ORIGINAL RESEARCH PAPER

Veterinary Science

COMPARATIVE STUDY OF IN VITRO EFFICACY OF METARHIZUIM ANISOPLIAE AND BEAUVERIA BASSIANA AGAINST RHIPICEPHALUS MICROPLUS TICK INFESTATION IN CATTLE

KEY WORDS: Ticks, Biopesticide, Entomopathogenic, Fungi

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ABSTRACT

Entomopathogenic fungi (EPF) is one of the biopesticide agent which is widely used to manage agricultural and forest pests. *In vitro* efficacy of commercially available EPF *Metarhizuim anisopliae* and *Beauveria bassiana* was observed against *Rhipicephalus microplus* ticks collected from cattle in comparison with commercially available deltamethrin and control group treated with sterile distilled water. It was observed that the fungal products exhibited acaricidal activity against both unfed nymphs and mature engorged female ticks. At a concentration of n x 10^8 spores mL^{-1}, the significant 83.33 % mortality of female *Rhipicephalus microplus* ticks was noted 15 days after treatment with *Metarhizuim anisopliae* and *Beauveria bassiana*. The unfed nymphal stages of *Rhipicephalus microplus* ticks showed 100 percent mortality after treatment with both the EPF at the concentration of n x 10^8 spores mL^{-1} on 15 days after treatment. The significant reduction in Egg Mass Weight (EMW) and Egg Production Index (EPI) was reported in engorged female ticks after treatment with *Metarhizuim anisopliae* and *Beauveria bassiana*. Following treatment with *Metarhizuim anisopliae* and *Beauveria bassiana*., engorged female ticks showed a noticeably lower Egg Mass Weight (EMW) and Egg Production Index (EPI).

INTRODUCTION

Ticks are transient, obligatory, and external parasites that feed on blood in order to survive, and they infect birds, mammals, and reptiles. In particular, *Rhipicephalus microplus* (*R. microplus)* is the tick with the biggest economic impact because of its widespread distribution, ability to operate as a vector, blood sucking tendencies, and the quantity of cattle that negatively impact animal health and productivity. (Benavides and Romero, 2001; Roger *et al*., 2014; Domínguez *et al*. 2016; Eskezia and Desta, 2016;). The careless and hazardous use of synthetic acaricides for tick control leads to environment pollution and acaricidal resistance. (Abbas *et al*., 2014; Klafke *et al*., 2017). To defeat this danger, entomopathogenic fungi (EPF) are piquing the interest of biological control agents in the form of commercial pest management treatments. (Jaihan *et al*. 2016; Ríos-Moreno *et al*. 2016). The ability of EPF to have an insecticidal toxicity which infect and kill arthropods, making them useful for environmentally friendly and no reports of pest resistance yet been documented. EPF have high safety for mammals, birds, aquatic animals and plants and no effects on human health. (Kirkland *et al*., 2004; Zimmermann, 2007, Hadi *et al*. 2013, Jaihan *et al*. 2016; Ríos-Moreno *et al*. 2016; Lovett and Leger 2017). The present study was planned to investigate the biological potential of entomopathogenic fungi against *Rhipicephalus microplus* ticks infesting cattle and to investigate the possibility of including entomopathogenic fungi in integrated tick management programs.

MATERIALS AND METHODS

Commercially available ready to use fungal conidial suspensions of *Metarhizuim anisopliae* and *Beauveria bassiana* were procured from market. The products were marketed as biopesticide, used for controlling Root weevils, Plant hoppers, Japanese beetle, Stem borer, Black vine weevil, Spittlebug, and white grubs infecting crops with recommended dose rate of 5 ml/ liter of water in the spray form containing conidial concentration of 1 x 10^8 spores mL^{-1}.

The ticks were collected from villages of Kuhi taluka of Nagpur district of Maharashtra, India. According to the procedure outlined by Angelo *et al*. (2010), cuticle antisepsis was performed. Each adult female tick was weighed and ticks with homologous weight were selected for treatment.

The company recommended fungal concentration of 10^8 spores ml^{-1} was prepared by adjusting the volume of sterile distilled water. According to (FAO, 1984), adult immersion method (AIT) was applied to both engorged female ticks and unfed nymphs. The ticks were placed in a desiccator with 29 degrees Celsius and 75% relative humidity and examined up to 15 days for tick mortality by observing their pedal reflexes and oviposition or until the end of oviposition. (Quinelato *et al*., 2012). Same way the unfed nymph ticks were subjected to AIT and nymphal mortality was examined. The egg mass laid by each female tick was weighed, and the Egg Mass Weight (EMW) and Egg Production Index (EPI) were assessed. Eggs were incubated in a BOD incubator at 28°C and 85 ± 5% RH. and were observed for larval hatchability. (Bennett, 1974; Faranandes *et al*., 2010).

