



Perspective

Unleashing nature's defenders: Farmer-managed natural enemies field reservoirs (NEFRs) enhance management of the invasive papaya mealybug (*Paracoccus marginatus*) in coastal Kenya

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HIGHLIGHTS

- Establishment of farmer-managed Natural Enemies Farm Reservoirs (NEFRs) led to an increase in the absolute and average count of *A. papayae* per leaf.
- The rise in *A. papayae* populations per leaf elevated parasitism rates, leading to an overall reduction in the infestation levels of PMB per leaf.
- The presense of NEFRs resulted in an increase in the type (species) and number (abundance) of predatory arthropods in the respective treatments.

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ABSTRACT

The papaya mealybug (PMB), *Paracoccus marginatus*, infests a wide range of plant species, including economically important crops, like papaya, citrus, cassava, and avocado, leading to significant economic losses. The parasitoid, *Acerophagus papayae* has been shown to manage the pest and was introduced in three coastal counties of Kenya from 2021. Natural Enemies Field Reservoirs (NEFRs), a technology that serves as in-situ production of *A. papayae*, were established in farmers' fields in the three counties to assess their effectiveness in controlling the papaya mealybug in Kenya. Three treatments were set up including a. 'No prior *A. papayae* release + NEFR', b. 'Prior *A. papayae* release + NEFR', and c. 'Prior *A. papayae* release but no NEFR' (control). PMB populations decreased by 49.12 % and 62.8 % in treatments a and b, respectively, but increased by 37.6 % in the control. On the other hand, the absolute count of *A. papayae* increased by 456 %, 190 % and 51.6 % in treatments a, b and c, the control, respectively. Consequently, the parasitism rates increased by 116.7 % and 17.8 % in treatments a, and b, respectively but declined by 10.3 % in the control. The most abundant predators out of ten recovered genera were *Cryptolaemus montrouzieri* (44.0 %), *Tenuisvalvae notata* (23.9 %) and *Exochomus* sp. (19.9 %). The highest abundance and diversity of predators was recorded in treatment b 'Prior *A. papayae* release + NEFR' and the least abundance and diversity in the control. This study sheds light in the critical role of NEFRs in the management of PMB and its underscored potential as an effective, low-cost, farmer managed technology is discussed.

1. Introduction

The papaya mealybug (PMB), *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), is a versatile polyphagous pest able to attack over 250 plant species belonging to 189 genera and about 58 plant families (García Morales et al., 2016). Some of its preferred hosts are economically significant crops such as *Carica papaya* L. (papaya), *Citrus* spp. L. (citrus), *Manihot esculenta* (cassava),

and *Persea americana* P. Mill. (avocado) (Miller & Miller, 2002; Okeke et al., 2019). Infestation by *P. marginatus*, along with its feeding activities, induce physiological changes in the host plant. It extracts cell sap from plant tissues and injects a toxic substance, resulting in a depletion of vital resources, thereby subjecting the plant to stress. This stress has a cascading impact on the plant's overall health, ultimately influencing its fitness and growth (Mani et al., 2012; Sharma & Muniappan, 2022). The pest also secretes honeydew on plant surfaces, promoting the growth of

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sooty mold and impairing the plant's photosynthetic efficiency (Sharma & Muniappan, 2022; Williams & Granara, 1992). Crop losses of up to 91 % has been reported on papaya in Kenya (Macharia et al., 2017), 65 % yield loss on papaya in Ghana (Goergen et al., 2011) and yield losses on different crops in the range of 10 to 60 % (Myrick et al., 2014). Such losses have been associated with serious economic consequences including loss of employment and revenue. In Bangladesh, for example, an average economic loss of approximately US\$700 per hectare per year was recorded (Khan et al., 2015) and loss of employment for 1700 individuals was reported in Ghana (Goergen et al., 2011).

Paracoccus marginatus is believed to be native to Mexico and Central America, and has become an invasive pest in different regions including: the Caribbean islands and Florida, USA (1994–2002); the West and Central Pacific islands (2002–2006); South-East Asia and the Indo-Pacific islands (2008–2010); West Africa (2010–2016); East Africa from 2015; and Israel in 2016 (Finch et al., 2021; Watson, 2019). The pest was first reported in Kenya in 2016 with devastating losses to papaya (Macharia et al., 2017). In most of these regions, the expansion of *P. marginatus* has been partially curbed and infestations brought under control through the introduction of biocontrol measures such as *Acerophagus papayae* Noyes and Schauff, *Anagrus loecki* Noyes and *Pseudeptomastix mexicana* Noyes and Schauff (Hymenoptera: Encyrtidae) (Amarasekare et al., 2009; Lyla et al., 2012; Mani et al., 2012; Meyerdirk et al., 2004; Muniappan et al., 2006; Myrick et al., 2014; Sakthivel, 2013). In Tamil Nadu, for example, the introduction of the three parasitoids *A. papayae*, *A. loecki* and *P. mexicana* resulted in the reduction of *P. marginatus* population in mulberry by 96.6 % in 6 months (Sakthivel, 2013).

In Kenya, at the onset of papaya mealybug outbreaks, farmers relied on synthetic pesticides to manage the papaya mealybug despite their lack of registration for use on papaya (Kansiime et al., 2020). This, however, did not yield positive results. Consequently, CAB International (CABI), through its PlantwisePlus programme, in collaboration with the Kenya Agricultural and Livestock Research Organization (KALRO), the Kenya Plant Health Inspectorate Services (KEPHIS) made concerted efforts to undertake classical biological control of the pest by introducing the parasitoid *A. papayae* in three coastal counties (Kwale, Mombasa and Kilifi) of Kenya (Opisa et al., 2023). Initial field releases of the parasitoid were carried out in papaya farms in Kwale, Kilifi and Mombasa counties in late 2021 and 2022. To complement the initial field releases and enhance the conservation and naturalization of the parasitoid, CABI sought to support farmers in establishing Natural Enemies Field Reservoirs (NEFRs) in their fields.

The rationale behind NEFR is rooted in the ecological principle of biological control, where natural enemies play a crucial role in suppressing pest populations, thereby reducing the need for synthetic pesticides and promoting sustainable pest management practices. The original concept of NEFR pertained to the strategic management of agricultural landscapes to promote the conservation and augmentation of natural enemies of crop pests (Mahmood et al., 2011). Thus, NEFR involved creating and enhancing habitats within agricultural ecosystems (planting reservoir crops) to support populations of beneficial organisms such as predators and parasitoids that naturally regulate pest populations. This has been supported by several studies which have shown that non-crop habitats, such as field margins rich in floral resources, can attract and support natural enemies, leading to increased natural enemy densities in adjacent crop fields (Lagerlöf and Wallin, 1993; Mahmood et al., 2011; Gardarin et al., 2018; Pollier et al., 2018). However, in various farming ecosystems, the trade-off between the farming space occupied by the non-crop habitats/ non-production vegetation and the protection offered as a result of biodiversity conservation has raised concerns (Power, 2010).

In 2018, Mahmood et al. (2018) modified the NEFR system previously used in the management of cotton mealybug involving the use of reservoir crops in addition to the metallic trays in a shed where infested cotton leaves were kept (Mahmood et al., 2011). This modification

involved the omission of reservoir crops and utilizing only the metallic trays in a shed and infested papaya leaves as source of both pest and natural enemies. Mahmood et al. (2018) demonstrated that construction of NEFRs not only enhanced the reduction of papaya mealybug populations through parasitism but also promoted the proliferation of other natural enemies in Pakistan.

