Biological and Biomedical Journal

Journal homepage: https://bbj.journals.ekb.eg

Ultrastructural and effectiveness of the fungus *Metarhizium anisopliae* for controlling the cattle tick *Rhipicephalus annulatus* (Acari: Ixodidae)

Nabawia M. Elhadidy[1, *], Eman A. Elkelesh[2], Basma H. Amin [3] Aliaa A. Balegh[2]

[1]Zoology Department, Faculty of Science, Arish University, Egypt.

[2]Parasitology Department, Animal Health Research Institute, Agricultural Research Center, Egypt.

[3] The Regional Center for Mycology and Biosociology (RCMB), Al-Azhar University, Egypt.

ARTICLE INFO

Received: 30/12/2024

Accepted: 30/1/2025

Corresponding author:

Nabawia M. Elhadidy, Ph. D
E-mail: n.alhadidy@yahoo.com
Mobile: (+2)01120476750

P-ISSN: 2974-4334
E-ISSN: 2974-4324
DOI:
10.21608/bbj.2025.325405.1063

ABSTRACT

The work aimed to evaluate the effectiveness of the fungus *Metarhizium anisopliae* as a biological agent for controlling the *Rhipicephalus (Boophilus) annulatus* cattle ticks under laboratory conditions. This research could provide an alternative biological method to the chemical acaricides. *R. annulatus* ticks are a significant menace to the economic stability of the Egyptian cattle industry. *M. anisopliae* is a commercially available entomopathogenic fungus (EPF) that infects various insects. This research investigated the biological parameters, morphology, and ultrastructure of *R. annulatus* engorged female ticks treated with fungus using spraying and dipping applications (10^6 spores/ml). On the fifth day of treatment, morphological abnormalities in *Metarhizium anisopliae*-treated ticks were noticed, characterized by wrinkled, white patches, and darker cuticles. Furthermore, the treated ticks' eggs had a gloomy, lifeless appearance. By the tenth day of treatment, the mortality rate had reached 100% in the ticks in both applications. Compared to the control group, the treated *R. annulatus* engorged ticks revealed a significant reduction in egg mass, egg counts, and hatchability. The reproductive index was notably lower in the treated groups, with results at 0.324, and 0.320 in the dipping and spraying groups, respectively, compared to the control group of 0.815. Moreover, the dipping group exhibited 60.2% inhibition in oviposition, while the sprayed group showed a 60.7% reduction. The analysis of *M. anisopliae* infection using a transmission electron microscope revealed fungal penetration through the three cuticle layers. There were some outgrowing hyphae, and the treated cuticle was severely and violently damaged. The present findings suggest that *M. anisopliae* can infect and penetrate the cuticle of *R. annulatus* soon after treatment, making it a viable choice for biological tick control.

Key words: Biological control, Cuticle, *Metarhizium anisopliae* *Rhipicephalus annulatus*, TEM, Ultrastructure

1. Introduction

The most prevalent blood-sucking bovine external parasite is the tick, *Rhipicephalus (Boophilus) annulatus* (Acari: Ixodidae), which is found worldwide (Rajput et al., 2024). Cattle ticks seriously menace the economic stability of Egypt's livestock industry through Their obligatory blood feeding which affects animal productivity by decreasing milk and meat production and acts as a vector for transmitting various infectious agents, Such as viruses, rickettsia, and blood parasites (Eskezia and Desta, 2016). Furthermore, ticks have the potential to harm the skin and hide (Hend et al., 2019). The applicative approach for managing ticks is the utilization of chemical acaricides. However, improper application techniques significantly compromise their effectiveness and contribute to the development of acaricide resistance in tick populations (Vudriko et al., 2016). Additionally, chemical acaricides pose serious environmental risks to human and animal health due to harmful residues in cattle products, besides the high cost of acaricides (Klafke et al., 2017). Consequently, alternative biological control is intended as a promising global strategy for tick management, gaining significant attention in recent decades (Oundo et al., 2025). The most effective biocontrol agent is the fungus *M. anisopliae*. This fungus was extensively examined for its effectiveness in controlling external parasites, particularly ticks (Elhadidy, 2014; Beys-da-Silva et al., 2020). The Russian microbiologist Metchnikoff (1879) isolated *M. anisopliae* from a grain pest and applied it to control the beetle *Anisoplia austriaca*. In nature, *M. anisopliae* is widely distributed and can be found in soil, the rhizosphere, plant roots, and the carcasses of arthropods and exists as saprophytic, endophytic, or infectious (St Leger, 2008). *M. anisopliae* is particularly efficient in managing cattle ticks in the laboratory and field, which, makes *M. anisopliae* a promising candidate for wide commercial use (Kaaya, 2000; Schrank and Vainstein, 2010). Therefore, there is decreasing the drawbacks of chemical acaricides. This study plans to examine the capability of *M. anisopliae* fungus to control ticks and tick-borne diseases, by evaluating its effectiveness against *R. annulatus* engorged female ticks and assessing its impact on tick mortality and reproductive parameters. Additionally, scanning electron microscopy (SEM) was used to ensure information detailed about the fungus's ability to penetrate the tick cuticle and induce abnormalities in its cuticle.

2. Materials and methods

Ethics clearance

The Animal Health Research Institute in Egypt's Committee approved the protocol (Ethical Issue: ARC AHRI 42 24).

Metarhizium anisopliae source

M. anisopliae Bioranza (10^6 spores/ml), a commercial product from the Plant Protection Research Institute's Biopesticide Production Unit Dokki, Giza, Egypt, was used in this investigation as a source of *M. anisopliae* preparation. Conidial spores of *M. anisopliae* (10%) were prepared as a wettable powder in a glass container with Tween-80 (0.01%) as a surfactant. The mixture was mixed well using a vortex and left for 30 minutes before use (Aqueel and Leather 2013).

