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Activity of *Scopulariopsis brevicaulis* on *Hyalomma anatolicum* and *Amblyomma lepidum* (Acari : Ixodidae)

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Ticks and Tick-Borne Diseases (TBDs) are widespread in the Sudan causing substantial losses in terms of morbidity, mortality, reduction of production and costs of control and treatment. Control of ticks is based mainly on chemical acaricides. The present study was conducted to evaluate the use of fungi as an alternative means of controlling ticks in Sudan to overcome their known drawbacks. *Amblyomma lepidum* ticks were collected from El Damazin abattoir in the Blue Nile State, Central Sudan to establish laboratory colonies. Ticks developed fungal growth and subsequently died. *Scopulariopsis brevicaulis* was isolated from scrapings taken from the white mat that covered tick's integuments after death following incubation at 27°C, RH 85%. They were cultured on Sabouraud's and Brain heart infusion agar. Pure fungal culture was obtained. *S. brevicaulis* was isolated and its identification was based on macro and microscopic characteristics. Identification of the isolate was confirmed by biotechnical laboratory in Denmark. Metabolite profiling of the fungus culture filtrate was conducted on Thin Layer chromatography. Organic compounds were detected. Pathogenicity of the spore suspension and culture filtrate of the isolated fungus to larvae, nymph and adult stages of *Hyalomma anatolicum* and *A. lepidum* was investigated. High mortality to flat larvae and biotic potential of the adult were observed. Results obtained stimulate the use of *S. brevicaulis* metabolites as biological control agents for controlling ticks in Sudan. The study reports the first isolation of *S. brevicaulis* from *A. lepidum* in Sudan.

Key words: *Scopulariopsis*, *Hyalomma*, *Amblyomma*, metabolites, pathogenicity

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INTRODUCTION

Ticks (Acari: Ixodidae) are blood sucking ecto- parasites feeding obligatory on human and animals. They are known to transmit various diseases (Sonenshine, 1991). Malignant ovine theileriosis was transmitted by *Hyalomma anatolicum* (Salih *et al*., 2003). Genus *Amplyomma* has a very wide host range including most of the orders of terrestrial mammalian, particularly ungulates, birds are rarely attacked by the adult forms, but are frequently the hosts of the immature forms (nymphs and larvae) of certain species. Immature of many species will infest humans (Estrada-Pena and Jongejan, 1999). Some species of genus *Amblyomma* are important transmitters of human diseases such as Lyme and Crimean Congo Haemorrhagic Fever (Linthicum and Bailey, 1994). Ehrlichiosis, an emerging zoonotic infection is also, known to be transmitted by *Amblyomma* species (Piesman and Gray, 1994). Veterinary diseases, such as tick- bite fever (Richkettsia infection) is transmitted by *A. hebraeum* (Kelly *et al*., 1994). In Sudan, *A. leipdum* and *A. variegatum* were found as potential vectors of *Cowdria ruminantium* (Karrar, 1960; Jongejan *et al*., 1984; Abdel-Wahab *et al*., 1998).

Control of ticks with chemical acaricides induced environmental problems. Laborious technique of application besides high cost have stimulated research on alternative methods of control (Samish *et al*., 2004). Using fungi as biological control agents is due to their unique mode of action besides their ability to adhere to the cuticle, to germinate and penetrate enzymatically (Ferron, 1981).

Scopulariopsis is a large anamorphic genus of fungi contains hyaline and dematiaceous species and telemorphs which are classified in the genus *Microascus* (Guarro *et al*., 1999). There is a great diversity of microscopic fungi naturally found on the body surfaces of ticks. The majority of these fungi are anamorphs of ascomycetes like *S. brevicaulis*, capable of producing copious numbers of conidia (Abbott *et al*., 1998). In 1907, Bainier made the initial identification of *S. brevicaulis* (Barron, 1968). Since then several other species have been identified (De Hoog *et al*., 2000). *S. brevicaulis* is by far the commonest species with worldwide distribution in soil, plants and insects. It is a filamentous non dermatophyte fungus mainly associated with nail, hair and skin infections of both man and animals (Abdel- Gawad, 1998; Kozak *et al*., 2003; Ogawa *et al*., 2008). Although, it is the most common saprotroph species, it is frequently encountred in dogs, cats and large animals. The role of *Scopulariopsis* species is more significant in immunosuppressed and immunocompromised human (Krisher *et al*., 1995).

