



REVIEW ARTICLE

***Metarhizium anisopliae*: current status and future in hard ticks control in Asia**Azmi, N.F.A.M.¹, Yee, L.C.¹, Choong, S.S.¹, Peng, T.L.^{1,2*}, Syazwan, S.A.^{3,4}¹Faculty of Veterinary Medicine, Universiti Malaysia Kelantan City Campus, Pengkalan Chepa, 16100, Kota Bharu, Kelantan, Malaysia²Natural Products and Veterinary Ethno-medicine, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan City Campus, Pengkalan Chepa, 16100, Kota Bharu, Kelantan, Malaysia³Mycology and Pathology Branch, Forest Health and Conservation Programme, Forest Biodiversity Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia⁴Department of Forest Science and Biodiversity, Faculty of Forestry and Environment, 43400 Serdang, Selangor, Malaysia

*Corresponding author: li.peng@umk.edu.my

ARTICLE HISTORY

Received: 20 June 2024

Revised: 18 August 2024

Accepted: 19 August 2024

Published: 31 December 2024

ABSTRACT

Ticks exert a significant economic impact on the livestock industry, particularly in Asian regions. Presently, chemical acaricides constitute the primary method employed to combat tick infestations in livestock, but their use carries adverse environmental consequences. Overreliance on acaricides has contaminated milk and meat products with chemical residues while fostering tick resistance to these agents due to improper and intensive application. Various alternative methods have been explored, including using vaccines to manage tick populations. However, the efficacy of these treatments varies and is often limited when applied separately. Among these alternatives, entomopathogenic fungi like *Metarhizium anisopliae* appear to be a promising candidate for tick population control. This fungus can be used independently and in conjunction with other products. This review article explores the current and future prospects of *M. anisopliae*, where the existing gaps and future directives for using this fungus to control hard ticks in Asian countries are highlighted.

Keywords: Acaricide resistance; biocontrol; Ixodidae; livestock; entomopathogenic fungi.

INTRODUCTION

Asia is the largest continent globally, with tropical, subtropical, and temperate climates that host extensive species, contributing to a high degree of biodiversity. The agricultural sector plays a crucial role in the economic development of South and Southeast Asian countries, providing employment and reducing poverty within the community (Liu *et al.*, 2020). The exponential economic growth and urbanization of Asian countries over the last two decades have significantly increased the demand for cattle and buffalo products, including beef and milk (OECD-FAO, 2018). Asia has emerged as a major global contributor to cattle and buffalo production, representing approximately 39% of the global stock (Roche *et al.*, 2020). India leads the region in cattle and buffalo inventory, followed by China, Pakistan, Bangladesh, Myanmar, Indonesia, Nepal, Vietnam, Thailand, and the Philippines (FAO, 2022a). However, the persistent challenge of ticks and tick-borne diseases poses a constant threat to the economic stability of the livestock industry in Asian countries.

Ticks exert a tremendous financial impact on the livestock industry, particularly in Asian countries with subtropical and tropical regions (Iqbal *et al.*, 2022). *Rhipicephalus* ticks, particularly *Rhipicephalus (Boophilus) microplus* and *R. (B.) annulatus*, are major vectors transmitting cattle diseases in the livestock industry, resulting in significant economic losses (Hosseini-Chegeni *et al.*, 2019). Mortality, treatment costs, and acaricide applications contribute most to the economic losses associated with ticks and tick-borne

diseases, while milk and weight loss contribute to the financial burden to a lesser extent (Kivaria, 2006). A study by Ashfaq *et al.* (2015) assessed the impact of tick infestation by evaluating the value of milk loss, the cost of veterinary treatment, and the value of weight loss in affected animals, revealing that tick infestation is a primary factor causing financial loss among dairy cattle farmers. In India, cumulative losses of USD 595.07 million were attributed to milk loss, treatment costs, and leather loss due to tick infestation, with an additional total loss of USD 191.15 million due to tick-borne diseases (Singh *et al.*, 2022; Khan *et al.*, 2022).

Ectoparasite control is traditionally achieved by using chemical acaricides such as pyrethroids and organophosphates (Cella *et al.*, 2023). Asia has utilized large quantities of pesticides, averaging 0.65 million tons per year over the past three decades, with China and India recording the highest pesticide consumption (FAO, 2022b). There is a large-scale establishment of multi-acaricides resistant tick populations in tropical and subtropical regions been reported (Shanmuganath *et al.*, 2021). Studies on alternative approaches encompass biological control, such as the use of botanical extracts (Ajith Kumar *et al.*, 2016; Bravo-Ramos *et al.*, 2021), entomopathogenic nematode (de Mendonna *et al.*, 2019; Singh *et al.*, 2018), tick symbiont bacteria (Khoo *et al.*, 2016), and combination of entomopathogenic nematode with plant extract (Monteiro *et al.*, 2021) can play pivotal roles in achieving a comprehensive and sustainable management approach besides mitigating the selective pressures exerted on tick populations through chemical control.

Biological control of ticks using entomopathogenic fungi (EPF) such as *Metarhizium anisopliae* (MA), *Beauveria bassiana*, *Aspergillus oryzae*, and *Lecanicillium lecanii* has been evaluated (Angelo et al., 2010; Meirelles et al., 2023; Msangi et al., 2022; Sullivan et al., 2022). Among the EPFs, MA and *B. bassiana* are the most studied species, with several commercially available formulations developed (Weeks et al., 2020). A study conducted by Mesquita et al. (2023) reported that MA can affect the gut bacterial diversity of engorged female ticks and demonstrated that the mycoacaricidal efficacy of MA would not be affected if the host is under antibiotic therapy, suggesting MA as a promising biological acaricidal agent. Therefore, this review paper intends to draw attention to MA for its current status, challenges and future advancement towards hard tick control in Asian countries by employing the online database Clarivate™ and SCOPUS as the primary search engine.

***Metarhizium anisopliae*: Current Status of Livestock Tick Control in Asia**

Extensive research has been conducted globally to explore the pathogenicity of *Metarhizium* spp. as a biological control agent against ticks, with notable contributions from countries across various continents, such as Brazil and the USA, dating back to 1997 (Correia et al., 1998; Zhioua et al., 1997). In Asia, the inception of investigations into utilizing *Metarhizium* spp. for tick control was documented in 2001 in Israel (Gindin et al., 2001). Israel emerged

as a prominent contributor to research in this field in Asia during the early 20th century.

A comprehensive collection of 298 records on the biological control of ticks using *Metarhizium* spp. from around the world, spanning the years 1997 to 2023, was available in the online database Clarivate™ and SCOPUS. Among the 298 records, 142 articles were documented in the past 10 years. The primary *Metarhizium* spp. being studied previously include *M. anisopliae*, as evidenced by numerous recent publications (de Almeida et al., 2022; Barbieri et al., 2023; da Paixto et al., 2023; Guimapi et al., 2023; Lee et al., 2023; Marzouk & Ali, 2023; Meirelles et al., 2023; Saciloto-de-Oliveira et al., 2023), as well as *M. brunneum* (Ment et al., 2010; Sullivan et al., 2020; Sullivan et al., 2021; Sullivan et al., 2022), *M. robertsii* (Goldsmith et al., 2021; Marciano et al., 2021; Fiorotti et al., 2022), and *M. pempighi* (Lorenz et al., 2020).

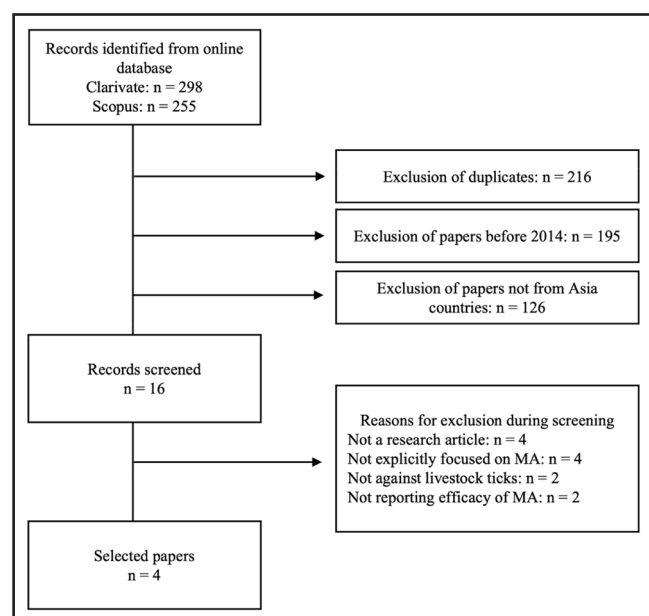
Despite Asia being the largest continent and hosting a substantial livestock industry, the records from the past 10 years indicate a disproportionately low representation. Out of the 142 global records, there were only 16 entries reporting the utilization of *Metarhizium* spp. as a biological control against ticks in Asia (Figure 1). Of these 16 entries, a mere four articles specifically addressed the efficacy of *M. anisopliae* against livestock ticks (Table 1). Criteria for the paper to be included for review were published within 10 years from 2023, a research article, reporting the efficacy of *M. anisopliae* against livestock ticks. The screening and selection process is described in Figure 2.



