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Pathogenicity of Different Isolates of Entomopathogenic Fungi on Cotton Mealybug, *Phenaccocus solenopsis* Tinsley

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ABSTRACT

Cotton mealybug, *Phenacoccus solenopsis* Tinsley is a destructive pest of cotton, ornamental plants and many other crops due to its polyphagous nature. A study was conducted to check the efficacy of different local isolates of entomopathogenic fungi on 2nd nymphal instar of *P. solenopsis* under laboratory conditions by immersion method. Three entomopathogenic fungi; *Beauveria bassiana* (isolates Bb-01, Bb-08), *Metarhizium anisopliae* (isolates Ma-11.1, Ma-2.1) and *Isaria fumosorosea* (isolates If-2.3, If-02) showed percent mortalities of (61.0%, 85.0%), (78.0%, 56.0%) and (52.0%, 54.0%) with LC_{50} values of (4.25×10^8, 2.54×10^8 spores/ml), (3.26×10^8, 5.08×10^8 spores/ml) and (6.22×10^8, 7.20×10^8 spores/ml) with LT_{50} (6.43, 4.80), (5.43, 6.74) and (6.66, 6.69) days at highest concentrations. Amongst all isolates, *B. bassiana* isolate Bb-08 was highly efficient against cotton mealybug with highest percent mortality and lowest LC_{50} and LT_{50} values. The study showed that Bb-08 can be used in the IPM of *P. solenopsis*.

INTRODUCTION

Cotton, *Gossypium hirsutum* also famous as white gold is the primary cash crop of Pakistan and it contributes approximately 1.5% of GDP. Cotton crop is attacked by many sucking and chewing insect pests (Saeed *et al*., 2007) and that is the reason of about 20-40% loss per annum (Ahmad, 1999). Cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has a large ecological pattern (Fuchs *et al*., 1991; Williams and Granara de Willink, 1992). The cotton mealybug, due to its polyphagous feeding behavior, is a severe insect pest worldwide including Taiwan, Thailand, India and Pakistan (Yousuf and Tayyib, 2007; Hodgson *et al*., 2008; Abbas *et al*., 2010). From the Eastern region of Sri Lanka, recent information due to the attack of cotton mealybug is originated (Prishanthini and Vinobaba, 2009) where it is found on vegetables, weeds and ornamental plants and in China, on China rose plants (Wang *et al*., 2009; Wu and Zhang, 2009). During the year 2005, for the first time in Pakistan (South Asia) this insect pest was reported and in Pakistan (Anonymous, 2008a) and India (Anonymous, 2008b; Nagrare *et al*., 2008) it has become a widespread pest causing severe damage to *Gossypium* fields in Punjab and Sindh Provinces (Anonymous, 2006, 2008c; Zaka *et al*., 2006; Kakakhel, 2007).

Large populations of mealybugs cause general weakening, yellowing and malformation of leaves and defoliation, dropping of fruits and death of susceptible plants if unable to control. Indirectly, it may also damage plants by serving as vectors of plant diseases. Moreover, the honeydew excreted by the mealybugs causes growth of sooty moulds (Saeed *et al*., 2007), and other secondary infections that decrease photosynthesis and reduces the marketability of plant products. The feeding by mealybugs influences the growing points resulting in smaller fruit or flowers, which eventually decreases seed production (Afzal *et al*., 2014).

A large number of synthetic pesticides belonging to carbamate, organophosphate, pyrethroid and new chemistry groups are being applied for the management of this insect pest. On the other hand, the cryptic habit and waxy body causes hindrance in the efficient control of *P. solenopsis* with conventional synthetic insecticides. Chemicals provide only short term control, repeated applications and due to injudicious use of these synthetic insecticides, resistance has become the main issue (Ramakrishnan *et al*., 1984).

Due to drawbacks of synthetic chemical pesticides, it is necessary to develop an alternative tactic such as biological control, which is safe and eco-friendly. Biological control involving insect pathogens, predators

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and parasitoids, have effectively suppressed mealybugs of majority of the crops, e.g., *Phenacoccus manihoti* (Herren and Neuenschwander, 1991), *Maconellicoccus hirsutus* (Kairo *et al*., 2000) and *Planococcus citri* (Singh, 2004).

Amongst the biocontrol agents, entomopathogenic fungi serve as mycoinsecticide (Faria and Wraight, 2007) and the use of different entomopathogenic microorganisms are gaining importance due to their target specificity and the environment safety. The pest control scenario primarily of insect pathogenic fungi including, *Beauveria bassiana, Metarhizium anisopliae* and *Isaria fumosorosea* (Marannino *et al*., 2006; Kaaya and Munyinyi, 1995) have been proved beyond doubt over the decades. Insect pathogenic fungi are the component of integrated control strategies for various insect pests of number of economic cash crops. These fungi are easy to cultivate for mass production and all over the world these microbes are being commercially prepared and effectively used in green houses and field conditions. Another important fact to be considered in favour of these fungi is that, to date there has no report of developing resistance. The present study was conducted to check the efficiency of local isolates of different insect pathogenic fungi against *P. solenopsis* under laboratory conditions.

