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Utilizing local plant extracts to control Diamondback moth in round cabbage

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Abstract
Diamondback moth (*Plutella xylostella* L.) with abbreviation DBM is a detrimental pest that mostly attacks cabbage and other vegetables in Brassicaceae family. The build-up of resistance in DBM due to continuous and indiscriminate use of synthetic insecticides have refocused farmer's perspectives to plant based extracts. Plant extracts are generally compatible with natural enemies of pests therefore are considered eco-friendly. This study was done to evaluate the push effect of Garlic (*Allium sativum* L.), Chili (*Capsicum frutescens* L.) and Seaweed extract on the activity of DBM. All three treatments; chili extract, garlic extract, and seaweed extract were generally effective in controlling DBM than the reference group (control treatment). Separation of means by Student-newman-keuls (SNK) test showed that Diamondback moth (DBM) abundance under seaweed extract was significantly low (2.18±0.32, $p < 0.05$) followed by chili extract (3.22±0.43, $p < 0.05$). There was no significant difference in DBM abundance under garlic extract and control treatment. For defoliation, only control treatment recorded a significant increase in DBM population (5.40±0.64, $p < 0.05$). The other three plant extracts had similar low non-significant abundances ($p > 0.05$). Data on LAI did not show any significant differences in all the treatments meaning treatments did not affect LAI ($p > 0.05$). This study found that both seaweed and chili extracts were effective in controlling *P. xylostella* in round cabbage. Both extracts are recommended for use since they differ in their mechanism pathways to protect crops.

Keywords: Diamondback moth, plant extracts, treatments, leaf area index, defoliation, abundance

Introduction

Diamondback moth (*Plutella xylostella* L.) with abbreviation DBM is a detrimental pest that mostly attacks cabbage and other vegetables in Brassicaceae family. DBM is an important economic pest that is widely distributed globally (Zhang *et al.* 2016) [55]. Indiscriminate application of synthetic pesticides in intensified agricultural systems have resulted in resistance build up, natural enemy suppression, human risks to contaminated residues and other environmental poisoning (Obeng-Ofori & Ankrah 2002; Timbilla & Nyarko 2004; Fening *et al.* 2011; Iamba & Malapa 2020) [38, 51, 16, 19].

The moth is a destructive pest at Vudal and throughout East New Britain Province (Iamba & Yoba 2019; Iamba & Waiviro 2021) [23, 21]. Due to bad reputation of synthetic insecticides, focus in controlling DBM has shifted from chemical to botanical pesticides that repels feeding or deters oviposition (Coulibaly *et al.* 2002) [12]. Many local spice plants have insecticidal properties and can be extracted with low-cost tactics to manage pests (Iamba & Masu 2020) [20]. Plant extracts are applied to deter gravid females, reduce herbivory and increase larval mortality of DBM in cabbage plants (Shin-Foon & Yu-Tong 1993; Chen *et al.* 1996; Charleston *et al.* 2005; Abbasipour *et al.* 2010) [50, 10, 1, 11]. Botanical pesticides derived from plants are an essential component of any pest management program since they are compatible with natural enemies to supress pests (Gentz *et al.* 2010; Iamba & Yoba 2019) [23, 17]. In a push-pull system, non-host plant extracts plays a push mechanism to reduce oviposition of insect pest while enhancing the activity of parasitoids in crops (Liu *et al.* 2007) [32]. Semiochemical cues are mediated in a push-pull system to repel pests, lure natural enemy populations or congregate both counterparts (Pyke *et al.* 1987; Miller & Cowles 1990; Pickett *et al.* 1997; Agelopoulos *et al.* 1999) [37, 2, 41, 43].

This research was done to evaluate the push effect of Garlic (*Allium sativum* L.), Chili (*Capsicum frutescens* L.) and Seaweed solution on the activity of DBM. We presumed that the application of these non-host plant extracts would reduce the population of DBM and repels them from either feeding or ovipositing on cabbage leaves.

