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An integrated approach of managing *Conopomorpha cramerella* Snellen: Application of plant extracts in a push-pull system

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Abstract
The push-pull strategy plays a vital role in management of pests in tropical agriculture. Extracts from two local plants, marigold (*Tagetes erecta* L.) and ginger (*Zingiber officinale* R.) were investigated for their potential to manage infestation of Cocoa pod borer (*Conopomorpha cramerella* S.). Bean weight under ginger extract in block 1 was relatively low (p<0.05, 65.13 ±6.65, σ =49.29) while highest in block 2 (p<0.05, 110.45±11.61, σ =73.41). Damage score under ginger extract in block 1 was relatively low (p<0.05, 1.49±0.19, σ =1.37). Control treatment produced the highest number of entry holes under block 2 (p<0.05, 8.38±0.73, σ =4.89). There was a positive correlation between bean damage and bean weight under ginger extract treatment (p<0.05, r= 0.42). Marigold extract produced non-significant results for all response variables (p>0.05). The findings from this study rectify that ginger (*Z. officinale*) extract was effective in managing *C. cramerella* infestation in cocoa (*Theobroma cacao* L.) through push strategy. Marigold (*T. erecta*) extract did not reduce the impact of *C. cramerella* therefore it was recommended for planting as intercrop rather than applying as extract.

Keywords: Push-pull strategy, *Tagetes erecta* L., *Zingiber officinale* R., *Conopomorpha cramerella* S., cocoa, extract

Introduction

The cocoa pod borer (*Conopomorpha cramerella* Snellen) is a major destructive pest of cocoa in East New Britain Province and Papua New Guinea. Since its emergence in 2006, the cocoa industry has suffered tremendous yield losses [1]. The problem of cocoa pod borer (CPB) persisted in South-east Asia since the mid-1980s and continues to be major threat to cocoa production [2, 3]. Integrated Pest Management (IPM) was developed from Integrated Pest and Diseases Management (IPDM) concept and introduced as an appropriate strategy to combat *C. cramerella* infestation [4]. IPM strategy incorporates pruning of cocoa and shade trees; manual and chemical weeding; chemical control of pests and insect vectors; application of NPK fertilizer and animal manure; and block sanitation [5]. Synthetic chemicals such as deltamethrin, cypermethrin or lindane have been recommended for spraying at resting sites of adult CPB [6]. Cocoa farmers are now facing financial constraints due to *C. cramerella* infestation, high cost of IPDM inputs, labour intensity and devaluation of local currency [7]. Recently there has been focus on the use of local plant extracts that contain either repellent or attractant properties [8]. Plants with insecticidal properties are locally abundant and this may set a way forward in finding low-cost method of managing *C. cramerella*. For this study, we focus on two local plants, (1) marigold (*Tagetes erecta* L.) and (2) ginger (*Zingiber officinale* R.). Extracts from both plants have shown to reduce pest infestation in vegetables and crops. Lower abundance of *Plutella xylostella* larvae and flea beetles were recorded on cabbages that were intercropped with *T. erecta* than on sole cabbage [9]. Intercropping of tomato plants with marigold reduced the infestation of glasshouse whitefly (*Trialeurodes vaporariorum* W.) [10]. Marigold reduced both oviposition ability of female and larvae abundance of *Helicoverpa armigera* (H) in intercropped tomato [11]. The infestation of flea beetle on foliar damage was significantly lower in intercrop marigold than in sole cabbage plots [12]. *Z. officinale* also possess insect repellent properties and has been effective in repelling Dipteran insects (i.e. *Culex tritaeniorhynchus* and *Anopheles subpictus*) [13]. A study by Saripah, Hajjar [14] showed that ginger extract inhibited the emergence of adult *C. cramerella*, therefore it was recommended as a promising botanical pesticide. The volatile chemical constituents of *Z. officinale* extract are known to be effective against several insect pests [15, 16].

Z. officinale showed significant repellent activity against both male and female maize weevil, *Sitophilus zeamais* [17]. This study aims to test the repellent efficacy of Z. *officinale* and *T. erecta* extracts in reducing the negative impact of *C. cramerella*. Since botanical pesticides are part of IPM strategy, both extracts were tested under managed and unmanaged cocoa blocks. Findings from this study would divulge the role of push-pull strategy under different management practices in cocoa.

