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Kari Iamba
Department of Forestry, School of Natural Resources, PNG University of Natural Resources & Environment, Vudal, Papua New Guinea, Oceania

Sandrina Malapa
Department of Agriculture, School of Natural Resources, PNG University of Natural Resources & Environment, Vudal, Papua New Guinea, Oceania

Corresponding Author:
Kari Iamba
Department of Forestry, School of Natural Resources, PNG University of Natural Resources & Environment, Vudal, Papua New Guinea, Oceania

Efficacy of selected plant extracts against diamondback moth (*Plutella xylostella* L.) on round cabbage in situ

Kari Iamba and Sandrina Malapa

Abstract
Conventional application of synthetic insecticides to control *Plutella xylostella* (Diamondback moth) has been unsuccessful due to resistance within the pest. Garlic extract, Chili extract and Seasol® seaweed solution was tested for their efficacy against DBM. Both garlic and chili are locally abundant, possess insecticidal properties, cost-effective and compatible with natural enemies. Three concatenated parameters; DBM abundance, Defoliation (%) and Leaf Area Index (LAI) were measured to test the efficacy of these botanical extracts. Concerning DBM abundance, both seaweed extract (p=0.00) and chili extract (p=0.01) showed significant results ($p< 0.05$) when compared with control treatment ($p>0.05$). Defoliation (%) was low under all extract treatments; seaweed (p=0.00), garlic (p=0.00) and chili (p=0.00). However, there were no significant differences in LAI results. There was significant interaction effect between DBM abundance and extract treatments (p=0.00). Reduced number of DBM were found under Seaweed and chilli extract treatments.

Keywords: Botanical extract, *Plutella xylostella*, defoliation, leaf area index, conventional

1. Introduction
The diamondback moth (DBM), *Plutella sxylostella* (L.) (Lepidoptera: Plutellidae) is a major pest of cabbage and other vegetables under the Brassicaceae family. DBM is cosmopolitan and important economic pest globally [1]. Due to imprudent use of synthetic pesticides over the years, DBM has developed resistance [2]. Therefore, researchers have gain special interest in Botanical extracts since they are locally abundant, possess insecticidal properties, cheaper to access, compatible with natural enemies and safer for human consumption. Application of leaf, flower, fruit and seed extracts from selected insecticidal plants resulted in larval mortality, oviposition deterrence and reduced feeding activity of DBM on treated cabbage leaves [3-6]. Most botanical pesticides when used in combination with effective natural enemies produces efficacious plant protection results in an IPM program [7]. Application of plant extracts is to develop a push-pull system in order to reduce oviposition of insect pest and concurrently enhancing parasitism by its parasitoids in crops [8]. Various semiochemicals are involved in push-pull strategies which act as repellent or attractant volatiles to orchestrate pests and natural enemy populations [9]. Pests can be ‘pushed’ out of the crop by repellents and deterrents, and natural enemies can be ‘pulled’ into the pervaded crop by foraging attractants ‘volatiles’ to suppress the pests [10]. This research was done to test the repellent effect of Garlic (*Allium sativum* L.), Chili (*Capsicum frutescens* L.), and Seaweed extract on DBM. It was hypothesized that the application of these selected plant extracts would lower the number of DBM and deters them from feeding on cabbage leaves. Evaluation of leaf damage was tested on two parameters; defoliation and LAI. K-K cross variety was chosen as the host subject since it is a common leafy vegetable that is cultivated locally. LAI can be a measure of yield in leafy vegetables like cabbage and defoliation quantifies damage due to DBM.

2. Materials and Methods
2.1 Location
The study was carried out at the experimental crop section of PNG University of Natural Resources and Environment (PNGUNRE) in Vudal, Papua New Guinea (PNG). The study was carried out in the Field Crops Section of PNG UNRE from September 2nd to 18th, 2019. The climate is classified as tropical with a great deal of rainfall experienced even in the driest

month. PNG UNRE is located 4º21'01.90" S and 152º00'33.44" E with elevation of 51 meters above sea level [2]. The experimental site is surrounded by crops, shrubs, grasses and cattle paddock to the north-east. The soil type is sandy loam with high pH level (alkaline) and more calcareous. Predominant vegetables grown here are cabbages, tomatoes and capsicum which grows all year round.

