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Effect of *Isaria fumosoroseus* on *Eretmocerus* sp. nr. *furuhashii* (Hymenoptera: Aphelinidae), a Parasitoid of *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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Abstract.-- Pathogenicity of *Isaria fumosoroseus* Wize against *Eretmocerus* sp. nr. *furuhashii* Rose & Zolnerowich (Hymenoptera: Aphelinidae), was investigated under laboratory conditions to determine if the fungal infection of the whitefly (*Bemisia tabaci*) host can effect some of the bioparameters of this parasitoid, i.e. survival rate, longevity and fecundity. The results indicated that the number of parasitized larvae surviving decreased with increasing concentrations of *I. fumosoroseus. I. fumosoroseus* treatment after 6 and 12 days of oviposition decreased with increasing concentrations of *I. fumosoroseus*. There were no significant differences on adult parasitoid survivorship after 7 days among all treatments. The results showed a non significant effect of *I. fumosoroseus* on longevity and next offsprings of *E. furuhashii* females. The percentage emergence of parasitoids from the whitefly nymphs produced by the females emerging from treated pupae was almost comparable; being greatest (70.7%) in control and lowest (64.1%) in fungal treatment of 1×10^7 conidia/ml. The longest longevity (4.27 days) was observed for control treatment, whereas the lowest one (4.10 days) was observed for fungal treatment of 1×10^8 conidia/ml.

Key words: *Bemisia tabaci*, *Eretmocerus* sp. nr. *furuhashii*, *Isaria fumosoroseus*, compatibility.

INTRODUCTION

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) has long been recognized as a serious pest in all tropical and subtropical regions of the world (Brown, 1994; Gould *et al*., 1992; Hoddle and van Driesche, 1996). Previous research works regarding biological control of whitefly have indicated that parasitic species belonging to genus *Eretmocerus* (Hymenoptera: Aphelinidae) are consistently the best performing biological control agents under laboratory as well as greenhouse conditions (Goolsby *et al*., 1996; Ren *et al*., 2001). *Eretmocerus* sp. nr. *furuhashii* (Hymenoptera: Aphelinidae), a parasitoid of sweet potato whitefly *B. tabaci*, has a widespread distribution worldwide (Qiu *et al*., 2003). Parasitoid females preferentially oviposit within 3rd and 4th instar nymphs of whitefly and the higher rates of development were observed within 3rd nymphal instar (Roudriguez *et al*., 1994; Gerling and Fried, 2000; Kirk *et al*., 2000).

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Isaria fumosoroseus (*Paecilomyces fumosoroseus* designated as *Isaria* clade, Luangsa -Ard *et al*., 2005) is one of the most important natural enemies of several insect species including *P. xylostella* (Altre *et al*., 1999), *Bemisia argentifolii* (Vidal *et al*., 1997) and *Diuraphis noxia* (Vandenberg, 1996). This fungus has been used as a mycopesticide for whitefly management in the United States, Europe and China (Osborne and Landa, 1992; Landa *et al*., 1994; Huang and Ren, 2004; Huang *et al*., 2006, 2007) both in greenhouse as well as in open environment.

The biological control agents may act synergistically, additively or antagonistically. Synergetic interactions between pathogens and insect natural enemies can enhance control efficacy, whereas antagonistic interactions can also reduce total control efficacy (Roy and Pell, 2000). Adult females of *Aphelinus asychis*, a common parasitoid of Russian wheat aphid (*Diuraphis noxia)* when exposed to *Paecilomyces fumosoroseus* significantly reduced host searching ability (walking time, speed, and distance) at high humidity and high pathogen dose (Lacey *et al*., 1997). Accordingly, interactions between specific entomopathogenic fungi and natural enemies should be examined before their use in wide scale field or greenhouse pest-control

systems.

To date, most of the studies on whitefly parasitoids show their ability to establish and maintain a high level of parasitism and generally secondary effects of entomopathogenic fungi on endoparasitic insects are little known (Goettel *et al*., 1990). Therefore, the aim of this work was to study the effect of different conidial concentrations of *Isaria fumosoroseus* on different development stages (pupa and adult) of *Eretomecrus* sp. nr. *furuhashii* presenting evidence of the capacity of these species to be used together with *I. fumosoroseus* against *B. tabaci*.

