Efficacy of *Verticillium lecanii* and *Beauveria bassiana* of commercial source against cattle tick, *Rhipicephalus (Boophilus) annulatus*

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Abstract: Two entomopathogenic fungi, *Verticillium lecanii* and *Beauveria bassiana*, were tested against *Rhipicephalus annulatus*. Mycotal® was the source of *Verticillium lecanii* while Biosect® was the source of *Beauveria bassiana*. Five concentrations (1×10^7, 5×10^8, 2.5×10^9, 1×10^{10} and 4×10^{10} spore/ml) of *Verticillium lecanii* as well as five different concentrations (5×10^7, 2×10^8, 8×10^9, 3.2×10^{10} and $12.8\text{x}10^{10}$/ml) of *Beauveria bassiana* were prepared and tested against adult female tick, eggs and larvae. The mortality in adult ticks was 60.60 to 72.00% after 2 weeks of application for *V. lecanii* at concentration ≥ $5\text{x}10^8$spore/ml, while *B. bassiana* showed no mortality at any concentrations. The treated tick revealed nutritional index significantly lower than control untreated one for both fungi. Furthermore, *V. lecanii* showed no effect on eggs, while, *B. bassiana* delayed and reduced the egg hatching. In addition, both fungi caused 100% mortality of larvae. The effective concentration was ≥ 10^8spore/ml for both fungi with no significant difference among the highest concentrations. Moreover, the fungal extract had no effect on adult tick. In conclusion, *V. lecanii* is lethal to adult tick and *B. bassiana* caused larvae mortality and reduced egg hatching. A prospective application of fungi in the pasture or animal farm is possible for tick control.

Key words: *Rhipicephalus annulatus*, *Verticillium lecanii*, *Beauveria bassiana*, reproductive index, egg, larvae

1. Introduction

Ticks are an important threat to livestock due to either their direct effect or their role as tick-borne diseases. The ticks control is commonly by using chemical products, which has several side effects. Therefore, using biological control agents has become urgent and safe alternative to reduce and to avoid these adverse effects (Samish et al. 2004; Reis-Menini et al. 2008).

Entomopathogenic fungi were commonly used in pest control of crops and forest pests (Kaaya et al. 1996). Recently, great attention is being paid to these fungi in control of arthropods borne disease of human and animals. These fungi can infect and often kill ticks and therefore can be used in ticks control (Gindin et al. 2002). *Beauveria bassiana* and *Lecanicillium lecanii (=Verticillium lecanii)* are among the most important species infecting ixoidid ticks in nature and different strains of *B. bassiana* are pathogenic to different kinds of tick (Fernandes et al. 2012; Camargo et al. 2012; Ren et al. 2016). The efficacy of used the fungi depend on the tick species and population and the fungal strain (Fernandes *et al.*, 2012; Campos et al. 2010; Sun et al. 2011; Perinotto et al. 2012). The biopesticide that contains *B. bassiana* (Balsamo, Vuillemin) is used to control flies on animal species (balEnce), and against crop pests' greenhouses and food crops (Met52) (Gonzalez et al. 2016). While, *Lecanicillium lecanii* (Zimmermann) Gams and Zare [*Verticillium lecanii* (Zimmermann) Vi´egas], are well known as pathogen of arthropods with a broad host range and applied for control of whiteflies in field crops (Ravensberg et al. 1990; Osborne and Landa, 1992). The development in experiments led to release of two commercial products “Vertalec” and "Mycotal" based on strains specifically selected for use against aphids and whiteflies (Gardner et al. 1984; Ramakers, 1989). The efficacy of these products was approved against larvae and adult of *Colorado potato* beetle and Aphids (Frournier and Brodeur 2000; Kim et al. 2005; Öztürk et al. 2015).

Therefore, the aim of the present study was to assess the *in vitro* efficacy of two fungi at different concentrations of commercial source against eggs, larvae and adult stages of *R. annulatus.*

2. Material and methods

2.1. Ticks collection and preparation of eggs and larvae

Boophilus annulatus ticks were collected from naturally infested cattle from veterinary units and clinics and cow farms in Fayoum governorate in the hot seasons during the period from September 2015 to June 2017. The collected ticks were kept in carton or plastic boxes with opening for ventilation and transported to the laboratory of Parasitology, Faculty of veterinary medicine, Beni-Suef University, Egypt. In the lab, tick were washed in distilled water and allowed to dry on filter paper then, weighted and divided into groups with 10 adult ticks in each group. Part of these ticks was kept in BOD incubator for egg ovi-position (14-18 day). The eggs were collected into glass tubes then sealed by cotton to be used for bioassay on eggs. Another part of eggs was incubated in BOD incubator until hatching to give larvae that were used for bioassay on larvae.

