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Intercropping of Pak choi (*Brassica rapa chinensis*) with Marigold flower (*Tagetes erecta* L.) and Onion (*Allium cepa* L.) to control foliar pests

Kari Iamba and Veronica Homband

Abstract
The public is now more aware of the negative impacts of synthetic pesticides to the natural enemies, beneficial insects, general human health and development of pest resistance. In this study, two (2) repellent plants, Marigold flower (*Tagetes erecta* L.) and Onion (*Allium cepa* L.) were intercropped with Pak choi (*Brassica rapa chinensis*) in an attempt to lower pest abundance and damage to foliar. Separation of treatment means by Tukey HSD test showed that marigold intercrop ($p<0.05$) was effective in lowering the population of two common cabbage pests, *Plutella xylostella* and *Psylliodes chrysocephala*. Onion intercrop and control treatment (sole Pak choi) did not show any significant differences in the response variables: (1) pest abundance, (2) defoliation (%) and (3) leaf area index (LAI) ($p>0.05$). Both temperature (°C) and relative humidity (%) are critical abiotic factors that determines the activity of natural enemies as well as pests. There was a positive correlation between temperature and LAI in control treatment (r=0.33, $p<0.05$), onion intercrop (r= 0.23, $p<0.05$) and marigold intercrop (r= 0.25, $p<0.05$). A negative correlation existed between relative humidity (%) and pest abundance (r= 0.18). These relationships contributed to variations in the response variables.

Keywords: Tagetes erecta, Allium cepa, Plutella xylostella, Psylliodes chrysocephala, pest abundance, defoliation, leaf area index

Introduction

The public is now more aware of the negative impacts of synthetic pesticides to the natural enemies, beneficial insects, general human health and development of pest resistance. These factors have contributed to the implementation of new ecological-friendly approaches to manage pests in crops [1, 2]. A novel strategy used in traditional cropping systems is the incorporation of companion plants to manage insect pests while supporting a natural enemy population through host plant diversification [3]. There are relatively two types of practices in companion planting; trap cropping (i.e. intercropping non-crop species that attracts pests) and repellent cropping (i.e. intercropping plant species that repels pests) [4, 5]. Repellent and trap cropping systems involves a push-pull system to discourage oviposition of insect pest while boosting activities of parasitoids and predators in crops [6]. *Desmodium uncinatum* was described as a repellent plant that contributed to fewer insect pests when it was intercropped with maize [7]. Different plant volatiles are involved in push-pull strategies which act as repellent or attractant volatiles to orchestrate pests and natural enemy populations [8]. Pests can be 'pushed' out of the crop by repellent plants, and natural enemies can be 'pulled' or attracted by 'semiochemicals' to suppress the pests [9].

In this study, two (2) repellent plants, Marigold flower (*Tagetes erecta* L.) and Onion (*Allium cepa* L.) were intercropped with Pak choi (*Brassica rapa chinensis*) to lower pest abundance and damage to foliar. In a previous study, intercropping of onion with cabbage was done to manage diamondback moth *Plutella xylostella* (L) and other pests of cabbage [10]. Due to its nutritional value and succulency, cabbage plant has attracted many insect pests to feed on it including *P. xylostella* [11-13]. Among all the other foliar pests of cabbage, *P. xylostella* is the most destructive one [14]. Apart from *P. xylostella*, flea beetle also causes defoliation to cabbage plants in tropical regions [15]. The cabbage flea beetle (*Psylliodes chrysocephala* L.) is attracted to the glucosinolate presence in cabbage which acts as a feeding stimulant [16]. Studies on intercropping cabbage with non-host crops such as onion and tomato had contributed to lower numbers of *P. xylostella* [17]. Onion (*Allium cepa*) and garlic (*Allium sativum*) are aromatic herbs that have been utilized in intercropping systems due to their ability to repel insects [18, 19]. Lower number of *P. xylostella* larvae and flea beetles were observed on cabbages that were intercropped with marigold flower hence was considered the most effective pest control on cabbage [20]. Intercropping of tomato plants with marigold protects the tomatoes' fruits from the glasshouse whitefly (*Trialeurodes vaporariorum* W.) [21]. Marigold reduced both eggs and larvae of *Helicoverpa armigera* (H) and fruit infestation in intercropped tomato [22]. The abundance of flea beetle and foliar damage was significantly lower in intercrop marigold-cabbage plots than sole cabbage plots [23]. This study aims to test the repellent effect of marigold and onion on abundance of *P. xylostella* and *P. chrysocephala.*