Objectives of the study are to clarify natural fungi infesting *A. lepidum*.; assess their pathological efficiency against target ticks; to be used as entomo-pathogenic fungi.

MATERIALS AND METHODS

Isolation of the fungus: Eggs, larvae and dead females of *Amblyomma lepidum* covered with whitish materials were brought to mycology department at Central Veterinary Research laboratory to determine natural occurrence of fungi infecting *A. lepidum*.

Following the method described by Milne (1989), the mummified female ticks were 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. Similarly, eggs and dead larvae were microscopically examined. Eggs and dead larvae were cultured in duplicate slopes and plates of Sabouraud Dextrose Agar (SDA) and Brain Heart Infusion Agar (BHIA) supplemented with 0.05 mg mL^{-1} chloramphenicol and 0.5 mg mL^{-1} cycloheximide. Cultures were then, incubated at 27°C and daily examined for two weeks. Surface disinfection of *A. lepidum* cadavers was carried out according to the method described by Lacey (1997) by placing the ticks in 70% alcohol for few seconds to facilitate wetting of the specimen then were dipped in 1% sodium hypochlorite (NaOCL) for one minute and then briefly rinsed in three changes of sterile water. They were, then, dried using sterile filter paper (Whatman, No. 4). Some of the ticks were cultured onto SDA plates, while others were homogenized by adding sterile distilled water and, then, crushed using sterile mortar and pestle before culture. Plates were, then, incubated at 27°C for two weeks. Colonies which developed were mounted in lactophenol cotton blue and microscopically examined. Subcultures on SDA were kept at 4°C for further studies.

Identification of the isolate: The isolated fungus was tentatively identified on the basis of its morphology according to the monograph by Clayton and Midgley (1989). Following the method described by Koneman *et al*. (1992), a small portion of mould colony was picked with a sterile culture needle, mounted in a drop of lactophenol cotton blue on a slide and was microscopically examined. The developed fungal colonies were later lyophilized according to the method described by Cruickshank (1975). Fungal colonies were covered with 3 mL sterile horse serum. Two mL of the colony suspensions were collected into sterile bijou bottles kept in a freezer overnight. The bottles were transferred to Lyotrap machine (Edwards (LTE)). The suspensions were rapidly dried in a vacuum. The Lyophilized cultures were

sent to the Centre for Microbial Biotechnology, Technical University of Denmark, for confirmation of the identification.

Preparation of spore suspension: Spore suspension was prepared according to method described by Maniania (1992). Young colonies (10-14 days) of *S. brevicaulis* was harvested by scraping from surface cultures. The harvest was collected in McCarteny bottles with glass beads. Ten milliliter of sterile water containing 0.1% tween-80 was added to the bottles. Glass beads were used to facilitate separation of the propagules and tween-80 to reduce the surface tension. Bottles were then agitated on vibrant shaker (Auto-vortex mixer, Stuart Scientific Co. Ltd., Great Britain) for 5 min to produce homogenous conidial suspension. The suspension was filtered through sterile glass wool packed into 10 mL disposable syringe. Centrifugation of the suspension at 3000 rpm for 10 min was carried out. The supernatant was discarded and the sediment was washed three times in sterile distilled water. A test sample of the suspension was examined microscopically to confirm that it contained microconidia only. Later, the sediment was re-suspended in sterile distilled water and kept at 4°C till use.

Preparation of culture filtrate of *S. brevicaulis*: *S. brevicaulis* was cultivated on SDA for two weeks at 28°C. Colonies were removed and transferred to 50 mL glucose broth in three 250 mL flasks on shaking incubator at 100 rpm and 26°C for three days. Flasks were kept at room temperature for further three days. The mycelia were removed by filtration and the solution was centrifuged three times at 3000 rpm for 10 min to remove mycelial fragments. It was then sterilized by passing through nitrocellulose filter pore- size (0.45 μ).