In the context of the present study, the NEFRs were further modified (i.e. constructed using locally available, cheap wooden poles and polythene instead of metallic trays and without cementing the floors) and without the need for reservoir crops. It is important to note, however, that papaya is mainly grown alongside other crops such as amaranth, maize, pumpkins among other crops but the main intercrop in coastal Kenya is amaranth. The NEFRs would act as in situ conservation structures for production of *A. papayae*, managed by the farmers as opposed to research officers. This would also encourage farmers to reduce their reliance on synthetic pesticides. It is assumed that parasitoids generated from the NEFR facilities would be fitter and better adapted to the natural conditions compared to laboratory reared parasitoids thus enhancing their establishment and spread (Hopper et al., 1993; Fauvergue et al., 2012; Szűcs et al., 2019; Istas and Szűcs, 2023). Whereas NEFRs utilized in previous studies were managed by the research officers (Mahmood et al., 2011, 2018), this study aimed to assess the effectiveness of low-cost, farmer managed NEFRs in controlling the papaya mealybug in Kenya.

2. Materials and methods

2.1. Identification of experimental sites

The Kenya Standing Technical Committee on Imports and Exports (KSTICIE) granted CABI permission in October 2021 to release *Acerophagus papayae* for the control of PMB in three coastal counties of Kenya: Kilifi, Kwale and Mombasa. These areas were known to produce papaya and were heavily affected by losses due to PMB. Farm visits were conducted in March 2023 to identify potential farms for the establishment of NEFRs. The study enlisted farms based on specific criteria, including a minimum farm size of 0.5 acres, a requirement of at least 40 trees per farm, the necessity for irrigation to ensure year-round crop availability, identification of farms with and without prior parasitoid releases for different treatments, and a preference for farmers who abstained from using synthetic pesticides in their practices. Based on these criteria, a total of 11 farms were selected spanning across Mombasa, Kilifi and Kwale counties Fig. 1. The distance between the sites was variable, ranging from 500 m to several tens of kilometers. Since the study was being conducted on farmer fields, it was technically impossible to standardize the distance between farms and still meet the inclusion criteria.

In Mombasa county, four farms were selected and three treatments assigned as follows:

- Treatment a: No prior *A. papayae* release + NEFR (2 farms): The farms had no prior release of the parasitoid and at least one NEFR was constructed on them. The parasitoid was introduced into these farms through mummy cards obtained from CABI's rearing facility at KALRO, Muguga.
- Treatment b: Prior *A. papayae* release + NEFR (1 farm): The parasitoid had been released on the farms at least once and at least one NEFR was constructed on them. Infested papaya leaves containing parasitized mealybugs (mummies) were placed in the NEFR as source of parasitoid inoculum.
- Treatment c: Prior *A. papayae* release but no NEFR (1 farm): The parasitoid had been released on this farms at least once but no NEFR was constructed. This treatment also served as the **control** in the study.

These treatments were replicated in Kwale and Kilifi counties with

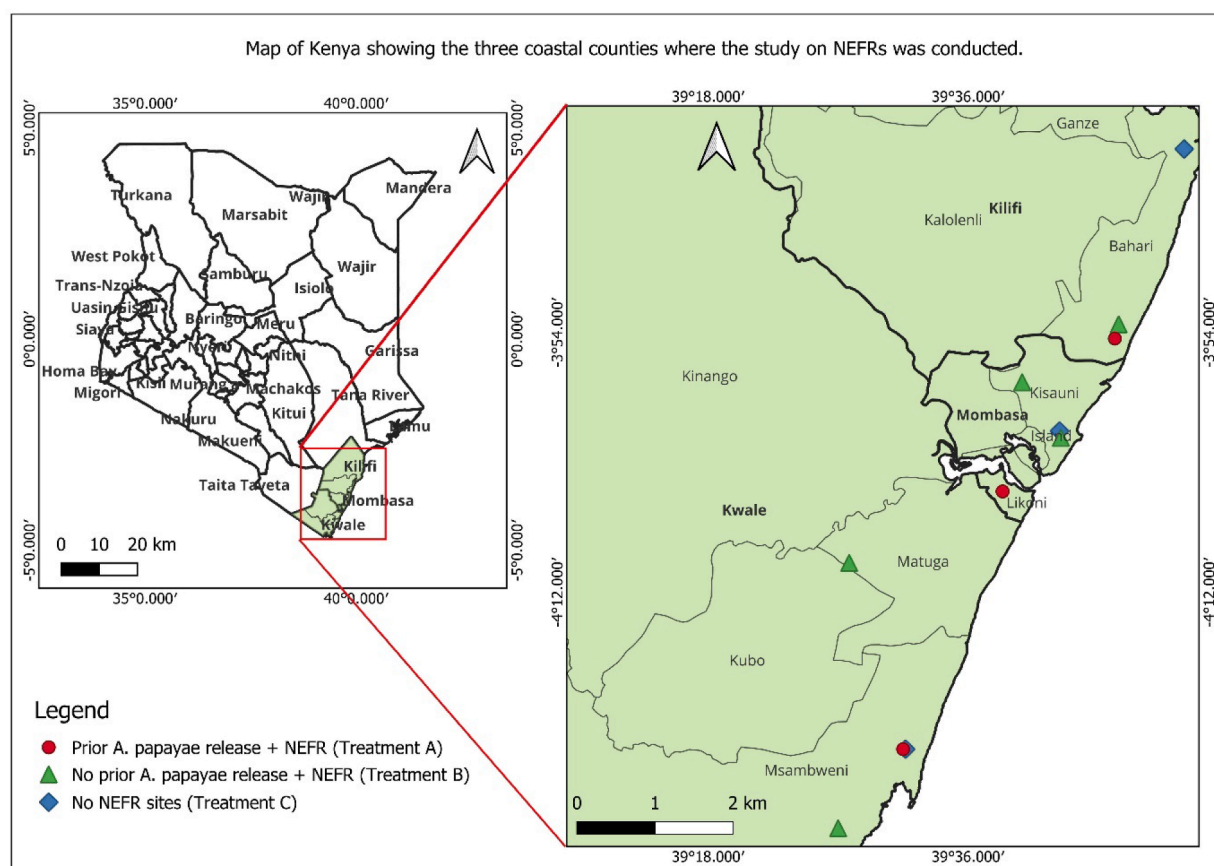


Fig. 1. The selected study sites for the establishment of NEFRs in coastal Kenya. The entire study was conducted between March and June 2023. Data collection was carried out between April and June 2023.

the same number of farms except Treatment a in Kilifi which had 1 farm. The parasitoids introduced in Treatment a were reared according to the procedure outlined in [Opisa et al. \(2023\)](#) with some modification. In this case, the developed *A. papayae* mummies were collected in jars and mounted in cards before transporting them to the field in cooler boxes instead of adults. Data collection was carried out between April and June 2023.

2.2. Setting up NEFRs

The procedure for setting up NEFRs in treatments a and b was modified from [Mahmood et al. \(2018\)](#) to incorporate the use of low-cost locally available materials including polythene and wooden poles rather than metallic sheets. The following steps were followed:

- 1) The position of setting up the NEFR was selected either at a side corner plot or at the centre of the papaya orchard. The corner plots were selected to be up-wind of the papaya trees to help the parasitoid to be blown in the right direction.
- 2) A low-cost temporary shed measuring 1.8 m (L) by 1.6 m (W) was made using poles and covered using 'makuti' (woven palm leaves) as shown in [Fig. 2](#). To ensure proper flow of water during rainy periods, the height of the shed was 2 m and 1.2 m on the longer and shorter ends, respectively ([Fig. 2A](#)).
- 3) A rectangular tray measuring 80 cm (length) x 60 cm (width) x 60 cm (height) was then constructed within the shed using wooden poles and 'makonge' (sisal stem) and lined all round using black polythene sheet ([Fig. 2B](#)). The tray was raised by 20 to 30 cm from the ground at the four corners.
- 4) To prevent ants and other crawling insects from getting into the trays, the supports were smeared with grease ([Fig. 2B](#)).