MATERIALS AND METHODS

Collection and rearing of P. solenopsis

The population of mealybug, *P. solenopsis* was collected from the cotton field of Bahauddin Zakariya University, Multan. Mealybugs were reared on fresh leaves of China rose, *Hibiscus Rosa sinensis* the preferred ornamental host plant of the mealybug. In order to develop the culture of mealybug, stems of the host plants attacked with adult females were brought to the Laboratory. Insects were separated and inoculated on China rose plants and reared in the lab. The female *P. solenopsis* settled on host plants leaves and twigs, started egg laying after 2 days. The newly hatched crawlers emerged out and started feeding on the China rose leaves which were not exposed to any insecticide applications previously and free from the infestation of *P. solenopsis*. Leaves and twigs were washed with tap water, dried with tissue paper and utilized as food source. The crawlers were placed on China rose leaves with fine camel hair brush. The culture was placed in plastic jars (13×22 cm). Fresh leaves were provided after every 1-2 days under lab. conditions at 27 ± 2˚C and 60 ± 5% RH with a 14:10 h light: dark photoperiod.

Propagation of media and culturing of insect pathogenic fungi

Six different isolates of entomopathogenic fungi were used to check the toxicity of entomopathogenic; *M. anisopliae* (Ma-11.1 and Ma-2.1), *I. fumosorosea* (If-02 and If-2.3), *B. bassiana* (Bb-01 and Bb-08). Potato dextrose agar (PDA) (potato 200g, glucose 20g, agar 20g and 1000ml water) was freshly prepared by means of distilled water and commercial ingredients. This media was transferred into the petri dishes which were introduced with the spores of different insect pathogenic fungi. Later, after inoculation, all petri dishes were incubated at 25°C for 14 days and after incubation, the spores were harvested solution in 0.05% Tween 80. The spore's concentration was determined by hemocytometer and afterwards, required concentrations *i.e.,* 2×10^8 to 7×10^8 spores/ml of each isolate was made by serial dilution. On the PDA plates (9 cm diameter), the spores from slant culture were inoculated which were placed for 14 days at 25°C in darkness at 70-75% RH for more propagation. Fungal spores were applied to the insects or stored at 4°C until utilized for bioassay of insect after 14 days of fungal growth.

Experimental method and bioassay

The experiment was conducted under completely randomized design (CRD) with six concentrations including control for each fungal treatment, while each concentration was replicated five times. The efficiency of different entomopathogenic fungi was assessed by immersion method on 2nd instar nymphs of *P. solenopsis*. 750 nymphs of *P. solenopsis* from the laboratory reared culture were exposed with the suspension of all fungal concentrations (2×10^8 - 7×10^8 spores/ml) with 25 individuals per replication. Nymphs of *P. solenopsis* were individually immersed in the concentration for least 8-10 sec. Treated insects were placed on the tissue paper to soak up the excess moisture, then transferred into petri dishes and provided with China rose leaves, while in the control treatment, nymphs were treated with 0.05% Tween 80 solution.

Mortality data was recorded on daily basis for continuous 7 days at 24 h interval. Dead nymphs were collected daily and placed in sterile Petri dishes containing damp filter paper. Morality was taken into account for those nymphs which sporulation was visible.

Data analysis

Percent mortality was calculated on each day. The mortality was corrected where necessary by Abbot's formula (Abbot, 1925). LC_{50} and LT_{50} values of each isolate were calculated for nymphs by using probit analysis (Finney and Stevens, 1948). Percent mortality was analyzed and compared by LSD test by using Statistics 8.1 statistical software.

RESULTS

Pathogenicity of entomopathogenic fungi against P. solenopsis

Six isolates of entomopathogenic fungi were found pathogenic to cotton mealybug. Significantly different mortalities were obtained at each conidial concentration tested. The highest mortality was found at concentration 7×10^8 conidia/ml in all the four isolates against second instar of mealybug (Table I). The entomopathogenic fungi (isolate Bb-08) was found to be more efficient causing highest mortality of second instar (85.0%) at concentration of 7×10^8 conidia/ml. Higher nymphal mortality with increase in spore concentration of entomopathogenic fungi shows that their efficiency was in proportion to the concentration of spores.

Concentration and time mortality response

Tables I and II indicate median lethal concentration (LC_{50} conidia/ml) and median lethal time (LT_{50} days), respectively for all entomopathogenic fungi against second instar nymphs of mealybug. The LC_{50} (2.54×10^8 spores/ml) was found to be lowest for isolate Bb-08 against 2nd instar. Similarly, the isolate Bb-08 also showed lowest LT_{50} values (4.80 days) in respect of second instar of *P. solenopsis*.

Accumulative percentage mortality

Accumulative percentage mortality of isolates of insect pathogenic fungi, *B. bassiana* (Bb-08 and Bb 01), *I. fumosorosea* (If-02, If 2.3) and *M. anisopliae* (Ma-2.1 and Ma-11.1) on mealybug are shown in Figure 1. Percent mortality caused by isolate Bb-08 range from 45.0 to 85.0%, isolate Bb-01 (34.0 to 60.0%), isolate If-02 (32.0 to 54.0%), isolate If-2.3 (32.0 to 52.0%), isolate Ma-2.1 (38.0 to 56.0%) and isolate Ma-11.1(36.0 to 78.0%), respectively over a period of 7 days at the concentrations from 2×10^8 to 7×10^8 spores/ml.