Materials and Methods

The study was done at PNG University of Natural Resources and Environment (PNG UNRE) campus in East New Britain, Papua New Guinea (PNG). PNG UNRE is situated at an elevation of 51 meters above sea level and approximately 4º21'01.90" S and 152º 54 00'33.44" E [24]. The soil is more calcareous in nature and relatively sandy loam with high alkalinity [25]. The climate is classified as tropical with a great deal of rainfall experienced even in the driest month. Three treatments (T); T_1=onion+pak choi, T_2=marigold+pak choi, and T_3=sole pak choi were replicated four times within a Randomized Complete Block Design (RCBD). Onions were planted at a spacing of 10cm x 30cm while marigold flowers at spacing of 45cm x 45xcm respectively within rows of pak choi. Both repellent plants had three (3) rows each within each plot. The pak choi seedlings were transplanted to the field after three weeks in nursery house. Cabbage seedlings were planted at spacing of 30cm x 40cm within rows and between plants. There was no synthetic insecticide applied as the aim was to test the potency of repellent plants alone. The data on pest abundance, defoliation (%) and leaf area index (LAI) were collected two times per week for a total of four weeks. Three plants were sampled per plot and all three variables measured for individual plants. A total of 289 samples were collected throughout the experiment time span. Local meteorological data on temperature, rainfall and relative humidity was obtain from university science lab. Mean values for each week was calculated and used as abiotic factors for constructing correlation with response variables.

We used the R function *aov* (R Core Team, 2013) for Analysis of Variance (ANOVA) to analyze effect of repellent plants on pest's abundance, defoliation (%) and leaf area index (LAI). For correlation between response variables (i.e. abundance, defoliation, LAI) and factors (i.e. treatments), we used function *cor. test* (package ggplot2) to produce pearson correlation values, and Tukey HSD test (package agricolae, function *HSD. test*) for construction of graphs with letters denoting level of significance. In addition, we used function *stat_cor* and *stat_regline_equation* (package ggpmisc) to draw linear regression equations between the response variables and factors, and calculating of p-values (i.e. treatments, sampling time, abiotic variables).

Results

A total of 289 samples from the three treatments were collected for 4 weeks. The data of response variables (i.e. pest abundance, defoliation, leaf area index) showed some significant results (fig. 1). There was significant difference between abundance of pests (i.e. *P. xylostella* and *P. chrysocephala*) and treatments (i.e. onion and marigold intercrops). Separation of treatment means by Tukey HSD test showed that the statistical difference came from marigold intercrop ($p<0.05$). Marigold intercrop plots had low number of pests when compared to onion intercrop and control plots (sole Pak choi). Onion intercrop and control plots did not show any significant differences ($p>0.05$).