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The aim is to determine the plant extract that can significantly suppress the activity of DBM on round cabbage (*Oleracea* var. *capitata*). Round cabbage (*O.* var. *capitata*) is highly susceptible to attack of DBM due to its nutritive value and succulency. From previous studies, chili (*C. frutescens*), garlic (*Allium sativum* L.), and seaweed extracts were successful in controlling DBM due their antifeedant and deterrent properties (Iorizzi *et al.* 2000; Khan *et al.* 2000; Khan *et al.* 2009; Iamba & Yoba 2019 [23]; Iamba & Malapa 2020) [26, 23, 19, 29, 30]. Cabbages that were treated with chili and other botanical extracts produced comparable yield (Begna & Damtew 2015) [6].

Materials and Methods
(a) Study site
The study was done at the academic crops section at Vudal agroecosystem. The agroecosystem is located within the campus of PNG University of Natural Resources & Environment (PNG UNRE) in Papua New Guinea (PNG). The experimental site is situated at 4º 21' 01.90" S and 152º 00' 33.44" E on an altitude of 51m above sea level (Iamba & Waiviro 2021; Iamba & Yaubi 2021) [21, 22]. The academic crops section is a research site where most agricultural *in situ* experiments are done on tropical crops. PNG UNRE is a natural resource university that supports the sustainable management of Agricultural resources as well as that of Fisheries, Forestry and Animal Sciences. The soil type is characterized as sandy loam that is well-drained, fertile, calcareous and relatively alkaline in nature (Iamba & Yoba 2020; Malagat & Iamba 2021) [24, 35].

Sandy loam soil is adequate for raising seedlings since it possess good binding properties (Howcroft 2002) [18]. Soil sterilization is not required for sandy loam soils except when there is evidence of pathogenic infection. Vudal enjoys a tropical climate with a great deal of rainfall that is experienced all year round even in the driest month. It is highly humid with an average rainfall of 2780 mm per year and a intervening mild dry season. The daily average humidity ranges from 77-79% with mean temperature of 27-29 °C (Iamba *et al.* 2021) [21]. These abiotic conditions are most preferable and conducive for plant cultivation (Howcroft 2002) [18].

(b) Nursery
K-K cross variety was chosen as the host subject since it is a common leafy vegetable cultivated locally and highly susceptible to pest attack. Seedlings were raised at the nursery house by direct sowing of cabbage seeds in nursery trays. The variety of round cabbage used for this pest control study was K-K cross (*Oleracea* var. *capitata*). K-K Cross is a medium-sized hybrid of *Oleracea* var. *capitata* that has been genetically improved to suit the tropical and sub-tropical countries due to its high heat tolerance and early maturity (58 days after transplant). Approximately 300 seeds were raised in the nursery while field experimental plots were established. Cabbage seedlings were given four (4) weeks to establish in the nursery house to ensure acclimatization before transplanting. Acclimatization process is vital to habituate the seedlings to the field abiotic conditions. Seedlings that are raised in nursery have high viability than direct sown seeds (Iamba & Waiviro 2021) [21]. Acclimatized seedlings can withstand the harsh weather conditions when transplanted into field experimental plots.

(c) Experimental design
Site clearance and plot preparation was done 3 weeks prior to nursery phase in order to allow for solarization. General work involved clearing of bushes, soil tillage and weed removal. Proper drainages were done to remove excess water in case if there would be possible waterlog conditions. The prepared plots were divided into experimental units (sub-plots) according to Complete Randomized Block design (CRBD). The plot size, planting distance and plant density followed the measurements of Iamba and Malapa (2020) [19]. Each plot had a dimension of 2x3m (6m^2) with a spacing of 40cm between plants and 50cm between rows. Therefore, each plot had a plant density of 30 plants per plot [(6m^2 / (0.4 x 0.5)]. There were four (4) treatments (T): T1=10ml of Chili extract L^{-1} *v/v*, T2=10ml of Control L^{-1} *v/v*, T3=10ml Garlic extract L^{-1} *v/v*, and T4= 10ml of Seaweed solution L^{-1} *v/v*. All extracts were mixed with tank water at concentration of 10mlL^{-1} v/v and applied using conventional method of hand spraying. Higher concentration doses have been suggested for plant extracts in order to be effective in the field (Charleston *et al.* 2005) [10]. Each treatment was replicated three (3) times giving a total of twelve (12) experimental plots. All plant extracts were selected based on their repellent and deterrent properties. A control treatment, sprayed with only tank water, was included and used as a reference group for comparison. There were no chemical insecticides nor chemical fertilizers applied because the objective was to test the sole effect of the plant extracts.