MATERIALS AND METHODS

Insects

Eretmocerus sp. nr. *furuhashii* and *Bemisia tabaci* were collected from the stock colony kept in greenhouse of the Engineering Research Center of Biological Control (ERCBC), South China Agricultural University (SCAU) on *Euphorbia pulcherrima* Willd (Poinsettia), respectively. Plants were grown in plastic pots (15cm diameter). Sufficient slow release fertilizer (N: P: K=13:7:15, Shenzhen Batian Ecotypic Engineering Co., Ltd. Xili Shenzhen, China) was added as required to maintain normal plant growth. After maintaining on host plants for more than ten generations, *B. tabaci* and *E.* sp. nr. *furuhashii* used in these studies were moved into an air-conditioned room (at 25±2ºC , 80-90% RH, 14:10 L:D). Parasitoids females were exposed to poinsettia plants infested with *B. tabaci* 3rd and 4th instar nymphs for parasitization. A large number of *B. tabaci* adults were put into plastic bags with small holes cased by needle, the leaves of poinsettia were fixed in plastic bags. *B. tabaci* adults were taken out after 6 hours of egg deposition. Nymphs of *B. tabaci* were kept on host plants in cages (60×60×60cm) in an air-conditioned room. When nymphs of *B. tabaci* developed into third instar, 90-100 third instar nymphs per leaf were reserved for treatment.

Fungi preparation

For all assays, the fungal strain PF01-N4 originally isolated from *B. tabaci* nymph (Huang and Ren, 2004), maintained in tubes containing Sabouraud dextrose agar (SDA) and deposited in ERCBC-SCAU, was cultured on potato dextrose agar (PDA) and incubated at 26±2^{o}C for 10 days. Conidia were harvested with distilled water containing 0.02% Tween 80 and sieved through filter paper into sterile vials. Conidia were counted in a compound microscope using a hemocytometer (0.0625m m^{2}; Fuchs-Rosenthal Merch Eurolab) to calibrate a suspension of 1×10^{8} conidia/ml of *I. fumosoroseus*. Lower concentrations of 1×10^{7} - 1×10^{4} conidia/ml were prepared by serial dilutions.

Before the preparation of fungal suspension, spore viability was determined by spreading 0.2 ml of 1×10^{4} conidia/ml suspension on PDA and the number of germinated propagules were estimated under compound microscope after 24 h of incubation at 25±2°C, RH 80-90%, L : D = 14 : 10. Propagules were considered viable when the germ tube lengths correspond to the width. The viability of conidia was assessed immediately before each experiment was started and percentage germination was estimated to > 95% for all experiments.

Influence of I. fumosoroseus *on young larvae and pupae of* E. *sp. nr*. furuhashii

Each leaf with 90-100 third instar of *B. tabaci* was put into leaf cage, three-mated females (2 days old) of *E.* sp. *furrhashii* were released into each leaf cage for oviposition 48 h. Plant materials were left in cages (60×60×60cm) in an air-conditioned room and the leaf cages with parasitoid were removed after 48 h.

Six days following third instar of *B. tabaci* treated with parasitoid adults (probably parasitioids were in the 1st larval stage) and after 12 days of parasitoid oviposition (parasitoids were in pupal stages), leaves were dipped into *I. fumosoroseus* suspension (1×10^{4}, 1×10^{5}, 1×10^{6}, 1×10^{7}, 1×10^{8} conidial/ml) for 15 seconds and they were then left to dry out and labeled. Control leaves were also immersed into 0.02%Tween 80 distilled water for 15 seconds. Following the fungal treatment leaves bearing *I. fumosoroseus* treated *B. tabaci* scales were sealed using plastic bags to maintain a relative humidity of 80-90%. After 24 h, plastic bags were removed and plants were kept under the aforementioned laboratory condition. After 6 days of parasitoid treatment with *I. fumosoroseus*, treated

leaves were examined and the number of healthy red-eyed pupae of *B. tabaci* as well as rate of emergence of *E*. sp. nr. *furuhashii* was recorded. For each concentration 20 leaves were treated at one time and each concentration was replicated 3 times at the same time.

