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Prospects of classical biological control of papaya mealybug in Kenya: Performance of its exotic parasitoid, *Acerophagus papayae*, under laboratory and field conditions

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A R T I C L E I N F O

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A B S T R A C T

Papaya mealybug (PMB), *Paracoccus marginatus*, native to Mexico and Central America, invaded Kenya in 2016 causing severe yield losses of between 57% and 91% and £2224/ha household economic losses annually. A classical biological program for PMB involved the importation of *Acerophagus papayae*, a koinobiont endoparasitoid, from Ghana into the quarantine facility at Kenya Agricultural and Livestock Research Organization, Muguga. Laboratory bioassays were conducted to evaluate the suitability of *A. papayae* to parasitize PMB. Parasitism rates, sex ratio and development time of the parasitoid were evaluated under choice and no-choice experimental conditions. High parasitism rates of 72.5 ± 5.9 and 75.0 ± 3.8% were recorded in third instar and adult female PMB, respectively, and lower parasitism rates of 43.8 ± 4.6 % were recorded in second instars, under no-choice test conditions. Significant differences in host choice were noted when *A. papayae* was offered several host stages, with third instars being preferred over second instars. Adult females were preferred over third instars. Adult parasitoids were released and monitored for their establishment at six papaya farms in the Coastal region of Kenya from December 2021 to November 2022. Parasitoid establishment was recovered within the first month of release. Parasitism levels varied across the sites with the highest parasitism of 72.89 % recorded in Kwale. Findings from this study highlight the potential of *A. papayae* as a good candidate for biological control of PMB in Kenya and Africa beyond.

1. Introduction

Papaya (*Carica papaya* L.) is a perennial fruit tree widely cultivated in tropical and subtropical climates for its nutritional and medicinal values. It is Kenya's fourth most important fruit crop, after oranges, mangoes and bananas (Rimbeira and Wamocho, 2014). The total volume of papaya fruit marketed in Kenya was 120 474 MT in 2020 (HCD, 2020). However, the production of papaya has been severely threatened by the invasive papaya mealybug (PMB), *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) (Kansiime et al., 2020; Macharia et al., 2017). The polyphagous invasive pest attacks plant species in more than 200 botanical families (Miller et al., 1999), where it sucks and injects toxic substances into the leaves, stems and fruits. Heavy infestations by PMB produce large volumes of honeydew and thick white waxy secretions, which provide a suitable medium for the growth and development of black sooty mould covering infected fruits, leaves and stems, making the affected fruits inedible and unmarketable (Kansiime et al., 2020; Macharia et al., 2017; Meyerdirk et al., 2004).

Papaya mealybug was first reported in Kenya in 2016 in the coastal

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counties and has since rapidly spread to over 21 counties across the country, reaching near epidemic levels, particularly in papaya-growing locations (Heya et al., 2020; Macharia et al., 2017). In 2017, practically no papaya crop was harvested in most parts of coastal Kenya (Macharia et al., 2017). Papaya mealybug is native to Mexico, where it is not considered a serious pest (Walker et al., 2006). From a survey carried out in 2019 in 8 counties in Kenya, namely Mombasa, Kwale, Kilifi, Makueni, Taita Taveta, Machakos, Embu, and Tharaka Nithi, 307 samples comprising 53 518 individual PMBs revealed only 0.004% parasitism (Nyasani et al., 2019), which is insufficient to reduce the pest populations below economic thresholds. Severe outbreaks often occur when a pest is accidently introduced outside of its endemic range without its natural enemies, which has happened in Kenya (Pfeiffer et al., 2013; Muniappan et al., 2008).

In Kenya, the control of PMB primarily relies on synthetic pesticides (Kansiime et al., 2020). However, the extensive use of synthetic pesticides poses health risks to humans and animals. In addition to acute toxicity concerns, accumulation of highly hazardous residues in the food chain can raise legitimate environmental concerns (Grewal, 2017; Jeyanthi and Kombairaju, 2005). Most of these pesticides are registered for this pest on papaya in Kenya (Kansiime et al., 2020). Managing PMB using synthetic insecticides is also challenging due to its cryptic habit, protective waxy coating over the body, and wide host range, making it difficult to target and control effectively (Sakthivel, 2013; Noyes &Schauff, 2003). Additionally, there have been reports of resistance to chemical insecticides in other parts of the world, indicating the potential for a similar scenario to occur in Africa (Finch et al., 2020; Mani et al., 2012; Sakthivel et al., 2012; Noyes &Schauff, 2003). This is further compounded by the high cost of pesticides and the limited use of personal protective equipment (PPE) by resource-limited farmers in Africa (Kansiime et al., 2020).

