Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

Journal of Entomology 9 (6): 343-351, 2012
ISSN 1812-5670 / DOI: 10.3923/je.2012.343.351

The Activity of *Aspergillus terreus* as Entomopathogenic Fungi on Different Stages of *Hyalomma anatolicum anatolicum* under Experimental Conditions

[1]Elham Abdelbasit Suliman and [2]Yassir Osman Mohammed
[1]Department of Mycology, Veterinary Research Institute, P.O. 8067, Amarat, Khartoum, Sudan
[2]Department of Entomology, Veterinary Research Institute, P.O. 8067, Amarat, Khartoum, Sudan

Corresponding Author: Elham Abdelbasit Suliman, Department of Mycology, Veterinary Research Institute, P.O. 8067, Amarat, Khartoum, Sudan Tel: +249912460405 Fax: +249912460405

ABSTRACT

Ticks are obligate blood sucking ectoparasites. They are regarded as a major constraint to improve cattle production in sub-Saharan Africa. They are vectors of many kinds of microorganisms than any other single arthropods. Fungi are capable to infect a wide range of living organisms including insects and acaroids. Numerous natural enemies of ticks including pathogens such as fungi were investigated. In the present study, the mould *Aspergillus terreus* was isolated from *Hyalomma anatolicum anatolicum* eggs. Spore suspension of *A. terreus* at different concentrations was tested against different developmental stages of *H. a. anatolicum*. Assessment of immature stages of *H. a. anatolicum* seven days post treatment showed that the unfed larval stage was more susceptible than other stages. Spore suspensions at different concentrations, induced no mortalities among unfed and fed females. It was also demonstrated that the treatment affected reproductive capacity of the both unfed and fed females by reducing egg-conversion factor and arresting oviposition. Hatchability of the treated eggs decreased with increasing the contact periods. The findings were assessed and implications on bio-control of ticks using *A. terreus* were discussed. Accordingly, *A. terreus* might play a substantial role in future IPM programmes for tick control.

Key words: *Aspergillus terreus, Hyalomma anatolicum*, fungal pathogen, biological control, reproductive capacity

INTRODUCTION

Ticks are obligatory temporary blood sucking parasites that mainly feed on vertebrates. This constantly poses higher risks of diseases transmission by ticks. Ticks and tick-borne diseases are of extreme economic importance hence represent a major constraint for livestock industry. In practice, ticks are controlled mainly by acaricides via two common application approaches; on-host and off-host. In both, environmental hazards are considerably stimulating. Hence, biological control is becoming an increasingly attractive approach to tick management (Bittencourt, 2000) because of; increasing concerns about environmental safety; cost of chemical control and emergence of acaricide-resistant strain of ticks. Numerous natural enemies of ticks (Kaaya, 2003), including pathogens (Hoogstraal, 1977; Chandler *et al*., 2000), parasitoids (Mwangi *et al*., 1997) and predators (Mwangi *et al*., 1991) have been documented, though only a few species have been evaluated as potential tick bio-control agents.

The interest and concept for using fungi to control vectors of veterinary and medical importance was established in the 19th century as a result of their successful use in controlling agricultural pests (Ferron, 1981; Hall and Papierok, 1982). Recently, fungal enzymes such as chitinase have been developed as biopesticides against plant pests (Sharma *et al*., 2011). Entomopathogenic fungi were found to infect wide range of insects and parasitoids. Pathogenicity of *Metarhizium anisopliae* and *Paecilomyces* species were assessed against the subterranean burrower bug *Cyrtomenus bergi* Froeschner (Jaramillo and Borgemeister, 2006). Nielsen *et al*. (2004) studied the effect of *Metarhizium anisopliae* on survival and reproduction of the filth fly parasitoid, *Spalangia cameroni*. Infection of malaria mosquito *Anopheles gambiae* with the entomopathogenic fungus *Metarhizium anisopliae* revealed reduction of blood feeding and fecundity (Scholte *et al*., 2006). Furthermore, an oil-based conidia formulation of three isolated fungi was evaluated against fourth instars larvae of hairy caterpillar (Sahayaraj and Borgio, 2012).