Materials and Methods

This quantitative study was done to measure the response of *C. cramerella* to marigold and ginger extracts. The study was done at PNG University of Natural Resources and Environment (PNG UNRE) campus in East New Britain Province (ENB). The cocoa blocks studied are planted with clonal varieties (*Theobroma cacao*) which are situated at an elevation of 51 meters above sea level and approximately 4º21'01.90" S and 152º 54 00'33.44" E [18]. Cocoa trees are mainly grown on Andisol which is more calcareous in nature and relatively sandy loam with high alkalinity [19, 20]. A great deal of rainfall is experienced all year round even in the driest month. Three treatments (T); T1=marigold extract, T2=ginger extract, and T3=control (no extract) was replicated five (5) times within a managed and unmanaged cocoa block. Plant compounds from ginger and marigold were obtained using ethanol extract method as described by Iamba and Malapa [19]. There were five cocoa trees treated with each treatment respectively in each block. So, the study had 10 trees per treatment, and a total of 30 trees for both blocks combined. High integrated pest management (IPM) practice was applied in cocoa block 1 where sanitation, pruning, manual weeding, manure application and weekly harvesting of pods was done [21]. There were no management practices applied to Block 2 as it represents the current practice for most local farmers and small-block holders.

Three important response variables were measured, (1) damage score, (2) bean weight (g), and (3) number of entry holes. These variables were taken into consideration since they pertain to the impact of *C. cramerella* on cocoa beans. Cocoa pods were harvested every week and data on the 3 variables were recorded per pod per tree. The damage score was estimated once the pod was split open with a bush knife. A score of 1 was given to apparently healthy beans (loose beans), 2- infested with CPB (infested regardless of severity), 3- highly infested (50% or more clustered beans) and 4- very highly infested (50% or more beans unextractable) [22]. A total of 147 pods weighing 50.8kg were sampled under managed block while 140 pods weighing 62.6kg came from unmanaged block. A digital mega desk scale with maximum load of 40kg was used to take weight of individual cocoa pods and beans. Once the beans and placenta have been removed completely from pods, number of exposed entry holes made by *C. cramerella* larvae were manually counted and recorded. The data from all three variables were recorded per pod per tree under both managed and unmanaged cocoa blocks. A total of 12708 data sets were collected under managed block and 14204 under unmanaged block. It takes about 2-man hour per day to collect all data pertaining to each treatment.

The R function aov (R Core Team, 2013) was used for Analysis of Variance (ANOVA) to analyze effect of plant extracts (treatments) and cocoa block management on bean damage (score), bean weight (g), and (3) number of entry holes. For correlation between response variables (i.e. bean weight, damage score, number of entry holes) and factors (i.e. treatments, blocks), we used function cor.test (package ggplot2) to produce pearson correlation values, and Tukey HSD test (package agricolae, function HSD.test) for separation of means and construction of graphs with letters denoting level of significance. In addition, we used function stat_cor (package ggpmisc) for calculating p-values of correlations and stat_regline_equation to produce linear regression equations between the response variables and factors (i.e. treatments/blocks).