Collection of *R. annulatus* ticks

From El Fayoum and Sharkia governorates, 360 engorged female ticks were collected from naturally infested cattle in labeled plastic containers with perforated lids for aeration and transfer the ticks to the Parasitology Department of the Animal Health Research Institute Dokki, Giza. After being cleaned with tap water, each tick sample was dried on filter paper and was identified according to Hoogstraal and Kaiser (1958), and Walker (2003), as *Rhipicephalus annulatus.*

Application of *M. anisopliae* on female ticks

After being weighed, the engorged adult female ticks were split up into three groups, each containing 120 engorged female ticks of uniform weight. The engorged female ticks in the dipping group were submerged in the *M. anisopliae* suspension at room temperature for ten minutes with gentle stirring to maintain homogeneity. The ticks of the spraying group were sprayed for two minutes with the prepared fungal suspension. In the control group, ticks were submersed in distilled water. After that, the ticks were dried on filter paper, and the solution was thrown away. Each female tick in all three groups was put in a customized glass tick-rearing tube sealed with cotton and a gauze tampon. The tubes were incubated at 27 °C (± 1 °C) and 80 to 85% relative humidity until the eggs hatched (Pirali-Kheirabadi et al., 2007).

Assessment of *M. anisopliae* against treated *R. annulatus*

The morphological abnormalities and the mortality of the ticks were observed and compared with the control ticks daily. The egg mass weight, egg numbers, and hatchability % were recorded. The following formulas, outlined by Drummond et al. (1973) and Haggag et al. (2017), were used to calculate the effectiveness of *M. anisopliae*.

Accurate mortality (%) = dead / Alive + dead x 100

Reproductive index (RI) = egg mass/engorged tick weight

Inhibition of oviposition (IO %) = RI (control)−RI (treated) /RI (control) x 100

Hatchability % = No. of hatched larvae/No. of eggs x 100

Transmission electron microscope

For TEM, cuticle slice samples were fixed in 3% glutaraldehyde buffered in 0.1M cacodylate buffer (pH 7.0), after which post-fixation was performed in 1% osmium tetroxide cacodylate buffer. Specimens were dehydrated in ethanol, treated with propylene oxide, and embedded in epon epoxy resin. Ultrathin sections (60-80 nm) were stained with uranyl acetate followed by lead citrate. They were examined by TEM (JEOL JEM 1010) at 70 KV, at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Egypt (Abdel-Aziz et al., 2017 and Yosri et al., 2022).

Statistical analysis

IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY, USA) was used for statistical analysis. The mean, standard deviation, median, and range were used to express numerical data. Using the Kolmogorov-Smirnov test, the data's normality of distribution was examined. The post-doc "Schefe test" was employed for pairwise comparison after the ANOVA test for normally distributed data was used to compare the three groups. In the case of a non-normal distribution, pairwise comparisons were conducted after the Kruskal-Walli's test was used to compare the three groups. Every test had two tails. A p-value of less than 0.05 was deemed significant.

3. Results

The efficacy of *M. anisopliae*

The efficacy of *M. anisopliae* was determined using three hundred and sixty engorged female ticks *R, annulatus* collected from cattle.

Morphological changes of treated engorged females and their egg masses

Daily observation of *R. annulatus* engorged female ticks revealed the presence of abnormalities in the treated ticks with *M. anisopliae*. These changes were noticed on the 5th day as the cuticles of ticks became dark in color with a wrinkled appearance and white spots were seen. The deformations and size of the white spots increased on the seventh day post-treatment. The eggs appeared dull and dark in color. On the 10th day, the cuticle had obvious noticeable degenerative changes. It was noticed that ticks treated by spraying showed these changes clearly and faster than those treated by dipping, Figs. (1 and 2).

Fig. 1. The Morphological changes of the treated *R. annulatus* females with *M. anisopliae* A, B, and C) dorsal cuticle surface at 5th, 7^{th,} and 10th-day post-treatment (blue arrows refer to white spots), D) ventral surface at 10th-day post-treatment) and E) Tick control (at 10x).

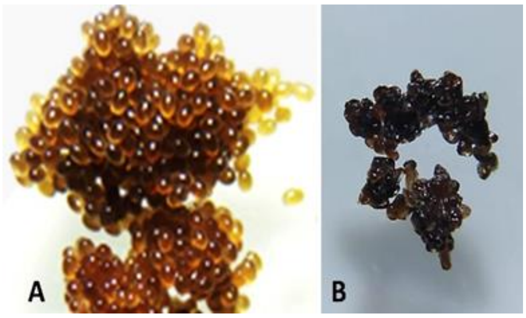

Fig. 2. Eggs of control Ticks (A), Egg mass of treated *R. annulatus* female with dark color and shrink (B).

Effect of *M. anisopliae* on *R. annulatus* engorged female tick mortality.

The effect of *M. anisopliae* on the engorged female ticks' mortality reached 100%, on the 10th day post-treatment in the dipping and spraying applications, compared to the control group (Zero%). Additionally, the mortality reached nearly 50% in both treated groups (dipping and spraying) on the 6th day post-treatment. Moreover, no mortality was recorded in the first two days in the treated groups, but most females showed weak tarsal reflexes. Generally, it was observed that all females in the treated groups died before they began laying eggs, except a small number died after they started laying eggs but did not complete oviposition. Also, the sprayed ticks showed a faster mortality response to the product than those of the dipping group (table 1and fig. 3).