(d) Sampling
Data was collected four days after spraying and four weeks after transplanting. Three (3) response variables were measured: (1) abundance, (2) defoliation (%) and (3) leaf area index (LAI). These variables were used to calculate the efficacy of each treatment on DBM activity. The abundance was basically the number of individual DBM caterpillars counted at each sampling time. With less disturbance, cabbage leaves were opened and DBM caterpillars counted and recorded. Both the leaf area index (LAI) and defoliation (%) were obtained using a mobile phone application called the BioLeaf® Foliar Analysis. A sample of leaf was held against a clean sheet of white paper, and a clear shot of 13-megapixel resolution was taken using the phone camera. All scanning and processing of defoliation (%) and LAI were done automatically by the app. Data pertaining to each response variable was collected 4 days after spraying for a total period of 5 weeks. Three plants were randomly selected per plot and measurements on the 3 variables were processed and recorded. There were 108 data points collected per sampling time (12plots x 3 plants per plot x 3 variables per plant). Since data was collected for 5 weeks, a total of 540 data points were recorded per week.

(e) Plant content extraction
The garlic cloves and chili fruits were bought from the local market. The seaweed solution used was bought from the store and manufactured by Seasol®. The seaweed extract is derived from a blend of three finest brown kelps grown around the world; *Durvillaea potatorum*, *Durvillaea antarctica* and *Ascophyllum nodosum*. Both garlic and chili fruits were chopped into smaller pieces and mixed well. The plant materials were dried under hot sun for 3 weeks to rid-off excess moisture. Then they were grounded separately

using hands and then passing them through a spice grinder. About 2.5±0.5 kg of each grounded material was obtained using an electronic balance and placed in a desiccator. Approximately 500ml of 75% ethanol (C_2H_6O) was added to the desiccator with the lid tightly fastened. The desiccator shielded the hygroscopic contents from reacting with water vapour in the air and prevented ethanol from evaporating. The mixture was left standing on a clear bench for 7 days (1 week) to allow the ethanol to degrade the cell walls of the materials so that phytochemicals are released into the solution.

After 1 week, the resultant mixture was filtered using filter paper and the filtrate was collected in a 500ml beaker. Filtrates were allowed to evaporate at room temperature (25°C) to remove excess ethanolic residues (Iamba & Malapa 2020) [19]. Then a few drops of natural oil and soap were added to the mixture to improve its adhesiveness to leaf surface (Fening *et al.* 2013) [15]. The extract was stored in a separate 1L container with a polyseal ('P') type lids to avoid evaporation. The extract was mixed at the ratio of 1ml extract to 1 L water in an hand sprayer and applied.

(f) Data analysis

The data on response variables showed asymmetric distribution implying they were not normally distributed according to Shapiro-Wilk test ($p<0.05$). We correct the skewness in data distribution using the Generalized Linear Model (glm). Two (2) glm models were tested for their quality and relevance to the data: Poisson and Gamma distributions.

Using the analysis of deviance, Gamma model had a low residual deviance and Akaike's information criterion (AIC) (*$G^2=51.78.2$, AIC =446)*. Poisson model had a higher residual deviance and AIC (*$G^2=177.84$, AIC =482)*. Use of residual deviance as a measure of Goodness of Fit for Poisson glm in normal linear model is $\sum(\gamma - \bar{\gamma})^2$ while Gamma, $2\sum(\gamma - \mu)/\gamma - \ln(\gamma/\mu)$ where γ is observed data, $\bar{\gamma}$ the mean value of y, and μ are the fitted values of γ from the maximum likelihood model (Crawley 2012) [13].

The Akaike information criterion (AIC) used to estimate the quality of the two glm models is derived AIC formular, *AIC = 2*ln(likelihood) + 2*k* where *ln* is the natural logarithm, *k* is the number of parameters in the statistical model and RSS is the residual sums of squares (Panchal *et al.* 2010) [39]. Therefore the Gamma model was selected as the best fit for analysing the count data. Data was fitted into this Gamma distribution: *$g(\mu_i) = \eta_i = x_i\beta$*, where *g* is the link function, *$\beta = (\beta_0,...,\beta_p)$* is the vector of mean regression parameters, *x_i* is the *i-th* vector value of the explanatory variables, and *η_i* is a linear predictor (Cepeda-Cuervo & Corrales-Bosio 2015) [8]. The response variables were analysed using the Gamma exponential family function (link = log) in RStudio (version 4.0.3). All graphs relating to DBM abundance, LAI and defoliation were constructed with ggplot2 package. The Student-newman-keuls test (SNK) was used to separate the treatments means since it was more sensitive to mean difference than Tukey HSD test. SNK test was also used in post hoc analysis to detect significant differences between treatments.

