Veterinary Parasitology 172 (2010) 305–310

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Metarhizium anisopliae (Ascomycota: Hypocreales): An effective alternative to chemical acaricides against different developmental stages of fowl tick *Argas persicus* (Acari: Argasidae)

S.H. Pourseyed[a], M. Tavassoli[a,*], I. Bernousi[b], K. Mardani[c]

[a] Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, PO Box 1177, Urmia, West Azarbaijan, Iran
[b] Department of Genetic and Biometry, Faculty of Agriculture Science, Urmia University, Urmia, West Azarbaijan, Iran
[c] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, PO Box 1177, Urmia, West Azarbaijan, Iran

ARTICLE INFO

Article history:
Received 9 January 2010
Received in revised form 28 April 2010
Accepted 12 May 2010

Keywords:
Argas persicus
Fowl tick
Chemical acaricides
Metarhizium anisopliae
Biological control

ABSTRACT

The fowl bloodsucking tick *Argas persicus* is of great medical and veterinary importance in tropical and subtropical regions because of its role as vector of certain parasitic, bacterial and viral pathogens. A variety of acaricides are used for the control of tick infestation in poultry, resulting in environmental contamination and the development of resistance. In order to develop an alternative control method, the efficacy of three strains (V245, 685 and 715C) of entomopathogenic fungus *Metarhizium anisopliae* against different life stages of *A. persicus* including eggs, larvae, unfed and engorged adult females was evaluated under laboratory conditions. Five concentrations of different strains of *M. anisopliae* ranging from 10^3 to 10^7 conidia/ml were utilized. The effects of fungal strains on egg hatchability and larva and adult female mortality were significant and dose-dependent compared to the control groups ($P < 0.05$). The mortality rates of larvae ranged from 92% to 100% for two different concentrations (10^3 and 10^4 conidia/ml) of *M. anisopliae* strains. Treated engorged females were more susceptible than the unfed females reaching mortality rate of 100% at the highest concentration (10^7 conidia/ml) at 18 days post-inoculation. Among strains used in this study, V245 was the most virulent strain regarding the LC_{50} values for adult females exposed to fungal conidia. The results demonstrate that the application of *M. anisopliae* as a biocontrol agent is a promising option in reducing the use of chemical acaricides, resulting in benefits to poultry and the environment.

1. Introduction

The soft tick, *Argas persicus*, was first recorded by Lorenz Oken in 1818 in Mianeh, East Azarbaijan of Iran (Fortescue, 1924). It has a worldwide distribution in warm climates and also is prevalent in different parts of Iran. Apart from causing anaemia, anorexia, weight loss and depressed egg output, *A. persicus* is the main vector of *Borrelia anserina* (the casual agent of the avian spirochetosis) and *Aegyptianella pullorum* (Leeflang and Ilemobade, 1977). It is also capable of transmitting *Mycobacerium avium*, *Pasteurella avicida/multocida*, *West Nile virus*, *Salmonella gallinarum/pullorum*, *Mycoplasma gallisepticum/meleagridis* to poultry (Stefanov et al., 1975; Hoogstraal, 1985; Soliman et al., 1988). Tick paralysis in chickens, a flaccid motor paralysis, may result from attacks by larval stage of *A. persicus* (Rosenstein, 1976). In addition to chickens and other domestic fowl, it also feeds on humans (Trager, 1940).

Application of chemical acaricides such as organophosphorus compounds (malathion, comaphous) and the carbamate carbaryl is the most common method for controlling tick populations (Rodriguez-Vivas et al., 2006), but

* Corresponding author. Tel.: +98 441 2972616; fax: +98 441 2771926.
E-mail address: mtavassoli2000@yahoo.com (M. Tavassoli).

0304-4017/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2010.05.014

they may be hazardous for the environment. Drawbacks to this strategy include environmental contamination (Pell et al., 2001), impacts on non-target organisms (Schulze et al., 2001), human health hazards due to chemical residues in food products (Ostfeld et al., 2006) and the development of resistance in ticks (Graf et al., 2004). These disadvantages have stimulated the search for alternative methods to control ticks.