Figure 1. Records of *Metarhizium* spp. against livestock ticks in Asia. (South Korea 5, Israel 3, China 3, Iran 2, Malaysia 2, Turkey 2, India 1).

Table 1. Current research of *Metarhizium anisopliae* as a biological control agent against livestock ticks in Asia

| Country | Tick Species | Host | Experiment | Conc. Tested | Formulation | Result | Reference |
|---------|--|----------------------|-----------------|---------------------------------------|---|---|-------------------|
| China | <i>Haemaphysalis qinghaiensis</i> (nymph) | Sheep | Laboratory | 1x10 ⁵ – 1x10 ⁹ | Conidia suspensions mixed with 0.05% Tween 80 | 62% – 100% mortality | Ren et al., 2016 |
| | <i>Haemaphysalis qinghaiensis</i> (engorged female) | | | 1x10 ⁶ – 1x10 ⁸ | | 73% – 100% mortality | Ren et al., 2014 |
| Korea | <i>Haemaphysalis longicornis</i> (nymph) | Collected from grass | Laboratory | 1x10 ⁶ – 1x10 ⁸ | Conidia suspensions added with 0.03% siloxane | 10% – 70% | Lee et al., 2019 |
| | | | Semi-field test | 1x10 ⁸ | | 75% – 90% (30 days) | |
| Turkey | <i>Hyalomma anatolicum excavatum</i> (nymph & engorged female) | Cattle | Laboratory | 1x10 ⁶ – 1x10 ⁸ | 0.03% aqueous Tween 80 | 55% – 100% mortality (nymph) Not susceptible toward MA (Engorged female) | Butt et al., 2016 |

**Figure 2.** The screening and selection process of articles for current research of *Metarhizium anisopliae* as a biological control agent against livestock ticks in Asia.

The previous studies on the efficacy of MA against livestock ticks, as delineated in four specific articles (refer to Table 1), spans three distinct countries – Korea, Turkey, and China. China contributed to two studies conducted by the same researcher in 2014 and 2016, with a focus on assessing the efficacy of MA against engorged female and nymph stages of *Haemaphysalis qinghaiensis*. The Chinese studies (Ren et al., 2014; Ren et al., 2016) specifically evaluated the impact of MA on engorged female and nymph stages of *H. qinghaiensis*, demonstrating mortality rates ranging from 50% to 100%. In 2016, a Turkish publication reported on the efficacy of MA against *Hyalomma anatolicum excavatum* nymphs, showcasing mortality rates ranging from 73.3% to 100%. The most recent Korean study focused on *Haemaphysalis longicornis* in both laboratory and simulated field trial conditions, with mortality rates exceeding

50%. Notably, nymphs under semi-field conditions exhibited lower susceptibility to MA, achieving maximum mortality of 90% after 30 days.

It is noteworthy that, to date, there are no documented instances in the literature of field trials or large-scale implementation of MA against livestock ticks in Asia. Meanwhile, on a global scale, initiatives have been undertaken to initiate investigations into the efficacy of MA through field trials, as documented by Webster et al. (2017) and Barbieri et al. (2023). The findings from these studies reveal that the application of MA can eradicate more than 50% of the targeted tick populations. While extant literature indicates the potential of MA to induce tick mortality, particularly in controlled environments (Fernandes et al., 2012; Fernández-Salas et al., 2016; Alonso-Díaz & Fernández-Salas, 2021); its adoption as an alternative control method in Asia remains comparatively limited, lagging behind other countries as indicated in the literature search in Figure 2. The promising early findings highlight the need for additional research and exploration to fully realize the promise of MA for tick management in Asian regions. This underscores the significance of conducting further scientific research and developing novel techniques to maximize the benefits of MA. While current research indicates MA's potential, much more remains to be learned regarding its uses and efficacy. Thorough research is required to determine how MA can effectively meet the specific tick control challenges unique to Asia.

Chemical Control: An Overview of Acaricides Resistance in Asia

Acaricides, chemical agents specifically formulated for the management of ticks and mites, have constituted a pivotal component in pest control strategies. Despite the efficacy demonstrated by certain acaricides in rapidly mitigating tick populations, the protracted utilization of a singular acaricide has engendered a surge in tick resistance (Beys-da-Silva et al., 2020; Githaka et al., 2022; Barbieri et al., 2023). This phenomenon of acaricide resistance is not a recent development, as historical records indicate its recognition as early as 1937, with resistance surfacing after more than four decades of Arsenic employment (Newton, 1967). Acaricide resistance extends beyond the mere inability to eradicate ticks, encompassing a multifaceted challenge involving the proliferation of tick populations into non-endemic regions (Yawa et al., 2022).

Over the preceding half-decade, a limited number of countries, specifically four globally apart from Asia, have undertaken investigations into acaricide resistance in livestock, yielding significant insights from the United States, Brazil, South Africa, and Nigeria. Research originating from the United States has divulged disparate mortality rates (ranging from 0% to 59%) in engorged female *R. microplus* when exposed to pyrethroids, as documented by Klafke et al. (2019). In stark contrast, there is a heightened susceptibility (88% to 100% mortality) observed in response to a pyrethroid-organophosphate combination, and an increased mortality trend in *R. microplus* larvae upon exposure to ivermectin, as elucidated by Villar et al. (2019).

Comparable findings have been documented in Brazil, demonstrating elevated resistance levels in engorged female *R. microplus* to pyrethroids (cypermethrin), with mortality rates recorded at 26.2%. Additionally, substantial resistance in *R. microplus* larvae to pyrethroids has also been observed, as highlighted in the same research conducted by Higa et al. (2020). Recent South African research findings, as reported by Yawa et al. (2022), indicate the emergence of resistance in *R. decoloratus* larvae to amidines, organophosphates, and pyrethroids. A parallel study in Nigeria, conducted by Akande et al. (2020), underscores low resistance in engorged female *R. microplus* to formamidine, with emerging resistance patterns observed against pyrazoles, macrocyclic lactones, and pyrethroids.

Within the Asian context, a noticeable gap or missing information when it comes to discussing acaricide resistance in livestock in Asia. Recognizing the urgent need to fill this information gap, the subsequent table aims to furnish a comprehensive elucidation of the prevailing status of acaricide resistance within tick populations across the Asian region. This effort aims to assess the current status of acaricide resistance in livestock ticks across

Asia. By providing comprehensive data on the resistance levels, we hope to gain clearer insights into the extent and severity of acaricide resistance. This knowledge is crucial for devising effective strategies to manage and mitigate acaricide resistance, thereby enhancing livestock health and productivity in Asia.

In this section, keywords: (chemical OR chemicals OR acaricide OR acaricides) AND (resistant OR resistance) AND (tick OR ticks) AND (livestock) were used for the literatures search. Initially, the search yielded 238 results, but this number was narrowed down to 173 after excluding papers published before 2013. Of these, 119 research papers were selected. Among these, only 13 Asian countries with papers that related to the subject, with 33 studies matching our keyword criteria. Comprehensive studies on acaricide resistance in ticks have been conducted primarily in two Asian countries – India and Pakistan (Table 2). The research focused on two predominant tick species: *R. microplus* and *H. anatolicum*. The findings revealed that *R. microplus* exhibited a higher resistance level, particularly demonstrating a level 4 resistance to Deltamethrin (Godara et al., 2019), whereas the majority of others ranged from susceptible to level 2. *Hyalomma anatolicum*, a significant etiologic agent of tropical theileriosis (Jongejan & Uilenberg, 2004), displayed resistance levels falling between susceptible and level 1. A notable trend emerged, indicating an increasing resistance of ticks in these two Asian countries to various chemical classes. The primary classes affected include pyrethroids (Deltamethrin and Cypermethrin) (Shyma et al., 2013; Singh et al., 2015; Godara et al., 2019; Solanki et al., 2020; Sagar et al., 2020; Sindhu et al., 2022), followed by pyrazoles (Fipronil) (Gupta et al., 2020; Kamran et al., 2020), organophosphorus (Coumaphos and Diazinon) (Shyma et al., 2013; Sagar et al., 2020), and, lastly, macrocyclic lactones (Ivermectin) (Sagar et al., 2020; Kamran et al., 2020).