The interaction plots were iteratively executed with cowplot and ggplot2 packages in RStudio. Faceted graphs involving the *z-axis* was computed using the flexplot function in RStudio to produce three LAI size ranges; small (2.3-4.9), medium (4.9-6.6) and big (6.6-15.8).

Results

A total sum of 338, 101 counts and average of 3.88 DBM larvae were quantified during this study. For leaf area index (LAI), a total sum of 1093.63, 177 counts and average of 6.21 were recorded. A total sum of 515.21, 174 counts and average of 2.98 defoliation (%) measurements were measured. Summary statistics on DBM abundance, LAI and defoliation (%) were quantified for the four treatments (Table 1). Separation of means by Student-newman-keuls (SNK) test yielded varied results for each treatments. Abundance of DBM larvae under seaweed extract was significantly low (2.18±0.32, $p< 0.05$) followed by chili extract (3.22±0.43, $p< 0.05$). There was no significant difference in DBM abundance under garlic extract and control treatment. For defoliation, only control treatment recorded a significant increase in DBM population (5.40±0.64, $p< 0.05$). The other three plant extracts had similar low non-significant abundances ($p> 0.05$) (Table 1). Data on LAI did not show any significant differences in all the treatments ($p> 0.05$).

Table 1: Summary statistics on DBM abundance, LAI and defoliation (%) were computed for each of the four treatments. The counts of DBM abundance, and values of LAI and defoliation are expressed as mean±SE.

TREATMENTS	SUMMARY STATISTICS			
	N	DBM abundance	SD	CI
Chilli extract	23	322±0.43 ab	2.07	0.89
Control	31	5.09±0.62 a	3.46	1.27
Garlic extract	24	4.50±0.67 a	3.28	1.39
Seaweed extract	22	2.18±0.32 b	1.50	0.67
	N	Defoliation (%)	SD	CI
Chilli extract	23	2.89±0.54 b	2.59	1.12
Control	31	5.40±0.64 a	3.56	1.31
Garlic extract	24	2.68-0.49 b	2.39	1.01
Seaweed extract	22	2.89±0.47 b	2.19	0.98
	N	LAI	SD	CI
Chilli extract	23	6.57±0.61 a	2.91	1.26
Control	31	5.92±0.41 a	2.26	0.83
Garlic extract	24	6.31±0.49 a	2.38	1.01
Seaweed extract	22	5.74±0.41 a	1.93	0.86

Mean±SE with same letters are not significantly different at p=0.05 (Tukey HSD test). Standard deviation (SD) of both pest and NE abundance. N is the number of samples per treatment (3 data collection per treatment x 4 sampling times). 95% Confidence interval (CI) with values not containing zero are significant (p< 0.05).

The DBM abundance (mean±SE) was significantly low under seaweed treatment ($p< 0.05$) followed by chili extract ($p< 0.05$). Only control treatment had a high significant defoliation ($p< 0.05$) while other three treatments were non-significant ($p> 0.05$). No significant trend was detected for LAI in all treatments ($p> 0.05$). Both seaweed and chili extracts had proven potency to control the population and reduce the detrimental impact of DBM to round cabbage. Garlic extract harboured high DBM abundance and was similar to control treatment (Figure 1).

DBM abundance and defoliation were similarly low under chili extract treatment ($p> 0.05$) while LAI was significantly high ($p< 0.05$) (Figure 2). All three variables did not differ significantly from each other under control treatment ($p> 0.05$). The three variables significantly differed from each other under garlic extract treatment. LAI significantly increased ($p< 0.05$) while defoliation

decreased significantly ($p < 0.05$). DBM abundance also decreased ($p < 0.05$) however it was higher than that of chili and seaweed extract (Figure 2). Seaweed extract treatment recorded a significant increase in LAI while DBM abundance and defoliation were similarly very low ($p > 0.05$). Post hoc analysis of DBM abundance by SNK test detected a high significant difference between control and seaweed extract treatment (p= 0.002**). A significant difference was also detected between garlic extract and seaweed extract (p=0.01*). Post hoc analysis of defoliation was highly significant between chili extract and control (p=0.002**); control and garlic extract (p=0.005**); and control and seaweed extract (p=0.006**).