2.2. The source of entemopathogenic fungi in this study

Mycotal® (as source of *Verticillium lecanii* preparation) is a commercial product produced by Koppert Biological Systems (Veilingweg, the Netherlands) and used for biological control in agriculture field. This product contains 10^{10} spores/gram entomopathogenic fungus *V. lecanii*. A Stock suspension of this product was prepared by adding 20 gm of the product powder in 50 ml of distilled water in a plastic tube then mixing well using vortex and wait 30 min before use (Aqueel and Leather 2013**)**. The following concentrations were prepared from this stock; 1×10^7, 5×10^8, 2.5×10^9, 1×10^{10} and 4×10^{10} spore/ml**.** These dilutions were 10ml solution diluted with distilled water.

Biosect® as source of Beauveria bassiana preparations

Biosect® is a commercial product manufactured by Organic Bio-Technology (S.A.E), Egyptian Company and used for biological control in the agriculture field. Biosect® contains *B. bassiana* 32×10^6spore per mg. A stock suspension was prepared by adding 20gm of Biosect® product to 50ml distilled water then agitated by vortex to prepare the suspension. Five concentrations were prepared from this stoke; 5×10^7, 2×10^8, $8\times10^{9,}$ 3×10^{10} and 12×10^{10}spore/ml DW.

2.3. Viability of fungi in the used products

Entomopathogenic fungi formulations of Mycotal® and Biosect® were prepared as mentioned above. An inoculation from each suspension was inoculated on sabourauds dextrose agar (SDA) plates, and then incubated in dark incubator at 26-28°C and 80% relative humidity for 7-10 days. Slides stained with cotton blue (Lactophenol blue stain) were prepared and examined under a microscope for viability assay (Hasan et al. 2013).

2.4. Application on adult engorged females tick

The previously prepared suspensions of *V. lecanii* and *B. bassiana* were tested on engorged female ticks of the same size. The ticks were immersed in 10ml of the fungal suspension for 2 minutes then dried and incubated in BOD for 21 days in Petri dishes (Sun et al. 2011). Five replicates each one of 10 ticks was done for each suspension. A control group was treated by immersion in 10ml DW for 2 minutes. The mortality was observed daily and

the biological parameters were recorded for each group. In addition, the reproductive efficiency (RE) and other biological parameters were calculated (Bennett, 1974).

2.5. Application on the eggs

The eggs were collected in 10 days of oviposition to be used for application of fungal suspensions. The collected eggs were subdivided into test tubes each contained 50mg. Then, 1ml of the prepared suspension was poured into each tube and kept for 3min. The tubes were turned upside down to remove any excess of the suspension with a cotton plug to absorb it. The tubes were incubated at 27 ± 1 °C and RH ≥ 80%. The incubation period and hatching percentage were calculated. The control group was immersed in 1ml distilled water by the same technique (Angelo et al. 2010; Camargo, et al. 2012).

2.6. Application on the larvae

The larvae of 7-14 days age were used for bioassay. A filter paper was put in Petri dish then 1 ml of prepared fungi suspensions was added on it. The impregnated paper was allowed to dry. The treated papers were folded to form a packet and larvae were transferred by brush then sealed by bulldog clips. The treated packets were kept in controlled environment chamber at 26-28°C and 80% relative humidity for 7 days. In the control group, filter paper impregnated with 1 ml distilled water (Pirali-Kheirabadi et al. 2007).

2.7. Fungal extract production

The fungal suspensions of *V. lecanii* and *B. bassiana* were prepared as above. An inoculation was done on sabourauds dextrose agar (SDA) plates then it was incubated in dark incubator at 26-28 °C and 80% relative humidity for 2 weeks. The grown colonies were sub-cultured on sabourauds dextrose broth in glass tubes for another 2 weeks. After fungal growth in broth, the tubes were centrifuged at 3000 rpm for 10 min to precipitate fungal hyphae. Then, the collected supernatant was applied on adult tick and larvae by the same techniques as mentioned previously.

2.7. Statistical analysis

Data of tick biological parameters were analyzed statistically using Statistical Package for Social Science (SPSS for Windows (IBM), version 22, Chicago, USA) to determine if variables differed between treatments. In addition, ANOVA tests were applied to determine the differences between means. Results are expressed as means ± SE. Probability values of less than 0.05 ($P < 0.05$) were considered significant.

3. Results

3.1. Confirmation the fungal viability of the used products

Verticillium lecanii (Mycotal®) and *Beauveria Bassiana* (Biosect®) were grown on the culture media that confirmed fungal viability (Fig.1).

