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Entomopathogenic fungi for the management of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) on gerbera (*Gerbera jamesonii*) in polyhouses

K. GOPALAKRISHNA PILLAI* **and P. N. GANGA VISALAKSHY**

Division of Entomology & Nematology, ICAR-Indian Institute of Horticultural Research,
Hesaraghatta Lake Post, Bengaluru - 560089, India
***E-mail:** pillaig@iihr.res.in

ABSTRACT: The experiment was carried out in polyhouse condition to study the potency of entomopathogenic fungi for the management of whiteflies on gerbera. Studies showed *Beauveria bassiana* and *Lecanicillium lecanii* to effect 70.81 and 85.65% and 69.54 and 84.90% mortality over control in the first and second trial respectively. *M. anisopliae* caused 77.86 and 74.35% mortality while spirotetramat caused 79.41 and 96.93% and buprofezin 72.67 and 99.95 % mortality. The differences among the treatments were larger during the first 14 days, but decreased thereafter, becoming insignificant after the second spray. On 14th day of second spray, the mortality caused by fungal pathogens was reduced to 23-45% while chemicals caused 31- 49% and were significantly on par with each other. This study shows that entomopathogens can be components in an IPM under moderate levels of whitefly infestations on gerbera in polyhouses.

Keywords: Gerbera, polyhouse, whitefly, entomopathogenic fungi

INTRODUCTION

Gerbera (*Gerbera jamesonii* Bolus) is the third most preferred cut flower in the world. Presently the cut flower industry is one of many businesses in India and gerbera is widely grown in polyhouses across the country. The predominant species of whiteflies are *Bemisia tabaci* (either B or Q-biotype) and *Trialeurodes vaporariorum* and are primary pests on gerbera under polyhouse condition. It feeds on plant sap, deposit honeydew and inhibit photosynthesis with the development of sooty mold. Its multivoltine development and high rate of multiplication with increased temperatures in polyhouse make the growers rely heavily on chemical insecticides to control the pest (Costa *et al*., 1993; Cahill *et al.,* 1996; Prabhaker *et al.*, 1997 and Palumbo *et al.,* 2001). To control whiteflies in greenhouses, insect growth regulators (IGR) and neonicotinyl group of insecticides have been used as a relatively efficient option. Buprofezin, a thiadiazine insect growth regulator acting as moulting inhibitor, Spirotetramat, an innovative ambimobile insecticide, a tetramic acid derivative, inhibits lipid biosynthesis, whereas acetamiprid and imidacloprid are second generation neonicotinoid insecticides, affect the nicotinic acetylcholine receptor, leading to paralysis and death of pest organisms are effective for controlling sucking insect pests such as aphids, whiteflies, leafhoppers, planthoppers and thrips (Tomizawa and Casida, 2005; Elbert *et al.,* 2008; Matsuda *et al.,* 2009; Nauen *et al*., 2008 and Jeschke *et al.,* 2011). In china *B. tabaci* (B-biotype and Q-biotype) has developed resistance to these three insecticides. Moderate to high levels of resistance to two neonicotinoids were established in both biotypes (28–1900-fold to imidacloprid, 29–1200-fold to thiamethoxam (Wang *et al.,* 2010 and Yuan *et al*., 2012). Because of frequent applications of pesticides (1 time/week) to confined populations, the potential for developing pesticide resistant or tolerant strains is very high. This has been lead to the development of resistance to this group of insecticides (Nauen and Denholm 2005). Therefore, it is necessary to find sound alternative control methods as part of integrated pest management programmes (Kunimi, 2007). The ability of biological controls agents to manage whiteflies on various ornamental plants has been well documented, whereas commercially viable IPM and resistance management programs with explicit directions for ornamental crops have not. With respect to the current situation in many commercial polyhouses, there is a critical need for developing resistance management programs for whiteflies. Numerous strategies were tested for controlling greenhouse whiteûy using an array of beneûcial species and species combinations either in succession or without applications of compatible insecticides. There is also a critical need for integrating biological control agents with existing cultural and chemical controls. Entomopathogenic fungi (EPF) have been recognized as important biocontrol agents to control pests, especially sucking insects (Wraight *et al*., 1998). Unlike other biocontrol agents, EPF penetrate the host's external cuticle and need not be ingested to initiate disease. Fungal infection depends on numerous biological events including adhesion of fungal spores to the insect cuticle, spore germination, hyphal growth, etc. Among the entomopathogenic fungi, *Paecilomyces fumosoroseus*, *Lecanicillium lecanii* and *Beauveria bassiana* have had been most widely studied. In the last two decades entomopathogenic fungi, such as *L. lecanii, Paecilomyces spp., B. bassiana* etc. have shown potential as control agents against greenhouse whitefly, *Trialeurodes vaporariorum*, and silverleaf whitefly, *Bemisia argentifolii* (Meade and Byrne, 1991 and Lacey *et al*., 1996). Thirty isolates of six species of entomopathogenic fungi were pathogenic to second instar nymphs of *B .tabaci* biotype Q and two isolates of *B. bassiana, L. lecanii* and *I. fumosorosea* exhibited the highest virulence (Zhu and Kim, 2011). Several oil-based emulsifiable preparations of *P. fumosoroseus* and *B. bassiana* with or without low application rates of imidacloprid 10% WP were reported for control of *T. vaporariorum* populations on lettuce grown under polyethylene film-covered greenhouse conditions (Faria and Wraight, 2001 and Feng *et al*., 2004).