Table 2. Current acaricides resistance status of ticks in Asia

| Country | Tick | Test | Acaricide | Resistance Factor | Resistance Level* | Reference | | |
|----------------------|----------------------|----------|--------------|-------------------|-------------------|----------------------|--------------------|---------------------|
| India | <i>H. anatolicum</i> | LPT | Cypermethrin | 0.18 – 2.25 | S – 1 | Shyma et al., 2013 | | |
| | | | Deltamethrin | 1.05 – 4.65 | | | | |
| | | | Diazinon | 1.82 – 4.46 | | | | |
| | <i>R. microplus</i> | | Cypermethrin | 0.98 – 3.90 | S – 2 | | | |
| | | | Deltamethrin | 4.50 – 10.80 | | | | |
| | | | Diazinon | 4.20 – 12.00 | | | | |
| | <i>H. anatolicum</i> | LPT | Amitraz | 12.23 | 1 | | Singh et al., 2015 | |
| | | | Cypermethrin | 3.51 | 2 | | | |
| | <i>R. microplus</i> | AIT | | Cypermethrin | 0.32 – 13.18 | | S – 2 | Godara et al., 2019 |
| | | | | Deltamethrin | 0.94 – 51.71 | | S – 4 | |
| <i>H. anatolicum</i> | LPT | | Fipronil | 0.23 – 3.80 | S – 1 | Gupta et al., 2020 | | |
| | | | | 2.40 – 28.20 | 1 – 3 | | | |
| <i>R. microplus</i> | AIT | | Cypermethrin | 0.77 – 18.66 | S – 2 | Solanki et al., 2020 | | |
| | | | | Deltamethrin | 0.73 – 22.59 | | S – 2 | |
| <i>R. microplus</i> | AIT | | Coumaphos | 8.52 – 13.20 | 2 | Sagar et al., 2020 | | |
| | | | | Cypermethrin | 7.05 – 13.20 | | 2 | |
| | | | | Deltamethrin | 3.40 – 24.02 | | 1 – 2 | |
| | | | | Diazinon | 1.60 – 3.81 | | 2 | |
| | | | | Ivermectin | 0.91 – 9.88 | | S – 2 | |
| Pakistan | <i>H. anatolicum</i> | AIT | Fipronil | 11.12 | 2 | Kamran et al., 2020 | | |
| | | | Ivermectin | 9.97 | | | | |
| | LIT | Fipronil | 13.27 | 2 | | | | |
| | | | Ivermectin | 7.78 | | | | |
| <i>R. microplus</i> | LPT | | Cypermethrin | 0.80 – 17.46 | S – 2 | Sindhu et al., 2022 | | |

*Resistance level based on resistance factor (RF). Susceptible: RF ≤ 1.4; Level 1: 1.5 – 5; Level 2: 5.1 – 25; Level 3: 25.1 – 40; Level 4: RF > 40.1.

Challenges and Advancements of *Metarhizium anisopliae* as an Alternative Ticks Control

The relative slow killing speed and inconsistent performance of the entomopathogenic fungus as microbial biocontrol agents are seemingly not something new since the limitations were stated few decades ago in a review paper by Moore & Prior (1993). Many factors can be contributed to the limitations and challenges of adopting the entomopathogenic fungus to control ticks. It is notable that the inconsistency performance is linked directly to the short shelf-life of this living organism (Sarma et al., 2023). The shelf life of entomopathogenic fungus is an important feature to consider because it increases the microorganism's commercial feasibility (Meirelles et al., 2023). It became one of the main obstacles to getting this biological control on the market since under storage, the viability is decreasing (Meirelles et al., 2023). Moore & Prior (1993) have stated that the estimated range for MA viability was only range from 3 to 18 months and this has been supported with a study from Sarma et al. (2023) that MA could be viable until 180 days but it started to decrease the virulence from day 20 until day 180.

In addition, the diverse strains of MA display variations in their virulence, efficacy, and adaptability to different insect hosts (Hussien et al., 2021). Selecting the most suitable strain for mass production and maintaining consistent quality and effectiveness of the produced inoculum can be challenging. It requires careful strain selection, monitoring of genetic stability, and maintaining a repository of well-characterized and high-quality strains. Overcoming these challenges is critical for optimizing the use of MA as an acaricide in the field, requiring a meticulous focus on refining the conidial production process and selecting suitable adjuvants for formulations, ensuring product stability, cost-effectiveness, and efficacy (Barbieri et al., 2023). The impediment posed by the high cost of the substrate in MA mass production technology can potentially be mitigated by adopting the alternative approach suggested by Barra-Bucarei et al. (2016), advocating for the use of recycled rice substrate from MA production instead of relying on new parboiled rice.

Drawing insights from the information presented in Table 1, a conspicuous gap in research becomes evident, particularly in the absence of *in vivo* studies in Asia that examine the efficacy of MA under field conditions. This research void aligns with observations by Chen et al. (2014), who proposed that MA exhibits diminished virulence in field environments compared to laboratory settings due to soil conditions influencing its pathogenicity. The microclimatic sensitivity of this fungus is underscored by its susceptibility to various environmental factors, including soil moisture, air temperature, soil temperatures, air relative humidity, and solar UV radiation (Chen et al., 2014; Barbieri et al., 2023). Many pest insects dwell in geographic regions with considerable temperature changes; thus, effective biological control agents must not only endure extreme temperatures in these areas but also resume growth during periods of favourable conditions (Keyser et al., 2014). To advance MA as a biopesticide, comprehensive field efficacy data is the key, serving as the foundational information for further research and development.

In general, advancements in MA predominantly centre on mass production and formulation, key prerequisites for commercialization and widespread adoption in control programmes. The primary method for large-scale production is solid-state fermentation (SSF), leveraging various grains like rice, wheat, chickpea, pigeon pea, black gram, green gram, groundnut, sorghum, soybean, and maize (Agale et al., 2018). SSF is recognized for its efficiency, with different substrates yielding varying conidia counts. Green mass and maize demonstrated the highest conidia counts, while green gram and black gram produced comparatively lower spore yields (Agale et al., 2018). Additionally, liquid culture in bioreactors emerges as an alternative method for mass production, offering advantages in scalability, process control, and consistent product quality. Liquid fermentation, as highlighted by Barra-Bucarei et al. (2016), facilitates the generation of blastospores and mycelium forms (Riaz et al.,

2013; Kruger et al., 2014). Different media play a pivotal role in this process, with Sabouraud Dextrose Broth exhibiting the highest conidial count, closely followed by Potato Dextrose Broth (Agale et al., 2018). The liquid culture approach not only ensures efficiency and precision but also underscores *M. anisopliae*'s adaptability to different fermentation methods, enhancing the versatility of large-scale cultivation practices.

In terms of formulation, in the early stage, the formulations were relatively basic by mixing the conidia with carrier materials like talc or clay. The recent advancements in formulation techniques have been pivotal in enhancing the pathogenicity of this fungus. Conidia formulations emerge as a promising biological tool in effectively managing ticks. Achieving practical formulations necessitates comprehensively considering factors such as the duration of conidia viability, sustained virulence, and the overall efficacy of entomopathogenic fungi when deployed in field conditions. The creation of an appropriate formulation is critical to the successful use of mycoinsecticides. For commercialization to occur, the formulation's biological and physical qualities must be stable for at least a year, preferably for more than 18 months (Daoust & Roberts, 1983; Alvest et al., 2002). Crucially, successful formulations must incorporate ingredients that facilitate conidia adherence to the tick surface, ensuring targeted action, alongside elements that protect against adverse environmental conditions. Table 3 summarises 34 papers on some of the formulations of *Metarhizium* that have been tested and used globally. The references used were based on the literature search using the keywords: (metarhizium) AND (biological AND control) AND (formulation OR formulations) with 277 research papers being screened. The search was not restricted by year and arthropod hosts, ensuring a broad scope of inclusion. This approach allows for a comprehensive analysis of the development of formulations over time, providing insights into trends and advancements in formulation improvements across various arthropod hosts and different regions from year to year.

Advancements in *Metarhizium* formulation have been ongoing for three decades, aiming to optimize the practicality of this fungus. Among various types of formulations, oil formulations have gained popularity, as highlighted by data from Table 3. According to Kaaya & Hedimbi (2012), oil formulations outperform water-based ones due to their superior blending with the lipophilic cuticle of ticks. Additionally, oils provide a longer-lasting moisture source for fungus germination compared to water, which evaporates rapidly. Rice et al. (2020) further support this idea, suggesting that oil-based emulsions offer advantages over purely aqueous solutions, including enhanced adhesion of conidia to lipophilic insect cuticles and increased persistence of conidia. The choice of oil is crucial, as certain oils can inhibit spore germination, affecting effectiveness, while others may not be compatible with conidia formulations (Seye et al., 2012). Hence, selecting the appropriate oil is essential for maximizing the effectiveness of the fungus in mycoinsecticide formulations.