Correlation was done to assess the relationship between defoliation (%) and DBM abundance under each treatment. Prior to computation of correlation statistics, the z-axis of the graph was faceted using flexplot() function in RStudio to separate LAI into three size range: small (2.3-4.9), medium

(4.9-6.6) and big (6.6-15.8) leaves. All variables were taken into consideration for the correlation analysis. There is a non-significant negative correlation between defoliation and abundance under chili extract on big leaves (r= -0.42, $p > 0.05$) (Figure 3). Positive correlation trend was detected on small and medium leaf sizes however these were not significant. Control treatment had a very high significant positive correlation between defoliation and abundance on medium (r= 0.93, $p < 0.001***$) and big (r= 0.96, $p < 0.001***$) cabbage leaves. A negative correlation was detected on small leaf size however it was non-significant. Garlic extract showed positive correlation between defoliation and abundance on medium and big leaf sizes but were not significant. Seaweed extract showed a positive correlation on both small and big leaf sizes while negatively correlated on medium leaf size. Regardless of these positive and negative trends, the correlations were non-significant.
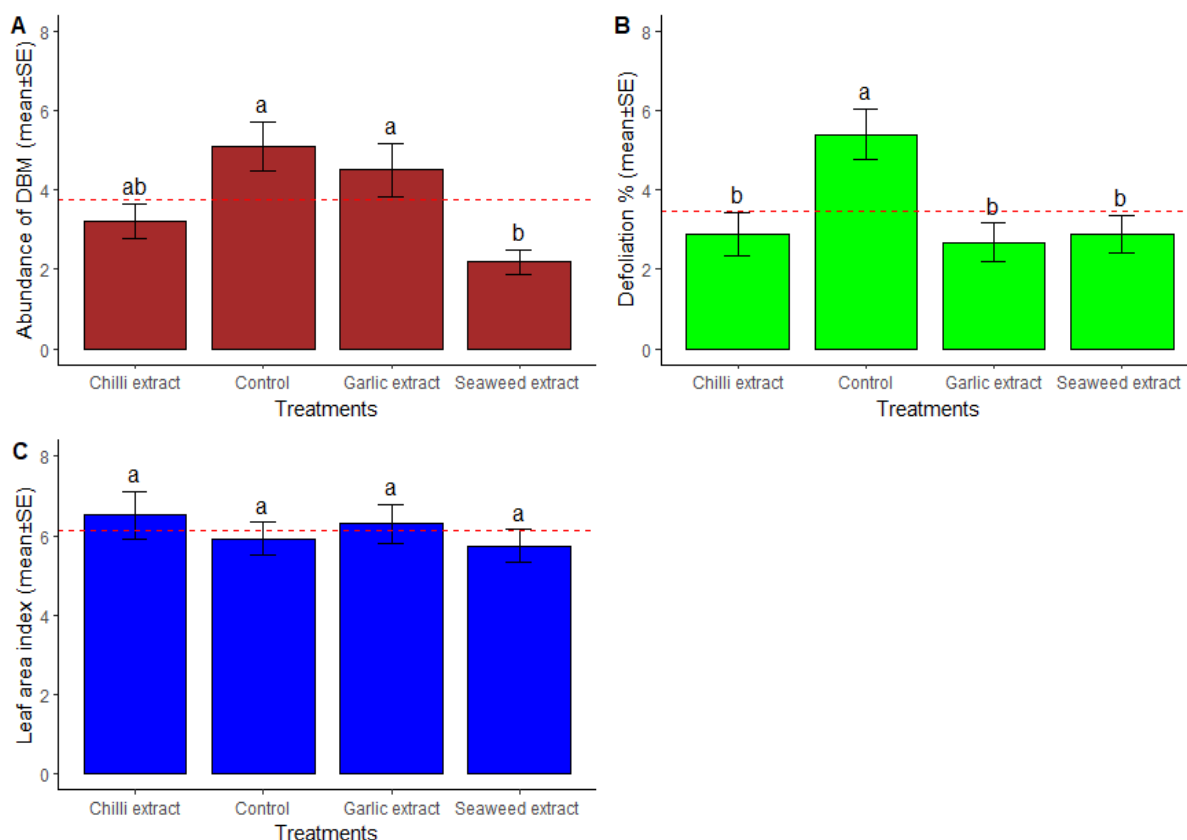