Metabolite profiling of *S. brevicaulis*: Thin Layer Chromatography (TLC) was used to determine *metabolite* profiling of *S. brevicaulis* culture filtrate. Following Frisvad *et al.* (1989) method. The method was used without metabolite standard as it was a profile. Instead a broad spectrum antifungal griseofulvin was used. Two agar plugs from SDA colony were cut using Pasteur pipette. The mycelium side was gently pressed for a few seconds onto TLC plate coated with silica gel 60, Merck Art 5721 with the agar side facing down towards the plate 2.5 cm from the bottom line. One agar plug from BHIA was also placed on the plate with the agar side down towards the plate placed 1 cm from where the SDA plugs were placed. The liquid was extracted from the agar plugs and was visible on the TLC. The plugs were then removed. Griseofulvin (100 mg mL^{-1} in methanol) was placed in the middle lane as internal standard. The plate was then eluted for 15 min in CAP (chloroform/acetone/2-propanol; 85, 15, 20). The plate was dried for 5 min. Another plate was prepared in the same way as the above one but eluted for 15 min in TEF (Toluene/ethyl acetate /90% formic acid; 5/4/1). Then the TLC plate was dried for 1 h and seen under UV light (366 and 265 nm) and in day light. Later, the plate was sprayed with $AlCl_3$ (20% $AlCl_3.6\,H_2O$ in 60% ethanol) and then heated at 130°C for 8 min. The secondary metabolites were examined under UV and day light. Finally, the first plate was sprayed with $Ce(SO_4)_2$(1% in 3MH_2SO_4). Similarly the second plate was sprayed with ANIS (0.5% p-anis aldehyde in ethanol/ con-acetic acid/con-sulphuric acid; 17/2/1). The plate was heated at 130°C for 8 min. The secondary metabolites were examined under UV and day light. The separated spots were then scraped, dissolved in 5 mL of acetone, filtered through Whatman No.1 filter paper. The solution was transferred to screw-capped bottles and injected in GC-MS (Gas chromatography Mass spectrometer) to determine the volatile compounds which might be present in the culture filtrate of *S. brevicaulis.*

Pathogenicity of *S. brevicaulis*: Clean laboratory reared larvae and engorged females of *H. a. anatolicum* and *A. lepidum* were subjected to infection with *S. brevicaulis.* Percentages mortality, female fecundity, moulting and hatchability of eggs were determined.

Treatment of flat larvae: Three replicates of about (150) *H. a. anatolicum* and *A. lepidum* flat larvae were subjected to treatment with spore suspension and culture filtrate of *S. brevicaulis.* Concentration of 10^7 spores mL^{-1} in distilled water was prepared according to Kaaya (1989) method. Inoculation was performed by dipping larvae into the spore suspension for 1 min according to the method described by Mwangi *et al.* (1995). Larvae were dried with sterile filter paper Whatman No. 4 and then kept in sterile plastic test tubes tightly covered with nylon mesh. Three replicates of 150 flat larvae were treated in the same way with sterile distilled water to serve as control. Both treated and controls were maintained at 27±1°C and 85% Relative Humidity (R.H). They were monitored every two days for two weeks. Infection and mortality were recorded.

Treatment of engorged larvae: Engorged larvae of *H.a. anatolicum* and *A. lepidum* were treated by dipping into the spore suspension and culture filtrate of *S. brevicaulis* for 3 min. Three replicates each of 150 engorged larvae were tested following Kaaya (1989) method Inoculation was performed by dipping larvae into the spore suspension and culture filtrate for 1 min

according to the method described by Mwangi *et al.* (1995). Three replicates of 100 engorged larvae served as controls that were treated in the same way with sterile distilled water. Both treated and control larvae were maintained at 27±1°C and 85% RH. They were monitored every two days for two weeks. Infection and mortality were recorded.