Over the all species of entomopathogenic fungi have been reported only few species of fungi have been accounted to be associated with ticks in nature (Kaaya *et al*., 1996; Zhioua *et al*., 1997; Saquis *et al*., 2002), the most promising fungi appear to be *Metarhizium anisopliae* and *Beauveria bassiana*. *M. anisopliae* has been tested as a biological control agent against different insects and tick species (Nielsen *et al*., 2004; Frazzon *et al*., 2000; Bittencourt, 2000).

In the Sudan ticks and tick-borne diseases represent a key constriction for intensive livestock industry due to great economic losses induced. Prevalence of *Hyalomma anatolicum anatolicum*, the potential vector of *Theileria annulata*, in the most important region (central Sudan) for raising livestock consequently hinders the execution of policies aimed at improving livestock due to considerable losses caused by *T. annulata* infection (Osman, 1976; Latif, 1994; Salih *et al*., 2004). Conversely systemic tick control has not been applied in the country, yet acaricides have been in use. Reliance on acaricides together with their abuse has led to emergence of resistant-tick strains (Latif, 1984; Mohammed, 2003). Hence, new approach for tick control is needed, even so, to date bio-control agents against ticks of medically or veterinary important in the Sudan have not been tried.

Biological control is likely to play a substantial role in future of tick control. Because of fungi diversity taxa that show high potential as tick bio-control agents. Estrada Pena *et al*. (1990) addressed the pathogenicity of *Aspergillus ochraceus* on *Rhipicephalus sanguineus* adult females. The present study is carried out to isolate and identify the naturally occurring fungi associated with *H. a. anatolicum* in the Sudan, develop them as bio-control agents, establish their effectiveness on the survival and biotic potential of the target tick and devise production strategies to bring them to practical use. Thus, mycoinsecticides are being used for the control of many insect pests as an environmentally acceptable alternative to chemical insecticides (Bittencourt, 2000; Leger *et al*., 1996). Vertalec was found effective against lettuce Aphids (Fournier and Brodeur, 2000).

MATERIALS AND METHODS

Ticks collection: Ticks used in this study were randomly collected from different cattle breeds found in the central region of the Sudan including Khartoum, Gezira and North Kordofan States. About 600 engorged female ticks were collected, transferred to the Laboratory and identified according to Hoogstraal (1956). Only engorged female ticks of *H. a. anatolicum* of each State were selected and separately kept into sterilized test tubes. Then they were incubated at 27±2°C and 75-80% Relative Humidity (RH) in order to lay eggs.

Tick maintenance: Feeding of the flat phase of the different stages of *H. a. anatolicum* was done according to the method of Bailey (1960). Eggs for hatching and the engorged phases for moulting were incubated under optimum laboratory conditions of 27±2°C and 75-80% RH (Yassir *et al*., 1992).

Fungal isolation: Eggs yielded from each tick group were surface disinfected as described by Mwangi *et al*. (1995), consequently ground and cultured onto Sabouraud's dextrose agar medium (SDA, Oxoid) containing chloramphenicol 0.05 g L^{-1}. Slant and plate culture were incubated at 27±2°C and observed daily for fungal growth for two weeks. Growth obtained was sub-cultured onto Malt extract Agar (MEA, Oxoid). The isolated fungus was identified as *Aspergillus terreus* according to Raper and Fennell (1973) and preserved for infectivity trials and further studies.

Experimental infection of ticks: Spore-suspension stock of *Aspergillus terreus* was prepared (Maniania, 1993; Kaaya and Hassan, 2000) by suspending the spores in 50 mL of distilled water with a small amount of the dispersing agent Triton x-100 into sterilized bottle. The concentration of the stock suspension was assessed using a counting-chamber. Then serial dilutions of *A. terreus* spore-suspension of varying concentrations were prepared and tested against different developmental stages of a laboratory colony of *H. a. anatolicum* by dipping method (Maniania, 1994; Mwangi *et al*., 1995). The treated ticks were observed daily.

Seven days post treatment the tick percentage mortality corresponding to spore concentrations used was determined. Data obtained were analyzed using a probit analysis programme (Steel and Torrie, 1986) and the probit values of LC_{50} for each developmental stages tested were assessed. Surface disinfection and re-culture of cadavers were carried out according to Kaaya *et al*. (1996) method to determine the cause of death.

Effect of the treatment on biotic potential of the treated fed and unfed *H. a. anatolicum* females were assessed and measured by percentage inhibition of oviposition or Egg Conversion Factor (ECF) as described by Drummond *et al*. (1973):

$$\text{Inhibition (\%)} = \frac{ER_{control} - ER_{treated}}{ER_{control}} \times 100$$

where, ER is estimated reproductive factor.