2.2 Plant material
Fruits from Chili (*C. frutescens*) and Garlic cloves (*Allium sativum* L.) were procured and dried. The dried materials were then crushed and grounded using a mini blender. Grounded contents were placed in desiccators and ethanol added to halfway with all contents totally submerged. Procedures involved in the ethanolic extraction followed those used by Adeniyi and Ayepola [11]. Desiccators were tightly fastened and left on the Lab bench for 7 days with good ventilation. Contents were then filtered through filter paper and fluid contents collected in glass beakers. Filtered solutions were allowed to evaporate at room temperature (25ºC) to dryness and stored in airtight containers with appropriate labels. The seaweed extract used in this study was manufactured by Seasol® and is derived from a blend of three finest brown kelps around the world; *Durvillaea potatorum, Durvillaea antarctica* and *Ascophyllum nodosum.*

2.3 Experimental design
A factorial design was used with four treatments and each treatment had three levels. Four weeks old seedlings of K-K cross were transplanted onto experimental plots according to treatments: T1=Garlic extract, T2=Seaweed solution, T3=Chili extract, and T4=Control. Each treatment was replicated 3 times within a 2x3m plot. Data was collected at 4 days interval from time of spraying with a total of 5 sampling times. All extracts were mixed with water at concentration of 10ml/1L and applied using conventional method of spraying. This concentration was used in accordance with findings from Charleston [5] which suggested higher doses of plant extracts. No extract spraying was applied to control plots but rather used pure water.

2.4 Parameters and sampling
Three parameters or levels measured in this study were; DBM abundance, defoliation (%) and Leaf Area Index (LAI). The abundance was simply the counting of DBM larvae on the cabbage leaves including the underside. Defoliation (%) was measured using BioLeaf®-Plant Image Analysis application which allows for rapid and precise calculation in the field. The BioLeaf® also calculates the LAI of cabbage leaf to complement the defoliation and DBM abundance data. Three cabbage plants were randomly selected per plot and each parameter was measured and recorded in field data sheet. The experiment had 12 plots with 3 measurements per parameter per plot which amounted to a total of 108 measurements per sampling date. Since there were 5 dates of data sampling, the overall data collected amounted to 540. All data were tabulated and subjected to data analysis to generate statistical summary, ANOVA, interaction effect and correlation.

2.5 Data analysis
Field raw data was filtered to produce tabulated summary statistics such as mean ($\bar{x}$), standard deviation (SD) and standard error (SE). ANOVA (p-value) was used to detect significant differences between treatments and separation of means was done in Tukey HSD Test ($\alpha = 0.05$). Pearson correlation (r) was also included to test the correlation between two continuous variables i.e. DBM abundance to defoliation (%) or LAI. For interaction effect, General Linear Model (GLM) was used to detect any significant interaction between DBM abundance and defoliation or LAI under different extract treatments. These statistical tests were utilized to evaluate and concatenate the results of plant extract efficacy against DBM. All analysis, graphs and tables were done in ggplot2 package of R version 3.6.1.

3. Results
A total count of 180 cabbage plants and a sum of 388 DBM larvae were sampled during the experimental period from 6th - 22 September, 2019. Results pertaining to defoliation (%) and LAI showed varying information in relation to DBM abundance. Table 1 shows the values of mean ($\bar{x}$), standard error (SE) and standard deviation (SD) for different parameters under selected plants extracts.

Table 1: The treatments (plant extracts) were tested on three parameters; LAI, defoliation and DBM abundance. Results of each plant extract was compared to control treatment (no extract) which served as a reference group. All plant extracts showed significance differences (p>0.05) in defoliation (%) while DBM abundance was only significant in seaweed solution and chili extract. There were no statistical differences in LAI under each treatment when compared to control treatment.

