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Entomopathogenic fungi (*Aspergillus oryzae*) as biological control agent of cattle ticks in Tanzania

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Ticks are the most important ectoparasites that are responsible for severe economic losses in livestock industry. The use of chemical acaricides is the most common method used to control ticks in livestock. This study was conducted to determine the efficacy of *Aspergillus oryzae* as an alternative biological agent in controlling ticks to enhance livestock productivity. The efficacy of *A. oryzae* at different concentrations was evaluated against larvae and adults of the hard tick genera *Rhipicephalus, Boophilus,* and *Amblyomma* using an immersion test under laboratory conditions. Field trials were conducted in two purposively selected cattle herds in Monduli district, northern Tanzania. *A. oryzae* at a concentration of 1×10^6 conidial/ml was sprayed on all cattle tick-infested areas. The results demonstrated a concentration-related increase in mortality for both larvae and adult female engorged ticks. The mean mortality of larvae and female engorged ticks was statistically significant at $p < 0.05$ and $p < 0.001$, respectively. Egg production was found to decrease with increased *A. oryzae* concentration. Additionally, there was a statistically significant difference in egg production index and oviposition reduction ($p = 0.009$) while there was no significant difference in egg hatching and product effectiveness at $p = 0.089$ and $p = 0.004$, respectively between the tested ticks' genera. Under field conditions, the bio-acaricide demonstrated a statistically significant tick reduction in all the treated cattle. This study concludes that *A. oryzae* has good acaricidal activity against ticks and hence, is one of the potential tick control methods for sustainable tick control schemes.

Key words: *Aspergillus oryzae*, bio-acaricide, entomopathogenic fungi, cattle, ticks, Tanzania.

INTRODUCTION

Among the arthropods of economic importance in the livestock industry, ticks are the most common ones found in the order *Acari* under class *Arachnida*. Ticks impose severe economic losses by being both blood-sucking

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ectoparasites and important pathogenic vectors of livestock diseases (Almazan et al., 2018; Jongejan and Uilenberg, 1994; Jongejan and Uilenberg, 2004; Mohammed et al., 2017; Rodriguez et al., 2018; Sumbria et al., 2018). Ticks not only transmit diseases of economic importance in livestock especially diseases caused by, protozoans, rickettsia and viruses, but also are important vectors with adverse socio-economic impacts on humans (Almazan et al., 2018; Benelli et al., 2016; de la Fuente, 2018; Jongejan and Uilenberg, 2004; Lynen et al., 2007; Salih et al., 2015; Sumbria and Singla 2017). Examples of severe economic losses caused by ticks in the livestock industry include great losses, particularly in the cattle production. In addition, tick infestations lead to poor quality of animal by-products especially hides and skins for processing industries (Adehan et al., 2018; Jongejan and Uilenberg, 1994; Lew-Tabor and Valle, 2016; Nagagi et al., 2020).

Tanzania is among the countries with experience of economic burden, caused by high costs incurred for cattle production, due to impacts of diseases spread by ticks. These include high mortalities, decreased production, treatment and control costs. A study carried out by Kivaria (2006) showed that Tanzania incurs an estimated direct cost of up-to 364 million USD due to tick-borne diseases annually. In addition, approximately, 71.4% of all cattle mortalities are due to tick-borne diseases with high mortality in calves especially in pastoral and agro-pastoral communities (Kerario et al., 2018; Kivaria, 2006; Laisser et al., 2017).

Tanzania has been using acaricides for managing and controlling ticks for decades (Adrian, 2012; Kerario et al., 2018; Laisser et al., 2017; Nagagi et al., 2020). However, these chemicals (acaricides) are neither environmentally friendly nor economically affordable by livestock keepers. Besides, apart from retaining toxic residues in meat and milk (Almazan et al., 2018; Drummond, 1976; Jongejan, 1999; Kaaya and Hassan, 2000; Lew-Tabor and Valle, 2016), their long term use leads to tick-drug resistance, high production costs, and thus posing more challenges in managing tick-borne diseases (Almazan et al., 2018; de la Fuente et al., 2016; Kaaya and Hassan, 2000; Laisser et al., 2017; Wharton, 1983). The need to explore more alternatives such as, the use of bio-acaricides, which are cost-effective and environmentally friendly, is regarded as a paramount innovative intervention for managing and controlling ticks in Tanzania.

Entomopathogenic fungi are fungi that specifically infect and often kill insects and other anthropods (Ghany, 2015; Skinner et al., 2014). They are host-specific hence are nonpathogenic to plants and leave no toxic residue on crops. Additionally, they are non-toxic to animals and humans and are environmentally friendly as compared to chemicals (Skinner et al., 2014).

