Tropical Biomedicine 32(3): 463–470 (2015)

Toxicity of entomopathogenic fungi, *Beauveria bassiana* and *Lecanicillium muscarium* against a field-collected strain of the German cockroach *Blattella germanica* (L.) (Dictyoptera: Blattellidae)

Davari, B.[1], Limoee, M.[2*], Khodavaisy, S.[3], Zamini, G.[4] and Izadi, S.[5]
[1]Department of Medical Entomology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; Kurdistan Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
[2]School of Public Health, Research Center for Environmental Determinants of Health (RCEDH), Kermanshah University of Medical Sciences, Kermanshah, Iran
[3]Department of Medical Mycology and Parasitology, Kurdistan University of Medical Sciences, Sanandaj, Iran; Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Medical Parasitology and Mycology, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
[5]Department of Parasitology and Mycology, Tehran University of Medical Sciences, Tehran, Iran; Isfahan National Institute of Health Research, Isfahan, Iran
[*]Corresponding author email: mojtabalimoee@yahoo.com
Received 12 January 2014; received in revised form 26 December 2014; accepted 26 December 2014

Abstract. The German cockroach, *Blattella germanica* (L.) has been recognized as a serious health problem throughout the world. Control failures due to insecticide resistance and chemical contamination of environment have led some researchers focus on the other alternative strategy controls. Microbial insecticides such as those containing entomopathogenic fungi could be of high significance. *Lecanicillium muscarium* and *Beauveria bassiana* grow naturally in soils throughout the world and act as a parasite on various arthropod species, causing white muscardine disease. Thus, these two species could be considered as entomopathogenic fungi. The current study conducted to evaluate the toxicity of *Beauveria bassiana* and *Lecanicillium muscarium* against German cockroach, *Blattella germanica*. Conidial formulations of *L. muscarium* (PTCC 5184) and *B. bassiana* (PTCC5197) were prepared in aqueous suspensions with Tween 20. Bioassays were performed using two methods including submersion of cockroaches in conidial suspension and baiting. Data were analyzed by Probit program and LC_{50} and LC_{90} were estimated. The obtained results indicated that both fungi species were toxic against German cockroach however; *Beauveria bassiana* was significantly 4.8 fold more toxic than *L. muscarium* against German cockroach using submersion method.

INTRODUCTION

The German cockroach, *Blattella germanica* (L.) is a world-wide pest in households, hospitals and residential areas that can cause serious health problem because of its potential to transmit human infectious diseases (Roberts, 1996; Pai *et al.*, 2003, 2005; Pai, 2013).

Extensive use of insecticides against the German cockroach has led to the development of insecticide resistance in this insect pest. Resistance to different classes of insecticides including chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids has been frequently reported in the German cockroach, *Blattella germanica* (Cochrane, 1995; Lee *et al.*, 1996;

Valles, 1998; Valles *et al*., 2000; Wei *et al*., 2001; Prigdeon *et al*., 2002; Limoee *et al*., 2006; Limoee *et al*., 2011). Nevertheless, the development of insecticide resistance in the German cockroach has resulted in control failures of some populations (Valles and Yu, 1996; Dong *et al*., 1998). The concerns about of control failures of German cockroach and, as well as, chemical contamination of environment due to insecticide application have led some researchers to focus on the other alternative strategy controls.

