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Bio-Pesticide Control of the Brown Dog Tick (*Rhipicephalus sanguineus*) in Egypt by using Two Entomopathogenic Fungi (*Beauveria bassiana* and *Metarhizium anisopliae*)

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ABSTRACT

The current study examined the potential use of entomopathogenic fungi to control infestation of *Rhipicephalus sanguineus*. Examination of 514 dogs admitted to veterinary clinics in Egypt revealed that 67.5% were infested with *R. sanguineus*. Two hundred and sixty adult ticks were collected. *Beauveria bassiana* and *Metarhizium anisopliae* were then tested for their effect on these ticks. The *in vitro* effect of different concentrations of *B. bassiana* on engorged females, unfed females, fed males, eggs, larvae, and nymphs was strong for all three concentrations of *B. bassiana* compared with controls ($P<0.05$) and white fungal colonies grew on the surface of the ticks. The B1 of *B. bassiana* (10^8 conidia/ ml) was the most pathogenic on adult and developmental stages of ticks. The impact of different concentrations of *M. anisopliae* on adult and developmental stages in comparison with controls was similar, with the growth of green hyphae around eggs and adult ticks which prevented hatching and resulted in tick death. The M2 suspension (10^7 conidia/ml) had the most potent effect on adult ticks and developmental stages. The efficacy of this suspension was higher than that of the B1 concentration of *B. bassiana* (98 and 100% respectively). Therefore, 10^7conidia/ml of *M. anisopliae* seems to be the most effective fungus to use as bio-pesticide to control different developmental stages of *R. sanguineus* and may be a reasonable alternative to chemical treatment.

Key words: Bio-pesticides, Entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, *Rhipicephalus sanguineus*

INTRODUCTION

Ticks are obligate blood-sucking arachnids which feed on vertebrates. A huge number of emerging vector-borne infectious disease could be transmitted by ticks to domesticated and wild dogs such as *Babesia*, *Ehrlichia*, *Hepatozon* and *Rickettsia* species which transmitted by *R. sanguineus* (Walker *et al*., 2000). Ticks have a global distribution because of their ability to survive under different climatic conditions and ecological housing, and so their control is still a major dare for veterinarians and pet owners. Since long time ago the use of chemicals considered the main line of defense against ticks (Zhioua *et al*., 1997), although acaricides have been powerful in suppressing tick populations and incidences of tick-borne diseases, their main obstacles is the accumulation of its toxic residues in milk and meat products resulting in negative effects on the human health besides its high production costs (Latif and Jongejan, 2000).

Environmental and food contamination by these products and their residues considered another major disadvantage (Norval *et al*., 1992). Tick resistance to acaricides is an increasing problem and real economic intimidation to different animal species and allied industries. For tick's control, most stockholders depend completely on acaricides, but the misuse or the ignorance on how to make a profit from their tick control program or how to detect and resolve problems with resistance to acaricides (George, 2000). As well adverse experiences encompass some veterinary chemical products have been reported in the form of skin reactions, neurological signs, lethargy, anorexia and in some cases death in dogs (Pesticides and Authority, 2003). More importantly, these commercial synthetic insecticides have a lethal effect on many invertebrates, including non-target ones as beneficial insects and arthropod predators (Echegaray, 2009). So, the scientific community has motivated interest in developing substitution ways to their control. One of the most successful methods to control various agricultural and pasture pests is the use of entomopathogenic fungi. In Brazil, they used to control sugar cane pests via spraying with airplanes in large fields (Gillespie and Claydon, 1989).

