ACARICIDAL ACTIVITY OF COMBINED COMMERCIAL STRAINS OF ENTOMOPATHOGENIC FUNGI AGAINST *Amblyomma mixtum* AND *Rhipicephalus microplus* TICKS †

[ACTIVIDAD ACARICIDA DE CEPAS COMBINADAS DE HONGOS ENTOMOPATÓGENOS COMERCIALES CONTRA LAS GARRAPATAS *Amblyomma mixtum* Y *Rhipicephalus microplus*]

D. Romero-Salas[1], J.L Bravo-Ramos[1*], D.S. Sanchez-Montes[2], C. Cardenas-Amaya[1], J. Gamboa Prieto[1], A. Cruz-Romero[1] and A. Olivares-Muñoz[1]

[1]Laboratorio de Parasitología, Rancho Torreón del Molino, Facultad de Medicina Veterinaria y Zootecnia, Universidad Veracruzana, Circunvalación y Yáñez s/n, 91710 Veracruz, México. E-mail: jbravo@uv.mx

[2] Facultad de Ciencias Biológicas y Agropecuarias región Tuxpan, Universidad Veracruzana, Carretera Tuxpan Tampico Kilómetro 7.5 Universitaria 92870 Tuxpan de Rodríguez Cano, Veracruz, México

**Corresponding author*

SUMMARY

Background: *Rhipicephalus microplus* and *Amblyomma mixtum* are the most important tick species affecting livestock in tropical areas. They can have a direct harmful effect on cattle and humans due to transmission of pathogens of tick-borne diseases (TBD). Entomopathogenic fungi have an important role in crop pest control. However, data concerning the efficacy of use of entomopathogenic fungi combinations are scarce. **Objective:** For this reason, in this study, the efficacy of commercial fungal combinations was assessed: Trishok® (*Trichoderma harzianaum* and *Trichoderma virens*) and Esporomax® (*Beauveria bassiana*, *Metarhizium anisopliae*, *Paecelomyces fumosoroseus*). **Methodology:** Adult immersion tests were performed with six different concentrations of fungal solutions. **Results:** In this study, Trishok® and Esporomax® showed potential efficacy against *R. microplus*. Different concentrations of fungal application tested on *A. mixtum* did not show any effect. The observed mortality of *R. microplus* was 85. 0 ± 2.0 and 65.0 ± 2.0 for Trishok® and Esporomax®, respectively. The estimated LC_{50} for *R. microplus* immersed in solutions of Trishok® and Esporomax® thereof were: $6.5x10^4$ and $3.9x10^5$ conidia mL^{-1}, respectively. **Implications:** These results should be considered in the design and implementation of alternatives based on the biological control of ticks. **Conclusion:** We can conclude that combined commercial strains of entomopathogenic fungi have *in vitro* acaricidal activity against *R. microplus*.

Key words: Biological control; Fungal combinations; *Trichoderma harzianaum; Trichoderma virens;* Arthropods.

RESUMEN

Antecedentes: *Rhipicephalus microplus* y *Amblyomma mixtum* son las dos principales especies de garrapatas que infestan al ganado bovino en áreas tropicales. Estos ectoparásitos, pueden causar un efecto nocivo directo en el ganado y también en los seres humanos debido a la transmisión de patógenos de enfermedades transmitidas por garrapatas (TBD). Por otro lado, los hongos entomopatógenos tienen un papel importante en el control de plagas de los cultivos. Sin embargo, los datos sobre el uso de formulaciones combinadas de hongos entomopatógenos, así como de su eficacia son escasos. **Objetivo:** Por esta razón, en este estudio se evaluó la efectividad de combinaciones de hongos comerciales. Trishok® (*Trichoderma harzianaum* y *Trichoderma virens*) y Esporomax® (*Beauveria bassiana*, *Metarhizium anisopliae*, *Paecelomyces fumosoroseus*). **Metodología:** Se realizaron pruebas de inmersión de adultas con seis concentraciones diferentes de soluciones fúngicas. **Resultados:** En este estudio, Trishok® y Esporomax® mostraron eficacia contra *R. microplus*. La aplicación de hongos en todas las concentraciones probadas en la garrapata *A. mixtum* no mostro efecto. La mortalidad observada de *R. microplus* fue 85,0 ± 2,0 y 65.0 ± 2.0 para Trishok® y Esporomax® respectivamente. Las CL_{50} estimadas para *R. microplus*

