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Susceptibility of Different Species of Ticks (Acari: Ixodidae) to an Entomopathogenic Fungus in Tanzania

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Abstract

Ticks and tick-borne disease cause severe skin damage on livestock as well as wildlife mortifying animal health and byproduct for processing and tourism industries. Management of ticks by conventional acaricidal is environmentally and economically unaffordable in Tanzania. This study evaluated the effectiveness of a novel entomopathogenic fungi *Aspergillus oryzae* (TZ/P/2018/000035) against three species of ticks (Acari: Ioxididae); *Rhipicephalus appendiculatus, Hyalomma anatolicum* and *Amblyomma gemma* by spraying 0.2 mL/tick of 1×10^6, 1×10^7, 1×10^8 conidia/mL of *A. oryzae* and control (water and 0.5% triton x-100) in 35.5°C and 85% RH repeated at 20.5°C and 70% RH in the laboratory conditions at Nelson Mandela African Institution of Science and Technology, Arusha. Results showed that at 1×10^8 conidial/mL, *A. oryzae* caused high mortality rate averaging 88.2%, 72.5% and 67.9% within 6.25 ± 0.75 days, 7.55 ± 0.59 days and 11.9 ± 0.65 days in *H. anatolicum R. appendiculatus* and *A. gemma* respectively, whereas in control the highest mortality rate reached 12.5%, 11.0% and 6.5% after 22.50 ± 1.2, 24.6 ± 0.9 and 28 ± 2.9 days in *R. appendiculatus, H. anatolicum* and *A. gemma* respectively at 20.5°C and 70% RH. It was also revealed that at 1×10^8 conidial/mL of *A. oryzae* reduced oviposition rate in *A. gemma* whereby 94.8 ± 10.74 eggs/female were laid compared to control that laid 354.15 ± 42.65 egg/female. Again, eggs averaging 166.20 ± 7.5 eggs/female were laid in *H. anatolicum* treated with *A. oryzae* at 1.0×10^8 conidia/mL compared to control that laid eggs averaging 416.25 ± 21.71/female in cold. This study revealed that *A. oryzae* was effective for control of ticks could be applied in agricultural fields to protect animal from tick's damage consequently improving animal products in processing industry in Tanzania.

Keywords: Animal Health; *Aspergillus oryzae*; Entomopathogenic fungi; Hides and skin damage; Tick diseases; Tanzania

Introduction

The occurrence and diversity of ticks' species and associated predicaments affect animal husbandry, wildlife and allied industry in Africa [1-3]. Hard ticks are reported as key parasites of cattle, goat and sheep causing high economic loss in Africa including Tanzania [4-6]. The most common and serious ticks of East Africa are *Amblyomma gemma*, *Rhipicephalus appendiculatus* and *Hyalomma anatolicum* affecting both domesticated and wild animals [7,8]. They affect animal health by direct parasitism and transmission of Tick Borne Diseases (TBDs) leading to low quality of animal products including milk, meat poor hides and skins due to lesion hindering utilization of animal products in industries [7,9-12]. The impact of hard ticks on skins is noticeably as they puncture direct through sucking blood leaving the skin wounded making hides unsuitable for tannery industry in Africa [12-14]. The deprived and reject hides and skins by tanning industry lower manufacturing capability and exportation of leather products affecting economy of the country [15-17]. Impacts of ticks do not end on large mammals but also small ruminant and birds including ostrich leading to severe skin damage and loss [13,18-21]. However, poor animal keeping and grazing method such as free range landraces especially in pastoralist communities have been reported to amplify the problem in Africa including Tanzania [22-24]. The hard ticks especially the *A. gemma* and *R. appendiculatus* are the main cause of skins and hides damage in Africa [25,26] whereas *H. marginatum* infest bird skin as well. The overall effect of tick on animals and birds lead to downgrading of hides and skins and finally rejection by processing industries [16,15]. In Somalia and Ethiopia for instance, Ticks and Tick-Borne Diseases (TTBDs) have been reported to have high impact on tannery industry leading to sluggish in leather industry [27-29].

