Article

Biological role of marigold (*Tagetes erecta* L.) in habitat manipulation and sustenance of natural enemy populations in upland rice

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Abstract
The re-designing of agricultural systems in integrated pest management (IPM) has been aimed at reducing the use of synthetic pesticides to manage pests. In conservation biological control, the cropping habitat is manipulated to provide vital resources that are required by natural enemies to suppress pest populations. Habitat manipulation is an example of conservation biological control where non-host plants are incorporated in a crop field to provide floral resources, refuges and alternate hosts for natural enemies. We tested the Mexican marigold (*Tagetes erecta* Linnaeus) in upland rice plots to attract natural enemies. The paradigm was to manipulate the rice habitat with marigold plants to provide vital resources that are needed by natural enemies to suppress pest populations. From the four marigold treatments applied, plots that had only *T. erecta* (T2) as barrier plants attracted the highest population of natural enemy complex (i.e. *Apanteles* sp., *Telenomus* sp., *Oxypes javanus* and *Coelophora inaequalis*). Conversely, the population of pest complex (i.e. *Spodoptera litura*, *Sirpophaga incertulas* and *Leptocorisa acuta*) was significantly low under T2. Plots that received a treatment combination of *T. erecta* and its extract (T4) had the second highest population natural enemies. Both control (T1) and *T. erecta* extract (T3) treatments had low natural enemy populations. This study has proven that *T. erecta* is a vital plant that maintains and sustains a high natural enemy biodiversity. More natural enemies are attracted to *T. erecta* plants resulted in a decline in pest population. By combining *T. erecta* and its extract, a duel mechanism for pests mortality is being utilized via utilization of plant material for botanical insecticide and floral resources for natural enemies.

Keywords conservation biological control; pest population; habitat manipulation; *Tagetes erecta* L.; natural enemy complex; pest complex.

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1 Introduction

The intensification of agricultural systems hasten by use of indiscriminate crop protection practices are affecting the balance between natural enemies and pests. The re-designing of agricultural systems in integrated pest management (IPM) has been aimed at reducing the use of synthetic pesticides to manage pests

(Amoabeng et al., 2019). Biological control forms the central component of IPM in which natural enemies including parasitoids, predators and pathogens are strongly embraced (Gurr et al., 2000a; Bale et al., 2008; Gurr et al., 2018). An emerging tactic known as conservation biological control focuses on building the natural enemy populations that are already present within an agroecosystem and utilizing them to control target pests with the aim of reducing use of synthetic pesticides (Ehler, 1998; Begg, 2017). In conservation biological control, the cropping habitat is manipulated to provide vital resources that are required by natural enemies to suppress pest populations (Gurr et al., 2000b; Fiedler et al., 2008; Gurr et al., 2017). Habitat manipulation involves incorporating plants that provide floral resources, refuges and alternate hosts for natural enemies (Altieri, 1994; IRRI, 1998; Griffiths et al. 2008; Zhang, 2011; Zhang et al., 2014; Gurr et al., 2017). Focus of habitat manipulation has been on non-host plants that have been proven to provide and sustain a rich biodiversity of natural enemies (Baggen et al., 1999; Balzan et al., 2014).

The rice plant (*Oryza sativa* L.) is succumb to attacks of more than 100 species of insects in which 20 of them can cause serious economic loss (Rahaman et al., 2016). One of the major pest of rice is the yellow stem borer, *Scirpophaga incertulas* W. (Rahaman et al., 2016). The rice stem borers are the principal devastators and are responsible for economic crop losses under field condition (Mahar and Hakro, 1979). *S. incertulas* seems to be one of the common serious pests causing serious damage to the rice crops in Asian countries including Papua New Guinea (PNG). Heavy infestation may cause yield loss up to 80% (Rubia-Sanchez et al., 1997). Rice stem borers causes more damage by boring the stem of rice plants hence destroying the vascular bundles to cause dead heart at vegetative and maturity stage, resulting in white heads (Rahaman et al., 2016). Another common rice pest in PNG is the *Leptocorisa acuta* T., known as rice bug. The rice bug feeds on the endosperm in seeds through its prominent sucking mouthpart, causing either small grains, unfilled grains or pecky rice (Litsinger et al., 1993). Another frequent pest of rice in PNG is the polyphagous tobacco cutworm, *Spodoptera litura* F., which affects rice sporadically. The cut worm mainly causes problem in upland rice because it needs dry soil for pupation (Zaidi et al., 2009). Problems in lowland rice fields occurred occasionally when the larvae migrate to new planted fields to feed on young rice plants (Rao et al., 1993; Dale, 1994).

For this study, we incorporated the African marigold or Mexican marigold (*Tagetes erecta* Linnaeus) as a non-host plant in upland rice plots to attract natural enemies. The paradigm was to manipulate the rice habitat with marigold plants to provide vital resources that are needed by natural enemies to suppress pest populations. Marigold is known to maintain and sustain high natural enemy biodiversity (Silveira et al., 2003, 2004, 2009). Marigold extracts possess antibacterial, antimicrobial, larvicidal, mosquitocidal, nematicidal and insecticidal properties (Gopi et al., 2012). A combination of *T. erecta* and its extract is presumed to exert duel impact via utilization of plant extracts and floral resources for natural enemies to enhance pest mortality (Amoabeng et al., 2019).