- 5) The trays were then filled halfway with mealybug infested papaya leaves and fruits collected from the farm ([Fig. 2C](#)). These infected fruits and leaves contained mealybugs, that had been parasitized while on the plant, immature predators and unparasitized mealybugs at different stages of development. The detached leaves worked as 'parasitoid cards' since the parasitoid still completed its lifecycle without flow of sap to the mealybug. Furthermore, it took at least 3 days for the leaves to dry during which the mealybugs could still draw sap from the leaves.
- 6) The leaves were left to dry, as leaving moist leaves in the NEFRs led to fungal growth and contamination. The petioles of the leaves were cut off and the leaves partly shredded as they were introduced into the NEFR to hasten drying and prevent dampening.
- 7) For treatment a, five cards containing pupae of the parasitoid *A. papayae* were introduced into the NEFR along with the infested papaya leaves for parasitization ([Fig. 2D](#)).
- 8) The infested leaves and other plant parts were kept in the trays in the NEFR for 15 to 30 days.
- 9) After 15 to 30 days, the trays were emptied and dry leaves discarded. The trays were then cleaned and refilled with freshly infested papaya leaves.

2.3. Assessing the effect of NEFRs on the populations of papaya mealybug

Pre-treatment count of papaya mealybug was conducted following the procedure described by [Meyerdirk et al. \(2004\)](#) with a few modifications. In each of the selected 11 farms, four trees were selected at random in each quadrant for sampling. Three mature leaves per tree from the lower and mid strata were selected at random for PMB density counts. Only the lower surface of the leaves was examined using a hand lens. Mealybugs were counted on the entire leaf, however, only one



Fig. 2. The NEFR construction process: (A) NEFR shed covered with 'makuti' and the NEFR tray; (B) The NEFR tray lined with a black polythene and grease smeared on the supports to prevent ants and other crawling insects from climbing into the structures; (C) Papaya mealybug infested leaves placed in the NEFR; (D) Parasitoid mummy cards placed in the NEFR.

lateral half of each leaf was counted for samples with high mealybug densities. The later counts were then adjusted by multiplying the counts by two for a complete leaf estimate. On trees bearing fruits, 2 fruits were sampled alongside the leaves. The fruits sampled were picked at random from the bottom and mid sections of the tree. The fruits had a sizeable diameter ranging between 5 and 10 cm. The stages of the PMB that were counted include: egg masses with eggs alone as a single unit; egg masses with eggs and crawlers; second and third instars; adult male and female mealybugs. Mummies (parasitized mealybugs) observed on the leaves were also counted.

Mechanical counters were used to tally the total number of mealybugs per stage of development. The average number of mealybugs per leaf/fruit were then calculated. Post-treatment counts of PMB were

conducted following the same procedure after the first month and thereafter weekly until the eighth week.

2.4. Assessing the effect of NEFRs on the parasitism rates of *a. Papayae*

To determine the levels of parasitism, 100 individual mealybugs were collected from each of the farms sampled. These included late second and third instars and adult female papaya mealybugs. The mealybugs were removed gently from the leaves using a soft camel hair brush and kept in 20 ml vials in groups of 20. The vials were labelled and incubated for 30 days at room temperature 25°C – 27°C and 60–70 % RH. The vials were examined weekly to record the number of emerged parasitoids and any mummies that did not eclose. The emerged

parasitoids were subsequently identified to species level.

2.5. Effect of NEFR on diversity and abundance of beneficial insects within the farming ecosystem

The leaves and fruits that were sampled for assessment of PMB population were also examined for presence of other natural enemies and their numbers established. The adult stages of predators observed on the leaf were collected by tapping the leaf above a white tray. These were then collected and preserved in 95 % ethanol solution. Immature stages of the predators encountered were carried along with fresh PMB infested leaves into the laboratory for eclosion.

2.6. Data analysis

The data on abundance of the papaya mealybug and absolute counts of *A. papayae* was analyzed using the Generalised Linear Model (GLM) with the quasipoisson family and the log link function. The incidence of *A. papayae* was analyzed using GLM with the binomial family and the logit link. Based on the count of parasitoids on the leaves (both mummies and adults), the apparent parasitism was calculated as a percentage of the ratio of observed parasitoids to the total count of mealy bugs on the leaf i.e.

$$\text{Apparent parasitism}(\%) = \frac{\text{Total count of } A. \text{papayae} \times 100\%}{\text{Total count of PMB} + \text{total count of } A. \text{papayae}}$$

The actual parasitism was calculated as a percentage of the mummies recovered from sampled mealybugs to the total number of mealybugs sampled (in all cases, 100 mealybugs were sampled) i.e.

$$\text{Actual parasitism}(\%) = \frac{\text{Total count of } A. \text{papayae} \times 100\%}{\text{Total count of PMB sampled}}$$

Pearson correlation was used to measure the linear dependence between the apparent and actual parasitism. One-way analysis of variance

(ANOVA) was employed to assess the variation in parasitism rates over time and between treatments, with a focus on temporal differences rather than comparing apparent and actual parasitism rates. Mean separation was done using Tukey's HSD test. These analyses were conducted using R version 4.3.1 statistical software (R Development Core Team 2023). Species diversity of predators of papaya mealybug in each treatment during the period of the study was determined using Shannon diversity index and Evenness (Magurran, 2004).

3. Results

3.1. Papaya mealybug infestations

A clear preference for leaves over fruits was evident, with significantly higher densities observed on leaves for all stages of the papaya mealybug, including adults ($P < 0.001$; Odds Ratio (OR) = 11.8), second and third instars ($P < 0.001$; OR = 12.8), egg masses ($P < 0.001$; OR = 7.0), and the combined total of all stages ($P < 0.001$; OR = 10.1) (see Fig. 3). It was observed that as leaves began to senesce, newly hatched crawlers uniformly migrated toward the leaf stalk and stem, eventually reaching other leaves or fruits. Due to the substantial variation in mealybug numbers between fruits and leaves, subsequent analyses and comparisons focused exclusively on counts from leaves.

During the initial assessment (baseline), varying levels of papaya mealybug (PMB) infestation were observed on papaya leaves across the three treatment groups, with significantly higher PMB populations in farms previously exposed to *A. papayae* ($P < 0.001$; $df = 2, 129$; $\chi^2 = 3486.4$). Following the establishment of NEFRs and over the 8-week period, PMB populations decreased by 49.12 % in treatment a and by 62.8 % in the treatment b (see Fig. 4). Conversely, in the control (treatment c), PMB populations increased by 37.6 %.