Biological pesticides are natural, more environmentally friendly, potentially less expensive, and more effective than chemical pesticides, as problems with resistance are less likely to occur (Whipps and Lumsden, 2001). Among biocontrol agents, entomopathogenic fungi received major attention in recent years (Briggs et al., 2006; Abolins et al., 2007; Tavassoli et al., 2008). One of the most pathogenic fungal species examined for pathogenicity against ticks under laboratory and field conditions was found to be *Metarhizium anisopliae* (Samish et al., 2004; Ostfeld et al., 2006). It has shown high pathogenic activity against the ixodid ticks *Amblyomma maculatum* and *Amblyomma americanum* (Kirkland et al., 2004), *Ixodes scapularis* (Benjamin et al., 2002; Hornbostel et al., 2005a), *Rhipicephalus appendiculatus* and *Amblyomma variegatum* (Kaaya and Hassan, 2000) and *Boophilus microplus* (Alonso-Diaz et al., 2007). In Iran, some indigenous strains of *M. anisopliae*, *Beauveria bassiana* and *Lecanicillium psalliotae* have been isolated with promising results to control different life stages of *Rhipicephalus* (*Boophilus*) *annulatus* under laboratory conditions (Pirali-Kheirabadi et al., 2007). However, only few studies have been reported about the control of argasid ticks by *M. anisopliae* or other entomopathogenic fungi (Sewify and Habib, 2001; Zabalgogeazcoa et al., 2008).

The aim of the present study was to evaluate the susceptibility of different developmental stages of *A. persicus* to three strains of *M. anisopliae* (one from UK, one from Finland and one from Iran) in order to finding out novel potent strains.

2. Materials and methods

2.1. M. anisopliae strains and preparation of fungal suspensions

Three strains of *M. anisopliae*, including strain 685 isolated from *Ixodes ricinus* in the UK, strain V245 obtained from Hayfield soil in Finland and strain 715C isolated from a locust in Iran, were used in this study. The first two strains were part of the fungal culture collection at the University of Wales Swansea, UK while the Iranian strain was held at the University of Tehran, Iran.

Fungal virulence was maintained by passaging twice through *A. persicus* ticks before being cultured on PDA (potato dextrose agar; E. Merck, Germany) in Petri dishes (90 mm × 15 mm) in a dark incubator at 25 °C. Conidia were harvested 14 days post-inoculation (DPI) by adding sterile distilled water (dH_2O). After that, suspensions were poured into sterile glass tubes and homogenized on a vortex mixer. The number of conidia was determined by direct count using a Neubauer haemocytometer and adjusted to final concentrations of 10^3, 10^4, 10^5, 10^6 and 10^7 conidia/ml by dilution with sterile aqueous 0.05% Tween 80 solution as described by Butt and Goettel (2000). Conidia viability for each strain was determined by placing three droplets of the conidial suspension on PDA and counting conidia with protruding germ tubes under a light microscope at 40× magnification 48 h later (Goettel and Inglis, 1997).

2.2. Tick

The *A. persicus* ticks are active during the night. They spend the daytime hidden in cracks and crevices of the walls of chicken houses or wooden materials such as windows or doors of poultry-roosting areas. Therefore, these places were examined for the presence of ticks in some villages of Urmia City, West Azarbaijan of Iran. Special attention was paid to feces of the ticks which were in the form of black and red grains like blood clots in the tick habitats. At each infested site, several specimens at different developmental stages were found, and then were transferred to the Department of Parasitology, Faculty of Veterinary Medicine, Urmia University where their species and sex have been determined using morphological characteristics (Wall and Shearer, 1997). In order to reduce fungal contamination on the ticks, they were consequently immersed in 70° alcohols for 3 s in vertical laminar flow chamber (Tavassoli et al., 2009). Then, they were dried on sterile filter paper and transferred to Petri dishes and incubated at 28 °C with 80% relative humidity (RH). Unfed and engorged female ticks were used in the experiments without considering their age.

Some adult engorged female ticks were kept in desiccators at room temperature (22–25 °C) with 80% RH for egg production. The eggs laid during 2 weeks were collected and kept in desiccators to obtain larvae. During this period, eggs were checked two or three times a week for eclosion. Each 50 larvae were placed in a sterile vial and treated 15 days after eclosion.