2 Materials and Methods

2.1 Study site

The study was done at the academic crops section at PNG University of Natural Resources and Environment (PNG UNRE) campus in Papua New Guinea (PNG). The experimental site is located at 4°21′01.90″S and 152°00′33.44″ E with an altitude of 51m above sea level (Iamba and Waiviro, 2021; Iamba and Yaubi, 2021). The academic crops section is an experimental site where most agricultural researches on tropical crops are done. PNG UNRE is a natural resource university that supports the sustainable management of natural resource in Agriculture, Fisheries, Forestry and Animal Sciences. The soil type is predominantly sandy loam which is well-drained, fertile, calcareous and relatively alkaline in nature (Malagat and Iamba, 2021). Sandy loam soil has good binding properties that are adequate for raising seedlings (Howcroft, 2002). Soil sterilization is not necessary for sandy loam soils except if infection occurs. The tropical climate here supplies a great deal of rainfall that is experienced all year round even in the driest month. The atmosphere can be described as highly humid with an average rainfall of 2780 mm per year and an intervening mild dry season. The daily average humidity can range from 77-79% with mean temperature of 27-29°C (Iamba et al., 2021). These environmental conditions are most favorable for plant cultivation (Howcroft, 2002).

2.2 Experimental design

A total of 121 m^2 land area was ploughed prior to plotting and left exposed to sunlight for 1 week to rid out any infectious plant pathogens. Plots were constructed following a Complete Randomize Block Design (CRBD). There were four treatments used: T1=control, T2=Eco-manipulation, T3=Marigold extract and T4= Marigold+Eco. No treatments were applied to control (T1) as it was considered as a reference group and contains only rice plants. Eco-manipulation treatment (T2) consists of sole marigold plants that were planted around the plot borders as barrier plants. Marigold extract (T3) treatment composed of plots that were only sprayed with marigold extracts. The fourth treatment (T4) was a combination of marigold plants and its extract. In the same plots, marigold grown as barrier plants and its extract were applied concurrently. Each of these 4 treatments was replicated three times. Each plot had an estimated plant density of 144 seedlings/plot that were planted at a plant spacing of 30 cm between rows and 20 cm between plants. A total of 18 marigold seedlings (*T. erecta*) were planted as barrier plants surrounding T2 and T4 plots. Out of the 12 plots, 6 plots were planted with marigold plants.

2.3 Plant content extraction

The leaves and flowers of *T. erecta* were collected from an established garden. Both materials were then chopped into smaller pieces and mixed together. Plant materials were dried under hot sun for 3 weeks in order to remove excess moisture content. Then they were grounded separately using hand power and then passing it through a spice grinder. About 2.5±0.5 kg of the grounded material was obtained using an electronic balance and placed in a desiccator. About 500 ml of 75% ethanol (C_2H_6O) was added to the desiccator with the lid tightly fastened. The desiccator protected the hygroscopic contents from reacting with water in the atmosphere and prevented ethanol from evaporating. The mixture was left on a clear bench for 7 days (1 week) to allow the ethanol to digest the cell walls of the materials so that chemical constituents are released into the solution. After 1 week, the resultant mixture was filtered through a filter paper and the filtrate collected in a 500ml beaker. Filtered solutions were allowed to evaporate at room temperature (25^{o}C) to remove volatilized ethanolic residues (Iamba and Malapa, 2020). Then a few drops of natural oil and soap were added to the mixture to enhance its delivery and adhesiveness on the leaf surface (Fening et al., 2013). The extract was stored in a separate 1L container with polyseal ('P') lids to prevent evaporation. The extract was mixed at the rate of 5ml extract to 1 L water in a hand sprayer and applied to T3 and T4 plots.

2.4 Arthropods sampling

The population of pests and their natural enemies were estimated by visual counts. The aim was not to disturb the insect complex on the rice plants. Both insect complex were randomly counted on three hills per plot. To assist with visual counts, clear photographs were taken to assist with species identification. Visual counts were done between 07.00 and 08.00 h while the insects were inactive. Predators and parasitoids of rice pests were identified using a checklist from Wongsiri et al. (1981). The population of the immature stem borer stages were estimated by randomly checking for ‘white heads’ and hills showing premature drying. The tillers/spikelets displaying ‘white heads’ were manual dissected to expose the stem borer larvae. Clear photographs of the larva were taken to aid the identification process.

Population of natural enemies and pests were counted for both adults and larvae using absolute estimates (number per hill) from hill. Within the natural enemy complex, Hymenopterans were classified as parastoids. Two predominant and most frequent parasitoids encountered during this study were *Apanteles* sp. S. (Braconidae) and *Telenomus* sp. N. (Platygastridae). *Apanteles* species are larval parasitoids (Srivastava et al., 2019) while *Telenomus* species are egg parasitoids (Laumann et al., 2009). For predators, both Arachnida and Coleoptera were considered as important natural enemies. Two common and frequent predators sampled were *Oxyopes javanus* T. (Oxyopidae) and *Coelophora inaequalis* F. (Coccinellidae). For the rice pest complex, three herbivorous insects were identified as economic pest of rice. The pest complex includes the tobacco cutworm (*Spodoptera litura* F.), yellow stem borer (*Sirpophaga incertulas* W.) and the rice bug (*Leptocorisa acuta* F.). Count sampling of each insect complex were done at all growth stages of rice: Seedling, Tillering, Booting and Milking stages. Data were collected 2 weeks after transplanting (AT) at seedling stage, 4 weeks AT (tillering stage), 9 weeks AT (booting stage) and 12 weeks AT (milking stage).