There was an initial surge in the overall populations of PMB in treatment a in the first 4 weeks before a sharp decline in the subsequent weeks (Fig. 4). On the other hand, there was a sharp and steady decline in the populations of PMB in treatment b (Fig. 4). At week 8, the

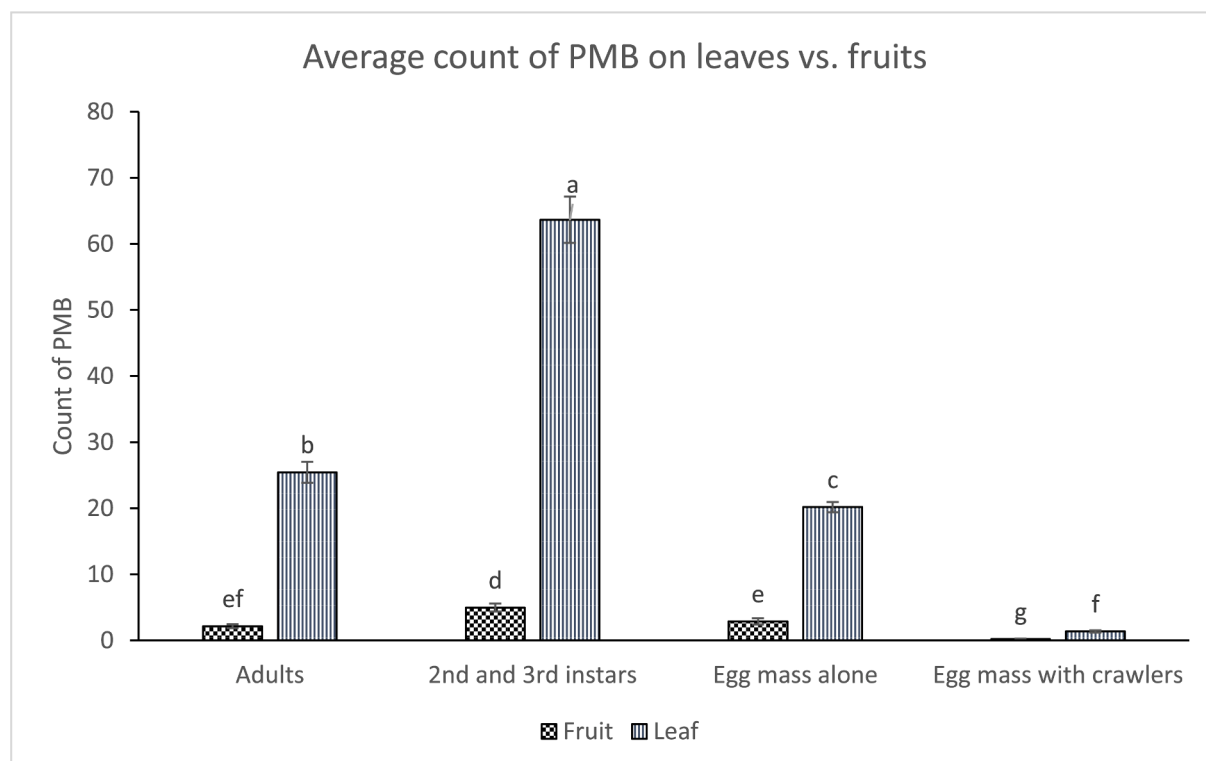


Fig. 3. The average count of the different stages of Papaya mealybug (PMB) per leaf and per fruit across all the treatments in the three coastal counties.

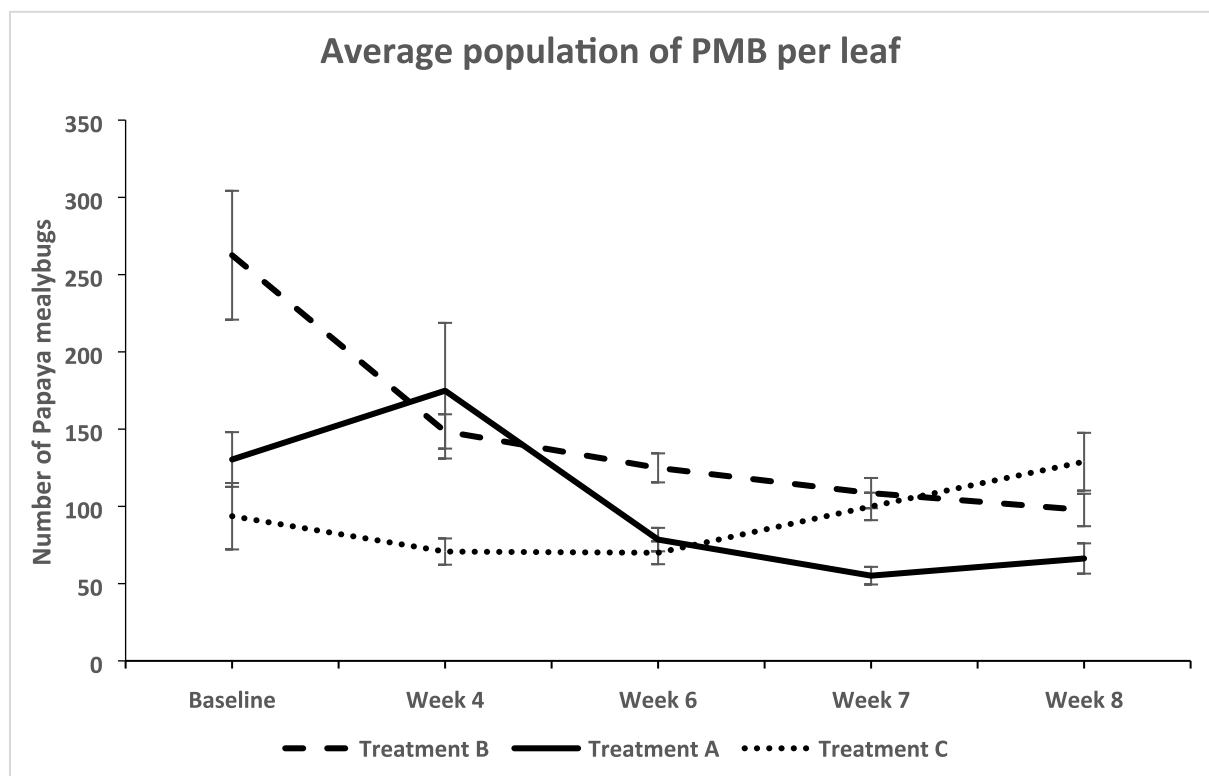


Fig. 4. The mean infestation per leaf of Papaya mealybug in the 3 treatments over time. The trend shows a decline in the populations of PMB in the treatments with NEFRs and an increase in the control (Treatment A = No prior *A. papayae* release + NEFR, Treatment B = Prior *A. papayae* release + NEFR, Treatment C = No NEFR/Control).