Results

A total of 1407 measurements on different response variables were taken from September to November 2020. The managed block had a total bean weight of 11.492kg while unmanaged block had 12.943kg. The sum of bean damage due to CPB was slightly higher in unmanaged block (337) than in managed block (328) (fig. 1). Both marigold and ginger extracts had high bean weight when compared to control treatment. Treatment 1 (marigold extract) recorded a total bean weight of 4.320kg under managed block while 5.316kg under unmanaged block. Response of bean weight under Treatment 2 (ginger extract) was 3.582kg in managed block and 4.418kg in unmanaged block. Treatment 3 (no extract) recorded slightly higher bean weight in managed (3.590kg) block than in unmanaged (3.209kg) blocks. The level of bean damage under marigold extract (124) and control treatment (122) was quite similar in managed block while ginger had the lowest damage (82) values (fig. 2). To test the efficacy of the extracts, three (3) response variables were measured: (1) damage score, (2) bean weight (g), and (3) entry hole number. Since three treatments were applied in two levels of block management, the experimental design is considered as factorial. Interactions between the two factors, treatment and block, for each response variable are displayed in table 1. For this section, only the treatments with significant interaction effects are discussed. There is a significant interaction between ginger extract and block 1 (managed) for bean weight (g) ($p<0.05$). A significant interaction also existed between ginger extract and block 2 (unmanaged) for bean weight ($p<0.05$) (table 1.). Bean weight under ginger extract in block 1 was relatively low (65.13 ±6.65, σ =49.29) while highest in block 2 (110.45±11.61, σ =73.41). It shows that ginger extract increased the bean weight in unmanaged cocoa block than managed block. There is a significant interaction between ginger extract and block 1 (managed) for damage score ($p<0.05$) (table 1). Damage score under ginger extract in block 1 was relatively low (1.49±0.19, σ =1.37). However, there was no interaction between ginger extract and block 2 (1.90±0.19, σ =1.26). Bean damage was the lowest in block 1 (1.49±0.19, σ =1.37) when compared to marigold extract (2.07±0.16, σ =1.22) and control (2.35±0.16, σ = 1.17). Even though ginger extract produced the lowest bean damage in unmanaged block, the interaction was not significant ($p>0.05$). There is a significant interaction between ginger extract and block 1 (managed) for number of entry holes per pod ($p<0.05$) (table 1). Ginger extract had the lowest number of entry holes (3.85±0.51, σ = 3.81) compared to marigold extract (4.85±0.48, σ =3.68) and control (7.40±0.64, σ =4.62). There is a significant interaction between control treatment (no extract) and block 2 (unmanaged) for number of entry holes ($p<0.05$) (table 1). Control treatment produced the highest number of entry holes under block 2 (8.38±0.73, σ =4.89).

Table 1: Three response variables; bean weight (g), damage score (1, 2, 3, 4), and number of entry holes (per pod) were measured to test the efficacy of ginger and marigold extracts against control treatment (no extract). The frequency, mean±SE (x̅), standard deviation (σ), confidence interval (CI) and interaction p-value (α=0.05) were calculated for each response variable under respective treatments and blocks (block 1=managed, block 2 = unmanaged). Significant means are denoted with a single asterisk *($p<0.05$).

Variables	Cocoa block	Parameters	Treatments		
			Marigold extract	**Ginger extract**	**Control**
Bean weight (g)	Block 1	Frequency	60	55	52
		Mean (x̅)	72 ±5.26	65.13 ±6.65	69.04 ±7.43
		SD (σ)	40.76	49.29	53.59
		CI	10.53	13.33	14.92
		P-value (interaction)	p >0.05	p <0.05*	p >0.05
	Block 2	Frequency	54	40	45
		Mean (x̅)	98.44±8.69	110.45±11.61	71.31±9.80
		SD (σ)	63.91	73.41	65.75
		CI	17.44	23.48	19.75
		P-value (interaction)	$p>0.05$	$p<0.05$*	p >0.05
Damage score (1,2,3,4)	Block 1	Frequency	60	55	52
		Mean (x̅)	2.07±0.16	1.49±0.19	2.35±0.16
		SD (σ)	1.22	1.37	1.17
		CI	0.32	0.37	0.33
		P-value (interaction)	$p>0.05$	$p<0.05$*	p >0.05
	Block 2	Frequency	54	40	45
		Mean (x̅)	2.63±0.16	1.90±0.19	2.64±0.19
		SD (σ)	1.2	1.26	1.32
		CI	0.33	0.4	0.39
		P-value (interaction)	$p>0.05$	$p>0.05$	$p>0.05$
Number of entry holes (per pod)	Block 1	Frequency	60	55	52
		Mean (x̅)	4.85±0.48	3.85±0.51	7.40±0.64
		SD (σ)	3.68	3.81	4.62
		CI	0.95	1.03	1.29
		P-value (interaction)	$p>0.05$	$p<0.05$*	$p>0.05$
	Block 2	Frequency	54	40	45
		Mean (x̅)	6.19±0.46	5.33±0.74	8.38±0.73
		SD (σ)	3.35	4.68	4.89
		CI	0.92	1.49	1.47
		P-value (interaction)	$p>0.05$	$p>0.05$	$p<0.05$*