Fig 1: The DBM abundance (mean±SE) was significantly low under seaweed treatment ($p < 0.05$) followed by chili extract ($p < 0.05$) (A). Only control treatment had a high significant defoliation ($p < 0.05$) (B). No significant trend detected for LAI in all treatments ($p > 0.05$).
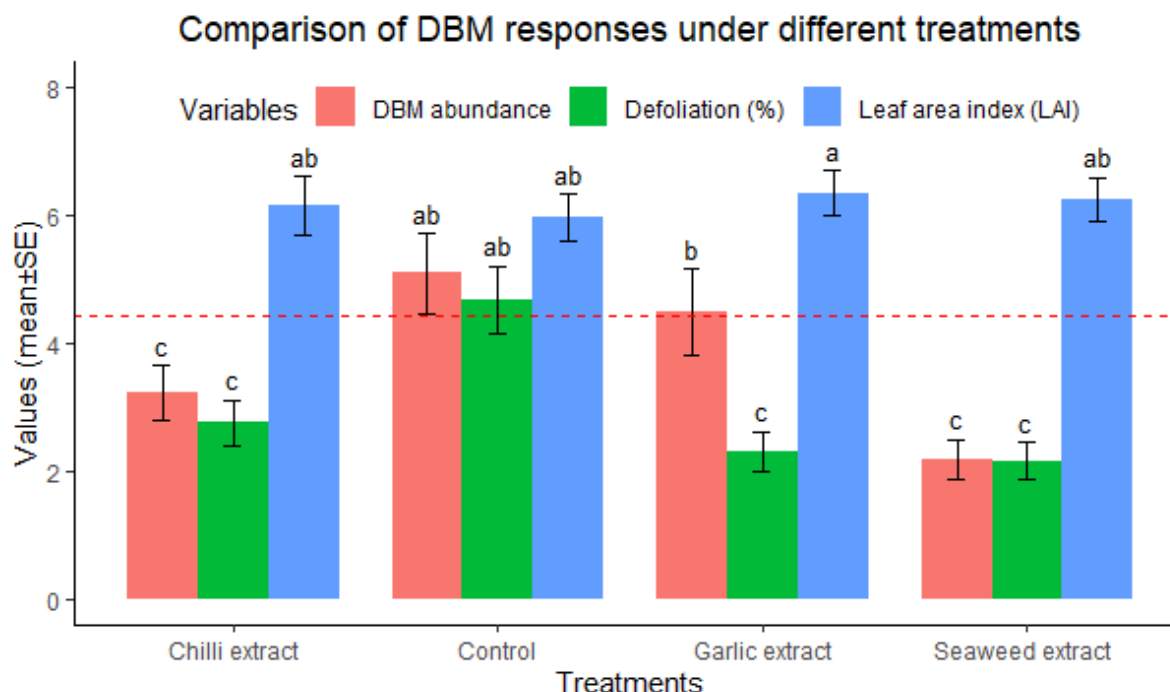
Comparison of DBM responses under different treatments

Fig 2: The values (mean±SE) on y-axis pertains to the three variables. The variables were compared to each other under each treatment. Variables having same letters are not statistically significant according to SNK test (*p*> 0.05).