3.2. Effect of V. lecanii and B. bassiana on the adult female of R. annulatus

V. lecanii application at concentration of 1×10^7spore/ml DW on the ticks adult female revealed no any effect. While, the concentrations of ≥ $5x10^8$ spore/ml DW processed a lethal effect on adult tick with mortality percent ranged from 60.60 to 72.00%. The fungal growth was obvious on the dead ticks after 2 weeks post application (PA) (Fig. 2 B and C). In addition, these concentrations affected the biological parameters of the ticks, where NI of the treated ticks was significantly lower than control untreated one ($p < 0.05$). Moreover, the control percent (CP) of the treated ticks at the previous concentrations was better and higher than control

untreated ones. It was noticed that the concentrations of ≥ $5x10^8$spore/ml DW have the same effect with no significant difference in between ($p < 0.05$) (Table 1). In addition, the pre-oviposition, oviposition and incubation periods of the produced eggs were in the normal time as that of control untreated ticks (nearly 14 days). Meanwhile, *B. bassiana* application on adult ticks revealed no lethal effect at any concentrations. Furthermore, the lowest concentration of $5x10^7$spore/ml DW has no effect on the treated ticks. While, the highest concentrations of ≥ $2x10^8$spore/ml DW showed significant effects on the biological parameters. Where the NI of the treated ticks was significantly lower than those of the control untreated ones. Moreover, CP was higher for the treated ticks than control untreated ones ($P < 0.05$) with no significant difference in between these concentrations (Table 2). Additionally, we did not observe any fungal growth on adult tick. Additionally, the pre-oviposition, oviposition and incubation periods of the produced eggs were in the normal time same as of the control untreated ticks.

3.3. Effect of V. lecanii and B. bassiana on eggs and larvae

The application of *V. lecanii* on the egg of ticks showed no significant effect at all concentrations on either hatchability percentage or period of hatchability. As well as, *B. bassiana* showed no significant on eggs at the concentration of 5×10^7spore/ml DW where it hatched after nearly 14 days of application. While, the concentrations of ≥ $2x10^8$spore delayed the egg hatching to 21.0 ± 0.41days post application. In addition, the hatchability percent ranged from 65.00 to 72.50% to the treated eggs at these highest concentrations. Furthermore, there was no significant difference among the effect of highest concentrations (Fig. 2 D) (Table 3). Regarding the treatment of larvae with *V. lecanii*, the lowest concentration 1×10^7spore/ml DW was of no effect on larval mortality and no significant difference with the control untreated larvae. The mortality effect was appeared at the concentrations of ≥ $5x10^8$spore after 4 days post application. The mortality rate 100% was achieved at the seventh day PA (Table 4) with obvious fungal growth on the larvae (Fig. 2, E). Furthermore, *B. bassiana* effect appeared at the third day PA and 100% larvae mortality was encountered at the fifth day PA (Table 4). This effect appeared at the highest concentrations of ≥ 2×10^8spore (Table 4).

3.4. Fungal extracts evaluation

The supernatants that obtained from fungal broth were applied on adult tick and showed no any efficacy with biological parameters same as these of control untreated tick.

4. Discussion

The bioinsecticides are commercial products containing entomopathogenic fungi and are widely used in control of insects in agriculture. These products are available and effective. Therefore, these products could be a source for fungi that can be tested against the most threatening ectoparasites of cattle in the tropical area, *R. annulatus*. Mycotal® is a commercial product containing *Verticillium lecanii* and is currently used in agriculture for control of whiteflies in crops (tomato, bean). The other product is Biosect® that is the source of *Beauveria bassiana*, which is commonly used against white fly in tomato.

In the present study, the *in vitro* effects of *V. lecanii* on adult *R. annulatus*, eggs and larvae were evaluated. The concentration of 1×10^7spore/ml did not show any effect on the adult ticks. While the highest concentrations $\geq 5\times10^8$spore/ml showed significant effect which appeared as death and decrease in the reproductive efficacy of ticks. After 14 days, post application (PA) 60.6-72% of treated ticks was died and fungal growth was detected on ticks. The reproductive index of the treated ticks was significantly lower than those of the control untreated group and the group of ticks treated by $1x10^7$spore. In the same way after 7 days PA, the treated larvae died at the highest concentrations $\geq 5x10^8$ spores and fungus grow well of on the treated larvae. Meanwhile, no difference in the eggs hatching was observed between the treated eggs and the control ones at all concentrations. Therefore, *V. lecanii* has no effect on *R. annulatus* eggs. It is worthy to mention that there was a clear growth for the fungal hyphae on adult tick, which may be the main cause for death of the adult tick. The mechanism of tick infection by entomopathogenic fungi has described through germination on cuticle then penetration of it using enzymatic and physical activities of the fungus, proliferation of fungal mycelia in tick's hemocoel and production of toxic metabolites. These led to breakdown of integument and tick death (Kirkland et al. 2004; Arruda et al. 2005; Leemon and Jonsson 2008). In the present study, the mortality rate in the treated ticks was 60.60-72.00%, which closely similar to those reported by Pirali-Kheirabdi et al. (2007) as they recorded 56.6% mortality rate in *Boophilus annulatus* ticks after application of *V. lecanii* at concentration of 10^7conidia/ml. In contrary to our results, Pirali-Kheirabdi et al. (2007) found that, the application of *V. lecanii* on tick eggs decrease the egg hatchability by 59.74%. Furthermore, our rate of mortality of ticks differed from those reported by Angelo *et al.* (2010) who applied *V. lecanii* on engorged females of *Rhipicephalus microplus* and recorded 97.6% mortality at concentration 1×10^8 conidia/ml oil suspension. This difference may be due to different tick species and they used oil suspension rather than water that used in our study. In addition, Anglo et al. (2010) used *L. lecanii*, isolate CG 420 (laboratory strain). Furthermore, the pre-oviposition period was same as the control untreated group at all used conidial concentrations, which is similar to the results of Anglo et al. (2010) at conidia (10^8) aqueous suspension. In addition, the oviposition and egg incubation periods for treated engorged females were same as control in our study but it was significantly decreased for females treated in Anglo's study. Also, the period of hatching and the percentage of hatching did not differ from the control group in both studies. Regarding the application on the larvae, both studies showed the same effects but the larvae highest mortality percent 100% was at day 7 after treatment in our study while it was at day 10 post-treatment in Anglo's study. Furthermore, Anglo et al. (2010) found that conidia in oil suspension caused death of adult *R. microplus*, stopped the egg hatching and led to 100% mortality of larvae. While, our findings are somewhat differ from the findings of Gindin et al. (2001). The later found that 1×10^7 conidia ml−1 of *L. lecanii* caused reduction in the egg production of *R. annulatus, Rhipicephalus sanguineus and Hyalomma excavatum* with no significant mortality in larvae of *R. annulatus*. Nevertheless, Gindin et al. (2001) have same results like us upon