The inhibition hatchability of treated eggs with larval LC_{50} level of concentration at 6.7×10^5 spore mL^{-1} based on exposure period was assessed.

RESULTS

Effect of *A. terreus* spores on 7 day-old immature stages of *H. a. anatolicum* assessed 7 days post treatment: Table 1 shows the susceptibility levels of the immature stages of *H. a. anatolicum* to *A. terreus* spores. Based on LC_{50} value it was apparently evident that the unfed larval phase was more susceptible than the unfed and fed nymphal phases.

Table 1: Probit values of LC_{50} for immature stages of *H. a. anatolicum* treated with different concentrations of *A. terreus* spore-suspension

Tick stages	LC_{50} value (spore mL^{-1})
Unfed larvae	6.7×10^5
Unfed nymph	1.5×10^7
Fed nymph	2.9×10^8

Table 2: Treatment efficiency of *A. terreus* spore-suspension on fed females of *H. a. anatolicum* under laboratory conditions

Concentration (spores mL^{-1})	Estimated hatchability (Mean±SE)	Inhibition (%)
0.00	93.93+28.17	0.0
7.2×10^{6}	79.73+31.02^{ns}	15.1
4.4×10^{7}	73.90+20.47*	21.2
2.0×10^{8}	50.90+28.17*	45.7

*Significant ($p<0.05$), ns: Not significant ($p>0.05$)

Table 3: Effect of *A. terreus* spore-suspension on reproductive capacity of unfed females of *H. a. anatolicum* under laboratory conditions

Concentration spore mL^{-1}	Z (Mean±SE)	X (Mean±SE)	Y (Mean±SE)	ER	IO (%)
Control	0.52±0.28	0.35±0.01	93.85	1263365.4	00.00
5×10^{6}	0.34±0.03**	0.22±0.01**	51.10	0661294.1	47.00
4×10^{8}	0.40±0.01**	0.13±0.03***	71.65	0465725.0	64.14

**High significant ($p<0.01$), ***Very high significant ($p<0.001$), X: Weight of eggs produced in grams, Y: Estimated percentage hatchability, Z: Weight of engorged female in grams, ER: Estimated reproductive factor, IO: Inhibition of oviposition

Table 4: Effect of fungal treatments on 12 h-old egg masses of *H. a. anatolicum*

Contact period h^{-1}	Hatchability (%)	Calculated hatchability (%)	Calculated inhibition (%)
Control	80.82	82.64	-
12	72.40	73.84	8.64
24	67.20	42.15	47.84
36	47.30	40.39	50.02
48	27.20	38.63	52.20
72	19.60	36.86	54.38

Effect of *A. terreus* spore-suspension on oviposition of treated fed females of *H. a. anatolicum*: No moralities for female fed stage at the all levels of the concentration used were induced. Although, the treated and untreated control females succeeded to oviposit, yet, there were significant ($p<0.05$) reductions in hatchability of the eggs laid by ticks treated with highest concentrations of the spore-suspension. There was a strong positive correlation ($r = 0.9981$) between the concentrations used and resultant percentage inhibition of egg hatchability. The probit value of the concentration able to induce 50% (LC_{50}) inhibition of egg hatchability was 5.3×10^{8} spore mL^{-1}. The results obtained are summarized in Table 2.

Effect of *A. terreus* spore-suspension on biological performance capacity of unfed females of *H. a. anatolicum*: The results are presented in Table 3. The treatment induced high significant reductions in engorgement weight gained ($p<0.01$) and the total egg-mass ($p<0.001$) laid by the treated flat female ticks. Consequently reduction induced in Egg Conversion Factor (ECF) for such ticks coincided with potency of spore concentration used. Moreover, the treatment has inhibited eggs hatchability, as a result, arrested oviposition of the treated female ticks.

Effect of *A. terreus* spores on *H. a. anatolicum* eggs: The result obtained indicated that the susceptibility of the treated eggs increased with increasing contact periods (Table 4). Consequently there was a strong positive linear correlation ($r = 0.99$) between exposure period and percentage inhibition of egg hatchability.