2.5 Data analysis

The data on response variables showed asymmetric pattern meaning they were not normally distributed according to Shapiro-Wilk test ($p<0.05$). To correct the skewness in data distribution, we used the Generalized Linear Model (glm). Two glm models were tested for their validity and relevance to the count data: Linear and Poisson distributions. Using the analysis of deviance, Poisson model had a low residual deviance and Akaike's information criterion (AIC) (G^2=220.2, *AIC* =472.8). Linear model had a higher residual deviance and AIC (G^2=744.9, *AIC* =487.1). 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 Poisson, $2 \sum \gamma \ln(\gamma/\mu)-(\gamma - \mu)$, 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). The Akaike information criterion (AIC) used to estimate the quality of the two glm models was derived from this formula, $AIC = 2*\ln(likelihood) + 2*k$ 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). Therefore the Poisson model was selected as the best fit for analysing the count data. Using the Poisson distribution, the response variable was log transformed: $\log(y)=\alpha + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_p x_p$, where y is the response variable, α and β are numeric coefficients, α being the intercept (sometimes by β_0), and x is the predictor/explanatory variable (Hinde, 1982). The response variables were log transformed and analysed using the Poisson exponential family function (link = log) in RStudio (version 4.0.3). All graphs relating to population abundance of pests and natural enemies were constructed with ggplot2 package. Tukey Honest Significant Difference test (Tukey HSD) was used to separate the means of populations at each growth stages. The Student-Newman-Keuls test (SNK) was used for mean separations of the treatments since it was more sensitive to mean difference than HSD test. SNK test was also used in post hoc analysis and to determine the interactions of pests and natural enemy populations under different treatments and growth stages. The interaction plots were executed iteratively with cowplot and ggplot2 packages in RStudio.

3 Results

A total count of 149 natural enemies (NE) and 166 pests of upland rice (var. TCS10) were quantified during this study. The mean abundance (mean ± SE) of these two insect complex varied to certain degree in each treatment. Post hoc testing of abundances along with respective confidential intervals (CI) were used to detect level of statistical significances between treatments. Marigold (*T. erecta*) is an important non-host plant that is utilized locally to control vegetable pests (Iamba and Homband, 2020).

3.1 Pest and NE populations under different marigold treatments

Different marigold treatments utilized as extract application, border plants (eco-manipulation) and their combination were used to study their push-pull effect on pest and NE populations. The pest population under control treatment was significantly higher (5.50±1.06, CI=2.34, p<0.05) than NE abundance (1.67±0.38, CI=0.83, p>0.05) (Table 1). For the Marigold extract treatment, there was no significant differences between NE (1.25±0.35, CI=0.77, p>0.05) and pest (2.83±0.37, CI=0.81, p>0.05) populations. The eco-manipulation treatment (i.e. border marigold plants) attracted significant populations of NE (5.17±1.51, CI=3.32, p<0.05) which resulted in a significant drop in pest abundance (3.00±0.55, CI=1.21, p<0.05). The fourth treatment which was a combination of marigold extract and eco-manipulation (marigold+eco), also had a significant increase in NE population (4.33±1.11, CI=2.44, p<0.05) and a decrease in pest numbers (2.50±0.56, CI=1.23, p<0.05). According to these analysis, both Eco-manipulation and combination (marigold+eco) treatments attracted more NE (Table 1). Conversely, more NE attracted to both treatments led to a decline in pest population.

In a cross analysis, the population of each insect complex varied between treatments. The population of NE was statistically similar under control and marigold extract treatments. There was an increase in NE abundance under eco-manipulation treatment (5.17±1.51, CI=3.22, p<0.05) when compared to marigold+eco treatment (4.33±1.11, CI=2.44, p<0.05). The pest population under control treatment was significantly higher (5.50±1.06, CI=2.34, p<0.05) than the other three treatments. The abundance of pests were non-significant for marigold extract, eco-manipulation and marigold+eco treatments (p>0.05) (Table 1). The population of rice pests was relatively low under all treatments except control. For NE, high populations were found in eco-manipulation and marigold+eco plots (p<0.05). Therefore we presumed that plot manipulation with marigold plants attracted more Arthropod Natural Enemies (ANE) and significantly increased biological control of rice pests. Marigold plants provided biological stability by lowering the pest population and provided better protection to rice plants.

Table 1 The population abundance of both pests and natural enemies (NE) were quantified for each of the four treatments. The abundances are further analysed by the standard error (SE), standard deviation (SD) and confidential interval (CI).

Treatments	**Insects**	**Summary statistics**			
		‡N	**Abundance †mean±SE**	**SD**	**CI**
Control	NE	12	1.67 ± 0.38c	1.30	0.83
	Pest	12	5.50 ± 1.06a	3.68	2.34
Marigold extract	NE	12	1.25 ± 0.35c	1.22	0.77
	Pest	12	2.83 ± 0.37bc	1.27	0.81
Eco-manipulation	NE	12	5.17 ± 1.51a	5.22	3.32
	Pest	12	3.00 ± 0.55bc	1.91	1.21
Marigold+Eco	NE	12	4.33 ± 1.11ab	3.85	2.44
	Pest	12	2.50 ± 0.56bc	1.93	1.23

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

3.2 Pest and NE populations at different growth stages of upland rice

Statistical comparison was done between pest and NE populations within each treatment at different growth stages. The aim was to test whether the increased NE populations attracted to marigold treatments were able to lower pest abundance. There was no significant increase in the population of both complex under control treatment and seedling stage. Similar non-significant trend was also noted for tillering stage (Table 2). While booting stage of control treatment showed a significant increase in pest population (9.67±1.76, $p<0.05$), NE population was notably low (1.33±0.33, $p>0.05$). The milking stage of control treatment recorded a significant increase in NE populations (3.33±0.33, $p<0.05$) while pest abundance dropped (6.67±1.20, $p<0.05$). Student-newman-keuls test (SNK) of NE detected a significant difference between booting and milking ($p<0.05$*) , and, milking and seedling ($p<0.05$*) stages under control treatment (Table 2). Other pairwise comparisons between stages were deemed non-significant for NE. The population of NE increased from booting and peaked at milking stage while concurrently reducing the pest population. SNK test of pests detected a significant difference between booting and seedling ($p<0.05$*), and, booting and tillering ($p<0.05$*) stages (Table 2). The population of pests decreased from booting to milking stage as a function of an increase in NE abundance under control treatment.

Table 2 The population abundance of both pests and natural enemies (NE) were quantified for each of the four treatments at different growth stages. A complex pairwise comparison was done to test the significance level of each treatment means.