Future Direction and Conclusion

Given recent advancements, the future of *Metarhizium anisopliae* (MA) is promising. Urgent attention is needed to improve MA's pest control efficacy, which is slower than chemical treatments. Enhancing its effectiveness could make it a competitive biopesticide and a sustainable alternative. Understanding how MA interacts with other species will help develop formulations that target pests while minimizing harm to beneficial organisms. For successful incorporation into agriculture, advancements in formulation research, commercial production, regulatory approval, and public awareness are essential. Improving stability, shelf life, and application efficiency is critical to making MA useful and affordable. Streamlining production processes and regulatory assessments, along with educating farmers about acaricide resistance and MA strategies, can reduce reliance on chemical acaricides and promote sustainable alternatives.

Table 3. Different formulations of *Metarhizium* as mycoinsecticides

| Formulations | Improvements | Ingredients | References |
|--|--|--|---------------------------|
| Alginate formulations | Protect fungi from solar radiation and high-temperature | Sodium alginate + calcium chloride | Pereira & Robert, 1991 |
| Oil formulations | Protect fungi from solar radiation and high-temperature | Cornstarch oil | Pereira & Robert, 1991 |
| | Increase persistence of oil and water formulation of conidia | ShellSol T | Inyang et al., 2000 |
| | Increase virulence | Coconut/soybean oil | Batta, 2003 |
| | Increase virulence | Neem oil | Okumu et al., 2007 |
| | Increase virulence | Canola oil | Lemon et al., 2008 |
| | Increase virulence | Sunflower oil | Brito et al., 2008 |
| | Increase virulence and persistence | Neem oil | Paula et al., 2019 |
| | Enhance ovicidal activity | Sunflower oil | Albernaz et al., 2009 |
| | Increase virulence and persistence | ShellSol T | Bukhari et al., 2011 |
| | | Ondina oil 917 | |
| | Suppress on-host and off host tick population | Sunflower oil | Kaaya & Hedimbi, 2012 |
| | Increase virulence | Mineral oils | Camargo et al., 2012 |
| | Increase virulence | Neem oil | Seye et al., 2012 |
| | Increase virulence | Mineral oil | Rodrigues et al., 2019 |
| | Increase virulence and persistence | Oil in water | Muniz et al., 2020 |
| Increase virulence | Mineral oil + diatomaceous earth Silicon oil | Barbieri et al., 2023 | |
| Dust formulations | Increase virulence and persistence | Talcum | Gupta et al., 2020 |
| | Increase virulence and persistence | Diatomaceous earth | Michalaki et al., 2006 |
| | Increase virulence | Bad wheat flour | Sharriford et al., 2014 |
| Dried-powdered conidia | Better protection Increase virulence | Kaolin | Hendrawan & Ibrahim, 2006 |
| | | Tapioca flour | |
| | | Talc | |
| Fungus mixed with acaricide formulations | Increase virulence | Imidacloprid | Brito et al., 2008 |
| | Increase virulence | Amitraz Deltamethrin Pyrethroids Organophosphate Monoterpenoid | Carneiro et al., 2022 |
| Aqueous formulations | Suppress on-host and off host tick population | Peanut oil | Kaaya & Hedimbi, 2012 |
| | Enhance ovicidal activity | Vegetable oil | Luz et al., 2015 |
| Leaf extract formulation | Increase virulence | <i>Calpurnia aurea</i> extract | Nana et al., 2016 |
| Encapsulation | Enhance shelf life | Microencapsulated | Rodrigues et al., 2019 |
| | Increase virulence and persistence | Bioencapsulatio | Przyklenk et al., 2017 |
| | Improved survival and shelf life | Calcium gluconate | Humbert et al., 2017 |
| | Increase virulence | Calcium-alginate encapsulation | Shah et al., 2022 |
| | Increase virulence and persistence | Ionic gelation | Meirelles et al., 2023 |
| Enhance shelf life and bioactivity | Alginate-based encapsulation | Sarma et al., 2023 | |
| Air-dried blastospore | Increase virulence | Diatomaceous earth | Iwanicki et al., 2018 |
| Granular formulations | Increase virulence Increase virulence | Organic millet | Sullivan et al., 2021 |
| | | Microsclerotia | Marciano et al., 2021 |
| | | Blastospores-based | |
| Conidial emulsion formulations | Enhance shelf life | Emulsion | Lei et al., 2022 |
| Modified atmosphere packaging | Enhance shelf life | Millet grain | Jeong et al., 2022 |

This review explores the potential of *Metarhizium anisopliae* (MA) as a biocontrol agent against ticks and the current state of tick control in Asia. MA is promising for integrated tick management due to its low environmental impact and flexibility. Its use can improve control methods and prevent tick resistance to chemical acaricides. While promising, further studies and field trials are needed to optimize its effectiveness. MA should complement, not replace, chemical control, highlighting its role in integrated tick management. Current literature indicates that livestock ticks in Asia are beginning to develop resistance to acaricides, though resistance levels are still modest. This low level of resistance and the continued effectiveness of acaricides may limit the adoption of alternatives like MA. However, it is crucial to monitor this situation as resistance evolves, and proactive measures will be necessary to ensure sustainable pest management in the future.

ACKNOWLEDGEMENT

We express our gratitude for the support provided by the members of the Faculty of Veterinary Medicine at Universiti Malaysia Kelantan and the Mycology and Pathology Laboratory of the Forest Research Institute Malaysia. We would also want to express our gratitude to the Ministry of Higher Education Malaysia for providing financing for this research.

Funding

The Ministry of Higher Education Malaysia funded this study through the Fundamental Research Grant Scheme (FRGS/1/2022/WAB04/UMK/02/9).

Credit Authorship Contribution Statement

Nurul Fatin Amirah Mohd Azmi: Conceptualization, Writing – original draft. Investigation, Formal analysis. **Lim Chien Yee:** Conceptualization, Investigation, Writing – original draft. **Siew Shean Choong:** Supervision, Writing – Review & editing. **Tan Li Peng:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. **Samsuddin Ahmad Syazwan:** Validation, Visualization, Writing – review & editing.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

No data was used for the research described in the article.