2.3. Bioassay procedure

Fungal virulence against eggs, larvae and adult female ticks of *A. persicus* was determined in three different bioassays using suspensions of varying conidia concentrations of three fungal strains. Randomly selected specimens were submerged for 5 s in suspensions ranging from 10^3 to 10^7 conidia/ml prepared in 0.05% aqueous Tween 80 in the bioassays as indicated. For control treatment, the specimens were only immersed in sterile dH_2O. Trials for each concentration and control group were performed in three replicates. Treated specimens were placed into Petri dishes containing moist filter paper to retain humidity for fungal growth in a dark incubator. Fungal treatment groups were periodically examined for fungal growth with changes recorded every 2–3 days during the time course of the experiments. The most virulent strain was further studied for its median lethal concentration (LC_{50}) in adult susceptibility bioassay.

2.3.1. Egg susceptibility

To evaluate the effect of fungal strains on egg hatchability, randomly selected eggs without considering their age were inoculated with the conidia suspension

(1×10^3 conidia/ml). In addition, 1-day-old eggs were tested with the same concentration in another experiment to evaluate the impact of fungal strains on fresh eggs. A total of 600 eggs (50 per Petri dish) were used in each experiment. All treatment and control groups in both experiments were checked at 2, 4, 6, 8, 10, 12, and 14 DPI to calculate the hatchability rate of eggs.

2.3.2. Larval susceptibility

A total of 1050 larvae (50 per Petri dish) were infected with two different concentrations (10^3 and 10^4 conidia/ml) of the strains studied. Mortality was recorded at 2, 4, 6, 8, 10, 12, and 14 DPI in both treatment and control groups, and the percentage of mortality was calculated.

2.3.3. Adult susceptibility

Because of the importance of female ticks in spreading the diseases by laying eggs, only adult females were selected for use in two separate experiments. The virulence of each fungal strain (10^5, 10^6 and 10^7 conidia/ml) was tested by immersing engorged and unfed adult females in conidial suspensions. A total of 300 ticks (5 per Petri dish) were used in the bioassay. Both treatment and control groups were examined at 3, 6, 9, 12, 15, 18, and 21 DPI to detect dead ticks and signs of mycosis.

2.4. Determining infection

Seven days after death, fungal hyphae started to emerge and sporulate and dead larvae and adult ticks appeared swollen with greenish areas on their body and legs. Mortality due to *M. anisopliae* was then confirmed by microscopic examination of hyphae and spores on the surface of fungal treatment specimens.

2.5. Statistical analysis

Statistical analysis of data was performed using general linear model and repeated measure analysis (three-factor mixed design) using Statistical Package for the Social Sciences (SPSS, Version 17, Chicago). Values of $P < 0.05$ were considered significant. Probit analysis was conducted to calculate the LC_{50} of the fungi with 95% confidence limit using MINITAB (Version 15, State College, PA).

3. Results

3.1. Effect of fungal strains on egg hatchability

All three strains of *M. anisopliae* with the conidia viability of more than 96% significantly decreased the hatchability of randomly selected eggs compared to the control group ($P < 0.05$). Only 17.3% of the eggs infected with strain V245 were hatched after 2 weeks, while the hatchability rate was 20% and 23.3% for the eggs infected with strains 715C and 685, respectively. In contrast, the hatching rate in the control group was 90%. On the other hand, 1-day-old eggs showed higher susceptibility to fungal strains compared to randomly selected eggs. The hatchability rate for 1-day-old eggs were 0%, 2.6% and 4% for strains V245, 715C and 685, respectively, whereas in the control group the hatchability rate was 86% (Fig. 1).

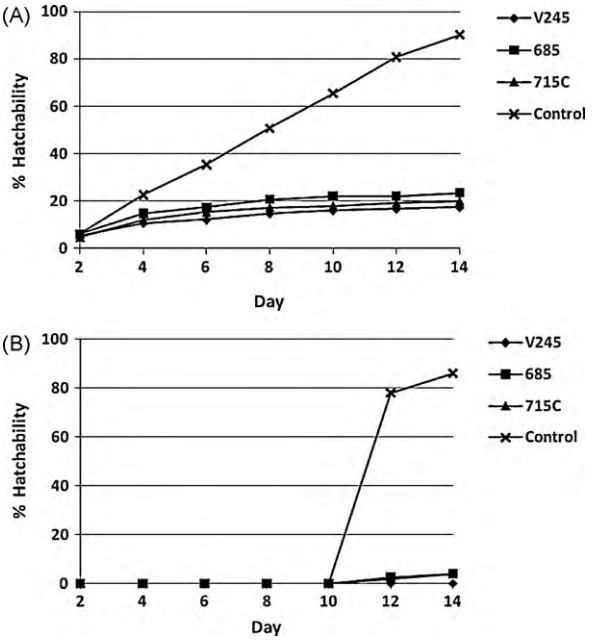

Fig. 1. Hatchability percentage of *A. persicus* eggs infected with three strains of *M. anisopliae* after different days post-inoculation with 10^3 conidia/ml. Randomly selected eggs without considering their age (A) and 1-day-old eggs (B).