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Whereas in Indonesia and Malaysia, they are used to control a serious pest of oil palms, the rhinoceros beetle. In Australia, field tests have produced promising results with other Australian pests (Milner *et al*., 1994; Hooper *et al*., 1995). They used to control the subterranean pasture pest (Rath 1992; Rath *et al*. 1995a; Rath *et al*., 1995b). Higher mortalities in tsetse flies also have been found to induce by entomopathogenic fungi (Kaaya and Munyinyi, 1995) and evidence of horizontal transmission from infected to uninfected flies has been demonstrated (Kaaya and Okech, 1990). Some laboratory experiments demonstrated high virulence of entomopathogenic fungi against ticks. Among fungal genera and species that have been tested, *Metarhizium anisopliae* and *Beauveria bassiana* found to have the highest virulence; and therefore, these are the most investigated entomopathogenic fungi regarding their potential for the control of tick species worldwide. Avoidance the introduction of exotic fungal isolates requires the isolation of indigenous entomopathogenic fungi for proper biological control of ticks in certain environments. Moreover, indigenous isolates may be better adapted to the natural conditions (e.g., tolerance to heat, cold activity, UV-radiation) of their geographical origins, suggesting that these isolates could reach higher efficacy in tick population control (Fernandes *et al*., 2007; Fernandes *et al*., 2008). *M. anisopliae* act by penetration into the cuticle of *R. microplus* and *R. sanguineus* (Magalhães *et al*., 1998). This process involves secretion of enzymes such as proteases and chitinases and is assisted by mechanical processes of the appressorium infection peg (Charnley and Leger, 1991). After penetration, the fungus invades the internal organs with the production of mycotoxins that kills the host (Kaaya *et al*., 1991). However, certain tick species may display differential susceptibility to entomo-pathogenic fungi tick due to fungistatic compounds present in the epicuticle (Kirkland *et al.*, 2004). An increased number of conidia on the ticks' cuticle lead to increased mortality rate (Kaaya *et al*., 1996). Possibly, there is cooperation between neigh boring germinating conidia on the arthropod cuticle (Zhioua *et al*., 1997). Our research was conducted in vitro to demonstrate the effectiveness of entomopathogenic fungi on different stages of an Egyptian strain of *R. sanguineous* as a way to be used as an alternative to chemicals.

MATERIALS AND METHODS

Tick sampling

Five hundred fourteen (514) dogs of different breeds were examined. Two hundred and sixty adult ticks were collected from naturally infested dogs admitted to veterinary clinics in Cairo and Giza governorates of Egypt. Ticks were identified based on morphological features (Walker, 2003).

Ticks rearing

The engorged females *R. sanguineous* collected from different infested dogs (no previous application of pesticides along more than 30 days) was used for breeding. Ticks were reared at the Department of Parasitology, Faculty of Veterinary Medicine, Cairo University, Giza. Each full fed female tick was well kept and maintained alive in a single glass tube with 16 mm of diameter and a height of 75 mm were used for maintenance of ticks, tubes were locked tightly with a cotton tampon, labeled with data and incubated at 27°C and 80% relative humidity (Cafarchia *et al*., 2015).These female ticks were daily observed till oviposition and hatching of eggs, then the hatched larvae were fed on ears of male Newzeland white rabbits (2-2.5 kg body weight and with no previous history of exposure to ticks and mites) and were protected by an ear bag till molt into nymphs, the ear bag method described by Abuowarda *et al*., 2015 were used. Rabbits were reared according to the role of ethical of institutional animal care and use committee, Cairo University.

Entomopathogenic fungi culture

The entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* were native isolates, each was isolated from soil (Ali and Moharram, 2014), in Bio-insecticide production unit, plant protection research institute and identified in Mycological center, Faculty of Science Assuit University. These isolates were cultivated on Sabouraud dextrose agar (SDAY) with 1% yeast extract plates and were full grown at 25±1°C and 100% RH (Figure 1). The conidia were picked by scraping the surface of 14-15 days old culture slightly with inoculation needle. The conidia were suspended in a sterile distilled water containing 0.1% Tween-80. The mixture was stirred with a magnetic shaker for 10 min (Kaaya and Hedimbi, 2012; Ali and Moharram, 2014).

In vitro biological control of ticks

R. Saunguines were subjected for *B. bassiana* and *M. anisopliae* as a method used for biological control. The experiment was performed from 2016 to 2018 in the laboratory of Parasitology Department, Faculty of veterinary medicine of Cairo University.

Adult ticks

Three hundred and 60 male, unfed and fed female *R. Saunguines* (120 ticks each) were collected from the laboratory tick colony. Ticks were cleaned from the dorsal and ventral surface with a brush soaked in sterile distilled water then dried with filter paper. Each male and female tick was kept in a sterile glass tube and closed with a sterile cotton plug. These ticks were divided into two equal groups (60/group) for subjected to both *B. bassiana* and *M. anisopliae*.