Management of ticks has been solely based on chemicals through spray and dipping of animals in chemical such as organophosphate and organochlorine where resistance has been reported as well as negative impact to animals and environment [30-32]. Several studies have conducted is searching for new acaricidal compound for effective control of tick [33]. However, use of alternative methods such as botanicals and biocontrols has been reported to improve the quality of animal skin [18,31]. A study by Kalala W, et al. [34] reported the potential of *Commiphora swynnertoni* on control of tick in Tanzania. Other studies reported the effect of entomopathognic fungi such as *Beauvaria bassiana, Metarhizium anisopliae, Lecanicillium salliotae* and *Lecanicillium lecanii* against ticks [35-40] whereas *A. oryzae* has been reported to be effective against Camel tick eggs [41]. However, none of the study reported the acaricidal activity of Aspergillus oryzae on three deadly species of ticks namely; *Amblyomma gemma Rhipicephalus appendiculatus* and *Hyalomma anatolicum* (Korch, 1844) in Tanzania. It is in this vein that new isolates of *A. oryzae* was screened for is efficacy against three species of ticks in Tanzania.

Material and Methods

Collection of ticks and identification

Ticks were collected at Oldoinyo was village in Arusha region and identification by the Tropical Pesticides Research Institute (TPRI) in which three species namely as *Amblyomma gemma,* the biggest female tick, *Rhipicephalus appendiculatus and Hyalomma marginatum* were identified. Fungal isolate; *Aspergillus oryzae* (TZ/P/2018/000035) previously isolated for management of *Tuta absoluta* was offered by Plant Biodefender Limited, Moshi-Tanzania

Preparation of *A. oryzae* concentration

Working concentration were prepared and selected based on [42] methods. Fungal isolate was sub-cultured on Potato Dextrose Agar (PDA) in Petri dishes to confirm its viability. After 5 days of full maturation, spores were gently scrapped from the media by suspending into 10 mL sterile distilled water with 0.1% Triton X-100 per Petri dish to make a stock suspension. After mixing suspension in a flask, the concentration of stock solution was accessed by a haemocetometer neubauer (Manfield, German) that was 1.1×10^9 conidial/mL which was further diluted to get working concentrations of 1.0×10^6 conidia/mL, 1.0×10^7 conidia/mL and 1.0×10^8 conidia/mL by the addition of sterile distilled water.

Acaricidal activity of *A. oryzae* on adult ticks

Twenty ticks, ten (10) engorged female and (10) male adults' ticks with an average weight of (2.62 ± 0. 60g) *A. gemma*, (1.5 ± 0.27g) *R. appendiculatus* and (0.9 ± 0.25g) *H. anatolicum* were sprayed by 0.5 ml containing 1×10^7 and 1×10^8 conidial/mL of *A. oryzae* and control (containing water and 0.5% triton X-100). Treated ticks were placed in plastic lunch box (21 cm × 12 cm × 7 cm) lined with moist paper towel. All treated ticks were fed once a day by fresh blood by using plastic syringe. Tick mortality was recorded after every 48 hours whereas dead ticks were removed and incubated on moist petri dish for observation of fungal growth. The experiment was replicated four times. For the female ticks' bioassay, mortality was recorded post treatment to observe effect of treatment on oviposition until death.

Effect of *A. oryzae* on oviposition

Ovicidal effect of biopesticides was conducted according to [41] method with minor modification whereby fully engorged female ticks were exposed to at 1×10^6, 1×10^7 conidial/mL, 1×10^8 conidial/mL and control by spraying 0.5 ml of suspension on ticks. Oviposition inhibition rate was determined by counting number of eggs laid per day dividing by number of treated engorged female ticks to obtain average eggs laid. Weight of ticks before and after laying eggs was recorded to establish relationship between weight and oviposition rate.