3.2. Pathogenicity of fungal strains to A. persicus larvae

The results showed that all three strains of fungi examined were effective on the larvae at 4 DPI ($P < 0.05$). At 6 DPI strain V245 had the highest effect on larval stage with a mortality rate more than 50% for two conidial concentrations which have been used. All strains caused more than 80% mortality at 10 DPI for 10^3 and 8 DPI for 10^4 conidia/ml. Strain V245 caused 100% mortality after 2 weeks for the concentration of 10^4 conidia/ml in the larvae examined (Fig. 2). It was observed that an increase in the mortality rate of larvae was proportional to the concentration of conidia used, i.e., the greater the concentration of conidia, the higher the percentage mortality of larvae.

3.3. Effect of fungal strains on adult ticks

All strains of *M. anisopliae* examined in this study increased the mortality rate in the engorged ticks significantly ($P < 0.05$) at 6 DPI except for 10^5 conidia/ml that was 9 DPI comparing to the control group (Fig. 3). Strain V245 was the most effective strain with LC_{50} value of 2.8×10^5 conidia/ml at 12 DPI followed by 715C with LC_{50} value of 3.8×10^5 conidia/ml at 12 DPI (Table 1).

Unfed female ticks showed a lower susceptibility than engorged female ticks to different fungal strains (Fig. 4). The mortality rate was significantly increased at 9 DPI except for the lowest concentration (10^5 conidia/ml) that it was at 12 DPI ($P < 0.05$). The results revealed that strain V245

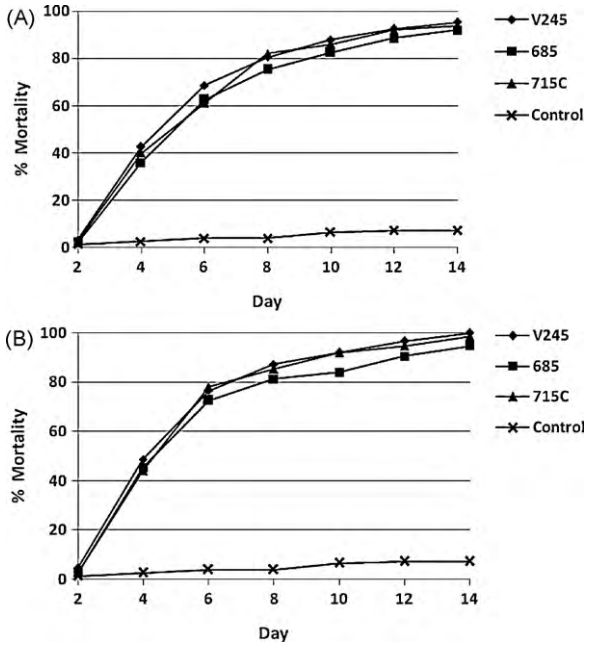

Fig. 2. Mortality percentage of *A. persicus* larvae infected with three strains of *M. anisopliae* after different days post-inoculation with 10^3 conidia/ml (A) and 10^4 conidia/ml (B).

was the most effective strain on unfed ticks same as results obtained for engorged ticks. The LC_{50} values for all strains have been calculated at 9 and 12 DPI (Table 1).

4. Discussion

The mortality percentages and the LC_{50} values showed that strain V245 was the most pathogenic strain against different developmental stages of *A. persicus* for all tested concentrations, while 685 and the indigenous strain, 715C, had almost similar effect. Similar results were found in a previous study on the pathogenicity of these fungal strains on different life stages of *Dermanyssus gallinae* under laboratory conditions. It was also reported that the pathogenicity of *M. anisopliae* on different life stages of *D. gallinae* was correlated with conidial concentration (Tavassoli et al., 2008).

