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Efficacy and Mode of Application of Local *Beauveria bassiana* Isolates in the Control of the Tea Weevil

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EC, FMEW, JW and LM designed the study, wrote the protocol and interpreted the data. Author EC anchored the field study, gathered the initial data and performed preliminary data analysis. Authors EC, FMEW, VS and RN managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

The tea crop in Kenya ubiquitously is exposed to biotic and abiotic stresses which can be devastating. This includes damage by five species of tea weevils. Tea weevils reported to occur in Kenya include, the Tea Root Weevil (*Aperitmetus brunneus*) [Hust], Nematocerus Weevil (Nematocerus sulcalus), Systates Weevil (Systates sp.), Kangaita/Kimari Weevil (*Entypotrachelus meyeri*) [micans/Kolbe] and Nyambene Weevils (*Sprigodes mixtus*) [Hust], among others, Adult

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weevils damage tea by defoliating nursery, newly established and mature tea orchard. Kimari/Kangaita weevils are documented to occur throughout the tea growing areas of Kenya. Occasional isolated epidemic outbreaks occur causing variable level of damage by defoliating mature tea bushes. Studies were conducted to determine the efficacy of two *Beauveria bassiana* Isolate compared with a pesticide, Karate, which contains Lambdacyholothrin as the active ingredient, in two different major tea weevils occurring geographic regions namely; Kenya Tea Development Agency (KTDA) Igembe Factory catchment, Giciaro tea Farm of Meru District and Kenya Tea Development Agency (KTDA) Mununga Factory catchment. Mr. Njogu Kiruki's farm. The fields were laid out in a random complete block design (RCBD) with three replicates. The treatments were of two efficacious locally isolated *B. bassiana* isolates applied at a rate of $1x10^{13}$ conidia/ha applied with two mode of application; a Solid state fermented mixture in wheat bran and a spores liquid formulation, Karate which was sprayed on trash and the control. The effect of weevils on productivity of tea was significant with reduction in yield of between 30-33%. Similarly, Data for damage scores, percentage damaged leaves and canopy cover varied significantly ($P<0.05$) with the control having the highest damage (57%compared to 52%) and reduced canopy cover. Mature leaves showed a higher percentage of damaged leaves compared to pluckable leaves The performance of *B. bassiana* isolates, applied in a solid substrate or sprayed as conidia on the foliage, was comparable to that of Karate at the rate of 2L/ha. This study suggests the possibilities of the use of *B. bassiana* isolates to control tea weevils using either mode of application.

Keywords: Tea weevils; Beauveria bassiana; control; application mode.

1. INTRODUCTION

Besides the favourable environmental conditions prevalent in the tea growing zones of Kenya, successful tea cultivation has largely been attributed to focused research and development through adequate funding of commodity specific Tea Research Foundation and statutory marketing through auctions since 1920. However, owing to global climatic changes and non-adherence to good agricultural practices, the crop has become exposed to biotic and abiotic stresses. The devastating biotic stresses include damage by tea mites (red crevice mites, red spider mites and purple mites), scale insects (*Aspidiotus sp*, green soft scales and brown scales), weevils (Systates weevils, tea root weevils, *Entypotrachelus meyeri*), tea thrips (*Scirtothrips kenyensis*), termites, tea mosquito bug (*Helopeltis sp*), tea Crickets and chafer grubs among others arthropods [1]. The two main diseases include one major wood rot, *Hypoxylon serpens*, and a sporadic root rot caused by *Armillaria* s*pp* of fungi. The most prevalent weeds are grass weeds such as couch grass (*Penisetum sp*) and *Cynodon dactylon* [1]. Arthropods appear to be the main pest determinants of reduced tea yields

1.1 Literature Review

Tea weevils comprise about 27 species [2]. Tea weevils reported to occur in Kenya include, the Tea Root Weevil (*Aperitmetus brunneus*) [Hust], Nematocerus Weevil (*Nematocerus sulcalus*), Systates Weevil (*Systates* sp.), Kangaita/Kimari Weevil (*E meyeri*) [micans/Kolbe] and Nyambene Weevils (*Sprigodes mixtus*) [3]. Adult weevils damage tea by defoliating nursery, newly established and mature tea orchards [2,4,5]. Kimari/Kangaita weevils are documented to occur throughout the tea growing areas of Kenya. Occasional epidemic/isolated outbreaks occur causing variable level of damage [6]. Weevils are polyphagus and feed on over 14 plant families [2] crossing commodity groups making the weevil a high risk pest [7]. Presently, the management strategies against tea weevils include cultural practices and spraying with an insecticide, Karate, a synthetic pyrethroid which contains lambdacyhalothrin as the active ingredient. Hand picking is applied where the weevil population is low [8]. Biological control may offer better potential against the tea weevils [9]. Sudoi et al. [6] isolated entomopathogenic fungus, *Hirsutella* species which gave high mortality rate on Kangaita weevil. Preliminary investigation at Tea Research Foundation of Kenya (TRFK) has indicated that locally isolated *Beauveria bassiana* showed good prospects in the control of the Kangaita tea weevil (*E. meyeri*) and the tobacco cricket [10,11,3].