application on eggs. Several authors attributed the contrast in the results to the fungal isolates, manipulation, and cultivation of these organisms in the lab (Fernandes and Bittencourt 2008; Sun et al. 2011; Perinotto et al. 2012; Ren et al. 2012; Ren et al. 2016). Similarly, the fluctuation in our results in comparison with the previous studies might be due to the nature of the source of fungi that it is of commercial product lyophilized powder and using aqueous suspension as recommended by the manufacturer.

Beauveria bassiana is one of the important entomopathogenic fungi used in biological control in agriculture field. In the present study *B. bassiana* showed no lethal effect on adult female of *R. annulatus* at any concentration. However, a significant ($p < 0.05$) reduction in biological parameters and significant reduction in the eggs hatchability percentage with prolonged hatchability period when compared with the control untreated group (21.2 ± 0.47 day) were noticed at concentrations $\geq 2\times10^8$spore/ml. Furthermore, treatment of larvae caused significant mortality at concentrations $\geq 2x10^8$spore and the mortality percentage reached to 100% after 5days PA. In contrast to our study, Kaaya et al. (1996) recorded 30% and 37% mortality of *Rhipicephalus appendiculatus* and *Amblyomma variegatum* respectively after application of *B. bassiana*. In addition, Kaaya and Hassan (2000) reported 80-90% mortality in adults *Rhipicephalus appendiculatus, Amblyomma variegatum* and *Boophilus decoloratus* using aqueous and oil-based formulations of *Beauveria bassiana*. In the same way, Ren et al. (2011) recorded 100% mortality rate at 10^8 conidia ml^{-1} in *Haemaphysalis qinghaiensis* ticks in china. In addition, Ren et al. (2012) reported that three *B. bassiana* isolates caused up to 100% mortality for the ticks at concentrations of 10^8 and 10^9 conidia mL–1. Moreover, Cafarchia, et al. (2015) estimated high significant mortality rate in the adult of *Rhipicephalus sanguineus sensu lato*. Similarly, Murigu et al. (2016) tested *B. bassiana* against amitraz-resistant and amitraz-susceptible strains of *Rhipicephalus decoloratus* and they found a significant reduction in the number of ticks and larval mortality (10-100% and 12.1-100%) in both respects. Consequently, this discrepancy with our findings may be due to the virulence of the fungus strain used in the present study, which is isolated from Egyptian soil as in the label of manufacturing. In addition, Fernandes et al. (2011) tested the virulence of sixty *Beauveria*-like isolates on *R. microplus* larva and verified that larvae from different origins had different susceptibilities to the fungal isolates. Moreover, Perinotto (2010) reported variation in susceptibility of *R. microplus* larval stages to *B. bassiana and M. anisopliae* when the ticks were collected from different locations. Additionally, the difference in susceptibility of *R. microplus* to entomopathogenic fungi related to genetic and physiological characteristics of the tick strains and species (Fernandes et al. 2012). Moreover, Polar et al. (2005) reported that ticks might be physically and structurally tolerant to infection by entomopathogenic fungi. Besides, the previous exposure to entomopathogenic fungi cannot discard in nature where these fungi present in the soil but there is no record of epizootic naturally in ticks (Prinitto et al. 2012).