Growth stages of TCS10	**TREATMENTS**							
	†Control		**†Marigold extract**		**†Eco-manipulation**		**†Marigold+Eco**	
	NE	**Pests**	**NE**	**Pests**	**NE**	**Pests**	**NE**	**Pests**
Seedling	0.67±0.67 b	1.67±0.33 b	0.33±0.33 a	3.00±0.00 a	0.00±0.00 b	2.67±0.67 a	0.33±0.33 c	1.00±0.58 b
Tillering	1.33±0.67 b	4.00±1.53 b	0.67±0.67 a	1.00±0.58 b	1.67±0.88 b	1.33±0.88 a	2.33±0.67 bc	0.67±0.67 b
Booting	1.33±0.33 b	9.67±1.76 a	2.33±0.33 a	3.67±0.33 a	10.00±1.15 a	4.33±1.20 a	8.33±1.86 a	4.33±0.33 a
Milking	3.33±0.33 a	6.67±1.20 ab	1.67±0.88 a	3.67±0.33 a	9.00±3.06 a	3.67±1.20 a	6.33±1.76 ab	4.00±0.58 a
Post hoc comparisons using Student-newman-keuls test								
#Booting – Milking	*p = 0.02**	*p = 0.15*	*p = 0.46*	*p = 1.00*	*p =0.69*	*p=0.65*	*p=0.32*	*p=0.68*
#Booting – Seedling	*p = 0.66*	*p = 0.01**	*p = 0.16*	*p = 0.45*	*p=0.01**	*p= 0.51*	*p=0.01**	*p=0.00***
#Booting – Tillering	*p = 1.00*	*p = 0.03**	*p = 0.18*	*p=0.00***	*p=0.02**	*p=0.23*	*p=0.03**	*p=0.00***
#Milking – Seedling	*p = 0.02**	*p = 0.07*	*p = 0.31*	*p=0.24*	*p=0.01**	*p=0.51*	*p=0.03**	*p=0.00***
#Milking – Tillering	*p = 0.06*	*p = 0.19*	*p = 0.27*	*p=0.00***	*p=0.02**	*p=0.29*	*p=0.07*	*p=0.00***
#Seedling – Tillering	*p = 0.39*	*p = 0.25*	*p = 0.71*	*p = 0.00***	*p=0.51*	*p=0.38*	*p=0.32*	*p=0.68*

†Mean±SE with same letters are not significantly different at α=0.05 (Tukey HSD test).
#Pairwise comparison of populations at different growth stages.
Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 (values > α=0.05 are significant).

There was no significant increase in the population of both insect complex under marigold extract treatment and seedling stage. However, tillering stage recorded a significant decline in pests population. The abundance of NE was relatively low at all four growth stages ($p>0.05$) (Table 2). The pest population was low at seedling, booting and milking stages except in tillering stage where it declined extremely (1.00±0.58, $p<0.05$). SNK test of NE detected no significant difference at all four stages under marigold extract treatment ($p>0.05$) (Table 2). We presumed that application of marigold extract as conventional spraying was not effective in attracting sufficient populations of NE. While for pest populations, high significant differences were recorded between booting and tillering ($p<0.01$**), milking and tillering ($p<0.01$**), and, seedling and tillering ($p<0.01$**). There was a huge difference between tillering and all other four stages (Table 2). The pest population in marigold extract treatment was generally low suggesting that phytochemicals from extract might have repelled both the pests and NE.

In eco-manipulation treatment, there was significant difference between NE (0.00±0.00, $p<0.05$) and pest (2.67±0.67, $p<0.05$) populations at seedling stage. At tillering stage, NE populations increased while pest numbers decreased. At booting stage, the abundance of NE increased sharply (10.00±1.15, $p>0.05$) in response to an increase in pest numbers (4.33±1.20, $p>0.05$) (Table 2). A similar trend was also noted at milking stage with NE population maintained at high numbers. The volatiles from marigold plants (i.e. florescence) attracted and maintained high population of NE. High population of NE provided a stable biocontrol of the rice pests. SNK test for pests did not detect any significant differences at all growth stages under eco-manipulation treatment. While for NE populations, significant differences were recorded between booting and seedling ($p<0.05$*), booting and tillering ($p<0.05$*), milking and seedling ($p<0.05$*), and, milking and tillering ($p<0.05$*) (Table 2). Booting and milking stage under eco-manipulation plots attracted and maintained a high population of NE. The population of pests was non-significant under eco-manipulation treatment implying that they were effectively maintained at low numbers by NE.

In marigol+eco treatment, the population of NE (0.33±0.33, $p<0.05$) was significantly lower than that of pests (1.00±0.58, $p<0.05$) at seedling stage. The abundance of NE increased significantly at tillering stage (2.33±0.67, $p<0.05$) while pest population decreased (0.67±0.67, $p<0.05$). The population of both complex did not differ significantly at booting stage although NE was maintained at high numbers. There was a drop in the population of both complex at milking stage. SNK test for NE detected significant differences between booting and seedling ($p<0.05$*), booting and tillering ($p<0.05$*), and milking and seedling ($p<0.05$*). High population of NE was recorded at Booting and milking stages under marigol+eco treatment with corresponding drop in pests abundance. SNK test for pest detected high significant differences between booting and seedling ($p<0.01$**), booting and tillering ($p<0.01$**), milking and seedling ($p<0.01$**), and, milking and tillering ($p<0.01$**). The population of pests at seedling and tillering stages were greatly reduced by increasing NE abundance under marigol+eco treatment.