Treatment of nymphs: Both Flat and engorged nymphs of *H. a. anatolicum* and *A. lepidum* were subjected to infection with 10^7 spores mL^{-1} *S. brevicaulis* and its filtrate by dipping for 2 min following the same procedure applied for larvae. Three replicates each of 100 flat nymphs and 50 engorged nymphs were used. Control groups were treated with sterile distilled water. Treated engorged nymphs were observed for moulting and development of mycosis and Mortality. Dead engorged nymphs were surface disinfected according to method described by Mwangi *et al.* (1995). Disinfected ticks were then cultured into moist chamber, kept at room temperature and observed for fungal growth.

Treatment of flat adult *H.a. anatolicum* and *A. lepidum*: Flat adult female *H. a. anatolicum* and *A. lepidum* were subjected to treatment with *S. brevicaulis* following the procedure described above. Spores of 1×10^7sp mL^{-1} and culture filtrate were used. The control group was treated with sterile distilled water. The experiment was repeated three times. Mortality of treated ticks was recorded.

Treatment of engorged *A. lepidum* females by immersion: Fifty engorged *A. lepidum* females were used to assess pathgenicity of *S. brevicaulis*. Weights of ticks were taken before treatment. They were then treated by immersion method for 5 min using 3.5×10^7spores mL^{-1}. Control groups were immersed in sterile distilled water for the same period. They were maintained at 27±1°C and 85% RH and monitored for mortality, pre-oviposition period, eggs laying and hatchability.

Treatment of engorged *A. lepidum* females by injection: Engorged females of *A. lepidum* were surface disinfected with 70% alcohol followed by sterile distilled water. A volume of 0.1 mL of 10^4 spore mL^{-1} *S. brevicaulis* spore suspension was injected using a fine 1 mL insulin syringe. Injection was made between the third and fourth coxae following Ignoffo *et al.* (1982) method. Ticks were then kept at room temperature (30°C) in desiccators with water to give 85% RH. They were daily observed for developing of mycosis, mortality, oviposition and hatching of eggs. Control groups were injected with sterile distilled water in addition to non injected control group that were kept under the same conditions of incubation. Three replicates each of 10 females were used.

RESULTS

Mycological findings: Cultures from contaminated eggs, larvae and scrapings of dead *A. lepidum* females revealed fungal growth after 4 days. The colonies on SDA were glabrous, velvety and radially folded. The fungus was easily propagated on both SDA and BHIA but the growth was better on BHIA. Heavier growth was obtained with chloramphenicol without addition of cycloheximide.

The isolate was identified as *S. brevicaulis* according to morphological and cultural characteristics using monograph of Clayton and Midgley (1989) (Fig. 1). Lyophilized cultures sent to the Centre for Microbial Biotechnology Laboratory in Denmark were confirmed as *S. brevicaulis*.

Metabolites profiling: Different substances with various Rf values (ratio between distance of substance to distance of the solvent) were obviously seen separated on TLC plates under 265 nm (Fig. 2). Analysis of TLC

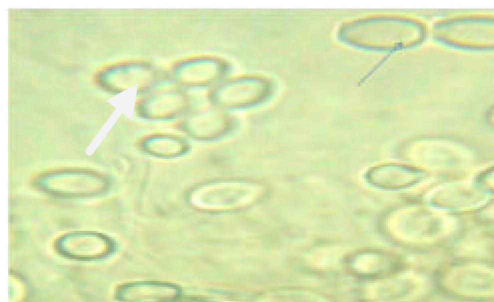

Fig. 1: Barreled-shaped conidia of *S. brevicaulis* on lactophenol cotton- blue stain growing in culture of scraping of mummified *A. lepidum* females