Herein, the mortality of larvae was 100% at 5 days PA. It was known that tick larvae were the most susceptible than other stages (Camargo et al. 2012).The present result is

similar to those reported by Cafarchia et al. (2015) but they encountered 100% mortality of larvae at 15 days PA. On other hand, several studies reported lower mortalities rates than the rate of present study (Kaaya and Hassan 2000; Reis et al. 2005; 2012; Campos et al. 2010). We think that the technique of application, larval age and tick species may be the causes of this variation. The results of the application on eggs led to reduction in the percentage of hatching and delaying in the hatching. These results are similar to that obtained by Kaaya and Hassan (2000) and Cafarchia et al. (2015). In the present study, the application of *B. bassiana* of commercial origin was greatly effective on larvae. This is in agreement with González *et al.* (2016) who found that the application of *B. bassiana* commercial strain (Balsamo, Vuillemin) on wild rabbit burrows under field conditions in aqueous solutions of the product reduced the infestation by 63.28 =78.63% % on day +30 and +60, respectively.

In the present study, the effective concentrations of fungi of the significant effect were ≥ 2×10^8spore/ml while the concentration of 1×10^7spore/ml was not effective. This in agreement with Polar et al. (2005) who found that high concentration of fungal conidia is necessary to cause its lethal effect on tick. Moreover, Prette et al. (2005) found that the application of 10^9 conidia/ml of *B. bassiana* isolates was significantly reduced the hatching of larvae compared to the concentrations of 10^7 conidia mL-1. In addition, ticks seem to be naturally more tolerant to fungal infection than many other arthropods, therefore, high conidial concentrations are needed to achieve the lethal effect (Polar et al. 2005b). Moreover, Maniania et al. (2007) reported that tick control needs very high concentrations in comparison with the fungal concentration used to control important agricultural insect pests. Similarly, Ren et al. (2012) reported that concentrations of 10^8 and 10^9 conidia was the effectiveness of *B. bassiana*, while the concentration 10^7spores mL–1 reduced only the reproductive efficacy. However, in the present study, the concentrations of 10^9 and 10^{10}spore/ml showed no significant difference with the result of concentration of 10^8spore. This may be due to fungi occupy all body of the tick at the concentration of 10^8 spore and any increase in conidia concentration do not find a place to act or grow on the tick. Regarding, the fungal extracts application on adult tick had no effect. This may support that the action of fungi on tick mainly due to its growth and not to its metabolites. This in agreement with Moon et al. (2008) who found that *M. acridum* has very low destruxin production even though this isolate is very effective against grasshopper/locust. Moreover, Golo et al. (2011) found that different concentrations of destruxin A did not cause any effect on engorged female of *R. microplus*. Additionally, Fernandes et al. (2012) concluded that the fungal production of toxic metabolites might not be crucial for pathogenicity to arthropods, including ticks. In conclusion, *V. lecanii* had a lethal effect on adult *R. annulatus* and larvae but had no effect on eggs. This finding is interest and need field application. While *B. bassiana* had no lethal effect on adult *R. annulatus* but reduced its reproductive efficacy and its eggs hatchability and caused mortality for the larvae. Concurrently, a recommendation for using these fungi in its commercial products and as aqueous suspensions on tick-infested pasture may be of significant effect. This issued by Ojeda-Chi et al. (2010) who applied this strategy previously.

Therefore, regular application of the concentration 10^8conidia/ml as a spray in the pasture and animal farms may play an effective role in the control of tick larvae and eggs in the pasture.

Conflict of interest

None

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Conc.	DF	FWBT	FWAT	EPF	RI	EPI	HP	RE	NI	CP
$1x10^7$	00 ± 00	0.079 ± 0.004	0.047 ± 0.002	0.023 ± 0.001	0.478 ± 0.029	28.74 ± 2.507	96.00 ± 1.000	27.62 ± 2.659	72.41 ± 9.091	000 ± 000
$5x10^8$	6.60 ± 0.400*	0.076 ± 0.003	0.035 ± 0.003	0.003 ± 0.001*	0.010 ± 0.034*	4.194 ± 1.189*	92.20 ± 0.97	3.881 ± 1.111*	3.58 ± 0.200*	91.89 ± 0.180*
2.5x10	6.80 ±	0.089	0.039 ±	0.002 ±	0.072 ±	2.305 ±	91.50 ±	2.107 ±	3.578 ±	93.89 ±

^{9}	0.374*	± 0.006	0.009	0.000*	0.014*	0.072*	1.322	0.061*	0.201*	0.178*
$1x10^{10}$	7.000 ± 0.316*	0.076 ± 0.005	0.033 ± 0.002	0.003 ± 0.001*	0.089 ± 0.016*	3.885 ± 0.678*	92.80 ± 1.157	3.620 ± 0.659*	7.104 ± 1.215*	89.50 ± 1.911*
$4x10^{10}$	7.200 ± 0.374*	0.106 ± 0.005	0.045 ± 0.001	0.005 ± 0.000*	0.101 ± 0.007*	4.228 ± 0.134*	93.80 ± 0.583	3.966 ± 0.132*	7.384 ± 0.263*	88.50 ± 0.382*
Control	00 ± 00*	0.072 ± 0.009	0.027 ± 0.004	0.024 ± 0.003*	1.041 ± 0.130*	35.71 ± 3.104*	96.50 ± 0.401	34.51 ± 3.062*	62.94 ± 9.290*	000 ± 000*