† *Submitted August 13 2021 Accepted July 12, 2022. http://doi.org/10.56369/tsaes.3919*

ISSN: 1870-0462.

utilizando Trishok® y Esporomax® fueron: $6.5x10^4$ y $3.9x10^5$ conidias mL^{-1} respectivamente. **Implicaciones:** Estos resultados deben ser considerados en el diseño e implementación de alternativas basadas en el control biológico de garrapatas. **Conclusión:** Los resultados sugieren que las cepas de hongos comerciales pueden considerarse como agentes potenciales para el control biológico de *R. microplus.*
Palabras clave: Control biológico; *Trichoderma harzianaum;* Artrópodos; *Trichoderma virens.*

INTRODUCTION

Ticks are haematophagous arthropods, which are distributed worldwide, mainly in tropical and subtropical areas. They can have a direct harmful effect on livestock and also humans due to pathogen transmission (Miraballes and Riet-Correa, 2018). Chemical compounds are the most commonly used methods to control tick populations. However, these methods have several side effects including the appearance of acaricide-resistant ticks, food contamination, increasing costs of treatments and impacts on non-target organisms (Rodríguez-Vivas *et al*., 2018). The use of entomopathogenic fungi against ticks has become a more sustainable and promising alternative due to their capacity to penetrate the arthropod cuticle (Shang *et al*., 2012). However, the successful use of entomopathogenic fungi to control arthropods depends not only on pathogenicity levels but also on the interactions between the fungus and its target host and their environment (Fernandez-Salas *et al*., 2017).

Beauveria bassiana and *Metarhizium anisopliae* are the most commonly assessed fungi in alternative control of *Rhipicephalus microplus*, *Ixodes scapularis*, *Dermacentor variabilis* and *Amblyomma variegatum* ticks (Ub and Narladkar, 2018). However, several entomopathogenic fungi as *Trichoderma harzianaum*, *T. virens* and *Paecelomyces fumosoroseus*, have been reported as acaricidal and nematicidal against *Cimex hemipterus*, *Acanthoscelides obtectus*, *Meloidogyne javanica*, *Toxocara canis*, *Geotrogus deserticola* and *Tetranychus kanzawai* (Kiriga *et al*., 2018, Rodríguez-González *et al*., 2018, de Souza *et al*., 2017, Zahran *et al*., 2017, Harizia and Lazreg, 2016, Sanjaya, 2016). For this reason, due to the potential acaricidal activity of several species of entomopathogenic fungi**,** the aim of this study was to evaluate the *in vitro* effectiveness of combined commercial strains against *Amblyomma mixtum* and *R. microplus* ticks.

MATERIALS AND METHODS

Engorged female ticks

Ticks were collected from naturally infested cattle in the municipality of Laguna de Farfan, Veracruz, Mexico. Collected ticks were placed in plastic tubes and kept in a refrigerator until they were used for the bioassay (not exceeding 1 week). Ticks were identified by taxonomic keys according to Guzman-Cornejo *et al*. (2011) for *A. mixtum* and Walker (2003) for *R. microplus*.