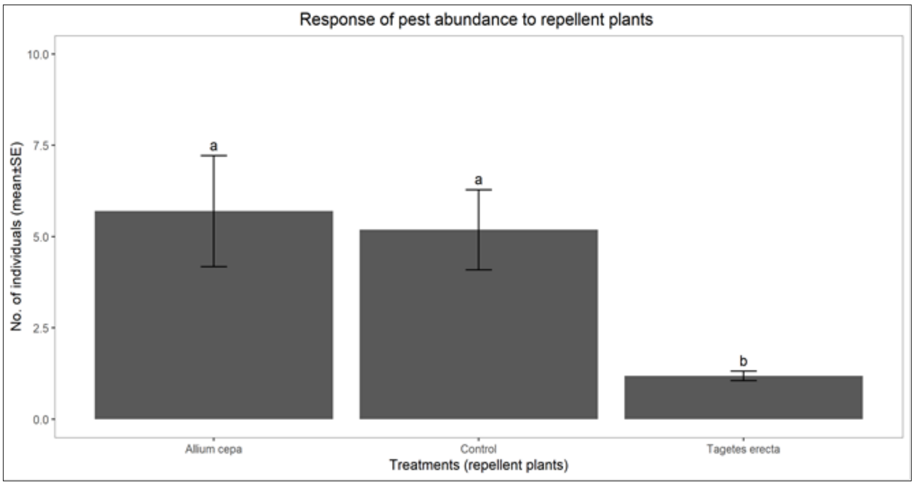

Fig 1: The mean number of pests were low under marigold (T. erecta) intercrop treatment ($p<0.05$). Both onion (A. cepa) intercrop and control plots had no significant differences on pest abundance ($p>0.05$). All treatments are grouped according to probability of their mean differences at alpha level (0.05). Treatments with the same letter are not significantly different

A similar trend was observed from the response variable, leaf defoliation (%) (fig. 2). Figure 2 showed significant differences when defoliation was observed under different treatments. The significant difference was produced by both onion and marigold intercrop ($p<0.05$). Higher defoliation of Pak choi leaves were recorded under onion intercrop plots while lower defoliation was observed in marigold intercrops. Control treatment did not display any significant results and had medium defoliation ($p>0.05$). Marigold intercrop plots gave significant results for both pest abundance and defoliation by having the lowest values ($p<0.05$).

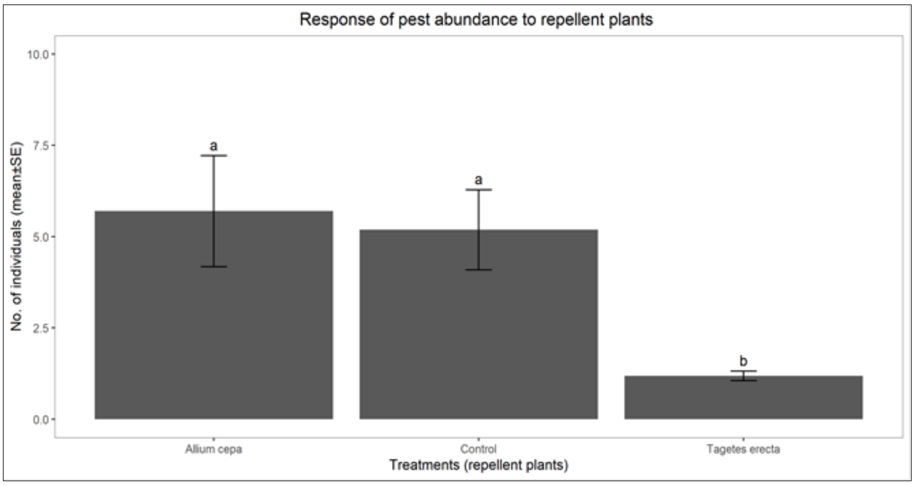

Fig 2: The mean number of defoliation (%) of Pak choi leaves was low under marigold (T. erecta) intercrop treatment ($p<0.05$). Onion (A. cepa) intercrop recorded the highest defoliation. The control plots had no significant difference on leaf defoliation ($p>0.05$). All treatments are grouped according to probability of their mean differences at alpha level (0.05). Treatments with the same letter are not significantly different

The leaf area measured as leaf area index (LAI) did not gave any significant differences among the treatments ($p>0.05$) (table 1). Although marigold intercrop plots had high leaf areas than onion and control, the results were not statistically significant. We presume that extrinsic factors could have influenced the ability of marigold intercrops to produce significant leaf areas.