A recent ecological model study on the potential distribution of *P. marginatus* has predicted the expansion of the pest into unexplored areas in Central and East Africa (Finch et al., 2020). This highlights the urgent need to look for sustainable measures to mitigate its spread. Classical biological control using host-specific natural enemies such as *Anagyrus loecki* Noyes and Menazes, *Acerophagous papayae* Noyes and Schauff, and *Pseudleptomastrix mexicana* Noyes and Schauff (Hymenoptera: Encyrtidae) has been reported to be effective in many parts of the world, such as Guam, Palau, Sri Lanka, Puerto Rico, Dominican Republic and Florida (Sakthivel, 2013; Muniappan et al., 2006; Meyerdirk et al., 2004). For instance, the release of *A. papayae* in India saved farmers and consumers $121 to $309 million, respectively, in the first year alone (Myrick et al., 2014). In Ghana, the classical biological control intervention of PMB using *A. papayae* benefited producers and consumers to the tune of £1.1 million and £0.97 million over 3 years (2011–2013), respectively, at an estimated intervention cost of £0.12 million (Thiombiano &Solal-Céligny, 2015).

Leveraging the significant effectiveness results obtained in West Africa by controlling PMB using *A. papayae* led to the selection of this parasitoid as a candidate biological control agent against the pest in Kenya. To ensure the success of classical biological control, careful evaluation of host specificity, acceptability, and suitability are critical factors that must be considered before releasing an exotic natural enemy (Kuhlmann et al., 2006; Van Lenteren et al., 2006). Therefore, in this study, we evaluated the host development stage preference and suitability of *A. papayae* to parasitize PMB under laboratory conditions for its consideration as a potential agent for a classical biological control program in Kenya. Following approval from the Kenya Standing Technical Committee on Imports and Exports (KSTCIE) to release *A. papayae* in three coastal counties (Kwale, Mombasa, Malindi) in November 2021, we released adult parasitoids from December 2021 to November 2022 at six research sites in the Coastal regions and assessed their efficiency of establishment in papaya farms in Kenya.

2. Materials and methods

2.1. *Laboratory performance of Acerophagus papayae on papaya mealybug*

2.1.1. *Host plants*

Potato (*Solanum tuberosum*) plants of Shangi cultivar were produced from tubers previously purchased from the local market and sorted to remove damaged and cut ones. Selected potato tubers were disinfected with 5% sodium hypochlorite for 10 min and rinsed thrice with sterile water. Cleaned potato tubers were then cut into smaller sizes within 2–3 nodes (eyes) and treated with 100 ppm gibberellic acid for half an hour to enhance sprouting and root establishment. The tubers were then transferred into 154-cell seedling trays filled with moistened coco peat (SeedPro Kenya, LTD). The trays were staked together and covered using dark-plastic polythene paper and kept in a controlled room for seven days at a temperature of 30 ± 1 °C and relative humidity (RH) of 62 ± 2%. The sprouted potatoes were transplanted individually into 2.5 L plastic pots filled with a mixture of sterile manure and soil in a ratio of 1:4 and watered daily. A 100 g of NPK fertilizer (N: 22%, P: 6%, K: 12%) (MEA Ltd. Kenya) was applied to each pot at planting. Three-week-old host plants were used for rearing PMB and for conducting laboratory experiments with *A. papayae*.