Source of entomopathogenic fungi and viability

Trishok® (sourced from combinations of *T. harzianaum* and *T. virens*) is a commercial product produced by Bioproductora de Jardines Sostenibles (Jalisco, Mexico) and used as a biological control method in agricultural pest control. This product contains $7x10^6$ spores/g. A stock suspension of this product was prepared by adding 4 g of the product powder to 50 ml of distilled water in a plastic tube according to the manufacturer's instructions. This, was mixed well using a vortex and allowed 30 min before use (Aqueel and Leather, 2013). After homogenisation, the conidia concentration was determined with a haemocytometer and adjusted to concentrations of $5x10^5$, $2.5x10^5$, $1.25\ x10^5$, $7.5x10^4$, $3.5x10^4$ and $1.75\ x10^4$ conidia mL^{-1}. These dilutions were in a 10 ml solution diluted with distilled water.

Esporomax® (sourced from combinations of *B. bassiana*, *M. anisopliae* and *P. fumosoroseus*) is a commercial product manufactured by Bioproductora de Jardines Sostenibles (Jalisco, Mexico) and used for biological control of agriculture pests. Esporomax contains $5x10^6$ spores/g. A stock suspension of this product was prepared by adding 8 g of the product powder to 50 ml of distilled water in a plastic tube according to the manufacturer's instructions. This was mixed well using a vortex and allowed 30 min before use. After homogenisation, the conidia concentration was determined with a Neubauer chamber and adjusted to concentrations of $5x10^5$, $2.5x10^5$, $1.25\ x10^5$, $7.5x10^4$, $3.5x10^4$ and $1.75\ x10^4$ conidia mL^{-1}. These dilutions were in a 10 ml solution diluted with distilled water. Each suspension tested was inoculated on potato dextrose agar plates, and then incubated in a dark incubator at 26–28 °C and 80% relative humidity for 7–10 days. Next, the slides were stained with cotton blue (lactophenol blue stain), prepared and examined

under a microscope for the viability assay (Hasan *et al*., 2013).

Adult immersion test of engorged female ticks

Previously prepared suspensions of Trishok® and Esporomax® were tested on engorged female ticks of the same size. The ticks were washed in distilled water, dried and immersed in the fungal suspension for 3 minutes then dried and incubated for 14 days in Petri dishes (Drummond *et al*., 1973). Five replicates, each of ten ticks, were done for each suspension. Each bioassay was repeated on a different day, and the results reported are averages of both repetitions. A control group was treated by immersion in 10 ml of distilled water for 3 minutes. The ticks were observed daily over 15 days and the paralysis of ticks (straightened legs, no response to CO_2 stimuli) was recognized as a mortality effect.

Data analysis

LC_{50} values were calculated using the probit analysis method with the LC_{50}/LD_{50} calculator (Finney, 1952). Mortality data were analysed using a one-way analysis of variance (ANOVA) with STATISTICA software, V.10. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Mortalities caused by Trishok® and Esporomax® in engorged female ticks are shown in Table 1. Trishok® showed the highest acaricidal effect (85.0 ± 2.0%), followed by Esporomax® (65.0 ± 2.0%). Mortality was significantly different after engorged female ticks were exposed to fungal dilutions in a dose dependent manner, compared with the control group exposed to diluent ($p < 0.05$). On the other hand, Trishok® and Esporomax® did not cause mortality at any concentration in *A. mixtum* ticks. The fungal growth was observed on *R. microplus* ticks after two weeks post application (Fig. 1). The LC_{50} values of Trishok® and Esporomax® are shown in Table 2.

DISCUSSION

Studies have shown that combinations of entomopathogenic fungi isolated from soil or bought from a commercial source are potential pathogens for use in the control of ticks (Szczepańska *et al*., 2020, Aboelhadid *et al*., 2018, Ren *et al*., 2016). Thus, in this study, we examined the *in vitro* acaricidal effectiveness of combined commercial strains Esporomax® (*B. bassiana*, *M. anisopliae* and *P. fumosoroseus*) and Trishok® (*T. harzianaum* and *T. virens*). The results demonstrated the resistance of *A. mixtum* to Esporomax and Trishok®. Thus, these results are different to previous reports on the susceptibility of *Amblyomma* sp. Resulting from fungal suspensions of *B. bassiana* and *M. anisopliae*, which indicated that the fungal combination induced higher mortalities than each fungus alone (Maranga *et al*., 2005). On the other hand, Ren *et al*. (2016) reported a higher mortality

Table 1. Mean percentage mortality of *Rhipicephalus microplus* and *Amblyomma mixtum* adult females obtained after treatment with commercially sourced fungi at different concentrations.