DISCUSSION

Ticks of *Hyalomma anatolicum anatolicum* examined for susceptibility to artificial infection with *Aspergillus terreus* in the present study is of common occurrence (Osman, 1976) in the central

region of the Sudan. Where it has been approved to be the potential efficient vector of tropical theileriosis (Salih *et al*., 2007), which causes economic losses that hinder livestock up-grading programmes (Latif, 1994).

However, a number of *H. a. anatolicum* engorge females randomly collected from central region of the Sudan including Khartoum, Gezira and North Kordofan States were found naturally infected with *A. terreus* according to Raper and Fennell (1973). In nature, a higher percentage of adult ticks seem to be infected by fungi than their immature stages and engorged females seem to be most readily infected (Zhioua *et al*., 1999). The percentage of ticks infected by fungi in nature varies considerably according to tick stage and species, season and to ecological conditions at the sample sites (Kalsbeek *et al*., 1995; Mwangi *et al*., 1995).

In the present study, *A. terreus* spores to be tested against different stages of the target tick species were prepared as suspension formulation since in most laboratory tests the spores were suspended only in water with a small amount of dispersing agent (Kaaya and Hassan, 2000). Evidently, the formulation in which the spores are applied is critical to the level of control obtained, but very little has been published as yet on the subject.

The dipping method applied in this work has been used basically for assessing efficiency of fungal infection against various tick species and other arthropod pests (Maniania, 1994; Mwangi *et al*., 1995). Usually fungi take several days to kill ticks. For instance, the LT_{50} of the majority of the entomopathogenic fungi so for tested against unfed and fed stages of different tick species generally ranged from few days to few weeks (Hall and Papierok, 1982). For this reason in current work the tick percentage mortality corresponding to spore concentrations used was determined 7 days post treatment.

Both larvae and nymphs of *H. a. anatolicum* were affected by *A. terreus* spores treatment, which resulted in death. Death of ticks was attributed to penetration of their soft cuticle by the fungus which rapidly invades their internal organs and ultimately kills them (Kaaya and Essuman, 1995; Kaaya *et al*., 1996). Recently, adhesion of fungal conidia to cuticle of insect larvae and germination were demonstrated to facilitate penetration of the cuticle (Altre *et al*., 1999; Altre and Vandenberg, 2001). Data obtained were analyzed (Steel and Torrie, 1986) and the concentrations that caused 50% mortality of the various developmental stages of *H. a. anatolicum* were assessed. The values of LC_{50} obtained could be regarded as base line data for susceptibility of this tick species, as it is the first times to be tested against *A. terreus* infection. Similarly, in a study carried by (Gindin *et al*., 2001) under laboratory conditions Ixodid tick species showed unpredictable susceptibility level to entomopathogenic fungi (Gindin *et al*., 2001). Free-living larval, nymphal, and adult *Ixodes scapularis* showed high infection rate when treated with entomopathogenic fungi (Zhioua *et al*., 1999).

Based on the calculated value of LC_{50} for both unfed and fed phases of the immature stages of *H. a. anatolicum*, the unfed larval phase could be considered more susceptible to *A. terreus* infection. This finding is in agreement with Munshi *et al*. (2008) who recorded LC_{50} values in a range of 1.9×10^{8}-1.3×10^{11} on using *Fusarium* species as a bio-control agent against caterpillar larvae.

Variation in susceptibility levels observed for the examined immature stages was also reported by Kaaya and Okech (1990) and such phenomenon might be attributed to ticks physiological factor. Comparison between the susceptibility of the unfed stages of *Rhipicephalus appendiculatus* and *Amblyomma variegatum* or of *H. excavatum* and *R. sanguineus* demonstrated decreasing susceptibility to fungi in progression through the larval, nymph and adult stages (Kaaya, 2000;

Samish, 2000) and unfed stages seem to become more resistant after engorgement (Reis *et al*., 2001). High mortality of immature stages might be due to their incubation condition as they had not been incubated individually a matter that enhances infection via tick to tick contact in the tube.