Table. 1. Cumulative mortality percentage of *R. annulatus* females treated with the fungus *M. anisopliae* by two different application methods

Days	Mortality (%)	
	Dipping Group (n=120)	**Spraying Group** (n=120)
1st day	0	0
2nd day	0	0
3rd day	14 (11.7%)	19 (15.8%)
4th day	20 (16.7%)	37 (30.8%)
5th day	24 (20.0%)	48 (40.0%)
6th day	56 (46.7%)	69 (57.5%)
7th day	100 (83.3%)	99 (82.5%)
8th day	110 (91.7%)	109 (90.8%)
9th day	120 (100.0%)	119 (99.2%)
10th day	120 (100.0%)	120 (100.0%)

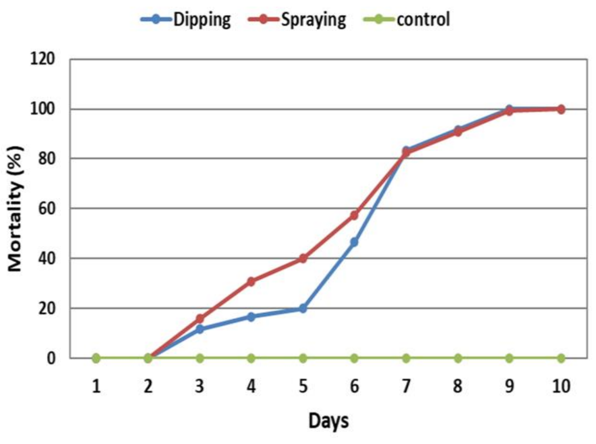

Fig. 3. Cumulative mortality percentage of *R. annulatus* females treated with the fungus *M. anisopliae* by dipping and spraying applications.

Effect of *M. anisopliae* on the reproduction of *R. annulatus* engorged female ticks.

Table 2. demonstrates the impact of *M. anisopliae* on the reproductive performance of *R. annulatus* engorged female ticks. The mean weight of the egg mass in the dipping and spraying groups were 0.051±0.017 and 0.049±0.017, respectively, and that was significantly lower than that of the control group (0.130±0.018) (Fig. 4). The average number of eggs in the dipping (433±155) and spraying (423±157) groups was significantly lower than that of the control group (2807±381) (Fig. 5). As a result, the reproductive index in the dipping and spraying were 0.324 and 0.320, respectively, which were significantly lower than that of the control group (0.815). *M. anisopliae* exhibited suppression of egg laying in the sprayed group (60.7%) and dipping group (60.2%) compared to zero in control ticks (Fig. 6). Moreover, the control group showed an 87.02% hatchability (2438), as opposed to 6.01% and 7.60% for ticks in the dipping and spraying application, respectively.

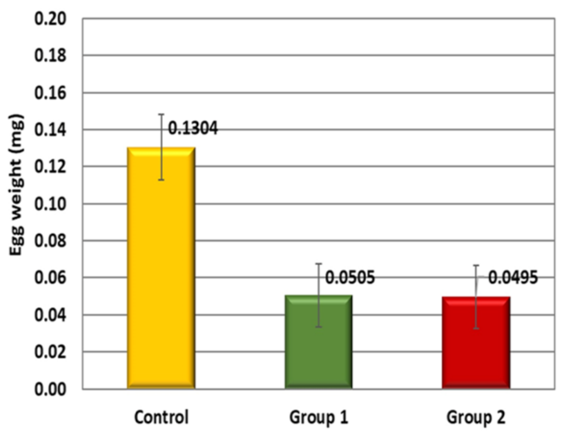

Fig. 4. The effect of *M. anisopliae* on the egg weight (mg) of *R. annulatus engorged* females.Group 1: Dipping method, Group 2:Spraying method.

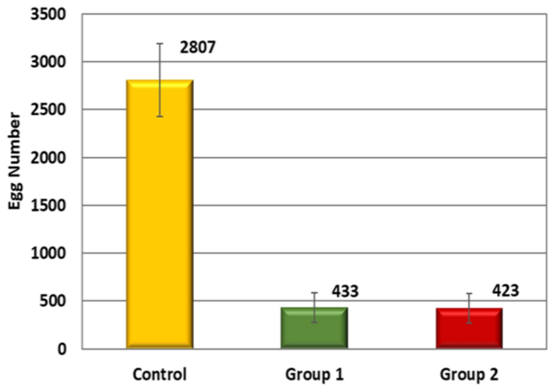

Fig. 5. The effect of *M. anisopliae* on the egg numbers of *R. annulatus engorged* females. Group 1: Dipping method, Group 2:Spraying method.

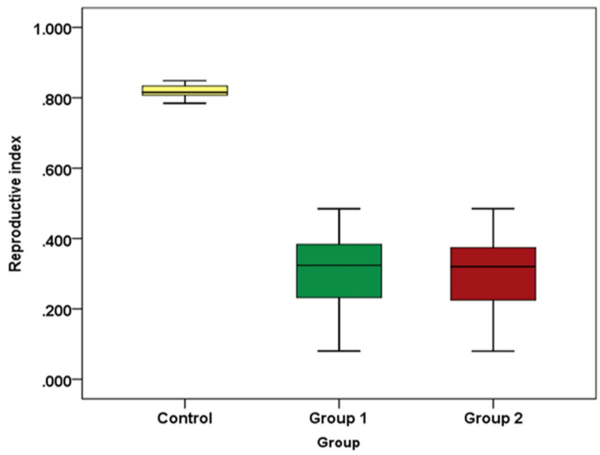

Fig. 6. The effect of *M. anisopliae* on the reproductive index of *R. annulatus* engorged females. Group 1: Dipping method, Group 2:Spraying method.

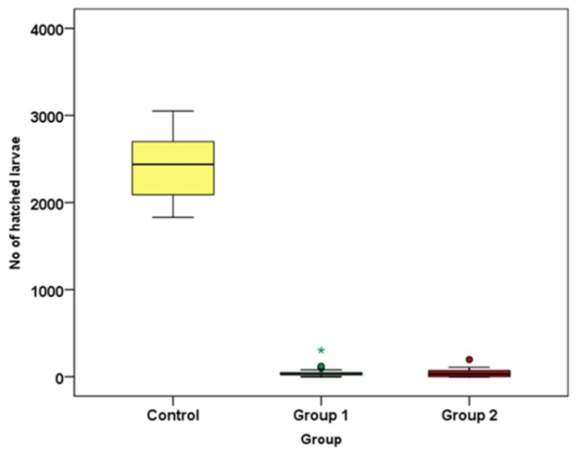

Fig. 7. The effect of *M. anisopliae* on the number of hatched larvae of *R. annulatus* engorged females. Group 1: Dipping method, Group 2:Spraying method.