Effect of Isaria fumosoroseus *on survival of adult* E. *sp. nr*. furuhashii

Test tubes were treated with different conidial concentrations (1×10^4 to 1×10^8 conidia/ml) of *I. fumosoroseus* suspension. After drying, 12 adults of *E*. sp. nr. *furuhashii* were introduced in the test tubes having cotton boll imbibed with honey for each conidial concentration and each treatment was replicated 3 times. The tubes were incubated in an air-conditioned room. After 24 h the alive insects were transferred to untreated test tubes with leaves and provided with honey as a food source. The tubes were incubated and mortality of insects was assessed at 3, 5 and 7 days intervals. Dead insects, *i.e.* those which presented reddish atypical coloration, were removed and immediately surface sterilized with 70% ethanol followed by 3 rinse with distilled water. Then the cadavers of each of the treatments (including the controls) were placed on sterile wet filter paper in Petri dishes sealed with parafilm and examined under a stereomicroscope 5 days later to determine if there was any mycelial growth or conidiation of *I. fumosoroseus* on the insects.

Effect of I. fumosoroseus *on* Eretmocerus *sp. nr*. furuhashii *adult*

The female parasitoids developing from the treated pupae (after 12 days of parasitoid oviposition) were used to evaluate adult longevity, their offspring and adult emergence of next offspring. Adults emerging from the pupal treatment or control were paired in small glass tubes (2×0.5cm). After 24 h of mating, each pair of adults was transferred to plastic bags attached to leaves bearing 90-100 third instars of *B. tabaci*. After 48h fresh leaves with third instars of *B. tabaci* were provided. The leaves together with plant after parasitoid oviposition were kept in cages (60cm × 60cm × 60cm) in an air-conditioned room at 25±2°C, 85±5% R.H and 14:10 (L:D) for the development of brown pupae. The number of brown pupae was observed and then adult emergence was recorded in later period. There were five concentrations and the control, and for each concentration 10 adults were treated. This experimental procedure was replicated 3 times. Observations for longevity from the day of adult emergence up to the death of adult parasitoids were also recorded at the same time.

Statistical analysis

All obtained data were analyzed using one way-ANOVA. When F value was significant, means were separated using Duncan's Multiple Range test at 5% level of significance. All statistical analysis was performed using SAS 8.1 (SAS, 2000).

RESULTS

Influence of I. fumosoroseus *on young larvae and pupae of* E. *sp. nr*. furuhashii

The percentage emergence of *E.* sp. nr. *furuhashii* against different conidial concentrations after 6 and 12 days of parasitoid oviposition is shown in Table I. The emergence of parasitoids from the *B. tabaci* nymphs treated with *I. fumosoroseus* spores and control after 6 days of *E.* sp. nr. *furuhashii* parasitization showed significant differences among different treatments and the control (DF=5, F=19.65, P=0.0001). The results indicated that the number of parasitized larvae surviving decreased with increasing concentrations of *I. fumosoroseus*. Successful parasitoid emergence was greatest (57.9) in the control and lowest (36.0) at a conidial concentration of 1×10^8 conidial/ml.

Immatures of *E.* sp. nr. *furuhashii* 12 days postoviposition were not affected by fungal application and no significant differences were observed for the mean number of parasitoids emerging at concentrations of 1×10^4, 1×10^5, 1×10^6 , 1×10^7 conidial/ml and the control, 1×10^7 and 1×10^8 conidial/ml (DF=5, F=0.4361, P=0.024). Maximum percentage emergence of adult parasitoid (70.7) was observed in the control while significantly similar rates of emergence were observed at 1×10^4, 1×10^5 and 1×10^6 conidia/ml treatments (Table I).

Table I.- Percent emergence of *Eretmocerus* sp. nr. *furuhashii* adults following application of different conidial concentrations of *Isaria fumosoroseus* 6 and 12 days post parasitization at 25±2°C .

Treatments (conidia/ml)	6 days			12 days		
	Parasitized host	Emerged adults	Percentage emergence	Parasitized host	Emerged adults	Percentage emergence
Control	110	79	57.9±12.4 a	110	98	70.7 ±1.1 a
1×10^4	110	76	56.2±9.2 a	110	96	69.1 ±1.2 a
1×10^5	110	67	51.3±8.7 ab	110	95	68.3±1.1 a
1×10^6	110	59	47.1±7.9 b	110	94	67.6±1.2 a
1×10^7	110	50	42.4±9.1 b	110	90	64.8±1.5 a
1×10^8	110	38	36.0±7.5 c	110	89	64.1 ±1.2 a

Means compared by one way ANOVA, number within the same column followed by the same letter are not significantly different (DMRT, $P>0.05$). Data on mean (±SE) percentage emergence were subjected to arcsine square root transformation prior to computation.

