported during relative humidity of 64.27 to 80.82 percent (Ahmad & Ansari, 2010). This aforementioned study closely supports the current data as the *P. xylostella* population peaked during moderate RH (67.5-69.8%) and was also significant at high RH (69.8-88%).

4. Conclusion

The methods chosen for controlling pests in developing countries should be effective, cheap, and simple to be adopted by farmers. Since insecticidal plants can be found locally and near farms, they should be much cheaper to source than synthetic insecticides. The only cost to be incurred would be labor for the collection, preparation, and application of plant extracts in cabbage (Amoabeng 2014). Using water as a solvent to produce chili extract is one of the simplest methods to control *P. xylostella* in cabbage. Although ethanol is frequently used as an extraction solvent for phytochemicals, the procurement and process of producing it can be quite tedious, spacious, technical, expensive, and unadoptable by farmers. Through a series of field experiments over three years, this study is confident to conclude that chili extracted through hot water is effective to control *P. xylostella* in cabbage.

Acknowledgments

A special thank you to the final-year students from 2019 to 2021 for your tireless effort in collecting the data. Extended appreciation to Mr. James Aipa and the staff of the UNRE academic nursery section. Your positive response to our request for cabbage seedlings has contributed significantly to this study. And finally, we dedicate this publication to one of our colleagues, Mr. Jordan Sangi, who passed away in 2021. Though his passing was truly shocking, we appreciate Jordan for his contribution to data collection.

Conflict of interest

The authors have no conflict of interest to disclose.

Author contributions

Kari Iamba conceived the ideas and designed the methodology; Tony Fukatine carried out chili extraction, collected the data, and contributed to the write-up; Wendy Wanio and Rudolph Tarue assisted with laboratory work and extraction protocols; Kari Iamba analyzed the data, prepared the figures; and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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