REFERENCES

- Agale, S.V., Gopalakrishnan, S., Ambhure, K.G., Chandravanshi, H., Gupta, R. & Wani, S. (2018). Mass production of entomopathogenic fungi (*Metarhizium anisopliae*) using different grains as a substrate. *International Journal of Current Microbiology and Applied Sciences* **7**: 2227-2232. <https://doi.org/10.20546/ijcmas.2018.701.268>
- Ajith Kumar, K.G., Sharma, A.K., Kumar, S., Ray, D.D., Rawat, A.K.S., Srivastava, S. & Ghosh, S. (2016). Comparative *in vitro* anti-tick efficacy of commercially available products and newly developed phyto-formulations against field collected and resistant tick lines of *Rhipicephalus (Boophilus) microplus*. *Journal of Parasitic Diseases* **40**: 1590-1596. <https://doi.org/10.1007/s12639-015-0736-3>
- Akande, F., Adenubi, O. & Garba, A. (2020). In vitro analysis of the efficacy of selected commercial acaricides on the cattle tick *Rhipicephalus (Boophilus) annulatus* (Acari: Ixodidae). *Egyptian Journal of Veterinary Sciences* **51**: 153-161. <https://doi.org/10.21608/ejvs.2020.21560.1144>
- Albernaz, D.A.S., Tai, M.H.H. & Luz, C. (2009). Enhanced ovicidal activity of an oil formulation of the fungus *Metarhizium anisopliae* on the mosquito *Aedes aegypti*. *Medical and Veterinary Entomology* **23**: 141-147. <https://doi.org/10.1111/j.1365-2915.2008.00792.x>
- Alonso-Díaz, M.A. & Fernández-Salas, A. (2021). Entomopathogenic fungi for tick control in cattle livestock from Mexico. *Frontiers in Fungal Biology* **2**: 657694. <https://doi.org/10.3389/ffunb.2021.657694>
- Alves, R.T., Bateman, R.P., Gunn, J., Prior, C. & Leather, S.R. (2002). Effects of different formulations on viability and medium-term storage of *Metarhizium anisopliae* conidia. *Neotropical Entomology* **31**: 91-99. <https://doi.org/10.1590/s1519-566x2002000100013>
- Angelo, I.C., Fernandes, E.K.K., Bahiense, T.C., Perinotto, W.M., Moraes, A.P.R., Terra, A.L. & Bittencourt, V.R.E.P. (2010). Efficiency of *Lecanicillium lecanii* to control the tick *Rhipicephalus microplus*. *Veterinary Parasitology* **172**: 317-322. <https://doi.org/10.1016/j.vetpar.2010.04.038>
- Ashfaq, M., Razzaq, A., Hassan, S. & Haq, S.U. (2015). Factors affecting the economic losses due to livestock diseases: a case study of district Faisalabad. *Pakistan Journal of Agricultural Sciences* **52**: 503-508.
- 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 and Tick-Borne Diseases* **14**: 102147. <https://doi.org/10.1016/j.ttbdis.2023.102147>
- Barra-Bucarei, L., Vergara, P. & Cortes, A. (2016). Conditions to optimize mass production of *Metarhizium anisopliae* (Metschn.) Sorokin 1883 in different substrates. *Chilean Journal of Agricultural Research* **76**: 448-454. <https://doi.org/10.4067/s0718-58392016000400008>
- Batta, Y.A. (2003). Production and testing of novel formulations of the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes). *Crop Protection* **22**: 415-422. [https://doi.org/10.1016/s0261-2194\(02\)00200-4](https://doi.org/10.1016/s0261-2194(02)00200-4)
- Beys-da-Silva, W.O., Rosa, R.L., Berger, M., Coutinho-Rodrigues, C.J., Vainstein, M.H., Schrank, A., Bittencourt, V.R.P. & Santi, L. (2020). Updating the application of *Metarhizium anisopliae* to control cattle tick *Rhipicephalus microplus* (Acari: Ixodidae). *Experimental Parasitology* **208**: 107812. <https://doi.org/10.1016/j.exppara.2019.107812>
- Bravo-Ramos, J.L., Flores-Primo, A., Paniagua-Vega, D., Sánchez-Otero, M.G., Cruz-Romero, A. & Romero-Salas, D. (2021). Acaricidal activity of the hexanic and hydroethanolic extracts of three medicinal plants against southern cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *Experimental and Applied Acarology* **85**: 113-129. <https://doi.org/10.1007/s10493-021-00654-y>
- Brito, E.S., de Paula, A.R., Vieira, L.P., Dolinski, C. & Samuels, R.I. (2008). Combining vegetable oil and sub-lethal concentrations of Imidacloprid with *Beauveria bassiana* and *Metarhizium anisopliae* against adult guava weevil *Conotrachelus psidii* (Coleoptera: Curculionidae). *Biocontrol Science and Technology* **18**: 665-673. <https://doi.org/10.1080/09583150802195965>
- Bukhari, T., Takken, W. & Koenraadt, C.J.M. (2011). Development of *Metarhizium anisopliae* and *Beauveria bassiana* formulations for control of malaria mosquito larvae. *Parasites & Vectors* **4**: 23. <https://doi.org/10.1186/1756-3305-4-23>
- Butt, T.M., Wood, M., Taylor, J.W.D., Bakirci, S., Hazir, C., Ulug, D. & Hazir, S. (2016). Differential susceptibility of *Hyalomma excavatum* adults and nymphs to the entomopathogens *Metarhizium anisopliae* ARSEF 4556 and *Steinernema carpocapsae*. *International Journal of Pest Management* **62**: 261-266. <https://doi.org/10.1080/09670874.2016.1181287>
- Camargo, M.G., Golo, P.S., Angelo, I.C., Perinotto, W.M., Sá, F.A., Quinelato, S. & Bittencourt, V.R.E.P. (2012). Effect of oil-based formulations of acaripathogenic fungi to control *Rhipicephalus microplus* ticks under laboratory conditions. *Veterinary Parasitology* **188**: 140-147. <https://doi.org/10.1016/j.vetpar.2012.03.012>
- Carneiro, A.D.S., Mesquita, E., Meirelles, L.N., Bittencourt, V.R.E.P. & Golo, P.S. (2022). Compatibility of different *Metarhizium* spp. propagules with synthetic acaricides for controlling *Rhipicephalus microplus*. *Revista Brasileira De Parasitologia Veterinária* **31**: e018221. <https://doi.org/10.1590/s1984-29612022018>
- Cella, W., Rahal, I.L., Silva, G.C.C., Jacomassi, E., Piau Junior, R., Gonnalves, J.E., Gonçalves, D.D. & Gazim, Z.C. (2023). Activity of essential oils from leaves, flower buds and stems of *Tetradenia riparia* on *Rhipicephalus (Boophilus) microplus* larvae. *Revista Brasileira De Parasitologia Veterinária* **32**: e013522. <https://doi.org/10.1590/s1984-29612023011>
- Chen, Z.H., Xu, L., Yang, F.L., Ji, G.H., Yang, J. & Wang, J.Y. (2014). Efficacy of *Metarhizium anisopliae* isolate MAX-2 from Shangri-la, China under desiccation stress. *BMC Microbiology* **14**: 4. <https://doi.org/10.1186/1471-2180-14-4>