Treatment	Parameters	Statistics		
		Mean ± SE	**SD**	***p-value***
Garlic extract	LAI	5.23 ± 1.25	5.22	0.49
	Defoliation (%)	3.06 ± 1.71	3.82	0.000***
	DBM abundance	4.80 ± 2.33	5.22	0.17
Seaweed solution®	LAI	3.17 ± 0.89	2.95	0.60
	Defoliation (%)	3.77 ± 1.72	3.84	0.000***
	DBM abundance	1.80 ± 1.32	2.95	0.000***
Chili extract	LAI	5.74 ± 0.53	2.68	0.60
	Defoliation (%)	2.21 ± 0.61	1.37	0.005**
	DBM abundance	1.20 ± 1.20	2.68	0.005**
Control	LAI	6.01 ± 2.04	4.60	0.58
	Defoliation (%)	4.69 ± 2.01	4.50	0.005**
	DBM abundance	4.80 ± 2.06	4.60	0.03*

Values from the treatments were compared with reference group (control treatment)
The asterisks (*) indicates the level of statistical significance.
Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1'' 1

Analysis of Variance (ANOVA) for all the treatments were computed in R. Significant differences were detected in the number of DBM (p=0.000, s^2=50.16) and defoliation (%) (p=0.000, s^2=54.85) under the four treatments. A small variance indicates that the data points tend to be very close to the mean, and to each other. A high variance indicates that the data points are very spread out from the mean, and from one another. Due to these significant differences, Tukey HSD Test was used to separate their means. No significant differences were detected in LAI values (p=0.91, s^2=1.23) therefore no further separation of means was required.

Mean separation of DBM and Defoliation were compared at 95% family-wise confidence level.

There is significant difference in the number of DBM between control and chili extract (p<0.05*), and highly significant difference between seaweed solution and control (p< 0.05***). The difference (diff) in number of DBM between control and chili extract is 1.87 with control averaging 1.87 higher. The 95% confidence interval of that difference is between 0.33 and 3.41 DBM. There is significant difference in Defoliation (%) between control & chili extract (p< 0.05**), highly significant difference between garlic extract & control (p< 0.05**), and seaweed solution & control (p< 0.05***). Control treatment is averaging 1.84 higher than chili extract, 2.22 higher than garlic extract, and 2.41 higher than seaweed solution. There is no significant difference in LAI between all the treatments (p>0.05). Control treatment is averaging 0.31 lower than chili extract, 0.37 lower than garlic extract, and 0.28 lower than seaweed solution.

Table 2: The Interaction ANOVA table shows that there is interaction in Defoliation (%) between DBM and treatment (p< 0.05**, r = -0.11) (A). There is interaction in LAI between DBM and defoliation (%) (p< 0.05***, r = 0.56) (B). There is also interaction in the number of DBM between defoliation (%) and LAI (p< 0.05**, r =-0.39) (C).

A. Interaction response in Defoliation (%)					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
DBM	1	402.95	402.95	91.4280	< 2.2e-16 ***
Treatment	3	67.47	22.49	5.1027	0.002085 **
DBM: Treatment	3	69.83	23.28	5.2813	0.001654 **
Residuals	172	758.05	4.41		
B. Interaction response in Leaf Area Index (LAI)					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
DBM	1	8.50	8.501	1.623	0.2044
Defoliation	1	220.64	220.638	42.122	8.424e-10 ***
DBM: Defoliation	1	87.05	87.049	16.619	6.910e-05 ***
Residuals	176	921.90	5.238		
C. Interaction response in DBM abundance					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Defoliation	1	479.72	479.72	85.4300	< 2e-16 ***
LAI	1	35.01	35.01	6.2354	0.01344 *
Defoliation: LAI	1	42.61	42.61	7.5890	0.00649 **
Residuals	176	988.30	5.62		

The asterisks (*) indicates the level of statistical significance.
Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