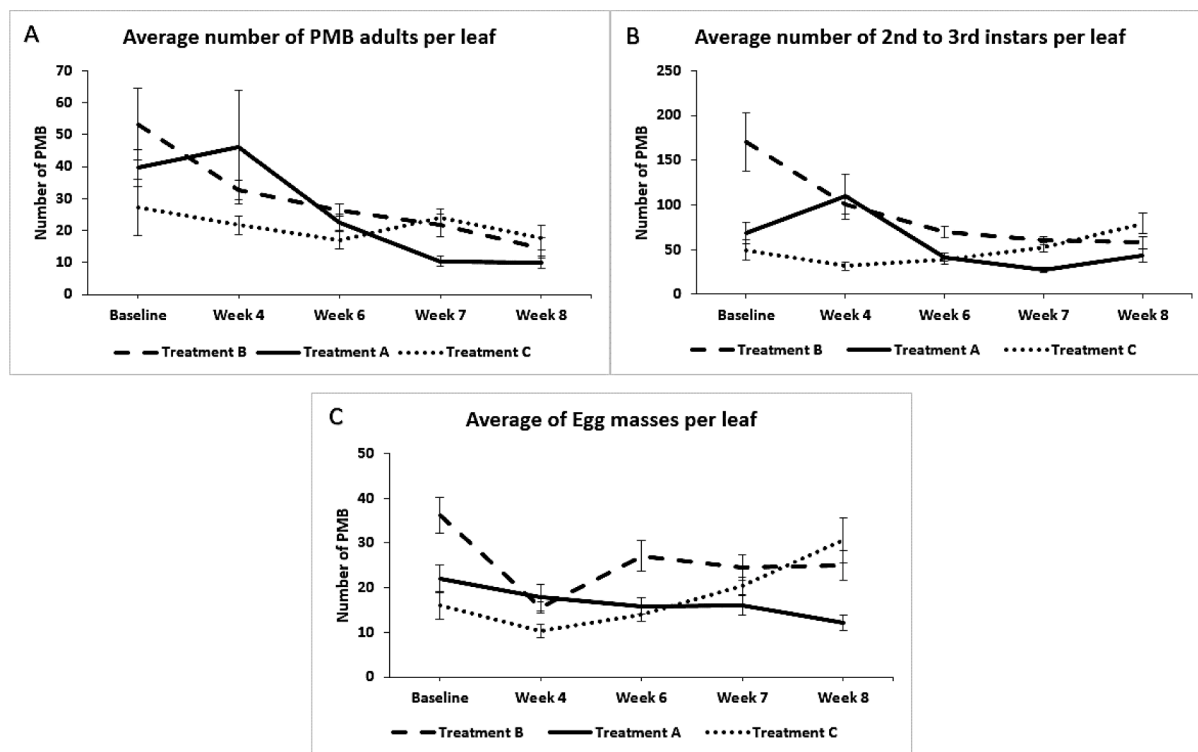


Fig. 5. The average populations of the different life stages of the papaya mealybug per leaf with time. A) Population of adults per leaf, B) Population of 2nd and 3rd instar stages per leaf, C) Average count of egg masses on each leaf. (Treatment A = No prior *A. papayae* release + NEFR, Treatment B = Prior *A. papayae* release + NEFR, Treatment C = No NEFR/Control).

population of PMB was significantly lower in either of the treatments with NEFRs (a and b) compared to the control ($P < 0.001$; $df = 2,81$; $\chi^2 = 609.8$). This trend was observed in both adults (Fig. 5A) and second to third instar stages (Fig. 5B) of PMB which also constituted the highest proportion of the PMB population on leaves. There was also a decrease in the number of egg masses of PMB in the sites where NEFRs were constructed but an increase in number of egg masses on leaves from the sites with no NEFRs (Fig. 5C).

The incidence of *A. papayae* varied across treatments at the onset (baseline) of the trial with treatments a, b and c recording 41.67 %, 97.2 %, and 75 % incidence, respectively. At week 8, the incidence of *A. papayae* had risen to 69.4 %, 100 %, and 95.8 % in the treatments a, b and c, respectively.

The absolute count of *A. papayae* that could be observed in the leaves either as mummies or adults increased steadily over time until the 6th to 7th weeks then declined in all the treatments (Fig. 6). A total of 8,445 individual parasitoids were observed in the leaves and fruits of papaya throughout the period of the study across all the treatments. The highest number of *A. papayae* was recorded in the 6th week with 1,305 individuals in treatment b. Treatment a recorded the highest count of *A. papayae* in week 7 with a total of 884 individuals compared to only 159 at the onset of the study representing more than 400 % increase. Comparatively, there was a 51.6 % increase in the counts of *A. papayae* in treatment c compared to baseline (Fig. 6). There were significant variations in the average count of *A. papayae* per leaf across different treatments and time ($P < 0.001$; $df = 14,525$; $\chi^2 = 2944.6$) (Fig. 6). There was a 3-fold increase in the average number of parasitoids per leaf in treatment b by the 6th week and a 2-fold increase in the 8th week. Similarly, treatment a had a 4-fold increase in the average number of parasitoids per leaf at the end of the 8 weeks. The control had a 1.7-fold increase in the average number of parasitoids per leaf at 8 weeks.

At baseline, the apparent parasitism rates were similar in both treatments that received an initial release of the parasitoid (treatment b (6.1 %) and c (6.5 %)) but significantly lower in treatment a (1.7 %) ($P < 0.001$; $df = 2, 129$; $F = 16.8$). Over the course of eight weeks, the treatment a exhibited a remarkable six-fold increase in apparent

parasitism compared to baseline ($P < 0.001$; $df = 4,235$; $F = 16.52$). In comparison, treatment b ($P < 0.001$; $df = 4,151$; $F = 18.58$) and c ($P = 0.004$; $df = 4,139$; $F = 4.05$) showed three-fold and two-fold increase, respectively (Fig. 7).

There was no correlation between the actual parasitism (accounting for parasitized yet not mummified mealybugs) and apparent parasitism (estimated by counting the mummified mealybugs on the leaves) and the actual parasitism levels exceeded the apparent parasitism. Initially, farms with prior releases of *A. papayae* exhibited similar parasitism rates, with 36.9 % and 32.3 % in treatments b and c, respectively, at the commencement of the trials. In contrast, treatment a started with a lower parasitism rate of 14.4 % during the same period. By the eighth week, the same treatment experienced a doubling of parasitism rates to 31.2 %, while treatment b saw a 17.8 % increase. Conversely, the control witnessed a 10.3 % decline. Notably, treatment b consistently exhibited significantly higher parasitism rates ($P < 0.001$; $df 2, 124$; $F = 11.68$) compared to both a and c (Fig. 8).

Numerous predators spanning 10 genera, including but not limited to *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), *Exochomus* sp., *Tenuisvalvae notata*, *Hyperaspis* sp., Scymninae, *Ortalia* sp., Coniopterygidae, Chrysopidae, and Hemerobiidae, were sampled from both infested leaves and fruit and successfully identified. The most abundant predators were *C. montrouzieri* (44.0 %), *T. notata* (23.9 %) and *Exochomus* sp. (19.9 %). The highest abundance of predators was recorded in treatment b (927 individuals) and the least abundance in the control (366 individuals). There was a significantly higher diversity of predators on the treatments b ($H = 1.427$) and a ($H = 1.406$) compared to the control ($H = 1.13$) (Table 1). Whereas the highest abundance of predators was found in week 6 (620 individuals), a higher diversity of predators was recorded in week 8 ($H = 1.308$) and the least at baseline ($H = 0.663$) across the different treatments.

4. Discussion

The natural enemies field reservoirs (NEFRs) constructed in the farmer fields, with an aim of enhancing the proliferation of *A. papayae* in