Fungal product	Concentration (conidia mL^{-1})	Mortality %	
		R. microplus	*A. mixtum*
Negative control (Distilled water)	0.0	0.0	0.0
Trishok®	$5x10^5$	85. 0 ± 2.0[a]	0.0
	$2.5x10^5$	82.5 ± 3.0[a]	0.0
	$1.25\ x10^5$	75.0 ± 1.5[b]	0.0
	$7.5x10^4$	55.2 ± 2.5[c]	0.0
	$3.5x10^4$	45.3 ± 1.5[d]	0.0
	$1.75\ x10^4$	30.0 ± 1.0[e]	0.0
Esporomax ®	0.0	0.0	0.0
	$5x10^5$	65.0 ± 2.0[a]	0.0
	$2.5x10^5$	55.4 ± 1.9[b]	0.0
	$1.25\ x10^5$	49.0 ± 2.1[c]	0.0
	$7.5x10^4$	35.5 ± 1.5[d]	0.0
	$3.5x10^4$	25.0 ± 1.5[e]	0.0
	$1.75\ x10^4$	15.0 ± 2.0[f]	0.0

Different letters in the same column represent a difference between concentrations ($p \leq 0.05$) within the fungal product.

Table 2. Lethal concentrations (LC_{50}) of *Rhipicephalus microplus microplus* infected with different commercially sourced fungal combinations.

Fungal product	LC_{50}	Confidence limits (95%) (upper-lower)
Trishok®	$6.5x10^{\wedge}4$	$4.2x10^{\wedge}4 – 1.8\ x10^{\wedge}5$
Esporomax ®	$3.9x10^{\wedge}5$	$2.1\ x10^{\wedge}5 – 5.2x10^{\wedge}5$

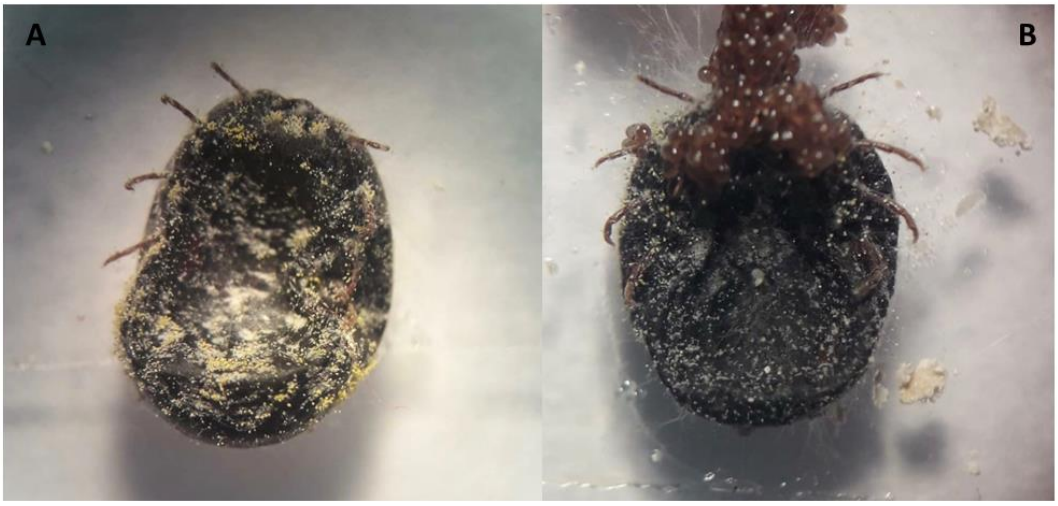

Figure 1. Fungal mycelia growth on the cuticle of *Rhipicephalus microplus* female. A Trishok®; B Esporomax®.