Table (1) Effect of *Verticillium lecanii* of commercial source at different concentrations on adult ticks *Rhipicephalus annulatus* at day 14 post applications

*Significant $p \leq 0.05$
Data expressed as (Mean ± Standard Error), conc. (spores/ml DW).
DF= Number of deaths of adult female, FWBT= Female weight before treatment, FWAT=Female weight after treatment, EPF= Egg mass per female, RI= Reproductive index, EPI= Egg production index, HP= Hatchability percentage, RE= Reproductive efficacy, NI= Nutritional index, CP= Control percent

Table (2) Effect of *Beauveria bassiana* of commercial source at different concentrations on adult ticks *Rhipicephalus annulatus* at day 14 post applications

Conc.	DF	FWBT	FWAT	EPF	RI	EPI	HP	RE	NI	CP
$5x10^{7}$	0000 ± 0000	0.047 ± 0.004	0.013 ± 0.001	0.022 ± 0.002	1.096 ± 0.086	34.03 ± 2.852	93.50 ± 1.190	36.77 ± 3.812	58.31 ± 11.62	000 ± 000
$2x10^{8}$	0000 ± 0000	0.059 ± 0.002	0.013 ± 0.001	0.003 ± 0.001*	0.235 ± 0.037*	4.990 ± 0.628*	91.20 ± 0.750	4.560 ± 0.602*	6.403 ± 0.785*	87.51 ± 1.649*
$8x10^{9}$	0000 ± 0000	0.055 ± 0.006	0.013 ± 0.001	0.005 ± 0.000*	0.041 ± 0.037*	10.05 ± 1.172*	93.50 ± 1.190	9.383 ± 1.091*	13.87 ± 2.293*	74.30 ± 2.989*
$3.2x10^{10}$	0000 ± 0000	0.052 ± 0.007	0.014 ± 0.001	0.005 ± 0.000*	0.353 ±	9.625 ± 1.319*	92.00 ± 1.224	8.840 ±	13.77 ±	75.78 ±

					0.027*			1.181*	2.679*	3.238*
$12.8x10^{10}$	0000 ± 0000	0.067 ± 0.004	0.029 ± 0.002	0.008 ± 0.003*	0.259 ± 0.069*	11.47 ± 3.414*	92.00 ± 0.707	10.52 ± 3.119*	12.16 ± 3.704*	84.15 ± 2.012*
Control	0000 ± 0000	0.055 ± 0.004	0.018 ± 0.001	0.020 ± 0.001*	1.163 ± 0.089*	37.81 ± 3.966*	96.50 ± 0.422	36.46 ± 3.826*	57.77 ± 7.633*	000 ± 000*

*Significant at $p \leq 0.05$
Data expressed as (Mean ± Standard Error), conc. (spores/ml DW).
Egg per female= 000 (Significant)

Table (3) Effect of *Verticillium lecanii and Beauveria bassiana* on *Rhipicephalus annulatus* eggs

V. lecanii Conc.	Hatchability percentage	Period of hatching (Days)	Comment
$1x10^7$	98.2 ± 0.73	14.6 ± 0.24	Normal hatching
$5x10^8$	97.8 ± 0.37	14.8 ± 0.20	Normal hatching
$2.5x10^9$	98.4 ± 0.51	14.2 ± 0.37	Normal hatching
$1x10^{10}$	97.8 ± 0.37	15.0 ± 0.00	Normal hatching
$4x10^{10}$	98.2 ± 0.37	14.6 ± 0.24	Normal hatching
Control	98.8 ± 0.37	14.8 ± 0.20	Normal hatching
B. bassiana Conc.	Hatchability percentage	Period of hatching (Days)	Comment
$5x10^7$	98.5 ± 0.62	14.7 ± 0.25	Normal hatching
$2x10^8$	68.2* ± 3.11	21.2* ± 0.47	Delayed hatching
$8x10^9$	65.0* ± 3.67	21.2* ± 0.25	Delayed hatching
$3.2x10^{10}$	71.0* ± 1.22	21.0* ± 0.41	Delayed hatching
$12.8x10^{10}$	72.5* ± 1.50	21.0* ± 0.41	Delayed hatching
Control	98.2* ± 0.63	14.7* ± 0.25	Normal hatching

*Significant at $p \leq 0.05$
Data expressed as (Mean ± Standard Error), conc. (spores/ml DW).