- Correia, A.D.C.B., Fiorin, A.C., Monteiro, A.C. & Veríssimo, C.J. (1998). Effects of *Metarhizium anisopliae* on the tick *Boophilus microplus* (Acari: Ixodidae) in stabled cattle. *Journal of Invertebrate Pathology* **71**: 189-191. <https://doi.org/10.1006/jjpa.1997.4719>
- da Paixão, F.R.S., Muniz, E.R., Catão, A.M.L., Santos, T.R., Luz, C., Marreto, R.N., Mascarin, G.M. & Fernandes, E.K.K. (2023). Microsclerotial pellets of *Metarhizium* spp.: thermotolerance and bioefficacy against the cattle tick. *Applied Microbiology and Biotechnology* **107**: 2263-2275. <https://doi.org/10.1007/s00253-023-12467-7>
- Daoust, R.A. & Roberts, D.W. (1983). Studies on the prolonged storage of *Metarhizium anisopliae* conidia: effect of temperature and relative humidity on conidial viability and virulence against mosquitoes. *Journal of Invertebrate Pathology* **41**: 143-150. [https://doi.org/10.1016/0022-2011\(83\)90213-6](https://doi.org/10.1016/0022-2011(83)90213-6)
- de Almeida, I.B., Calvo Duarte, F., Lucena Cassiano, L., Costa Fiorini, L., Mello Morán, L., Martins, A.M.C.R., Eduardo Marcondes de Almeida, J. & Cristina Mendes, M. (2022). New field technique to evaluate the action of the fungus *Metarhizium anisopliae* on *Rhipicephalus (Boophilus) microplus* tick. *Biological Control* **171**: 104934. <https://doi.org/10.1016/j.biocontrol.2022.104934>
- de Mendonça, A.S., Moreira, R.G., da Penha Henriques do Amaral, M., de Oliveira Monteiro, C.M., de Mello, V., Vilela, F.M.P., Mendonça Homem, F.C., Furlong, J., Dolinski, C., de Azevedo Prata, M.C. et al. (2019). Entomopathogenic nematodes in pharmaceutical formulations for *Rhipicephalus microplus* (Acari: Ixodidae) control: *In vitro* evaluation of compatibility, thermotolerance, and efficiency. *Ticks and Tick-Borne Diseases* **10**: 781-786. <https://doi.org/10.1016/j.ttbdis.2019.03.012>
- FAO. (2022a). World food and agriculture statistical yearbook 2022. FAO. <https://openknowledge.fao.org/server/api/core/bitstreams/0c372c04-8b29-4093-bba6-8674b1d237c7/content>
- FAO. (2022b). Pesticides use, pesticides trade and pesticides indicators – Global, regional and country trends, 1990–2020. FAOSTAT Analytical Briefs, no. 46. Rome. <https://doi.org/10.4060/cc0918en>
- Fernandes, É.K.K., Bittencourt, V.R.E.P. & Roberts, D.W. (2012). Perspectives on the potential of entomopathogenic fungi in biological control of ticks. *Experimental Parasitology* **130**: 300-305. <https://doi.org/10.1016/j.exppara.2011.11.004>
- Fernández-Salas, A., Alonso-díaz, M.A., Alonso-morales, R.A., Lezama-gutiérrez, R., Rodríguez-rodríguez, J.C. & Cervantes-chávez, J.A. (2016). 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**: 36-43. <https://doi.org/10.1111/mve.12203>
- Fiorotti, J., Urbanová, V., Gôlo, P.S., Bittencourt, V.R.E.P. & Kopáček, P. (2022). The role of complement in the tick cellular immune defense against the entomopathogenic fungus *Metarhizium robertsii*. *Developmental & Comparative Immunology* **126**: 104234. <https://doi.org/10.1016/j.dci.2021.104234>
- Gindin, G., Samish, M., Alekseev, E. & Glazer, I. (2001). The susceptibility of *Boophilus annulatus* (Ixodidae) ticks to entomopathogenic fungi. *Biocontrol Science and Technology* **11**: 111-118. <https://doi.org/10.1080/09583150020029790>
- Githaka, N.W., Kanduma, E.G., Wieland, B., Darghouth, M.A. & Bishop, R.P. (2022). Acaricide resistance in livestock ticks infesting cattle in Africa: current status and potential mitigation strategies. *Current Research in Parasitology & Vector-Borne Diseases* **2**: 100090. <https://doi.org/10.1016/j.crvbd.2022.100090>
- Godara, R., Katoch, R., Rafiqi, S.I., Yadav, A., Nazim, K., Sharma, R., Singh, N.K. & Katoch, M. (2019). Synthetic pyrethroid resistance in *Rhipicephalus (Boophilus) microplus* ticks from north-western Himalayas, India. *Tropical Animal Health and Production* **51**: 1203-1208. <https://doi.org/10.1007/s11250-019-01810-8>
- Goldsmith, A., Loftin, K., Steinkraus, D., Szalanski, A., Cleary, D. & Castrillo, L. (2021). Isolation of *Metarhizium guizhouense* and *Metarhizium robertsii* strains from soil-exposed *Amblyomma americanum* (Acarina: Ixodidae) from Northwest Arkansas, USA. *Florida Entomologist* **104**: 205-212. <https://doi.org/10.1653/024.104.0309>
- Guimapi, R.A., Klingen, I., Tonnang, H.E. & Nana, P. (2023). Linking spatial distribution of *Rhipicephalus appendiculatus* to climatic variables important for the successful biocontrol by *Metarhizium anisopliae* in Eastern Africa. *Acta Tropica* **238**: 106800. <https://doi.org/10.1016/j.actatropica.2022.106800>
- Gupta, S., Gupta, S. & Kumar, S. (2020). Emergence of fipronil resistance in cattle ticks *Rhipicephalus microplus* and *Hyalomma anatolicum* collected from Haryana, India. *International Journal of Tropical Insect Science* **41**: 401-407. <https://doi.org/10.1007/s42690-020-00218-4>
- Hendrawan, S. & Ibrahim, Y. (2006). Effect of dust formulations of three entomopathogenic fungal isolates against *Sitiphilus oryzae* (Coleoptera: Curculionidae) in rice grain. *Journal of Biosains* **17**: 1-7.
- Higa, L.D.O.S., Barradas Piña, F.T., Rodrigues, V.D.S., Garcia, M.V., Salas, D.R., Miller, R.J., de Leon, A.P., Barros, J.C. & Andreotti, R. (2020). Evidence of acaricide resistance in different life stages of *Amblyomma mixtum* and *Rhipicephalus microplus* (Acari: Ixodidae) collected from the same farm in the state of Veracruz, Mexico. *Preventive Veterinary Medicine* **174**: 104837. <https://doi.org/10.1016/j.prevetmed.2019.104837>
- Hosseini-Chegeni, A., Nasrabadi, M., Sadat Hashemi-Aghdam, S., Oshaghi, M.A., Lotfi, A., Telmadarray, Z. & Sedaghat, M.M. (2019). Molecular identification of *Rhipicephalus* species (Acari: Ixodidae) parasitizing livestock from Iran. *Mitochondrial DNA Part A* **30**: 448-456. <https://doi.org/10.1080/24701394.2018.1546298>
- Humbert, P., Przyklenk, M., Vemmer, M. & Patel, A.V. (2017). Calcium gluconate as cross-linker improves survival and shelf life of encapsulated and dried *Metarhizium brunneum* and *Saccharomyces cerevisiae* for the application as biological control agents. *Journal of Microencapsulation* **34**: 47-56. <https://doi.org/10.1080/02652048.2017.1282550>
- Inyang, E.N., McCartney, H.A., Oyejola, B., Ibrahim, L., Pye, B.J., Archer, S.A. & Butt, T.M. (2000). Effect of formulation, application and rain on the persistence of the entomogenous fungus *Metarhizium anisopliae* on oilseed grape. *Mycological Research* **104**: 653-661. <https://doi.org/10.1017/s0953756200002641>
- Iqbal, Z., Kayani, A.R., Akhter, A. & Qayyum, M. (2022). Prevalence and distribution of hard ticks and their associated risk factors in sheep and goats from four agro-climatic zones of Khyber Pakhtunkhwa (KPK), Pakistan. *International Journal of Environmental Research and Public Health* **19**: 11759. <https://doi.org/10.3390/ijerph191811759>
- Iwanicki, N.S., Ferreira, B.D.O., Mascarin, G.M. & Júnior, T.D. (2018). Modified Adamek's medium renders high yields of *Metarhizium robertsii* blastospores that are desiccation tolerant and infective to cattle-tick larvae. *Fungal Biology* **122**: 883-890. <https://doi.org/10.1016/j.funbio.2018.05.004>
- Jeong, S.G., Kim, H.M., Kim, J., Kim, J.S. & Park, H.W. (2022). Effect of storage conditions on the shelf-life extension of fungus-colonized substrates based on *Metarhizium anisopliae* using modified atmosphere packaging. *Scientific Reports* **12**: 423. <https://doi.org/10.1038/s41598-021-04232-5>
- Jongejan, F. & Uilenberg, G. (2004). The global importance of ticks. *Parasitology* **129**: S3-S14. <https://doi.org/10.1017/s0031182004005967>
- Kaaya, G.P. & Hedimbi, M. (2012). The use of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, as biopesticides for tick control. *International Journal of Agricultural Sciences* **2**: 245-250.
- Kamran, K., Ali, A., Villagra, C.A., Bazai, Z.A., Iqbal, A. & Sajid, M.S. (2020). *Hyalomma anatolicum* resistance against ivermectin and fipronil is associated with indiscriminate use of acaricides in southwestern Balochistan, Pakistan. *Parasitology Research* **120**: 15-25. <https://doi.org/10.1007/s00436-020-06981-0>
- Keyser, C.A., Fernandes, V.K.K., Rangel, D.E.N. & Roberts, D.W. (2014). Heat-induced post-stress growth delay: a biological trait of many *Metarhizium* isolates reducing biocontrol efficacy? *Journal of Invertebrate Pathology* **120**: 67-73. <https://doi.org/10.1016/j.jip.2014.05.008>
- Khan, S.S., Ahmed, H., Afzal, M.S., Khan, M.R., Birtles, R.J. & Oliver, J.D. (2022). Epidemiology, distribution and identification of ticks on livestock in Pakistan. *International Journal of Environmental Research and Public Health* **19**: 3024. <https://doi.org/10.3390/ijerph19053024>
- Khoo, J.J., Chen, F., Kho, K.L., Ahmad Shanizza, A.I., Lim, F.S., Tan, K.K., Chang, L.Y. & AbuBakar, S. (2016). Bacterial community in *Haemaphysalis* ticks of domesticated animals from the Orang Asli communities in Malaysia. *Ticks and Tick-Borne Diseases* **7**: 929-937. <https://doi.org/10.1016/j.ttbdis.2016.04.013>
- Kivaria, F.M. (2006). Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. *Tropical Animal Health and Production* **38**: 291-299. <https://doi.org/10.1007/s11250-006-4181-2>
- Klafke, G.M., Miller, R.J., Tidwell, J.P., Thomas, D.B., Sanchez, D., Feria Arroyo, T.P. & Pérez de León, A.A. (2019). High-resolution melt (HRM) analysis for detection of SNPs associated with pyrethroid resistance in the southern cattle fever tick, *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *International Journal for Parasitology: Drugs and Drug Resistance* **9**: 100-111. <https://doi.org/10.1016/j.ijpddr.2019.03.001>
- Kruger, R.D., Posadas, J.B., Lewylle, M.A., Mini, J.I. & Lecuona, R.E. (2014). Solid substrate production and formulation of an isolate of *Metarhizium anisopliae* for biological control of stem bug *Tibraca limbativentris*. *World Applied Sciences Journal* **32**: 1242-1251.