Entomopathogenic fungi have been reported to be effective against ticks and appeared to be more promising than other potential biological control agents (Stafford and Allan, 2014). Entomopathogenic fungi can infect ticks, through fungal conidia attached to that penetrate into the tick cuticle leading to death (Alonso-Díaz and Fernández-Salas, 2021; Fernandes et al., 2012; Ghany, 2015; Jiang et al., 2020; Perinotto et al., 2012). For instance, fungi belonging to the species *Beauvena bassiana* and *Metarhizium anisopliae* are the most used entomopathogenic fungi for the biological control of ticks (Bittencourt, 2008; Fernandes et al., 2012; Ghany, 2015; Kaaya and Hassan, 2000; Kalsbeek et al., 1995; Perinotto et al., 2012). The use of entomopathogenic fungi may reduce the frequency of chemical acaricide application hence, reducing the cost of tick control as well as the development of tick resistance to acaricides (Jiang et al., 2020; Kaaya and Hassan, 2000; Murigu et al., 2016).

Recently, Zekeya et al. (2019), identified a new species of fungus *Aspergillus oryzae* with insecticidal activity against tomato leaf miner (*Tuta absoluta* Meyrick) and it is also considered to have bio-acaricidal activity under controlled laboratory conditions (Zekeya et al., 2020). The use of entomopathogenic fungi is reported as the eco-friendly and cheaper option that could contribute to overcoming the tick challenge in Tanzania. However, field studies for determining the susceptibility of the tick population to entomopathogenic fungi in Tanzania are limited. Therefore, the aim of the present study was to evaluate the efficacy of *A. oryzae* on eggs, larvae and engorged female ticks under controlled laboratory environment conditions and the application of bio-acaricide to rabbits as laboratory animals and cattle herds of Maasai pastoralists.

MATERIALS AND METHODS

The fieldwork for the present study was undertaken in Monduli district northern Tanzania and laboratory experiments were carried out at the Tropical Pesticide Research Institute (TPRI) located in Arusha region, Tanzania. Apart from having a bio-efficacy laboratory for livestock vector controlling, TPRI has many facilities for rearing ticks and thus, it was selected as the most convenient laboratory facility for this study Figure 1.

Tick collection and managing laboratory bioassay

Female engorged ticks of the three genera of livestock economic importance (*Rhipicephalus, Amblyomma* and *Boophilus*) were randomly collected from six cattle herds at Meserani Chini village in Monduli district. During collection, they were gently removed from cattle by bending the tick upward and forward and then exerting a steady pull. They were then placed in ventilated tubes with absorbent paper. Once in the laboratory they were incubated for 20 days to lay eggs at 28±1°C and 80% relative humidity. The eggs were kept in 2.5 cm diameter × 8 cm long test tubes, sealed with cotton wool and gauze plugs and left to hatch into larvae at 28±1°C and 80% relative humidity (Shyma et al., 2019). Tick collection was done in February 2020.

Figure 1. Map of Arusha region showing Monduli district (in red) where the fieldwork of this study was conducted.
Source: Adapted from *https://fr.wikipedia.org/wiki/District_de_Monduli.*

Bio-acaricide preparation

A. oryzae in aqueous suspension with patent number; TZ/P/2020/000119 was kindly donated by Plant Bio-defender Limited, Moshi Tanzania. The aqueous suspension of *A. oryzae* with a sample concentration of 1.0×10^8 conidial/ml was diluted using sterile distilled water to make a working solution of 2.0×10^6 conidial/ml. The working solution was further serially diluted to make test concentration as follows: 1.0×10^6, 5.0×10^5, 2.5×10^5, 1.25×10^5, 6.25×10^4, 3.125×10^4 and 1.5625×10^4 conidial/ml for efficacy testing against larva and adult stages of the tick life cycle of the three genera *Rhipicephalus, Amblyomma* and *Boophilus.*