Table (4) Effect of *Verticillium lecanii and Beauveria bassiana* on the larvae of *Rhipicephalus annulatus*

V. lecanii Conc.	% of Larval mortality at 24 hrs PA	% of Larval mortality at 48 hrs PA	% of Larval mortality at day 7 PA
$1x10^7$	2.30 ± 0.01	2.70 ± 0.03	3.20 ± 0.01
$5x10^8$	1.55 ± 0.05	2.06 ± 0.05	100* ± 0.00
$2.5x10^9$	2.40 ± 0.02	2.70 ± 0.03	100* ± 0.00
$1x10^{10}$	1.56 ± 0.05	2.46 ± 0.05	100* ± 0.00
$4x10^{10}$	2.30 ± 0.05	3.10 ± 0.03	100* ± 0.00
Control	2.50 ± 0.04	3.20 ± 0.04	4.10* ± 0.00
B. bassiana Conc.	% of Larval mortality at 24 hrs PA	% of Larval mortality at 48 hrs PA	% of Larval mortality at day 7 PA
$5x10^7$	3.40 ± 0.03	4.50 ± 0.03	5.01 ± 0.03
$2x10^8$	54.0* ± 1.68	75.7* ± 0.47	100* ± 0.00
$8x10^9$	55.2* ± 2.28	78.5* ± 0.95	100* ± 0.00
$3.2x10^{10}$	54.0* ± 1.68	76.5* ± 0.64	100* ± 0.00

$12.8x10^{10}$	55.5* ± 1.55	77.0* ± 1.08	100* ± 0.00
Control	3.20* ± 0.02	4.10* ± 0.01	4.70* ± 0.05

*Significant at $p \leq 0.05$
Data expressed as (Mean ± Standard Error), conc. (spores/ml DW).

