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Evaluation of Three Different Insect Pathogenic Fungi for the Control of *Dysdercus koenigii* and *Oxycarenus hyalinipennis*

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Abstract.- Red cotton bug, *Dysdercus koenigii* and dusky cotton bug, *Oxycarenus hyalinipennis* are progressively gaining the status of primary pests in Asia due to the overuse of chemical insecticides. For the use of biological control in pest management, this study assessed the effectiveness of three different entomopathogenic fungi *i.e., Beauveria bassiana* (Bb-08), *Isaria fumosorosea* (If-2.3) and *Metarhizium anisopliae* (Ma-2.3) on adults of *D. koenigii* and *O. hyalinipennis* using immersion method under laboratory conditions of 28±1°C, 60-70% RH and 10L:14D hrs photoperiod. Among these insect pathogenic fungi, *B. bassiana* divulged the lowest LC_{50} values (2.4×10^7 spores/ml, 2.5×10^7 spores/ml) on *D. koenigii* and *O. hyalinipennis*, with LT_{50} values of 5.09 and 4.32 days at higher concentrations of 3×10^8 spores/ml, respectively. Current study opens the new possibilities of using these entomopathogens as a substitute to chemical pesticides and their use in association with integrated pest management.

Keywords: Cotton strainers, entomopathogens, microbial control, *Beauveria bassiana*, insecticide alternatives.

INTRODUCTION

Insects pests are traditionally managed by insecticides which, however, eventually results in development of resistance, ecological pollution by chemicals, detrimental effects on non-target species and threats to human health (Hegedus and Khachatourians, 1995; Thomas and Read, 2007). The undesirable aspects of old-fashioned pest control have headed to the exploration of alternative methods such as biological control. Commonly available worldwide biological control agents (BCAs) for controlling agricultural pests are entomopathogenic fungi (Wraight *et al*., 2001) *i.e.* fungal pathogen, *Beauveria bassiana* (Clavicipitaceae: Hypocreales: Sordariomycetes) (Balsamo) Vuillemin (Li and Yang, 1988).

Cotton, *Gossypium hirsutum* L. is an important cash crop and the host of many primary and secondary insect pests. Due to the extensive cultivation of *Bt* cotton, there is a considerable increase in the number of sucking pests (Uthamasamy *et al*., 2004). One of the alarming pests of cotton in Southeast Asian countries is red cotton bug (RCB) or stainer bug, *Dysdercus koenigii* (Fabricius) (Hemiptera: Pyrrhocoridae), which is also an important pest of lady finger, *Abelmoschus esculentus* L., hollyhock, *Alcea rosea* L. etc. Both nymphs and adults severely damage cotton bolls and leaves (Kohno and Thi, 2004). The bug causes germination problem to the crop by feeding on the cotton seed and also reducing the quality of the lint by feeding and staining the fibers. Seed weight, oil contents and viability deteriorate as a result of RCB infestation, while severe damage on two weeks old bolls can kill developing seeds leading to boll shedding (Sahayaraj *et al*., 2011).

Another disturbing pest of cotton is *Oxycarenus hyalinipennis* (Costa) (Hemiptera: Lygaeidae) generally known as dusky cotton bug or cotton seed bug. It primarily feeds on plant seeds mostly of *Gossypium* spp. (Slater and Baranowski, 1994). During severe infestation, it causes considerable reduction in cotton yield, seed weight, oil contents and seed germination (Srinivas and Patil, 2004). *O. hyalinipennis* starts feeding, mating, and laying eggs when mature seeds of its host plants become available (Mensah and Kumar, 1977; Sweet, 2000).

Many synthetic insecticides are used to control these pests but due to high residual effects there is an immediate need to find other eco-friendly and economical ways. Other alternative strategies such as biological control could be promising (Soares and Almeida, 1998; Arruda *et al.,* 2005). The current research was carried out to evaluate the

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prospective of entomopathogenic fungi, *B. bassiana*, *I. fumosorosea* and *M. anisopliae* as bio-control of *D. koenigii* and *O. hyalinipennis*. The artificial application of these organisms results in depressing pest populations to satisfactory densities and keeping targets at a non-harmful level (Gronvold *et al*., 1996).