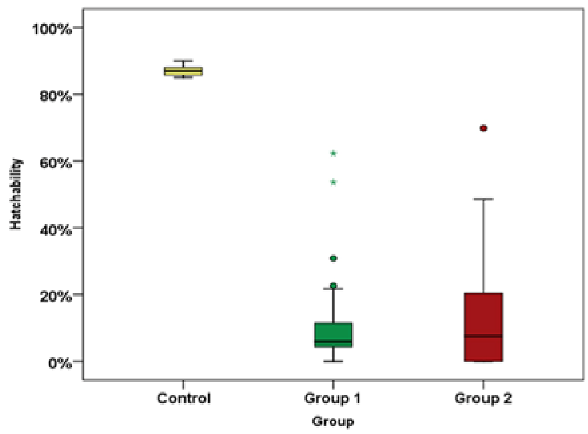

Fig. 8. The impact of *M. anisopliae* on the hatchability of *R. annulatus* engorged females. Group 1: Dipping method, Group 2:Spraying method.

Table. 2. Effect of *M. anisopliae* on reproductive biological parameters of the engorged female ticks

Parameters	**Control group** (n=120)	**Dipping group** (n=120)	**Spraying group** (n=120)	***p*-value***
Female weight (g)	0.160±0.023	0.164±0.022	0.164±0.022	0.137
Egg weight (mg)	0.130±0.018	0.051±0.017	0.049±0.017	< 0.001
Egg number	2807±381	433±155	423±157	< 0.001
Inhibition of oviposition (%)	0.0%	60.2%	60.7%	
Reproductive Index	0.82 (0.79-0.85)	0.32 (0.08-0.49)	0.32 (0.08-0.49)	< 0.001
Hatched larvae no.	2438 (1830-3050)	28 (0-305)	31 (0-197)	< 0.001
Hatchability (%)	87.02 (85.01-90)	6.01 (0.00-62.24)	7.60 (0.00-69.84)	< 0.001

Light and Transmission Electron Microscopy of *R. anunlatus* cuticle treated with the fungus *M. anisopliae* with two different applications

The effects of dipping and spraying ticks with *M. anisopliae* conidia are illustrated in Fig. 9. In the control ticks, the classical compact and dense cuticle layers were observed in an organized manner with smooth surfaces. The cuticle of the control group of *R. annulatus* consisted of an outer epicuticle, a lamellated procuticle (including the exocuticle and endocuticle), and a single epidermal layer. The epicuticle consists of a thin chitinous layer, while the procuticle comprises a series of laminar chitin fibers; each layer consists of sheets of microfibrils oriented in the same direction. In contrast, the treated ticks exhibited slight alterations in cuticle structures from the first to the third-day post-treatment, featuring an irregular outer surface and noticeable destruction on the outer layer. By the fifth to seventh day of treatment, fungal spores began to accumulate within the internal organelles of the cuticle. Finally, many fungal spores are aggregated within the internal structure of the ticks. Furthermore, a general disorganization of the cuticle resulted in a loss of differentiation between the epicuticle and procuticle. A significant separation between the epicuticle and the endocuticle was observed, accompanied by the appearance of vacuoles in the epidermis. Fragmented epidermal cells were also noted, as shown in Fig. 10.

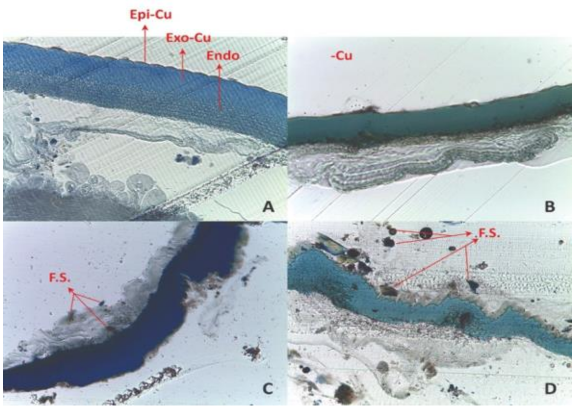

Fig. 9. Light microscopic micrograph of *R. annulatus* treated with *M. anisopliae*, A, B, C and D, 1st, 3rd,5th and 7th day post treatment, respectively. Ep-Cu (Epicuticle), Exo-cu (Exo-Cuticle) and En-Cu (Endocuticle)

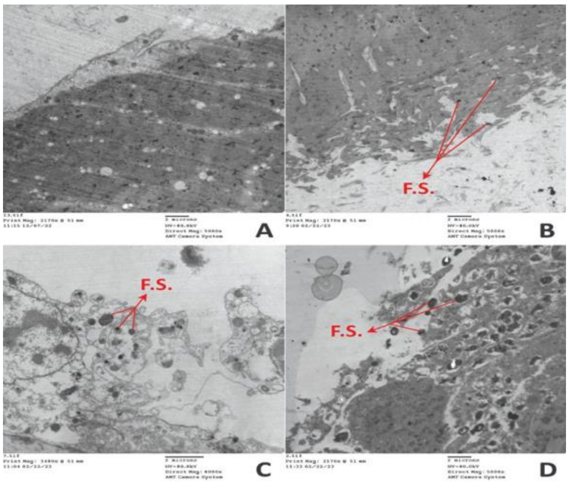

Fig. 10. TEM of *R. annulatus* treated with *M. anisopliae,* showed a large density of fungal spores scattered inside the three cuticle layers. A, B, C, and D at 1st,3rd,5th, and 7th day post-treatment, respectively, FS (fungal spores).

