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Evaluation of biological control option for Bagrada bug (*Bagrada hilaris* (Burmeister)) in Kenya

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Summary: Brassica production is important for economic development of Kenya. Bagrada bug, a significant pest of brassicas, affects their yields and quality, currently posing a threat to both local and commercial vegetable production in Kenya. Biological control of bagrada bug using natural enemies is a cheaper and environmentally friendly method. The study aimed to identify native egg parasitoid species in Kenya. A field prospection survey of the bagrada bug egg parasitoid was done by a series of bagrada bug egg exposure in different parts of the country. Freshly laid bagrada bug eggs in cards were exposed for possible parasitism in the field for three days. The eggs were later carried to the laboratory at NSRC to await hatching. Two egg parasitoid species *Trissolcus basalis* (Hymenoptera, Scelionidae) and *Gryon sp.* were identified during the study after a period of bagrada bug egg exposure in Machakos and Kisumu. Two cards with parasitized bagrada bug eggs were recovered from Machakos from which one card yielded four parasitoids of one species *Trissolcus basalis* and the other yielded two parasitoids *Trissolcus basalis* and *Gryon sp*. One card with two parasitized eggs by *Trissolcus basalis* was recovered from Kisumu, however, there were no parasitoids identified in Nanyuki, Naivasha and Kitengela where bagrada bug is also prevalent. Results showed parasitoid presence in fields with high bug populations compared to areas with few or no Bagrada bug infestations. Conducting trials in both laboratory and field settings is recommended to obtain clear data on the effectiveness of the identified egg parasitoid in managing the bagrada bug population.

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Introduction

Bagrada hilaris (Burmeister, 1835) (Hemiptera: Pentatomidae) is a stink bug native to Africa, that later spread to other Asian countries (Huang et al., 2014). The bug has been reported as an important pest of cruciferous crops (Brassicaceae), particularly in India and Pakistan (Malik et al., 2012). In Kenya, the Bagrada bug is emerging as a major pest of brassicas, important vegetable crops for both domestic and export markets.

In Kenya, brassicas are amongst the most commonly grown and consumed vegetables with cabbage (*Brassica oleracea var. capitata*) and kale (*B. oleracea var. acephala*) being ranked third and fourth after potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) (FAO STAT, 2021). Apart from cabbage and kale, other common brassicas grown in the country include, cauliflower (*B. oleracea var. botrytis*) and broccoli (*B. oleracea var. italica*) (FAO STAT, 2022). In 2020, Kenya exported 944,542 MT of brassicas valued at KES 351 million. This accounted for 12.6% of total exotic vegetable produce and 5.4% of the total horticultural crops FAO STAT (2022), thus contributing significantly to the national revenue. Besides foreign exchange, brassicas are valuable sources of minerals, dietary fiber, vitamins, health promoting compounds such as carotenoids, glucosinates as well as a source of cash income for small-scale farmers in rural and peri-urban areas.

Apart from Brassicaceae plants, the pest has also been observed feeding on other indigenous vegetables including spider plant (*Cleome gynandra*) in some fields (DAFF, 2013). In Kenya the pest has been identified in different areas including Nakuru, Machakos, Kajiado and Kiambu counties (Macharia, 2018).

Bagrada bug has piercing and sucking mouthparts (Shimat et al., 2014). It feeds on both parts of the leaves destroying the crop in just a few hours especially if not managed (Joseph et al., 2017). The pest affects young seedlings by feeding on the apical meristem and growing points leading to their death or blind terminals (Patel et al., 2017). Severe attacks during the early developmental stage cause forked or multiple heads, resulting in poor production (Shimat, 2017). In brassica crop production, loss of marketable crops is caused by the nymph and adult stages of bagrada bug which result in not only loss of quality of the crop but also the quantity (Palumbo et al., 2016). Concomitant with the damage to brassica crops, bagrada bug among other pests has also increasingly become problematic in community and private gardens in urban areas (Reed et al., 2013).