3.3 Comparison of eco-manipulated plots to other marigold treatments

The overall means of pests (μ=3.46) and NE abundance (μ=3.10) were quite similar. Control treatment recorded a significant increase in pest population ($p<0.05$, $\mu>3.46$) (Fig. 1). Pest abundance in the other three treatments were non-significant ($p>0.05$, $\mu<3.46$). Eco-manipulation treatment attracted the highest NE populations ($p<0.05$, $\mu>3.10$) followed by marigold+eco treatment ($p<0.05$, $\mu>3.10$) while low abundances were detected in control and marigold extract treatment ($p>0.05$, $\mu<3.10$) (Fig. 1). According to Fig. 1, rice plots that were surrounded by marigold plants were more effective in attracting high population of NE. In eco-manipulation treatment, there is a significant difference between NE (5.17±1.51, $p<0.05$) and pests population (3.00±0.55, $p<0.05$).

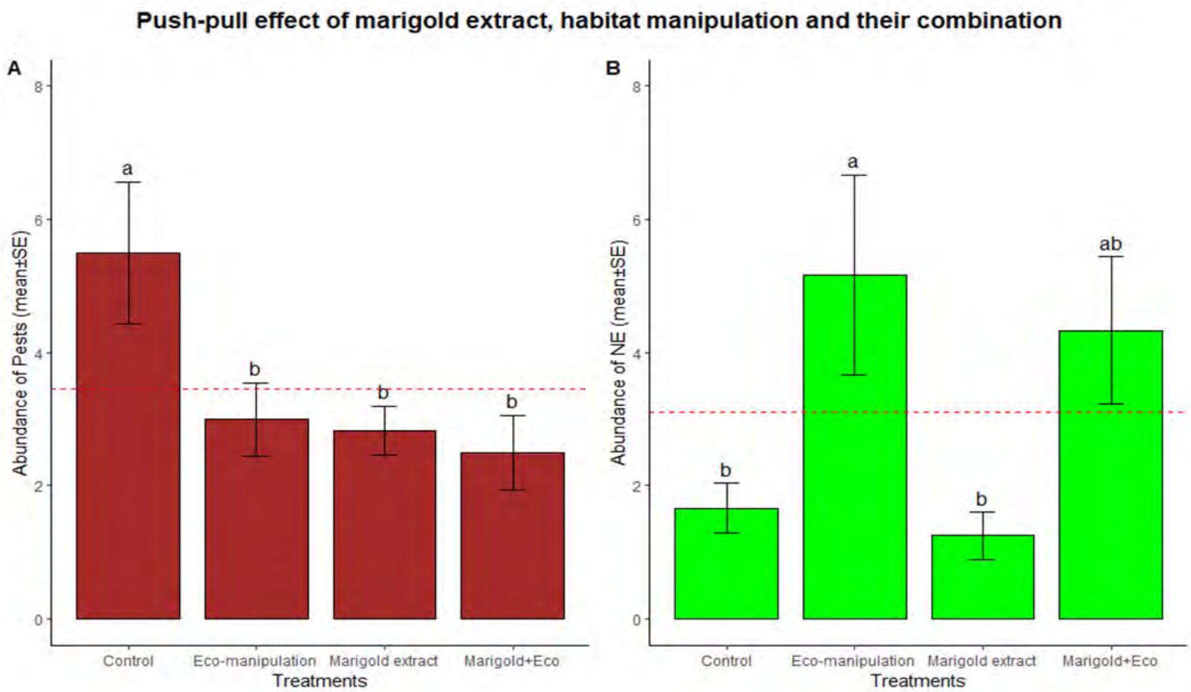

Fig. 1 The population of pests were significantly high under control ($p<0.05$, $\mu>3.46$) treatment while non-significant in other three treatments ($p>0.05$, $\mu<3.46$) **(A).** Corresponding populations of NE was the highest in eco-manipulation treatment followed by marigold+eco treatment ($p>0.05$, $\mu>3.10$) **(B).** NE abundance in eco-manipulation treatment was significantly the highest (5.17 ± 1.51, $p<0.05$).

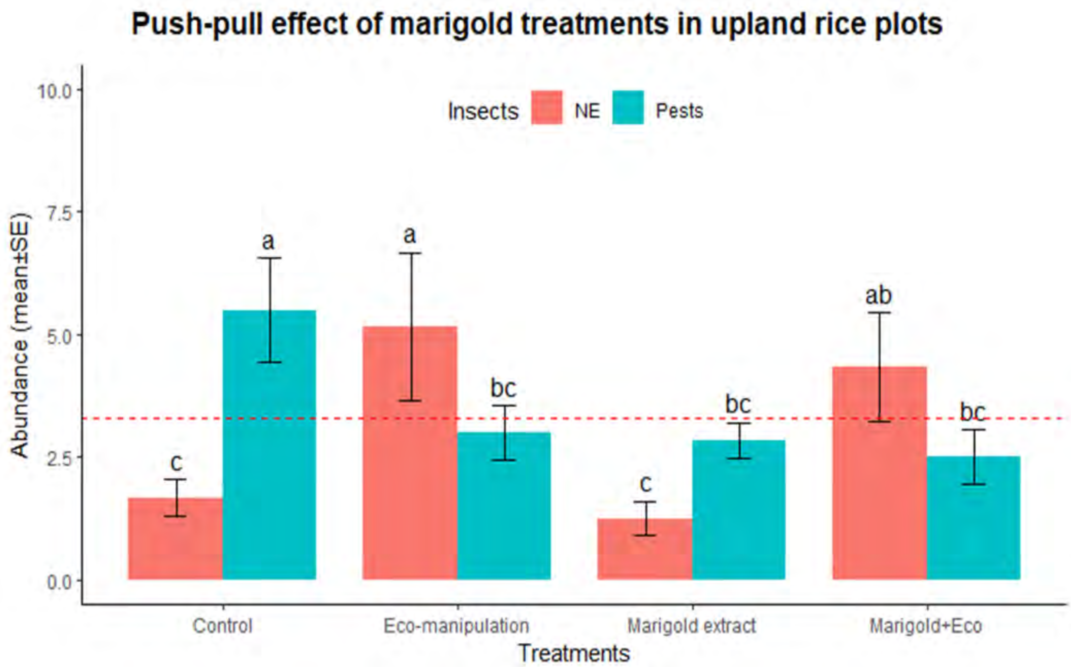

Fig. 2 Population of NE was significantly low ($p<0.05$) in control treatment with corresponding high pests abundance ($p<0.05$). While NE was significantly higher ($p<0.05$) than pests ($p<0.05$) under eco-manipulation treatment. The red dotted line is the overall mean ($\mu=3.28$).