Discussion

The effect of *M. anisopliae* on female *R. annulatus* ticks was investigated, revealing significant morphological changes in the ticks. By the fifth day after treatment, the observed alterations included blackened cuticles with wrinkles and white patches, besides the degeneration of the females and their eggs. These changes may be attributed to the action of the protease enzyme on the cuticles, as previously described by Harrison and Bonning (2010) and Sarodee et al. (2016) in their studies of *Aphis craccivora* and *Diatraea saccharalis* infected with *M. anisopliae.* This hypothesis is supported by the fact that proteins constitute about 70% of the cuticle in insects (Petrisor and Stoian, 2017). Further support for these findings was by Shahatha (2019), who observed that *Hyalomma anatolicum* ticks were sprayed with a formulation *of M. anisopliae* isolate at a concentration of 10^5 spores/ml exhibited a high rate of deformation, characterized by darkened, wrinkled cuticles with black patches. Additionally, many researchers have noted the same results using the *M. anisopliae* fungus, affecting eggs' size and color, leading to shrinking and darkening (Camargo et al., 2014; Perinotto et al., 2017).

This change might be due to the fungus covering all the surface of eggs and reducing gas exchange.

Mortality of *R. annulatus* after exposure to *M. anisopliae*

Regarding the impact of *M. anisopliae* on the survival of engorged *R. annulatus* female ticks, our data showed no mortality in the first two days of dipping and spraying groups. These results are compatible with Nogueira et al. (2020), who reported *M. anisopliae* conidia must germinate for 24 to 48 hours. Wadaan et al. (2023) found no mortality in the first three days of treated *Hyalomma* ticks with spore-free fungal culture filtrates from isolated *Alternaria sp.*, *Aspergillus*, and *Penicillium.* In this document, *M. anisopliae* caused a high mortality rate of engorged *R. annulatus* ticks in dipping and spraying applications, reaching 100% at 10 days of treatment. Moreover, the mortality rate in the treated groups (dipping and spraying) was nearly 50% on the sixth day after treatment. Related results were reported by Frazzon et al. (2000), who found the death rate reached 100% within 8–14 days after treatment of *R. microplus* engorged female ticks dipped in conidial solutions of *M. anisopliae* (10^8 spores/ml). Ojeda-Chi et al. (2010) demonstrate *M. anisopliae* Ma34 strain (10^8 conidia/ml) showed mortality of 100% at 12 days PT on engorged females of *R. microplus*. The longer time was remarked by Alcalá-Gómez et al. (2017) *M. anisopliae* isolates (10^8 / mL) caused a mortality of 99 - 100% at 20 days PT in engorged adult females of *R. microplus*; Webster et al. (2015) the effectiveness of *M. anisopliae* against females *R. microplus* reached 93.9% after 14 days of exposure, and Zhioua et al. (1997) observed that 100% mortality of adult *Ixodes scapularis* females occurred after 2 weeks from treatment with *M. anisopliae* spores (10^8/ml); The dissimilarity of mortality rate in this research and the previous studies may be due to the pathogenicity of the fungus depending on the strain of *M. anisopliae* fungi used as mentioned by Riaz et al. (2013). Several strains of this fungus are highly efficacious against a variety of insect pests; also the weather conditions and the season in which the conidia are deployed (temperature and humidity); the conidia concentration of fungal suspension as described by Zhioua et al. (1997), they found a spore concentration of 10^6 spores/ ml had a low effect, whereas a concentration of 10^7 spores/ ml induced 100% mortality among engorged adult females; and finally, the tick species that used according to Kaaya et al. (2011) observed 93-100%mortality of *R. evertsi* and *R. decoloratus*, treated by *M. anisopliae* respectively. There was no discernible difference in mortality between the two applications (dipping and spraying), indicating that *M. anisopliae's* primary mode of action was spore attachment to cuticle surfaces, accomplished in both applications.

Reproductive efficacy of *R. annulatus* treated with *M. anisopliae*

Compared to the control group, the reproductive performance of *R. annulatus* engorged females was distressed by *M. anisopliae*. This was supported by the significantly decreased egg mass and number in the treated groups (dipping and spraying) compared to the control group. This appropriately was comparable to the results by Suleiman et al. (2013), which revealed a significant reduction in eggs laid by the treated group of adult *H. anatolicum* ticks with *M. anisopliae* (10^7 spores/ml) with an average egg mass of 0.06 g. Significant reductions in egg mass weight and egg numbers of *R. microplus* due to the effect of *M. anisopliae* were reported by Marciano et al. (2013); Perinotto et al. (2017); Nogueira et al. (2020) and Barbieri et al. (2023)**.** While the egg production index was lower for *R. sanguineus* females treated with *M. anisopliae* (Barreto et al. 2016).

Reproductive index of *R. annulatus* Female ticks treated by *M. anisopliae*

The control group's reproductive index (RI) was 0.815 in this work, whereas the dipping and spraying groups were 0.324 and 0.320, respectively. These results appeared similar to those of Ojeda-chi et al. (2010) where the RI caused by the *M. anisopliae* Ma34 strain reached 0.25 in *R. microplus*. Ren et al. (2012) reported that the RI of *R. microplus* ticks reached 0.17 and 0.12 when exposed to *M.anisopliae* at concentrations 10^8 and 10^9, respectively. Barbieri et al. (2023) found an RI index of *R. microplus* tick was 0.121.

Inhibition of oviposition of *R. annulatus* treated by *M. anisopliae*

According to the present findings, females in the dipping group showed more significant inhibition of oviposition (60.2%) than those in the spraying group (60.7%), compared to the control group. These results align with earlier research: Ojeda-Chi (2010) found that *R. microplus* treated with *M. anispoliae* Ma34 had a 55.5% oviposition inhibition. Admes et al. (2011) found that *R. microplus* treated with *M. anispoliae* (10^7 conidia/ml) had a 77.09% oviposition inhibition; and Alcalá-Gómez et al., (2017) revealed that a 73% reduction in egg oviposition was seen in *R. microplus* treated with *M. anisopliae* isolate a136. These discrepancies in oviposition inhibition results are due to the conditions under which the engorged females were incubated throughout the study, such as temperature, humidity, or duration of light, which may impact the number of eggs laid by ticks.