The reproductive potential of ticks was assessed by egg laying capacity of treated female ticks and egg hatchability of treated eggs which was recorded in accordance with a formula to determine the effectiveness of the entomopathogenic fungi (Goncalves *et al*. 2007). The efficacy of EPF was compared in relation to a control group treated with sterile distilled water and standard treatment with deltamethrin @ 25 ppm.

RESULTS AND DISCUSSION

After being treated with EPF *Metarhizium anisopliae* at concentrations of n x 10^8 spores mL^{-1}, the mean mortality percentage of engorged *R. microplus* female ticks and unfed nymphs was 66.66 and 100 percent respectively at 15 days after treatment (DAT). Following the treatment with *Beauveria bassiana* at concentrations of n x 10^8 spores mL^{-1}, the mortality in engorged *Rhipicephalus microplus* female ticks and nymphs was demonstrated as 83.33 and 100 percent efficacy respectively on 15 DAT. No mortality of adult female as well as nymphs of *Rhipicephalus microplus* ticks was observed at lower concentrations (n x 10^6 spores mL^{-1} and n x 10^6 spores mL^{-1} and n x 10^7 spores mL^{-1}) of both the EPF. The treatment of adult female as well as nymphs of *Rhipicephalus microplus* ticks with deltamethrin resulted in 100 percent mortality at 15 DAT whereas no mortality was recorded in control group treated with distilled water.

The significant reductions in Egg Mass Weight (EMW) and Egg Production Index (EPI) in engorged female ticks after treatment with *Metarhizuim anisopliae* and *Beauveria bassiana* at recommended doses was recorded . The reproductive index (RI) of female ticks treated with *Metarhizuim anisopliae* and *Beauveria bassiana* at n x 10^8 spores mL^{-1} concentration was 0.0832 and 0.0283 respectively showing reduced

reproductive potential however RI in the control group was higher (0.631) as compared to treated female ticks. The percent inhibition of oviposition of female ticks treated with *Metarhizuim anisopliae* and *Beauveria bassiana* was 86.84 and 95.51% respectively. There was no evidence of suppression of oviposition in the control group. By counting the number of eggs in the treatment group and watching the larvae hatch, the hatchability of the eggs laid by treated female ticks was visually evaluated.

The percent hatchability of eggs of female treated with *Metarhizuim anisopliae* and *Beauveria bassiana* was 45 and 40 percent respectively. When compared to the treatment group, the hatchability in the control group was 90%, indicating a substantial difference.

The strains of EPF *Beauveria bassiana* are pathogenic to all stages of *Rhipicephalus appendiculatus* ticks whereas *Metarhizium anisopliae* was found to be slightly pathogenic. About 74 percent of *R. appendiculatus* female ticks were failed to lay eggs after immersing in spore suspension of *B. bassiana* as reported by Kaaya *et al.* (1996). *Beauveria* strain suspensions tested in laboratory and under field conditions against *Rhipicephalus microplus* causes significant reduction in egg weight and reproductive efficiency. (Campos *et al.,* 2010 ; Ming *et al.,* 2013 and Cafarchia *et al.,* 2015). The analysis of *in-vitro* effects of 12 isolates of *Metarhizium anisopliae* on engorged *Boophilus microplus* showed adult mortality of 100% in ticks with treatment of 10^7 spores/ ml of *M. anisopliae*. *M. anisopliae* and *Beauveria Bassiana* against developmental stages of *Boophilus annulatus* ticks under laboratory conditions caused a mortality of 85-100% within 7-10 days post-inoculation and reduced egg laying capability of the ticks up to 92%. (Gindin *et al.,* 2009 and Fernandes *et al.,* 2010). *M. anisopliae* can represent a sustainable non-chemical alternative for tick control (Ojeda-chi *et al.*, 2010). The mortality rate of *Boophilus microplus* treated with fungus *M. anisopliae* and *B. bassiana* ranged from 92 to 100% and 44 to 100% at concentrations of 10^8 and 10^9 conidia mL^{-1} suspension respectively. (Broglio-Micheletti *et al.,* 2012).