The comparison of the insect complex in each treatment can be best viewed in their interactions. There is a significant interaction between NE and pest populations under control treatment ($p<0.05$) (Fig. 2). The NEs are not responsive to their host (i.e. pests) under control treatment. The same scenario was noticed under marigold extract treatment where pests population was high while NE abundance was significantly reduced ($p<0.05$). There is a significant interaction between pests and NE numbers under marigold+eco treatment ($p<0.05$). The abundance of NE is significantly higher than pest population. The highest NE abundance was detected under eco-manipulation treatment ($p<0.05$) which is higher than that of marigold+eco treatment (Fig. 2). However, there was no significant difference between pests abundance of the two treatments ($p>0.05$).

3.4 Temporal populations at different growth stages

The upland rice (var. TCS10) reached seedling stage at approximately 2 weeks after transplanting (AT). At this stage, the pests abundance ($p>0.05$, 2.08±0.31) was significantly higher than that of NE ($p>0.05$, 0.33±0.19) (Fig. 3). The rice plants reached the next stage, tillering, at 4 weeks after transplanting. Tillering stage recorded similar populations of pests and NE. The booting stage (9 weeks AT) detected a drop in both the abundances of pests and NE (Fig. 3). At milking stage (12 weeks AT), the populations of both insect complex increased. These results presents the temporal overview of insect complex at each growth stage while ignoring the effect of treatments (Fig. 3).

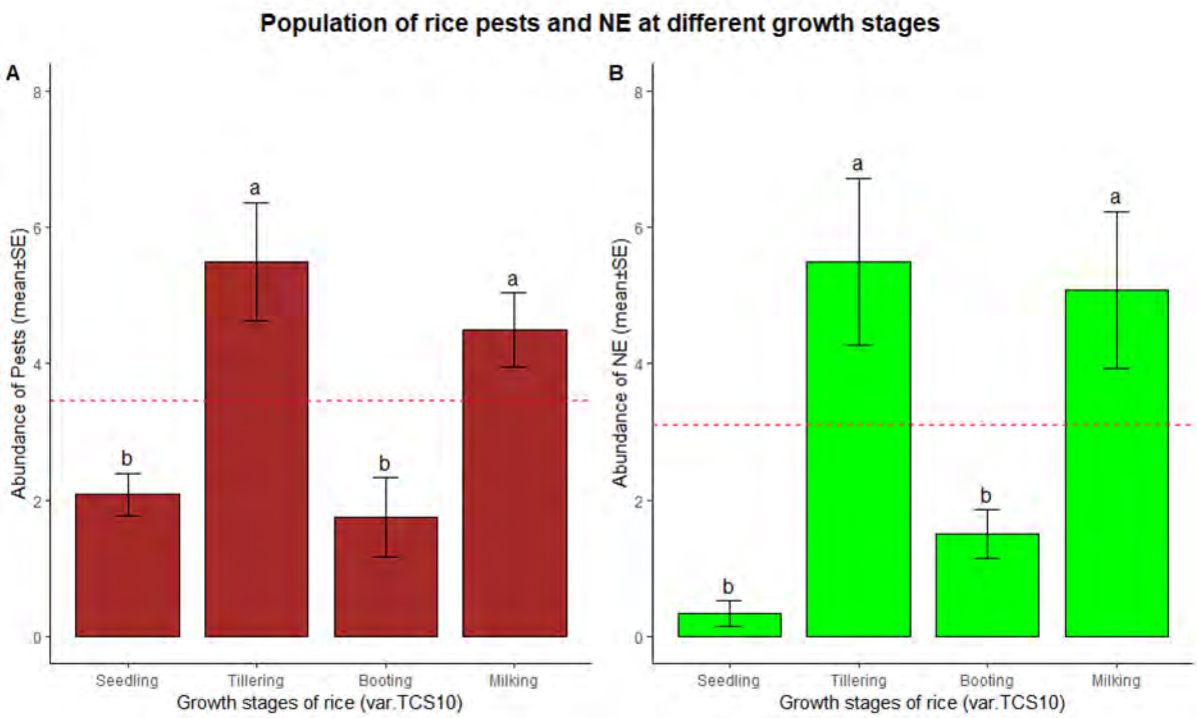

Fig. 3 Pest populations were similar between seedling and booting ($p>0.05$, $\mu<3.46$), and, tillering and milking stages ($p>0.05$, $\mu>3.46$) **(A).** The NE populations were similar between seedling and booting ($p>0.05$, $\mu<3.10$), and, tillering and milking stages ($p>0.05$, $\mu>3.10$) **(B).**

Comparison of interactions between pests and NE populations at each growth stage was also done. There was a significant interaction between pests and NE populations at seedling stage ($p<0.05$). The NE abundance was significantly lower than that of pests. Tillering stage did not have any significant difference between the two insect complex ($p>0.05$) (Fig. 4). Similar scenario was evident at booting stage although populations of both complex decreased (p>0.05). Even though there was a population increase at milking stage, no significant interaction between the two insect complex was detected (p>0.05). These results shows the temporal overview of insect complex at each growth stage while ignoring the effect of treatments (Fig. 4). The overview of growth stages showed that the difference between pests and NE populations occurred only at seedling stage.