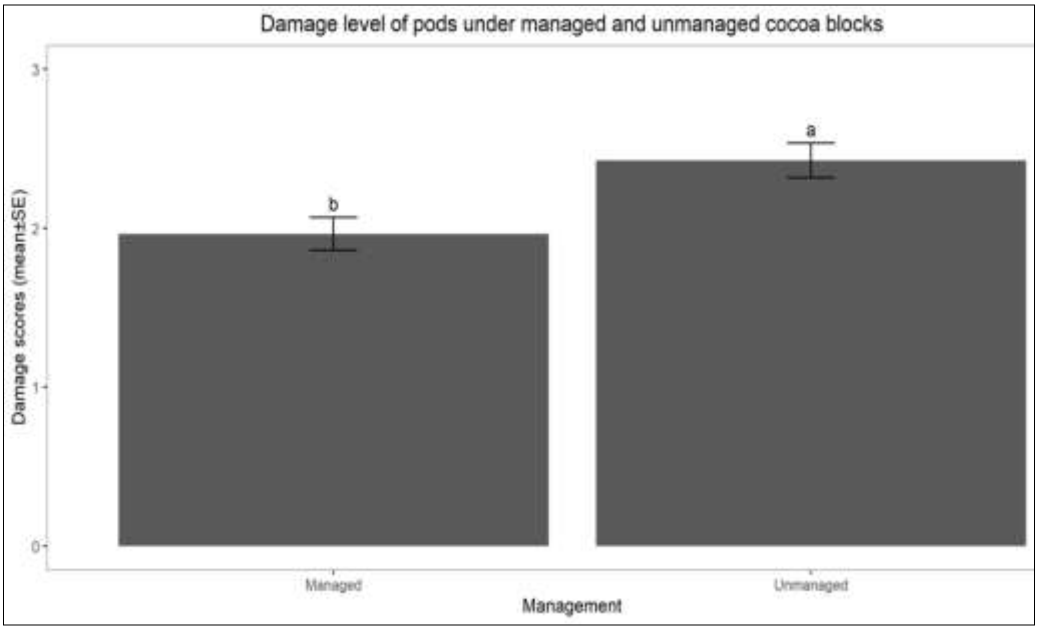

Fig 1: The level of bean damage was significantly higher in unmanaged cocoa block ($p<0.05$). Bean damage was significantly lower in managed block as anticipated ($p<0.05$). Both managed and unmanaged cocoa blocks are grouped according to probability of their mean differences at alpha level α = 0.05. Treatments with the same letter are not significantly different.

The separation of treatment means using Tukey HSD test shows that there is no significant difference between treatments 1 (marigold extract) and treatment 3 (no extract) ($p>0.05$). The test is done based on the probability of their mean differences at alpha level α = 0.05 with treatments having different letters are significantly different. However, the bean damage is significantly low in treatment 2 (ginger extract) ($p<0.05$) (fig. 2). We are confident that ginger extract was able to lower bean damage when compared to the other two treatments. Marigold extract did not provide evidence of repellence to CPB infestation even though it was proven to lower pest populations in vegetables (i.e. cabbages). Although ginger was not so effective in lowering pest infestation in vegetables, it has proven effective in controlling *C. cramerella*