The 1st group was divided into 4 subgroups (15 ticks each) as mentioned in table (1), which infected by different concentration of *B. bassiana* (B1= $1x10^8$; B2= $1x10^7$; B3=$1x10^6$ conidia/ml) and compared with control one (distilled water containing 0.1% TWEEN®80). The 2nd group infected by different concentration of *M. anisopliae* and used the same procedures in *B. bassiana*. Each female tick immersed in diluted conidia for 2 min, then dried by filter paper and kept in a sterile glass tube closed by a sterile cotton plug. These ticks were incubated at 27°C and 80 RH for 7 days waiting for lay eggs. After one week, each subgroup of ticks transferred to one petridish contained cotton soaked with sterile distilled water and closed with paraffin film and the female mortality and mold growth on dorsal surface daily for 20 days (Table 1).

In addition, the following biological parameters of ticks used to evaluate entomopathogenic fungi (*B. bassiana*

and *M. anisopliae)*: preoviposition period, oviposition period, egg mass [was calculated as the weight of eggs laid by individual females], engorgement weight [where adults dropped were weighted individually], the egg production index (EPI) was calculated by using the formula: EPI= [egg mass weight (mg)/ initial weight of engorged female (mg)]×100 (Alves *et al*., 2017), mortality rate of unfed female (%), DT%=100(1-NTV/NTC) [DT(%) is the percentage reduction of mean number of adult females, NTV the number of adult females in the treated group, NTC the number of adult females from control group as per the method of (Fragoso *et al.,* 1998), DO (%) = 100(1-PATV/PATC) [DO (%) is the percentage reduction of mean weight of eggs, PATV the mean weight of eggs of the treated group, and PATC the mean weight of eggs of the control group] and E (%) = 100[1- (CRT x CRO)] [E (%) is the efficacy of treated, CRT the reduction in the number of adult females NTV/NTC and CRO the reduction in egg laying capacity PATV/PATC]. Male *R. sanguineus* ticks were treated by using the same number and procedures as in fed female.

Stages of *R. sanguineus*

Eggs, larvae and nymphae were collected from the laboratory tick colony. Three subgroups of the first group of each stages (eggs, larva, and nymph) (Table 2) subjected to different concentrations of *B. bassiana (B1, B2, B3)* and the fourth groups were controlled, while the second groups were treated by *M. anisopliae (M1, M2, M3)* and divided as in *B. bassiana* previously. Eggs, larvae, and nymph incubated at 27ºC and 80%RH and assessed the Rate of hatchability (%), the percentage of living larvae after 13days, the Molting rate of (%) after 13 days, Mortality rate of nymph (%) and Time of molting (days).

Statistical analysis

Statistical analysis was performed using the LSD and Duncan tests in ANOVA (SPSS 17.0 statistical software) for determined significant differences between treated and control animals. Differences were considered significant at $P<0.05$ level (Freeman and Tukey, 1950).

RESULTS

During examination of 514 clinical cases admitted to the clinics, the percentage of tick infestations were 67.5%. The dogs <1 years of age were more prone to ticks (74.7%) followed by dogs from 1 to 3 years old (21.5%) then dogs >3 years old (3.8%). Female dogs were more prone to be affected with ticks than male.

Determination of impact of entomopathogenic fungi as in vitro biological control of ticks

The in vitro effect of different concentration of *B. bassiana* and *M. anisopliae* on engorged full feed females, male, egg, larva, and nymph in compared with control at *p* value<0.05 were shown (Fig. 2). The white fungal mycelium grown of developmental stages and adult of *R. sanguineus*.

The longest preoviposition and oviposition periods of adult full engorged female ticks were 15.85±1.45and 15.14±1.27 days respectively showed on B1 concentration (10^8 conidia/ ml) in compared with control (10.67±0.45 and 9.67±0.59 respectively). The lowest egg production index (EPI) was 10.91±0.03 observed on B1 concentration (10^8 conidia/ ml). The highest mortality rate of unfed female and fed male ticks was recorded on B1 concentration (73.21%±5.3 and 68.89%±2.22 respectively) in compared with control (10.96 %±1.48 and8.78%±2.1 respectively) (Table 3-4). Furthermore, the highest percentage reduction of the mean number of adult females (DT%), the percentage reduction of mean weight of adult females (DO%) and the efficacy of fungus against ticks (E%) were 88.84, 100 and 98% respectively recorded on B1 concentration (Table 3; Chart 1).