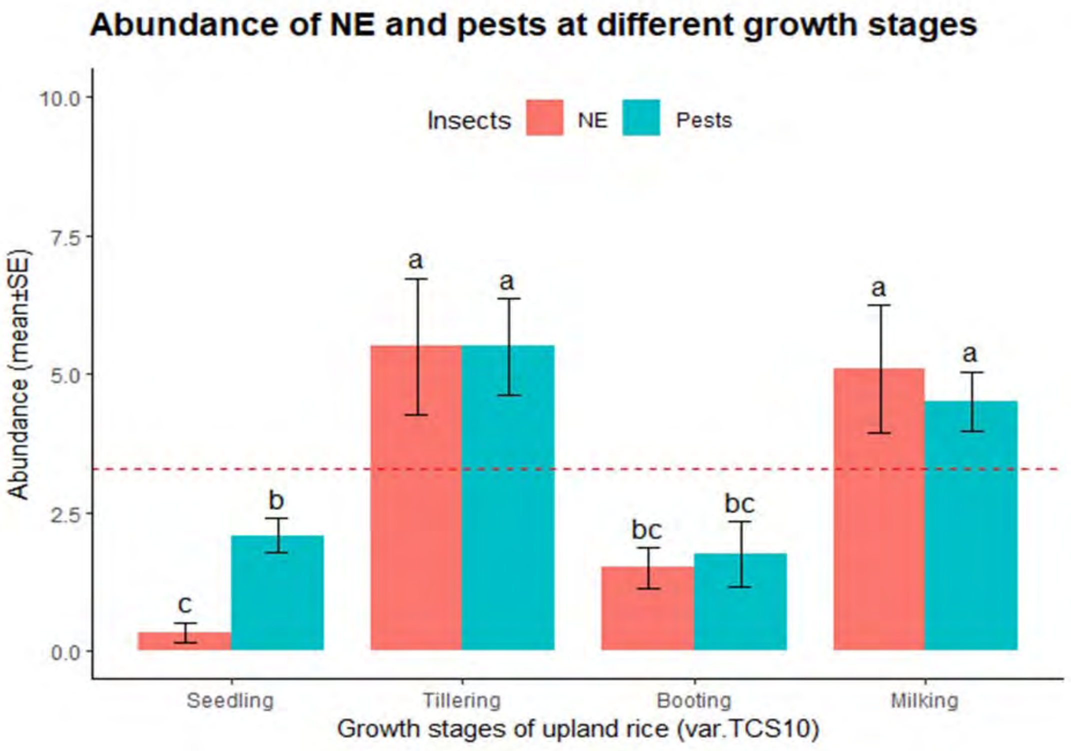

Fig. 4 The population of NE was significantly low (p<0.05) while pest was high (p<0.05) at seedling stage. The abundance of the two insect complex did not differ significantly at proceeding stages (p>0.05). The red dotted line represents the overall mean (μ =3.28).

3.5 Relationship between pests and NE population under different marigold treatments

All treatments showed a positive-relationship between pests and NE populations. As the population of pests increased, NE populations also increased. The correlation between pest and NE populations was highly significant under eco-manipulation treatment (r=0.73, p<0.01**) and marigold+eco treatment (r=0.75, p<0.01**) (Fig. 5). We assumed that marigold plants played a vital role in attracting both the natural enemies and pests. Marigold plants also attracts other phytophagous pests that are prey for predacious natural enemies (Silveira et al., 2009). Control (r=0.46, p>0.05) and marigold extract (r=0.21, p>0.05) treatments had no significant relationship between pests and NE populations. Due to the absence of marigold in control treatment as a pull effect, the relationship was not significant. Conversely, marigold extract treatment was also deem non-significant due to its push effect (Singh et al., 2003; Pérez Gutierrez et al., 2006).

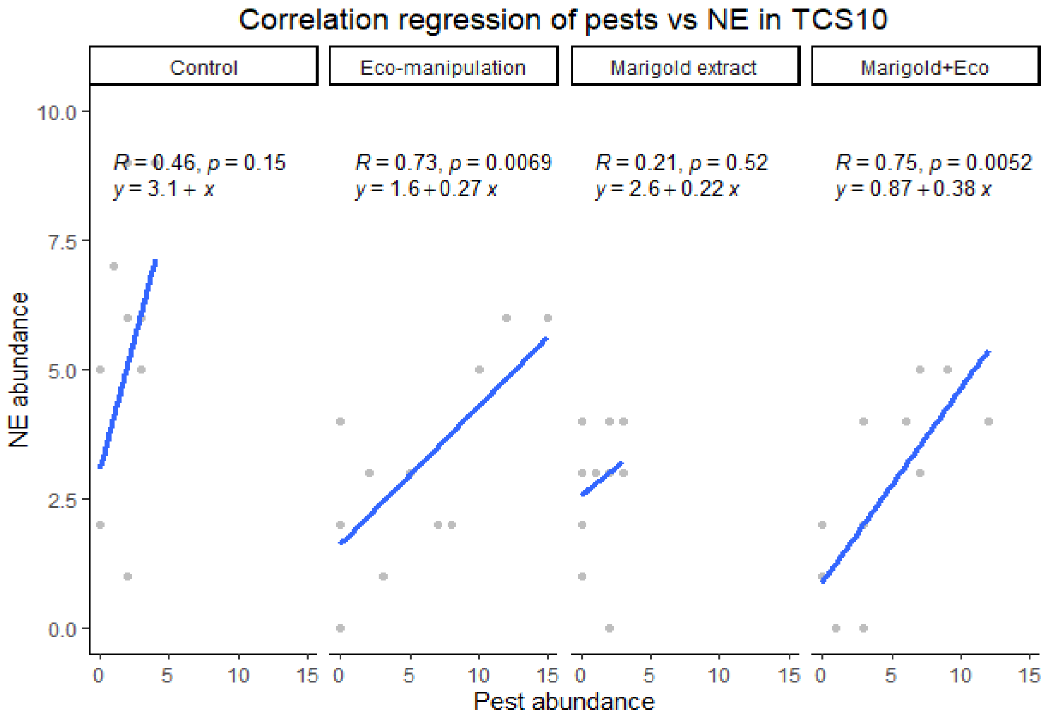

Fig. 5 The correlation between pest and NE populations was highly significant under eco-manipulation treatment (r=0.73, p<0.01**) and marigold+eco treatment (r=0.75, p<0.01**). Control and marigold extract treatment were not significant. All treatments showed a positive-relationship between pests and NE populations.