MATERIALS AND METHODS

Insect culture

The adults and nymphs of red cotton bug *D. koenigii* and dusky cotton bug *O. hyalinipennis* were collected from *Gossypium* fields of Bahauddin Zakariya University, Multan. The insects for bioassay were reared in transparent cages (30×30×30cm) maintained at 28±1°C, 60-70% RH, 10L-14D hr photoperiod and were fed on both mature and immature cotton bolls.

Media and culturing of entomopathogenic fungi

The isolates of entomopathogenic fungi used were obtained from the Insect Microbiology Laboratory of B. Z. University, Multan. Growth media *i.e.*, potato dextrose agar (PDA) was freshly made using commercial ingredients and distilled water. This media was poured in the petri plates which were then inoculated with the spores of different entomopathogenic fungi. After inoculation, all petri plates were incubated at 25°C for 14 days and after 14 days of incubation the spores were harvested and dissolved in 0.05% Tween 80 solution. The concentrations of the spores was measured by hemocytometer and later on required concentrations *i.e.* 1×10^6, 1×10^7, 1×10^8, 2×10^8 and 3×10^8 spores/ml of each isolate was prepared by serial dilution method.

Experimentation

For conducting tests on adults of *D. koenigii* and *O. hyalinipennis*, immersion method was used. Five adults per replication were treated against different concentrations of insect pathogenic fungi with six treatments including control and each treatment was replicated four times. Adults of *D. koenigii* and *O. hyalinipennis* were individually immersed in the concentration for about 8-10 seconds. Treated insects (*D. koenigii* and *O. hyalipennis*) were placed on the tissue paper to soak up the excessive moisture, transferred into transparent plastic jars and provided with cotton bolls, while in the control treatment both insect species were treated with Tween 80 (0.05%) solution and were provided with similar cotton bolls. Mortality data was recorded daily for consecutive seven days and cadavers were moved in sterilized petri plates with wet cotton pads to encourage sporulation.

Statistical analysis

The experiment was planned under completely randomized design (CRD). Percentage corrected mortality was calculated for every day and were separated by Duncan's Multiple Range Test (DMRT). All the statistical analysis was performed by using SAS (SAS, 2002), while LC_{50} and LT_{50} values of the respective entomopathogenic fungus were calculated by probit analysis (Throne *et al*., 1995).

RESULTS

Effect of entomopathogenic fungi

Red cotton bug

Virulence of three different entomopathogenic fungi *i.e., B. bassiana*, *I. fumosorosea* and *M. anisopliae* were evaluated against the adults of red cotton bug. The LC_{50} and LT_{50} values of these entomopathogenic fungi which exhibited more than 50.00% mortality are represented in (Tables I, II). Cumulative percentage mortality of *B. bassiana*, *I. fumosorosea* and *M. anisopliae* on red cotton bug were 95.00, 70.00 and 80.00 respectively over a period of 7 days at the concentrations of 3×10^8 spores/ml (Fig. 1). However, *B. bassiana* (Bb-08) with lowest LC_{50} value of 2.4×10^7 spores/ml (1.1×10^7- 5.8×10^8) and LT_{50} of 5.09 (3.92-6.60) days proved to be most virulent isolate against red cotton bug as compared to *I. fumosorosea* (If-2.3) with 7.1×10^7 spores/ml (2.1×10^7 – 2.4×10^8) and *M. anisopliae* (Ma-2.3) with 1.1×10^8 spores/ml (5.1×10^7- 2.4×10^8).

Dusky cotton bug

While testing against the adults of dusky cotton bug these entomogenous fungi *i.e., B. bassiana, I.*

Table I.- LC_{50} (spores/ml) values of *B. bassiana, I. fumosorosea* and *M. anisopliae* against adults of *D. koenigii* and *O. hyalinipennis* by immersion method.