Effect of bio-acaricide to different stages of ticks in the laboratory

***Sensitivity of A. oryzae* on larvae**

The sensitivity test procedures were carried out as described in the larvae immersion test but with minor modifications (De Sousa et al., 2020) in April 2020. Sterile distilled water was used as a control during the experiment. Prior to conducting each test, the bio-acaricide in the test concentration was agitated for 2 min using a vortex at 3000 rpm for uniformity. Thereafter, 10 ml of the bio-acaricide of each test concentration were used for testing its efficacy on larvae as follows: 3 ml of the bio-acaricide was drawn using a 10 ml pipette and transferred to a 15 cm Petri dish, then a 11 cm Whatman filter paper was placed above, and using number 3 painting brush, larvae of 14 to 21 days old of each genus were removed from the rim of the specimen tube and were distributed evenly on the Whatman filter paper in the petri dish. An aliquot of 4 ml of the bio-acaricide was then added over the larvae covered by another Whatman filter paper, creating a sandwich. Finally, 3 ml of the bio-acaricide was added and the whole experiment setup was covered by another Petri dish of the same size, and left to stand for 10 min at room temperature. After 10 min, the sandwich was opened and left to dry after initial absorption. The larvae were then taken from the Whatman filter paper and placed in the apex of a folded Whatman filter paper that was segmented and clipped using 'bulldog' clips of 5 cm long on the sides making a packet. The open end of the filter paper was closed with clips, placed in a rack and incubated at 28°C ±1 and RH≥80% in BOD incubator for 24 h. Thereafter, dead and alive larvae were counted, and the values were used for calculating percentage mortality. Larvae were deemed dead if no motility was observed. These procedures were repeated for each test concentration in triplicate for each of the selected genera.

Sensitivity of A. oryzae on adult ticks

The adult sensitivity test was carried out as described by Drummond et al. (1973) but with some small modifications. The test was done in two replicates for each concentration in each of the selected genera. Sterile distilled water was used as control. Groups of 5 engorged female ticks from each genus were weighed to get a uniform constant sample weight (De Sousa et al., 2020). The fully-engorged females were then immersed in the different concentrations of the bio-acaricide under the same procedure as for the larvae. Then, the ticks were dried using a paper towel, placed in the petri dish with the adhesive material to prevent their movements and incubated at 28°C±1, RH ≥ 80%. Mortality was monitored daily from the time of commencement of laying eggs up to finishing. The laid eggs were weighed and recorded. Failure to lay eggs was interpreted as an indication of death of the tick. The laid eggs were kept under the same conditions to hatch, and failure to hatch indicated that the egg was not viableand this was observed visually.

Then, egg production index, oviposition, product efficacy (De Sousa et al., 2020; Temba et al., 2018) and lethal dose (LC_{50} and LC_{99}) were calculated using prior calculated female's initial weight, egg mass weight and hatching percentage. Egg production index (EPI) was calculated using the equation:

$$\%EPI = (egg\ weight \div initial\ female\ weight) \times 100$$

Oviposition reduction (OR) was obtained according to the equation:

$$\%OR = ((EPI\ control\ group - EPI\ experimental\ group) \div (EPI\ control\ group)) \times 100$$

Reproductive efficiency (RE) was calculated using the formula:

$$RE = (weight\ of\ egg\ mass \times \%\ hatching\ egg \times 20000) \div weight\ of\ female$$

where 20,000 is the average number of eggs per gram.

Product efficiency (PE) was estimated by the formula (De Sousa et al., 2020; Temba et al., 2018):

$$\%PE = ((RE\ control\ group - RE\ experimental\ group) \div (RE\ control\ group)) \times 100$$

Application of bio-acaricide to laboratory animals

Rabbits were used for testing the safety of *A. oryzae* product. A total of 20 non-pregnant rabbits of both sexes were purchased for this activity and placed in cages. Four cages, each with five rabbits were supplied with water and food. Cages were named as A, B, C and D for easy identification. Each rabbit was ear-tagged for subsequent identification. Before being subjected to the experiment, rabbits were acclimatized for seven days. Prior to infecting with ticks, a collar was placed around the rabbit's neck to prevent it from scratching and removing the ticks during experimentation. Both ears were shaved to facilitate tick attachment. A total of 50 ticks were introduced into each ear and allowed to attach themselves to the rabbit's ears for two days. Once the ticks had attached themselves to the rabbits, they were treated with *A. oryzae*. Rabbits in cages A, B and C were sprayed with the bio-acaricide at a concentration of 1×10^4, 1×10^5 and 1×10^6 conidial/ml, respectively. These concentrations were used to test for any side effects of *A. oryzae* on the rabbits at the minimal, median and higher concentrations. Hand spraying was used and targeted the ears. Rabbits in cage D acted as controls and were sprayed with distilled water. Each rabbit was sprayed with 50ml of bio-acaricide (Cage A, B and C) and distilled water (Cage D), respectively. The experimental rabbits were monitored every morning for 14 days for any clinical signs and drop-off ticks.