Although native to Africa, the bagrada bug, which was not considered a serious pest, is now becoming a threat in conventional farming fields (Macharia, 2018). Despite the great damage caused by bagrada bug on brassicas, no specific management strategies for the pest have been developed in Kenya. Farmers depend on the use of synthetic pesticides, often applied on a calendar basis for the management of most brassica pests (Grasswitz, 2016). Continuous use of synthetic insecticides destroys the natural enemies besides posing great concern to the environment especially to humans and nature due to the negative effects associated with the use of such chemicals. Moreover, continuous use of synthetic insecticides increases chances of bagrada bug resistance to chemicals and development of pest resurgence if the pest is not properly managed (Jones et al., 2015). These negative impacts of insecticides have therefore stimulated an urgent need for alternative control options in order to reduce growers' dependence on synthetic pesticides (Shimat, 2017). Alternative control options for example predation and parasitism by natural enemies have proved effective components in integrated pest management approaches for many different insect pests of vegetable crops (Ganjisaffar et al., 2018). Research employing such techniques in the control of bagrada bug is however limited in Kenya. So far, studies on the control of bagrada bug have concentrated on chemical control, with baseline data on the potential impact of native natural enemies mostly lacking. Therefore, the study was carried out to identify native egg parasitoids of bagrada bug in different parts of Kenya where the pest is prevalent.

Materials and methods

To enable identification of bagrada bug egg parasitoids, bagrada bugs were collected and reared to lay eggs. The eggs were then used to attract and trap potential bagrada bug parasitoid(s) from fields grown with brassicas.

Collection and rearing of bagrada bug

Collection and rearing of bagrada bug were done as per the method described by Palumbo et al. (2016). Male and female bagrada bug samples were collected from various brassica growing areas in Kenya, including Naivasha, Machakos, Nanyuki, and Kisumu, where the bug is prevalent. Bugs were collected by picking method as described by Mahmood et al. (2015) where individual bugs were sucked using a modified aspirator ***(Figure 1)*** and then transferred to small plastic containers to hold them while still in the field. Kale and dark-colored paper towels were added to the containers ***(Figure 2)*** to provide food for the bugs and sustainable environment for laying eggs. Laid eggs were then carefully transferred from the paper towels in the container into protected cages in the laboratory where they hatched. The hatched bagrada bug nymphs were reared to mature bugs in the cages while being fed using cabbage and kale plants ***(Figure 3)***. During rearing of the nymphs to mature bugs, the environment inside the cages was maintained at 25±1 °C using a thermal heater and relative humidity at 50–60% by sprinkling some water on the lower surface, ranges that offer favorable conditions for establishment of new colonies and increase in the number of bagrada bugs (Shimat et al., (2017).

Eggs field exposure

Freshly laid bagrada bug eggs (≤ 24 hours old) from the reared colony in the cages were removed from the colored paper towel using a pair of scissors and mounted on glued sentinel cards measuring 25 cm^2. Eggs mounted on sentinel cards ***(Figure 4)*** were exposed in fields grown with kale and cabbage in Naivasha (Delamare), Machakos (Katumani), Nanyuki, Kitengela and Kisumu (Kibos) sites infested with bagrada bug, to help attract parasitoids according to the procedure by Ganjisaffar et al. (2018). Bagrada bug exposure was done three times for each location during the periods; February-April 2018, August-October 2018 and August-October 2019.

In the Nanyuki site, 56 bagrada bug eggs were exposed to brassica fields in exposure one (1) and two (2), whereas 88 bagrada bug eggs were exposed in exposure three (3). In the Naivasha site, 142, 41 and 17 bagrada bug eggs were exposed to brassica fields in exposure 1, 2 and 3, respectively. In Machakos, 105, 41 and 54 bagrada bug eggs were exposed in brassica fields in exposure 1, 2 and 3 respectively. Ninety-five (95), 53 and 52 bagrada bug eggs were exposed in brassica fields in Kitengela in the first, second and third exposures, respectively while in Kisumu, 65 bagrada bug eggs were exposed in first and second exposures each and 70 bagrada bug eggs were exposed in the third exposure period. The number of bagrada bugs in each exposure period varied depending on the availability of fresh bagrada bug eggs. In each location, 200 bagrada bug eggs were exposed in total.

Sentinel cards were attached to kale plants at each location using a wire positioned 30 cm above the ground to prevent egg predation by predators. Eggs mounted on the cards were exposed in the brassicas fields for 3 days then taken to the laboratory into a well-sealed chamber with black lining on the interior and lighting provided to promote hatching.

Figure 1: Collection of bagrada bug in the field using a modified aspirator.

Figure 2: Bagrada bug adults and nymphs held in a small plastic container with dark paper towel and kale leaves while in the field.

Figure 3: Rearing process of bagrada bug in the laboratory.

Figure 4: *Trissolcus basalis* (Hymenoptera, Scelionidae) bagrada bug egg parasitoid.

Figure 5: Gryon sp.