Organism	Isolates	LC_{50} (spores/ml)	FD[a]	Slope	Chi	n[b]
D. koenigii						
B. bassiana	Bb-08	2.4×10^7	1.1×10^7-5.8×10^8	0.75±0.16	4.94	100
I. fumosorosea	If -2.3	7.1×10^7	2.1×10^7- 2.4×10^8	0.49±0.15	0.20	100
M. anisopliae	Ma-2.3	1.1×10^8	5.1×10^7- 2.4×10^8	0.83 ±0.19	2.21	100
O. hyalinipennis						
B. bassiana	Bb-08	2.5×10^7	1.0×10^7- 6.5×10^7	0.66±0.15	2.05	100
I. fumosorosea	If -2.3	8.5×10^7	1.3×10^7 – 5.2×10^8	0.34±0.14	1.75	100
M. anisopliae	Ma-2.3	8.1×10^7	1.8×10^7- 3.6×10^8	0.41 ±0.14	2.06	100

a, Fudicial limit; b, number of insect exposed

Table II.- LT_{50} (days) values of *B. bassiana, I. fumosorosea* and *M. anisopliae* against adults of *D. koenigii and O. hyalinipennis* by immersion method

Organism	Isolates	Concentrations	LT_{50}	FD[a]	Slope	Chi	n[b]
D. koenigii							
B. bassiana	Bb-08	3×10^8	5.09	3.92-6.60	5.39±1.47	11.3	20
		2×10^8	6.01	5.01-7.21	3.85±0.83	3.62	20
I. fumosorosea	If-2.3	3×10^8	6.58	5.25-8.26	3.49±0.81	1.94	20
		2×10^8	6.60	5.18-8.39	3.24±0.75	0.37	20
M. anisopliae	Ma-2.3	3×10^8	5.92	4.99-7.01	4.10±0.86	4.31	20
		2×10^8	6.60	5.10-8.50	3.02±0.70	0.59	20
O. hyalinipennis							
B. bassiana	Bb-08	3×10^8	4.32	3.85-4.85	5.19±0.86	1.86	20
		2×10^8	5.42	4.82-6.08	5.50±1.05	1.13	20
		1×10^8	5.97	5.16-6.90	4.88±1.02	0.84	20
I. fumosorosea	If-2.3	3×10^8	4.90	4.04-5.93	2.98±0.59	1.27	20
		2×10^8	6. 64	5.53-7.97	4.60±1.07	0.72	20
M. anisopliae	Ma-2.3	3×10^8	6.07	5.30-6.94	5.30±6.94	3.27	20
		2×10^8	8.57	5.59-13.1	2.42±0.66	4.80	20

a. Fudicial limit; b, number of insect exposed

fumosorosea and *M. anisopliae* showed effective results. *B. bassiana* (Bb-08) with lowest LC_{50} value of 2.5×10^7 spores/ml (1.0×10^7- 6.5×10^7) and LT_{50} of 4.32 (3.85-4.85) days proved to be most virulent isolate against dusky cotton bug as compared to *I. fumosorosea* (If-2.3) with 8.5×10^7 spores/ml (1.3×10^7 – 5.2×10^8) and *M. anisopliae* (Ma-2.3) 8.1×10^7 spores/ml (1.8×10^7- 3.6×10^8) (Tables I and II). Accumulative percentage mortality of these three insect killing fungi, *B. bassiana*, *I. fumosorosea* and *M. anisopliae* on dusky cotton bug were 90.00, 75.00 and 75.00, respectively over a period of 7 days at the concentrations of 3×10^8 spores/ml (Fig. 2).

DISCUSSION

Entomogenous fungi are a unique group of insect pathogens in that assimilation by the host is not a requirement since infection results from direct penetration of the cuticle. In order to eradicate the insect pests there is an important phenomenon which includes dealing of specific disease of a particular pest caused by the pathogenic fungi. Entomogenous fungi have been extensively used for the control of agricultural and forest pests (Ferron, 1981; Anderson *et al*., 1988; Maniania, 1993).