Application of bio-acaricide on cattle under the field conditions

In the field, two pastoral cattle herds (herd size ≥50 cattle each) at DukaMbili and Shakape sub-villages in MeseraniJuu village, Monduli district were purposively selected for this study. Verbal consent was given by the farmers to use their animals. The tick population was assessed before treatment and monitored throughout the experimental period, and the data were used to determine tick reduction. Seven animals were randomly selected in each herd; five animals were marked and treated with bio-acaricide, while two animals were marked differently, in each herd were used as controls. Prior to bio-acaricide application, the number of ticks on each animal was established on the same day. The animals were restrained and sprayed with 500 ml of bio-acaricide at 1×10^6 conidial/ml on all areas infested with ticks. A higher concentration was used in the field because ticks are physically and structurally tolerant to fungal infection (Fernandes et al., 2012) and also, to overcome environmental challenges such as UV-light. The 1×10^6 conidial/ml of *A. oryzae* was used in the field because it has shown higher tick mortality in the laboratory. Close monitoring was done to establish drop-off ticks on the treatment day, the first three days consecutively and the seventh day after treatment, by counting the number of ticks before releasing the animals and after grazing A reduction in the number of ticks was interpreted to indicate tick drop-off. Similar monitoring was done for the control individuals. Re-infestation was monitored until the next spraying/application by counting ticks before and after the experiment on animals, any additional number of ticks on animal's body indicates re-infestation. The subsequent application was repeated on the 14th day to further monitor the performance of the bio-acaricide. Thereafter, a random picking of 30 engorged, female ticks from the animal's body was carried out by bending the tick upward and forward then exerting a steady pull, placed in ventilated tubes with absorbent paper and taken to the laboratory. In the laboratory, the ticks were treated under controlled environmental conditions as described earlier to assess mortality and hatching percentage. This experimentof applying bio-acaricide on cattle under field conditions was done in February and March 2021.

Data analysis

Descriptive statistics were used to summarize the data into means, percentages and standard deviations. A one-way analysis of variance (ANOVA) was used for analyzing tick mortality while the pairwise comparison test (Turkey test) was used to analyse variations between dose concentrations at a 5% significance level. Variables related to the production of eggs were subjected to Kruskal Wallis rank sum test. The probit regression analysis was used to analyze the lethal concentration 50(LC_{50}) and LC_{99} for the mortality of ticks. Normality test was done by using Shapiro test (log transformation). The critical probability level used was 0.05. All statistical analyses were carried out using R software (4.0.3).

Ethical consideration

The ethical clearance was obtained from Kibong'oto Infectious Disease Hospital, Nelson Mandela African Institution of Science and Technology, Centre for Educational Development in Health, Arusha (KIDH-NM-AIST-CEDHA) Health Research Ethics Committee (KNCHREC) as ethical clearance number KNCHREC0001 of 14th April 2020.

RESULTS

Effect of *A. oryzae* on the different life cycles of ticks

Larvae mortality

The mean larvae mortality for all genera was significant at $p<0.05$, with *Boophilus* having the highest mean mortality (Table 1). However, the difference in larvae mortality between *Rhipicephalus* and *Amblyomma* was notsignificant (p = 0.512). The effect of *A. oryzae* on larvae was so high that led to the lethal concentrations in larvae mortality to be at least 50 and 99% for the three

Table 1. Larvae mortality due to *A. oryzae* exposure after 24 h done at TPRI in April 2020.

Tick genus	Mortality (Mean ± SD)%
Boophilus	95.57^{b}±3.25
Rhipicephalus	84.95^{a}±10.71
Amblyomma	87.90^{a}±9.89

$F_{(2, 60)}$ =8.484, p = 0. 0006.Genera bearing the same letter (superscript) show non-significant variation while those with different letters show significant variation.
Source: Research data (2022)

Table 2. The lethal concentration (LC_{50} and LC_{99}) of *A. oryzae* against *Boophilus*, *Rhipicephalus* and *Amblyomma* larvae done at TPRI in April 2020.

Genus of tick	LC_{50} (conidial/ml)	95% CI	LC_{99} (conidial/ml)	95% CI	R^2
Boophilus	99.610	5.431-473.55	9.8×10^{5}	4.7×10^{4} -3.5×10^{6}	0.36
Rhipicephalus	5.0×10^{3}	2.1×10^{3}- 8.8×10^{3}	3.0×10^{6}	1.4×10^{6} -1.0×10^{7}	0.55
Amblyomma	3.9×10^{3}	2.2×10^{3} -6.0×10^{3}	1.6×10^{6}	1.0×10^{6}-3.0×10^{6}	0.50

Source: Research data (2022)

genera as shown in Table 2. A concentration related increase in mortality of larvae was also observed in all three genera (Figure 2).