rate (96%) of *B. bassiana* and *M. anisopliae* combinations against *Haemaphysalis qinghaiensis.* These mortality rates were similar to the results obtained in this study. The different results observed in this bioassay with fungal solutions and tick populations may be related to the reduction of fungal pathogenicity *in vitro* (Pérez-González *et al*., 2014) and also to differences in the susceptibility of tick populations to entomopathogenic fungi (Perinotto *et al*., 2012). This effect could be explained by the capability of fungi to produce hydrolytic enzymes, e.g. proteases, chitinases, lipases and other factors, which promote germination and growth of the fungus and subsequent penetration of the cuticular layers (Ortiz-Urquiza and Keyhani, 2013, Xiao *et al*., 2012, Zheng *et al*., 2011). Additionally, the lesser efficacy of entomopathogenic fungi against different tick strains may be due to cuticular wax, which can also promote or inhibit fungal attachment to the cuticle. Such attachment can be affected by nutritional requirements and could be enhanced via a formulation that can result in increased efficacy of the fungal agents against target ticks (Rodríguez-González *et al*., 2018, Akbar *et al*., 2004).

CONCLUSION

According to the results obtained, we can conclude that the combined commercial strains of entomopathogenic fungi Trishok® and Esporomax have *in vitro* acaricidal activity against adult *R. microplus*, which should be considered in the design and implementation of alternatives based on the biological control of ticks.

Funding. None.

Conflict of interest. The authors declare that they have no competing interests

Compliance with ethical standards. Does not applies.

Data availability. Data is available with corresponding author upon reasonable request

Author contribution statement (CRediT). D. Romero-Salas--Conceptualization; Data Curation; **J.L. Bravo-Ramos** – Conceptualization; Data Curation; Formal Analysis; Original Draft; and Writing., **D.S. Sanchez-Montes** – Data Curation; Supervision; Editing., **C. Cardenas-Amaya-** Methodology., **J. Gamboa Prieto-** Methodology., **A. Cruz-Romero-** Methodology., **A. Olivares-Muñoz-** Methodology

REFERENCES

Aboelhadid, S.M., Ibrahium, S.M., Arafa, W.M., Maahrous, L.N., Abdel-Baki, A.A.S. and Wahba, A.A., 2018. *In vitro* efficacy of *Verticillium lecanii* and *Beauveria bassiana* of commercial source against cattle tick, *Rhipicephalus* (boophilus) *annulatus*. *Advances in Animal and Veterinary Sciences*, 6, pp. 139–147. http://dx.doi.org/10.17582/journal.aavs/2018/6.3.139.147

Akbar, W., Lord, J.C., Nechols, J.R. and Howard, R.W., 2004. Diatomaceous earth increases the efficacy of

Beauveria bassiana against *Tribolium castaneum* larvae and increases conidia attachment. *Journal of Economic Entomology*, 34, pp. 273–280. http://dx.doi.org/10.1093/jee/97.2.273

Aqueel, M.A. and Leather, S.R., 2013. Virulence of *Verticillium lecanii* (Z.) against cereal aphids; does timing of infection affect the performance of parasitoids and predators. *Pest Management Science*, 69, pp. 493–498. https://doi.org/10.1002/ps.3398

Drummond, R.O., Ernest, S.E., Trevino, J.L., Gladney, W.J. and Graham, O.H. 1973. *Boophilus annulatus* and *B. microplus*: Laboratory tests of insecticides. *Journal of Economic Entomology*, 66, pp. 130–133. https://doi.org/10.1093/jee/66.1.130

Fernandez-Salas, A., Alonso Dıaz, M.A., Alonso Morales, R.A., Lezama Gutierrez, R., Rodrıguez Rodrıguez, J.C. and Cervantes Chavez, J.A., 2017. Acaricidal activity of *Metarhizium anisopliae* isolated from paddocks in the Mexican tropics against two populations of the cattle tick *Rhipicephalus microplus*. *Medical and Veterinary Entomology*. 31, pp. 36–43. https://doi.org/10.1111/mve.12203