Current study evaluated the virulence of three different entomopathogenic fungi, *i.e., B. bassiana*,

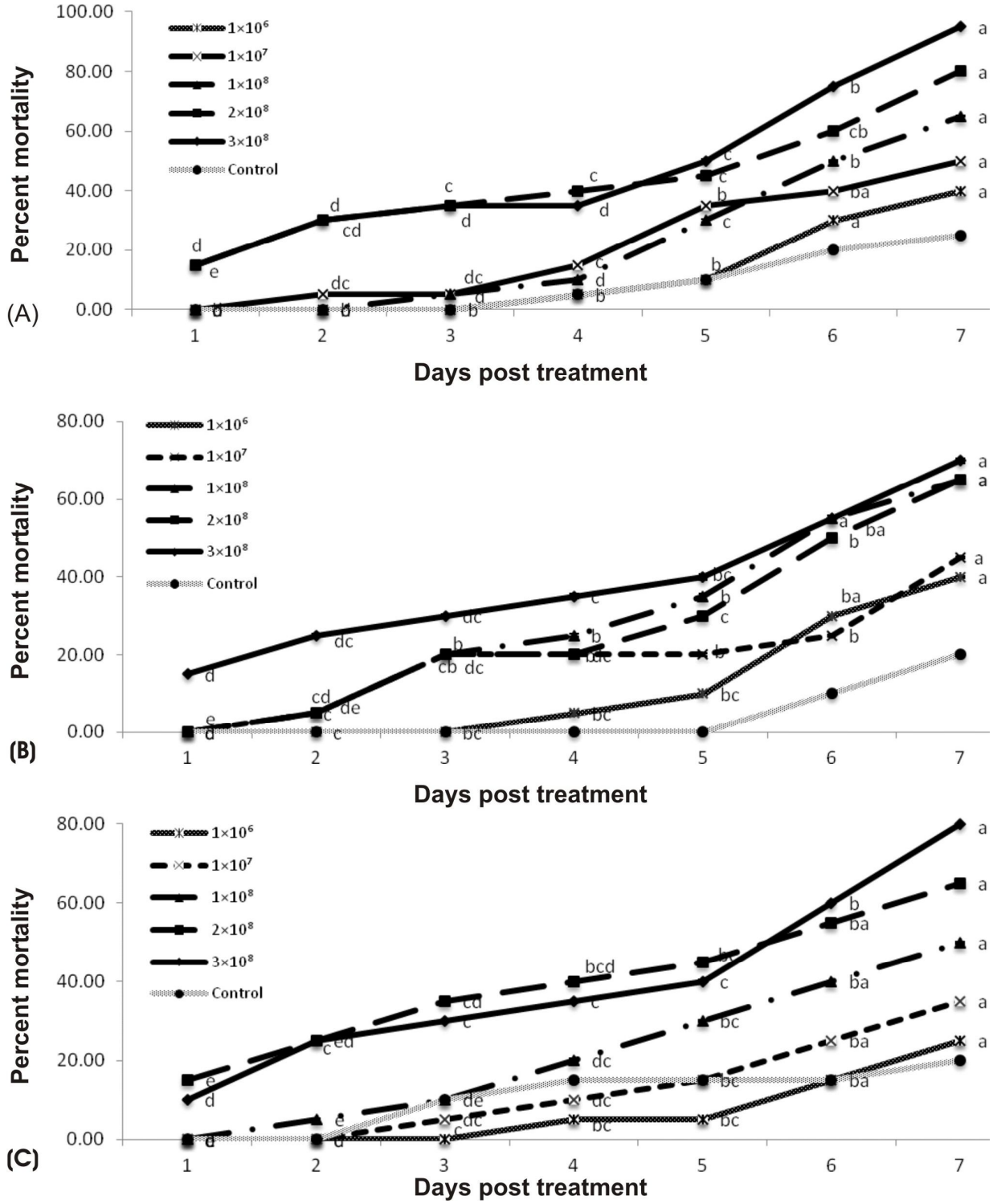

Fig. 1: Percent mortality of *D. koenigii* after exposure to different concentrations of (A) *B. bassiana* (Bb-08) (B) *I. fumosorosea* (If-2.3) (C) *M. anisopliae*(Ma-2.3). For each day the letters sharing similarity are not significant different ($P<0.05$) by Duncan's Multiple Range Test (DMRT). *The comparison of different concentrations was done on different days, respectively.