Table 1: Three response variables; pest abundance, defoliation (%), and leaf area index (LAI) were measured to test the efficacy of onion and marigold intercrops against control treatment (sole Pak choi). The total number of pests, mean±SE ($\bar{x}$), standard deviation (σ) and p-value (α=0.05) were calculated for each response variable under respective treatments

Variables	**Parameters**	**Treatments (repellent plants)**		
		Allium cepa	**Control**	**Tagetes erecta**
Pest abundance	Total abundance	547	498	114
	Mean abundance ($\bar{x}$)	5.94 ±1.63	5.23 ±1.12	1.17 ±0.13
	SD abundance (σ)	15.44	10.84	1.28
	P-value abundance	$p>0.05$	$p>0.05$	$p<0.05$
Defoliation (%)	Total defoliation	204.36	171.26	132.79
	Mean defoliation ($\bar{x}$)	2.29±0.32	1.82±0.21	1.41±0.17
	SD defoliation (σ)	3.05	2.06	1.69
	P-value defoliation	$p<0.05$	$p>0.05$	$p<0.05$
Leaf area index (LAI)	Total LAI	697.09	674.62	745.34
	Mean LAI ($\bar{x}$)	6.84±0.27	6.90±0.23	7.63±0.27
	SD LAI (σ)	2.52	2.22	2.63
	P-value LAI	$p>0.05$	$p>0.05$	$p>0.05$

Temperature (°C), relative humidity (%) and rainfall (mm) were plotted against LAI to detect any significant differences. A linear regression of temperature versus LAI showed that temperature significantly influenced LAI of Pak choi in all treatments: control (r=0.33, $p<0.05$), onion intercrop (r=0.23, $p<0.05$) and marigold intercrop (r=0.25, $p<0.05$) (fig. 3). There is a positive correlation between temperature and LAI in all treatments. As temperature increases, the LAI of Pak choi also increases in control (r=0.33), onion intercrop (r= 0.23) and marigold intercrop (r= 0.25). Marigold intercrop produced the highest Pak choi LAI when compared to control and onion intercrop. Pak choi intercropped with marigold produced relatively bigger leaves than control and onion intercrop.

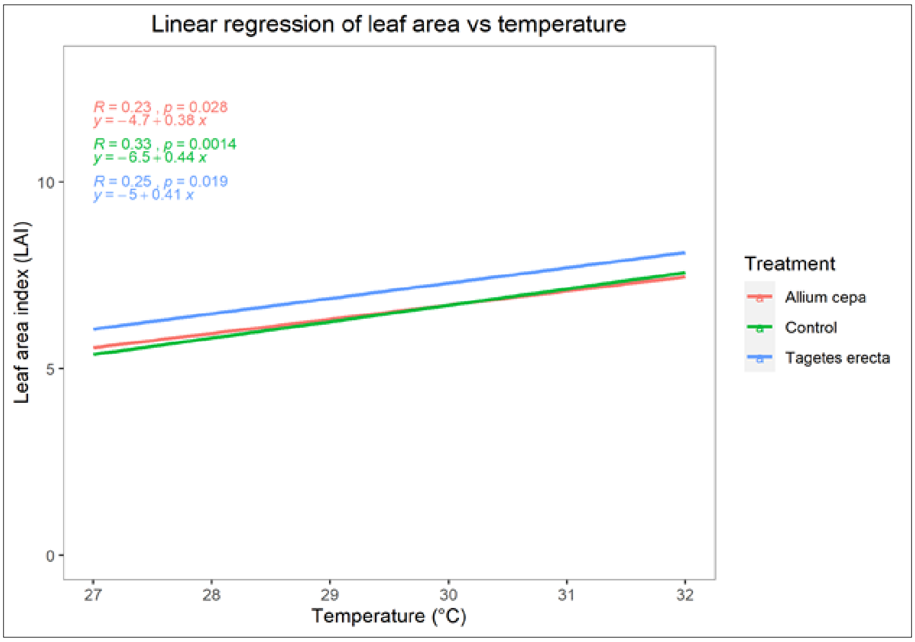

Fig 3: A linear regression of temperature versus LAI showed that temperature significantly influenced LAI of Pak choi in all treatments. There is a positive correlation between temperature and LAI in all treatments. As the temperature rises, the LAI of Pak choi also increases in control (r=0.33), A. cepa intercrop (r=0.23) and T. erecta intercrop (r=0.25)