The present study is to develop integrated pest management (IPM) packages, for whitefly pests, which will be highly effective and safe. ICAR- Indian Institute of Horticultural Research, Bengaluru holds isolates of indigenous *B. bassiana, M. anisopliae* and *L. lecanii* in the institute's microbial collections. This paper presents results of studies undertaken to evaluate the potential of these entomopathogenic fungal isolates to control whiteflies. Oil-based emulsion formulations of entomopathogenic fungi were tested under polyhouse conditions against whitefly, *B. tabaci* using insecticides as reference treatments. The results are discussed in the framework of selecting the best safe management practices of whiteflies under polyhouse conditions.

MATERIALS AND METHODS

Gerbera (*Gerbera jamesonii*), cv. Colosseo was grown in a naturally ventilated polyhouse (16mx6m). The polyhouse was divided into forty four quadrates of equal dimension (1mx1m), with 9 plants per quadrate planted at 30 cm ×30 cm spacing, arranged 11 rows by 4 columns. Gerbera was grown under drip irrigation without any pesticide application throughout the experiment. Relative humidity within the polyhouse ranged between 60-70% and temperature 32±2° C during the study. In order to get equally infested gerbera plants, after two months of planting (4-6 leaf stage), the plants were artificially infested with 50 newly emerged whitefly, *B. tabaci* collected from the stock colony cultured on *N. tabacum* in glasshouse. Such releases were made in the centre of each square and monitored for their establishment in polyhouse till the infestation reached the level that was intended for the experiment. Trials were conducted in August-September and November-December 2011.

Three indigenous ICAR- IIHR isolates of *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin, and *Lecanicillium lecanii* (Zimmerman) Viegas maintained in the laboratory were formulated with sunflower oil were tested for their potential to control whitefly, *B. tabaci*. To prepare each formulation, 10 g of conidia powder (CP) of fungus (equivalent to 10^9spores) was suspended in 10 L of tap water to which 3 ml of sunflower oil was added.

The insecticides investigated were as follows: 95% spirotetramat, (cis-3-(2,5dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro(4.5)dec-3-en-4-YL ethyl ester), 97% acetamiprid, ((E)-N((6-chloro-3-pyridinyl)methyl)-N2 -cyano-N-metyl-ethanimidamide, 95% imidacloprid, (1-((6-chloro-3-pyridinyl)methyl)-N-nitro-imidazolidinimine) and buprofezin, (EZ)-2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5- thiadiazinan-4-one, an insecticide that acts by the inhibition of chitin synthesis.

The experiments were laid out in randomized block design with treatments comprising of three mycopathogens in sunflower oil formulations. Three beds were selected randomly and received each of the treatments. Fungal formulations at the rate of $1x10^9$ spores/ml and insecticides at the recommended rates were applied and 250 ml of spray liquid was used to cover each of the 1 m^2 beds. To reduce interference of treatments, one buffer row that did not receive any spray was set up between every two beds. The treatments were imposed on the gerbera from 75 days of planting by using a hand sprayer after taking the pre-treatment count of the pest. Besides the treatments of three oil-based liquids containing *M. anisopliae*, *B. bassiana* and

L. lecanii, three insecticides and untreated check sprayed with Tween- 20 were also included as treatments for comparison. The second spray was provided 15 days after the first one. In the first experiment, three rounds of spray were done, whereas in the second experiment two rounds of spray were given and the observations were terminated after 7days of last spray. For recording observations, three plants were randomly selected from each plot. Sampling was initiated the day before the first spray and observations on whiteflies were recorded at frequent intervals after each spray. Observations were recorded on the population development on treated as well as the untreated control sprayed with Tween-20. The whitefly population was counted on one leaf in the middle part of the plants as nymphs are predominantly found on middle aged leaves. The number of nymphs was counted on a single gerbera leaf (having 50cm^2 area) with the help of a hand lens. The numbers were subjected to analysis of variance (ANOVA). Friedman tests were used to ascertain signiûcant differences within a treatment during the experimental time and were separated by DMRT at 0.05 levels.