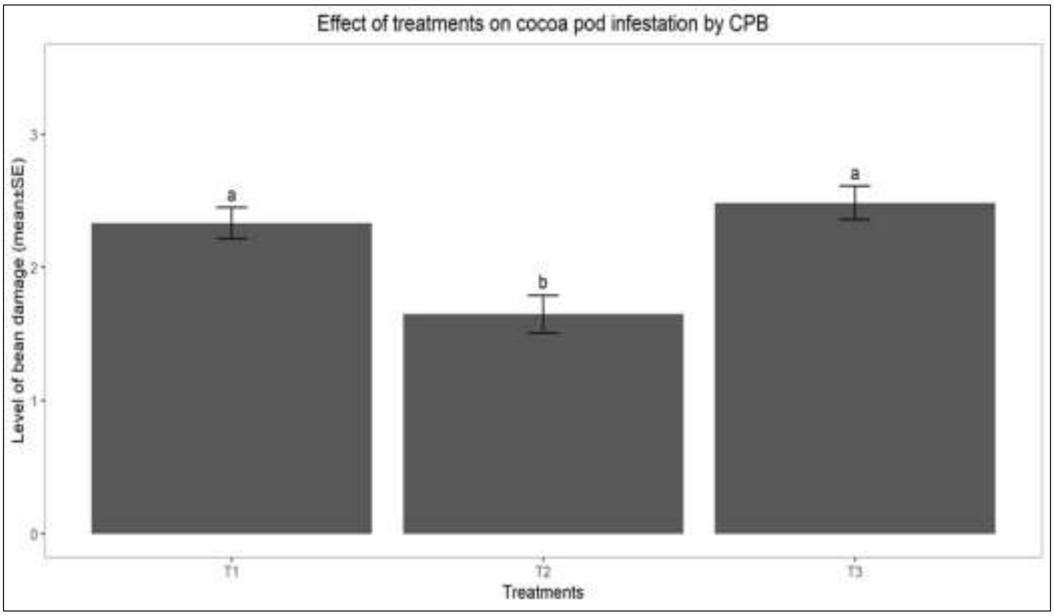

Fig 2: Treatment 2 (ginger extract) had the lowest bean damage ($p<0.05$) when compared to treatment 1 (marigold extract) and treatment 3 (control -no extract). The level of bean damage was not significantly different between treatment 1 and treatment 3 ($p>0.05$). All treatments are grouped according to probability of their mean differences at alpha level α = 0.05. Treatments with the same letter are not significantly different

In order to test the efficacy of the two plant extracts, statistical correlations, regression and p-values were calculated. From the three response variables, we compared two variables at a time against the two factors, (1) treatment and (2) management. We firstly analyzed the correlation between bean damage and bean weight. There was no significant correlation under all treatments in managed cocoa blocks ($p>0.05$) (fig. 3). Although there is a negative trend, the increasing level of bean damage did not significantly lower the bean weight. There is a negative correlation between bean damage and bean weight under both marigold treatment ($p<0.05$, r= -0.59) and control treatment (p≤0.05, r= -0.31) in unmanaged block. However, there is a significant positive correlation between bean damage and bean weight under ginger extract treatment ($p<0.05$, r= 0.42). Ginger extract did not lower the bean weight regardless of increasing bean damage under unmanaged cocoa block.

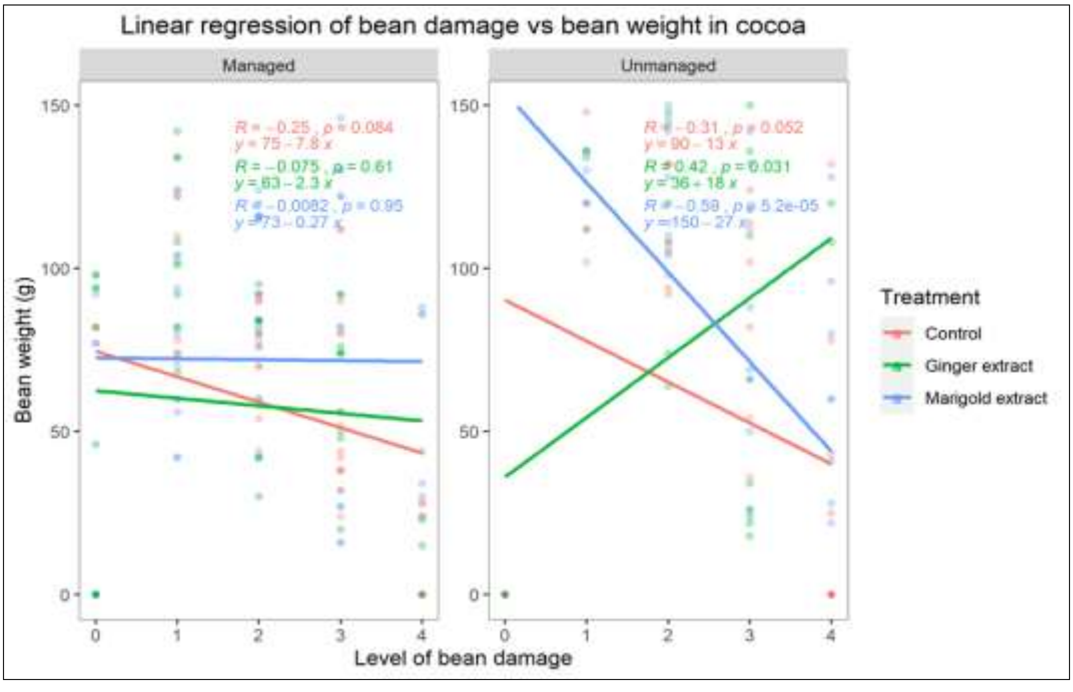

Fig 3: The facet line regressions show the correlation (r) between bean damage and bean weight. Managed cocoa block had non-significant correlations meaning the treatments were not compatible with application of management inputs. Ginger extract showed significant positive correlation ($p<0.05$, r= 0.42) while marigold extract had significant negative correlation ($p<0.05$, r= -0.59) under unmanaged cocoa block.

