Sudan Journal of Science and Technology (2013) 14(2)75-80

Sudan Journal of Science and Technology
Journal homepage: http://jst.sustech.edu/

Investigation On Fungi associated with *Hyalomma anatolicum* and *Amblyomma lepidum* (Acari=Ixodidae) in Khartoum State, Sudan

Elham Abdelbasit Suleiman[1]; Ali Siddig Mohammed[2]

1. Veterinary Research Institute (VRI), Mycology Department, Khartoum, Sudan.
2. Veterinary Research Institute (VRI), Entomology and Ticks Department P.O. Box: 8067 Al Amarat, Khartoum, Sudan.

ARTICLE INFO

Article history
Received:20February2014
Accepted: 7April 2014
Available online: 5 August 2014

KEYWORDS:
fungal pathogens,
Hyalomma anatolicum,
Amblyomma lepidum,
pathgenicity

ABSTRACT

The objective of this study was to demonstrate the occurrence of fungi on *Hyalomma anatolicum* and *Amblyomma lepidum*. Ticks were collected from different localities in the Sudan, Khartoum, Gezira, El Obeid and Ed Damazine in approach to biological control. Various fungal species were isolated from field collected and colony- contaminated tick. Most fungi were found to belong to Hyphomycetes in addition to yeasts. The study reported the first isolation of such fungi from *Hyalomma anatolicum* and *Amblyomma lepidum* in the Sudan. *Scopulariopsis bervicaulis,* the currently isolated fungus, was found to have significantly inhibited reproductive performance of the treated ticks. Thus, the use of naturally occurring fungi as biological means of tick control in the Sudan is highly recommended.

INTRODUCTION

Hard ticks (Ixodidae: Ixodoidea) are considered as the most important vectors of diseases affecting both humans and animals (Estrada-Pena and Jongejan, 1999). Many tick species are significant transmitters of human fatal diseases such as Ehrlichiosis, Crimean Congo Haemorrhagic Fever (CCHF) (Linthicum and Bailey, 1994) and Lyme disease (Piesman and Gray, 1994). Diseases transmitted by ticks to livestock constitute a major factor, which altered animal industry worldwide (Jongejan, and Uillenberg 1994; Kelly *et al*., 1994).

Ticks have numerous natural enemies consist of predators, pathogens and parasites (Mwangi *et al*., 1991). However, only entomopathogenic fungi have been comprehensively investigated (Butt *et al*., 2001; Samish *et al*, 2004). Several studies recorded natural infection of ticks with fungi and over 700 species of the genera *Aspergillus*, *Penicillium*, *Beauveria*, *Torrubiella, Cephalostomum*, *Paecilomyces, Mucor*, *Fusarium*, *Verticilium* and *Fumosoroseus* were isolated (Kalsbeek *et al*., 1995; Mwangi

et al., 1995). Yet few species have been developed for the control.

In the Sudan the problems posed by ticks and tick-borne diseases are the essential negative effects on livestock industry. There are over 70 species of ticks prevalent in the Sudan; but only few species are of economical importance (Osman and Hassan, 2003). The most essential cattle tick vectors in the country are *Hyalomma anatolicum* and *Amblyomma lepidum*; the principal vector of *Theileria annulata* and *Cowdria ruminantium*, respectively (Jongejan *et al*., 1984; Abdelwahab *et al*., 1998; Salih *et al.,* 2005). The present study was aimed to demonstrate the occurrence of fungi on *H. anatolicum* and *A. lepidum* ticks in approach to further biological control.

MATERIALS and METHODS

Ticks samples:

Ticks were collected from cattle found in El Obied (North Kordofan State) Wad Madane (Gezira State), Ed Damazine (Blue Nile State) and Khartoum (Khartoum State). Identification of ticks was done according to Hoogstraal (1956). Engorged female ticks of *Hyalomma anatolicum* and *Amblyomma lepidum* were selected, due to their economical importance. They were maintained under controlled conditions (27ºC± 1ºC and 80% RH) in order to lay eggs (Mohammed *et al*., 1992).

Fungal isolation:

Dead ticks were examined for fungi infections directly by slide wet mount. Following the method described by Milne (1989), each mummified female tick was gently scraped using a sterile wire loop. The harvested materials were examined by placing few scrapings in a drop of 20% KOH on a microscopic slide.