Effect of I. fumosoroseus *on survival of* E. *sp. nr.* furuhashii *adult*

Significant differences in adult parasitoid survivorship were observed among conidial concentrations of 1×10^6, 1×10^7, 1×10^8 conidial/ml and the control after 3 days of confinement in test tube (DF=5, F=2.8, P=0.027) (Table II). After 3 days, highest average parasitoid survivorship (76.4) was observed in the control, and it was lowest (61.9) at 1×10^8 conidial/ml.

Table II.- Mean (±SE) percentage survivorship of *Eretmocerus* sp. nr. *furuhashii* adults after 24 h exposure to different conidial concentrations of *Isaria fumosoroseus* and after different time intervals at 25±2°C .

Treatments (conidia/ml)	3 days	5 days	7 days
Control	76.4± 13.4a (34)	38.6 ±6.2a (14)	16.8 ±3.2a (3)
1×10^4	73.2± 9.8a (33)	36.9 ±5.6a (13)	16.8 ±2.8a (3)
1×10^5	70.5± 11.4a (32)	35.3 ±6.4a (12)	13.6 ±4.2a (2)
1×10^6	65.9± 10.1b (30)	35.3 ±4.9a (12)	13.6 ±3.6a (2)
1×10^7	63.8± 8.7b (29)	35.3 ±4.5a (12)	13.6 ±2.8a (2)
1×10^8	61.9± 9.5b (28)	33.6 ±7.3a (11)	13.6 ±3.7 a (2)

Means compared by one way ANOVA, number within same column followed by the same letters are not significantly different (DMRT, *P>0.05*). Data on mean (±SE) percentage survival were subjected to arcsine square root transformation prior to computation.

Maximum survival after 5 days (38.6) was observed in the control and it was lowest (33.6) at 1×10^8 conidial/ml. Similarly after 7 days, maximum survivorship (16.8) was observed in the control and it was minimum (13.6) at 1×10^8 conidia/ml but there were no significant differences in adult parasitoid survivorship after 5 (DF=5, F=1.03, P=0.5834) and 7 days (DF=5, F=1.08, P=0.6379) of confinement into test tubes treated with different conidial concentrations and control.

Effect of I. fumosoroseus *on next offspring and longevity of adult* E. *sp. nr*. furuhashii

I. fumosoroseus showed a non significant

effect on next offspring and longevity of female parasitoids (Tables III, IV). *E.* sp. nr. *furuhashii* females emerging from the treatment of *I. fumosoroseus* 12 days after oviposition, when tested for their reproductive capacity showed no significant differences (*DF*=5, *F*=1.39, *P*=0.2927). The percentage emergence of parasitoids from the *B. tabaci* nymphs produced by the females emerged from treated pupae was almost similar. Maximum emergence (73.1) was observed at control treatment, however, the lowest one (69.4) was observed in 1×10^7 conidia/ml.

There were no significant differences in the longevity of adult *Eretmocerus* emerging from *B. tabaci* nymphs treated 12 days of parasitization among the treatments and control (*DF*=5, *F*=1.39, *P*=0.4683). The longest longevity (4.27 days) was observed for control where as the lowest longevity (4.10 days) was observed in 1×10^8 conidia/ml.

Table III.- The number of progeny and their adult emergence produced by *Eretmocerus* sp. nr. *furuhashii* emerged from *Isaria fumosoroseus* treatment after 12 days of parasitoid oviposition.

Treatment (conidia/ml)	No. of parasitoid ♀	Total parasitized hosts	Progeny per ♀	Adult emergence of next offspring
Control	30	177	5.90±1.89 a	73.1±11.2 a (162)
1×10^4	30	175	5.83±2.67 a	73.0±10.7 a (160)
1×10^5	30	173	5.77±2.87 a	72.3±10.4 a (157)
1×10^6	30	171	5.70±2.19 a	71.6±9.8 a (154)
1×10^7	30	169	5.63±3.01 a	68.4±9.2 a (146)
1×10^8	30	170	5.67±3.49 a	69.4±8.9 a (149)

Means compared by one way ANOVA, number within the same column followed by the same letter are not significantly different (DMRT, *P*>0.05). Data on mean (±SE) adult emergence of next offspring were subjected to arcsine square root transformation prior to computation.