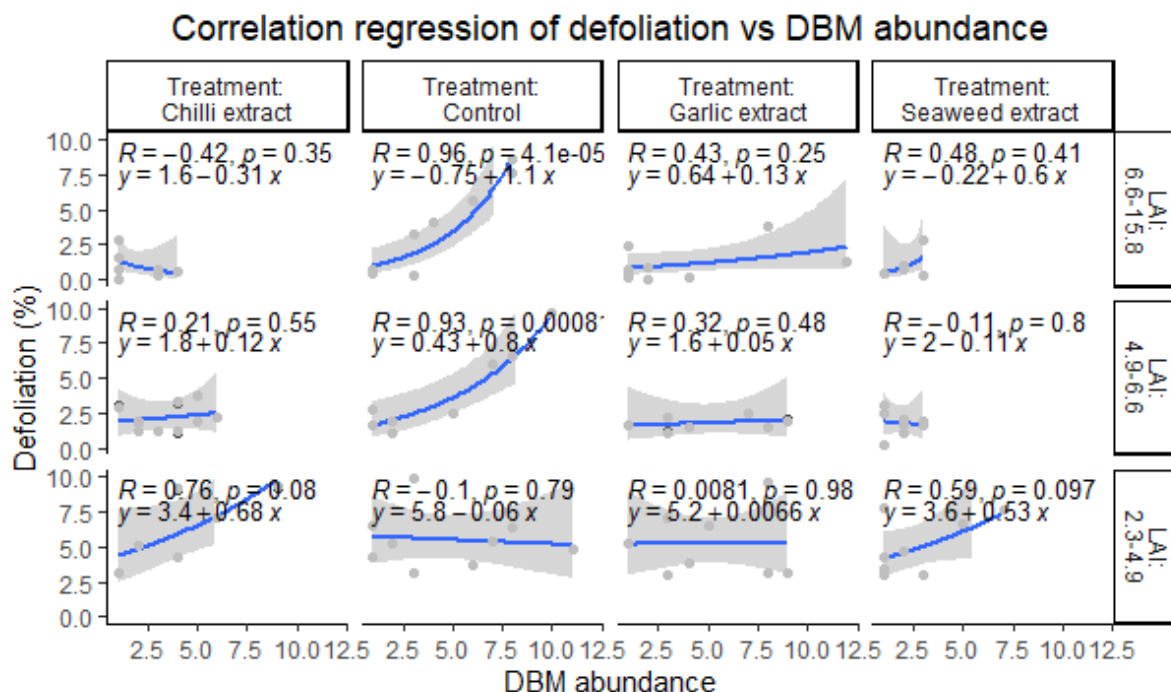## Correlation regression of defoliation vs DBM abundance

Fig 3: The correlation assessed the relationship between defoliation (%) and DBM abundance under each treatment. The z-axis was faceted by flexplot function in RStudio to produce three LAI ranges: small (2.3-4.9), medium (4.9-6.6) and big (6.6-15.8).

Discussion

All three plant extracts were generally effective in controlling DBM activity than the reference group (control treatment). The three measured variables; LAI, Defoliation (%) and DBM abundance also varied within the four treatments with control treatment experiencing more damage. Garlic extract deterred gravid female red spider mite, *Oligonychus coffeae*, from ovipositing (Roobakkumar *et al*. 2010) [47], and repelled *P. xylostella* besides other Lepidopteran pests (Yang *et al*. 1994; Samarasinghe *et al*.

2007; Machial *et al*. 2010; Ribeiro *et al*. 2015) [53, 48, 46]. Although garlic extract had low defoliations, the DBM abundance was higher than chili and seaweed extracts. Garlic essential oil suppressed *P. xylostella* eggs from hatching, however activity against larvae was generally less effective (Sangha *et al*. 2017) [49]. Applications of garlic extract to suppress Lepidoptera pest in situ showed mixed results (Endersby *et al*. 1992) [14]. The active ingredient of garlic, allicin, degrades rapidly when compared to most chemical insecticides that persist in the environment over

certain period of time (Koch & Lawson 1996) [31]. Garlic extract was not so effective against DBM due to rapid degradation of its active ingredient, allicin, when exposed to extreme weather conditions (Koch & Lawson 1996) [31]. Karavina *et al*. (2014) [28] noted a non-significant repellent effect between garlic intercropped cabbages and those treated with Malathion 25WP. The mean DBM abundance on cabbage was slightly higher under garlic extract than chili extract treatments (Baidoo & Mochiah 2016) [5]. Damages in cabbage heads was low under garlic extract, followed by chili extract and high under control treatment (Baidoo & Mochiah 2016) [5].