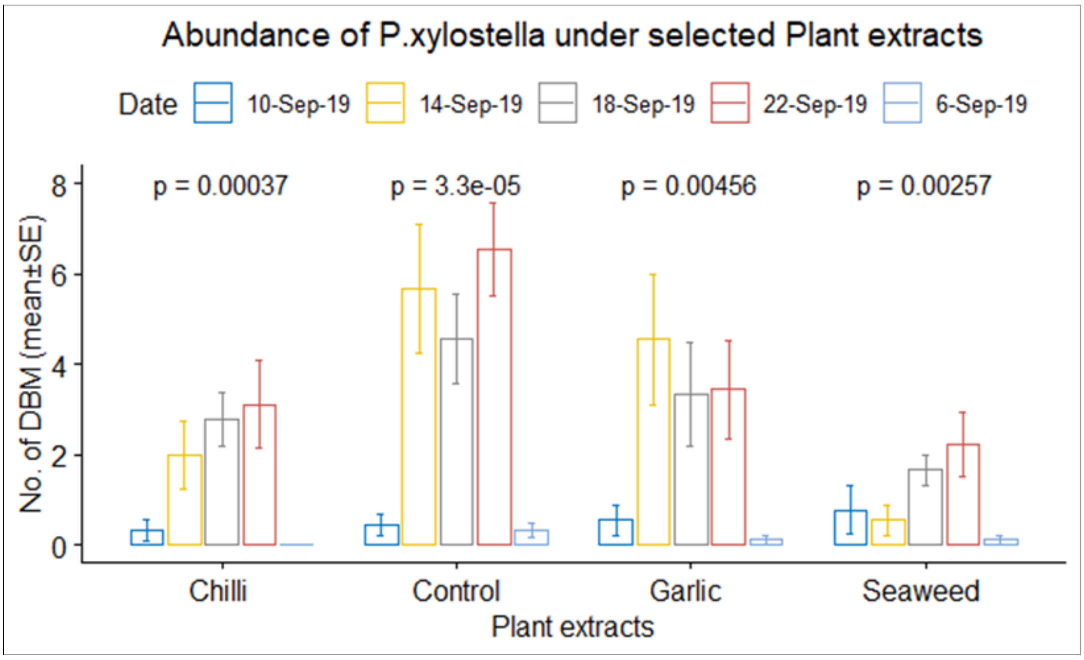

Fig 1: The selected plant extracts showed significant differences among sampling dates. All treatments were compared with control treatment as the reference group. There were five sampling dates of each parameter (DBM abundance, LAI, Defoliation) under each treatment. Control treatment had the highest number of DBM (mean±SE) on 22-Sep-19 (p< 0.05) followed by garlic extract (p>0.05). Chili and seaweed solution had low number of DBM (p< 0.05) at 22-Sep-19.

There is significant difference between the sampling dates and number of DBM under all treatments (fig. 1). However, low numbers were recorded under seaweed and chili extract treatments. Low DBM abundance was recorded on 6-Sep-19 with similar scenario across all the treatment. Chili, garlic and control treatment showed increased number of DBM on 14-Sep-19 while low number under seaweed treatment. DBM abundance increased on 18-Sep-19 under chili and seaweed treatments while decreasing in garlic and control treatments. Control treatment had the highest number of DBM on 22-Sep-19 followed by garlic extract. Both chili and seaweed solutions had low number of DM on 22-Sep-19 when the experiment was completed. A steady increase in each sampling date can be seen in chili treatment with the lowest at 6-Sep-19 and highest at 22-Sep-19. Similar scenario is shown under seaweed treatment with the exception on 14-Sep-19 where it recorded lower number of DBM. The significant differences were influenced by treatments and other factors with their interactions. High number of DBM would be expected to result in high defoliation of cabbage foliage (fig. 2) and wise versa. The selected plant extracts served as repellents and/or deterrents against DBM feeding and oviposition. Therefore, low number of DBM hence low defoliation rate would be expected under the selected plant extracts.