Adult tick mortality

Results showed that female engorged ticks respond differently when exposed to different concentrations of *A. oryzae*. Few female engorged ticks died before they began laying eggs. A concentration-dependent increase in larvae mortality rate was observed in all genera, although 100% mortality was not observed even at the higher tested concentration (Figure 3).

Variation in the mean female engorged tick mortality rate among all genera was significant at $p < 0.001$ (Table 3) although by using post hoc test of *Boophilus,* the mean mortality rate was found to be significantly higher than that of *Rhipicephalus* and *Amblyomma* ($p < 0.001$).

The lethal concentration of *A. oryzae* for female engorged tick mortality rate in all genera was established as shown in Table 4.

By visual observation, it was found that the hatching percentage lowered to 1% on 2.5×10^{5} conidial/ml concentration although the variation across all genera was not significant (P=0.89, Table 5). Similarly, there was a high reduction in oviposition with the increase in concentration of the bio-acaricide, which was significantly different (P=0.009) across the genera.

The egg production index was found to decrease as the product concentration increased with *Boophilus* having the lowest egg production index followed by *Rhipicephalus* and *Amblyomma*. Although variation in egg production index and oviposition reduction was significant at p=*0.009*, variation in egg hatching and product effectiveness across the general was not significant (*p=0.89* and *p=0.004,* respectively) (Table 5).

Response of *A. oryzae* application to animal behavior

Neither mortality nor signs of toxicity were observed in the rabbits subjected to all the tested concentrations of the bio-acaricide. Their behaviors as well as physiological performance were generally normal throughout the study period.

A delay in the feeding habits of the ticks was observed as the concentration increased. The ticks on the control group were fully engorged after three days of incubation and dropped from the rabbits' bodies whereas, it took 7, 10 and 14 days for the treated groups, respectively to be fully engorged. There was a decrease in the hatching of the laid eggs in the treated groups (1×10^{4}conidial/ml and 1×10^{5} conidial/ml) and no hatching for eggs laid by engorged ticks exposed to 1×10^{6} conidial/ml.

Effect of *A. oryzae* on ticks under field conditions

It was observed that the number of ticks gradually decreased from day one to day seven on animals treated with *A. oryzae* as compared to control groups. Variation