Eggs laid by each tick species were separately collected and randomly divided into two groups. Group one was allowed to hatch into larvae. The larval progeny as well as eggs of group two and female cadavers were surface disinfected. According to Mwangi *et al*., (1995), the specimens were immersed into 70% alcohol for few seconds; 5% sodium thiosulfate for 5 minutes; hypochloride for five minutes; and then three successive washing into sterile water each for three minutes. Subsequently each sample was ground and cultured in duplicate slopes and plates of Sabouraud Dextrose Agar (SDA) and Brain Heart Infusion Agar (BHIA) with 0.05 mg/ml chloramphenicol and 0.5 mg/ml cycloheximide.

Cultures were incubated at 27°C and daily examined for two weeks. Colonies which developed were mounted in lactophenol cotton blue and microscopically examined. The growth was identified according to Raper and Fennell (1973). Yeast colonies were identified by employing Germ tube test as well as API 20-C kits.

Pathogenicity test:

Spore-suspension of the isolated *Scopulariopsis bervicaulis* fungus at concentration of $3.3x10^7$ spores/ml was prepared according to Mwangi *et al*., (1995). method. The potency of the spore suspension was determined against laboratory clean engorged females of *H. anatolicum* and *A. lepidum* of known weight by immersing in the suspension for 3 minutes. Control groups were immersed in sterile distilled water for the same period. The ticks were incubated at 27 $^{\circ}$C ±1°C and 85% RH and daily monitored.

Statistical analysis:

The mean values were expressed as the Mean ± Standard Deviation (SD) and were analyzed using one-way ANOVA using the program SPSS 19.0 for Windows. Differences were considered significant at $P < 0.05$.

RESULTS

Hyalomma anatolicum collected from El Obeid and Khartoum showed diversity of fungal species. The recognized fungi isolated from El Obeid ticks were *Aspergillus terreus, A. niger*, *A. flavus, Penicillium* species besides yeasts. While Dematiaceous Hyphomycetes such as *Alternaria* species, *Aurobasidium pullulans* in addition to *Rhipzopus* and *Aspergillus* species were isolated from ticks collected from Khartoum. However, samples from Geizera State showed the least contaminated ticks. From Ed Damazine samples *A. lepidum* ticks were found to be infected with *A. niger*, *A. flavus*, *A. terreus*, *Penicillium* species and *Scopulariopsis bervicaulis*. Out of 540 collected ticks, 18.4% were found contaminated with fungi. The list of the isolated fungi is shown in Table (1). Fungal identification was based on macro and microscopic characteristics. The identification of the isolate was confirmed by biotechnical laboratory in Denmark.

Table 1: Percentages of fungi isolated from *Hyalomma anatolicum* and *Amblyomma lepidum* infesting cattle in four localities in Sudan

Isolated fungus	Localities				No. of isolates	%age
	ElObeid	Khartoum	Ed Damazine	Gezira		
A. terreus	18	3	1	1	23	33.3
A. flavus	7	5	2	1	15	21.7
A. niger	3	2	-	1	6	8.7
A. fumigates	-	-	1	-	1	1.4
Penicillium Sp.	-	3	1	1	5	7.2
Rhizopus SP.	2	3	-	-	5	7.2
Alternaria Sp.	-	1	-		1	1.4
Candida SP.	2	5	-	1	8	11.6
Cryptococcous laurentii.	-	1	-	-	1	1.4
Aurobasidium Sp.	-	1	-	-	1	1.4
Scopulariopsis brevicaulis	-	-	3	-	3	4.3
Total	32	24	8	5	69	99.6

All infected ticks with *S. brevicaulis* developed fungal infection yet succeeded to oviposit undersized batches of eggs with low hatchability. The fungus has significantly altered reproductive performance of the treated ticks. The control group did not show any fungal growth (Table 2).

Table 2: Mean (±SD) of the effect of *S. brevicaulis* spore suspension on engorged. *H. anatolicum* and *A. lepidum* females

Tick species	Hatchability (%)	Female fertility (%)	Egg mass (gm)	Lost weight of female
H. anatolicum	44.13^{x}±15.77	47.54^{x}±1.92	0.17^{xx}±0.07	0.22±0.99
Control	93.33 ±6.51	68.81±2.21	0.63±0.05	0.07±0.02
A. lepidum	40.63^{x} ±1.19	57.73^{x}±6.35	0.11^{x}±0.07	$0.15^{n.s}$±0.04
Control	77.78±0.58	81.55±5.87	0.58±0.05	0.04 ±0.02