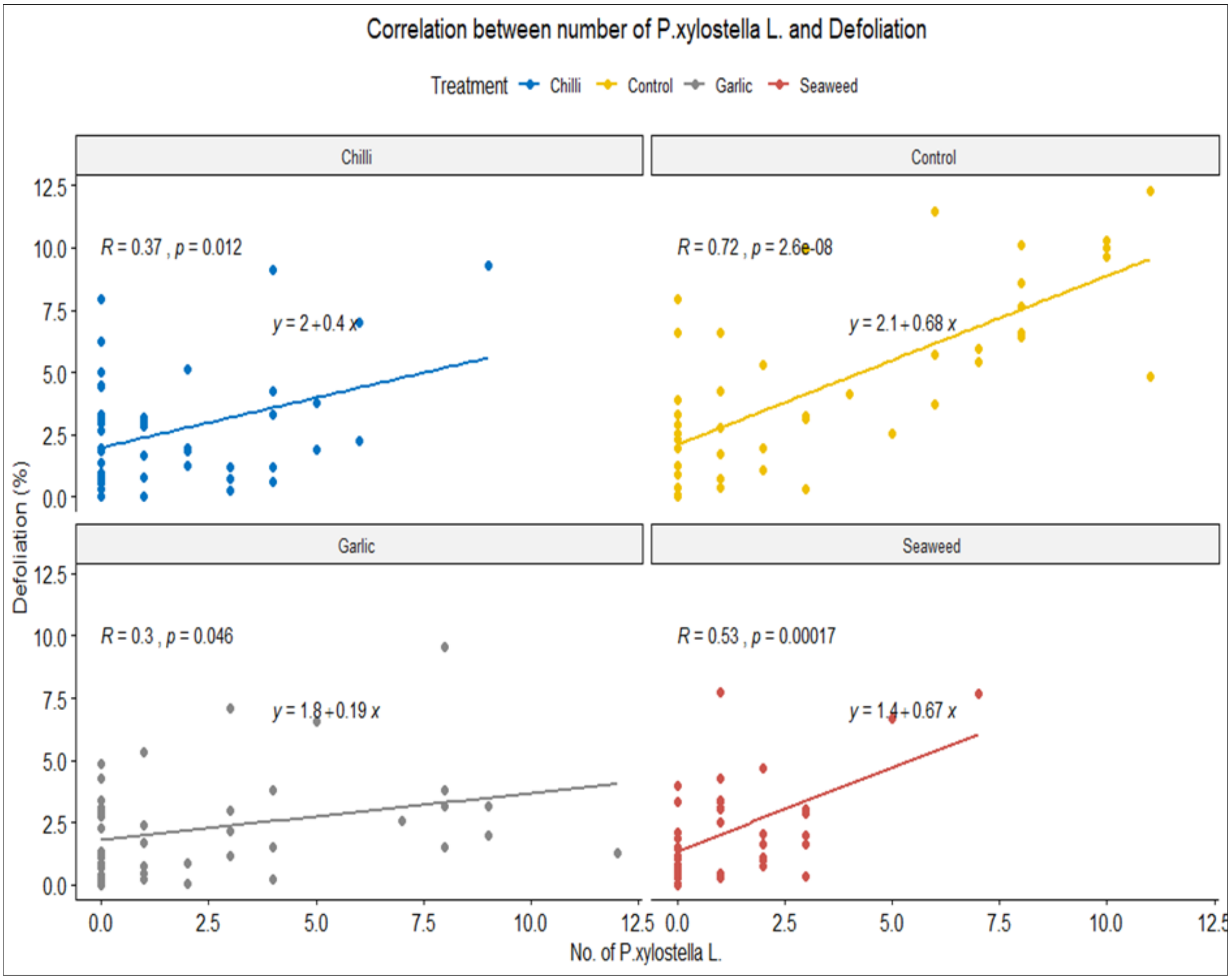

Fig 2: Correlation was done between two continuous variables, number of *P. xylostella* L. and defoliation (%). The abundance and feeding of DBM larvae on round cabbage foliage contributes to defoliation. According to the facet graph, all treatments showed positive correlation between number of DBM and defoliation ($p < 0.05$, r= (+)). As the number of DBM increased, defoliation of cabbage leaves also increased.