The data mentioned in the Table 3, 4 and Figure 1 illustrated that the B1 concentration of *B. bassiana* (10^8 conidia/ ml) was the most pathogenicto all stages of ticks whileB3 (10^6conidia/ ml) showed the lowest effect.

In addition to, the lowest rate of hatchability was 14.5±2.10 observed in B1 concentration while control one was 65±14.28. The percentage of lived larvae of *R. sanguineus* after 3, 4 and 13 days were decreased in groups treated by B1 concentration of *B. bassiana* (10.5±1.55, 6±2.16 and 0±0 respectively) than groups treated by B2 and B3 concentration. Meanwhile, 10.21±2.12 % was the lowest molting rate of larvae to nymphae after 13days was also showed in B1 concentration as well as the peak of the mortality rate of nymphae was 93.5±1.7 % plus the longest period of molting of nymph to adult stage (12±0.51days) reported in B1 concentration (Table 5).

On the other side, the impact of different concentrations of *M. anisopliae* (M1, M2 and M3) on a full engorged female, male, egg, larva, and nymph in compare with control at $P<0.05$ (Fig. 3). In addition, the treated eggs of *R. sanguineus* with *M. anisopliae* showed growth of green hyphae around egg colony led to prevent hatching of eggs. Moreover, both dorsal and ventral surface of male and female showed growth of green hyphae which led to impair the vitality of adult then death.

Furthermore, results indicated also that, no eggs were laying from adult female ticks treated with M2 concentration of *M. anisopliae* so, all biological parameters related to adult females such as preoviposition periodoviposition period, egg mass and egg productive index (EPI) were nil in compared with control female ticks at $P<0.05$. Meanwhile, the peak percentage of reduction of and the efficacy of fungus against ticks (E%) were reached to hundred percentage observed on M2 concentration (Table 3, Chart 1). Moreover, the M2 concentration (10^7conidia/ml) of *M. anisopliae* was the most potent effect on the adult female,male, egg, larva, and nymph in compared with other concentration. Results showed that the lowest rate of hatchability (7.25±2.49%), a percentage of lived larvae after three, four and thirteen days (22.25±2.52, 7.5±0.64 and 0±0 respectively) were observed on groups treated with M2 concentration as well as molting rate of larval to the nymphal stage reached to zero percentage after 13 days. Also, M2 concentration of *M. anisopliae* was the highest rate of mortality of unfed female, fed male and nymph (87.31±3.47, 82.22±2.2 and 96±1.82 respectively) as well as have the longest period of molting from nymphal stage to adult (14±0.435 days) in compared with control at $P<0.05$.

Finally, the efficacy (E%) of M2 concentration of *M. anisopliae* (10^7 conidia/ml) was higher than the E% of B1 concentration on *B. bassiana* (10^8 conidia/ ml) (98 and 100%

Table 1: Design of treatment of adult *R. sanguineus* with entomopathogenic fungi.

Groups	Subgroups (Concentration)	Concentration (conidia/ml)	Adult full engorged female ticks (120)	Adult unfed female ticks (120)	Adult fed male ticks (120)
1st group *Treated with B. bassiana*	B1	$1x10^8$	each subgroup has 15 female ticks	each subgroup has 15 female ticks	each subgroup has 15 female ticks
	B2	$1x10^7$			
	B3	$1x10^6$			
	Control	distilled water containing 0.1% TWEEN®80			
2nd group *Treated with M. anisopliae*	M1	$1x10^8$			
	M2	$1x10^7$			
	M3	$1x10^6$			
	Control	Distilled water containing 0.1% TWEEN®80			

Table 2: Design of treatment of different developmental stages of *R. sanguineus* with entomopathogenic fungi.

Stages	Groups	No. of Subgroup	No. of replicate	No. of glass tubes	No. of stages	Total No. of stages
Eggs	1st	4	Each subgroup 4 replicate	16	100 eggs/tube	1600 eggs
	2nd	4				
Larvae	1st	4		16	150 larvae /tube	2400 larva
	2nd	4				
Nymph	1st	4		16	10 nymph /tube	160 nymph
	2nd	4				

No.: number.