% Female fertility (F. F) = (Egg batch weight / engorged female weight) 100.
Lost weight of female (L.W.F) = Engorged female weight – (female weight after oviposition + weight of laid egg batch).
n.s= not significant P> 0.05
x = significant P<0.05
xx = highly significant P<0.005

DISCUSSION

In the present study various fungal species were found naturally associated with ticks of *H. anatolicum* and *A. lepidum*. The fungi of *Aspergillus* , *Rhizopus* and *Penicillium* were isolated from both tick species collected from different location in the country, this finding is similar to that of previous workers (Oliver *et al.,* 1991). Where *Aspergillus* species were found growing on numerous ticks that died in a humid chamber. These fungi are of world wide spread in nature and caused mortality among different tick species (Mwangi *et al.*, 1995). Similar investigation on entomogenus fungi associated with *Ixodes scapularis* was carried by Zhioua *et al*., 1999).
Mwangi *et al.*, (1995) investigated the biocide potency of *Beauveria bassiana* and *Metarhizium anisopliae* fungus against *Rhipicephaus appendiculatus*. Efficacies of some *Curvularia* species were tested on reproductive performance of *Rhipicephalus sanguines* (Oliver *et al.,* 1991).
However, in this study *Scopulariopsis brevicaulis* was isolated only from *A. lepidum* ticks where the recovery of *S. brevicaulis* from the American dog tick**,** *Dermacentor variabilis,* is reported by Jay *et al.,* (2003) when they topically applied to the dogs its spore suspension.

Several investigators worldwide studied the pathogenicity of fungi on different tick stages [Mwangi *et al.,* 1995; oliver *et al*., 1991; kaaya *et al*., 1996; Estroda-pena *et al*., 1990). In the present study, pathogenicity of the isolated fungus, *S.brevicaulis* revealed direct effect on reproductive capacity of both *H. anatolicum* and *A. lepidum*. It is the first time to address the pathogenicity of fungi on ticks in the Sudan. Thus, the present study encourages the use of naturally occurring fungi as biological means of tick control in the Sudan.

CONCLUSION

The present study was conducted to demonstrate the natural occurrence of fungi associated with *H. anatolicum* and *A. lepidum.* Ticks were collected from different localities of Sudan. The result revealed occurrence of various fungi. Pathogenicity of *S. brevicaulis* on both ticks showed impairment to reproductive performance. Thus, the potential biological control of such fungi should be considered.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Prof. Yassir Osman Mohammed (Entomology and Ticks Department,

Khartoum, Sudan) for his critical comments on the manuscript.

REFERECES

Abdelwahab, M.B., Musa, M.T., Kheir, S.M., and El gadal, A.A. (1998). Association of heartwater with outbreak of theileriosis in cattle in Western Sudan. *The Sudan Journal of Veterinary Research,* **15**:21-24.

Butt, T. M., Jackson, C. W. Mgan, N. (2001). *Fungi as Biocontrol Agents Progress, Problems and Potentia*l. Oxford, UK, CABI.

Estrada-Pena, A., and Jongejan, F. (1999) Ticks feeding on humans: a review of records on human- bitting Ixodoidea with special reference to pathogen transmittion. *Expermental and Applied Acarology*, **23**: 685-715.

Estroda-pena, A., Gonazole, J. and Casasolas, A. (1990). The activity of *Aspergillus ochraceus* on replete female, of *Rhipicephalus sanguineus* (Acari: Ixodidae) in natural and experimental conditions. *Folia parasitologica,, Prague*. **37**(4): 331-335.

Hoogstraal, H. (1956). African Ixodoidea. Ticks of the Sudan (with special reference to Equatorial province and with preliminary reviews of the genera *Boophilus, margaroplus* and *Hyalomma*) Head Department of Medical Zoology, USA Naval Medical Zoology Research Unit no 3 Cairo, Egypt. 1-1101.

Jay, A. Yoder, Peter E. Hanson, Lawrence W. Zettler, Joshua B. Benoit, Fiorella Ghisays, and Kurt A. Piskin. (2003). Internal and External Mycoflora of the American Dog Tick, *Dermacentor variabilis* (Acari: Ixodidae), and Its Ecological Implications. *Applied and Environmental Microbiology,* **69**(8): 4994–4996.