2.1.2. *Papaya mealybug colony*

An initial culture of PMB was field-collected from papaya plantations in Mwala, Machakos County. The collection areas ranged from latitude 0.610562°S to 1.377331°S and longitude 37.377487°E to 37.455608°E with elevation ranging between 1183 and 1288 m above sea level. Field-infested fruits, stems, and leaves were transferred onto each potato plant using a soft camel brush. Infested plant parts with ovisacs and small crawlers of PMBs that could not be transferred were carefully placed on the potato leaves or on the planting pot for PMB to move from the infested plant part to the vegetative potato for feeding. Each potato plant was placed individually into cages (43 cm × 43 cm x 60 cm) made at the Kenya Agricultural Livestock Research and Organization (KALRO), Muguga, Nairobi, Kenya. Each cage had an aluminium frame enclosed with white insect-proof netting cloth on both sides except the base with a khaki cloth material. The netting material had one opening for placing the potato plants in the cage, which was closed by tying it with rubber bands. The potatoes were watered every 48 h, and PMBs from heavily infested potato plants were transferred to new ones in cages as needed. Papaya mealybugs were reared and maintained up to the fifth generation before being used for experiments. The colony was monitored for emergence of any parasitoids. The colony was maintained at 25 ± 2 °C, 65%–90% RH and a photoperiod of 12:12 (L:D) h, using a humidifier and thermostatic heater. The genetic vigour of the already established colony was enhanced by field collections of PMBs from Kilifi, Kwale and Mombasa counties after four months of establishment and subsequently at four months interval from Tharaka Nithi, Embu, Kirinyaga and Machakos counties.

2.1.3. *Acerophagus papayae colony*

The colony was established from adult *A. papayae* that were obtained from the Centre for Agriculture and Bioscience International (CABI)-West Africa, in Ghana, in December 2020. A total of nine (30 ml)-plastic tubes, each containing 20 to 40 adults *A. papayae* were imported and reared on potatoes plants with second and third-instar PMBs in a BugDorm-6E (NHBS Ltd, England) insect rearing cage measuring 60 cm × 60 cm x 120 cm. New parasitoids that emerged were transferred to clean cages for further rearing. The parasitoids were fed on a 1:1 solution of honey and water (Mastoi et al., 2018; Amarasekare et al., 2012) and maintained at 23 ± 5.7 °C, 61% ± 14.4 RH and 12:12 (L:D) h photoperiod using a humidifier and thermostatic heater for six generations before being used for bioassays. At each generation, the *A. papayae* was monitored for emergence of any hyperparasitoids. Across the six generations, no hyperparasitoid emerged from the colony.

2.1.4. Assessment of host instar-stage preference, susceptibility, sex ratio and development time of A. papayae

To determine the preferred host instar stage for parasitisation by *A. papayae*, as well as the susceptibility of different host stages, the parasitoid sex ratio and the development time of *A. papayae*, choice and no-choice tests were conducted. We did not assess the efficiency of *A. papayae* on the first instar stage due to previous studies (Mwanauta et al., 2021; Simo et al., 2021) indicating that this stage is not susceptible to parasitisation.

2.1.5. Host susceptibility to parasitisation, sex ratio and development time of A. papayae

No-choice tests were conducted to determine the PMB host stages that are susceptible to parasitism and the sex ratio and development time of *A. papayae.* The experiment was conducted under the same environmental and physical conditions described in 2.1.3 for *A. papayae* rearing. Three to four-leaved potato plant stems were infested with 10 of each second-instar, third-instar and adult PMBs and then inserted separately into 30 ml glass vials held upright using moist cotton wool. The setup was then placed separately in 1.5 L glass jars that had a lid with a fine netting material for aeration. One newly emerged male and female *A. papayae* were held for 2-d to mate in the 30 ml glass vial. The 2-day-old presumably mated female *A. papayae* was introduced into a single jar containing either 10 s-instars, third-instars, or adult PMBs, with a ratio of 1 parasitoid to 10 host instars mentioned above that were held for 24 h. Each jar was considered as a replicate. The experiment was replicated eight times for each of host instar stages exposed for 24 h to a mated female parasitoid. After 24 h, the parasitoid was removed from the jar whereas the PMBs in the jars were then were carefully transferred onto new potato plants and placed into separate BugDorm-6E insect-rearing cages (60 cm × 60 cm x 60 cm) for host and parasitoid development. Mummified mealybugs were individually placed into 30 ml glass vials covered with a piece of fine white chiffon cloth and secured with a rubber band to avoid parasitoid escape. Time to adult parasitoid emergence and their sex and number were recorded to estimate the developmental time, sex ratio, and proportion of parasitism. For each replicate, the parasitism rate was calculated as follows:

$$\text{Parasitism rate} = \frac{\text{PMB host instars} - \text{emerged mummified PMB with } A.papayae}{\text{Total NO. of PMB host instars exposed for 24h}} \times 100$$

The sex ratio was expressed as the percentage of male and female *A. papayae* parasitoids that emerged from the total number of PMB host instars that were exposed to one mated *A. papayae* for parasitisation.