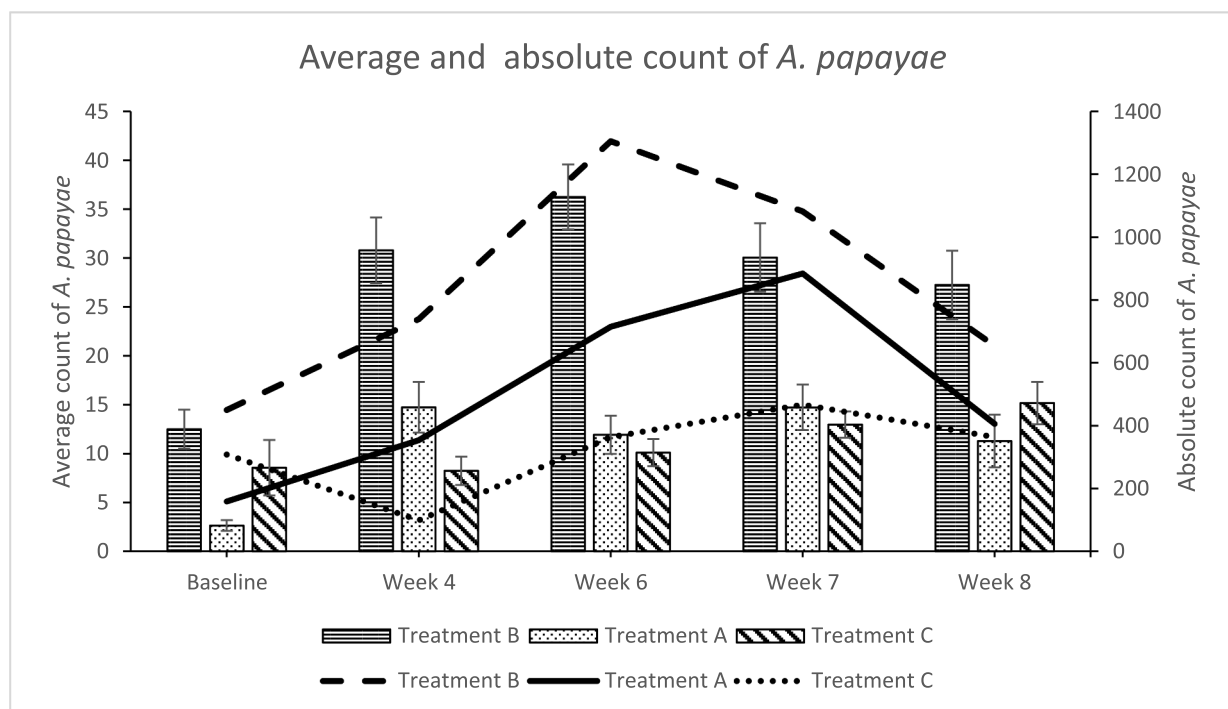


Fig. 6. The average count of *A. papayae* per leaf (bar graph) and the absolute count of the parasitoid *A. papayae* (line graph) observed in all the treatments at different times during the study. (Treatment A = No prior *A. papayae* release + NEFR, Treatment B = Prior *A. papayae* release + NEFR, Treatment C = No NEFR/Control).

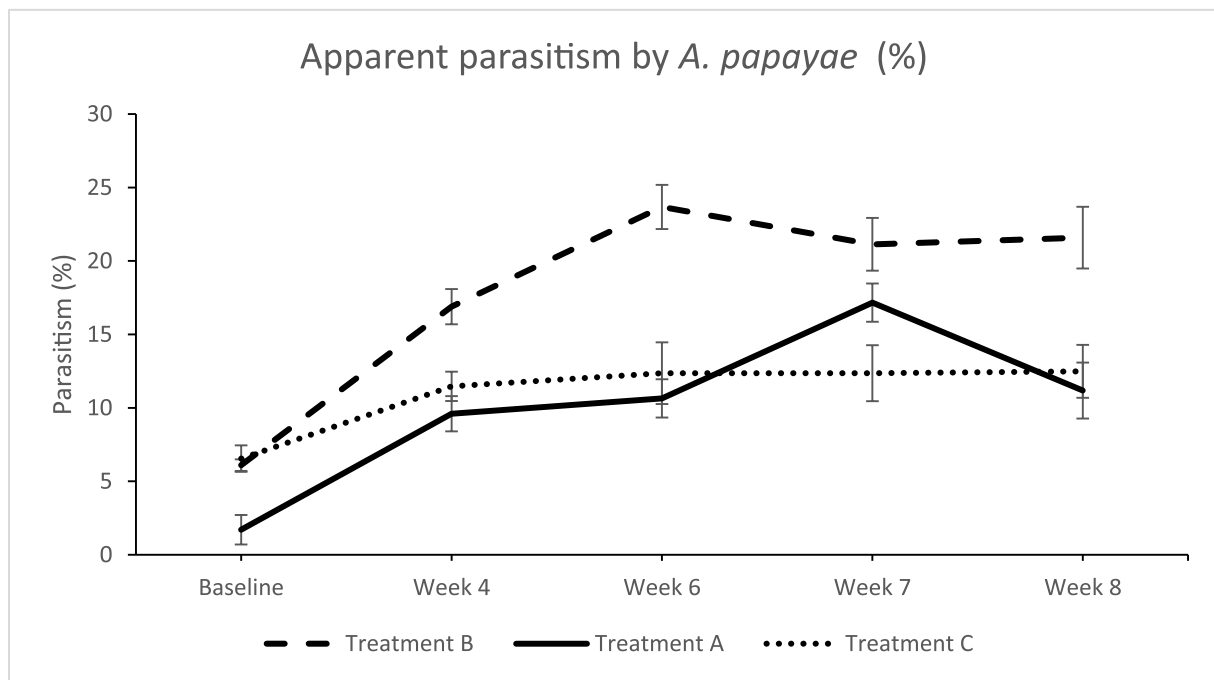


Fig. 7. Apparent parasitism (%). (parasitism based on observed mummies on leaves of papaya) caused by *A. papayae* over time across the three treatments. (Treatment A = No Prior *A. papayae* release + NEFR, Treatment B = Prior *A. papayae* release + NEFR, Treatment C = No NEFR/Control).

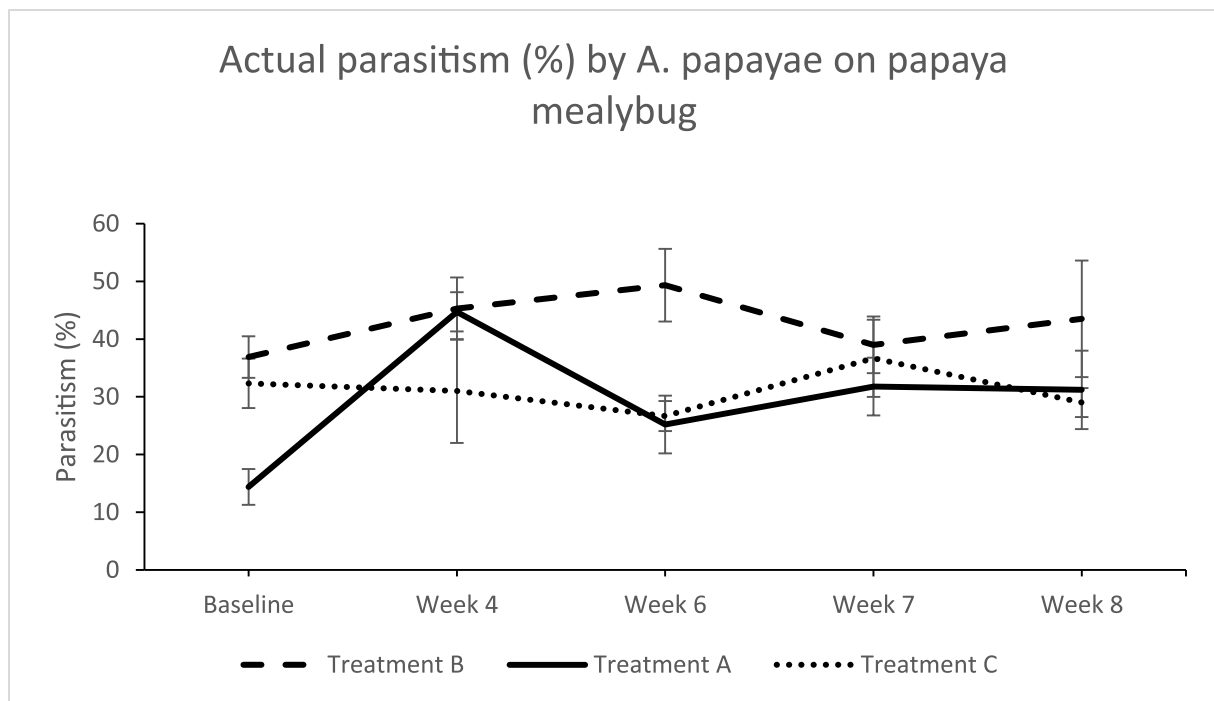


Fig. 8. Actual parasitism (%) (based on samples collected from the field for observation in the lab) caused by *A. papayae* across the three treatments over time. (Treatment A = No prior *A. papayae* release + NEFR, Treatment B = Prior *A. papayae* release + NEFR, Treatment C = No NEFR/Control).

the coastal region of Kenya, demonstrated the potential of such low-cost technologies in the management of PMB in papaya orchards. Our results show that the NEFRs led to a significant reduction in the populations of PMB coupled with an increase in the populations of *A. papayae* in the orchards. In addition, there was an influx of other natural enemies, mainly generalist predators, in the NEFR treatments feeding on PMB. Coupled with the low cost involved in its construction, using materials available to the farmer, incorporation of NEFR in IPM of PMB leads to

reduced usage of pesticides thereby revitalizing biodiversity and promoting sustainability in farming ecosystems.