Jongejan, F., Morzaria, S.P., Shariff, O.E. and Abdalla, H.M. (1984). Isolation and transmission of *Cowdria ruminantium* (Causitive agent of heartwater disease) in Blue Nile Province, Sudan. *Veterinary Research communications*, **8**: 141-145.

Jongejan, F. and Uilenberg, G. (1994). Ticks and control methods. *Rev. Sci. Tech. Off. Int. Epiz,* **13**(4): 1201-1226.

Kaaya, G.P., Mwangi, E.N.,Ouna, E.A. (1996). Prospects for Biological Control of Livestock Ticks, *Rhipicephalus appendiculatus* and *Amblyomma Variegatum*, Using the Entomogenous Fungi *Beauveria bassiana* and *Metarhizium anisopliae. Journal of Invertebrate Pathology*. 67: 15-20.

Kalsbeek, V., Fradsen, F. and Steenberg, T. (1995). Entomo-pathogenic fungi associated with *Ixodes ricinus* ticks. *Exp. Appl. Acarol*., **19**: 45-51.

Kelly, P.J., Beati, P.J., Mathewman, L.A., Masson, P.R., Dasch, G.A., and Raoult, D. (1994). A new pathogenic spotted fever group rickettsia from Africa. *Journal of Tropical Medicine and Hygiene*, **97**: 129-137.

Linthicum, K.J., and Bailey, C.L. (1994). Ecology of Crimean – Congo haemorrhagic fever. In: *Ecological dynamic of tick-*

borne zoonoses (Sonenshine and Mather, eds), pp. 392-437. Oxford University Press, Oxford.

Milne, L.J.R. (1989). Processing of Specimens and the Interpretation of the Results. Keratinous tissue. In: *Medical Mycology a Practical approach*. 1st ed. Edited by E.G.V Evans and M.D. Richard. IRL Press. Oxford University Press. Oxford, New York, Tokyo. Pp:24.

Mohammed, Y. O., O.M. Osman, T. H. El Amin. (1992). "Life cycle studies of the tick species, *Amblyomma lepidum*, *Hyalomma anatolicum anatolicum* and *Rhipicephalus evertsi evertsi* under laboratory conditions." *Insect Science and its Appli*cation, **13**(4): 565-568.

Mwangi, E.N. Dipeolu, O.O., Newson, R.M., Kaaya, G.P. and Hassan, S.M. (1991). Predators, Parasites and pathogens of ticks: a review-Biocontrol science and technology. **1**: 147-156.

Mwangi, E.N., Kaaya, G.P. and Essuman, S. (1995). Experimental infections of the tick *Rhipicephalus appendiculatus* with entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* and natural infections of some ticks with bacteria and fungi. *Journal of African Zoology*. **109**(2): 1-11.

Oliver, A.C., Estroda-pena, A. and Gonzales Cabo, J. (1991). Activity of *Rhizopus thailandensis*, *Rhizopius arrhizus* and *Curvularia lunata* on reproductive efficacy of *Rhipicepalus sanguineus* (Ixodidae). *Modern Acarology*, **2**: 633-3637.

Osman, A.M., Hassan, S. M. (2003). The ecology and distribution of the East African tick *Amblyomma lepidum*: A review. *Sudan Journal of Veterinary Science and Animal Husbandry,* **42**(1&2): 45-53.

Piesman, J., and Gray, J.S. (1994). Lyme disease/Lyme borreliosis. In: Ecological dynamic of tick-borne zoonoses (Sonenshine and Mather, eds), pp. 327- 350. Oxford University Press, Oxford.

Raper, K.B. and Fennell, D.I. (1973). The Genus *Aspergillus*, Ist ed. Robert, E. Krieger publishing company Huntington. New York.

Salih, D.A., Shariff, O.E., Lazarus, A.G., Hassan, S.M., El Hussein, A.M. .(2005). Natural infection rates and transmission of *Theileria annulata* by *Hyalomma anatolicum anatolicum* ticks in the Sudan. *Onderstepoort Journal of Veterinary Research*, **27**: 303-307.

Samish, M. Ginsberg, H. and Glazer, I. (2004). Biological control of ticks. *Parasitology,* **129**. Supp. **1**(S): 389-403.

Zhioua, E., Ginsberg,H. S., Humbard, R.A., Lebrun, R.A. (1999). Preliminary survey for entomopathogenic fungi associated with *Ixodes scapularis* (Acari: Ixodidae) in Southern New York and New England, U.S.A. *Journal of Medical Entomology*, **36**(5): 635-637.