There is interaction between number of DBM and Treatments (p=0.002) implying that the effect of DBM abundance on Defoliation (%) depends on treatments and vice versa. The correlation lines between number of DBM and defoliation are positive for each treatment: garlic (r=0.3, p=0.046), seaweed (r=0.53, p=0.000), chili (r=0.37, p=0.01) and control (r=0.72, p=0.000). It can be concluded that, as the number of DBM increase, defoliation also increase.

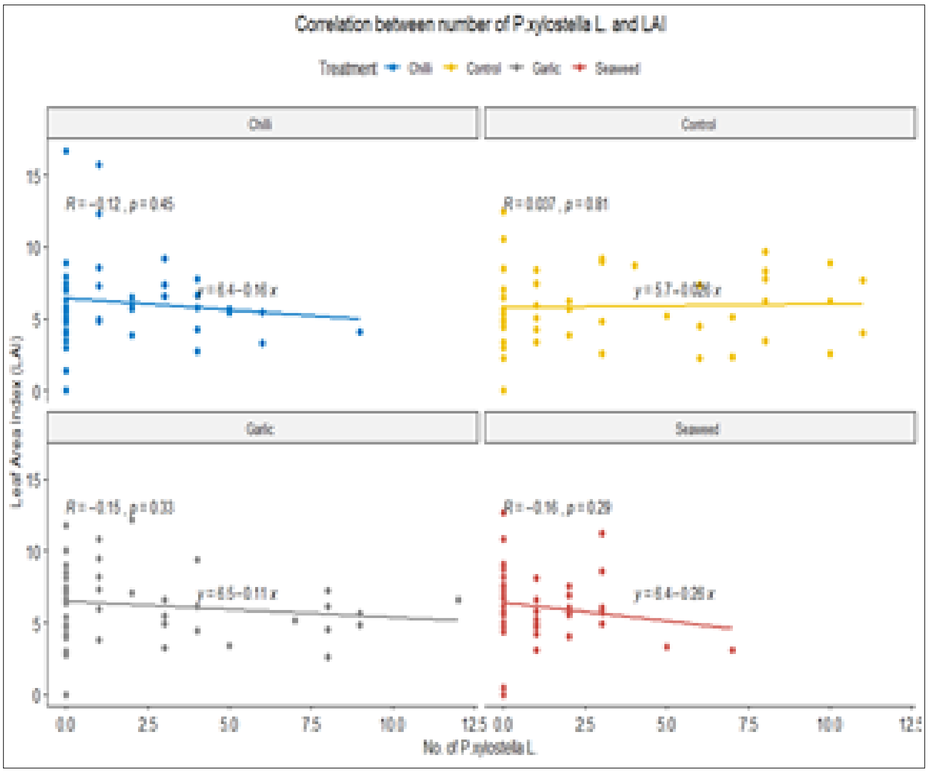

Fig 3: Correlation was done between another continuous variables (LAI) with number of *P. xylostella* L. The abundance and feeding of DBM larvae on round cabbage foliage was expected to decrease LAI. According to the facet graph above, all plant extracts showed slightly negative correlation between number of DBM and LAI ($p>0.05$, r=(-)). As the number of DBM increased, LAI of cabbage leaves also decreased. A slightly positive correlation is evident in control treatment (r=0.04).

The interaction term between number of DBM and LAI is generally not significant ($p>0.05$). The effect of DBM abundance on LAI depends on defoliation (%) and vice versa. There is negative correlation between DBM and LAI for each treatment: garlic (r=-0.15, p=0.33), seaweed (r=-0.16, p=0.29), chili (r=-0.12, p=0.45), with exception in control treatment (r=0.04, p=0.81). Increase in the number of DBM led to decrease in LAI, although non-significant differences suggested no interaction between DBM and LAI.