Barbieri *et al*. (2023),reported the significant reduction in the *R. microplus* tick count on day 21, reaching approximately 55% efficacy after application of *Metarhizium anisopliae* oil formulations. In 2018, Rao and Narladkar discovered that the effects of the *Metarhizium anisopliae* fungus was concentration-dependent and included adult tick mortality, a decrease in egg laying capacity, and hatchability of the treated eggs of *R. microplus* compared to the control.

Kaaya and Hassan (2000) after using fungal oil formulations (10^8 conidia per ml) of *Beauveria bassiana* and *Metarizium anisopliae* observed induction of 100% mortality in *Rhipicephalus appendiculatus* larvae however the mortality rates in nymphs and adults ranged from 80–90%. The reports of various researchers suggested that entomopathogenic fungi can be used as biocontrol agent as an alternatives to currently available chemical acaricides for tick control.

CONCLUSIONS

It is concluded that *Metarhizuim anisopliae* and *Beauveria bassiana* can be an effective biocontrol agent for control of ticks. Further studies on field application of EPF are needed.

REFERENCES:

[1] Abbas R.Z., Zaman M.A., Colwell D.D., Gilleard J, Iqbal Z. (2014). "Acaricide resistance in cattle ticks and approaches to its management: the state of play". Vet Parasitol. 203(1-2):6-20.

[2] Angelo, I.C., Everton, K.K., Fernandes, Thiago C. Bahiense, Wendell M.S., Perinotto, Ana Paula R. Moraes, Andréia L.M. Terra and Vânia Rita Elias Pinheiro Bittencourt. (2010). "Efficiency of *Lecanicillium lecanii* to control the tick *Rhipicephalus microplus*." Journal of Veterinary Parasitology. 172(3–4):317-322.

[3] Barbieri A, Rico I B, Silveira C , Feltrin C , Dall Agnol B , Schrank A , Lozina L , Klafke G M , Reck J. (2023). "Field efficacy of *Metarhizium anisopliae* oil formulations against *Rhipicephalus microplus* ticks using a cattle spray race". Ticks Tick Borne Dis. 14(3):102147

[4] Benavides E, Romero N. Consideraciones para el. (2001). "Control integral de parásitos externos del ganado. Revista Carta Fedegán." : 70:64-86

[5] Bennett G F. (1974). "Oviposition of *Boophilus microplus* (Canestrini) (Acarida: Ixodidae). Influence of tick size on egg production". *Acarologia*. 16(1):52-61.

[6] Broglio-Micheletti Sônia M. F. , Leilianne A. de Souza, Ellen C. N. Valente, Mário J. C. de Araújo, Nivia da Silva Dias and Mariuxi Lorena Gómez-Torres. (2012). "Evaluation of entomopathogenic fungi as biological control agents *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae)". IDESIA (Chile) 30(1): 93-99.

[7] Cafarchia, C., Davide, .I, Roberta, .I, Rafael, ANR, Riccardo, .P.L. and Daniele, P. (2015). "Native strains of *Beauveria bassiana* for the control of *Rhipicephalus sanguineus sensulato"*. Parasites and Vectors; 8 (1):80.

[8] Campos R.A., Boldo J.T. Pimentel I.C., Dalfovo V., Araujo W.L., Azevedo J.L., Vainstein M.H. and Barros N.M .(2010). "Endophytic and entomopathogenic strains of *Beauveria sp*. to control the bovine tick *Rhipicephalus (Boophilus) microplus"*, Genetics and Molecular Research. 9 (3): 1421-1430.

[9] Domínguez D, Torres F, Rosario-Cruz R. (2016). "Evaluación económica del control de garrapatas *Rhipicephalus microplus* en México". Revista Iberoamericana de las Ciencias Biológicas Agropecuarias:5(9)

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

[11] FAO (1984). "Ticks and Ticks Borne Disease Control". A Practical Field Manual. Vol. 1. Rome.

[12] Fernandes, E.K.K., Keyser, C.A., Chong, J.P., Rangel, D.E.N., Miller, M. P. and Roberts, D.W. (2010). "Characterization of *Metarhizium species* and varieties based on molecular analysis, heat tolerance and cold activity". Journal of Applied Microbiology. 108: 115–128.

[13] Gindin, G., Dana, M., Asael, R., Itamer, G. and Michael, S. (2009). "Pathogenicity of *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) to tick Eggs and the effect egg Cuticular Lipids on Conidia". Journal of Medical Entomology. 46(3):531-538.