2.1.6. Host instar preference

Two choice tests of different PMB host-instar combinations were conducted to determine the *A. papayae* host-instar preference as follows: (i) Second and third instar of PMB; and (ii) Third instar and adult female stage. For each combination, five PMBs of each stage, i.e., second-instar and third-instar; third instar and adult females, were artificially infested on a 3 to 4 –leaved potato stem making a total of 10 PMBs for each replicate. Each host-instar choice combination was replicated ten times. The setup was then placed separately in 1.5 L glass jars that had a lid with a fine netting material for aeration. A 2-day-old mated female adult of *A. papayae* was introduced into the jar and removed after 24 h. The PMBs of each host instar stage were carefully transferred onto a new potato plant and placed into separate BugDorm-6E insect rearing cages (60 x 60 × 60 cm). PMBs for each host stage were examined daily for mummies which were collected and monitored for parasitism rates and development times from parasitized egg to mummy and from mummy to adult parasitoid were monitored as described in section 2.1.5.

2.2. Release of Acerophagus papayae in papaya fields at the research sites and parasitism assessment

Six papaya farms, two in each of the three counties, namely, Kilifi, Mombasa and Kwale, were selected for the study (Fig. 1) since they had at least 100 papaya trees spaced at 3 m × 3m, with no chemical insecticides used. In preparation for field-release, a minimum of 50 adult *A. papayae* were collected from the colony using an aspirator into a separate aerated clear plastic jar. A single jar contained 10 parasitoids that were supplied with honey droplets as food source during transportation. We characterised PMB infestations on individual papaya using a scale of 0–4, where 0 = no damage, 1 = less than 25% of plant organs covered by mealybugs; 2 = mealybug coverage ranging from 25 to 50% of plant organs, 3 = mealybug coverage ranging from 50 to 75% of plant organs; 4 = more than 75% of plant organs covered by mealybugs (Galanihe et al. (2011). We used those in categories 3 and 4 for parasitoid releases. During parasitoid releases at the farms, single jars with 10 parasitoids each were tied randomly on individual papaya trees. The jars were tied on trees using rubber bands facing downwards to avoid water collection if it rained but still allowing the parasitoids to leave the jars. Details on numbers of parasitoid released at each of the six farms, duration in months between release and sampling period are reported in Table 4. The trees that had the parasitoid jars tied on them were labelled with ribbons for easy identification and were referred to as 'treated trees' and those that did not have parasitoid jars tied on them were referred to as 'untreated trees'. Parasitoids were released in two rounds at all the six farms as reported in Table 4. After the first release, eight PMB-infested fruits were randomly sampled from 4 treated and 4 untreated trees at all six farms. The second sampling was done after the