There was no significant correlation between number of entry holes and bean weight under all treatments in managed cocoa blocks ($p>0.05$) (fig. 4). In managed block, both marigold ($p>0.05$, r= -0.14) and control treatment ($p>0.05$, r= -0.26) showed negative correlation between bean damage and bean weight. Ginger extract had a positive correlation even though it was not statistically significant ($p>0.05$, r= -0.05) under managed block. In unmanaged block, the correlation line of control treatment was relatively flat ($p>0.05$, r= -0.001), assuming an increase in number of entry holes did not decrease the bean weight (fig. 4). Marigold extract showed positive correlation (r= 0.15) under unmanaged block however it was not statistically significant ($p>0.05$). It implies that marigold extract was not able to prevent female *C. cramerella* from ovipositing and that led to decrease in bean weight. It is very clear from this study that marigold does not perform well when it is applied as an extract (i.e. solution). Marigold produce better results when it is grown as a plant in an intercropping system

Ginger extract produced exceptional results under unmanaged cocoa block. There was a strong significant correlation between number of entry holes and bean weight ($p<0.05$, r= 0.55). An increase in female oviposition did not lower the bean weight of cocoa (fig. 4). The application of ginger extracts on pods would have disrupted entry of *C. cramerella* larvae into the beans.

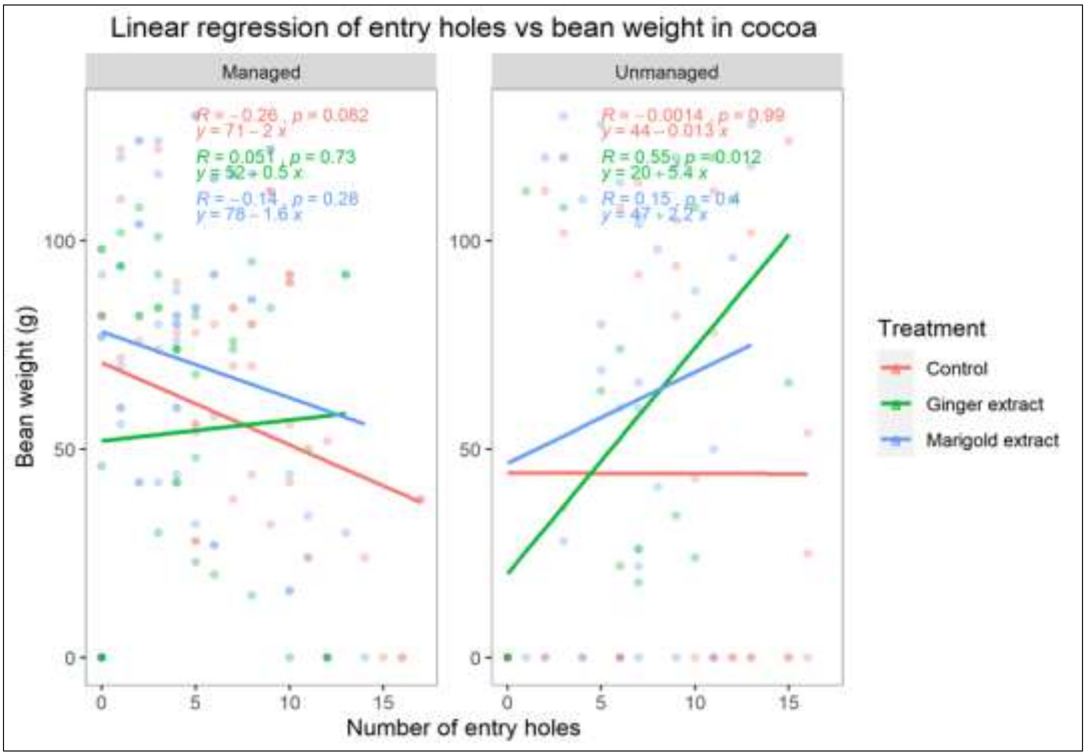

Fig 4: There were no significant correlations between number of entry holes and bean weight under managed block ($p>0.05$). Marigold extract had a positive correlation but it was not significant either ($p>0.05$, r= 0.15). Ginger extract showed a significant positive correlation under unmanaged cocoa block ($p<0.05$, r= 0.55). Control treatment had no significant correlations pertaining to an almost flat regression line ($p>0.05$, r= -0.001)