Thus, biological control measures of this pest including microbial insecticides such as those containing entomopathogenic fungi could be of high significance. In addition to some parasitoids belonging to different families of insects that are capable for biological control of cockroaches (Lebeck, 1991; Shamim *et al*., 2001; Hwang & Chen, 2004), a species of bacteria also reported that could be an effective agent in controlling of these insects (Payne *et al*., 1994). Insect pathogens particularly bacteria and entomopathogenic fungi such as *Beauveria bassiana* have a long history in biological control of various pests (Lacey & Kaya 2007). On the other hand, habitats of cockroaches are generally warm and moist so are favorable conditions for being infected cockroaches by different genera of fungi. For instance, four genera of fungi including *Metarhizium*, *Paecilmyces*, *Verticillium* and *Aspergillus* have been reported as potential agents for biological control of cockroaches (Pathak & Kulshrestha, 1998). Lopes & Alves, 2011, suggested that application of a powdery formulation of *Metarhizium anisopliae* to provide a contaminated surface to control German cockroach, may be more effective than using bait (Lopes & Alves, 2011). Another potential alternative in controlling the German cockroach has been the integration of insecticides and pathogenic fungi so, Wakil *et al*., 2012 have evaluated the efficacy of *Metarhizium anisopliae* in combination with sublethal doses of some insecticides against American cockroach. They concluded that combined use of insecticides and fungus, enhanced the lethal effect of *Metarhizium anisopliae* (Wakil *et al.*, 2012).

Although attempts have been made to evaluate the pathogenicity of a few species of fungi against some species of insects, for example, Sharififard *et al*., 2011, evaluated the pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to larvae and adult of house fly, *Musca domestica* (Sharififard *et al*., 2011), very little is known about toxicity of entomophatogenic fungi against German cockroach, *Blattella germanica*, in Iran. Thus, the current study conducted to evaluate the toxicity of *Beauveria bassiana* and *Lecanicillium muscarium* against a hospital collected strain of German cockroach, *Blattella germanica*.

MATERIALS AND METHODS

Cockroaches

Cockroaches were collected from residential areas and hospitals in Sanadaj and transferred to the insectary. All cockroaches were maintained in the glass rearing jars at 30ºC, 55–60% (RH), with a photoperiod of 12:12 h (L:D). The insects were provided cat food and water. Tests were conducted on third and forth instars and adult males and females.

Fungal isolates, cultivation and preparation of conidial suspension

All fungal isolates of *Lecanicillium muscarium PTCC 5184* and *Beauveria bassiana PTCC5197* were obtained from the Iranian research organization for science and technology. The fungi were cultured on potato dextrose agar (PDA) at 25±1ºC and relative humidity (RH) ≥ 80% for 15 days. Conidia were harvested from culture plates by gently scarping the medium surface with a plastic loop. A 30ml suspension was prepared with harvested conidia in sterile aqueous 0.1% Tween 80 solution in a 50-ml- falcon TM test tube. The conidial suspension was homogenized for 3 min using a vortex mixer. Conidia were also suspended in an oil solution of 15 ml sterile mineral oil with 84 ml sterile distilled water and 1 ml Tween 80. Conidial suspensions were quantified in hemacytometer and adjusted to 1 x 10^9 conidia ml^{-1}. The suspensions 1 x 10^7, 10^6 and

10^5 conidia ml^{-1} were prepared by serial dilutions. Conidia viability was determined by plating 100µl of conidial suspensions on PDA and incubating at 25±1ºC (Scholte *et al.*, 2007).

Bioassays

Toxicity of *Beauveria bassiana* and *Lecanicillium muscarium* against German cockroach was determined by two methods including submersion of cockroaches in conidial suspension and baiting.

Submersion method

The stock aqueous conidial suspension adjusted to 1 x 10^9 conidia ml^{-1} was serially diluted to prepare 6 doses including 1 x 10^4, 10^5, 10^6, 10^7, 10^8 and 10^9 conidia ml^{-1}. Cohort groups of 40 cockroaches were simultaneously submerged in the six aqueous treatments consisting of above concentrations and a control prepared with 0.1% Tween 80 aqueous solution for 5 *sec*. Each treatment contained 4 replicates of 10 cockroaches. Each group of 10 treated cockroaches transferred to the plastic containers provided with food and water. All containers were incubated at 28±2ºC and 75±5% RH. The containers were monitored and died cockroaches were removed daily. After the sterilization of surface, all cadavers transferred to the sterile Petri dishes. Cadavers with growing muscardine on their surface were recorded as mortality due to fungal infection. These tests were replicated four times in groups of ten cockroaches.