B. bassiana is a fungus that is found naturally in soils throughout the world and acts as a parasite on various insect species, causing white muscardine disease *B. bassiana* strains and races have successfully been used to control several pests such as: -Tobacco spider mites (*Tetranychus evensi*) Pritchard (*Acinina tetraychidae)* infesting tomatoes [12]. Botanigard an imported formulation of *B. bassiana* is being used commercially in Kenya as a biological insecticide to control a number of sucking insects including thrips, aphids and whiteflies on French beans and snow peas [13]. This fungus has shown success also in management of diamond back moth *Plutella xylostella* [14] and potato beetle *Leptimotarsa decemlinaeta*, [15,16] suggested that *B. bassiana* had a potential to persist in the environment and for horizontal transmission between weevils if used. He proposed more investigations on its ability to control populations of white pine weevil *Pissodes strobi*. *B. bassiana* has proved to be competitive with chemical insecticides for protection of forests and farms against pests [17-21] However; no proven documented report has been published on the fungus potential in the control of the tea weevils using different modes of application in Kenya. Therefore the objective of this study was to assess the efficacy and mode of application to indigenous *B. bassiana* isolates in control of the tea weevils compared to a conventional insecticide.

2. MATERIALS AND METHODS

2.1 Experimental Sites

This study to determine field efficacy of *B. bassiana* on tea weevils in Eastern tea growing region of Kenya was conducted from June 2012 to November 2013 in two different major tea weevils occurring geographic regions; (i) Kenya Tea Development Agency (KTDA) Igembe Factory catchment, Giciaro tea Farm of Meru District at an altitude of 1650 m.a.s.l. and coordinates at Lat. 0°08'S, Long.37° 54'E. Giciaro tea farm is located in Igembe sublocation, North Meru location of Meru County and about 18 km west of Maua Town. Giciaro tea farm is planted with a pure clone of TRFK 31/8 (ii) Kenya Tea Development Agency (KTDA) Mununga Factory catchment. Mr. Njogu Kiruki's farm, in Kirinyaga County is at an altitude of 1784 m.a.s.l. and coordinates at Lat. 0°27'S, Long.37° 13'E. The farm is located in Kaguyu Sub location, Mutira location of Kirinyaga Central District about 1½ kms from Mununga KTDA Tea Factory The farm (Mr Njogu's) is planted with about 3,398 tea bushes of mixed clones. The farms was previously having coffee plantation before tea establishment in years 1994 and 2003 for Njogu's and Giciaro farms respectively.

The soils in the two areas consist of well-drained, deep reddish brown to dark red friable clay with acidic humic top soil (humic nitosol) and a pH range of 4.4-5.6 [22]. The mean maximum and minimum temperatures are 23°C and 13°C respectively. It receives an average rainfall of about and above 1000 mm per year. The rainfall is bimodal and long rains are received between October and December while the short rains fall between March and June.

2.2 Experimental Design and Treatments

Experiments were set up in each site starting in June 2012 for one and one and a quarter years. The tea fields in Giciaro Farm Igembe that hosted the plots comprised of mature tea bushes of clone TRFK 31/8, which were established in 2003 with a spacing of 4 X2 ft. Mr. Njogu farm comprised of mature tea bushes of mixed clones of TRFK 6/8, PMC 51 and TRFK 31/8 which were planted with a spacing of 5 x2½ ft. Field efficacy of the isolates against the tea weevil was evaluated using three replicates in a randomized complete block design. Each replicate consisted of 5 (five) plots associated with treatments and one control: Each plot consisted of at least 20 bushes and surrounded by two rows of tea bushes as buffer zone; The treatments were of two efficacious *B. bassiana* isolates at a rate of $1x10^{13}$ conidia/ha (either applied with wheat bran on trash or sprayed on foliage) viz-a viz Isolates Bb7a &6a which were originally isolated locally in early 2012 [23] and maintained in Tea Research Foundation of Kenya (TRFK) laboratory, Karate, a synthetic pyrethroid insecticide which contains Lambdacyhalothrin 25% EC as the active ingredient was used as a standard and sprayed on trash and a control which was the formulation that contain no *B. bassiana* propagules.

NPK fertilizer application was done twice in a year at a rate of 100 kg N /ha /year. Plucking rounds were between 7-12 days. The fields were maintained weed free for the duration of the study by manual removal (either hand hoeing or hand manual removal). Pruning time was done as per KTDA schedule which is normally in July of the pruning year with either 3 or 4 pruning year cycle.