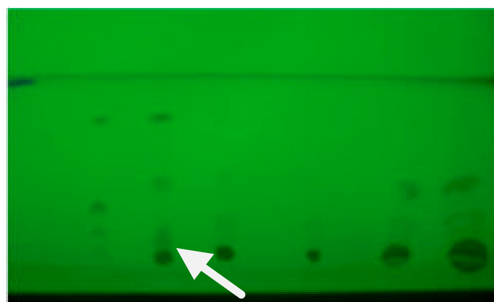

Fig. 2: TLC showing separation of the metabolites of *S. brevicaulis* cultured on SDA and BHIA under UV (265 nm)

product of culture filtrate on GC-MS (Gas chromatography mass spectrometry) Revealed detection of ketone and fatty acid compounds like 2-Pentanone, 4-hydroxy-4-methyl (formula: $C_6H_{12}O_2$ with molecular weight 116), n-hexadeconic acid($C_{16}H_{32}O_2$ with M.W 256), 6-octadecenoic acid ($C_{18}H_{34}O_2$ with M.W 282, (Z), 1, 2-Benzene dicarboxylic acid ($C_{16}H_{22}O_4$ having molecular weight of 278, bis (2-methylpropyl) ester and ethanone, 2, 2-dimethoxy-1, 2-diphenyl ($C_{16}H_{16}O_3$ with M.W 256).

Pathogenicity of *S. brevicaulis*

Effect of *S. brevicaulis* on flat larvae, nymphs and adult: High mortality of flat larvae of *H. a. anatolicum* and *A. lepidum* 3-5 days post treatment was observed. Treatment was highly significant (p = 0.01). Similarly, spore suspension of *S. brevicaulis* induced high mortality to flat nymphs as compared with the Filtrate. Infected nymphs developed fungal infection with mycelia obviously seen covering their bodies. Treatment of *A. lepidum* and *H. a. anatolicum* flat adults with *S. brevicaulis* induced mortality to flat adults (Table 1). Mortality was observed to be dose response. Infected ticks developed fungal infection 7 days post treatment. Fungal hyphae were seen covering their bodies. Microscopic examination of a needle mount of surface disinfected flat ticks revealed conidia of *S. brevicaulis*. *S. brevicaulis* filtrate-treated group induced mortality a little bit higher than *S. brevicaulis*-treated group.

Treatment by immersion of *A. lepidum* flat adults by *S. brevicaulis* also, induced mortality to flat adults. Infected ticks developed fungal infection and subsequently died 7 days post treatment. Fungal hyphae were seen covering their bodies (Fig. 3).

Engorged larvae: *S. brevicaulis* caused low moulting percentage of engorged larvae. *S. brevicaulis*-treated groups of engorged larvae started moulting on day 7 post treatment. Filtrate- treated group started moulting two weeks after infection. Moulting period was 10 days. *S. brevicaulis*-treated groups showed high mortality due to high rate of infection while the filtrate groups showed a moderate mortality rate where fungal mycelia seen covering their integuments. Moulting per cent was significantly reduced (p = 0.005) compared with the control. *S. brevicaulis*-treated groups have same moulting per cent but filtrate- treated group showed a higher per cent than spore- treated groups. Table 2 represents the differences. Most *A. lepidum* engorged larvae treated *S. brevicaulis* failed to moult and infection seen 2 days post treatment. Percentage morbidity was 70%.

Engorged nymphs: Treated engorged nymphs of *A. lepidum* developed fungal infection. Mycelium were seen covering their integuments 7 days post treatment. Moulting to flat adults started 21 days after dropping. All control groups succeeded to moult while only 45% of the treated groups moulted to adults. Surface disinfection of treated engorged nymphs that failed to moult and cultured on moist chamber, developed fungal mycelia. Control and treated groups of *H. a. anatolicum* engorged nymphs started moulting 18 days post treatment and after 16 days in filtrate-treated groups. Infected nymphs died on day 3 and 5 days after infection. Mycelia were seen covering their integuments. There was insignificant differences (p = 0.05) in moulting percentage as shown in (Table 2). The 73% of *S. brevicaulis*- treated died 21 days post infection.