Table I. LC_{50} (spores/ml) values of different isolates of *B. bassiana*, *M. anisopliae* and *I. fumosorosea.*

Fungi	Isolates	LC_{50} (spores/ml)	FL[a]	Slope	Chi	n[b]
B. bassiana	Bb-01 Bb-08	4.25×10^8	3.56×10^8-5.08×10^8	1.31±0.25	0.15	750
		2.54×10^8	1.98×10^8-2.97×10^8	1.71±0.26	6.49	750
M. anisopliae	Ma-11.1	3.26×10^8	2.81×10^8-3.66×10^8	1.97±0.26	2.51	750
	Ma-2.1	5.08×10^8	3.97×10^8-7.79×10^8	0.88±0.25	0.99	750
I. fumosorosea	If-02	7.20×10^8	5.55×10^8 -1.36×10^8	0.99±0.25	2.60	750
	If-2.3	6.22×10^8	4.96×10^8 -9.90×10^8	1.03±0.25	0.51	750

a: Fudicial limit, b: number of insects treated

Table II. LT_{50} (days) values of different isolates of *B. bassiana*, *M. anisopliae* and *I. fumosorosea* against 2nd instar nymphs of *P.solenopsis.*

Fungus	Isolates	Concentrations (spores/ml)	LT_{50}	FL[a]	Slope	Chi	n[b]
B. bassiana	Bb-01	7×10^8	6.431	5.915-6.993	3.67±0.33	3.42	125
		6×10^8	6.425	5.885-7.014	3.47±0.31	0.86	125
		5×10^8	6.979	6.324-7.702	3.51±0.34	2.15	125
	Bb-08	7×10^8	4.802	4.281-5.386	4.61±0.50	11.2	125
		6×10^8	5.674	5.342-6.027	4.38±0.36	3.74	125
		5×10^8	6.006	5.598-6.443	4.01±0.34	2.34	125
		4×10^8	6.036	5.634-6.466	4.13±0.35	1.65	125
		3×10^8	6.626	6.037-7.274	3.40±0.31	0.28	125
M. anisopliae	Ma-2.1	7×10^8	6.747	6.162-7.388	3.64±0.34	1.27	125
		6×10^8	6.735	6.087-7.451	3.16±0.30	3.28	125
	Ma-11.1	7×10^8	5.436	4.479-6.598	4.10±0.58	15.76	125
		6×10^8	6.061	5.682-6.465	4.47±0.38	9.62	125
		5×10^8	6.088	5.616-6.601	3.50±0.30	2.64	125
		4×10^8	6.953	6.319-7.652	3.62±0.35	0.93	125
I. fumosorosea	If-2.3	7×10^8	6.665	6.050-7.343	3.28±0.30	0.69	125
	If-02	7×10^8	6.698	6.077-7.382	3.29±0.31	0.95	125

a: Fudicial limit; b: number of insects treated.