Assessing eggs for parasitism

The eggs were checked daily for hatching into bugs or emergence of wasps. Healthy unparasitized eggs hatched within 7 days while parasitized eggs were expected to take longer than 7 days to hatch (Ganjisaffar et al., 2018). Parasitized eggs took 17 to 25 days to hatch into a parasitoid. Upon emergence, data was collected on the number of different parasitoid species that emerged. The parasitoids (wasp) hatched were stored in a vial containing absolute ethanol then sent to project partners at the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) European Biological Control Laboratory (Montpellier, France) for further identification. The remaining parasitoids were then transferred using a fine brush into a separate box measuring 200cm^2 to establish new colony. Drops of honey were added into the box to provide food for the bugs to allow them survive and reproduce.

Results

Majority of the eggs exposed in the brassica fields for the three exposure periods (February-April 2018, August-October 2018 and August-October 2019) either hatched into bagrada bug or were damaged while a few of the exposed eggs died or were parasitized.

Among the host sites, only bagrada bug eggs exposed in Machakos and Kisumu showed incidences of parasitism. Out of 105 bagrada bug eggs exposed during the period February-April 2018 in Machakos (Katumani), four (4) had parasitoid emerged, which was later identified as *Trissolcus basalis* (Hymenoptera, Scelionidae) ***(Figure 4)***. Ninety-one (91) eggs hatched to bagrada bug nymph while 10 eggs were damaged. In the second field exposure of bagrada bug eggs in Machakos, 41 fresh bagrada bug eggs were exposed in the brassica fields. One parasitoid emerged from the bug egg also later identified as *Trissolcus basalis* and another parasitoid identified as *Gryon sp*. adult ***(Figure 5)*** was found dead in another egg after the egg was opened. Thirty-nine (39) eggs hatched to bagrada bug nymphs and no eggs were recorded as having been damaged. The third exposure period in Machakos did not show any parasitoid emergence ***(Table 1)***.

In Kisumu (KALRO-Kibos), sixty-five (65) bagrada bug eggs were exposed in both the first and second exposure periods while 70 bagrada bug eggs were exposed in the third exposure period. The first and the third exposure period did not record any parasitoid emergence. Fifty-three (53) eggs hatched in to bagrada bug nymphs, 10 eggs were damaged and two eggs were dead in the first exposure period. Sixty-nine (69) eggs hatched into bagrada bug nymphs and one bagrada bug egg was found dead in the third period of exposure. From the second field exposure, parasitoids emerged from two eggs and were later identified to be *Trissolcus basalis* while 63 eggs hatched into bagrada bug nymphs. No egg was found dead or damaged during this exposure period ***(Table 1)***.

In the Nanyuki site, 56 Bagrada bug eggs exposed in exposures one (1) and two (2), and 88 eggs in exposure three (3), resulted in 25, 16, and 76 eggs hatching into Bagrada bug nymphs, respectively.; 18, 19 and 5 bagrada bug eggs were damaged and 13, 21 and 7 bagrada bugs eggs were dead in the three exposure periods, respectively ***(Table 1)***. In Naivasha site, during exposures one (1), two (2), and three (3), out of 142, 41, and 17 fresh bagrada bug eggs exposed to brassica fields, 37, 9 and 16 eggs hatched into bagrada bug nymphs, and 105, 32, and 1 egg were damaged respectively. None of the eggs was found dead in the site. In Kitengela, during exposures one (1), two (2), and three (3), 95, 53, and 52 fresh bagrada bug eggs were exposed, resulting in 93, 46, and 49 eggs emerging into bagrada bug nymphs, respectively. Two eggs were reported damaged in exposure 1, three eggs in exposure 2, while four and 3 three eggs were found dead in exposure two (2) and three (3) respectively. In all the exposures in Nanyuki, Naivasha and Kitengela sites, none of the eggs was parasitized hence no parasitoids were obtained.

Discussion

In this study the parasitoids *Trissolcus basalis* and *Gryon sp* were obtained on parasitized eggs of bagrada bug, in the farm sites of Katumani in Machakos County and Kibos research station in Kisumu County. Unlike Nanyuki and Naivasha, the conditions for the two farms fore-mentioned are relatively warmer, as Machakos is on the leeward side of Mt. Kenya and Kisumu being influenced by L. Victoria. In consistence with results of the present study, three species of bagrada bug parasitoid wasps; *Trissolcus hyalinipennis* Rajmohana & Narendran (Hymenoptera, Scelionidae, Telenominae) (Rajmohana 2006; Ganjisaffar et al. 2018), *Gryon gonikopalense* Sharma (Scelionidae, Scelioninae) (Sharma 1982; Martel et al. 2019) and *Ooencyrtus sp*. (Hymenoptera, Encyrtidae) were recovered in Parkistan (Mahmood et al., 2015). It could probably be postulated that climatic condition and favorable farm practices have more influence for the existence of these parasitoids in Kenya. Although, few cases of parasitism of *T. basalis* on bagrada bug eggs have been reported in California, USA, (Ganjisaffar et al., 2018) and Mexico (Felipe-Victoriano et al., 2019), the occurrence of *Trissolcus basalis* as a biological control agent on bagrada bug in the current study marks the first time the species parasitism on bagrada bug eggs is reported in Kenya. Given that the parasitoid is not specific to the widely spread *N. viridula*, it is