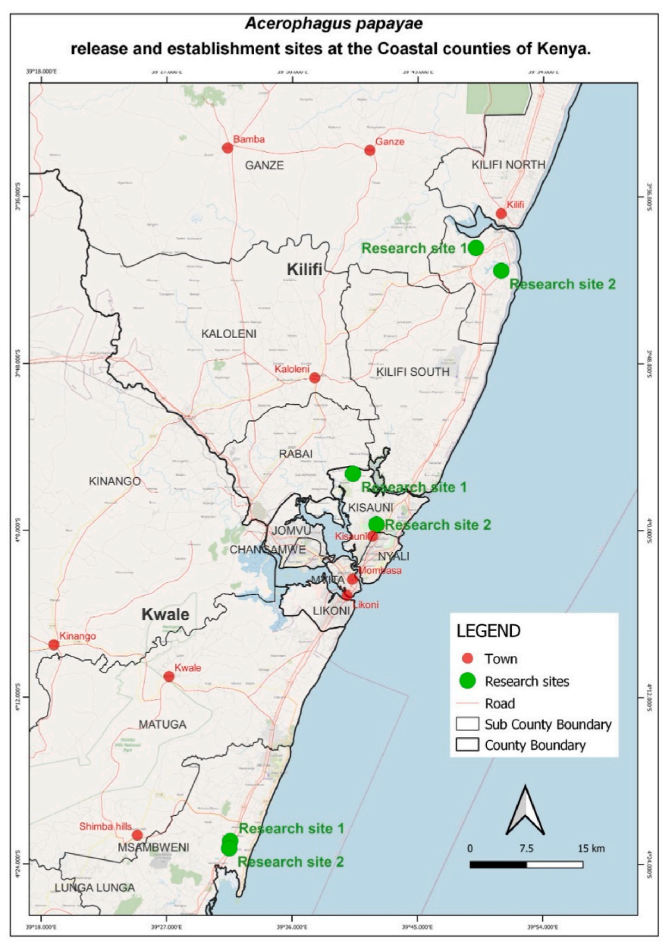

Fig. 1. *Acerophagus papayae* research sites at the Coastal region, Kenya.

first release at all six farms at different months as indicated in Table 4. It involved sampling of eight PMB-infested fruits from 4 treated and 4 untreated trees which were different trees from the ones sampled after the first release. Third sampling was done after the two releases at all six farms as reported in Table 4. Eight PMB infested fruits were randomly sampled from 4 treated and 4 untreated trees that were different trees from the ones sampled after the first and the second release.

For the first, second and third field sampling in all six farms as explained above and indicated in Table 4, sampling of the PMB infested fruits to check for the recovery of the parasitoid was done four times from randomly selected 4 treated and 4 untreated trees. Thereafter, an average of 1 kg of infested fruit from each sampling date was placed in a labelled ventilated transparent rearing jar and transported to the laboratory. Each of the four infested fruits from the two treatments were then monitored for parasitoid recovery. *A. papayae* mummies on the papaya fruits were incubated in separate aerated vials for their emergence. Parasitism rate was determined as indicated in the formula below:

$$\text{Parasitism rate} = \frac{\text{Number of } A.papayae \text{ emerged}}{(\text{Total number of PMB}) + (\text{Number of } A.papayae \text{ emerged})} \times 100$$

Population counts of PMB at each site were determined by taking counts of mealybug instar stages (i.e., egg masses with eggs and crawlers, second and third instars, adult male and female mealybugs) from 8 selected individual infested fruits, 4 from treated trees and 4 from untreated trees that were different from the ones the infested fruits for parasitoid recovery were collected during the three sampling periods explained above.

2.2.1. Data analysis

Data on the developmental time, percentage parasitism rates and sex ratios of F1 parasitoids on the second and third PMB instars and adult females were arcsine transformed to stabilise their variance (Kasuya, 2004). The transformed data were then analyzed using a one-way analysis of variance and means separated using the Student–Newman–Keuls (SNK) test if a significant difference was detected. Data on parasitism rates in the two-choice tests were analyzed using the Wilcoxon test because they were not normally distributed. Data on percentage of parasitism and papaya mealybug counts from the treated and untreated trees at the six research sites were pooled and tested for normality before comparing using Wilcoxon test. All analyses were performed in R version 4.1.0 (R Core Team, 2021).

3. Results

3.1. Host susceptibility to parasitisation

All PMB instars evaluated under no-choice test conditions were susceptible to parasitism. Parasitism rates of 75.0 and 72.5 % were recorded in adult females and third instars, respectively, while a lower parasitism rate (43.8 %) was recorded in the second PMB instar ($F_{2,\ 21} = 12.\ 87$, $P < 0.05$) (Table 1).

3.2. Influence of the host instar on the sex ratio of *Acerophagus papayae*

There was a significant difference in the numbers of females that emerged from the host instars, with third instar and adult female hosts producing a higher female-biased sex ratio compared to the second instars ($F_{2,\ 21} = 21.74$, $P < 0.05$) (Table 2) (see Table 3).

3.3. Development time of *Acerophagus papayae*

Differences were not detected among PMB stages for times for mummies to develop after host stages were exposed to a mated female *A. papayae* for 24 h ($F_{2,\ 21} = 0.565$, P = 0.577), with 10.26 ± 0.8, 8.11 ± 0.45, 8.99 ± 1.15 days for second instar, third instar and adult female, respectively. Development times of adult *A. papayae* from mummies did not vary among PMB stages ($F_{2,\ 21} = 0.777$, P = 0.473), with 11.58 ± 1.82, 11.91 ± 1.94, 14.44 ± 1.01 days for second instar, third instar, adult female, respectively.