The hatchability percentage of *R. annulatus* ticks treated by *M. anisopliae*

According to the current study, the fungus reduced the hatchability percentage for the dipping and spraying groups to 6.01% and 7.2%, respectively, compared to the control group's 87.02%. In addition to these findings, Suleiman et al. (2013) reported that *Hyalomma anatolicum* engorged females treated with *M. anisopliae* (10^7 spores/ml) were unable to hatch their eggs; Perinotto et al. (2017) found that the *M. anisopliae* CG 629 isolate reduced the hatchability of *R. microplus* larvae by 62%; and Nogueira et al. (2020) found that hatchability decreased by up to 61% of *R. microplus* (10^8 conidia/mL).

Transmission electron microscope of the cuticle *R. annulatus* ticks treated by *M. anisopliae*

When *R. annulatus's* cuticle was treated with the fungus, *M. anisopliae*, the epicuticle layer became discontinuous, and the endocuticle layer looked haphazard. Along with the lack of the cuticle's distinct layers, the cuticle layer was separated from the epidermal layer. Germinated conidia were visible on the ticks' surface on the first day after treatment. They developed penetration pegs, which began to pierce through the various layers of the cuticle and laminae. Subsequently, these penetration pegs formed invasive hyphae, allowing some to reach the epidermal cells through the cuticle. The tick cuticle showed significant and severe disturbance by the third, fifth, and seventh days after treatment. Our findings indicate that *M. anisopliae* can infect and penetrate *R. annulatus* beginning from the first day following inoculation. Our results align with previous studies, including the work of Arruda et al. (2005), who described the infection process of *M. anisopliae* in *B. microplus* by using transmission electron microscopy, twenty-four hours after infection, conidia were noticed attached to the surface of the tick, and germination began, the fungus then penetrated the layers of the tick's cuticle, and at 72 hours post-infection, extensive penetration was observed leading to significant morphological changes to the tick's cuticle. Pirali-Kheirabadi et al. (2016) used scanning electron microscopy to examine the binding, germination, and penetration of *Metarhizium anisopliae* on *Ixodes ricinus* ticks. They explained that conidial germination on the ticks occurs as the fungus produces a thin, amorphous mucilage layer that firmly adheres both the conidia and germ tubes to the tick's integument. Similarly, Meirelles et al. (2023) discovered all fungal particles of *M. anisopliae* displayed a spherical shape with cavities on their surfaces, and the germinated conidia showed a few hyphae beneath the surface of *Rhipicephalus microplus* ticks using scanning electron microscopy (SEM). Previous studies on the fungus *M. anisopliae* have shown its detrimental effects on insect cuticles. For instance, Eid et al. (2009) reported that adult desert locusts (*S. gregaria*) treated with *M. anisopliae* exhibited germinated spores on their cuticle surfaces, and the fungus successfully penetrated the insect cuticle within 24 hours of treatment. Furthermore, Salem et al. (2023) observed ultrastructural changes in the cuticles of 3rd instar *C. pipiens* larvae, specifically the loosening of the pro-cuticular lamellae, 48 hours after treatment with *M. anisopliae*. These findings support the results of the current study, confirming that *M. anisopliae* effectively infects female ticks and rapidly germinates, penetrating their cuticle and causing significant damage.

In conclusion

According to the present study findings, biological control is already a viable substitute for acaricides and may be used in a tick management plan. This emphasizes how urgently fungal products for veterinary application need to be developed. Furthermore, outdoor tests may produce fewer striking results. Therefore, to validate the results of laboratory experiments, further study is necessary to evaluate the effects of field myco-acaricides.

Acknowledgment

We express our gratitude to everyone who helped with the tick sample Health collection. The Parasitology Department of the Animal Health Institute in Dokki, Giza, Egypt, offered grants and support, for which the authors are thankful. The authors further acknowledge Professor Ahmed Mohammed Azzazy of the Plant Protection Research Institute's help in locating the bioagent source.

5. Reference

Abdel-Aziz MM, Yosri M, Amin BH, 2017. Control of imipenem-resistant Klebsiella *pneumoniae* pulmonary infection by oral treatment using a combination of myco-synthesized Ag-nanoparticles and imipenem. J. Radiation Research and Applied Sciences. 10:353-360.

Adames M, Fernández-Ruvalcaba M, Peña-Chora G, Hernández-Velázquez VM, 2011. Effects of passages through a suitable host of the fungus, *Metarhizium anisopliae*, on the virulence of acaricide-susceptible and resistant strains of the tick, *Rhipicephalus microplus*. J. Insect. Sci. 11:21.

Alcalá-Gómez J, Cruz-Vázquez C, Fernández-Ruvalcaba M, Ángel-Sahagún C, Vitela-Mendoza I, Ramos-Parra M, 2017. Virulence of *Metarhizium anisopliae* and *Beauveria bassiana* isolates and the effects of fungal infection on the reproduction potential of *Rhiphicephalus microplus* engorged females. Biocontrol Sci. Technol. 27: 931–939.

Aqueel MA, Leather SR, 2013. Virulence of *Verticillium lecanii* (Z.) against cereal aphids; does timing of infection affect the performance of parasitoids and predators. Pest. Manag. Sci. 69: 493–498.

Arruda W, Lübeck I, Schrank A *et al.*, 2005. Morphological Alterations of *Metarhizium anisopliae* During Penetration of *Boophilus microplus* Ticks. Exp. Appl. Acarol. **37**: 231–244.