Table 1: Status distribution of bagrada bug eggs upon exposure in the selected host sites

Bagrada bug eggs exposure period	Host site	Number of bagrada bug eggs					Identified Parasitoid
		Initial number exposed	Hatched	Parasitized	Damaged	Dead	
February-April 2018	Nanyuki	56	25	0	18	13	
	Naivasha	142	37	0	105	0	
	Machakos	105	91	4	10	0	*Trissolcus basalis*
	Kitengela	95	93	0	2	0	
	Kisumu	65	53	0	10	2	
August-October 2018	Nanyuki	56	16	0	19	21	
	Naivasha	41	9	0	32	0	
	Machakos	41	39	2	0	0	*Trissolcus basalis* *Gryon sp.*
	Kitengela	53	46	0	3	4	
	Kisumu	65	63	2	0	0	*Trissolcus basalis*
August-October 2019	Nanyuki	88	76	0	5	7	
	Naivasha	17	16	0	1	0	
	Machakos	54	53	0	1	0	
	Kitengela	52	49	0	0	3	
	Kisumu	70	68	0	0	1	

possible that *T. basalis* was able to detect or locate its host in this case bagrada bug eggs due to its high chemosensory ability to locate food, mates, hosts and other resources in a complex environment as reported by Chen et al (2021).

In the case of *Gryon sp*., not much was established because of the lack of enough material for identification of the species since the parasitoid was recovered dead and destroyed from bagrada bug egg parasitoid exposed in Machakos after dissection. However, *Gryon myrmecophilu* (Ashmead) and *Gryon aetherium* Talamas (Hymenoptera, Scelionidae) have been reported with high parasitism rates on bagrada bug eggs (Felipe-Victoriano et al., 2019; Hogg et al. 2021).

Concerning the various exposure sites in this study, Kitengela, Nanyuki and Naivasha had fewer bagrada bug compared to Kisumu and Machakos where parasitoids were found. As reported by Vet (2001), the finding implies that parasitoid populations depend on their ability to deal efficiently with specific distributions of their host population and its food plants. The survival of the parasitoid generally depends on the presence of the parasitized pest. Natural enemies are important in integrated pest management strategies. Their survival is however, hampered by excessive use of pest control products thus reducing their population. From the study, Kisumu and Machakos host fields had history of organic farming practices where the use of synthetic pesticides was not intense as in Naivasha, Nanyuki and Kitengela. Organic farming is one of the major ways natural enemies such as parasitoids can be conserved. It is likely that different parasitoid species might have existed in Naivasha, Nanyuki and Kitengela, as bagrada bug host fields but continuous use of synthetic pesticides eradicated parasitoids in a bid to control the pest. Therefore, due to the presence of the host bagrada bug and limited use of synthetic pesticides in Machakos and Kisumu gave the parasitoids conducive environment for their existence. These results are in agreement with earlier report by Kareiva and Odell (1987) that natural enemies respond to host distributions and density levels.

The incubation period for bagrada bug eggs ranges from four to eight days, depending on temperature, which may impact parasitoid populations in host fields. This is in line with Reed & Perring (2012), who reported that bagrada bug nymphs can emerge after approximately three to four days at optimal temperature conditions. Further, study by Singh & Malik (1993) reported that bagrada bug eggs incubate after 3 days at 35.8 °C and after 6 days at 25.6 °C. In consistence with the current study, warmer weather condition in Machakos and Kisumu could have favoured bagrada bug population. Adult activity tends to peak during the warmer parts of the day when the temperatures are above 25 °C and below 41 °C (Reed et al., 2013). When the temperatures are outside of the range (> 41 °C or < 25 °C), bagrada bug seeks shelter near the soil surface hence difficult for the parasitoid to locate their eggs for parasitization.

Conclusions

The study results show the presence of native egg parasitoids in Kenya. While mass rearing of the parasitoid could provide an effective bagrada bug management strategy, efficacy trials on the identified egg parasitoid should be carried out in both laboratory and field setting to provide clear data on the effectiveness of the parasitoid in management of bagrada bug.

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