2.3 Formulation and Fungi Applications for Spraying

B. bassiana isolates were applied as a spray liquid using a modified formulation basing on modified version as in Burges [24] review. Long-term effects of the formulation ingredients on the viability and virulence of the *B. bassiana* are unknown, so the final mixing of the formulation was conducted immediately prior to application, as recommended by Goettel et al. [25,26]. All treatments were applied at intervals of once every month. The foliar sprays were applied taking care to thoroughly wet both sides of the foliage. A knapsack sprayer was used at a constant pressure of 40 psi and a Low volume flow rate of 200l/ha.

2.4 Formulation and Fungi Applications with Wheat Bran

The fungal isolates were first cultured on potato dextrose agar (PDA) at 25℃ for 10 days and stored at 4℃ until use. In preparation of the seeding inoculums, aerial conidia taken from a stock culture growing on a PDA agar plate was suspended in sterile water. The number of conidia in the suspension was counted using a haemocytometer, followed by dilution to 1.0×10^8 conidia/ml. One milliliter of the conidium suspension was inoculated into a 250 ml Erlenmeyer flask containing 100 ml of liquid media, followed by culturing at 25±0.1℃ using a rotary shaking incubator operated at 200 rpm. The resulting suspension was then used as the seeding inoculum for Solid-state fermentation (SSF) for aerial conidia production. In preparation of SSF, moistened wheat bran medium was transferred into a container and autoclaved at 121℃ for 25 min. The container with moistened solid media was then cooled, after which the seeding culture was added at 10% inoculum size (10^7 conidia/g wet medium) to each container, followed by thorough mixing using a sterilized spoon. The specimens were then incubated at room temperature and relative humidity for a period of 60 days from where it was removed mixed and broadcasted in to the fields. All the treatment applications were done once every month.

2.5 Data Collection and Analysis

Data on number of weevils was not possible because of logistic issues. Therefore, Percentage damage was collected by using a grid of 40 cm X 40 cm and the number of damage pluckable leaves versus undamaged were counted and later converted to percent damaged pluckable leaves. Similarly data for mature maintenance leaves was also collected to determine the type of leaves the weevils prefer. Data on damage score as per Weigand and bishara [27], photosynthetically active radiation (PAR) intercepted by the plant canopy as per Squire [28], ground cover following method used by Burghess [29] and harvestable fresh green weight were collected for a year. The yield was determined in the experimental plots by regular conventional plucking of two leaves and a bud. Yields of green leaf were converted to kilograms of made tea per hectare (mt/ha/year) by multiplying by a standard factor of 0.225 [8]. Data on damage score, percentage damage, PAR and yield were subjected to analysis of variance using SAS Version 9.0 and means were compared using Least significant difference (LSD) at $P \leq 0.05$.

3. RESULTS

a) Giciaro Site

Effect of Tea Weevils on Productivity of tea and Efficacy *B. bassiana* Isolates Compared to an Insecticide

The weevil species found to be occurring in Giciaro area were predominantly the *S. mixtus* (Nyambene weevils). In Giciaro trial. The cumulative made tea yield data indicated that the effect of weevils on yield was significant. Yield of tea at Giciaro tea field was significantly higher in all the plots treated with B*. Bassiana* isolates (2922.4-3214 kgs compared with plots treated with Karate (2518.5 kg) and the control (2469 kg), 30 % higher yields in isolate Bb 7a treated plots. Similarly, mean monthly damage scores on pluckable leaves varied significantly (P≤0.0075) between treatments. Plots treated with, Karate (1.11) Isolates Bb Ke 6a(1.16) and Bb Gi 7a(1.15) had lower damage score compared with the control plots(1.4). Percentage of damaged leaves varied significantly (P≤0.05) between treatments. Percentage pluckable damaged leaves on plots sprayed with Karate, and the four isolates treatments were similar and had lower number of damaged leaves compared to the control. Similar trends were portrayed by the canopy cover data and mature leaves damage score and damage percentage (Table 1), it was also observed that Infestation of *S. mixtus* in the fields seems to be sporadic i.e. A field may be infested while a neighbouring field with the same clone is not. Despite the above results, it shows that the beetle don't disperse extensively once they have found a site.

b) Mununga Site

Effect of Tea *Weevils sp*. on Tea Production and Efficacy of *B. bassiana* Compared to an Insecticide in Mununga 1st Trial from July 2012 –Sept 2013

Yield effects varied significantly (P<0.04) among treatments (Table 2). Plots treated with the Isolates (both when sprayed and applied with wheat bran as a carrier) and those sprayed with Karate had significantly (33%) higher than the control. Canopy cover varied significant among the treatments P<0.001 with the control having significantly low canopy cover. The treatments had no significant (P≤0.05) effect on Photosynthetic active radiation (PAR) although a similar trand as in canopy cover was observed. Damage score on leaves varied significantly with the different treatments (P≤0.0001). Plots treated with sprays of isolates Bb 6a, Bb7a and Karate had lower damage score compared with plots treated with the same isolates in wheat bran (wb) substrate and the control. Percentage damaged leaves varied significantly with the different treatments (P≤0.016). Percentages of both pluckable and mature damaged leaves on plots treated with Karate, both mode of application of isolates 6 and 7 were significantly (P≤0.05) lower compared with the control (Table 2).