There was an overall reduction in the population of PMB in the NEFR treatments but a net increase in the treatment without NEFR. Although the reduction in PMB populations in these treatments is noteworthy, it is not unprecedented, as existing studies using a combination of 3 parasitoid species have documented similar decreases in PMB populations. In Palau, there was a reduction in PMB populations below detectable levels

Table 1
Diversity indices of predators of PMB across different treatments with NEFRs in Coastal Kenya.

Treatment/ Time	Treatments*			Time				
	Treatment b	Treatment a	Treatment c	Baseline	Week 4	Week 6	Week 7	Week 8
Diversity indices								
Taxa_S	9	10	8	5	5	7	9	9
Individuals	927	721	366	154	261	620	580	399
Shannon index (H)	1.406	1.427	1.13	0.663	0.915	1.239	1.192	1.308
Evenness	0.64	0.62	0.544	0.412	0.569	0.637	0.542	0.595
Variance	0.0003	0.001	0.004	0.01	0.005	0.001	0.002	0.004
Richness	2.33E-06	4.33E-06	1.306E-05	4.22E-05	1.47E-05	3.9E-06	5.95E-06	1.26E-05

* Treatment a = No prior *A. papayae* release + NEFR, Treatment b = Prior *A. papayae* release + NEFR, Treatment c = No NEFR/Control.

after release of a combination of parasitoids consisting *A. papayae*, *A. loecki* and *P. mexicana* (Muniappan et al., 2006). Similarly, in Guam, there was a 61.4 % – 94.4 % reduction in the population of PMB after introduction of the parasitoids *A. papayae*, *A. loecki* and *P. mexicana* (Meyerdirk et al., 2004). As with our study, where the drop in PMB populations corresponded with increase in *A. papayae* populations and parasitism rates, Mahmood et al. (2018) observed a 93.15 % reduction in the population of PMB following the establishment of NEFRs in Pakistan within 6 months. These results suggest that the NEFRs supported the proliferation of *A. papayae* leading to a steady decline in PMB populations through parasitism.

In classical biological control, it has been observed that sometimes there is a prolonged period ‘lag period’ between the establishment of the control agent and the point at which its population rises rapidly to control the pest (Waage & Greathead, 1988). This could partly explain the observed increase in PMB populations in treatment c despite having prior release of *A. papayae*. A similar short term increase in the populations of PMB by up to 500 % was reported in Guam after introduction of *A. papayae*, *A. loecki* and *P. mexicana* (Meyerdirk et al., 2004). In smallholder landscapes such as Kenya, where farmers frequently anticipate immediate outcomes from pest control interventions (Constantine et al., 2023; Kansime et al., 2020) often expect instant results from an intervention (Constantine et al., 2023; Kansime et al., 2023), an extended ‘lag period’ may be inaccurately interpreted as the ineffectiveness of the control agent. Such a conclusion would be inaccurate because complete control of PMB (i.e. pest reduction below economic thresholds) was realized after 1 year in Guam including sites which had increased pest populations in the initial 4 months (Meyerdirk et al., 2004). In the present study, a 51.6 % increase in absolute count of *A. papayae* in treatment c is an indication that the parasitoid populations were on the rise albeit at a slower pace compared to the pest populations. This means that it would take a much longer time for the effect of the parasitoid in this treatment to be felt, a luxury that most smallholder farmers do not have. This ‘lag period’ was either shortened or missing altogether in the NEFR treatments as there was up to 4-fold increase in *A. papayae* populations in these treatments within the 8 weeks. The NEFRs therefore appear to have played a critical role in conserving and multiplying *A. papayae* and other natural enemies to the point of preventing growth in PMB populations.

Apart from *A. papayae*, a higher abundance of predators of PMB and a higher Shannon diversity index (a measure of species diversity in a community) were reported from the treatments with NEFRs compared to those without NEFRs. These findings, along with those from other studies (Mahmood et al., 2018; Ullah et al., 2022), show that NEFRs promote the conservation and proliferation of not only *A. papayae* but also many other species of natural enemies consisting both predators and parasitoids which contribute additively to the management of PMB and other pests. According to Meyerdirk et al. (2004), generalist predators are considered incapable of suppressing PMB populations because their populations fluctuate irregularly in space and time. This

observation, however, may not hold in the context of NEFRs where significant numbers (thousands) of these predators are conserved (cultured) and released back into the farming ecosystem to manage the pest (Mahmood et al., 2011, 2018). Furthermore, generalist predators such as *C. montrouzieri* and *T. notata* observed in this study were introduced into Kenya for the management of *Planococcus kenyae* (coffee mealybug) in 1924 and *Phenacoccus manihoti* (cassava mealybug) in 1990, respectively (CABI, 2022; Kairo et al., 2013; Neuenschwander, 2001). It has been observed that *C. montrouzieri* has many of the attributes of an effective natural enemy, including a rapid development rate, high reproductive potential, good adaptation to a range of tropical and subtropical climates, high prey consumption rates by both adults and larvae and ease of rearing (Kairo et al., 2013). This suggests that these predators, in adequate numbers, can be able to suppress the populations of PMB in the farmer fields. The NEFRs managed by farmers can effectively preserve and boost the populations of these predators which in conjunction with *A. papayae* can collectively contribute to keeping the PMB populations in check. An additional advantage to preserving the generalist predators is that they could also keep other pests of vegetables often grown alongside papaya in Kenya. Further studies are recommended to establish the relationship between these generalist predators and the host-specific parasitoids such as *A. papayae*.

5. Conclusions

NEFR can be thought of as an elegant, self-sustaining, non-polluting and inexpensive technology with a great potential to manage PMB. The establishment of farmer-managed NEFRs across the 3 coastal counties led to an increase in the absolute count and average populations of *A. papayae* per leaf in the respective farms. This in turn led to an increase in the parasitism rates in the farms resulting in an overall decrease in the infestation levels of PMB in these farms. In addition, there were more predator species and higher abundance of predators in the NEFR treatments suggesting the effectiveness of this low-cost technology not only in conservation of these natural enemies but also its significant role in the management of PMB.

