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Development of *Trissolcus basalis* (Woll.) egg parasitoid bulking protocol for management of macadamia stink bug *Bathycoelia distincta* (Dist.)

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Abstract

An upsurge of macadamia stink bug (*Bathycoelia distincta* Distant) in Kenya has resulted in severe damage to macadamia nuts. Its management is best achieved through use of egg parasitoid, *Trissolcus basalis*, commonly used across the globe. To manage the pest, this study was carried out to determine rearing potential of the parasitoid, for long term bulking and field releases. It was carried out in the lab using fresh eggs of *Nezara viridula*, a stink bug that is easier to rear, to provide alternative rearing media. Eggs were exposed to target parasitoid for parasitization. About 143 freshly laid *N. viridula* egg masses were collected from the laboratory-reared colonies. Each egg batch contained 44 to 135 eggs per egg mass. The results show that 59.7% were fully parasitized, 24.8% egg masses had over 50% parasitism while the remaining 15.5% egg masses recorded less than 50% parasitism. The findings also show that the discovery efficiency of the parasitoid was not influenced by egg mass size ($F=2.34$, $df=2$, $p> 0.05$). Further, it was noted that parasitism efficiency decreased significantly as number of host eggs per egg mass increased ($F=3.23$, $df=2$, $p< 0.05$). This study confirmed that it is possible to mass produce the egg parasitoid, *Trissolcus basalis,* for long term sustainable management of macadamia stink bug pest in Kenya. Further, it is recommended that the parasitoid be mass produced for classical biological control of the pest to reduce loss of the crop and enhance the country's access to global market as a result of producing quality nuts.

Keywords: Parasitism, search efficiency, *Nezara viridula*, *Bathycoelia distincta, Trissolcus basalis*

Introduction

Increased global demand for macadamia nuts has made the crop to gain popularity worldwide (Yan *et al.,* 2018). It is considered the most lucrative cash crop in Kenya after tea (Quiroz *et al.,* 2019). Macadamia is considered the most important nut crop in Kenya (Waithaka,

2001) and its production is mostly done by small scale farmers (Quiroz *et al.,* 2019). Constraints including insect pests affect nuts and lower post-harvest nut quality in macadamia (Schoeman, 2013). Macadamia stink bug (*Bathycoelia distincta* Hemiptera: Pentatomidae) is a major pest causing nut yield loss and nut quality reduction in macadamia (Muthoka *et al.,* 2008). It is monophagous and major pest of macadamia across plantations in South Africa (Fourie *et al.,* 2022). It causes premature nut fall and development of lesions on mature kernels of macadamia leading to dropping off of undeveloped nuts (Schoeman, 2013; Van den Berg Steyn, WP & Greenland, J, 1999). Probing action during feeding by stink bugs causes wounds on nuts acting as entry points for infectious fungi leading to moldy kernels (Jones & Caprio, 1990).

Repeated use of pesticides for control of *B. distincta* has resulted in high resistance levels of the pest (Schoeman, 2017). Biocontrol, which involves use of naturally occurring living organisms (Wang *et al.,* 2019), has also been explored. Several attempts to introduce and establish parasitoids into new infested areas in Australia and Hawaii have been successful due to the immigrant nature of stink bugs (Conti *et al.,* 2021). *Trissolcus basalis* is a scelionid egg parasitoid of both *Bathycoelia distincta* and *Nezara viridula* and is one of the most common egg parasitoids in North America (Moraglio *et al.,* 2021).

The populations of egg parasitoids in normal environs are fairly low. It is therefore necessary to increase their ability to invade and successfully suppress pest populations by mass rearing and releasing them into agricultural holdings (Harris *et al.,* 1991). Upon release, the parasitoids use chemical signals to seek for mating partners and later for their searching of the host to lay eggs for progressing their generations. *Trissolcus* recognizes stinkbug's eggs by presence of secretions on the egg chorion, a mechanism that enhances the kairomonal relationship between the two organisms for effective biocontrol (Colazza & Wajnberg, 1998). Previous laboratory studies of *T. basalis* revealed that it is highly efficient in host finding. It has been shown to effectively utilize volatile compounds released by the host's oviposition-induced synomones, as well as volatile and contact kairomones emitted by adult bugs (Cusumano *et al.,* 2016).