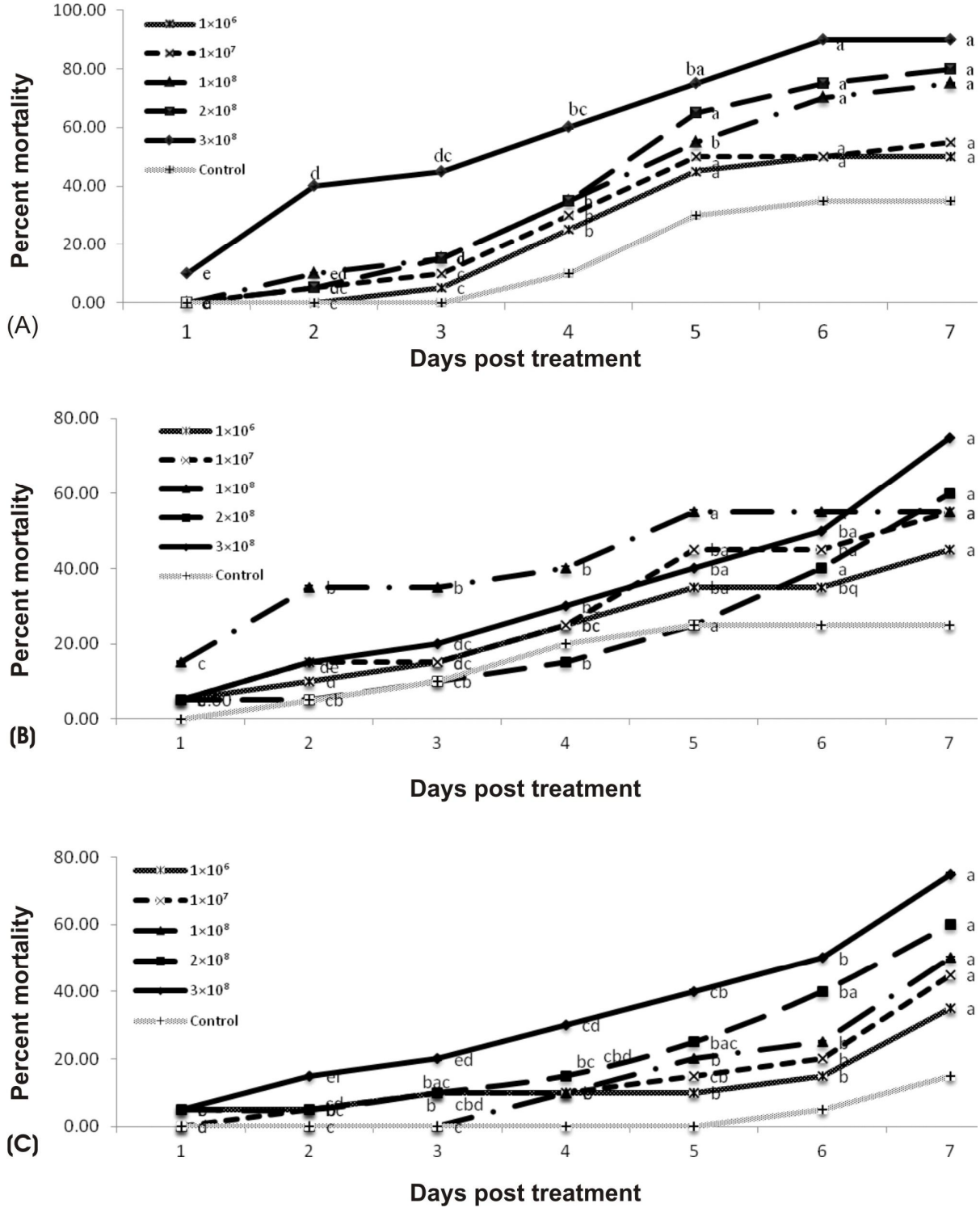

Fig. 2. Percent mortality of *O. hyalinipennis* after exposure to different concentrations of (A) *B. bassiana* (Bb-08) (B) *I. fumosorosea* (If-2.3) (C) *M. anisopliae* (Ma-2.3). For each day the letters sharing similarity are not significant different (P<0.05) by Duncan's Multiple Range Test (DMRT). *The comparison of different concentrations was done on different days, respectively.

I. fumosorosea and *M. anisopliae* on red cotton bug and dusky cotton bug under the laboratory conditions. The insect pathogenic fungi have been reported to control number of important pests like *Aedes aegypti* (Darbro *et al*., 2011), *Plutella xylostella*, *S. exigua* (Freed *et al*., 2012a, b), Maize army worm, *Mythimna separata* (Malik *et al*., 2013), different aphid species (Akmal *et al*., 2013) and on stored-grain beetles (Lord, 2001). These fungi were found highly virulent when tested against *D. koenigii* and *O. hyalinipennis*. Among the fungi tested *B. bassiana* isolate (Bb-08) showed the highest percent mortality as compared to (I.f-2.3) and (M.a-2.3). Similar to the present research Steinkraus and Tugwell (1997) used *B. bassiana* (virulent isolate) against tarnished plant bug, *Lygus lineolaris* nymphs and adults as this isolate produced large numbers of conidia which propagate rapidly and the hyphae produced thrive quickly at temperatures that commonly occur under field conditions. Novel isolate of *I. fumosorosea* was effective against the microbial control of adult Asian citrus psyllids, *Diaphorina citri* (Kuwayama) (Marjorie *et al*., 2010). Associated to current study the lowest LC_{50} was obtained from Bb-08 when treated against *Dysdercus cingulatus* (Fabricius) (Moorthi *et al*., 