4 Discussion

The marigold plant (*Tagetes erecta* L.) is usually intercropped with vegetables to control insect pests in traditional subsistence farming. Rice plots that were surrounded by *T. erecta* attracted the highest abundance of natural enemies (NE). To help protect crops from herbivorous insects, habitat manipulation is done to provide additional resources and semiochemicals to attract natural enemies (i.e. predators and parasitoids) (Soloneski and Larramendy, 2012). Marigold plant readily produces highly volatilized compounds that attracts natural enemies and repels pests (Khan et al., 2016; Bhattacharyya, 2017). Natural enemies as well as pests are responsive to volatile chemicals that are produced by *T. erecta* such as benzaldehyde, linalool, myroxide, piperitone, limonene, ocimene, lagetone and valeric acid (Burguiere et al., 2001; Meshkatalsadat et al., 2010). Marigold is a vital plant that maintains and sustains a high natural enemy biodiversity (Silveira et al., 2003, 2004, 2009). Conversely, more natural enemies attracted to *T. erecta* plants resulted in a decline in pest population. The practice of intercropping marigold with onions not only reduces aphids and whiteflies but nematodes as well (Martowo and Rohana, 1987; Abid and Maqbool, 1990; Zavaleta and Gómez, 1995). Marigold plants also attract other herbivorous insects that are prey for predacious natural enemies (Silveira et al., 2009). Marigold flowers provide both pollen and nectar that enhances the fecundity, longevity and survival of natural enemies (Baggen et al., 1999).

From our results, *T. erecta* provided biological sustenance of NE populations that kept the pest abundance below injury level and provided better protection to rice plants. Lower population of cabbage pests, diamondback moth and flea beetles, were detected on cabbages that were grown with marigold flower (Jankowska et al., 2009; Iamba and Homband, 2020). Incorporation of marigold with tomatoes provided better protection to tomato fruits against the glasshouse whitefly (*Trialeurodes vaporariorum* W.) (Conboy et al., 2019). The fecundity of the pest, *Helicoverpa armigera* H., and fruit infestation in tomato-marigold intercrop was effective (Srinivasan et al., 1994). The population of flea beetle and corresponding foliar damage was significantly reduced in marigold intercrop plots than in sole cabbage plots (Latheef et al., 1984). Plots that received a combination of *T. erecta* and its extract had lower pests and NE abundance than sole *T. erecta*. Essential oil of *T. erecta* contains β-caryophyllene, limonene, methyl eugenol, (E)-ocimene, piperetone, piperitenone and ?-terpinolene that has been used as treatment of fungal infections and to deter house flies (Pérez Gutierrez et al., 2006). Terpinolene, ocimene, piperitone and limonene phytochemicals in *T. erecta* oil had strong insecticidal activity against the white termite (Singh et al., 2003). The repellency of stored grain pest, *Tribolium castaneum*, was significant when *T. erecta* essential oil was applied (Ray et al., 2000). By combining *T. erecta* and its extract, a duel mechanism for pests mortality is being utilized via utilization of plant material for botanical insecticide and floral resources for natural enemies (Amoabeng et al., 2019).

The booting growth stage (63 days AT) had the highest pests abundance followed by milking stage (84 days AT) under control treatment. These findings support a study by Didonet et al. (2001) in upland rice where the population of two stink bug species, *Oebalus poecilus* and *Tibraca limbativentris*, peaked at 83 and 76 days after plant emergence (DPE) while *Spodoptera frugiperda* and *Mocis latipes* peaked at 69 and 41 DPE, respectively. The control treatment represents a natural system with no manipulation or augmentation applied to the rice's habitat. We presumed that application of marigold extract as conventional spraying repelled both the natural enemies and pests (Ray et al., 2000; Singh et al., 2003; Pérez Gutierrez et al., 2006). Marigold extracts possess antibacterial, antimicrobial, antioxidant, hepatoprotective, larvicidal, mosquitocidal, nematicidal, wound healing and insecticidal activities (Gopi et al., 2012). The tillering stage recorded a low number of both larval and adult pests. The rice leaffolder, *Cnaphalocrocis medinalis* (Guenée) oviposit most eggs in the tillering crop (Fukamachi, 1980, 1983). Since moth and egg density are poor indicators of larval density (de Kraker et al., 1999), we had to establish correlations of adults and larvae collectively under different marigold treatments. There was stability at tillering, booting and milking stages between pests and natural enemy populations (Fig. 4). This stability would not be significant if plant defensive traits resulted in rapid death of the herbivorous pests hence natural enemies would have no role to play (Price et al., 1980). The presence of abundant flowers in *T. erecta* provided complex kairomone cues and nectar as nutritive food resource for the natural enemies. The French marigold (*Tagetes patula* L.), a close species of *T. erecta*, showed high secretion rates and availability of nectar in standing crops (Comba et al., 1999). The availability of nectar directly dictates the presence and abundance predators and parasitoids (Gilbert, 1975; Gilbert and Smiley, 1978; Smiley, 1978). The top-down effect of marigold significantly influences the population of natural enemies in this study. The secretion of extrafloral nectaries in black cherry is maintained at the maximum in order to attract ants to prey upon its major herbivore, *Malacosoma americanum* (Tilman, 1978).

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