This study was carried out to identify the protocol of bulking of the egg parasitoid, *Trissolcus basalis* for management of *Bathycoelia distincta* which is a serious pest of macadamia orchards in Kenya. The end goal is to develop a sustainable classical biological control programme for the country to bring the pest to low population which have no economic impact to the crop. Such efforts will result to increased quality nuts available for trade and enhanced market access for Kenyan macadamia nuts.

Materials and methods

The research work was carried out at KALRO, National Sericulture Research Centre in Murang'a which lies within coordinates 0°59' S, 37° 04' E and 1548 m above sea level. It was carried out from July - December 2021.

Cultures of *N. viridula* were obtained from macadamia orchards at the KALRO SEEDS (formerly the Practical Training Centre, PTC). Nymphs and adults of the pest were reared separately, under controlled conditions (25 ± 2°C, 70 ± 10% RH, 14L:10D photoperiod), inside clear plastic food containers (300 × 195 × 125 mm-high) with 5-cm diameter mesh-covered holes in the laboratory. All stages were fed with seed and vegetative parts of their preferred food (soy beans and ground nuts) which was changed every 2–3 days. Ground nuts and soy bean at 50:50 ratio were used to feed *N. viridula* and water was provided throughout in soaked cotton wool balls.

Presence of *Trissolcus* in the macadamia orchards at KALRO SEEDS was determined by checking for parasitized eggs of *Bathycoelia distincta*; Freshly laid eggs are light green in colour, turning cream to pink with development (Bruwer, 1992), and black when parasitized with nymphal stages of parasitoids developing in the egg.

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Naturally oviposited *Nezara viridula* egg masses were collected biweekly. No insecticide treatments were used on the experimental sites being monitored. Egg sampling involved visual examinations of leaf surfaces of macadamia plants for approximately 3 hours. Egg masses found were labeled, taken to the laboratory for incubation to either hatch into nymphal stages or emergence of parasitoid. Eggs that remained intact after incubation were dissected to determine their contents. The following information were recorded for each egg mass collected: number of eggs per egg mass; number of nymphs emerged and number of eggs parasitized. *T. basalis* was collected from macadamia orchards at the KALRO SEEDS in Murang'a using *N. viridula* sentinel egg clusters. The collected parasitoids were reared in 85-ml glass tubes (30 mm diameter × 150 mm length) and incubated at (25 ± 1°C, 80 ± 5% RH, 15L:9D photoperiod). Selected eggs were ≤ 72 hours old. A total of 143 freshly laid *N. viridula* egg masses were collected in the lab rearing process with 18 having 40-70 eggs per mass, 84 having 71-100 eggs per mass and 41 having 101-135 eggs per mass and used during the study. Grouping of egg masses into clusters was done to determine whether egg mass size would influence parasitism efficiency. After every three days, collected egg masses were mounted, taken out and exposed in the macadamia orchards at KALRO SEEDS for parasitism by *T. basalis* for 48 hours. The 48-hour exposure allowed for complete parasitism. Egg masses were retrieved from the orchards, placed in petri dishes with moist filter paper and kept in incubators in the laboratory under controlled temperature (25±2°C) and relative humidity (65±10%) until emergence. Emerged parasitoid wasps were mass reared on honey syrup soaked in cotton wool balls and placed in petri dishes in the laboratory.

Adult wasps were fed on parasitoid diet of a solution of sugar (10%), honey (10%), benzoic acid (10%), yeast and water (Safavi, 1968). In the laboratory, 24 hours before the experiments started, 2–5 day old mated parasitoid females were isolated in small glass vials (10 mm diameter × 25 mm length) containing a small drop of the (Safavi, 1968) diet and kept in an incubator (25 ± 1°C, 80 ± 5% RH, 15L:9D photoperiod). Freshly laid (0–24 h old) *N. viridula* eggs were exposed to parasitoid females in the laboratory for 48 hours for maximum parasitism. Female parasitoids were removed and eggs incubated for hatching. Emerged male and female parasitoids were kept together to allow mating. Tally sheets of *Nezara* eggs laid, exposed to parasitism, parasitized and incubated and parasitoids emerged were recorded to track on parasitism and possibility for mass production of the parasitoid.