CRediT authorship contribution statement

Stephen T.O. Othim: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Selpha Opisa:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Ivan Rwomushana:** Supervision, Methodology, Funding acquisition, Conceptualization. **Belinda Luke:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Amarasekare, K.G., Mannion, C.M., Epsy, N.D., 2009. Efficiency and establishment of three introduced parasitoids of the mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae). *Biol. Control* 51 (1), 91–95.
- CABI. (2022). *Phenacoccus manihoti* (cassava mealybug) (Publication no. <https://doi.org/10.1079/cabicompendium.40173>). Retrieved 16 October 2023, from CABI <https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.40173>.
- Constantine, K., Makale, F., Mugambi, I., Rware, H., Chacha, D., Lowry, A., Williams, F., 2023. Smallholder farmers' knowledge, attitudes and practices towards biological control of papaya mealybug in Kenya. *CABI Agriculture and Bioscience* 4 (1), 1–15.
- Fauvergue, X., Vercken, E., Malausa, T., Hufbauer, R.A., 2012. The biology of small, introduced populations, with special reference to biological control. *Evol. Appl.* 5 (5), 424–443.
- Finch, E.A., Beale, T., Chellappan, M., Goergen, G., Gadratagi, B.G., Khan, M.A.M., Wyckhuys, K.A., 2021. The potential global distribution of the papaya mealybug, *Paracoccus marginatus*, a polyphagous pest. *Pest Manag. Sci.* 77 (3), 1361–1370.
- García Morales, M., Denno, B.D., Miller, D.R., Miller, G.L., Ben-Dov, Y., Hardy, N.B., 2016. ScaleNet: a literature-based model of scale insect biology and systematics. *Database* 2016. <https://doi.org/10.1093/database/bav118>.
- Gardarin, A., Plantegenest, M., Bischoff, A., Valantin-Morison, M., 2018. Understanding plant–arthropod interactions in multitrophic communities to improve conservation biological control: useful traits and metrics. *J. Pest. Sci.* 91 (3), 943–955.
- Goergen, G., Tamò, M., Kyofa-Boamah, M.E., Boknon-Ganta, A.H., Neuenschwander, P., 2011. Papaya mealybug: a New Invading Pest in West Africa. *Biocontrol News and Information* 32 (2), 9N–16N.
- Hopper, K.R., Roush, R.T., Powell, W., 1993. Management of genetics of biological-control introductions. *Annu. Rev. Entomol.* 38 (1), 27–51.
- Istas, O., Szűcs, M., 2023. Biological control potential of a laboratory selected generalist parasitoid versus a co-evolved specialist parasitoid against the invasive *Drosophila suzukii*. *Evol. Appl.* 16 (11), 1819–1829.
- Kairo, M., Paraiso, O., Gautam, R.D., Peterkin, D.D., 2013. *Cryptolaemus montrouzieri* (Mulsant) (Coccinellidae: Scymninae): a review of biology, ecology, and use in biological control with particular reference to potential impact on non-target organisms. *CABI Reviews* 8 (5), 1–20.
- Kansime, M.K., Rwomushana, I., Mugambi, I., Makale, F., Lamontagne-Godwin, J., Chacha, D., Day, R., 2023. Crop losses and economic impact associated with papaya mealybug (*Paracoccus marginatus*) infestation in Kenya. *International Journal of Pest Management* 69 (2), 150–163.
- Khan, M., Biswas, M., Ahmed, K., Sheheli, S., 2015. Outbreak of *Paracoccus marginatus* in Bangladesh and its control strategies in the fields. *Progress. Agric.* 25, 17–22.
- Lagerlöf, J., Wallin, H., 1993. The abundance of arthropods along two field margins with different types of vegetation composition: an experimental study. *Agr. Ecosyst. Environ.* 43 (2), 141–154.
- Lyla, K., Sinish, M., Vidya, C., Mani, C., 2012. Classical biocontrol of papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink in Kerala using the parasitoid, *Acerophagus papayae* Noyes and Schauf (Hymenoptera: Encyrtidae). *J. Biol. Control.* 26 (4), 386–388.
- Macharia, I., Kimani, E., Koome, F., Kosiom, T., Heya, H., Otira, M., Oronje, M., 2017. First report and distribution of the papaya mealybug, *Paracoccus marginatus*, in Kenya. *Journal of Agricultural and Urban Entomology* 33 (1), 142–150.
- Magurran, A.E., 2004. *Measuring Biological Diversity*. Blackwell Science Ltd., Malden, MA, p. 272 pages.
- Mahmood, R., Aslam, M. N., Solangi, G. S. & Samad, A. (2011). Historical perspective and achievements in biological management of cotton mealy bug *Phenacoccus solenopsis* Tinsley in Pakistan. In *Proceedings of 5th meeting of ICAC's Asian cotton research and development network* (pp. 23–25).
- Mahmood, R., Keerio, I.D., Rehman, A., Rashid, K., 2018. Role of natural enemies field reservoir (NEFR) in farmer fields for controlling papaya mealy bug *Paracoccus marginatus* at Karachi. *Pakistan Entomologist* 40 (1), 7–11.
- Mani, M., Shivaraju, C., Shylesha, A., 2012. *Paracoccus marginatus*, an invasive mealybug of papaya and its biological control-an overview. *J. Biol. Control.* 26 (3), 201–216.
- Meyerdirk, D., Muniappan, R., Warkentin, R., Bamba, J., Reddy, G., 2004. Biological control of the papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae) in Guam. *Plant Prot. Q.* 19, 110–114.
- Miller, D.R., Miller, G.L., 2002. Redescription of *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Coccoidea: Pseudococcidae), including descriptions of the immature stages and adult male. *Proc. Entomol. Soc. Wash.* 104 (1), 1–23.
- Muniappan, R., Meyerdirk, D., Sengebau, F., Berringer, D., Reddy, G., 2006. Classical biological control of the papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae) in the Republic of Palau. *Fla. Entomol.* 89 (2), 212–217.
- Myrick, S., Norton, G.W., Selvaraj, K., Natarajan, K., Muniappan, R., 2014. Economic impact of classical biological control of papaya mealybug in India. *Crop Prot.* 56, 82–86.
- Neuenschwander, P., 2001. Biological control of the cassava mealybug in Africa: a review. *Biol. Control* 21 (3), 214–229.
- Okeke, A., Omoloye, A., Umeh, V., Goergen, G., 2019. Food host preference and life cycle characteristics of the Papaya mealybug, *Paracoccus marginatus* Will. and Granara de Willink in Ibadan. *Southwest Nigeria. Nigerian Journal of Entomology* 35, 135–143.
- Opisa, S., Makale, F., Nyasani, J.O., Muvea, A., Kabole, M., Chacha, D., Luke, B., 2023. Prospects of classical biological control of papaya mealybug in Kenya: Performance of its exotic parasitoid, *Acerophagus papayae*, under laboratory and field conditions. *Crop Prot.* 106476.
- Pollier, A., Tricault, Y., Plantegenest, M., Bischoff, A., 2018. Sowing of margin strips rich in floral resources improves herbivore control in adjacent crop fields. *Agricultural and Forest Entomology* 21 (1), 119–129.
- Power, A.G., 2010. Ecosystem services and agriculture: tradeoffs and synergies. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 365 (1554), 2959–2971.
- Sakthivel, N., 2013. Effectiveness of three introduced encyrtid parasitic wasps (*Acerophagus papayae*, *Anagyrus loecki* and *Pseudoleptomastix mexicana*) against papaya mealybug, *Paracoccus marginatus*, infesting mulberry in Tamil Nadu. *J. Biopest.* 6 (1), 71–76.
- Sharma, A., Muniappan, R., 2022. Ecology and management of *Paracoccus marginatus* (Papaya Mealybug) (Hemiptera: Pseudococcidae) in the Indian subcontinent—achievements, and lessons. *Indian Journal of Entomology* 82 (2), 475–482.
- Szűcs, M., Vercken, E., Bitume, E.V., Hufbauer, R.A., 2019. The implications of rapid eco-evolutionary processes for biological control—A review. *Entomol. Exp. Appl.* 167 (7), 598–615.
- Ullah, F., Farooq, M., Zada, N., 2022. Parasitism potential of biological control agents of fruit fly under natural enemy field reservoirs (NEFR) technology. *Bulgarian J. Agr. Sci.* 28 (5), 876–881.
- Waage, J., Greathead, D., 1988. Biological control: challenges and opportunities. *Philosophical Transactions of the Royal Society of London. Biological Sciences* 318 (1189), 111–128.
- Watson, G. (2019). *Paracoccus marginatus* (papaya mealybug) (Publication no. <https://doi.org/10.1079/cabicompendium.39201>). Retrieved 16th October 2023, from CABI <https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.39201>.
- Williams, D. J. & Granara, D. W. (1992). *Mealybugs of Central and South America*. CAB International, Wallingford, Oxon, UK. 635 pages.