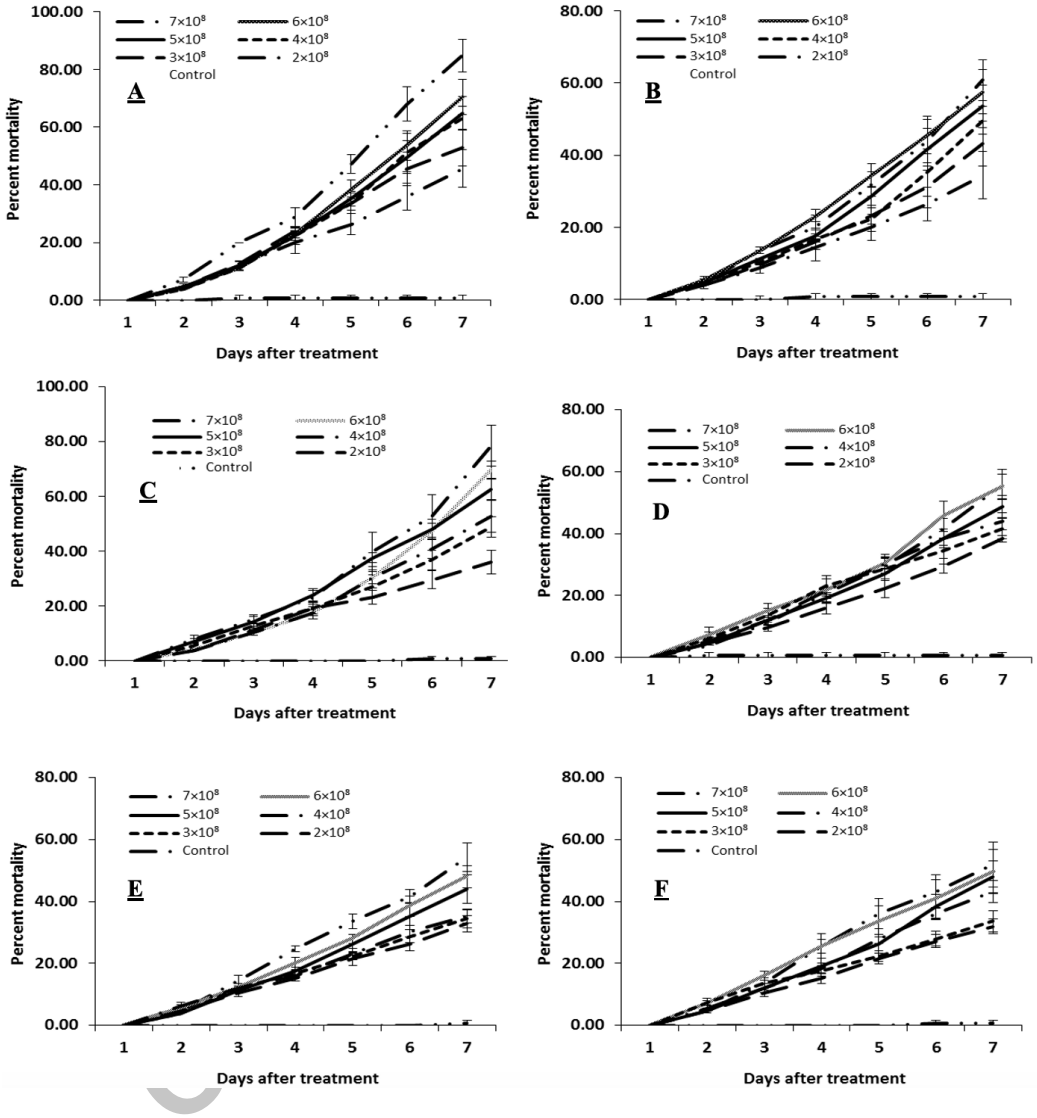

Fig. 1. Percentage mortality of 2nd instar aymphs *P.* solenopsis after application of different concentration of (A) *B. bassiana* (Bb 0b), (Bb 01), (c) *M. anisopliae* (Ma 11.1), (D) (Ma 2.1), (E) *I. fumosorosea* (if 02) and (F) (If 2.3). The assessment was done on different days.

DISCUSSION

Information is not only needed on the biology and feeding activity of the control agent but also on the most susceptible stage of pest species for the successful initiation of a fungal biocontrol program (Cuthbertson *et al*., 2003). For the period of fungal infection, the first step prior to penetration is the adhesion of fungi to the host cuticle (Boucias *et al*., 1984). It was suggested that adhesion occurs at three succeeding phases: (a) adsorption of the propagules of fungi to the surface of cuticle; (b) adhesion of the edge between epicuticle and propagules; (c) germination and development of fungi at the surface of cuticle in insects, until appresoria are grown to begin the stage of penetration (Fragues, 1984). During penetration of fungus through the cuticle of host, hydrolytic enzymes such as chitinases, lipases and proteases are produced that are suggested to be significant for the beginning of the infectivity process leading to cuticle transposition. The conidia of insect pathogenic fungi are found to produce on cuticle of termites through the penetrating germ tube and develop a systemic infection which eventually eradicates the insect (Milner and Staples, 1996).

During the current research, the spores of fungi were

able to develop and penetrate the exposed nymphs of *P. solenopsis*. It has been reported that temperature and the relative humidity are known to be limiting factors for growth of fungus on insects (Glare and Milner, 1991; Ferron, 1981). High rates of infectivity and a rapid kill of bugs by the hyphomycetous fungi were attained at humidity close to saturation (Silvia and Messias, 1985; Romana and Fergues, 1987; Luz, 1990; Romana, 1992; Luz *et al*., 1994).

Earlier, it has been reported that pathogenicity of *Verticillium lecanii* and *Aschersonia aleyrodis* to silver leaf whitefly, decreased with developmental stage and the older instars were less vulnerable and adults were rarely contaminated (Gindin *et al*., 2000; Fransen *et al*., 1987), while in the case of *Helicoverpa* spp, early stages were found less vulnerable to *Nomuraea rileyi* (Mohamed *et al*., 1977). The vulnerability of various stages of the *P. solenopsis* to the pathogen and its ability to transmit infection among different stages of development and generations, support the potential of entomopathogenic fungi for biocontrol of cotton mealybug.

Screening of entomopathogenic fungi to determine their effectiveness against *Ceratothripoides claratris*, *Pseudococcus cryptus*, and *Bemisia tabaci* showed *M. anisopliae* to be more efficient against mealy bug causing 73% mortality. The efficiency of *I. fumosorosea* and *Paecilomyces lilacinus* was not as that of *M. anisopliae* (Panyasiri *et al*., 2007). It was reported that *Isaria farinosa* caused high mortality, up to 90% at 95% RH and 1×10^8 conidia ml^{-1}. *I. fumosorosea* caused 87.76% mortality on *Aphelinus asychis* a parasitoid of *Diuraphis noxia*. Additionally, it was highlighted that the mortality was associated with the fungal concentration. Spores concentration is a significant aspect on the pathogenicity of the insect pathogenic fungi (Lacey *et al*., 1977). The application of *I. farinose* to *Eurygaster integriceps* on pine litter and wheat plants at different concentrations showed that the percent mortality was significantly higher at higher concentration i.e., 1×10^8 conidial concentrations (Parker *et al*., 2003). An association between the conidial concentration of *I. farinosa* and mortality percentages of *Pissodes punctatus* has also been determined. Our results are parallel to those in which *I. farinosa* caused significant mortality at 1×10^8 conidia ml^{-1} of *P. citri* ovisacs (Yang *et al*., 2009).