2012). Similar to the present investigations Sahayaraj and Borgio (2010) used *M. anisopliae* for the control of *D. cingulatus* and found 75.00% mortality, while the addition of synergists like soya bean oil with *M. anisopliae* increased the infectivity on both nymphs and adults of *Dysdercus peruvianus* (Fabricius) (Santi *et al*., 2011).

Lubeck *et al*. (2008) reported for the first time the use of *M. anisopliae* as a potential biopesticide against *D. peruvianus* in which females were more sensitive to fungal infection than males. Moreover, the entomopathogenic fungi showed extreme virulence against the caterpillars of *Spodoptera litura* (Fabricius) (Gayathri *et al*., 2010; Joseph *et al*., 2010; Malarvannan *et al*., 2010; Suganya and Selvanarayanan, 2010). Wraight *et al*. (1998) evaluated pathogenecity of three species of insect pathogenic fungi against pre imaginal *Bemisia argentifolii* (Gennadius). *B. bassiana* isolates of diverse origins proved to be highly pathogenic to the whitefly nymphs and the median lethal doses of four of the thirteen *B. bassiana* isolates ranged between 50 and 150 conidia/mm^2. Various isolates of insect pathogenic fungi signifying six species including *B. bassiana, M. anisopliae, I. fumosorosea, Isaria farinosa, Isaria flavovirescens,* and *Lecanicillium* spp. were screened as potential biological control agents of *A. aegypti*. Out of these, two isolates of *B. bassiana* appeared to be the most promising for the control of *A. aegypti* (Darbro *et al.*, 2011). The current study demonstrates the excellent bio-control potential of *B. bassiana* towards the emerging threats i.e., *D. koenigii* and *O. hyalinipennis* to cotton crop.

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