Relative humidity (%) did not have any significant influences on LAI of Pak choi in marigold (r= -0.09, *p*>0.05) and onion (r= -0.12, *p*>0.05) intercrops. Rainfall (mm) followed the same trend, having no significant influences on LAI of Pak choi in marigold (r= 0.04, *p*>0.05) and onion (r = -0.11, *p*>0.05) intercrops. However, both relative humidity (r= -0.23, *p*<0.05) and rainfall (r= -0.31, *p*>0.05) significantly influenced the LAI of Pak choi in control treatment.

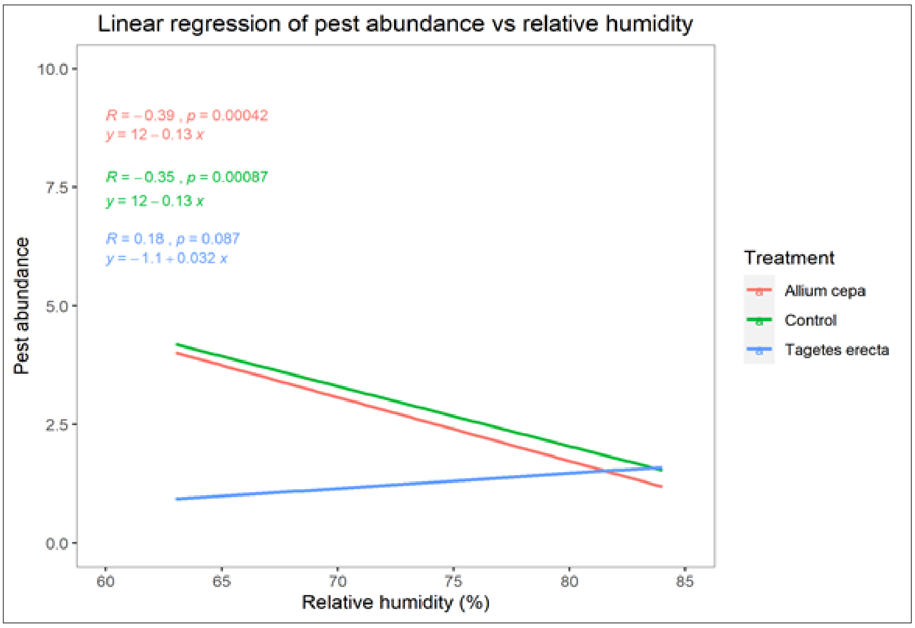

Fig 4: The pest abundance showed a negative relationship with relative humidity (%) in control and onion (A. cepa) intercrop. An increase in relative humidity resulted in decrease pest abundance and vice versa. However, marigold intercrop (T.erecta) showed a positive correlation between pest abundance and relative humidity. An increase in relative humidity is causing an increase in pest abundance

There is a positive correlation between pest abundance and relative humidity in marigold intercrop (r= 0.18, *p*>0.05) (fig. 4). An increase in relative humidity resulted in an increase in the number of pests. An opposite trend was seen in the control treatment and onion intercrop. Relative humidity was a key factor in determining the pest abundance in control treatment and inion intercrop. Increase in relative humidity caused a decrease in pest abundance under onion intercrop (r= -0.39, *p*<0.05) and control treatment (r= -0.35, *p*<0.05).

Discussion

Intercropping of repellent plants with vegetables such as cabbages (i.e. Pak choi) is a cost-effective way of controlling insect pests in gardens. The results from this study rectify that marigold (*T. erecta)* is an effective plant that produces different plant volatiles which act as attractant to natural enemies and repellent to pests [8, 9]. Natural enemies as well as pests responds to volatile chemicals such as benzaldehyde, linalool, myroxide, piperitone, limonene, ocimene, lagetone and valeric acid which are produced by *T. erecta* [26, 27].