There are few reports on the susceptibility of eggs of argasid ticks to biocontrol agents in the literature. In a study, the potential activity of three varieties of

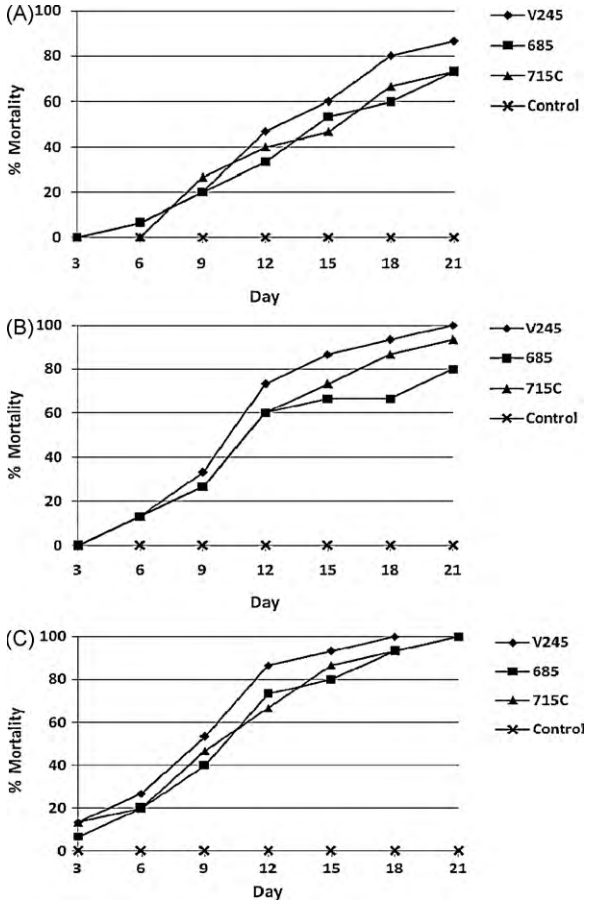

Fig. 3. Mortality percentage of engorged adult females of *A. persicus* infected with three strains of *M. anisopliae* after different days post-inoculation with 10^5 conidia/ml (A), 10^6 conidia/ml (B) and 10^7 conidia/ml (C).

Bacillus thuringiensis (*kurstaki*, *israeliensis* and *thuringiensi*) against *A. persicus* eggs revealed that eggs were mostly affected at 16 days after deposition, and the susceptibility decreased gradually for the 10-day and freshly deposited eggs (Hassanain et al., 1997). In contrast, in egg susceptibility bioassay, we found that 1-day-old eggs were more susceptible to fungal strains than eggs selected at random without considering their age. *A. persicus* eggs naturally hatch about 2 weeks post-deposition (Kettle, 1995), but no 1-day-old eggs hatched during this period when treated by

Table 1
Values of LC_{50} for three strains of *M. anisopliae* on *A. persicus* engorged and unfed adult females and their respective confidence limits.

Fungal strain	Day	LC_{50} (95% confidence interval)[a]	
		Engorged females	Unfed females
V245	9	2.4×10^7 (1.2×10^6 to 5.1×10^8)	8.5×10^5 (1.1×10^5 to 6.4×10^6)
	12	2.8×10^5 (6.4×10^4 to 1.2×10^6)	1.8×10^5 (1.5×10^4 to 2.2×10^6)
685	9	8.5×10^7 (4.4×10^4 to 1.6×10^{11})	8.1×10^6 (2.3×10^5 to 2.8×10^8)
	12	5.2×10^5 (9.1×10^4 to 2.9×10^6)	8.2×10^5 (6.6×10^4 to 1.0×10^7)
715C	9	4.0×10^7 (4.3×10^4 to 3.6×10^{10})	3.2×10^6 (5.1×10^5 to 2.1×10^7)
	12	3.8×10^5 (2.3×10^4 to 6.1×10^6)	1.0×10^5 (6.9×10^2 to 1.6×10^7)

[a] LC_{50}s were calculated using probit analysis.