Table 3: In vitro treated on *R. sanguineus* full engorged female ticks by *B. bassiana* and *M. anisopliae* compared with control at P<0.05.

Beauveria bassiana (mean±SE)				
Biological parameters of Ticks	Control	B1(10^8 conidia/ml)	B2(10^7 conidia /ml)	B3(10^6 conidia /ml)
Preoviposition period (day)	$10.67±0.45^c$	$15.85±1.45^b$	$14.53±1.51^b$	$12.2±0.64^a$
Oviposition period (day)	$9.67±0.59^c$	$15.14±1.27^b$	$14.33±0.1.48^b$	$11.6±0.32^a$
Egg mass (mg)	$0.0424±0.05^c$	$0.011±0.056^a$	$0.020±0.01^b$	$0.013±0.039^a$
Initial weight of engorged female(mg)	$0.1095±0.02^c$	$0.1008±0.032^b$	$0.1010±0.045^a$	$0.1021±0.042^a$
Egg productive index (EPI)	$38.72±0.033^c$	$10.91±0.03^a$	$19.80±0.06^b$	$12.73±0.036^a$
Mortality rate of unfed female (%)	$10.96±1.48^c$	$73.21±5.3^a$	$60.68±4.8^b$	$58.91±3.5^b$
DO %	-	88.84	51.21	48.75
DT %	-	100	92	90
E%	-	98	96	92
Metarhizium anisopliae (mean±SE)				
Biological parameters of Ticks	Control	M1(10^8 conidia/ml)	M2(10^7 conidia /ml)	M3(10^6 conidia /ml)
Preoviposition period (day)	$10.67±1.76^b$	$16.75±1.55^c$	N/A	$14±0.63^c$
Oviposition period (day)	$9.67±2.28^b$	$15.65±1.33^c$	N/A	$13±0.23^c$
Egg mass(mg)	$0.039±0.21^c$	$0.013±0.01^b$	N/A	$0.016±0.023^c0$
Initial weight of engorged female(mg)	$0.1052±0.011^c$	$0.1032±0.027^{ab}$	$0.1028±0.036^a$	$0.1036±0.051^b$
Egg productive index (EPI)	$37.07±1.20^d$	$12.59±0.05^b$	N/A	$15.44±0.029^c$
Mortality rate of unfed female (%)	$10.96±2.31^d$	$72.31±4.17^c$	$87.31±3.47^a$	$82.31±2.99^b$
DO %	-	90.87	100	88.83
DT %	-	100	100	94.93
E%	-	98	100	97

SE= Standard error, a, b, c Different superscripts within the same row of mean treated ticks by fungus indicate significant difference at P<0.05; DT%: the percentage reduction of mean number of adult females, DO%: the percentage reduction of mean weight of adult females and E%: the efficacy of fungus against ticks.

Table 4: In vitro treated on fed male *R. sanguineus* by *B. bassiana* and *M. anisopliae* compared with control at P<0.05.

Male ticks	*B. bassiana* (conidia/ml) (mean±SE)			*M. anisopliae* (conidia/ml) (mean±SE)		
Mortality rate (%)	B1(10^8)	B2(10^7)	B3(10^6)	M1(10^8)	M2(10^7)	M3(10^6)
	$68.89±2.22^c$	$53.33±3.84^b$	$51.11±4.4^b$	$13.33±0.0^a$	$82.22±2.2^c$	$71.11±4.4^b$
Control	$8.78±2.1^a$			$8.98±2.2^a$		

SE= Standard error, a, b, c Different superscripts within the same column of mean treated ticks by fungus indicate significant difference at P<0.05.

respectively) (Chart 1). So, M2 of *M. anisopliae* (10^7 conidia/ml) was the most potent concentration and effective fungus used as biopesticide control on adult and different developmental stages of *R. sanguineus*.