Barbieri A, Rico IB, Silveira C, Feltrin C, Dall B, Schrank A, Reck J, 2023. Field efficacy of Metarhizium anisopliae oil formulations against Rhipicephalus microplus ticks using a cattle spray race. Ticks and Tick-borne Diseases. *14*: 102147.

Barreto LP, Luz C, Mascarin GM, Roberts DW, Arruda W, Fernandes EKK, 2016. Effect of heat stress and oil formulation on conidial germination of *Metarhizium anisopliae* s.s. on tick cuticle and artificial medium. J. Invertebr. Pathol. 138: 94-103.

Beys-da-Silva WO, Rosa RL, Berger M, Coutinho-Rodrigues CJB, Vainstein MH, Schrank A, Bittencourt VREP, Santi L, 2020. Updating the application of Metarhizium anisopliae to control cattle tick Rhipicephalus microplus (Acari: Ixodidae). Exp. Parasitol. Jan; 208:107812.

Camargo M G, Marciano AF, Sá FA, Perinotto W M, Quinelato S, Gôlo PS, Bittencourt VR, 2014. Commercial formulation of *Metarhizium anisopliae* for the control of Rhipicephalus microplus in a pen study. Vet. Parasitol. 205: 271-276.

Drummond RO, Crust SF, Trevino JL, Gladney WJ, Graham OH, 1973. *B. annulatus* and *B. decoloratus;* laboratory tests of insecticides. J. Econ. Entomol. 66: 130–133.

Eid M A A, Sewify G H, Hanan Hamada M, Abdel-Fattah T A, Mohamad E S M, 2009. The effect of entomopathogenic fungus metarhizium anisopliae var acridum on flight muscles in the desert locust,schistocerca gregaria (forskal).J. Agric. Sci. Mansoura Univ, 34: 2211 - 2224.

Elhadidy NM, 2014. Biological effects of some microbial insecticides against locusts under laboratory conditions. Ph D Thesis, Suez Canal University, Faculty of Science, Zoology Department

Eskezia B, Desta A, 2016. Review on the impact of ticks on livestock health and productivity. Journal of Biology, Agric. and Healthcare. 6(22):1-7.

Frazzon APG, Vaz Junior I da S, Masuda A, Schrank A, Vainstein MH, 2000. In vitro assessment of Metarhizium anisopliae isolates to control the cattle tick Boophilus microplus. Vet. Parasitol. 94: 117–125.

Haggag YN, Hamed Abd El- Tawab Samaha, Mohammad Al- Sayed Nossair, Heba mohammed habib, 2017. Comparative in Vitro Study on the Efficacy of Some Commercial Insecticides against Rhipicephalus (Boophilus) annulatus Ticks. Alex. J. Vet. Sci. 54:158-167.

Harrison R L, Bonning B C, 2010. Proteases as insecticidal agents. Toxins 2: 935−953.

Hend HAM, Abdullaha, Amal, El-Mollab, Fayez A, Salibb Alaa A. Ghazya, Nesreen AT, Allam, Sobhy, Abdel-Shafy, 2019. Preliminary detection of Rickettsiae using PCR targeting OmpA gene among dogs and horses in Cairo, Egypt. Egypt J. Vet. Sci. 50: 1- 8.

Hoogstraal H, Kaiser MN, 1958. Observations on Egyptian *Hyalomma* ticks (Ixodiea Ixodidae) I. Parasitism of Lizards by nymphs. Ann. Fnt. Soc. Amer. 51 (1) 7-12.

Kaaya GP, Samish M, Hedimbi M, Gindin G, Glazer I, 2011. Control of tick populations by spraying Metarhizium anisopliae conidia on cattle under field conditions. Exp. Appl. Acarol. 55:273-81.

Kaaya GP, 2000. Laboratory and field evaluation of entomogenous fungi for tick control. Ann. N. Y. Acad. Sci. 916: 559-564.

Klafke G, Webster A, Dall Agnol B, Pradel E, Silva J, de La Canal LH, Becker M, Osório MF, Mansson M, Barreto R, Scheffer R, Souza UA, Corassini VB, dos Santos J, Reck J, Martins JR, 2017. Multiple resistance to acaricides in field populations of Rhipicephalus microplus from Rio Grande do Sul state, Southern Brazil. Ticks Tick Borne Dis. 8: 73–80.

Marciano AF, de Paulo JF, de Souza LA, Camargo MG, de Souza Perinotto WM, da Costa Angelo I, Gôlo PS, de Sá FA, Rodrigues CJBC, Quinelato S, Bittencourt VREP, 2013. Eficiência in vitro de uma formulação oleosa de Metarhizium anisopliae sensu lato no controle de Rhipicephalus microplus. Braz. J. Vet. Med. 35: 28-34.

Meirelles LN, Mesquita E, Corrêa T A, Bitencourt R d O B, Oliveira J L, Fraceto L F, Camargo M G, Bittencourt V R E P, 2023. Encapsulation of entomopathogenic fungal conidia: Evaluation of stability and control potential of *Rhipicephalus microplus. Ticks. Tick*. Borne. Dis. 14: 102184..

Nogueira MRdS, Camargo MG, Rodrigues CJBC, Marciano AF, Quinelato S, de Freitas MC, Fiorotti JdSFA, Perinotto WMdS, Bittencourt VREP, 2020. *In vitro* efficacy of two commercial products of *Metarhizium anisopliae* s.l. for controlling the cattle tick *Rhipicephalus microplus*. Rev. Bras. Parasitol. Vet. 29: 1–8.

Ojeda-Chi MMR, Rodriguez-Vivas E, Galindo-VelascoR, Lezama-Gutiérrrez, 2010. Laboratory and field evaluation of *Metarhizium anisopliae* (*Deuteromycotina: Hyphomycetes*) for the control of *Rhipicephalus microplus* (*Acari:ixodidae*) in the Mexican tropics. Vet. Parasitol., 170: 348-354.