- Lee, M.R., Kim, J.C., Park, S.E., Kim, W.J. & Kim, J.S. (2023). Detection of viral genes in *Metarhizium anisopliae* JEF-290-infected longhorned tick, *Haemaphysalis longicornis* using transcriptome analysis. *Journal of Invertebrate Pathology* **198**: 107926. <https://doi.org/10.1016/j.jip.2023.107926>
- Lee, M.R., Li, D., Lee, S.J., Kim, J.C., Kim, S., Park, S.E., Baek, S., Shin, T.Y., Lee, D.H. & Kim, J.S. (2019). Use of *Metarhizium anisopliae* s.l. to control soil-dwelling longhorned tick, *Haemaphysalis longicornis*. *Journal of Invertebrate Pathology* **166**: 107230. <https://doi.org/10.1016/j.jip.2019.107230>
- Lei, C.J., Halim, N.A., Asib, N., Zakaria, A. & Azmi, W.A. (2022). Conidial Emulsion Formulation and thermal storability of *Metarhizium anisopliae* against red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Dryophthoridae). *Microorganisms* **10**: 1460. <https://doi.org/10.3390/microorganisms10071460>
- Liu, J., Wang, M., Yang, L., Rahman, S. & Sriboonchitta, S. (2020). Agricultural productivity growth and its determinants in South and Southeast Asian countries. *Sustainability* **12**: 4981. <https://doi.org/10.3390/su12124981>
- Lorenz, S.C., Humbert, P. & Patel, A.V. (2020). Chitin increases drying survival of encapsulated *Metarhizium pemphigi* blastospores for *Ixodes ricinus* control. *Ticks and Tick-Borne Diseases* **11**: 101537. <https://doi.org/10.1016/j.ttbdis.2020.101537>
- Luz, C., D'Alessandro, W.B., Rodrigues, J. & Fernandes, E.K.K. (2015). Efficacy of water- and oil-in-water-formulated *Metarhizium anisopliae* in *Rhipicephalus sanguineus* eggs and eclosing larvae. *Parasitology Research* **115**: 143-149. <https://doi.org/10.1007/s00436-015-4729-z>
- Marciano, A.F., Mascarin, G.M., Franco, R.F.F., Golo, P.S., Jaronski, S.T., Fernandes, E.K.K. & Bittencourt, V.R.E.P. (2021). Innovative granular formulation of *Metarhizium robertsii* microsclerotia and blastospores for cattle tick control. *Scientific Reports* **11**: 4972. <https://doi.org/10.1038/s41598-021-84142-8>
- Marzouk, A.S. & Ali, A.A.B. (2023). A comparison between the effectiveness of the fungi *Beauveria bassiana* and *Metarhizium anisopliae* for the control of *Argas persicus* with the emphasis of histopathological changes in the integument. *Veterinary Parasitology* **317**: 109906. <https://doi.org/10.1016/j.vetpar.2023.109906>
- Meirelles, L.N., Mesquita, E., Corrêa, T.A., Bittencourt, 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 and Tick-Borne Diseases* **14**: 102184. <https://doi.org/10.1016/j.ttbdis.2023.102184>
- Ment, D., Gindin, G., Rot, A., Soroker, V., Glazer, I., Barel, S. & Samish, M. (2010). Novel technique for quantifying adhesion of *Metarhizium anisopliae* conidia to the tick cuticle. *Applied and Environmental Microbiology* **76**: 3521-3528. <https://doi.org/10.1128/aem.02596-09>
- Mesquita, E., da Costa, D.P., Meirelles, L.N., Camargo, M.G., Corrêa, T.A., Bittencourt, V.R.E.P., da Silva Coelho, I., Santos, H.A., Humber, R.A. & Golo, P.S. (2023). Entomopathogenic fungus treatment changes the gut bacterial diversity of *Rhipicephalus microplus* ticks. *Parasites & Vectors* **16**: 185. <https://doi.org/10.1186/s13071-023-05790-5>
- Michalaki, M.P., Athanassiou, C.G., Kavallieratos, N.G., Batta, Y.A. & Balotis, G.N. (2006). Effectiveness of *Metarhizium anisopliae* (Metschnikoff) Sorokin applied alone or in combination with diatomaceous earth against *Tribolium confusum* Du Val larvae: Influence of temperature, relative humidity and type of commodity. *Crop Protection* **25**: 418-425. <https://doi.org/10.1016/j.cropro.2005.07.003>
- Monteiro, C., de Assis Lage, T.C., Marchesini, P., Vale, L., de Souza Perinotto, W.M., Lopes, W.D.Z., Fernandes, S.A., Bittencourt, V.R.E.P., Furlong, J. & de Azevedo Prata, M.C. (2021). Combination of entomopathogenic nematodes with acaricides or essential oil of *Lippia triplinervis* against *Rhipicephalus microplus* (Acari: Ixodidae). *Veterinary Parasitology: Regional Studies and Reports* **23**: 100526. <https://doi.org/10.1016/j.vprsr.2020.100526>
- Moore, D. & Prior, C. (1993). The potential of mycoinsecticides. *Biocontrol News and Information* **14**: 31N-40N.
- Msangi, S.S., Zekeya, N., Kimaro, E.G., Kusiluka, L. & Shirima, G. (2022). Entomopathogenic fungi (*Aspergillus oryzae*) as biological control agent of cattle ticks in Tanzania. *Journal of Veterinary Medicine and Animal Health* **14**: 52-61. <https://doi.org/10.5897/jvmah2022.0985>
- Muniz, E.R., Paixão, F.R.S., Barreto, L.P., Luz, C., Arruda, W., Angelo, I.C. & Fernandes, E.K.K. (2020). Efficacy of *Metarhizium anisopliae* conidia in oil-in-water emulsion against the tick *Rhipicephalus microplus* under heat and dry conditions. *BioControl* **65**: 339-351. <https://doi.org/10.1007/s10526-020-10002-5>
- Nana, P., Ekesi, S., Nchu, F. & Maniania, N.K. (2016). Compatibility of *Metarhizium anisopliae* with *Calpurnia aurea* leaf extracts and virulence against *Rhipicephalus pulchellus*. *Journal of Applied Entomology* **140**: 590-597. <https://doi.org/10.1111/jen.12289>
- Newton, L.G. (1967). Acaricide resistance and cattle tick control. *Australian Veterinary Journal* **43**: 389-394. <https://doi.org/10.1111/j.1751-0813.1967.tb04891.x>
- OECD-FAO. (2018). OECD-FAO Agricultural Outlook 2018-2027. https://doi.org/10.1787/agr_outlook-2018-en
- Okumu, F.O., Knols, B.G. & Fillinger, U. (2007). Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae*. *Malaria Journal* **6**: 63. <https://doi.org/10.1186/1475-2875-6-63>
- Paula, A.R., Ribeiro, A., Lemos, F.J.A., Silva, C.P. & Samuels, R.I. (2019). Neem oil increases the persistence of the entomopathogenic fungus *Metarhizium anisopliae* for the control of *Aedes aegypti* (Diptera: Culicidae) larvae. *Parasites & Vectors* **12**: 163. <https://doi.org/10.1186/s13071-019-3415-x>
- Pereira, R.M. & Roberts, D.W. (1991). Alginate and cornstarch mycelial formulations of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*. *Journal of Economic Entomology* **84**: 1657-1661. <https://doi.org/10.1093/jee/84.6.1657>
- Przyklenk, M., Vemmer, M., Hanitzsch, M. & Patel, A. (2017). A bioencapsulation and drying method increases shelf life and efficacy of *Metarhizium brunneum* conidia. *Journal of Microencapsulation* **34**: 498-512. <https://doi.org/10.1080/02652048.2017.1354941>
- Ren, Q., Chen, Z., Luo, J., Liu, G., Guan, G., Liu, Z., Liu, A., Li, Y., Niu, Q., Liu, J. et al. (2016). Laboratory evaluation of *Beauveria bassiana* and *Metarhizium anisopliae* in the control of *Haemaphysalis qinghaiensis* in China. *Experimental and Applied Acarology* **69**: 233-238. <https://doi.org/10.1007/s10493-016-0033-6>
- Ren, Q., Sun, M., Guan, G., Liu, Z., Chen, Z., Liu, A., Li, Y., Ma, M., Yang, J., Niu, Q. et al. (2014). Susceptibility of the tick *Haemaphysalis qinghaiensis* to isolates of the fungus *Metarhizium anisopliae* in China. *Experimental and Applied Acarology* **64**: 253-258. <https://doi.org/10.1007/s10493-014-9790-2>
- Riaz, A., Shah, F.A. & Butt, T.M. (2013). Intra-specific variability among *Metarhizium anisopliae* strains in their ability to produce blastospores in liquid culture media. *Pakistan Journal of Botany* **45**: 1099-1103.
- Rice, S.J., Baker, D.K., Mayer, D.G. & Leemon, D.M. (2020). Mycoinsecticide formulations of *Beauveria bassiana* and *Metarhizium anisopliae* reduce populations of lesser mealworm, *Alphitobius diaperinus*, in chicken-broiler houses. *Biological Control* **144**: 104234. <https://doi.org/10.1016/j.biocontrol.2020.104234>
- Roche, X., Rozstalnyy, A., Tago Pacheco, D., Kamata, A., Pittiglio, C., Kamata, A., Beltran Alcrudo, D., Bisht, K., Karki, S., Kayamori, J. et al. (2020). Introduction and spread of lumpy skin disease in South, East and Southeast Asia: Qualitative risk assessment and management. In: FAO animal production and health. Rome, FAO: pp, 183. <https://doi.org/10.4060/cb1892en>
- Rodrigues, J., Borges, P.R., Fernandes, E.K.K. & Luz, C. (2019). Activity of additives and their effect in formulations of *Metarhizium anisopliae* s.l. IP 46 against *Aedes aegypti* adults and on post mortem conidiogenesis. *Acta Tropica* **193**: 192-198. <https://doi.org/10.1016/j.actatropica.2019.03.002>
- Saciloto-de-Oliveira, L.R., Broetto, L., Alves, C.I., da Rosa, R.L., Calegari Alves, Y.P., da Silva, R.C., Berger, M., Macedo, A.J., Dalberto, P.F., Bizarro, C.V. et al. (2023). *Metarhizium anisopliae* E6 secretome reveals molecular players in host specificity and toxicity linked to cattle tick infection. *Fungal Biology* **127**: 1136-1145. <https://doi.org/10.1016/j.funbio.2023.06.006>
- Sagar, S.V., Saini, K., Sharma, A.K., Kumar, S., Kumar, R., Fular, A., Shakya, M., Upadhya, D., Nagar, G., Shanmuganath, C. et al. (2020). Acaricide resistance in *Rhipicephalus microplus* collected from selected districts of Madhya Pradesh, Uttar Pradesh and Punjab states of India. *Tropical Animal Health and Production* **52**: 611-618. <https://doi.org/10.1007/s11250-019-02048-0>
- Sarma, B.D., Puzari, K.C., Dutta, P. & Pandey, A.K. (2023). An alginate-based encapsulation enhances shelf life and bioactivity of the entomopathogenic fungus, *Metarhizium anisopliae*. *Egyptian Journal of Biological Pest Control* **33**: 69. <https://doi.org/10.1186/s41938-023-00714-y>