DISCUSSION

Ticks are the most important acarine that harm the animals and human through bloodsucking and also pathogens transmission as viruses, bacteria including rickettsiae, protozoa (Babesiosis) and filarial nematodes (Onchocerciasis) to other animals and humans (Fournier *et al*., 2003; Chaligiannis *et al*., 2009). The present study illustrated the percentage of dogs infested with *R. sanguineus*to be 67.5% this percentage nearly matched to Heukelbach *et al*., 2012 findings who explained that 89.7% of dogs were infested with *R. sanguineus in* southeast Brazil. The highest tick infestation rate was recorded

Table 5: Different stages of *R. sanguineus* ticks (eggs, larvae and nymph) subjected to *B. bassiana* and *M. anisopliae* compared with control at P<0.05.

	Treatment mean ± SE						
B. bassiana (conidia/ml)	Rate of hatchability (%)	% of lived larvae after 3 d	% of lived larvae after 4 d	% of lived larvae after 13d	Molting rate (%) after 13 d	Mortality rate of nymph (%)	Time of molting (Days)
B1(10^8)	14.5±2.10^a	10.5±1.55^a	6±2.16^a	0±0^a	10.21±2.12^a	93.5±1.7^b	12±0.51^a
B2(10^7)	23.75±4.26^a	19.75±1.7^a	9.5±3.86^a	8.25±0.85^a	15.3±1.35^b	90.5±3.86^b	10±0.46^b
B3(10^6)	38.75±4.47^a	89.5±0.64^c	81.25±1.31^b	10±4.24^a	17.15±5.51^c	89.75±4.21^b	10±0.34^b
Control	65±14.28^b	73.75±5.5^b	65±6.45^b	60±11.5^b	76±1.95^d	40±11.54^a	7±0.088^c
			M. anisopliae (conidia /mL)				
M1(10^8)	10.75±1.65^a	69.75±2.09^b	14±0.91^a	0±0^a	8.75±0.47^b	86.5±10.24^b	13±0.091^a
M2(10^7)	7.25±2.49^a	22.25±2.52^a	7.5±0.64^a	0±0^a	0±0^a	96±1.82^b	14±0.435^a
M3(10^6)	29.5±3.52^b	62±3.58^b	26.5±0.517^b	9±1.08^b	11.25±1.79^b	83.5±12.01^b	11±0.562^a
Control	88.75±4.61^c	84.75±4.13^c	82.5±3.75^c	69.5±2.21^c	76±1.95^c	24.5±4.57^a	7±0.035^b

SE= Standard error, a, b, c Different superscripts within the same column of mean treated ticks by fungus indicate significant difference at P<0.05, d: day.

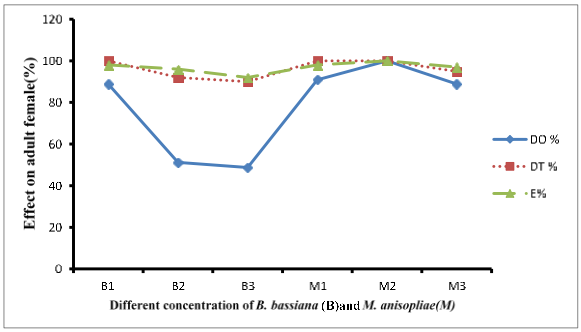

Chart 1: In vitro treated on *R. sanguineus* full engorged female ticks by *B. bassiana* and *M. anisopliae* compared with control at P<0.05.; *B: B. bassiana* (B1=10^8 , B2=10^7 , B3=10^6 (conidia/ml)), M: *M. anisopliae (M1*=10^8 , M2=10^7 , M3=10^6(conidia/ml)),DT %: the percentage reduction of mean number of adult females, DO % : the percentage reduction of mean weight of adult females and E %: the efficacy of fungus against ticks.

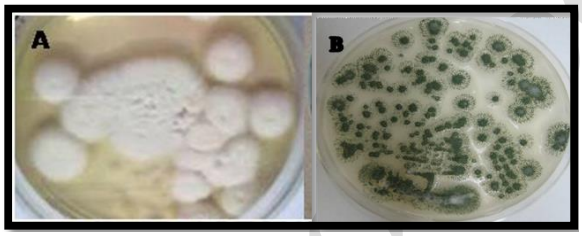

Fig. 1: Macroscopic growth on Sabaroud dextrouse agar media *appeared* white colonies *of B. bassiana* appeared (A) and green colonies of *M. anisopliae* (B).