The current outcomes are according to earlier studies reporting *B. bassiana* to cause highest mortality and *M. anisopliae* to be least efficient than *B. bassiana* in controlling mealybugs (Lacey *et al*., 2001; Lemawork, 2008). Foliar spray of *V. lecanii* or *B. bassiana* (2×10^8 CFU/ml) @ 5 g / mL per L of water is efficient during high humid months in minimizing the population of mealybugs (Tanwar *et al*., 2007). *B. bassiana* @ 5g / L decreased invasion of *P. marginatus* from 90 to 57.78% after exposure under field conditions (Suresh *et al*., 2010).

CONCLUSION

In conclusion, the results obtained in these experiments establish the pathogenicity of entomopathogenic fungi, *B. bassiana* and *M. anisopliae, I. fumosorosea* on 2nd nymphal instar of mealybug as biocontrol agents. All entomopathogenic isolates showed high cumulative mean mortality to second instar mealybugs but *B. bassiana* isolate (Bb-08) caused higher mortality. Moreover, isolate Bb-08 was superior in terms of lower LC_{50} and LT_{50} values and that makes it ideal candidate for commercial exploitation. However, detailed study of entomopathogenic fungi on cotton mealy bug, *P. solenopsis* should to be undertaken to ascertain its virulence.

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Statement of conflict of interest

The authors declare there is no conflict of interest.

REFERENCES

Abbas, G., Arif, M.J., Ashfaq, M., Aslam, M. and Saeed, S., 2010. Host plants, distribution and overwintering of cotton mealybug (*Phenacoccus solenopsis*; Hemiptera: Pseudococcidae). *Int. J. Agric. Biol.,* **12**: 421-425.

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.,* **18**: 265-267.

Afzal, M.B.S., Shad, S.A., Abbas, N., Ayyaz, M. and Walker, W.B., 2014. Cross-resistance, the stability of acetamiprid resistance and its effect on the biological parameters of cotton mealybug, *Phenacoccus solenopsis* (Homoptera: Pseudococcidae). *Pest Manage. Sci.,* **71**: 151-158. https://doi.org/10.1002/ps.3783

Ahmad, Z., 1999. Key Paper, pest problems of cotton-A regional perspective. In: *Proc. ICAC-CCRI, Regional Consultation, Insecticide Resistance Management in Cotton*. June 28 to July 1, Multan, Pakistan. pp 5-20.

Anderson, T.E., Roberts, D.W. and Soper, R.S., 1988. Use of *Beauveria bassiana* for suppression of Colorado potato beetle in New York State

(Coleoptera: Chrysomelidae). *Environ. Ent.,* **17**: 140–145. https://doi.org/10.1093/ee/17.1.140

Anonymous, 2006. *Economic survey of Pakistan*. Government of Pakistan, Ministry of Food, Agriculture and Livestock. Islamabad.

Anonymous, 2008a. *Emerging pests in North Indian cotton*. NCIPM, New Delhi and CICR, Regional Station, Sirsa. http://www.ncipm.org.in/Mealybugs/Emerging Pest.doc.

Anonymous, 2008b. Mealybug: A new threat to cotton production in Pakistan and India. *The ICAC Recorder, Tech. Inf. Sect.,* **26**: 15-19.

Anonymous, 2008c. *Pakistan statistical year book*. Ch. 2. Federal Bureau of Statistics. Agriculture. Statistics Division, Ministry of Economic Affairs and Statistics, Govt. of Pakistan. http://www.statpak.gov.pk/depts/fbs/publications/yearbook2008/yearbook2008.htm.

Boucias, D.G., Bradford, D.L. and Barfield, C.S., 1984. Susceptibility of the velvetbean caterpillar and soybean looper (Lepidoptera: Noctuidae) to *Nomuraea rileyi*: effects of pathotype, dosage, temperature and host age. *J. econ. Ent.,* **77**: 247–253. https://doi.org/10.1093/jee/77.1.247

Cuthbertson, A.G.S., Flemming, C.C. and Murchie, A.K., 2003. Detection of *Rhopalosiphum insertum* (apple-grass aphid) predation by the predatory mite *Anystis baccarum* using molecular gut analysis. *Agric. Ent.,* **5**: 219-225. https://doi.org/10.1046/j.1461-9563.2003.00181.x

Daniel, C. and Wyss, E., 2010. Field applications of *Beauveria bassiana* to control the European cherry fruit fly *Rhagoletis cerasi*. *J. appl. Ent.,* **134**: 675-681. https://doi.org/10.1111/j.1439-0418.2009.01486.x

Darbro, J.M., Graham, R.I., Kay, B.H., Ryan, P.A. and Thomas, M.B., 2011. Evaluation of entomopathogenic fungi as potential biological control agents of the dengue mosquito, *Aedes aegypti* (Diptera: Culicidae). *J. biol. Contr. Technol.,* **21**: 1027-1047. https://doi.org/10.1080/09583157.2011.597913

Fargues, J., 1984. Adhesion of the fungal spore to the insect cuticle in relation to pathogenicity. In: *Infection processes of fungi* (eds. D.W. Roberts and J.R. Aist), The Rockefeller Foundation. New York. pp. 90e110.