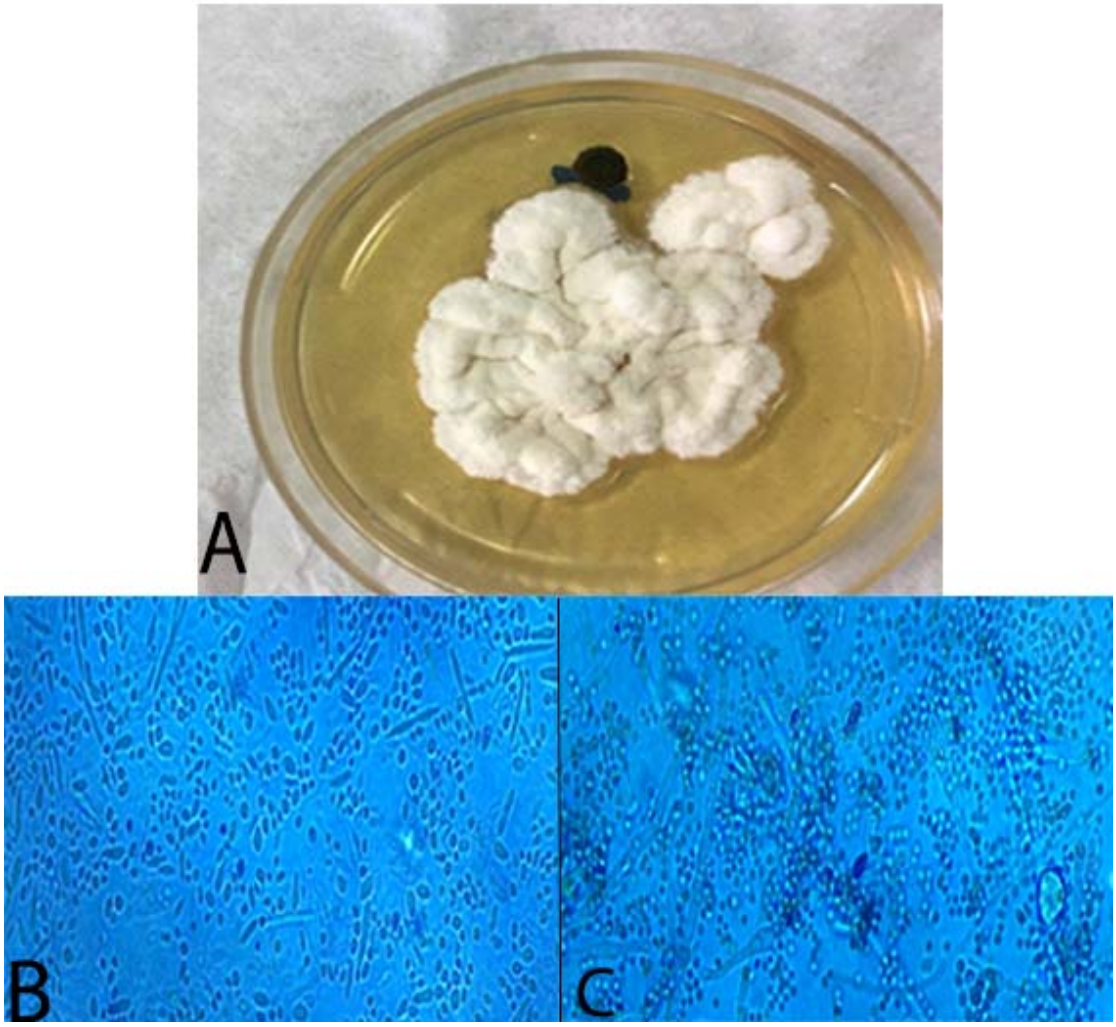

Fig. 1. A. Fungal growth on Sabaroud dextrose agar

B. *Verticiulium lecanii* **conidia** **C.** *Beauveria bassiana* **conidia**

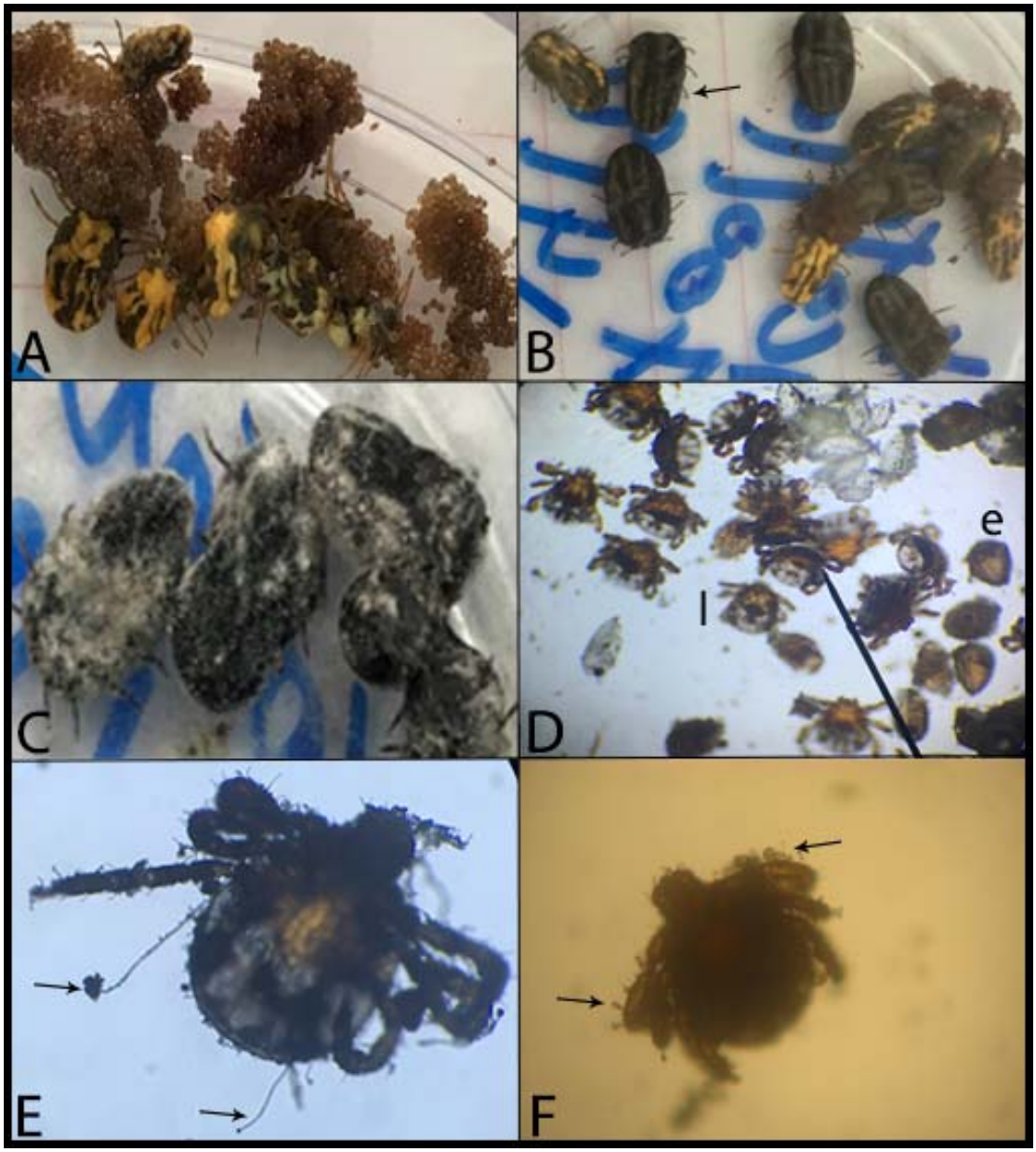

Fig. 2. A. Untreated tick females deposited eggs
B. Treated ticks with arrow referring to dead tick in black color
C. Dead ticks with fungal growth
D. Treat eggs showing hatched larvae (l) and un-hatched eggs (e)
E. Larvae with fungal hyphae of *V. lecanii* (arrow)
G. Larvae with fungal hyphae of *B. bassiana* (arrow)