Baiting Method

Each 5g bait contained 3g sugar, 2g powder milk and 2 ml distilled water. For better dispersion of fungus, 1 ml of each stock aqueous conidial suspension was dispersed on the surface of 5g baits to give seven concentrations of treated baits including 1x10^3, 1x10^4, 1x10^5, 1x10^6, 1x10^7, 1x10^8 and 1x10^9 conidia/g. Treated baits were left in the cages provided with water and cat food at 28±2ºC and 75±5% RH. Each cage contained a 9 cm diameter petridish with Whatman filter paper and 5g bait. Each treatment contained 4 replicates of 10 cockroaches. The cages were monitored and died cockroaches were removed daily. Cadavers with growing muscardine on their surface were recorded as mortality due to fungal infection.

Data analysis

Bioassay data were pooled and subjected to probit analysis (Finny, 1972) using a statistical Software (SAS 1998). The lethal concentrations LC_{50} and LC_{90} with confidence limits 95% were estimated. Comparisons between toxicities of two fungi by two methods against cockroaches were performed by calculating the ratios of LC_{50} values (LC_{50} value of the less toxic fungus divided by LC_{50} value of the more toxic one). The differences between LC_{50} values were considered statistically significant only when the 95% confidence limits did not overlap.

RESULTS

Submersion method

Both of two fungi species showed toxicity against German cockroaches when these insects were submerged in the aqueous conidial suspensions compared with the control group. Comparisons made between the 95% confidence limits of the LC_{50} values of *Beauveria bassiana* and *Lecanicillium muscarium* indicated that there was no significant difference between the toxicity of two fungi against German cockroach by submersion method (Table 1).

Baiting method

Bioassay tests using bait showed that both of two fungi species were toxic against German cockroach compared with the control group (Table 2) however, comparisons made between the 95% confidence limits of the LC_{50} values indicated that *Beauveria bassiana* was significantly 4.8 fold more toxic than *Lecanicillium muscarium* based on LC_{50} ratio (LC_{50} value of the less toxic fungus *Lecanicillium muscarium* divided by LC_{50} value of the more toxic one *Beauveria bassiana*) (Table 3) whereas; comparison made between the toxicity of fungi based on application methods indicated that there was no significant difference between submersion and baiting methods (Table 3).

Table 1. Probit analysis parameters for mortality results of submerged German cockroach in aqueous conidial suspensions (conidia/ml^{-1}) of *Beauveria bassiana* and *Lecanicillium muscarium*

Fungus	n	y-intercept	Slope(SE)	X^2(df)	LC_{50}(95%CL) conidia/ml[a]
Beauveria bassiana	240	1.44	0.49± 0.05	1.692(4)	1.5x10^7(3.5-6.3)
Lecanicillium muscarium	240	0.84	0.52±0.06	1,58(4)	8.1x 10^7(1.9-3.6)

[a]Conidia of fungus/1 ml of aqueous suspension

Table 2. Probit analysis parameters for mortality results of German cockroach using baiting method

Fungus	n	y-intercept	Slope(SE)	X^2(df)	LC_{50}(95%CL) conidia/g[a]
Beauveria bassiana	280	1.79	0.44±0.04	3.22 (5)	2x1o^7 (5- 8.2)
Lecanicillium muscarium	280	2.48	0.32±0.04	4.05 (5)	9.6x10^7(2.9 - 4)

[a]Conidia of fungus/1g of bait

Table 3. Comparisons between two bioassay methods and toxicity of two fungi based on LC_{50} values of *Beauveria bassiana* & *Lecanicillium muscarium* against German cockroach

Toxin - Method	Submersion *Lecanicillium muscarium*	Bait *Lecanicillium muscarium*	Bait *Beauveria bassiana*
Submersion *Beauveria bassiana*	5.6 Nonsig	—	1.33 Nonsig
Bait *Beauveria bassiana*	—	4.8 sig	—
Bait *Lecanicillium muscarium*	1.19 Nonsig	—	—