Fig. 3: *A. lepidum* flat adults colonized by *S. brevicaulis* 7 days post infection by immersion under laboratory conditions

Table 1: Mortality of flat larvae, nymphs and adult of *Hyalomma anatolicum* caused by *S. brevicaulis* 10^7 spores mL^{-1} and culture filtrate

Treatment	Mortality (%) (mean±SD) flat larvae	Mortality (%) (mean±SD) flat nymphs	Mortality (%) (mean±SD) flat adult
S. brevicaulis	95.7±7.5**	40.12±7.54	75±11.79**
S. brevicaulis filtrate	60.0±6.6**	22.15±4.04	77.5±3.54**
Control	3.50±3.17	4.22±1.15	3.77±8.26

**p = 0.01

Table 2: Effect of *S. brevicaulis* on moulting of engorged *H. a. anatolicum* larvae and nymphs

Fungus	Moulting (%) (mean±SD) of larvae	Moulting (%) (mean±SD) of nymphs
S. brevicaulis spore suspension	19.47±2.75**	74.10±9.41$^{n.s}$
S. brevicaulis filtrate	22.50±3.54**	70.00±4.00$^{n.s}$
Control	100.0±0.00	83.00±4.24

**p = 0.01, n.s: insignificant

Table 3: Mean (±SD) of the effect of *S. brevicaulis* on engorged female *H. a. anatoilicum*

Group	Hatchability (%)	Female fertility (%)	Egg mass (g)
S. brevicaulis	44.13±15.77	47.54±1.92	0.21±0.12
S. brevicaulis Filtrate	39.90±7.38**	50.02±5.85	0.22±0.10
Control	93.33±6.51	68.81±2.21	0.63±0.05

Table 4: Means (±SD) of the effect of *S. brevicaulis* on engorged *A. lepidum* females

Parameter	Control groups (Immersed in distilled water)	Treated groups (Immersed in 10^7 spores mL^{-1})
Female fertility (f.f) (%)	81.55±5.87	57.73±6.35*
Hatching of eggs (%)	70±6.49	40±2.99
Loss of weight of female (l.w.f) (%)	0.04±0.02	0.15±0.04
Weight of Egg batch (g)	0.58±0.0 5	0.11±0.07

*p = 0.05, f.f: Weight of egg batch/engorgement weight before oviposition x100, L.w.f: Engorgement weight before oviposition-(weight after oviposition+weight of egg batch)

Treatment of engorged females *H. a. anatolicum*: *S. brevicaulis* spore suspension and filtrate affected the biotic potential of the treated females. Hatchability, female fertility and eggs laid were significantly reduced (p = 0.01) compared with the control (Table 3). *S. brevicaulis* treated-groups and the control groups started oviposion 4 days from dropping time while filtrate treated-groups started to lay eggs 7 days post infection. *S. brevicaulis* - treated groups also showed infection of eggs. Dead, newly hatched larvae were seen colonized with fungal conidia. There was difference in oviposition period. It was 21 days in control *H. a. anatolicum* and 30 days in the *S. brevicaulis* and filtrate - treated groups.

Effect of *S. brevicaulis* on of engorged *A. lepidum* females treated by immersion: method: Infection started on day 3 post treatment. Ticks were black, immobile, showed no response to light stimuli. Mycelia were seen covering their first pairs of legs, mouth parts and later the whole integument. Death observed 15 days post treatment. However, 70% of treated ticks laid eggs normally and their eggs hatched. But, all larvae died 3 days after hatching. Treated groups showed fertility with values significantly (p = 0.05) lower than the control group. Weight loss was also, greater in treated groups than in the control (Table 4). Surface-sterilized treated engorged adult that failed to moult and cultured on moist chamber, developed fungal mycelia.

Infection by injection of engorged *A. lepidum* females with 10^4 spore mL^{-1} suspension of *S. brevicaulis* revealed high level of infection 3 days post treatment. However, 66.7% of the treated groups developed fungal infection. Two weeks later, the cadavers were colonized by the fungus (Fig. 4). All control groups succeeded to oviposit and 100% of their eggs hatched while only 46.2% of the treated group oviposited and 33.3% of the eggs hatched. Pre-oviposition period was 7 days for control and a range of 7-21 days for the treated groups. The oviposition period of the treated groups was 47 days.