Data and statistics analysis

Data on efficacy biopesticides against three species of ticks were presented as adult mortality rate, adult survival duration and oviposition rate that were analysed with the Proc GLM procedure of SAS, version 9.1 (SAS Institute, Cary, NC, USA) and tested for normality and homogeneity of variance. Mortality rate was transformed to log-10 for obtaining normally distributed data sets with equal variance. Adult survival duration and oviposition rate were subjected to analysis of variance (ANOVA) whereas adult mortality rate was subjected to Kruskal Wallis. Bonferroni was used to separate mean difference of adult mortality rate whereas Tukey's Honest Significant difference (HSD) was used to separate mean differences of Adult survival duration and oviposition rate at 5% level of significance.

Results

Time effect of *A. oryzae* on species of ticks

Effect of treatment on survival days of ticks varied significantly (p=<0.0001) between species of ticks at cold (20.5°C and 70% RH) and warm (35.5°C and 85% RH) conditions, with high activity in all concentrations of *A. oryzae* compared to control. *A. oryzae* treated ticks had lower survival duration in which survival time was reduced up to 6.25 ± 0.75 days in *H. anatolicum* than in *A. gemma* that survived for 11.9 ± 0.65 days whereas *H. anatolicum* and *A. gemma* in control survived for 31.95 ± 2.17 days and 41.35 ± 1.66 respectively (Table 1). However, the effect of treatment on species of ticks was insignificant (p=0.30) although *A. gemma* survived longer compared to *R. appendiculatus* and *H. anatolicum* in both cold and warm conditions (Table 1).

Effect of *A. oryzae* on oviposition rate of engorged female ticks

The effect of treatment on mortality of engorged female ticks was evaluated by species due to variation in species fecundity. The effect of treatment on oviposition rate of engorged female *R. appendiculatus* varied significantly (p=<0.0001) in both warm and cold in which at warm temperature high oviposition rate was observed.

A. gemma treated with *A. oryzae* at 1.0×10^8 conidial/ml laid few eggs in cold temperature in 94.8 ± 10.74 compared to control that laid up to 354.15 ± 42.65 egg/female whereas an average of 166.20 ± 7.5 eggs/female were laid by *H. anatolicum* treated with *A. oryzae* at 1.0×10^8 conidial/ml compared to control that laid eggs averaging 416.25 ± 21.71/female in cold condition (Table 2). In *H. anatolicum* the oviposition rate was significantly (p=<0.0001) different between treatments in warm and cold condition respectively, whereas *A. oryzae* at all concentration treated ticks laid few eggs than control (Table 2).

Effect of *A. oryzae* on mortality of female engorged ticks

The effect of treatment on mortality of engorged female ticks was evaluated by species due to variation in species morbidity. The effect of treatment on mortality of engorged female was significantly (p=<0.0001) warm and cold condition respectively, in which *A. oryzae* at 1.0×10^8 conidia/ml caused mortality within few days compared to lower doses 1.0×1^6 conidia/ml and 1.0×10^7 conidia/ml and control (Table 3). The effect of treatment on mortality of *H. anatolicum* varied significantly in both warm and cold condition (p=<0.0001), respectively (Table 3). There was also a significant (p=<0.0001) effect of treatment on survival of engorged female in warm and cold condition whereas *A. oryzae* at 1.0×10^8 conidia/ml induced up to 88.2%, 72.5% and 67.9%

in *R. appendiculatus*, *H. anatolicum* and *A. gemma* respectively within 3 to 6 days compared to control where 12.5% mortality was reached in warm condition (Table 3).

Virulence of *A. oryzae* on ticks

From dead ticks treated with *A. oryzae* was observed and reisolated by placing dead ticks on moist petri dishes incubated at 30°C. After 3 to 10 days, mycelia developed on ticks cuticle treated with *A.oryzae* and sporulated covering the dead tick indicating the pathogenic activity on ticks. However, none fungal spore was observed to germinate from dead ticks in control plates (Figure 1).