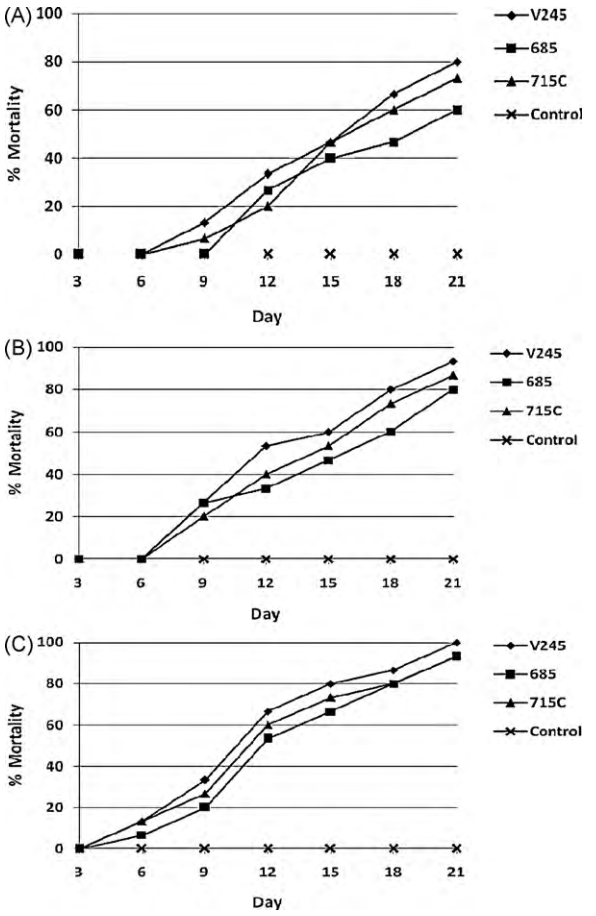

Fig. 4. Mortality percentage of unfed adult females of *A. persicus* infected with three strains of *M. anisopliae* after different days post-inoculation with 10^5 conidia/ml (A), 10^6 conidia/ml (B) and 10^7 conidia/ml (C).

strain V245, whereas the hatchability rate was 17.3% for randomly selected eggs. It seems that fungal strains need enough time for growth and inducing pathogenicity.

The effect of fungal infection on larval mortality was highly significant, even at the lower fungal concentration. The mortality rate of larvae at 2 weeks post-treatment with strains V245, 715C and 685 was calculated as 95.3%, 94% and 92% for 10^3 conidia/ml and 100%. 98.6% and 94.6% for 10^4 conidia/ml respectively, whereas the mortality of the control group was only 7.3%. Several studies using *M. anisopliae* to infect broad mite *Polyphagotarsonemus latus* (Maketon et al., 2008) and *B. microplus* (Bahiense et al., 2006) also found that larval mortality was dose-dependent.

In the third bioassay, 100% mortality of engorged female ticks was observed at 21 DPI except for strain V245 which occurred at 18 DPI at the highest concentration. However, unfed females mortality to strains V245, 715C and 685 at 21 DPI was 100%, 93.3% and 93.3%, respectively. The difference between engorged and unfed adult ticks in susceptibility to fungal strains could be due to the fact that the mouthparts, intersegmental folds or spiracles are more easily penetrated in engorged adult ticks comparing to the unfed ticks. The results indicated that the effect of *M. anisopliae* on both engorged and unfed female ticks was concentration dependent. Our results are in agreement with the result reported by Sewify and Habib (2001), who evaluated the susceptibility of *A. persicus* adult females to entomopathogenic fungi *M. anisopliae* and *B. bassiana* at laboratory and field bioassays. They also observed that 3 weeks after spray of heavily infested chicken houses with fungal spores, mortality rate increased to 100%.

Entomopathogenic fungi do not have to be ingested and can invade their hosts directly through the exoskeleton or cuticle. Therefore, they can infect non-feeding stages such as eggs and larvae (Hajek and St. Leger, 1994). However, virulent fungal strains selected on the basis of laboratory bioassays need to be assessed before they can be used as a commercial product. For example, the environmental stability (e.g., UV resistance, temperature tolerance), improved sporulation during mass production, ability to initiate infection at low humidity and their potential for damage to non-target invertebrates are characteristics which could be taken into account. One drawback to fungal pathogens is that they are slow acting especially in severe infestations (Briggs et al., 2006). However, the horizontal transmission of infection from fungus-infected to uninfected ticks is a positive point for using this biocontrol strategy especially in moist environments (Kaaya and Okech, 1990). In addition, acaricides with synergistic effects can be used in combination with fungal pathogens to improve the level of tick control (Hornbostel et al., 2005b).