Ferron, P., 1981. Pest control by the fungi *Beauveria* and *Metarhizium*. In: *Microbial control of pests and plant diseases* 1970–1980 (ed. H. D. Burges.), Academic Press, London. 465-482.

Feng, M., Poprawski, T. and Khachatourians, G., 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control current status. *Biocontr. Sci. Technol.,* **4**: 3-34. https://doi.org/10.1080/09583159409355309

Finney, D.J. and Stevens, W.L., 1948. A table for the calculation of working probits and weights in probit analysis. *Biometrika,* **35**: 191-201. https://doi.org/10.1093/biomet/35.1-2.191

Fransen, J.J., Winkelman, K. and Van Lenteren, J.C., 1987. The differential mortality at various developmental stages of the greenhouse whitefly, *Trialeurodes vaporariorum* (Homoptera:Aleyrodidae), by infection with the fungus *Aschersonia aleyrodis* (Deuteromycotina: Coelomomycetes). *J. Inverteb. Pathol.,* **50**: 158–165. https://doi.org/10.1016/0022-2011(87)90116-9

Faria, M.R.D. and Wraight, S.P., 2007. Mycoinsecticides and mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. *Biol. Cont.,* **43**: 237-256.

Fuchs, T.W., Stewart, J.W., Minzenmayer. R. and Rose, M., 1991. First record of *Phenacoccus solenopsis* Tinsley in cultivated cotton in the United States. *Southw. Entomol.,* **16**: 215-221.

Gayathri, G., Balasubramanian, C., Vinayaga M.P. and Kubendran, T., 2010. Larvicidal Potential of *Beauveria bassiana* (Balsamo) Vuillemin and *Paecilomyces fumosoroseus* (Wize,) Brown and Smith on *Culex quinquefasciatus*. *J. Biopestic.,* **3**: 147-151.

Glare, T.R. and Milner, R.J., 1991. Ecology of entomopathogenic fungi. In: *Handbook of applied mycology* (eds. D.K. Arora, L. Ajello and K.G. Mukerji) Vol. 2, Humans, Animals and Insects, Marcel Dekker Inc., New York. pp. 547-612.

Gindin, G., Geschtovt, N.U., Raccah, B. and Brash, I., 2000. Pathogenicity of *Verticillium lecanii* to different developmental stages of the silverleaf whitefly, *Bemesia argentifolli*. *Phytoparasitica,* **28**: 229-239. https://doi.org/10.1007/BF02981801

Hajek, A.E. and Leger, S.T., 1994. Interaction between fungal pathogens and insect hosts. *Annu. Rev. Ent.,* **39**: 293-322. https://doi.org/10.1146/annurev.en.39.010194.001453

Herren, H.R. and Neuenschwander, P., 1991. Biological control of cassava pests in Africa. *Annu. Rev. Ent.,* **36**: 257-283. https://doi.org/10.1146/annurev.en.36.010191.001353

Hodgson, C., Abbas, G., Arif, M.J., Saeed, S. and Karar, H., 2008. *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Coccoidea: Pseudococcidae),

an invasive mealybug damaging cotton in Pakistan and India, with a discussion on seasonal morphological variation. *Zootax,* **1913**: 1-35. https://doi.org/10.11646/zootaxa.1913.1.1

Joseph, I., Edwin, D. and Singh, R., 2010. Studies on the influence of *Beauveria bassiana* on survival and gut-flora of groundnut caterpillar *Spodoptera litura* (Fab). *J. Biopestic.,* **3**: 553–555.

Kaaya, G.P. and Munyinyi, D.M., 1995. Biocontrol potential of the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae* for tsetse flies (*Glossina* spp.) at developmental sites. *J. Inverteb. Pathol.,* **66**: 237-241. https://doi.org/10.1006/jipa.1995.1095

Kairo, M.T.K., Pollard, G.V., Peterkin, D.D. and Lopez, V.F., 2000. Biological control of the hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera; Pseudococcidae) in the Caribbean. *Integ. Pest Manage. Rev.,* **5**: 241-254.

Kakakhel, I., 2007. *Mealy bug attack affects cotton crop on 150,000 acres*. http://www.dailytimes.com.pk/default News story 23-8-2007. pp. 54.