- Seye, F., Ndiaye, M., Faye, O. & Afoutou, J.M. (2012). Evaluation of entomopathogenic fungus *Metarhizium anisopliae* formulated with suneem (neem oil) against *Anopheles gambiae* s.l. and *Culex quinquefasciatus* adults. *Malaria Chemotherapy, Control and Elimination* **1**: 235494. <https://doi.org/10.4303/mcce/235494>
- Shah, S., Ash, G.J. & Wilson, B.A.L. (2022). Resporulation of calcium alginate encapsulated *Metarhizium anisopliae* on Metham®-Fumigated soil and infectivity on larvae of *Tenebrio molitor*. *Journal of Fungi* **8**: 1114. <https://doi.org/10.3390/jof8101114>
- Shanmuganath, C., Kumar, S., Singh, R., Sharma, A.K., Saminathan, M., Saini, M., Chigure, G., Fular, A., Kumar, R., Juliet, S. et al. (2021). Development of an efficient antitick natural formulation for the control of acaricide-resistant ticks on livestock. *Ticks and Tick-Borne Diseases* **12**: 101655. <https://doi.org/10.1016/j.ttbdis.2021.101655>
- Shyma, K.P., Kumar, S., Sangwan, A.K., Sharma, A.K., Nagar, G., Ray, D.D. & Ghosh, S. (2013). Acaricide resistance status of *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* collected from Haryana. *Indian Journal of Animal Sciences* **83**: 591-594.
- Sindhu, Z.U.D., Naseer, M.U., Raza, A., Aslam, B., Ahmad, J., Abbas, R.Z., Khan, M.K., Imran, M., Zafar, M.A. & Khattak, B. (2022). Resistance to cypermethrin is widespread in cattle ticks (*Rhipicephalus microplus*) in the province of Punjab, Pakistan: *In vitro* diagnosis of acaricide resistance. *Pathogens* **11**: 1293. <https://doi.org/10.3390/pathogens11111293>
- Singh, K., Kumar, S., Sharma, A.K., Jacob, S.S., RamVerma, M., Singh, N.K., Shakya, M., Sankar, M. & Ghosh, S. (2022). Economic impact of predominant ticks and tick-borne diseases on Indian dairy production systems. *Experimental Parasitology* **243**: 108408. <https://doi.org/10.1016/j.exppara.2022.108408>
- Singh, N.K., Gelot, I.S., Jyoti, Bhat, S.A., Singh, H. & Singh, V. (2015). Detection of acaricidal resistance in *Hyalomma anatolicum* from Banaskantha district, Gujarat. *Journal of Parasitic Diseases* **39**: 563-566. <https://doi.org/10.1007/s12639-013-0397-z>
- Singh, N.K., Goolsby, J.A., Shapiro-Ilan, D.I., Miller, R.J., Thomas, D.B., Klafke, G.M., Tidwell, J.P., Racelis, A.E., Grewal, P.S. & Perez de León, A.A. (2018). Efficacy evaluation of six entomopathogenic nematode species against engorged females of southern cattle fever tick, *Rhipicephalus (Boophilus) microplus*. *Southwestern Entomologist* **43**: 1-17. <https://doi.org/10.3958/059.043.0119>
- Solanki, V., Singh, V., Shyma, K.P., Sharma, N., Parsani, H.R. & Prakash Gupta, J. (2020). Resistance status of one host tick *Rhipicephalus microplus* of arid and semi-arid region of Gujarat, India. *International Journal of Tropical Insect Science* **41**: 1633-1641. <https://doi.org/10.1007/s42690-020-00364-9>
- Sullivan, C.F., Parker, B.L. & Skinner, M. (2022). A review of commercial *Metarhizium*- and *Beauveria*-based biopesticides for the biological control of ticks in the USA. *Insects* **13**: 260. <https://doi.org/10.3390/insects13030260>
- Sullivan, C.F., Parker, B.L., Davari, A., Lee, M.R., Kim, J.S. & Skinner, M. (2020). Evaluation of spray applications of *Metarhizium anisopliae*, *Metarhizium brunneum* and *Beauveria bassiana* against larval winter ticks, *Dermacentor albipictus*. *Experimental and Applied Acarology* **82**: 559-570. <https://doi.org/10.1007/s10493-020-00547-6>
- Sullivan, C.F., Parker, B.L., Kim, J.S. & Skinner, M. (2021). Effectiveness of granular formulations of *Metarhizium anisopliae* and *Metarhizium brunneum* (Hypocreales: Clavicipitaceae) on off-host larvae of *Dermacentor albipictus* (Acari: Ixodidae). *Biocontrol Science and Technology* **31**: 1113-1127. <https://doi.org/10.1080/09583157.2021.1926428>
- Villar, D., Klafke, G.M., Rodríguez-Durán, A., Bossio, F., Miller, R., Pérez de León, A.A., Cortés-Vecino, J.A. & Chaparro-Gutiérrez, J.J. (2019). Resistance profile and molecular characterization of pyrethroid resistance in a *Rhipicephalus microplus* strain from Colombia. *Medical and Veterinary Entomology* **34**: 105-115. <https://doi.org/10.1111/mve.12418>
- Webster, A., Pradel, E., Souza, U.A., Martins, J.R., Reck, J., Schrank, A. & Klafke, G. (2017). Does the effect of a *Metarhizium anisopliae* isolate on *Rhipicephalus microplus* depend on the tick population evaluated? *Ticks and Tick-Borne Diseases* **8**: 270-274. <https://doi.org/10.1016/j.ttbdis.2016.11.012>
- Weeks, E.N.I., Allan, S.A., Gezan, S.A. & Kaufman, P.E. (2020). Auto dissemination of commercially available fungal pathogens in a laboratory assay for management of the brown dog tick *Rhipicephalus sanguineus*. *Medical and Veterinary Entomology* **34**: 184-191. <https://doi.org/10.1111/mve.12426>
- Yawa, M., Nyangiwe, N., Jaja, I.F., Marufu, M.C. & Kadzere, C.T. (2022). Acaricide resistance of *Rhipicephalus decoloratus* ticks collected from communal grazing cattle in South Africa. *Journal of Advanced Veterinary and Animal Research* **9**: 33-41. <https://doi.org/10.5455/javar.2022.i566>
- Zhioua, E., Browning, M., Johnson, P.W., Ginsberg, H.S. & LeBrun, R.A. (1997). Pathogenicity of the entomopathogenic fungus *Metarhizium anisopliae* (Deuteromycetes) to *Ixodes scapularis* (Acari: Ixodidae). *The Journal of Parasitology* **83**: 815-818. <https://doi.org/10.2307/3284273>