Sig.: Significant
Nonsig: Nonsignificant

DISCUSSION

The concerns about insecticides resistance and environmental impacts of pesticides application have involved the biological control agents of the pests. Biological control measures such as some bacteria and entomopathogenic fungi *Beauveria bassiana* have a long history in controlling of various pests (Lacey & Kaya, 2007) so that, some researchers have frequently focused on the potential effectiveness of entomopathogenic fungi in controlling of cockroaches (Pathak & Kulshrestha, 1998; Lopes & Alves, 2011; Wakil *et al.*, 2012). Many entomopathogenic fungi species including *Beauveria bassiana* and *Lecanicillium muscarium* have been recognized as saprophytic soil fungi that could be used for natural control of agricultural pests (Wakil *et al.*, 2013). According to Vega *et al.*, 2009, for the first time a fungus caused the muscardine disease of silkworm was identified as *Botrytis bassiana* (Wakil *et al.*, 2013). At present,

regards to the great diversity of the entomopathogenic fungi, they have been considered as the potent agent for biological control of various insect pests so that, a variety of fungal formulations of insecticidal effects have been developed from several species of fungi including *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosorsea*, *B. brongniartii* (Faria & Wraight, 2007). Based on results obtained in our study it can be concluded that *Beauveria bassiana* and *Lecanicillium muscarium* were toxic against German cockroach similarly, Wakil *et al.*, 2013, reported that *Beauveria bassiana* and *B. brongniartii* were the most pathogenic fungal species against the larvae of wax moth Galleria mellonella L. (Lepidoptera: Pyralidae) compared with the other fungi (Wakil *et al.*, 2013).

Our obtained results under laboratory condition indicated that the fungi *Beauveria bassiana* and *Lecanicillium muscarium* could provide a good control of *B. germanica* . However, it is necessary to conduct several additional researches at field conditions to assess the real effectiveness of these fungi in controlling of the German cockroach. Field studies could demonstrate the efficacy of the fungi in an integrated pest management of cockroaches.

Hence, some efforts have been made to develop the most appropriate formulations of these fungi providing more effective control of the pests. For instance, some researchers suggested that the development of powder or similar formulations of *M. anisopliae* to control *B. germanica* may provide faster and better results than some of the strategies based on baits currently available (Lopes & Alves, 2011).

The other workers revealed that the appropriate formulations could be of high importance in controlling of the pests for example, *B. bassiana* formulated in invert emulsion was more effective than unformulated conidia of this fungus (batta, 2007) and conidial oil-based suspension of *L ecanicillium lecanii* was more effective than oil-aquos suspension (Angello *et al.*, 2010).

Furthermore, as it was mentioned above, the fungi can be used in an integrated pest management program thus, we could also integrate the insecticide and pathogenic fungi against German cockroach. Some researchers have made efforts to enhance pathogenicity of different species of fungi by integrating them with sublethal doses of insecticides (Pachamuthu & Kamble, 2000; Zurek *et al.*, 2002; Wakil *et al.*, 2012).

Briefly, the researches suggesting the most effective application methods of fungi were described below.

Although two fungi were pathogenic against German cockroach nevertheless, attempts were made to identify the effective application method. It was revealed that application method of fungus was of high significance in controlling of cockroaches so that using of bait was significantly more effective than submersion method. This might be due to either loss of activity of the aqueous conidial of fungi (Bata, 2007) or cuticle barrier resulting in limited delivered insecticidal molecules to target sites (Grula *et al.*, 1978). On the other hand, it has been frequently confirmed that temperature and relative humidity under field conditions affected on the efficacy of the unformulated conidia of *B. bassiana* (Luz *et al.*, 1998; Luz & Fargues 1999; Haraprasad *et al.*, 2001; Lecuona *et al.*, 2001). For instance, Behle 2006, mentioned that the aqueous conidial suspension of *B. bassiana* became inactive under field conditions because of a loss of conidial viability so they showed no insecticidal activity (Behle, 2006). In general it can be concluded that the efficacy of entomopathogenic fungi against the targeted insect pest might be depend on their appropriate formulation for instance, according to Batta 2007, *B. bassiana* formulated in invert emulsion showed a high efficacy against the adult of almond dark beetle *Scolytus amygdali* (Batta, 2007). Angelo *et al.*, 2010 evaluated the efficacy of *Lecanicillium lecanii* in controlling of a hard tick *Rhipicephalus microplus*. Conidial formulation of *L. lecanii* in both oil and aqueous suspensions were applied. The authors suggested that conidial oil-based