Lacey, L.A., Frutos, R., Kaya, H.K. and Vails, P., 2001. Insect pathogens as biological control agents: Do they have future? *Biol. Contr.,* **21**: 230-248. https://doi.org/10.1006/bcon.2001.0938

Lacey, L.A., Mesquita, A.L.M., Mercadier, G., Debire, R., Kazmer, D.J. and Leclant, F., 1977. Acute and sublethal activity of the entomopathogenic fungus *Paecilomyces fumosoroseus* Deuteromycotina: Hyphomycetes) on adult *Aphelinus asychis* (Hymenoptera: Aphelinidae). *Environ. Ent.,* **26**: 1452–1460. https://doi.org/10.1093/ee/26.6.1452

Lemawork, S. 2008. *Evaluation of entomopathogenic fungi and hot water treatment against enset root mealybug*, Cataenococcus ensete, *Williams and Matile-Ferrero (Homoptera: Pseudococcidae) on enset*. M.Sc., thesis, Department of Plant Sciences, Awassa College of Agriculture, School of Graduate Studies Hawassa University, Awassa, Ethiopia. pp. 87.

Lord, C.J., 2001. Desiccant dusts synergize the effect of *Beauveria bassiana* (Hyphomycetes: Moniliales) on stored-grain beetles. *J. econ. Ent.,* **94**: 367-372. https://doi.org/10.1603/0022-0493-94.2.367

Lubeck, I., Arruda, W., Souza, B.K., Uaski, F., Carlini, C.R., Schrank, A. and Vainstein, M.H., 2008. Evaluation of *Metarhizium anisopliae* strains as potential biocontrol agents of the tick *Rhipi cephalus* (Boophilus) *microplus* and the cotton stainer *Dysdercus peruvianus*. *J. Fungal Ecol.,* **1**: 78-88. https://doi.org/10.1016/j.funeco.2008.09.002

Luz, C., 1990. Zur pathogenitat von beauveria bassiana (fungi imperfecti) gegenuber mebreren raubwanzenarten (Reduviidae: triatominae) und einfluss der relative luftfuechtigeik auf dic infekiton von *Rhodinius prolixus*. *Mitt. Dtsh. Ges. allg. angew. Ent.,* **7**: 510-511.

Luz, C., Fargues, J., Romana, C.A., Moreno, J., Goujet, R., Rougier, M. and Grunewald, J. 1994. Potential of entomopathogenic hyphomycetes for the control of triatomine vectors of Chaga's disease. *Proc. 6 Int. Coll. Inveteb. Path. Microbiol. Contr.,* **1**: 272-26.

Maniania, N.K., 1993. Effectiveness of the entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. for control of the stem borer *Chilo partellus* (Swinhoe) in maize in Kenya. *Crop Prot.,* **12**: 601–604. https://doi.org/10.1016/0261-2194(93)90123-Z

Malarvannan, S., Murali, P.D., Shanthakumar, S.P., Prabavathy, V.R. and Nair, S., 2010. Laboratory evaluation of the entomopathogenic fungi, *Beauveria bassiana* against the tobacco caterpillar, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). *J. Biopestic.,* **3**: 126-131.

Marannino, P., Santiago-Álvarez, C., De Lillo, E. and, Quesada-Moraga, E., 2006. A new bioassay method reveals pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* against early stages of *Capnodis tenebrionis* (Coleoptera: Buprestidae). *J. Inverteb. Pathol.,* **93**: 210-213. https://doi.org/10.1016/j.jip.2006.08.002

Milner, R.J. and Staples, J.A. 1996. Biological control of termites: results and experiences within at Csrip project in Australia. *Bicontr. Sci. Technol.,* **6**: 3-9.

Mohamed, A.K.A., Sikorowski, P.P. and Bell, J.V. 1977. Susceptibility of *Heliothis zea* larvae to *Nomuraea rileyi* at various temperatures. *J. Inverteb. Pathol.,* **30**: 414-417. https://doi.org/10.1016/0022-2011(77)90152-5

Nagrare, V.S., Kranthi, S., Kumar, R., Jothi, B.D., Amutha, M., Deshmukh, A.J. and Kranthi, K.R., 2011. *Compendium of cotton mealybugs*. Central Institute for Cotton Research, India.

Panyasiri, C., Attathom, T. and Poehling, H.M., 2007. Pathogenicity of entomopathogenic fungi-potential candidates to control insect pests on tomato under protected cultivation in Thailand. *J. Pl. Dis. Protect.,* **114**: 278–287. https://doi.org/10.1007/BF03356230

Parker, B.L., Skinner, M., Costa, S.D., Gouli, S., Reid, W. and Bouhssini, M., 2003. Entomopathogenic fungi of *Eurygaster integriceps* Puton (Hemiptera:

Scutelleridae): collection and characterization for development. *Biol. Contr.,* **27**: 260–272. https://doi.org/10.1016/S1049-9644(03)00017-3

Prishanthini, M. and Vinobaba, M.L., 2009. *Phenacoccus solenopsis daily news*. Srilanka's National Newspaper, Srilanka. http://www.dailynews.lk/2009/07/01/fea30.asp.

Ramakrishnan, N., Sexena, S.V. and Dhingra, S., 1984. Insecticide resistance in the population of *Spodoptera litura* (F.) in Andhra Pradesh. *J. Pestic. Sci.,* **18**: 23-27.