3.4. Host instar stage preference of *Acerophagus papayae*

A. papayae preferred to parasitize on the third compared to the second instar (W = 12.5, P = 0.0035). The adult female was also parasitized more than the third instar (W = 85, P = 0.005)(See Table 3).

3.5. Parasitism rates after release of parasitoids at the research sites

There were no significant differences in parasitism rates between treated and untreated trees (W = 2934, P = 0.08582) The parasitoid established at all six research sites on treated and untreated trees (Table 4), with some being recovered one month after the releases. For example, at the Kilifi research sites, the parasitism rates of above 30 % were recorded at both sites in July one month after releases in June (Table 5). Evaluation of parasitism after three months showed high parasitism rates of above 50% at some sites; for example, in Mombasa at research site 1, monitoring in May showed 51.22 ± 9.81% parasitism. High rates of parasitism were also recorded at Kwale research site 1 with 72.89 ± 16.32% parasitism 4 months post release from March to July (Table 5).

3.6. Population counts of papaya mealybug on papaya trees

There was no significant difference in the number of papaya mealybug counts on treated and untreated trees post release of the parasitoids (W = 2404, P = 0.7739).

There was notable decrease over time in the number of PMB recorded at research site 1, in Kwale, as the number of mealybugs decreased from 436 ± 95.56 on untreated plants to 2.5 ± 1.73 from March to July 2022 (Table 5). A significant decrease in the number of mealybugs recorded was also noted in most research sites after 2 releases. For example, after two releases were made at Research site 2 in Kwale, a decrease of mealybugs was noted from 78.8 ± 18.91 during the first release to 13.7 ± 7.57 for the second release then to 5.75 ± 1.79 after the two releases on treated plants. Similar decrease was also noted on untreated plants from 114.75 ± 7.67 after first release to 17.3 ± 7.44 after second release then to 2.25 ± 0.85 after the two releases (Table 5).

Table 1
Parasitism rate of three PMB instars exposed to *A. papayae* for 24 h in no-choice tests.

PMB developmental stage	% parasitism ±SE
Second instar	43.8 ± 4.6b
Third instar	72.5 ± 5.9a
Adult female	75.0 ± 3.8a

Means within a column followed by the same letter were not significantly different by Student–Newman–Keuls test (P < 0.05).

Table 2
Influence of papaya mealybug instars on the sex-ratio (percentage of females) of *A. papayae* in no-choice tests.

PMB developmental stage	Sex-ratio (%±SE) females)
Second instar	40.83 ± 5.65b
Third instar	72.95 ± 2.46 a
Adult female	74.6 ± 3.1 a

Means within a column followed by the same letter were not significantly different by Student–Newman–Keuls test (P < 0.05).

Table 3
Mean percent parasitism (±SEM) by *A. papayae* in a combination of two host instar stages of PMB to evaluate host instar preference using choice tests.

Host stage test combination	Host stage	% parasitism ±SE
Second instar vs. third instar	Second instar	30.10 ± 3.3a
	Third instar	56.18 ± 5.81b
Third instar vs. adult female	Third instar	46.21 ± 6.7a
	Adult female	72.10 ± 3.27b

Means within a test followed by the same letter are not significantly different by Student–Newman–Keuls test ($P < 0.05$).

4. Discussion

To establish an effective classical biological control program, determining the most suitable host instar for parasitisation by a parasitoid is essential for efficient mass rearing and optimal timing for field-release of the parasitoid. In our study, *A. papayae* successfully parasitized all three host stages in choice and non-choice scenarios. However, there was a higher level of parasitism observed in the third instar and adult female stages. Previous studies showed that the size of the host strongly influenced the fitness of the parasitoid (Mwanauta et al., 2021). Larger hosts provide an ample quantity of nutrient resources for the development of the parasitoid and can enhance the fitness of its offspring (Mastoi et al., 2018). As a result, higher parasitism rates were observed in the third instar and adult female host stages compared to second instar hosts, which are smaller in size (Mastoi et al., 2018).