suspension of *L. lecanii* was more effective than aqueous suspension of this fungus as biological control agent. They implied that oil assisted in the process of conidial adhesion to the cuticle, which was the first step of infection (Angelo, *et al*., 2010).

In present study, our findings under laboratory condition implied that using bait against cockroaches were more effective than the emersion method. Although, these findings consistent with the results obtained by Sharififard *et al.*, 2011which indicated this method of application of fungi against house fly was a suitable method (Sharififard *et al.*, 2011), it has been reported that Biopath Cockroach Chamber bait product commercially available in the USA in 1990s was not effective against German cockroach under field conditions. It could be suggested that the Biopath product had an important limitation of infection so that a long period of time was necessary for the fungi to control German cockroach (Kaakeh *et al*., 1997; Pachamuthu *et al*., 1999; Lopez & Alvez, 2011).

Based on our results and the reports mentioned above, we can suggest that using the bait could be an effective application method of *B. bassiana* against German cockroaches, *Blattella germanica* in the absence of properly formulated entomopathogenic fungi against this insect species.

REFERENCES

Angelo, I.C., Fernandes, E.K.K., Bahiense, T.C., Perinoto, W.M.S., Moraes, A.P.R., Terra, A.L.M. & Bittencourt, V.R.E.P. (2010). Efficiency of Lecanicillium lecanii to control the tick Rhipicephallus micropolus. *Veterinary Parasitology* **172**: 317-322.

Batta, Y.A. (2007). Biocontrol of almond bark beetle (*Scolytus amygdale* Geurin-Meneville, Coleoptera: Scolytidae) using *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes). *Journal of Applied Microbiology* **103**: 1406-141.

Behle, R.W. (2006). Importance of direct spray and spray residue contact for infection of *Trichoplusia ni* larvae by field application of *Beauveria bassiana*. *Journal of Economic Entomology* **99**: 1120-1128.

Cochran, D.G. (1995). Misuse of the tarsal-contact method for detecting insecticide resistance in the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology* **90**: 1441-1444.

Dong, K., Valles, S.M., Scharf, M.E., Zeichner, B. & Bennet, G.W. (1998). The knockdown resistance (kdr) mutation in pyrethroid-resistant German cockroach. *Pest. Biochem. Physiol.* **60**: 195-204.

Faria, M.R. & Wraight, S.P. (2007). Myco-insecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control* **43**: 237-256.

Finny, D.J. (1972). Probit analysis, third ed., Cambridge University, London. 333pp.

Grula, S.C., Burton, R.L., Smith, R., Mapes, T.,L., Cheung, P.Y.K., Pekrul, S., Champlin, F.R., Grula, M. & Abegaz, B. (1978). Biological basis for the pathogenicity of Beauveria bassiana. Proceedings of the first joint USA/USSR Conference on the production, selection and standardization of entomophatogenic fungi, Ed. By C.M. Lgnoffo, ED.; Washington DC; *Amer. Soc. Microbial.* 192-216.

Haraprasad, N., Niranjana, S.R., Prakash, H.S., Shetty, H.S. & Wahab, S. (2001). *Beauveria bassiana* – a potential mycopesticide for the efficient control of coffee berry borer, *Hypothenemus hampei* (Ferrari) in India. *Biocont. Sci. Technol.* **11**: 251-256.