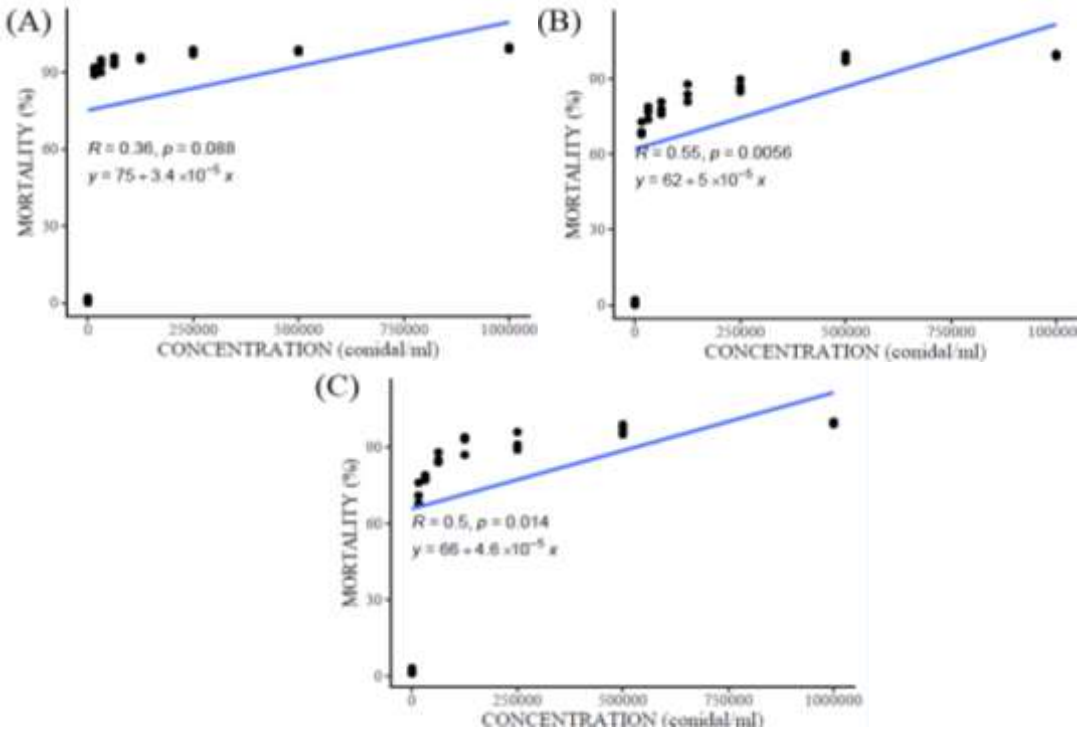

Figure 2. Effectiveness of *A. oryzae* on larva mortality at different concentrations. *Boophilus* (A), *Rhipicephalus* (B) and *Amblyomma* (C) done at TPRI in April 2020.
Source: Authors

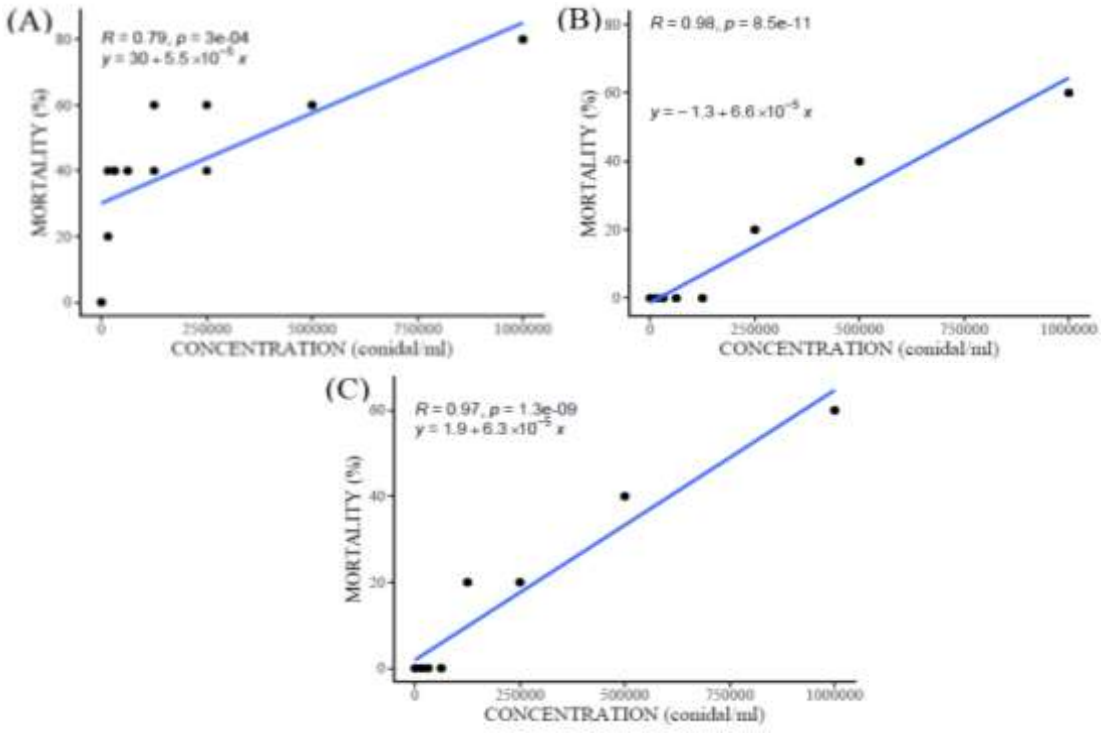

Figure 3. *A. oryzae* sensitivity across all genera; *Boophilus* (A), *Rhipicephalus* (B) and *Amblyomma* (C).
Source: Authors

Table 3. Mean mortality of female engorged ticks of three genera after exposure to treatment done at TPRI in April 2020.

Genus of tick	Mortality (Mean ± SD) %
Boophilus	62.86^{b}±23.90
Rhipicephalus	21.90^{a}±24.42
Amblyomma	23.81^{a}±24.99

$F_{(2, 60)} = 18.78$, $p < 0.001$. Genera bearing the same letter (superscript) show non-significant variation while those with different letters show significant variation.
Source: Research data (2022).

Table 4. The lethal concentration (LC_{50} and LC_{99}) of *A. oryzae* against *Boophilus*, *Rhipicephalus* and *Amblyomma* engorged ticks done at TPRI in April 2020.