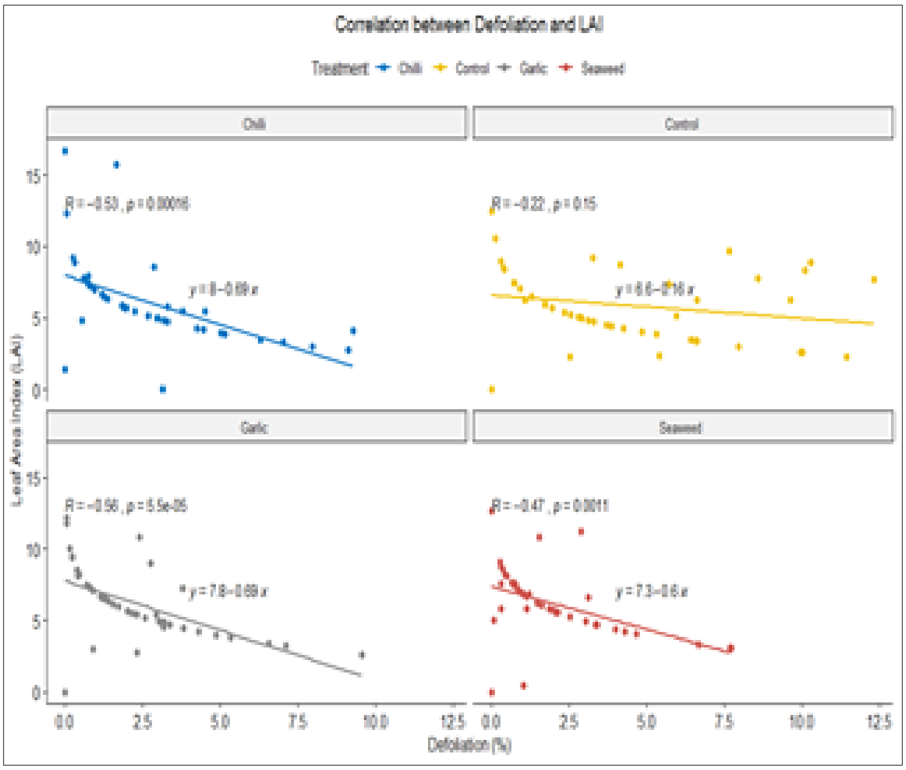

Fig 4: Correlations between Defoliation and number of *P. xylostella* L. are generally negative. Defoliation on round cabbage foliage was expected to decrease LAI. According to the facet graph above, all plant extracts showed negative correlation between number of Defoliation and LAI (r= (-)). As the number of Defoliation decreased, LAI of cabbage leaves increased.

The interaction term between Defoliation (%) and LAI is generally significant for the plant extracts ($p < 0.05$) while not significant for control treatment ($p > 0.05$). The effect of defoliation on DBM depends on LAI and vice versa. There is negative correlation between Defoliation and LAI for each treatment: garlic (r=-0.56, p=0.000), seaweed (r=-0.47, p=0.001), chili (r=-0.53, p=0.000) and control (r=-0.22, p=0.15). This inverse relationship shows that as defoliation increases, LAI decreases under each plant extract treatments.

4. Discussion

The selected plant extracts showed varying results when compared with the reference group (control treatment). Inferences drawn from the three parameters; LAI, Defoliation and DBM abundance differed amongst the treatments (plant extracts). Garlic extract deterred the red spider mite, *Oligonychus coffeae,* from ovipositing [12], and repelled other Lepidopteran pests, including *P. xylostella* [13-16]. Garlic essential oil was quite effective at suppressing *P. xylostella* egg hatch, however activity against larvae was generally less effective [17]. Endersby, Morgan [18] showed that applications of garlic extract for Lepidoptera pest suppression in situ have not always been effective. According to Koch and Lawson [19], the active ingredient of garlic, allicin degrades very fast when compared to most chemical insecticides that persist in the environment over certain period of time. Seaweed extract is an effective biostimulant due to the microelements and plant growth regulators such as cytokinin present in it which have shown to significantly increase the density of feeder roots in strawberry [20-22]. Soil fumigation via drench application increased the growth response of strawberry roots and other crops to control soil-borne pathogens and pests [23-25]. Chili extract treatment supported previous studies as a good control method of *P. xylostella* due to its antifeedant and repellent properties [2, 26, 27]. Cabbages treated with chili, and other botanical extracts produced comparable yield [28]. There was notable defoliation under control treatment due to absence of barrier against feeding larvae of *P. xylostella.* Reuben, Yahya [29] reported that control treatment of no botanical extract application resulted in highest *P. xylostella* severity.