Moreover, it was observed that *A. terreus* fungal infection affected reproductive potential of the treated both fed and unfed females of *H. a. anatolicum* rather than inducing mortality. These findings are in agreement with those observed by Kaaya *et al*. (1996) who tested *B. bassiana* and *M. anisopliae* on *R. appendiculatus* and *A. variegatum*. The treatment altered the development processes of *H. a. anatolicum* by reducing the egg-mass laid and inhibiting oviposition and eggs hatchability (Gindin *et al*., 2001). This suggests that there is a relatively long-lasting sub-lethal action of the fungi. Germination of the spores might have produced metabolites that affected females' reproductive ability. Oliver *et al*. (1991) suggested that a toxin produced by *Rhizopus thailandensis, R. arrhizus* and *Curvularia lunata* might affect reproductive efficiency of *Rhipicephalus sanguineus*. Further investigations in this point would be useful.

In this study, the ability of *A. terreus* to inhibit the treated egg hatchability increased with increase of the exposure period. This finding is in agreement with that reported by Mwangi *et al*. (1995). Tick eggs, in contrast to many insect eggs, are highly susceptible to fungi and up to 100% of the eggs exposed to fungi under laboratory conditions did not hatch (Kaaya, 2000).

Tick management is principally geared towards the prevention of development of the next generation (Bittencourt, 2000). This goal can be achieved either by destroying ticks or arresting their reproduction. Hence, *A. terreus* treatment verifies the goal of tick control strategy as it demonstrated mortality and arrested the reproduction of the treated ticks. Accordingly the fungi might be a successful candidate as a biological control agent and play a substantial role in future of tick control. Moreover, they can easily be produced in the laboratory (Soundarapandian and Chandra, 2007).

REFERENCES

Altre, J.A., J.D. Vandenberg and F.A. Cantone, 1999. Pathogenicity of *Paecilomyces fumosoroseus* isolates to diamondback moth, *Plutella xylostella*: Correlation with spore size, germination speed and attachment to cuticle. J. Invert. Pathol., 73: 332-338.

Altre, J.A. and J.D. Vandenberg, 2001. Penetration of cuticle and proliferation in hemolymph by *Paecilomyces fumosoroseus* isolates that differ in virulence against lepidopteran larvae *Plutella xylostella*. J. Inver. Path., 78: 81-86.

Bailey, K.P., 1960. Notes on the rearing of *Rhipiciephalus appendiculatus* and their infection with *Theileria parva* for experimental transmission. Bull. Epiz. Dis. Afr., 8: 33-43.

Bittencourt, V.R., 2000. Trials to control South American ticks with entomopathogenic fungi. Ann. Nat. Acad. Sci., 916: 555-558.

Chandler, D., G. Davidson, J.K. Pell, B.V. Ball, K. Shaw and K. D. Sunderland, 2000. Fungal biocontrol of Acari. Biocontrol Sci. Technol., 10: 357-384.

Drummond, R.O., S.E. Erns, J.R. Trevino, W.J. Gladney and O.H. Graham, 1973. *Boophilus annulatus* and *B. microplus*: Laboratory tests of insecticides. J. Econ. Entomol., 66: 130-133.

Estrada Pena, A., J. Gonzalez and A. Casasolas, 1990. The activity of *Aspergillus ochraceus* (fungi) on replete females of *Rhipicephalus sanguineus* (Acari: Ixodidae) in natural and experimental conditions. Folia Parasitol., 37: 331-336.

Ferron, P., 1981. Pest Control By Fungi *Beauveria* and *Metarhizium*. In: Microbial Control of Pests and Plant Diseases 1970-1980, Burges, H.D. (Ed.). Academic Press, London, pp: 465-482.

Fournier, V. and J. Brodeur, 2000. Dose-response susceptibility of pest aphids (Homoptera: Aphididae) and their control on hydroponically grown lettuce with the entomopathogenic fungus *Verticillium lecanii*, azadirachtin and insecticidal soap. Environ. Entomol., 29: 568-578.

Frazzon, A.P.G., I.S.V. Junior, A. Masuda, A. Schrank and M.H. Vainstein, 2000. *In vitro* assessment of *Metarhizium anisopliae* isolates to control the cattle tick *Boophilus microplus*. Vet. Parasitol., 94: 117-125.

Gindin, G., M. Samish, E. Alekseev and I. Glazer, 2001. The susceptibility of *Boophilus microplus* (Ixodiae) ticks to entomopathogenic fungi. Biocontrol. Sci. Technol., 11: 111-118.

Hall, R.A. and B. Papierok, 1982. Fungi as biological control agents of arthropods of agricultural and medical importance. Parasitology, 84: 205-240.