Discussion

Animals are good source of food, income through tourism whereas byproducts especially hides and skins are essential raw materials in leather processing industry [43,44]. Ticks injuries and flushes on animal hides and skins lead to massive economic losses and rejection of raw material in tannery industry [16]. In Tanzania, hard tick *A. gemma* is common in cattle and wild animals causing high rate of ricketisia whereas *R. appendiculatus* occur in several hosts infesting appendages and ears [45,46]. Although use of chemical acaricide is prominent in Tanzania, it is economically and environmentally expensive [5]. This study revealed the potential of new

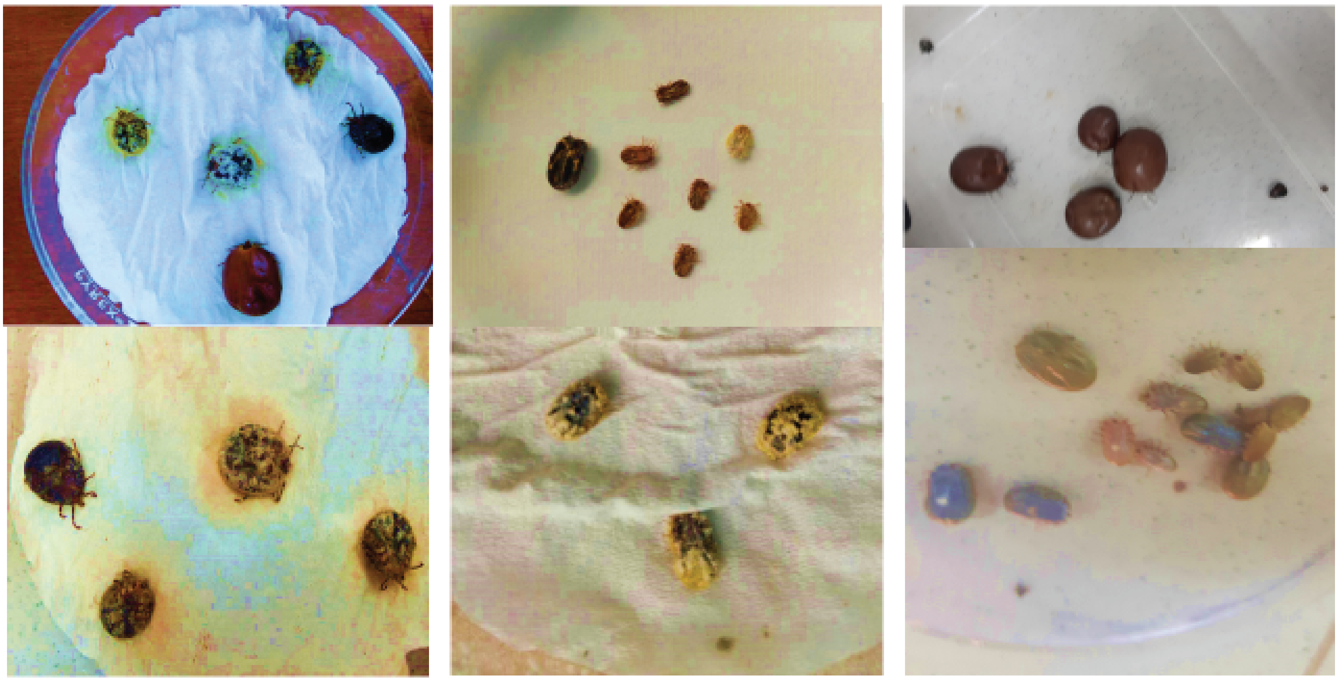

Figure 1: Comparison between *A. Oryzae* treated ticks (A and B) and Control (C) whereby fungal spore propagation on cuticles of *A. gemma* (A), *R. appendiculatus* and *H. anatolicum* (B) whereas no fungal sporulation on *A. gemma, R. appendiculatus* and *H. anatolicum* in control (C) after (C) 10 days post mortality.