among dogs less than 1 year old as the results mentioned by Shoorijeh *et al*., 2008; Chee *et al*., 2008 who reported that the prevalence of ectoparasite infestation was greatest in dogs less than one year old than other age in contrast with Omudu *et al*., 2010 who proved that 31.4% of ectoparasites were recovered from dogs within the age bracket of 2-3 years, 72.7% of the dogs above 8 years as well as Elom *et al*., 2015 who mentioned that dogs more than 1 year old were more susceptible than the younger ones. Moreover, the tick infestation was higher in females than males as the results of Elom *et al*., 2015 who found that female dogs were infected more than the males by ectoparasites. These observations with the mentioned authors might be as results of weak immunity among females (suffering from stress as pregnancy), young age as well as elder dogs.

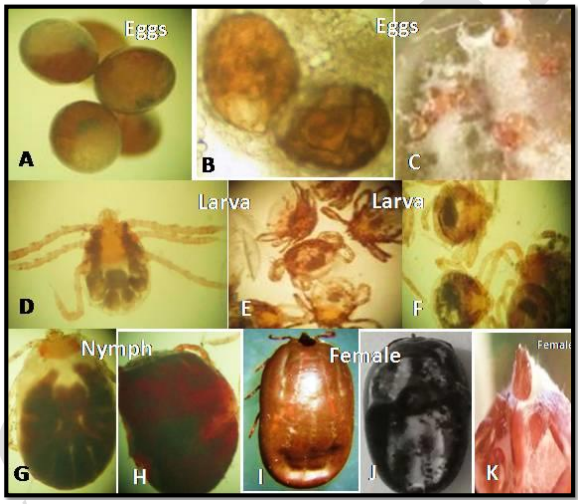

Fig. 2: Microscopic of different stages of *R. sanguineus* (eggs, larva, nymph, adult female); (A, D, G &I) control; (B, C, E, F, H, J &K) treated by *B. bassiana* fungus. (A, B & C at 40x), (I & J at 4x), (D, E, F, G, H & K at 10x).

Little data are available on biological control of ticks, especially on the use of tick pathogens. Entomopathogenic fungi have been investigated for their potential in the biological control of these arthropods due to their ability to penetrate the integument of ticks. In particular, *Beauveriab bassiana* and *Metarhizium anisopliae* fungi were effective in controlling several tick species including *R. microplus*, *R. sanguineus*, *Dermacentor nitens*, and *Amblyomma cajennense*. The susceptibility to fungi might vary according to tick species and population as well as to fungal strain (Fernandes *et al*., 2012). The present study aimed to use the concentrations of water suspension from *B. bassiana* and *M. anisopliae* isolate for the first time in vitro as a biological control on the native Egyptian strain of *R. sanguineus*. For *B. bassiana* the best results were recorded for B1 (concentration 10^8conidia/ml) which was the most pathogenic isolate in which *R. sanguineus* nymphs showed 93.5% mortality within 12 days post infection. These results were similar to those mentioned by Kirkland *et al*., 2004 who stated that >90% nymph mortality within 21-28 days post infection with the same tick species and fungus (*B. bassiana* at 10^8 conidia/ml). Furthermore, decreased the rate of hatchability lead to decrease tick population, which came similar to Kaaya and Hassan, 2000 who showed that, an aqueous formulation of *B. bassiana* (10^8 conidia per ml)