Oundo JW, Hartemink N, MartC M deJong , Constantianus JM, Koenraadt , Shewit Kalayou, Masiga, D, Bosch Q, 2025. Biological control of ticks in domestic environments: Modeling the potential impact of entomopathogenic fungi on the transmission of East Coast fever in cattle. Ticks and Tick-borne Diseases. 16: 1-12,

Perinotto W MS, Angelo IC, Golo PS, Camargo M G, Quinelato S, Sá FA, Bittencourt VRE, 2017. In vitro pathogenicity of different Metarhizium anisopliae s.l. isolates in oil formulations against Rhipicephalus microplus. Biocontrol Sci. Technol. 27: 338–347.

Petrisor C, Stoian G, 2017. The role of hydrolytic enzymes produced by entomopathogenic fungi in pathogenesis of insects. Romanian J. Plant Protect. X: 66−72.

Pirali-Kheirabadi K, Razzaghi-Abyaneh M, Eslamifar A, Halajian A, Nabian S, 2016. 'Scanning Electron Microscopy (SEM) analysis and biological control of *Ixodes ricinus* using entomopathogenic fungi. Mycologia Iranica. 3: 39-46.

Pirali-Kheirabadi K, Haddadzadeh H, Razzaghi-Abyaneh M, Bokaie S, Zare R, Ghazavi M, Shams-Ghahfarokhi M, 2007. Biological control of *Rhipicephalus (Boophilus) annulatus* by different strains of *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium psalliotae* fungi. Parasitol. Res. 100: 1297–1302.

Rajput M, Sajid MS, Rajput NA, George DR, Usman, M, Zeeshan M, Iqbal O, Bhutto B, Atiq M, Rizwan H M, Daniel IK, Sparagano OA, 2024. Entomopathogenic Fungi as Alternatives to

Chemical Acaricides: Challenges, Opportunities and Prospects for Sustainable Tick Control. *Insects*, *15*(12), 1017.

Ren Q, Liu Z, Guan G, Sun M, Ma M, Niu Q, Li Y, Liu A, Liu J, Yang J, Yin H, Luo J, 2012. Laboratory evaluation of virulence of Chinese *Beauveria bassiana* and *Metarhizium anisopliae* isolates to engorged female *Rhipicephalus* (*Boophilus) microplus* ticks. Biol. Control. 63: 98-101.

Riaz ABID, Shah FA, Butt TM, 2013. Intra-specific variability among Metarhizium anisopliae strains in their ability to produce blastospores in liquid culture media. Pak. J. Bot, 45(3), 1099-1103.

Salem HHA, Mohammed SH, Eltaly RI, Moustafa MAM, Fónagy A, Farag SM, 2023. Co-application of entomopathogenic fungi with chemical insecticides against *Culex pipiens*. J. Invertebr. Pathol. 198:107916.

Sarodee B, Pranab D, Joyarani P, Himadri K, Gogoi N, Puzari KC, Hazarika GN, 2016. SEM study on morphological changes in *Metarhizium anisopliae* infected Aphis craccivora Koch. J. of Biol. Control. 30: 29-33

Schrank A, Vainstein, MH, 2010. *Metarhizium anisopliae* enzymes and toxins. Toxicon. 56: 1267–1274.

Shahatha SSh, 2019. Evaluation the efficiency of the fungus Metarhizium anisopliae as biocontrol agent for adults of Hyalomma anatolicum. Iraqi J. Vet. Med. 33: 57-62.

Suleiman EA, Shigidi MT, Hassan SM, 2013. Metarhizium anisopliae as a Biological Control Agent Against Hyalomma anatolicum (Acari: Ixodidae). Pakistan J. Biolo. Sci. 16: 1943-1949.

St Leger, R.J., (2008). Studies on adaptations of *Metarhizium anisopliae* to life in the soil. J. Invertebr. Pathol. 98, 271–276.

Vudriko P, Okwee-Acai J, Tayebwa DS, Byaruhanga J, Kakooza S, Wampande E, Omara R, Muhindo JB, Tweyongyere R, Owiny DO, Hatta T, Tsuji N, Umemiya-Shirafuji R, Xuan X, Kanameda M, Fujisaki K, Suzuki H, 2016. Emergence of multi-acaricide resistant Rhipicephalus ticks and its implication on chemical tick control in Uganda. Parasites and Vectors. 9: 4.

Wadaan MA, Khattak B, Riaz A, Hussain M, Khan MJ, Fozia F, Iftikhar A, Ahmad I, Khan MF, Baabbad A, Ziaullah, 2023. Biological Control of Hyalomma Ticks in Cattle by Fungal Isolates. Vet Sci. 10:684.

Walker AR, 2003. Ticks of domestic animals in Africa: a guide to identification of species. *Biosci. Rep. Edinb.* 3:210.

Webster A, Reck J, Santi L, Souza U A, Dall'Agnol B, Klafke GM, 2015. Integrated control of an acaricide-resistant strain of the cattle tick *Rhipicephalus microplus* by applying *Metarhizium anisopliae* associated with cypermethrin and chlorpyriphos under field conditions. Vet.Parasitol.207: 302–308.

Yosri M, Elaasser MM, Abdel-Aziz MM, Hassan MM, Alqhtani AH, Al-Gabri N, Ali ABA, Pokoo-Aikins A, Amin BH, 2022. Determination of Therapeutic and Safety Effects of *Zygophyllum coccineum* Extract in Induced Inflammation in Rats. Biomed Res Int. 2022:7513155.

Zhioua E, Browning M, Johnson PW, Ginsberg HS, LeBrun RA. (1997). Pathogenicity of the entomopathogenic fungus Metarhizium anisopliae (Deuteromycetes) to Ixodes scapularis (Acari: Ixodidae). J Parasitol. 83:815-8. PMID: 9379283.