In conclusion, the present study has provided useful baseline data for *M. anisopliae* strain selection against different developmental stages of *A. persicus* as an important poultry tick. However, further investigations are needed to test the potential of isolates, especially strain V245, as safe and environmentally-friendly biocontrol agent for pest management in practice.

Acknowledgements

Authors would like to thank Mr. A. Aghapour for his technical assistance. We would like to sincerely thank the members of the Faculty of Veterinary Medicine and Urmia University Research Council for the approval and financial support of this research.

References

Abolins, S., Thind, B., Jackson, V., Luke, B., Moore, D., Wall, R., Taylor, M.A., 2007. Control of sheep scab mite *Psoroptes ovis* in vivo and in vitro using fungal pathogens. Vet. Parasitol. 148, 310–317.

Alonso-Diaz, M.A., Garcia, L., Galindo-Velasco, E., Lezama-Gutierrez, R., Angel-Sahagun, C.A., Rodriguez-Vivas, R.I., Fragoso-Sanchez, H., 2007. Evaluation of *Metarhizium anisopliae* (Hyphomycetes) for the control of *Boophilus microplus* (Acari: Ixodidae) on naturally infested cattle in the Mexican tropics. Vet. Parasitol. 147, 336–340.

Bahiense, T.C., Fernandes, E.K.K., Bittencourt, V.R.E.P., 2006. Compatibility of the fungus *Metarhizium anisopliae* and deltamethrin to control a resistant strain of *Boophilus microplus* tick. Vet. Parasitol. 141, 319–324.

Benjamin, M.A., Zhioua, E., Ostfeld, R.S., 2002. Laboratory and field evaluation of the entomopathogenic fungus *Metarhizium anisopliae* (Deuteromycetes) for controlling questing adult *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. 39, 723–728.

Briggs, L.L., Colwell, D.D., Wall, R., 2006. Control of the cattle louse *Bovicola bovis* with the fungal pathogen *Metarhizium anisopliae*. Vet. Parasitol. 142, 344–349.

Butt, T.M., Goettel, M., 2000. Bioassays of entomogenous fungi. In: Navon, A., Ascher, K.R.S. (Eds.), Bioassays of Entomopathogenic Microbes and Nematodes. CAB International, Wallingford, pp. 141–195.

Fortescue, L.S., 1924. The Western Elburz and Persian Azerbaijan. Geogr. J. 63, 301–315.

Goettel, M.S., Inglis, G.D., 1997. Fungi: hyphomycetes. In: Lacey, L. (Ed.), Manual of Techniques in Insect Pathology. Academic, San Diego, CA, pp. 213–249.

Graf, J.F., Gogolewski, R., Leach-Bing, N., Sabatini, G.A., Molento, M.B., Bordin, E.L., Arantes, G.J., 2004. Tick control: an industry point of view. Parasitology 129, 427–442.

Hajek, A.E., St. Leger, R.J., 1994. Interactions between fungal pathogens and insect hosts. Annu. Rev. Entomol. 39, 293–322.

Hassanain, M.A., El Garhy, M.F., Abdel-Ghaffar, F.A., El-Sharaby, A., Abdel Megeed, N., 1997. Biological control studies of soft and hard ticks in Egypt. Parasitol. Res. 83, 209–213.

Hoogstraal, H., 1985. Argasid and nuttalliellid ticks as parasites and vectors. Adv. Parasitol. 24, 135–238.

Hornbostel, V.L., Ostfeld, R.S., Benjamin, M.A., 2005a. Effectiveness of *Metarhizium anisopliae* (Deuteromycetes) against *Ixodes scapularis* (Acari: Ixodidae) engorging on *Peromnyscus leucopus*. J. Vector Ecol. 30, 91–101.

Hornbostel, V.L., Zhioua, E., Benjamin, M.A., Ginsberg, H.S., Ostfeldt, R.S., 2005b. Pathogenicity of *Metarhizium anisopliae* (Deuteromycetes) and permethrin to *Ixodes scapularis* (Acari: Ixodidae) nymphs. Exp. Appl. Acarol. 35, 301–316.

Kaaya, G.P., Okech, M.A., 1990. Horizontal transmission of mycotic infection in adult tsetse, *Glossina morsitans morsitans*. Entomophaga 35, 46–57.

Kaaya, G.P., Hassan, S.M., 2000. Entomogenous fungi as promising biopesticides for tick control. Exp. Appl. Acarol. 24, 913–926.