[14] Goncalves K, Toigo E, Ascoli B, Von Poser G, Ribeiro VS (2007). "Effects of solvents and surfactant agents on the female and larvae of cattle tick *Boophilus microplus"*. Parasitol Res. 100:1267–1270

[15] Hadi M.S., Himawan T., Aini L.Q. (2013). "The effectiveness of entomopathogenic fungi *Beauveria bassiana* with the addition of insect growth regulator lufenuron for controlling *Bactrocera carambolae"*. Journal of Tropical Life Science. 3(3):187–192

[16] Jaihan P, Sangdee K, Sangdee A. (2016). "Selection of entomopathogenic fungus for biological control of chili anthracnose disease caused by *Colletotrichum* spp". European Journal of Plant Pathology. 146:551–564.

[17] Kaaya Godwin P. and Hassan Shawgi. 2000. Entomogenous Fungi as Promising Biopesticides for Tick Control. *Experimental and applied acarology.* 24:913–926.

[18] Kaaya, G.P., Esther, N. Mwangi and Elizabeth, A. Ouna. (1996). " Prospects for Biological Control of Livestock Ticks, *Rhipicephalus appendiculatus* and *Amblyomma variegatum* using the Entomogenous Fungi *Beauveria bassiana* and *Metarhizium anisopliae"*. Journal of Invertebrate Pathology. 67(1):15-20.

[19] Kirkland, B. H., Cho, E. M., and Keyhani, O. N. (2004). "Differential susceptibility of *Amblyomma maculatum* and *Amblyomma americanum* (Acari: Ixodidea) to the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*. *Biological Control"*. 31, 414–421.

[20] Klafke G, Webster A, Dall Agnol B, Pradel E, Silva J, de La Canal LH. (2017). "Multiple resistance to acaricides in field populations of *Rhipicephalus microplus* from Rio Grande do Sul state, Southern Brazil." Ticks Tick Borne Dis ; 8(1):73-80

[21] Lovett B, Leger RJS (2017)."The insect pathogens". Microbiol Spectr. https://doi.org/10.1128/microbiolspec.funk-0001-2016

[22] Ming Sun, Qiaoyun Ren, Guiquan Guan, Yufeng Li, Xueqing Han, Chao Ma, Hong Yin and Jianxun Luo. (2013). "Effectiveness of *Beauveria bassiana sensu lato* strains for biological control against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in China". Parasitology international. 62 (.5) : 412-415

[23] Ojeda-Chi M.M., R.I. Rodriguez- Vivas, E. Galindo-Velasco and R. Lezama-Gutierrrez 92010). "Laboratory and field evaluation of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) for the control of *Rhipicephalus microplus* (Acari: Ixodidae) in the Mexican tropic". Journal of Veterinary Parasitology. 170(3-4):02.022.

[24] Quinelato Simone , Golo Patrícia S. , Perinotto Wendell M.S. , Fillipe A. Sá , Camargo Mariana G. , Angelo Isabele C. , Moraes Aurea M.L. , Bittencourt Vânia R.E.(2012). "Virulence potential of *Metarhizium anisopliae* s.l. isolates on *Rhipicephalus* (*Boophilus*) *microplus* larvae". Vererinary Parasitology: 556-565.

[25] Rao Greeshma U. B. Narladkar Babasaheb Wamanrao. (2018). "*In-vitro* evaluation of entomopathogenic fungus *Metarhizium anisopliae* against the cattle tick *Rhipicephalus (B.) microplus* (Acarina: Ixodidiae)". International Journal of Livestock Research. 8(7): 167-175

[26] Ríos-Moreno A, Garrido-Jurado I, Resquín-Romero G (2016). "Destruxin A production by *Metarhizium brunneum* strains during transient endophytic colonisation of Solanum tuberosum". Biocontrol Sci Technol 26:1574–1585.

[27] Roger Iván Rodríguez-Vivas, José Alberto Rosado-Aguilar, Melina Maribel Ojeda-Chi, Luis Carlos Pérez-Cogollo and Iris Trinidad-Martínez. (2014). "Integrated control of ticks in bovine". Livestock Manuel Emilio Bolio-González. 1 (3):295-308.

[28] Zimmermann Gisbert (2007). "Review on safety of the entomopathogenic fungus *Metarhizium anisopliae"*. Biocontrol Science and Technology. 17(9): 879-920.