Hwang, S.Y. & Chen, L.M. (2004). Effects of four physical treatments of oothecae of *Periplaneta americana* on paratistism and development of parasitic wasp *Evania appendigaster*. *Environmental Entomology* **33**(5): 1321-1325.

Kaakeh, W., Reid, B.L., Bohnert, T.J. & Bennet, G.W. (1997). Toxicity of imidacloprid in the German cockroach (Dictyoptera: Blattellidae) and synergism between imidacloprid and *Metarhizium anisopliae*. *J of Econ Entomol*. **90**: 473-482.

Lacey, L.A. & Kaya, H.K. (2007). Field manual of techniques in invertebrate pathology. *2nd edn. Springer. The Netherlands*.

Lebeck, L.M. (1991). A review of the hymenopterous natural enemies of cockroaches with emphasis on biological control. *Bio-Control* **36**(3): 335-352.

Lecuona, R.E., Edelstein, J.D., Berratta, M.F., La Rossa, F.R. & Arcas, J.A. (2001). Evaluation of *Beauveria bassiana* (Hyphomycetes) strains as potential agents for control of *Triatoma infestans* (Hemiptera: Reduviidae). *J Med Entomol* **38**: 172-179.

Lee, C.Y., Yap, H.H., Chong, N.L. & Lee, R.S.T. (1996). Insecticide resistance and synergism in field collected German cockroach (Dictyoptera Blattellidae) in Peninsular Malaysia. *Bulletin Entomological Research* **86**: 675-682.

Limoee, M., Ladonni, H., Enayati, A.A., Vatandoost, H. & Aboulhasani, M. (2006). Detection of pyrethroid resistance and cross-resistance to DDT in sven field-collected strains of the German cockroach *Blattella germanica* (L.) (Dictyoptera: Blattellidae). *Journal of Biological Sciences* **6**(2): 382-387.

Limoee, M., Enayati, A.A., Khassi, K., Salimi, M. & Ladonni, H. (2011). Insecticide resistance and synergism of three field-collected strains of the German cockroach *Blattella germanica* (L.) (Dictyoptera: Blattellidae) from hospitals in Kermanshah, Iran. *Tropical Biomedicine* **28**(1): 111-118.

Lopes, R.B. & Alves, S.B. (2011). Differential susceptibility of adults and nymphs of *Blattella germanica* (L.) (Blattodea: Blattellidae) to infection by *Metarhizium anisopliae* and assessment of delivery strategies. *Neotrop. Entomol* **40**(3): 368-374.

Luz, C. & Fargues, J. (1999). Dependence of the entomopathogenic fungus, *Beauveria bassiana*, on high humidity for infection of *Rhodnius prolixus*. *Mycopathologia* **146**: 33-41.

Luz, C., Tigano, M.S., Silva, I.G., Cordeiro, C.M. & Aljanabi, S.M. (1998). Selection of Beauveria bassiana and Metarhizium anisopliae isolates to control Triatoma infestans. Mem Inst Oswaldo Cruz, Rio de Janeiro **93**: 8.

Pachamuthu, P. & Kamble, S.T. (2000). *In vitro* study on combined toxicity of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) isolate ESC-1 with sublethal doses of chlorpyrifos, propetamphos and cyfluthrin against German cockroach (Dictyoptera: Blattellidae). *J Econ Entomol* **93**: 60-70.

Pachamuthu, P., Kamble, S.T. & Yuen, G.Y. (1999). Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) isolate ESC-1 to the German cockroach (Dictyoptera: Blattellidae) and its compatibility with insecticides. *J Econ Entomol* **92**: 340-346.

Pai, H.H., Chen, W.C. & Peng, C.F. (2005). Isolation of bacteria with antibiotic resistance from household cockroaches (*Periplaneta americana* and *Blattella germanica*). *Acta Tropica* **93**: 259-265.

Pai, H.H., Ko, Y.C. & Chen, E.R. (2003). Cockroaches (*Periplaneta Americana* and *Blattella germanica*) as potential mechanical dissemination of *Entamoeba histolytica*. *Acta Tropica* **87**: 355-359.