Kettle, D.S. (Ed.), 1995. Medical and Veterinary Entomology. International University Press, Cambridge, pp. 449–454.

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

Leeflang, P., Ilemobade, A.A., 1977. Tick borne diseases of domestic animals in northern Nigeria. Trop. Anim. Health Prod. 9, 211–218.

Maketon, M., Oroz-Coghlan, P., Sinprasert, J., 2008. Evaluation of *Metarhizium anisopliae* (Deuteromycota: Hyphomycetes) for the control of broad mite *Polyphagotarsonemus latus* (Acari: Tarsonemidae) in mulberry. Exp. Appl. Acarol. 46, 157–167.

Ostfeld, R.S., Price, A., Hornbostel, V.L., Benjamin, A.B., Keesing, F., 2006. Controlling ticks and tick-borne zoonoses with biological and chemical agents. Bioscience 5, 383–394.

Pell, J.K., Eilenberg, J., Hajek, A.E., Steinkraus, D.C., 2001. Biology ecology and pest management potential of Entomophthorales. In: Butt, T.M., Jackson, C., Magan, N. (Eds.), Fungi as Biocontrol Agents: Progress, Problems and Potential. CAB International, Wallingford, pp. 71–153.

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

Rodriguez-Vivas, R.I., Alonso-Dı'az, M.A., Rodrı'guez-Arevalo, F., Fragoso-Sanchez, H., Santamaria, V.M., Rosario-Cruz, R., 2006. Prevalence and potential risk factors for organophosphate and pyrethroid resistance in *Boophilus microplus* ticks on cattle ranches from the state of Yucatan. Vet. Parasitol. 136, 335–342.

Rosenstein, M., 1976. Paralysis of chickens caused by larvae of the poultry tick *Argas persicus*. Avian Dis. 20, 407–409.

Samish, M., Ginsberg, H., Glazer, I., 2004. Biological control of ticks. Parasitology 129, S389–S403.

Schulze, T.L., Jordan, R.A., Hung, R.W., Krivenko, A.J., Schulze, J.J., Jordan, T.M., 2001. Effects of an application of granular carbaryl on nontargets forest floor arthropods. J. Econ. Entomol. 10, 149–154.

Sewify, G.H., Habib, S.M., 2001. Biological control of the tick fowl *Argas persicus*. J. Pest Sci. 74, 121–123.

Soliman, A.M., Mousa, S.A., Gad, N., Desouky, U., Sokkar, I.M., 1988. Rodents and ticks, as a reservoir of *Mycoplasma* in poultry farms. Assiut Vet. Med. J. 9, 184–190.

Stefanov, V., Matev, I., Balimezov, I., 1975. Role of ticks of the species *Argas persicus* Oken, 1818, in the epizootology of pullorum disease in birds. Vet. Med. Nauki 12, 45–50 (in Bulgarian).

Tavassoli, M., Ownag, A., Pourseyed, S.H., Mardani, K., 2008. Laboratory evaluation of three strains of the entomopathogenic fungus *Metarhizium anisopliae* for controlling *Dermanyssus gallinae*. Avian Pathol. 37, 259–263.

Tavassoli, M., Ownag, A., Meamari, R., Rahmani, S., Mardani, K., Butt, T., 2009. Laboratory evaluation of three strains of the entomopathogenic fungus *Metrahizium anisopliae* for controlling *Hyalomma anatolicum anatolicum* and *Haemaphysalis punctate*. Int. J. Vet. Res. 3, 11–15.

Trager, W., 1940. A note on the problem of acquired immunity to argasid ticks. J. Parasitol. 26, 71–74.

Wall, R., Shearer, D. (Eds.), 1997. Veterinary Entomology. Chapman and Hall International Thompson Publisher Company, London.

Whipps, J.M., Lumsden, R.D., 2001. Commercial use of fungi as plant disease biological control agents: status and prospectus. In: Butt, T.M., Jackson, C.W., Magan, N. (Eds.), Fungi as Biological Control Agents—Progress, Problems and Potential. CAB International, Wallingford, UK, pp. 9–22.

Zabalgogeazcoa, I., Oleaga, A., Perez-Sanchez, R., 2008. Pathogenicity of endophytic entomopathogenic fungi to *Ornithodoros erraticus* and *Ornithodoros moubata* (Acari: Argasidae). Vet. Parasitol. 158, 336–343.