Fig. 4: An engorged *A. lepidum* female colonized by *S. brevicaulis* 14 days post infection by injection

DISCUSSION

The present study revealed isolation of *Scopulariopsis brevicaulis* from *Amblyomma lepidum*. The result showed great affinity of fungi with ticks as this fungus was known to be a common saprophytic filamentous fungi inhabiting soil (Abdel- Gawad, 1998). Association of soil fungi with different species of insects and ticks was reported. Honey bees and insects were found to be parasitized by *Scopulariopsis* species (Batra *et al*., 1973).

In this study, eggs, larvae and adult *A. lepidum* were found infected with *S. brevicaulis*. This finding is similar to that of Kalsbeek *et al*. (1995) who isolated fungi like *Verticillium lecanii* and *Paecilomyces farinosus* from dead and alive larvae, nymphs and engorged females of *Ixodes ricinus*.

Although, *S. brevicaulis* was known to be a common soil saprotrophs, Ascan *et al*. (2003) isolated it on Petri dishes of Rose Bengal Streptomycin Agar exposed to air for few minutes. Moreover, *Scopulariopsis* species were found to be common soil and plant saprotrophs and opportunistic pathogens that cause ringworm and dermatophytosis in human and animals (Aho, 1983; Kozak *et al*., 2003).

In this study, *S. brevicaulis* was successfully isolated from dead *A. lepidum*. It is considered as the fist report in the Sudan. Although, *S. brevicaulis* is a non

dermatophytic fungus it was reported to have a keratophytic function (Bagy, 1986; Marchisio *et al*., 2000). Aho (1983) reported hair and skin infections of laboratory animals like Guinea pigs as well as large animals like cows, donkeys besides dogs and cats. Moreover, S. *brevicaulis* was known to produce extracellular keratinase (Anbu e*t al*., 2006) which might facilitate penetration of tick integument. Furthermore, Yoder *et al*. (2003) identified *S. brevicaulis* in body contents of *Dermacentor variabilis* after topical application of fungal inoculum. Hence, pathogenicity of the fungus to various developmental stages of *A. lepidum* which was proved in this study might be due to ability of *S. brevicaulis* to assume parasitic form. Thus, death of *A. lepidum* which was observed in this study might be due to the extracellular enzymes or toxins produced by this fungus.

In the present study, detection of ketone compounds in culture filtrate of *S. brevicanlis* suggested presence of such microbial toxins which might lead to the death of ticks. Thus investigation on fungal metabolites and their use to control ticks could be further studied if biotechnology to produce such toxins is feasible. Further studies are recommended to elucidate these assumptions.

Furthermore, colonization of dead ticks might be due to fungal hyphae from germinating conidia which might be capable of penetrating the cuticle directly or may invade via the genital pore, anus, or spiracles, thereby preventing the hatching of eggs or causing tick death. This finding is also, observed by Samish and Rehacek (1999).

CONCLUSION

In the present study *S. brevicaulis* was found to be potent to *Hyalomma anatolicum* and *A. lepidum.* No previous studies been carried to evaluate its pathgenicity to these tick species. It is the first time to test its pathogenicity to such ticks. As virulence of a fungal pathogen is essential element in selection of a suitable candidate for microbial control.

S. brevicaulis showed ability to reduce fecundity and egg hatchability of treated ticks. This result is of great importance because a reduction in eggs laid and hatchability as shown in this study means a very significant reduction in the next generation of ticks when considering the large number of eggs laid. Hence, application of such fungi in vegetation will be effective as ticks spend 95-97% of their life in environment.

Culture filtrate of *S. brevicanlis* tested in this study highlighted the use of fungal toxins instead of live fungi especially when they considered as secondary pathogens. Thus, extraction of such microbial toxins and their use to control ticks could be investigated if biotechnology to produce toxins is feasible.

Small-scale farmers in developing countries like the Sudan may easily adopt microbial pesticides if appropriate technology of production and application is developed. In this study, *S. brevicanlis* was easily and cheaply mass produced. For large scale production a fermented can be used for production of large amount of fungal metabolites.

Drawbacks for the existing tick control methods such as high cost of acaricides, development of resistance to acaricides, adverse effect on environment, beneficial species and non target animals, encourage possibility of using such fungi as an alternative means to acaricides.

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