Pai, H.H. (2013). Multidrug resistant bacteria isolated from cockroaches in long-term care facilities and nursing homes. *Acta Tropica* **125**: 18-22.

Pathak, S.C. & Kulshrestha, V. (1998). Experimental aspergillosis in the German cockroach *Blattella germanica*: a histopathological study. *Mycopathologia* **143**(1): 13-16.

Payne, J.M., Kennedy, K.M., Randall, J.B. & Brower, D.O. (1994). *Bacillus thuringiensis* isolates active against cockroaches and genes encoding cockroach-active toxins. US. Patent No. 5302387.

Prigdeon, J.W., Appel, A.J., Moar, W.J. & Liu, N. (2002). Variability of resistance mechanisms in pyrethroid resistant German cockroach (Dictyoptera: Blattellidae). Pesticide Biochemistry and Physiology **73**: 149.

Quesada-Moraga, E., Santos-Quiros, R., Valverde-Garcia, P. & Santiago-Alvarez, C. (2004). Virulence, horizontal transmission, and sublethal reproductive effects of *Metharhizium anisopliae* (Anamorphic fungi) on the German cockroach (Blattodea: Blattellidae). *Journal of Invertebrate Pathology* **87**: 51-58.

Roberts, J. (1996). Cockroaches linked with asthma. *British Medical Journal* **312**: 1630.

Scholte, E., Takken, W. & Knols, B.G.J. (2007). Infection of adult *Aedes aegypti* and *Ae. Albopictus* mosquitoes with the entomopathogenic fungus *Metarhizium anisopilae*. *Acta Tropica* **102**: 151-158.

Shamim, S.A., Islam, W. & Mondal, K.A.M.S.H. (2001). Biological control potential of the cockroach parasitoid *Aprostocetus asthenogmus* (Waterson) (Hymenoptera: Eulophidae). *International Pest Control* **43**(2): 68-71.

Sharififard, M., Mossadegh, M.S., Vazirianzadeh, B. & Zarei Mahmoudabadi, A. (2011). Laboratory evaluation of pathogenicity of entomopathogenic fungi, *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metch.) Sorok. To larvae and adults of the house fly, *Musca domestica* L. (Diptera: Muscidae)

Valles, S.M. (1998). Toxicological and biochemical studies with field population of the German cockroach, *Blattella germanica*. *Pesticide Biochemistry and Physiology* **62**: 192-200.

Valles, S.M., Dong, K. & Brenner, R.J. (2000). Mechanisms responsible for cypermethrin resistance in a strain of German cockroach, *Blattella germanica* (L.). *Pesticide Biochemistry and Physiology* **66**: 195-205.

Valles, S.M. & Yu, S.J. (1996). Detection and biochemical characterization of insecticide resistance in the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology* **89**: 21-26.

Wakil, W., Ghazanfar, M.U., Riasat, T., Kwon, Y.J., Qayyum, M.A. & Yasin M. (2013). Occurrence and diversity of entomopathogenic fungi in cultivated and uncultivated soils in Pakistan. *Entomological Research* **43**: 70-78.

Wakil, W., Yasin, M., Qayyum, M.A. & Asim, M. (2012). Combined toxicity of *Metarhizium anisopliae* with sublethal doses of chlorpyrifos, fipronil and chlorantraniliprole against *Periplaneta americana* (Dictyoptera: Blattidae). *Pakistan Entomologist* **34**(1): 59-63.

Wei, Y., Appel, A.G., Moar, W.J. & Liu, N. (2001). Pyrethroid resistance and cross-resistance in the German cockroach, *Blattella germanica* (L.). *Pest Management Science* **57**: 1055-1059.

Zurek, L., Watson, D.W. & Schal, C. (2002). Synergism between *Metarhizium anisopliae* (Deuteromycoyina: Hyphomycetes) and boric acid against the German cockroach (Dictyoptera: Blattellidae). *Biol Control* **23**: 296-302.