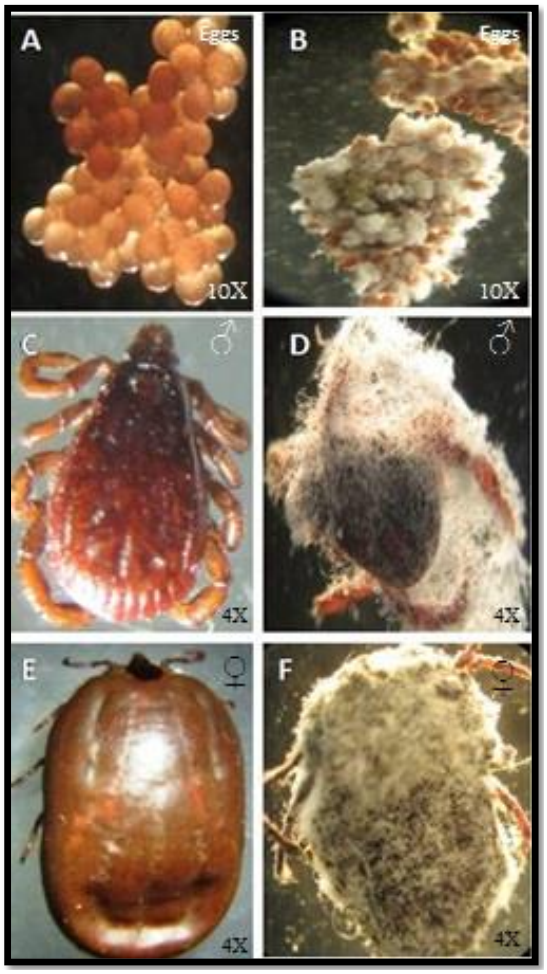

Fig. 3: *R. sanguineus* eggs, adult male and female; control (A, C & E); treated with *M. anisopliae* (B, D &F); (A, B, D, F observed under dark field microscope and C, E by binocular dissecting microscope).

induced higher mortalities, suppressed on-host populations of adult *R. appendiculatus* by 80%. In contrast, found that *B. bassiana* 10^7 conidia/ml was the most effective concentration on *R. sanguineus* and the eggs were more susceptible to fungal infection compared with adult females and unfed larvae.

Regarding *M. anisopliae*, the M2 concentration (10^7 conidia/ml) was the most virulent isolate recorded while M3 (10^6 conidia/ml) gave the lowest results. There was significance between the three different concentrations with control at $P<0.05$. The rate of hatchability of M2 concentration was 7.25%. These observationswere near to those mentioned by Pirali-Kheirabadi *et al*., 2007 who found that *M. anisopliae* (10^7 conidia/ml) capable of decreasing egg hatching to 14%. Moreover, Luz *et al.*, 2016 reported that no larvae hatched from eggs after direct applications of conidia regardless of the formulation and adult *R. sanguine* was the most susceptible stage among *R. e. evertsi*, *A.variegatum* and *R. appendiculatus* to *M. anisopliae*. The efficacy of M2 concentration reached to 100% as also mentioned by Kaaya *et al*., 2011 who illustrated that *M. anisopliae* fungus had significantly higher mortality ($P<0.05$) than the control groups on *R. evertsi evertsi*, and *Rhipicephalus (Boophilus) decoloratus*. In otherwise, Kaaya and Hassan 2000; Samish *et al.*, 2001; Magalhães *et al.*, 1998 showed that aqueous formulations of *M. anisopliae* 10^8 conidia /ml was effective with higher mortalities to adult *R. appendiculatus* (92%), *R. sanguineus* (100%) and *R. sanguineus* (50-70%) respectively. Finally, from the present results, the efficacy % of *M. anisopliae* (M2 concentration 10^7 conidia/ml) reached 100% and more potent than that of *B. bassiana* (B1concentration 10^8 conidia/ml) which was 98%, these findings were agreed with observations of Hedimbi *et al.*, 2011; Kaaya and Hedimbi, 2012.The variations between the present readings and that recorded previously may be regarded to the tick species, environment and laboratory animal used.

Conclusions

It could be concluded that *M. anisopliae* strain is higher effective and virulent toward full feed female, male and all developmental stages of *R. sanguineus* than *B. bassiana* strain. Thus, suggesting that may be using this fungus in vivo as Biological control of *R. sanguineus* to reduce ticks populations of animals. Nonetheless, further field studies require to determine the most effective route to apply and frequency of treatment for using *M. anisopliae* strain as a bio-control agent. Moreover, this fungus will lead to decrease drug resistance and the hazard of excessive use of the chemical product on animals and the environment.

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Authors contribution

Mai Abuowarda, Mounir Abdel Haleem and Magdy Elsayed contributed to the design of the study. Mai Abuowarda and Sara Magdy performed the investigation, collection, rearing and biopesticide control of ticks. Mai Abuowarda done the Statistical analysis of results. Heba Farag prepared different dilution of the entomopathogenic fungi. All authors drafted the manuscript and participated in the subsequent discussions and revisions of the entire text. All authors read and approved the final manuscript.

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