The host stage also influenced the sex ratio of *A. papayae*, with a higher proportion of female parasitoids emerging from third and adult female instars and more male parasitoids in the second instars. These findings are in agreement with previous studies that have reported similar patterns in the sex ratios of *A. papayae* (Simo et al., 2021; Mwanauta et al., 2021; Hintenou, 2015). It has been shown that female parasitoids in larger hosts live longer and have higher reproduction rates than in smaller hosts, which is an adaptive evolutionary sex ratio manipulation behaviour (Napoleon and King, 1999). When evaluating biological control agents, the sex ratio is a crucial parameter that can impact the success of a biological control program. A female-biased sex ratio is generally more advantageous than a male-biased ratio because

Table 4
Rates of parasitism at the 6 research sites of the Coastal region of Kenya after releases of *A. papayae* parasitoid at different monitoring periods.

County	Research sites	Number of *A.papayae adults* released before sampling period	Duration in months between release and sampling period	Parasitism rates (%) on treated papaya trees (±SE)	Parasitism rates (%) on untreated papaya trees (±SE)
Mombasa	Site 1	250	5 (December 2021–May 2022)	51.22 ± 9.81	0
		450	1 (May–June 2022)	40.97 ± 6.27	31.31 ± 1.55
		700[a]	**2 (June –August 2022)**	**42.39 ± 3.32**	**45.71 ± 10.89**
	Site 2	100	3 (February–May 2022)	30.73 ± 4.77	9.02 ± 5.93
		750	1 (May–June 2022)	10.42 ± 6.25	48.96 ± 10.54
		850[a]	**2 (June –August 2022)**	**37.18 ± 8.44**	**44.01 ± 5.75**
Kwale	Site 1	350	3 (December 2021–March 2022)	30.49 ± 9.87	5.19 ± 2.03
		1080	4 (March–July 2022)	72.89 ± 16.32	62.86 ± 14.68
		1430[a]	**4 (July –November 2022)**	**26.73 ± 4.3**	**44.49 ± 3.16**
	Site 2	200	1 (February–March 2022)	30.03 ± 8.97	13.66 ± 2.16
		840	1 (June–July 2022)	26.72 ± 4.53	40.84 ± 9.46
		1040[a]	**4 (July – November 2022)**	**43.53 ± 2.57**	**48.33 ± 6.87**
Kilifi	Site 1	250	1 (June–July 2022)	30.43 ± 3.86	23.87 ± 2.20
		550	2 (July–September 2022)	30.09 ± 5.16	17.95 ± 5.48
		800[a]	**1 (September – October 2022)**	**62.19 ± 14.73**	**50.85 ± 8.17**
	Site 2	305	1 (June–July 2022)	43.75 ± 10.75	42.86 ± 5.39
		600	2 (July–September 2022)	24.46 ± 4.23	28.19 ± 7.02
		905[a]	**1 (September – October 2022)**	**52.54 ± 6.79**	**42.47 ± 8.28**

[a] Cumulative number of parasitoids released at the research site.

Table 5
Mean number of papaya mealybug counted from papaya fruits from different monitoring periods at the six research sites.

County	Research site	Number of *A.papayae adults* released before sampling	Duration in months between release and Sampling period	Mean no. of PMB (±SE) counted from papaya fruits on trees with parasitoids released	Mean no. of PMB (±SE) counted from papaya fruits on non-released papaya trees (±SE)
Mombasa	Site 1	250	5 (December 2021–May 2022)	10.2 ± 5.96	11.8 ± 4.64
		450	1 (May–June 2022)	11.2 ± 2.14	13.8 ± 3.12
		700[a]	**2 (June –August 2022)**	**33.5 ± 8.50**	**34.3 ± 11.10**
	Site 2	100	3 (February–May 2022)	15.3 ± 4.99	4.4 ± 2.93
		750	1 (May–June 2022)	11.8 ± 9.45	4.5 ± 2.18
		850[a]	**2 (June –August 2022)**	**13.5 ± 2.25**	**13.3 ± 2.29**
Kwale	Site 1	350	3 (December 2021–March 2022)	59.5 ± 9.61	436 ± 95.56
		1080	4 (March–July 2022)	10.8 ± 9.78	2.5 ± 1.73
		1430[a]	**4 (July –November 2022)**	**16 ± 11.372**	**6.0 ± 3.03**
	Site 2	200	1 (February–March 2022)	78.8 ± 18.91	114.75 ± 7.67
		840	1 (June–July 2022)	13.7 ± 7.57	17.3 ± 7.44
		1040[a]	**4 (July – November 2022)**	**5.8 ± 1.79**	**2.3 ± 0.85**
Kilifi	Site 1	250	1 (June–July 2022)	15.3 ± 7.72	30.3 ± 11.74
		550	2 (July–September 2022)	20.8 ± 2.29	43.1 ± 12. 32
		800[a]	**1 (September – October 2022)**	**2.7 ± 0.67**	**6.8 ± 1.88**
	Site 2	305	1 (June–July 2022)	2.8 ± 0.85	12.3 ± 2.53
		600	2 (July–September 2022)	16.3 ± 4.23	11.3 ± 2.21
		905[a]	**1 (September – October 2022)**	**10.5 ± 6.01**	**6.8 ± 1.7**