Hoogstraal, H., 1956. African ixodoidea, ticks of the Sudan with special reference to equatorial province and preliminary reviews of the genera *Boophilus, margaropus* and *Hyalomma*. Research Report NM 00505029, Navy, Bureau of Medical Surgery, Washington DC., USA., pp: 1-1101

Hoogstraal, H., 1977. Pathogens of Acarina (ticks). Bull. World Health Organ., 55: 337-340.

Jaramillo, J. and C. Borgemeister, 2006. New bioassay method to assess the pathogenicity of Colombian strains of *Metarhizium anisopliae* (Metsch.) Sorokin and *Paecilomyces* sp. (Deuteromycotina: Hyphomycetes) against the subterranean burrower bug *Cyrtomenus bergi* Froeschner (Hemiptera: Cydnidae). J. Invertebr. Path., 91: 57-60.

Kaaya, G.P. and M.A. Okech, 1990. Horizontal transmission of mycotic infection in adult tsetse , *Glossina morsitans morsitans*. Entomophaga, 35: 589-600.

Kaaya, G.P. and S. Essuman, 1995. Experimental infections of *Rhipicephalus appendiculatus* with entomopathogenic fungi. Afr. Zool., 109: 151-160.

Kaaya, G.P., E.N. Mwangi and E.A. Ouna, 1996. Prospects for biological control of livestock ticks, *Rhipicephalus appendiculatus* and *Amblyomma variegatum*, using the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae*. J. Invert. Pathol., 67: 15-20.

Kaaya, G.P., 2000. Laboratory and field evaluation of entomogenous fungi for tick control. Ann. NY Acad. Sci., 916: 559-564.

Kaaya, G.P. and S. Hassan, 2000. Entomogenous fungi as promising biopesticides for tick control. Exp. Applied Acarol., 24: 913-926.

Kaaya, G.P., 2003. Prospects for innovative methods of tick control in Africa. Insect Sci. Application, 23: 59-67.

Kalsbeek, V., F. Frandsen and T. Steenberg, 1995. Entomopathpgenic fungi associated with *Ixodes ricinus* ticks. Exp. Applied Acarol., 19: 45-51.

Latif, A.A., 1984. Resistance to *Hyalomma anatolicum anatolicum* Koch (1844) and *Rhipicephalus evertsi* Neumann (1897) (Ixodoidea: Ixodidae) by cattle in the Sudan. Int. J. Trop. Insect Sci., 5: 509-511.

Latif, A.A., 1994. Economic losses in exotic breeds of cattle due to theileriosis in the Sudan. Proceedings of the Workshop on Bovine Tropical Theileriosis, May 4-5, 1994, Khartoum, Sudan.

Leger, R.S., L. Joshi, M.J. Bidochka and D.W. Roberts, 1996. Construction of an improved mycoinsecticide overexpressing a toxic protease. Proc. Natl. Acad. Sci. USA., 93: 6349-6354.

Maniania, N.K., 1993. Evaluation of three formulations of *Beauveria bassiana* for control of stem borer chilo partellus. J. Appl. Entomol., 115: 266-272.

Maniania, N.K., 1994. A laboratory technique for infecting adult tsetse with a fungal pathogen. Inset Sci. Applic., 15: 421-426.

Mohammed, Y.O., 2003. Base-line data on susceptibility of some ixodid tick species to Lindane in the Sudan. Sudan J. Vet. Res., 18: 93-98.

Munshi, N.A., B. Hussain, G.N. Malik, M. Yousuf and N. Fatima, 2008. Efficacy of entomopathogenic fungus *Fusarium pallidoroseum* (Cooke) Sacc. Against gypsy moth (*Lymantria obfuscata* Walker). J. Entomol., 5: 59-61.

Mwangi, E.N., O.O. Dipeolu, R.M. Newson, G.P. Kaaya and S.M. Hassan, 1991. Predators, parasitoids and pathogens of ticks: A review. Biocontrol Sci. Technol., 1: 147-156.

Mwangi, E.N., G.P. Kaaya and S. Essuman, 1995. Experimental infection of the tick *Rhipicephalus appendiculatus* with entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* and natural infections of some ticks with bacteria and fungi. J. Afr. Zool., 109: 1-11.

Mwangi, E.N., S.M. Hassan, G.P. Kaaya and S. Essuman, 1997. The impact of *Ixodiphagus hookeri*, a tick parasitoid, on *Amblyomma variegatum* (Acari: Ixodidae) in a field trial in Kenya. Exp. Appl. Acarol., 21: 117-126.