Table 1: Effect of A. oryzae on survival duration of three species of ticks after exposure to treatment conditions. Different letters show the significant difference where as similar letters show no difference between treatments at p<0.005.

Experimental conditions			Tick species		
Treatment	Temperature and RH (%)	Concentration (conidia/mL)	*Amblyomma gemma*	*Hyalomma anatolicum*	*Rhipicephalus appendiculutus*
Control	20.5°C+70	0.0	41.35 ± 1.6a	31.95 ± 2.17a	27.75 ± 1.4a
	35.5°C+85	0.0	28 ± 2.9ab	24.6 ± 0.9ab	22.50 ± 1.2a
A. oryzae	20.5°C+70	1.0×10^6	16 ± 0.8b	11.0 ± 0.5b	13.07 ± 0.5b
	35.5°C+85	1.0×10^6	12.9 ± 1.4bc	9.5 ± 0.3b	12.6 ± 0.3b
A. oryzae	20.5°C+70	1.0×10^7	12.3 ± 1.5c	9.05 ± 0.4b	12.0 ± 0.3b
	35.5°C+85	1.0×10^7	12.6 ± 0.5c	8.3 ± 0.6b	11.6 ± 0.4b
A. oryzae	20.5°C+70	1.0×10^8	11.1 ± 0.9c	7.9 ± 0.3b	7.7 ± 0.4c
	35.5°C+85	1.0×10^8	11.9 ± 0.6c	7.5 ± 0.5b	6.25 ± 0.75c
P-value			<0.0001	<0.0001	<0.0001

Table 2: Effect of *A. oryzae* on oviposition rate of three species of ticks after exposure to treatment conditions. Different letters show the significant difference where as similar letters show no difference between treatments at p<0.005.

Experimental conditions			Tick species		
Treatment	Temperature and RH (%)	Concentration (conidia/mL)	*Amblyomma gemma*	*Hyalomma anatolicuma*	*Rhipicephalus appendiculutus*
Control	20.5°C+70	0.0	354.15 ± 42.6a	416.25 ± 21.71a	322.95 ± 44.25a
	35.5°C+85	0.0	377.5 ± 33.7a	428.55 ± 11.90a	355.95 ± 51.11a
A. oryzae	20.5°C+70	1.0×10^6	306.9 ± 1.40b	367.25 ± 11.50b	301.25 ± 15.20b
	35.5°C+85	1.0×10^6	375 ± 13.75b	359.50 ± 9.39b	310.6 ±12.0b
A. oryzae	20.5°C+70	1.0×10^7	218.2 ± 1.51bc	322.25 ± 8.00bc	227.9 ± 22.2c
	35.5°C+85	1.0×10^7	276.6 ± 19.7bc	212.31 ± 7.28bc	254.6 ± 21.96c
A. oryzae	20.5°C+70	1.0×10^8	94.8 ± 10.74c	166.20 ± 7.52c	105.7 ± 13.67d
	35.5°C+85	1.0×10^8	138.8 ± 12.5bc	198.5 ± 6.20bc	110.16 ± 11.46d
P-value			<0.0001	<0.0001	<0.0001

Table 3: Effect of *A. oryzae* mortality rate of three species of ticks after exposure to different treatment conditions. Different letters show the significant difference where as similar letters show no difference between treatments at p<0.005.