Romana, C.A. 1992. *Recherches sur les potentialities des Hyphomycetes Entomopathogenes (Fungi imperfecti) dans la ladte contre les Triatominae (Heteroptera)*. These dc Doctorat, Montpellier. pp. 192.

Romana, C.A. and Fargues, J., 1987. Sensibilite des larves de la hemiplere hematophage *Rhodnius prokixus* (Triatominae) aus hyphomycetes entomopathogens. *Entomolophaga*, **32**: 167-179. https://doi.org/10.1007/BF02373128

Saeed, S., Ahmad, M. and Kwon, Y.J., 2007. Insecticidal control of the mealybug *Phenacoccus gossypiphilous* (Hemiptera: Pseudococcidae), a new pest of cotton in Pakistan. *Ent. Res.,* **37**: 76-80. https://doi.org/10.1111/j.1748-5967.2007.00047.x

Sahayaraj, K. and Borgio, J.F., 2010. Virulence of entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin on seven insect pests. *Indian J. agric. Res.,* **44**: 195–200.

Santi, L., Silva, L., Silva, W., Corrêa, A., Rangel, D., Carlini, C., Schrank, A. and Vainstein, M., 2011.Virulence of the entomopathogenic fungus *Metarhizium anisopliae* using soybean oil formulation for control of the cotton stainer bug, *Dysdercus peruvianus*. *World J. Microbiol. Biotechnol.,* **27**: 2297–2303. https://doi.org/10.1007/s11274-011-0695-5

Silva, J.C. and Messias, C.L. 1985. Virulencia de *Metarhizium anisopliae* var. *acridum* a *Rhodnius prolixus*. *Cienc. Cult.,* **7**: 37-40.

Singh, S.P., 2004. *Some success stories of classical biological control of agricultural pests in India*. Asia-specific Association of Agricultural Institutions, FAO Regional office for ASIA and the Pacific. APAARI Publication 2004/2, Bangkok, Thailand, pp. 73.

Steinkraus, D.C. and Tugwell, N.P., 1997. *Beauveria bassiana* (Deuteromycotina: Moniliales) effects on *Lyguslineolaris* (Hemiptera: Miridae). *J. entomol. Sci.,* **32**: 79-90. https://doi.org/10.18474/0749-8004-32.1.79

Suresh, S., Jothimani, R., Sivasubramanian, P., Karuppuchamy, P., Samiyappan, R. and Jonathan, E.I., 2010. Invasive mealybug of Tamil Nadu and their management. *Karnataka J. agric. Sci.,* **23**: 6 -9.

Tanwar, R.K., Jeyakumar, P. and Monga, D., 2007. *Mealybugs and their management.* Technical Bulletin 19, National Centre for Integrated pest Management, New Delhi. pp.12

Torrado, L.E., Lerma, M.J. and Pizo, V.E., 2006. Sub lethal effects of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) on the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) under laboratory conditions. *Mycopathologia,* **162**: 411–419. https://doi.org/10.1007/s11046-006-0077-y

Wang, Y.P., Wu, S.A. and Zhang, R.Z., 2009. Pest risk analysis of new invasive pest, *Phenacoccus solenopsis* to China. *Bull. Ent.,* **46**: 101-106.

Wraight, S.P., Carruthers, R.I., Jaronski, S.T., Bradley, C.A., Garza, C.J. and Wraight, S.G., 2000. Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of the silver leaf white fly, *Bemisia argentifoli*. *Biol. Contr.,* **17**: 203–217. https://doi.org/10.1006/bcon.1999.0799

Wraight, S.P., Jackson, M.A. and Kock, S.L., 2001. Production, stabilization and formulation of fungal biological agents. In: *Fungi as biocontrol agents* (eds. T.M. Butt, C. Jackson and N. Magan), CABI, Wallingford, USA. 253-287. https://doi.org/10.1079/9780851993560.0253

Williams, D.J. and Willink, M.C.G.D., 1992. *Mealybugs of Central and South America*. C.A.B. International Wallingford. pp. 635.

Wu, S.A. and Zhang, R.Z., 2009. A new invasive pest *Phenacoccus so1enopsis* threatening seriously to cotton production. *Chinese Bull. Ent.,* **46**: 159-162.

Yang, S., Zhuang, H., Li, Y. and Kuang, R., 2009. Insecticidal efficacy of *Isaria farinosa* in different life stages of *Pissodes punctatus* (Coleoptera: Curculionidae). *J. Pestic. Sci.,* **82**: 321–325. https://doi.org/10.1007/s10340-009-0256-y

Yousuf, M. and Tayyib, M., 2007. Mealybug problem on cotton in Pakistan. *Pak. Entomol.,* **29**: 49- 50.

Zaka, S.M., Saeed, S., Bukhari, S.A. and Baksh, E., 2006. *Mealybug, Phenacoccus solenopsis (Homoptera: Pseudococcidae): A novel pest of cotton in Pakistan*. In: Proceedings of 34 Pakistan (SAARC) Countries Science Conference. University of Veterinary and Animal Sciences, Lahore, Pakistan, 2006. pp. 32.