A mixture of garlic+chili extract at 0.5 and 1% had low effect on larval weight and feeding deterrence [30]. Karavina, Mandumbu [31] found out that there were no significant differences in the repellent effect between garlic intercropped cabbages and those treated with Malathion 25WP. The mean number of DBM found on cabbage after treatment application was slightly higher under garlic extract than chili extract [32]. Evaluation by Baidoo and Mochiah [32] demonstrated that the percent damaged cabbage heads was low under garlic extract, followed by chili extract and high under control treatment. According to Badenes-Perez, Shelton [33], total leaf area does not seem to be a major factor in determining *P. xylostella* ovipositional preference across cabbage and glossy and waxy collards having similar leaf areas.

Chili extract produced comparable yield since it contains active phytochemical family of capsaicinoids, diterpenoids, flavonoids, saponins, and phenolic compounds which possess lethal effects, antifeedant properties and parasite repellence [26, 28, 34]. Seaweed extract is an effective biostimulant that has shown to significantly increase the density of secondary roots and enhances the uptake of micronutrients to build up resistance against pests and pathogens [20-22]. Chili extract had the lowest number of DBM and second lowest defoliation which can be attributed to its compatibility with natural enemies of DBM such as predators [2]. Plant extracts are applied to develop a push-pull system in order to reduce oviposition and concurrently enhancing parasitism of insect pests by parasitoids in crops [8]. Seaweed extract had the second lowest number of DBM and defoliation which is mainly due to the systematic absorption and accumulation of biostimulant [20]. Seaweed extracts also enhance disease resistance in cucumber by inducing and triggering of defence genes or enzymes [35]. Garlic extract was not considered as an effect control of DBM due to rapid degradation of its active ingredient, allicin, when exposed to adverse weather conditions [19]. Total leaf area is not a major factor in determining the ovipositional and/or feeding preference of *P. xylostella* [33].

A study by Akter, Mendez [36] showed that the head weight of cabbage plants were significantly lower in the presence of 15 beet armyworms per plant compared to no larvae per plant. *P. xylostella* consumed 3.6 cm^2 of canola leaf area and defoliation increased as number of the pest increased [37]. A study by Pick, Van Dyk [38] showed that 2 to 5 mm of simulated rain washed off 50% or more of the chemical compounds applied 1 h after spraying. Another study from Reddy and Locke [39] suggested that imazaquin compounds (herbicide) are easily lost to foliar wash off. Seaweed extract is less vulnerable to rain because it has been uptaken by the plants via assimilation process and converted into physiological resistance [20-22]. Chili extract also proved to withstand rainy days because of its adhesive phytochemicals that contains active phytochemicals; capsaicinoids, diterpenoids, flavonoids, saponins, and phenolic compounds that can persist under adverse weather conditions [26, 28, 34].

5. Conclusion

Farmers are now aware of the adverse effects of continuous use of synthetic insecticides. As a result of lesson learnt, they are now resorting to botanical pesticides. Plant extracts are very cheap and locally abundant for farmers to utilized and use in crop protection. This study had found out that out from the selected plant extracts used, Seaweed and Chili extracts have proven to be effective in controlling *P. xylostella.* Interestingly, their mechanisms of control differ and both are recommended for farmers to use. Chili possess insecticidal phytochemicals that act as effective repellent and deterrent of *P. xylostella.* Seaweed extract on the other end is absorbed by the plant through assimilation and the biostimulants present along with micronutrients enhance the physiological resistance of the plant against *P. xylostella.* Both plant extracts are recommended to be used by famers and utilized in an Integrated Pest Management (IPM) program to ensure a better crop performance.

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