Discussion

The results from this study can be interpreted based on push-pull strategy in both bitrophic and tritrophic interactions. Ginger extract (*Z. officinale*) showed distinct results under both managed and unmanaged cocoa blocks. Ginger is considered a repellent plant that can be incorporated into a push system to discourage oviposition of female lepidopterans such as *C. cramerella* [23]. Ginger extract was able to produce the highest bean weight under unmanaged block which can be attributed to its active insecticidal properties [24]. Extracts from *Z. officinale* contains arcurcumene, b-myrcene, 1,8-cineole, citral, and zingiberene as major compounds [25, 26]. Our findings support the work of Saripah, Hajjar [14] where ginger extract was able to inhibit the emergence of adult *C. cramerella*. Ginger extract recorded the lowest bean damage (score) under managed block suggesting that *Z. officinale* can significantly reduce *C. cramerella* infestation when it is applied with high IPDM inputs [4]. The number of entry holes made by *C. cramerella* larvae were also significantly low under managed blocks therefore we presume there is compatibility between these two factors. Extract from *Z. officinale* have shown to act as oviposition deterrent, insect repellent and larval antifeedant [27, 28]. However, *Z. officinale* produced the lowest bean weight under managed block when compared to marigold (*T. erecta*) and control treatment. This finding shows that most active ingredients of botanical extracts degrade much faster than synthetic insecticides under extreme field conditions [29]. Since all cocoa trees under managed block were thoroughly pruned, pods sprayed were readily exposed to adverse weather conditions such as rain, temperature and humidity. Marigold extract (*T. erecta*) did not produce any significant results under both managed and unmanaged cocoa blocks (table 1.). Unlike *Z. officinale*, marigold utilizes pull strategy rather than push mechanism [23]. *T. erecta* produce different plant volatiles to attract natural enemies rather than repelling pests [30, 31]. Marigold is often recognized for attracting and maintaining a high biodiversity of natural enemy [32-34]. It is recommended to intercrop marigold plants within cocoa block rather than applying it as extract solution [35-38]. Intercropping marigold flowers with crops (i.e. cocoa) would provide pollen and nectar for natural enemies to survive and concurrently reducing the population

of pests (i.e. *C. cramerella*) [11, 12, 39]. Marigold plants can also attract other herbivorous insects (i.e. pests) that are prey for entomophagous natural enemies [32].
Botanical pesticides such as *Z. officinale* and *Capsicum frutescens* (chili) extracts are generally compatible with arthropod natural enemies (ANE) and environmentally friendly [18]. Due to negative impacts of synthetic pesticides to natural enemies and human health, emphasis is now on non-chemical options such as botanical pesticides and natural enemies [40-42]. According to our findings, bean damage of cocoa was low in managed block while high in unmanaged blocks. Ginger extract was able to repel *C. cramerella* effectively under high IPM input suggesting a synergic relationship between these two factors [18]. Ginger extract also reduced the impact of *C. cramerella* in unmanaged blocks by impeding its ovipositional ability and inhibiting larval development [14, 15, 17]. This study underlines the importance of incorporating plant chemical compounds in an IPM program [43]. As botanical extracts had been proven to be significantly compatible with ANE than synthetic insecticide, future study should focus on the pull strategy [44]. Due to expensive IPM inputs, rural cocoa farmers can now utilize local repellent plants like *Z. officinale* to manage *C. cramerella* with low IPM inputs [7, 8]. Findings from this study suggest that *Z. officinale* utilizes the push strategy under both high and low IPM practices.

Conclusion
The findings from this study rectify that ginger (*Z. officinale*) extract was effective in managing *C. cramerella* infestation in cocoa. Ginger extract was able to lower the impact of *C. cramerella* in a push strategy [23]. The high bean weight under unmanaged cocoa block signifies that *Z. officinale* extract is suitable for local cocoa farmers and small-block holders. Most cocoa farmers rarely apply high IPDM management practices since they are labour intensive and expensive. However, to maximize yield, farmers are encouraged to apply medium IPDM inputs and incorporate botanical pesticides (i.e. *Z. officinale* extract) concurrently. This study paves way for future research in botanical extracts that can benefit local cocoa farmers.

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