According to Ray, Walia [28], *T. erecta* showed significant repellency against the stored grain pest, *Tribolium castaneum*. The active constituents; terpinolene, ocimene, piperitone and limonene in *T. erecta* oil showed insecticidal activity against the white termite [29]. Marigold has been described as a plant vital in maintaining a high natural enemy biodiversity [30-32]. Intercropping of marigold plants with onion crops have proven to reduce aphids and whiteflies as well as nematodes [33-35]. In addition, marigold plants attracts other phytophagous insects that are prey for entomophagous natural enemies [30]. Some farmers plant marigold flowers to provide pollen and nectar which intend increase the fecundity and survival of natural enemies [36]. Lower abundance of diamondback moth and flea beetles were recorded on cabbages that were intercropped with marigold flower [20]. Intercropping of tomato plants with marigold protects the tomatoes' fruits from the glasshouse whitefly (*Trialeurodes vaporariorum* W.) [21]. Marigold reduced the fecundity of the pest, *Helicoverpa armigera* and fruit infestation in intercropped tomato [22]. The number of flea beetle and foliar damage in cabbage was significantly lower in marigold intercrop plots than in sole cabbage plots [23].

Onion intercrop plots did not produce significant results when compared to marigold. Onion produces phytochemical compounds such as allicin that have shown to repel insect pests in some studies [18, 37]. Onion intercrops produced cabbages with lower leaf area across all treatments [10, 18, 38]. Our results support findings of Baidoo, Mochiah [10] that onion intercrops had lower abundance of *P. xylostella*, and were not significantly different from sole cabbage. Intercropping of onion with cabbage such as Pak choi would not be recommendable since it is not reliable in controlling *P. xylostella* [39]. A recommendation to boost the efficacy of onion against cabbage pests would be to intercrop with non-host crops with use of insecticides [17].

Insects are generally considered as ectotherms therefore their physiological activities are regulated by external factors including temperature [40-43]. As the temperature increases, it significantly increased the leaf area of Pak choi since most insect pests are physiologically inactive at high temperatures (fig. 3) [42, 44, 45]. Temperature is a critical and determining abiotic factor that has tremendous effect on insect physiology and biology [46]. A research by Soh, Kekeunou [47] found that the cabbage aphid, *Brevicoryne brassicae* failed to survive at 35°C.

However, an inversed relationship was recorded in marigold intercrop where the pest abundance increased with relative humidity. The pest infestation under control and onion intercrop decreased with increasing relative humidity. High relative humidity can lower nectar concentration via hygroscopy in a flowering plant thus making it unattractive to entomophagous parasitoids and predators [48, 49]. Nectar concentration and viscosity determines the suitability of a flower as nectar source for nectarivorous natural enemies [50]. Some flowering plants use indirect mechanisms to protect themselves from herbivores by releasing kairomone volatile compounds that attracts the entomophagous natural enemies [51]. Both abiotic (i.e. humidity, temperature, light) and biotic factors (i.e. cultivar, growth stage, fertilization rate) affects the emission of these herbivore-induced kairomones [52, 53]. Our results confirm the findings of Embaby and Lotfy [54] that population of cabbage pests decreased as a result of increasing relative humidity. The optimum relative humidity for release of induced odor ranges between 45% and 65% while volatiles released decreased at lower or higher air humidity [52]. Stomata opening increases at low relative humidity (dry air) but closes at higher humidity (limit) [52, 55]. There was no significant correlation between rainfall and abundance of *P. xylostella* under onion and marigold intercrops [45].

Conclusion
The core finding from this study highlights that marigold (*T. erecta)* is an effective plant with active volatiles that attracts natural enemies to lower pest population [8, 9]. It is an important plant that maintains a high natural enemy biodiversity [30-32]. Intercropping of marigold plants with crops provides an eco-friendly strategy to reduce pest population [33-35]. The efficacy of onion intercrop against cabbage pests can be boosted with incorporating non-host crops and use of insecticides preferably botanical extracts [17]. Both temperature and relative humidity are critical abiotic factors that determines the physiological activity and performance of natural enemies as well as pests.

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