Genus of tick	LC_{50} (conidial/ml)	95% CL	LC_{99} (conidial/ml)	95% CI	R^2
Boophilus	5.3×10^{4}	2.2×10^{4}-9.3×10^{4}	7.9×10^{6}	1.9×10^{6} -2.6×10^{8}	0.79
Rhipicephalus	5.7×10^{5}	4.5×10^{5}-8.0×10^{5}	1.0×10^{7}	5.1×10^{6} -3.2×10^{7}	0.98
Amblyomma	5.3×10^{5}	3.4×10^{5}-1.0×10^{6}	1.2×10^{7}	3.8×10^{6} -1.6×10^{8}	0.97

Source: Research data (2022)

Table 5. Effect of *A. oryzae* on variables related to the production of eggs done at TPRI in April 2020.

Variable	Genus of Tick			Kruskal-Wallis Rank Sum Test	
	Amblyomma	***Boophilus***	***Rhipicephalus***	**χ^2-value**	**P-value**
Egg production index	39.0^{a} ± 10.0	0.76^{b} ± 0.44	17.0^{ab} ± 19.0	9.3803	0.009185
Reduction of oviposition	39.0^{a}±16.0	99.0^{b}±0.7	72.0^{ba}±31.0	9.3864	0.009157
Egg hatching	9.4a±13.0	3.3a±3.3	5.7a±7.1	0.22476	0.8937
Product effectiveness	93.0a±10.0	100.0a±0.076	98.0a±3.0	10.879	0.004342

Descriptive statistics (mean±SD); Kruskal-Wallis Rank Sum Test followed by post hoc analysis using Bonferroni test at 5% confidence level, genera with different letters (superscript) show significant difference, with same letter indicate none significance.
Source: Research data (2022)

Table 6. Effect of *A. oryzae* product on ticks under field conditions done in February 2020 at Monduli district.

Treatment	Number of ticks (Mean±SD)
Before	107.0^{c}±35.39
After	-
Day 1	103.2^{c}±35.15
Day 3	42.4^{ab}±19.39
Day 7	1.6^{a}±2.07
Day 14	55.4^{b}±17.81

$F_{(4, 20)} = 15.3$, $p < 0.001$ Days bearing the same letter (superscript) show non-significant variation while those with different letters show significant variation.
Source: Research data (2022)

in ticks mortality across different herds after 14 days was significant at $P<0.001$ (Table 6) with day seven being observed to have the least number of ticks. After 14 days, new ticks were observed to re-infest the cattle although

the number was less than those on the control group.

Ten of the 30 engorged female ticks that were taken to the laboratory died after two days. The remaining 20 engorged female ticks under controlled laboratory conditions laid eggs but none of the eggs hatched.

DISCUSSION

The present study was carried out to evaluate the acaricidal activity of *A. oryzae* in three genera of ticks, namely: *Rhipicephalus, Boophilus* and *Amblyomma*. Findings from the study have revealed that *A. oryzae* induces mortality in tick larvae and adults. Additionally, *A. oryzae* was found to lower egg production capability and viability of eggs. The tick mortality rate was found to increase with an increase in the concentration of *A. oryzae* in all the three genera, and a 100% mortality of larvae was observed at 1×10^6 conidial/ml concentration of *A. oryzae*.The potential acaricidal activity of entomopathogenic fungi such as *B. bassiana* and *M. anisopliae* has also been reported by other workers on genus *Rhipicephalus* (*R. microplus)* (Bernardo et al., 2018; Fernandes et al., 2012; Perinotto et al., 2012; Pirali-Kheirabadi et al., 2007). The mortality of ticks in this study was attributed to the virulence effect of *A. oryzae,* as also reported by Zekeya et al. (2019).

This study further assessed the ability of *A. oryzae* to cause the mortality of tick larvae. Larvae mortality was seen to be *A. oryzae* concertation-dependent. It was revealed that the LC_{50} and LC_{99} values resulted in a higher activity of *A. oryzae* on larvae than in engorged female ticks. This finding agrees with observations in other studies on entomopathogenic fungi on their effect on tick larva (Fernandes et al., 2012; Fernandes and Bittencourt, 2008). This observation indicates that *A. oryzae* can be regarded as a promising bio-acaricide since it has shown acaricidal effects on all stages of the tick life cycle.