Nielsen, C., H. Skovgard and T. Steenberg, 2004. Effect of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on survival and reproduction of the filth fly parasitoid, *Spalangia cameroni* (Hymenoptera: Pteromalidae). Environ. Entomol., 34: 133-139.

Oliver, A.C., A. Estrada-Pena and J. Gonzalcz-Cabo, 1991. Activity of *Rhizopus thailandensis*, *Rhizopus arrhizus* and *Curvularia lunata* on reproductive efficacy of *Rhipicephalus sanguineus* (Ixodidae). Mod. Acarol., 2: 633-637.

Osman, Y.M., 1976. A review of ticks and tick-borne diseases and their control in the Sudan. Bull. Int. Epiz., 86: 81-87.

Raper, K.B. and D.I. Fennell, 1973. The Genus *Aspergillus*. Krieger Publishing Company, Huntington, New York.

Reis, R.C.S., D.R. Melo, E.J. Souza and V.R.E.P. Bitten Court, 2001. *In vitro* action of the fungi *Beauveria bassiana* and *Metarhizium anisopliae* Sorok on nymphs and adults of *Amblyomma canjenense* (Acari: Ixodidae). Arq. Bras. Med. Vet. Zoot., 53: 544-547.

Sahayaraj, K. and F.J. Borgio, 2012. Screening of some mycoinsecticide for the managing hairy caterpillar *Pericallia ricini* Fab. (Lepidopetra: Arctiidae) in castor. J. Entomol., 9: 89-97.

Salih, D.A., S.M. Hassan, A.M. El-Hussein and F. Jongejan, 2004. Preliminary survey of ticks (Acari: Ixodidae) on cattle in northern Sudan. Onderstepoort J. Vet. Res., 71: 319-326.

Salih, D.A., A.M. El Hussein, U. Seitzer and J.S. Ahmed, 2007. Epidemiological studies on tick-borne diseases of cattle in Central Equatorial State, Southern Sudan. Parasitol. Res., 101: 1035-1044.

Samish, M., 2000. Biocontrol of ticks. Ann. New York Acad. Sci., 916: 172-178.

Saquis, G., M.A. Demorales and V. Bettencaurt, 2002. Isolation of *Beauveria bassiana* and *Metarhizium anisopliae var. anisopliae* from *Boophilus microplus* tick in Rio de Janerio State, Brazil. Mycopathologia, 154: 207-209.

Scholte, E.J., B.C.J. Knols and W. Takken, 2006. Infection of the malaria mosquito *Anopheles gambiae* with the entomopathogenic fungus *Metarhizium anisopliae* reduces blood feeding and fecundity. J. Invertebrate Pathol., 91: 43-49.

Sharma, N., K.P. Sharma, R.K. Gaur and V.K. Gupta, 2011. Role of chitinase in plant defense. Asian J. Biochem., 6: 29-37.

Soundarapandian, P. and R. Chandra, 2007. Mass production of endomopathogenic fungus *Metarhizium anisopliae* (Deuteromycota; Hyphomycetes) in the laboratory. Res. J. Microbiol., 2: 690-695.

Steel, R.D. and J.H. Torrie, 1986. Principles and Procedures of Statistics: A Biometrical Appraoch. 2nd Edn., Fong and Sons Printers Ltd., Singapore, pp: 1-66.

Yassir, O.M., O.M. Osman and T.H. Elamin, 1992. Life cycle studies of the tick species, *Amblyomma lepidum, Hyalomma anatolicum* and *Rhipicephalus evertsi evertsi* under laboratory conditions. Insect Sci. Applic., 13: 565-568.

Zhioua, E., M. Browning, P.W. Johnson, H.S. Ginsberg and R.A. LeBrun, 1997. Pathogenicity of the entomopathogenic fungus *Metarhizium anisopliae* (Deuteromycetes) to *Ixodes scapularis* (Acari: Ixodidae). J. Parasitol., 83: 815-818.

Zhioua, E., H.S. Ginsberg, R.A. Humber and R.A. Lebrun, 1999. Preliminary survey for entomopathogenic fungi associated with *Ixodes scapularis* (Acari: Ixodidae) in Southern New York and New England, USA. J. Med. Entomol., 36: 635-637.