Finney, D.J., 1952. Probit analysis (2nd Ed). *Journal of the Institute of Actuaries*, 78, pp. 388–390. https://doi.org/10.1017/S0020268100052938

Guzman-Cornejo, C., Robbins, R.G., Guglielmone, A.A., Montiel-Parra, G. and Pérez, T.M., 2011. The *Amblyomma* (Acari: Ixodida: Ixodidae) of Mexico: Identification keys, distribution and hosts. *Zootaxa*, 2998, pp. 16–38. https://doi.org/10.11646/zootaxa.2998.1.2

Harizia, A. and Lazreg, F., 2016. Efficacy of the entomopathogenic fungus, *Paecilomyces fumosoroseus* (Wise) Brown and Smith (Ascomycota: Hypocreales) against *Geotrogus deserticola* (Coleoptera: Melolonthidae). *Journal of Entomological Research*, 40, pp. 223–228. 10.5958/0974-4576.2016.00041.4

Hasan, S., Ahmad, A., Purwar, A., Khan, N., Kundan, R. and Gupta, G., 2013. Production of extracellular enzymes in the entomopathogenic fungus *Verticillium lecanii*. *Bioinformation*, 9, pp. 238–242. http://dx.doi.org/10.6026/97320630009238

Kiriga, A.W., Haukeland, S., Kariuki, G.M., Coyne, D.L. and Beek, N.V., 2018. Effect of *Trichoderma* spp. and *Purpureocillium lilacinum* on *Meloidogyne javanica* in commercial pineapple production in Kenya. *Biological Control*, 119, pp. 27–32. https://doi.org/10.1016/j.biocontrol.2018.01.005

Maranga, R.O., Kaaya, G.P., Mueke, J.M. and Hassanali A., 2005. Effects of combining the fungi *Beauveria bassiana* and *Metarhizium anisopliae* on the mortality of the tick *Amblyomma variegatum* (Ixodidae) in relation to seasonal changes. *Mycopathologia*, 159, pp. 527–532. https://doi.org/10.1007/s11046-005-3374-y

Miraballes, C. and Riet-Correa, F., 2018. A review of the history of research and control of *Rhipicephalus* (*Boophilus*) *microplus*, babesiosis and anaplasmosis in Uruguay. *Experimental and Applied Acarology*, 75, pp. 383–398. https://doi.org/10.1007/s10493-018-0278-3

Ortiz-Urquiza, A. and Keyhani, N.O., 2013. Action on the surface: Entomopathogenic fungi *versus* the insect cuticle. *Insects*, 4, pp. 357–374. https://doi.org/10.3390/insects4030357

Pérez-González, V.H., Guzmán-Franco, A.W., Alatorre-Rosas, R., Hernández-López, J., Hernández-López, A., Carrillo-Benítez, M.G. and Baverstock, J., 2014. Specific diversity of the entomopathogenic fungi Beauveria and Metarhizium in Mexican agricultural soils. *Journal of Invertebrate Pathology*, 119, pp. 54–61. https://doi.org/10.1016/j.jip.2014.04.004

Perinotto, W.M., Angelo, I.C., Golo, P.S., Quinelato, S., Camargo, M.G., Sá, F.A. and Bittencourt, V.R., 2012. Susceptibility of different populations of ticks to entomopathogenic fungi. *Experimental Parasitology*, 130, pp. 257–260.

https://doi.org/10.1016/j.exppara.2011.12.003

Ren, Q., Chen, Z., Luo, J., Liu, G., Guan, G., Liu, Z. and Yang, J., 2016. Laboratory evaluation of *Beauveria bassiana* and *Metarhizium anisopliae* in the control of *Haemaphysalis qinghaiensis* in China. *Experimental and Applied Acarology*, 69, pp., 233–238. https://doi.org/10.1007/s10493-016-0033-6