Results

In total, 143 *N. viridula* egg masses were collected and subjected to parasitism in the laboratory. *N. viridula* egg masses varied greatly in size, from 44 to 131 eggs with a mean of 92.4 ± 21.62 eggs per mass. Of these, 129 contained at least 1 egg parasitized by *T. basalis*. Average parasitism efficiency was found to be 90.21% (Table 1). To determine whether egg mass size had influence on parasitism efficiency, egg mass cluster size were grouped into 3: 40-70 eggs, 71- 100 eggs, and 101-135 eggs (Table 1). Discovery efficiency was not influenced by egg mass size (>F= 2.34, df = 2, $p > 0.05$). Parasitism efficiency decreased significantly as number of host eggs per egg mass increased (F = 3.23, df = 2, $p < 0.05$), as described by the regression: $y = 99.88 - 0.34x$; $R^2 = 0.42$; $p < 0.05$. There was inverse relationship between parasitism efficiency and exposed egg mass sizes.

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Table 1: Average parasitism efficiency by *Trissolcus basalis* on *Nezara viridula* eggs

Range	No. of egg masses exposed	No. of egg masses parasitized	Discovery efficiency	Parasitism efficiency, Mean ± SD
40-70	18	17	38.61	96.89 ± 3.63a
71-100	84	77	43.23	81.63 ± 29.02ab
101-135	41	35	32.96	68.97 ± 30.76c
TOTAL	**143**	**129**		**90.21%**

Means followed by the same letter are not significantly different ($p > 0.05$).

Parasitized egg masses were 129 during the study as a result of the 90.21% parasitism efficiency realized on 143 exposed *N. viridula* egg masses. *Trissolcus basalis* females fully parasitized egg masses in 59.70% of cases, whereas in 24.80% of cases egg masses were parasitized at <100% but >50%. The remaining 15.50% egg masses were parasitized at < 50% which relates closely with previous results obtained in a similar study by (Colazza & Bin, 1995) where *Trissolcus basalis* females fully parasitized 60% of the discovered egg masses, 24.76% egg masses were parasitized <100% but >50% while 15.24% egg masses were parasitized at < 50%. (Table 2).

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Table 2: *Trissolcus basalis* percentage parasitism on *Nezara viridula* egg masses.

Parasitism %	Eggs masses parasitized	% egg masses parasitized
100%	77	59.7
51% - 99%	32	24.8
< 50%	20	15.5
TOTAL	**129**	**100.0**

Discussion

The results of parasitism efficiency recorded in this study corroborates with results from Correa-Ferreira, 1994 (92.5%) and Colazza & Bin, 1995b (92.6%). Use of biocontrol is necessitated by emergence of new phytophage pests, like *N. viridula* (Catalán & Verdú, 2005) appearing in greenhouses (Canton-Ramos & Callejón-Ferre, 2010). *T. basalis* gives potential biocontrol for *B. distincta*. (Catalán & Verdú, 2005; Laumann *et al.,* 2008).

The greatly varying egg mass sizes of *N. viridula* recorded in this study corroborated results from a similar study by (Colazza & Bin, 1995). Like many other scelionids, *Trissolcus basalis* (Orr, 1988), is proovigenic with an egg potential fecundity of about 61 eggs (Mattiacci *et al.,* 1991). This agrees with results obtained in a previous study explaining the inverse relationship between parasitism efficiency and exposed egg mass size attributed to lower parasitoid egg to pest egg ratio in case of high number of eggs per egg mass exposed as hypothesized for another egg parasitoid, *Edovum puttleri* Grissell (Ruberson *et al.,* 1991). Results reflect great parasitic affinity of *T. basalis* for *N. viridula* eggs as described by Laumann *et al.,* 2009. Although no previous work on successful biocontrol of the *Bathycoelia distincta* have been documented in Kenya, the study agree with previous works by (Cook & Baker, 1983; Greathead, 1986; Greathead & Greathead, 1992) indicating biological control of insect crop pests is viable.

Conclusion

This study showed that *Trissolcus basalis* can be reared using *Nezara viridula* eggs and the adult parasitoids supported by a diet of solution of sugar (10%), honey (10%), benzoic acid (10%), yeast and water to extend their lifespan while reproducing. Such diet gives the adult parasitoids energy and time to search for the host and hence increase their parasitism rate.

Recommendations

It is recommended that mass production of the parasitoid be initiated in order to support classical biological control of the pest, which is an economic pest of macadamia, causing massive losses and reduction of market for Kenyan macadamia nuts.

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