Mean no. of PMB = Average number of individual papaya mealybug stages counted from selected four papaya fruits on different 4 trees.
[a] Cumulative number of parasitoids released at the research site.

male parasitoids primarily focus on mating and do not contribute directly to pest mortality. The female parasitoids also play a pivotal role in pest control through host feeding or oviposition, leading to the reduction of pest populations. Therefore, a female-biased sex ratio is considered a positive attribute when assessing the effectiveness of biological control agents (Roitberg et al., 2001).

Under our rearing conditions, no significant difference in the development time of *A. papayae* was observed among the different host instar stages, with the parasitoid taking 14.4, 11.9 and 11.6 days to develop in the second, third, and adult female host instar stages, respectively. In comparison, the PMB typically takes an average of 25.5 days to complete its entire life cycle. Our findings indicate that *A. papayae* has a shorter development time than its PMB host, a desirable characteristic in classical biological control. This shorter development time will enable *A. papayae* to produce offsprings faster and parasitize PMB populations within a shorter timeframe (Hintenou, 2015; Amarasekare et al., 2012).

Field-released *A. papayae* established in all six research sites within the coastal counties, with a parasitism rate of 30% or higher at all research sites within a month. The highest parasitism rate (72.89%) was recorded after more than 1000 parasitoids were released on a farm, and parasitism was observed four months after releases. This outcome provides strong evidence of the exceptional efficiency of *A. papayae* in reducing PMB populations. Similar studies have also demonstrated the establishment of *A. papayae* within only one month of introduction (Meyerdirk et al., 2004). Most parasitism rates that were recorded to be above 40% in this study were reported after five months of establishment. In most of the research sites, the population counts of papaya mealybug reduced significantly after releases to a level where it was difficult to find the pest at the research sites. For successful establishment of *A. papayae*, papaya farmers in the coastal region were encouraged to reduce the use of pesticides in order to conserve parasitoids. Apart from the releases at the research sites, additional papaya farms, that were part of a survey on knowledge, attitude and practices perception of farmers towards classical biological control of papaya mealybug (Constantine et al., 2023), were supplied with more parasitoids. This was meant to ensure that the parasitoids established throughout the Coastal region and helped suppress the population of papaya mealybug.

5. Conclusion and recommendations

The high parasitism rates and female-biased sex ratios obtained with third and adult female host instars indicate that mass rearing of *A. papayae* should be done with these host instar stages. *Acerophagus papayae* establishment in the field and parasitizing the mealybug to levels not detectable in the field has proved to be the most effective control strategy that should be implemented in Kenya and other Africa countries.

Author contribution

Selpha Opisa: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing and visualization **Fernadis Makale:** Conceptualization, Methodology and Writing - Review & Editing, **Johnson Nyasani:** Conceptualization, Methodology and Writing - Review & Editing, **Alexander Muvea**: Conceptualization, Methodology, Investigation and Writing - Review & Editing, **Melon Kabole:** Conceptualization, Methodology and Writing - Review & Editing, **Duncan Chacha:** Methodology, Investigation and Writing - Review & Editing, **Lakpo Koku Agboyi:** Conceptualization, Investigation and Writing - Review & Editing, **George O. Asudi:** Conceptualization, Methodology and Writing - Review & Editing, **Abdul Rehman:** Conceptualization, Methodology, Resources, Writing - Review & Editing and Supervision, **Belinda Luke:** Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration and funding acquisition, **Ivan Rwomushana:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Project administration and funding acquisition.

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.

Data availability

Data will be made available on request.

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