Rodríguez-González, Á., Casquero, P.A., Suárez-Villanueva, V., Carro-Huerga, G., Álvarez-García, S., Mayo-Prieto, S. and Gutiérrez, S., 2018. Effect of trichodiene production by *Trichoderma harzianum* on *Acanthoscelides obtectus*. *Journal of Stored Products Research*, 77, pp. 231–239. https://doi.org/10.1016/j.jspr.2018.05.001

Rodriguez-Vivas, R.I., Jonsson, N.N. and Bhushan, C., 2018. Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance. *Parasitology Research*, 117, pp. 3–29. https://doi.org/10.1007/s00436-017-5677-6

Sanjaya, Y., 2016. Pathogenicity of three entomopathogenic fungi, *Metarhizium anisopliae*, *Beauveria bassiana*, and *Paecilomyces lilacinus*, to *Tetranychus kanzawai* infesting papaya seedlings. *Arthropods*, 5, p. 109.

Shang, Y., Duan, Z., Huang, W., Gao, Q. and Wang, C., 2012. Improving UV resistance and virulence of *Beauveria bassiana* by genetic engineering with an exogenous tyrosinase gene. *Journal of Invertebrate Pathology*, 109, pp. 105–109. https://doi.org/10.1016/j.jip.2011.10.004

de Souza Maia Filho, F., da Silva Fonseca, A.O., Persici, B.M., de Souza Silveira, J., Braga, C.Q., Pötter, L. and Pereira, D.I.B., 2017. *Trichoderma virens* as a biocontrol of *Toxocara canis*: *In vivo* evaluation. *Revista Iberoamericana de Micología*, 34, pp. 32–35. https://doi.org/10.1016/j.riam.2016.06.004

Szczepańska, A., Kiewra, D., Plewa-Tutaj, K., Dyczko, D. and Guz-Regner, K., 2020. Sensitivity of *Ixodes ricinus* (L., 1758) and *Dermacentor reticulatus* (Fabr., 1794) ticks to entomopathogenic fungi isolates: Preliminary study. *Parasitology Research*, pp. 1–5. https://doi.org/10.1007/s00436-020-06805-1

Ub, G.R. and Narladkar, B.W., 2018. Role of entomopathogenic fungi in tick control: A review. *Journal of Entomology and Zoology Studies*, 6, pp. 1265–1269.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-peña, A., Horak, I.G., Latif, A.A., 2003. *Ticks of Domestic Animals in Africa: A Guide to Identification of Species* [Internet]. 1st ed. Edinburgh Scotland, UK: Bioscience Reports.

Xiao, G., Ying, S.H., Zheng, P., Wang, Z.L., Zhang, S., Xie, X.Q., Shang, Y., St Leger, R.J., Zhao, G.P. and Wang, C., 2012. Genomic perspectives on the evolution of fungal entomopathogenicity in *Beauveria bassiana*. *Scientific Reports*, 2, p. 483. https://doi.org/10.1038/srep00483

Zahran, Z., Nor, N.M.I.M., Dieng, H., Satho, T. and Ab Majid, A.H., 2017. Laboratory efficacy of mycoparasitic fungi (*Aspergillus tubingensis* and *Trichoderma harzianum*) against tropical bed bugs (*Cimex hemipterus*) (Hemiptera: Cimicidae). *Asian Pacific Journal of Tropical Biomedicine*, 7, pp. 288–293. https://doi.org/10.1016/j.apjtb.2016.12.021

Zheng, P., Xia, Y.L., Xiao, G.H., Xiong, C.H., Hu, X., Zhang, S.W., Zheng, H.J., Huang, Y., Zhou, Y. and Wang, S.Y., 2011. Genome sequence of the insect pathogenic fungus *Cordyceps militaris*, a valued traditional Chinese medicine. *Genome Biology*, 12, pp. R116. https://doi.org/10.1186/gb-2011-12-11-r116