Experimental conditions			Tick species		
Treatment	Temperature and RH (%)	Concentration (conidia/Ml)	*Amblyomma gemma*	*Hyalomma anatolicum*	*Rhipicephalus appendiculutus*
Control	20.5°C+70	0.0	4.5%a	10.5%a	8.2%a
	35.5°C+85	0.0	6.0%a	11.0%a	12.5%a
A. oryzae	20.5°C+70	1.0×10^6	36.2%b	41.0%b	40.7%b
	35.5°C+85	1.0×10^6	39.9%b	51.5%b	53.2%b
A. oryzae	20.5°C+70	1.0×10^7	48.5%bc	57.0%bc	58.5%bc
	35.5°C+85	1.0×10^7	53.6%bc	57.3%bc	64.6%bc
A. oryzae	20.5°C+70	1.0×10^8	57.4%c	62.5%c	68.7%c
	35.5°C+85	1.0×10^8	67.9%bc	72.5%b	88.2%bc

isolate of *A. oryzae* for control of ticks. This study revealed that *H. anatolicum* and *R. appendiculatus* were more susceptible to fungal *A. oryzae* compared to *A. gemma* that laid higher number of eggs and survived longer. However, at high concentration of 1.0×10^8 conidial/mL both warm and cold conditions *A. oryzae* had high activity on three species of ticks whereas *R. appendiculatus* was more susceptible to than *H. anatolicum* and *A. gemma* [47]. Less susceptible of *A. gemma* at all treatment conditions could be due to high food reservoir keeping it firmer and more active for long time than other ticks and resist to acaricidal in most East African countries [26].

Other studies have also revealed the potential of entomopathogenic fungi for control of several species of ticks [38,48]. Acaricidal activity of entomopathogenic fungi including *Scopulariopsis brevicaulis* on *Hyalomma anatolicum* and *Amblyomma spp* has also been reported [48-50]. This study revealed that *A. oryzae* was more virulent compared to control on ticks although the infectivity increased with rise in concentration in all species of ticks. The mode of action was through cuticle fungal penetration and infection as *Aspegillus* species like other fungal spp developed a symbiotic relationship with host ticks and caused pathogenic effect [51].

Other studies reported the activity of *M. anisolpliae* and *B. bassiana* against the deadly *H. anatolicum* in laboratory [52]. However, more studies show that a combination of entomopathogenic fungi with other compounds increases virulence toward insect in which *B. bassiana* and acaricidal showed enhanced acaricidal activity [53]. In current study, the oviposition rate was very low in ticks treated with *A. oryzae* compared to control in which high number of eggs were laid this could be due to infertility effect caused by entomopathogenic fungi [51]. Despite of *A. gemma* having the highest weight than *R. appendiculatus* and *H. anatolicum*, high number of eggs were laid by *Hyalomma* species due to high fecundity rate at warm laboratory condition in which other studies revealed similar situation. Low oviposition rate was observed in fungal treated ticks in contrast to control which could be due to virulence effect of *A. oryzae* that caused death prior to oviposition [53]. Even though *A. gemma* possessed heavy weight than *H. anatolicum* and *A. appendiculatus*, its fecundity rate declined after treatment with *A. oryzae* showing that fungal pathogenesis extended and inhibited egg oviposition and finally ticks died with their heavy weights. *A. oryzae* exhibited mortality at all concentration against all species of ticks, however death on *A. gemma* was delayed compared to *H. anatolicum* and *R. appendiculatus* which could be due to have hard exoskeleton that absorb slowly spores. In most treatment appendages of ticks showed highest and early virulence than other parts indicating that *A. oryzae* attack first insect appendages (cuticles) to slow down movement and thereafter causes death. Despite the mortality in control after long time of exposure to treatment, no fungal mycelia were reisolated from incubated dead ticks showing the death occurred natural unlike in fungal treated ticks. This substantiate that, fungal biopesticides are the best are natural control of ectoparasite pest as could have dual application in agricultural farms or grazing lands as both plant and animal pest control if sprayed in pastoral areas [35].

Conclusion

A. oryzae was effective in controlling three species of ticks that threaten animal husbandry and leather industry in Tanzania. Hence this study recommends further field experiment on application of *A. oryzae* as direct spray on animals or soil in grazing environment could be potential for management of ticks and mosquitoes to control skin damage for health animals and protect from Vector Bone Diseases (VTBDs) for improving quality of hides and skins for tanning industry in Tanzania.

Declaration

Authors declare that no competing interest exist.

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