Both mean larva mortality and mortality of engorged ticks were significantly higher in *Boophilus* compared to other genera. In both cases, *Rhipicephalus* responded the least. This could be due to a low chitin composition of *Boophilus* cuticle thereby influencing the penetration of the fungi (Flynn and Kaufman, 2015; Hackman, 1975; Hackman and Goldberg, 1987), since the mode of action was through penetration of the fungus on the cuticle of the tick and infection due to symbiotic relationship of *A. oryzae* with the host causing it's death like for other entomopathogenic fungi (Alonso-Díaz and Fernández-Salas, 2021; Fernandes et al., 2012; Fernandes and Bittencourt, 2008; Jiang et al., 2020; Perinotto et al., 2012; Zekeya et al., 2020).

The present study also observed that the egg production index decreased as *A. oryzae* concentration increased, with *Boophilus* having the least index followed by *Rhipicephalus* and *Amblyomma,* respectively. Furthermore, the hatching percentage was found to relate inversely with product concentration. This may be attributed to infertility effects caused by entomopathogenic fungi (Ghany, 2015).Further it has been reported that the fungus impairs ovary development, thus, egg production rates (Ghany, 2015; Wasinpiyamongkol and Kanchanaphum, 2019). These findings suggest that the use of *A. oryzae* may reduce the tick population with subsequent reduction of the prevalence and burden of tick-borne diseases.

The response to changes in animal behavior after application of *A. oryzae* on their bodies was assessed by using experimental rabbits. There was no behavioral and physiological difference between the treated animals and the controls. Generally, the physiological activities of the animal were normal, which suggests that *A. oryzae* may not have toxic effects on the rabbits. This agrees with other studies which indicated that *A. oryzae* and other entomopathogenic fungi have no effect on non-target organisms and are environmentally-friendly (Fernandes and Bittencourt, 2008; Ghany, 2015; Kaaya and Hassan, 2000; Kalsbeek et al., 1995; Stafford and Allan, 2014; Zekeya et al., 2020, 2019; Zhang et al., 2015).

To the best of our knowledge, this is the first field trial to study the effect of *A. oryzae* in Tanzania. The bio-acaricide (*A. oryzae*) has shown to be effective in the field at a concentration of 1×10^6 conidial/ml which is lower than the recommended concentration by Fernandes and Bittencourt (2008), Alonso-Díaz and Fernández-Salas (2021) and Perinotto et al. (2012) that was 1×10^9 conidial/ml. These findings indicate that the bio-acaricide is effective in reducing the tick population on cattle under field conditions. Tick reduction on the observed herds was noticed up to day seven which had the least number of ticks on study animals. Tick re-infestation started to be noticed thereafter. This suggests that *A. oryzae* has a residual effect of up to seven days after application. This finding implies that the application of the *A. oryzae* in the field can be carried out twice or thrice a month for the effective control of ticks. Since *A. oryzae* has shown high efficacy against tick infestation, it may be considered as one of the bio-acaricides for integrated management of ticks in the livestock sector.

This study has also revealed that *A. oryzae* has a delayed acaricidal effect like other entomopathogenic fungi. It took up to three days before its effect was observed when compared to chemical acaricides which are rapid and take a few hours to show effect. This delay may cause some farmers not to have confidence in its efficacy. Further studies on the efficacy of *A. oryzae* need to be undertaken to explore the potential for improving the same by using molecular techniques, nanotechnology, or other technologies to enhance its uptake. Improvement of other entomopathogenic fungi has been pointed out in other studies (Fernandes et al., 2012; Leger and Wang,

2010; Maina et al., 2018; St Leger et al., 1996; Sun et al., 2011; Winder et al., 2003).

Unavailability of cattle herds for field studies due to drought season, which caused migration of herds to other places in search for pasture and water was a limiting factor, and as a result only two herds were used. This impacted on the volume of data collected for field studies.

Conclusion

Findings from this study indicate that, *A. oryzae* both in the laboratory and under field conditions had acaricidal effects and hence, it can be considered as a promising bio-acaricide. The fungal product has shown significant effects on eggs and larvae, thus breaking the tick's life cycle. The use of this product may reduce chemical acaricide application and thus, protect the environment from chemical residues accumulation, overcome tick resistance and reduce costs associated with the use of chemical acaricides, hence it has to be considered as an alternative in the integrated management of ticks.

Further field studies in different geographical locations should be carried out to further validate the bio-acaricidal of the *A. oryzae* as environmental conditions may affect the susceptibility of ticks to fungi. Moreover, more studies should be done on the virulence, safety, shelf life and efficacy of *A. oryzae* for the potential management of ticks. Targeting eggs and larvae of the three cattle tick genera may pave the way to managing tick-borne diseases and therefore more systematic field studies to further validate these findings are required.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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