Seaweed extract was effective in reducing defoliation and DBM population while producing high LAI. Seaweed extract has been appreciated for its biostimulant effect on plant growth as it contains microelements and plant growth regulators (Iamba & Malapa 2020) [19]. Seaweed extract contains cytokinin, a growth regulator that have proven to significantly increase the density of feeder roots in strawberry (Khan *et al*. 2009; Arioli *et al*. 2015; Mattner *et al*. 2018) [29, 3, 36]. Crop protection mechanism of seaweed extract is mainly due to the systematic absorption and accumulation of biostimulant (Khan *et al*. 2009) [29]. Seaweed extracts also induces disease resistance in cucumber by triggering defence genes or enzymes (Jayaraman *et al*. 2011) [27]. Seaweed extract is able to withstand extreme abiotic factors such as rain because biostimulants are systematically assimilated by plants and converted into physiological resistance (Khan *et al*. 2009; Arioli *et al*. 2015; Mattner *et al*. 2018) [29, 36]. Seaweed extract via soil fumigation increased the growth response of strawberry roots and suppressed soil-borne pathogens and pests (Wilhelm & Paulus 1980; Yuen *et al*. 1991; Porter *et al*. 2004) [54, 52, 42].

Chili extract was also effective in reducing DBM abundance and defoliation and concurrently producing high LAI. Chili extract has proven to be an effective control of *P. xylostella* due to its antifeedant and repellent properties (Iorizzi *et al*. 2000; Khan *et al*. 2000; Iamba & Yoba 2019) [26, 23, 30]. Cabbages that were treated with chili extract along with other botanical extracts produced comparable yield (Begna & Damtew 2015) [6]. Defoliation was notable under control treatment due to absence of push effect against the feeding *P. xylostella* larvae. Application of nil botanical extract in control treatment resulted in highest *P. xylostella* severity (Reuben *et al*. 2006) [45]. A mixture of garlic and chili extracts at 0.5 and 1% influenced larval weight and deterred feeding (Chandrashekharaiah *et al*. 2015) [9]. The total leaf area was not a major factor in determining *P. xylostella* ovipositional and/or feeding preference across cabbage and glossy and waxy collards (Badenes-Perez *et al*. 2004; Baidoo & Mochiah 2016) [5]. Vice versa, leaf area was not affected by any of the plant extracts. Chili extract produced comparable yield since it contains capsaicinoids, diterpenoids, flavonoids, saponins, and phenolic compounds have antifeedant and repellent properties (Iorizzi *et al*. 2000; Madhumathy *et al*. 2007; Begna & Damtew 2015) [26, 6, 34]. Chili extract had the low DBM abundance and defoliation which can be attributed to its compatibility with natural enemies such as predators (Iamba & Yoba 2019) [23]. Plant extracts as plant derived chemicals are deem to reduce oviposition and simultaneously boosting parasitism of insect pests by parasitoids in cropping systems (Bhattacharyya 2017) [7].

Extreme environmental conditions can affect the capacity of plant extracts to effectively control insect pests in crops. It has been experimentally tested that 2 to 5 mm of simulated rain can wash off 50% or more of the chemical compounds that are applied 1 h after spraying (Pick *et al*. 1984) [40]. Another study also showed that imazaquin compounds present in herbicides are easily lost to foliar wash off (Reddy & Locke 1996) [44]. However, chili extract is able to withstand rainy days because of its adhesive phytochemicals such as capsaicinoids, diterpenoids, flavonoids, saponins, and phenolics that can strive under extreme weather conditions (Iorizzi *et al*. 2000; Madhumathy *et al*. 2007; Begna & Damtew 2015) [26, 6, 34]. Seaweed extract was presumed to withstand extremes in weather conditions since stimulation of resistance is being systematically assimilated (Khan *et al*. 2009) [29].

Conclusion
The build-up of resistance in DBM due to continuous and indiscriminate use of synthetic insecticides have refocused farmer's perspectives to plant based extracts. Plant extracts are generally compatible with natural enemies of pests therefore are considered eco-friendly. Pesticide plants are locally abundant, easy to extract using simple methods and much cheaper for subsistence farmers to utilize. This study found that both seaweed and chili extracts were effective in controlling *P. xylostella* in round cabbage. Both extracts are recommended for use since they differ in their mechanism pathways to protect crops. Chili has insecticidal phytochemicals that effectively repelled and deter pests. While seaweed extract has to be absorbed along with biostimulants and micronutrients through assimilation to enhance the physiological resistance of the plant. Their compatibility to natural enemies fit in well with the concept of conservation biological control in Integrated Pest Management (IPM) program.

Acknowledgement

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