Table IV.- Longevity of emerged *Eretmocerus* sp. nr. *furuhashii* adults from parasitized pupae treated with *Isaria fumosoroseus* after 12 days of parasitoid oviposition.

Treatments (conidia/ml)	No. of parasitoid ♀	Longevity± SE (d)
Control	30	4.27±0.19 a
1×10^4	30	4.20±0.57a
1×10^5	30	4.17±0.22 a
1×10^6	30	4.17±0.26 a
1×10^7	30	4.13±0.42 a
1×10^8	30	4.10±0.40 a

Means compared by one way ANOVA, number within same column followed by the same letters are not significantly different (DMRT, *P>0.05*).

DISCUSSION

In this study, the effect of direct application of *I. fumosoroseus* on the survival of *Eretmocerus* sp. nr. *furuhashii* at two different developmental stages yielded different results. Fransen *et al.* (1987) suggested that an increase in survival of a parasitized host treated with an entomopathogen with increasing age of the parasitoid can be caused by a decrease in susceptibility of the last larval instars or pupal stage to fungal infection. The penetration of entomopathogenic fungus to parasitized host larvae at older stages is likely difficult as compared to unparasitized hosts, which is attributable mainly to the changes in host cuticle (El-Sufty and Führer, 1981).

Overall mortality of *E.* sp. nr. *furuhashii* in early larval stages is most likely attributed to direct host death, which harbors the parasitoid larvae that leads to parasitoid death. According to Brooks (1993), the premature death of the host is the most

frequent consequence of the host-parasitoid -pathogen interaction. Several examples are known where premature death of the host has also caused the death of parasitoid (Billioti, 1955). Keller (1975) reported the influence of *Entomophthora* spp. on the aphid parasitoid, *Aphidius* sp. He found that emerged parasitoids were small in size and sometimes with malformation. Most of the parasitoids died as older instar larva or as pupa, but colonization by the fungus was restricted to the tissues of insect host. The reduction in survival was significant but still some of the parasitoids were able to survive.

Application timing of a pathogen during the use of parasitoid is often an important factor contributing to the success of biological control. Furlong (2004) showed that *B. bassiana* was detrimental to *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) an endolarval parasitoid of *Plutella xylostella* L. (Lepidoptera: Plutellidae). Emergence of parasitoids was reduced and infection of parasitoid larvae by *B. bassiana* increased with an increase in the pathogen concentration. However, parasitoid mortality was avoided by careful application timing of pathogen. *B. bassiana* may be effectively applied at least one day before the predicted parasitoid cocoon formation, thus avoiding infection to parasitoids. Temporal separation also proved to be an important factor for the interaction of *Encarsia formosa*, and the entomopathogenic fungus *Aschersonia aleyrodis* (Fransen and van Lenteren, 1993, 1994).

The mean numbers of offspring (parasitized brown pupae) per parasitoid produced at 25º C were not significantly reduced after spore treatment in 12 days following the parasitization as compared to the control (Table I). Our findings are in conformity with those reported by Fransen and Lenteren (1994). They found that the mean number of *T. vaporariorum* pupae parasitized by *E. formosa* was not significantly different from the number of black pupae in the control after treatment with *A. aleyrodis* after four days of parasitization, but a significant decrease in emergence was recorded when spores were applied after 1-3 days of parasitization. The effect mechanism of fungal infection of the host insect on the fecundity of emerging parasitoids has not been well studied. The females of *E. formosa* that emerged from *Trialeurodes vaporariorum* infected by *A. aleyrodis* demonstrated a behaviour similar to that shown by the females emerged from the non-infected nymphs (Fransen and van Lenteren, 1994).

It can be concluded that control strategies tested are compatible to a greater extent and incorporation of these bioagents have promising prospect for control of *B. tabaci*. Further knowledge is needed about the timing adjustments for various releases of both biological control agents to obtain maximum additive effectiveness.

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