Table 1. The mean yield (kgmt/ha) canopy cover (cm^2), damage score, (pluckable and mature) leaves, and damage, percentage (pluckable and mature) leaves on weevil infested tea and treated with *B. bassiana* in Giciaro Farm (July 2012-June 2013)

Treatments	Cul. Yield (kgmt/ha)	Canopy cm^2	Damage score pluckable	Damage score mature	% damage pluckable	% damage mature
Isolate 7 spray	3215a	7073b	1.15	1.15b	16.40b	20.62b
Isolate 7 in wheat Bran	3199a	7134b	1.15	1.17ab	22.38b	24.35b
Isolate 6 spray	3094a	7699a	1.16	1.20ab	17.50b	24.32b
Isolate in wheat bran	2922ab	8176a	1.3	1.18ab	17.70b	22.78b
Karate	2519ab	7489ab	1.11	1.09b	16.05b	19.67b
control	2469b	6223c	1.4	1.30a	28.31a	29.83a
P value	0.05	0.0001	0.0075	0.0327	0.02	0.06
% CV	12	22.27	23.25	26.12	71.52	51.21
LSD	648	789	0.16	0.18	8.06	6.90

Table 2. The mean yield (kgmt/ha) canopy cover (cm^2), damage score, (pluckable and mature) leaves, and damage, percentage (pluckable and mature) leaves on weevil infested tea and treated with *B. bassiana* in Mununga (July 2012 –Sept 2013 -Trial)

Treatment	Yield in Kg mt/ha	Canopy Cm^2	PAR	Damage rate pluckable	Mature damage rate	% pluckable damage	% mature damage
Isolate 7 spray	2018a	151423b	30.25	1.30d	1..42b	47.37b	52.69b
Isolate 7 in wheat bran	2176a	14548b	30.23	1.60b	1.60b	50.08b	51.54b
Karate	2492ab	16964a	30.52	1.41cd	1.44b	48.24b	51.68b
Isolate 6 spray	2587ab	14957b	30.12	1.42cd	1.44b	46.74b	53.48b
Isolate 6 in wheat bran	2504bc	16359a	30.34	1.58b	1.58b	50.11b	53.97b
Control	1937c	12595c	29.25	2.2a	2.13a	53.94a	57.67a
P value	0.05	<.0001	0.4557	<.0001	<.0001	0.0016	0.0008
% CV	10.46	15.76	10.28	26.45	29.22	18.30	14.02
LSD (P<05	439.48	955.98	ns	0.168	0.19	3.634	3.01

4. DISCUSSION

The study showed that, compared to controls all the pesticides (chemical pesticide and biopesticides) used (except karate in Giciaro) demonstrated their efficacy on reducing either weevil damage or yield loss as a result of high weevil infestations. The poor performance of Karate insecticide in Giciaro farm in terms of yield can be attributed to plots having low yields in the start of the experiment. This is evident in the result of damage score, canopy cover and percentage damaged leaves. The study has also shown that weevils can cause economic damage with yield loss ranging between 30-33%. The reduction may be attributed to reduced photosynthetic activity due to reduced photosynthetic organs caused by defoliation as shown by reduced canopy cover. In addition, the low PAR must also have contributed to low yield. There is documented evidence that the rate of photosynthesis (dry matter production) is largely dependent on incoming solar radiation and is proportional to the amount of solar intercepted and the efficiency to which it is converted to dry matter [28].

This results corroborated those of [30,31,17,32, 24,33,25,16,34] where the results showed that chemicals such as imidacloprid, lambdacyhalothrin and flubendiamide and biologicals, *B. bassiana* and *M. anisopilae*, significantly reduced the density of pest compared to the controls and they concluded that there is a possibility of *B. bassiana* and *M*. *anisopilae* being used as alternatives to chemical insecticides for pests control.

5. CONCLUSION

In conclusion, *B. bassiana* isolates has the potential for use in the management of tea weevils. *B. bassiana* isolates Bb 7a and isolates Bb 6 can be recommended for the management of the weevils either as sprays or by broadcasting in solid substrate like wheat bran. There is therefore need to develop further an appropriate ready formulation of these isolates for use by the tea growers affected by the weevils in times of posterity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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