RESULTS AND DISCUSSION

Whiteûy established consistently on gerbera and density increased rapidly to reach a level of more than 30 whiteûies per leaf. The experiment was started after 7 weeks as the plants were heavily damaged by whiteûies feeding and large amounts of honeydew with subsequent growth of sooty mold fungi. In the first set of efficacy trials in 2011, during a 60-day period of observation from August 17, the three applications of the fungal formulations and three insecticides effectively protected gerbera from damage by *B. tabaci*. Table 1 shows the whitefly nymph population and percentage mortality caused by the different treatments. On 3rd day after 1st spray, whitefly density decreased more than 70 per cent which was significantly on par with that attributed to the application of spirotetramat and buprofezin and higher than that of imidacloprid. In all the three fungal formulations, the mortality ranged from 65.31 to 70.81 and was almost on par with buprofezin and spirotetramat recorded mortality of 66.59 and 79.41 per cent over the control. Imidacloprid affected 57.48 per cent mortality, which was less compared to other treatments (F range =14.36-27.46; df=6, 56; p value=0.0001). Based on the estimations of relative efficacy and per cent density decrease on day7, spirotetramat and imidacloprid treatments controlled the whiteflies better than the fungi. Although the initial whiteûy density was considerably lower in 3 days after the ûrst spray, whiteûy populations increased steadily. Seven days after the ûrst application, no significant differences in nymph number were observed between the treatments of buprofezin and *M. anisopliae* with decrease in whitefly population. Whitefly population in other plots treated with *B. bassiana* and *L. lecanii* were comparable to those treated with imidacloprid and spirotetramat (F= 68.24, df=6, 56; p value=0.0001). The differences among the treatments were larger during the first 14 days, but decreased thereafter, becoming insignificant after the second spray. On 14th day of second spray, the mortality caused by fungal pathogens was reduced to 23 to 45 per cent while chemicals caused 31 to 49 per cent and were significantly on par with each other (F range =18.44; df=6, 56; p value=0.0001). There was no statistically significant reduction in population levels among the treatments, which effectively had no visible reduction in whitefly population. Although in the control treatment, on day 3, whiteûy population was shown reduction, most likely the result of water sprays or irrigation of the experimental plants.

In the second trial, as per Table 2, on day 3 after the first spray did not result in conspicuous control. For all fungal treatments, relative efficacy and per cent whitefly density decline on day 7 after the first spray and were estimated at 52-85 and 65-97 in case of insecticides (F range =6.50-37.96; df =6, 56; p value =0.0001). On day 15, the two estimates were 66-73 and 61-65 per cent respectively F range =4.15; df=6, 56; p value =0.0001). However, variations in either relative efficacy or whitefly density decline were significant among the fungal treatments, particularly after the first spray with *L. lecanii* rates tended to be more effective than *M. anisopliae* and *B. bassiana*. On day 14, however, all fungicidal treatments had a whitefly density decline of more than 65 per cent, much more effective than those with the insecticidal applications. The second spray of insecticides obviously contributed to the increased efficacy on day 3 and delayed resurgence of the whitefly population, but was not conspicuous in plots treated with fungi. On the all sampling days considered, the estimates of the whitefly density decline from control were all negative, implying a virtual increase of whitefly densities in fungal treatments. This clearly indicates that the efficacies for whitefly control estimated above were attributed to actions of fungal preparations after the two sprays. In contrast, overall mortalities from control maintained at very low levels but did not differ significantly from that observed in insecticidal treatments.

Table 1. Management of whiteflies on gerbera in polyhouse during August - September 2011

Treatment	Mean number and percentage reduction (in parenthesis) over control of whiteflies per leaf after the spray								
		Spray 1			Spray 2			Spray 3	
	Pre spray	3DAS	7DAS	14DAS	3DAS	7DAS	14DAS	3DAS	7DAS
M. anisopliae @ 1x10[9] spores/ml + Sunflower oil	60.33	17.78ab (66.38)	34.00bc (8.11)	11.78a (64.42)	10.33a (67.82)	17.00ab (66.81)	15.00a (25.00)	8.00a (77.85)	15.67a (57.90)
B. bassiana @ 1x10[9] spores/ml + Sunflower oil	75.56	15.44a (70.81)	20.67ab (44.14)	14.67a (55.69)	11.22a (65.06)	24.11ab (52.93)	11.00a (45.00)	14.44a (60.01)	16.33a (56.13)
L. lacaenii @ 1x10[9] spores/ml + Sunflower oil	56.11	12.22a (65.31)	19.89ab (46.24)	12.22a (63.09)	9.78a (69.54)	19.44ab (62.05)	15.33a (23.35)	13.67a (62.14)	16.67a (55.21)
Spirotetramat 150 OD (O.OO5%)	62.78	10.89a (79.41)	16.33a (55.85)	8.00a (75.84)	13.44a (58.14)	15.78a (69.19)	10.67a (46.65)	19.00ab (47.38)	13.22a (64.48)
Buprofezin 25% SC (O.OO5%)	86.89	17.67ab (66.59)	35.11bc (5.11)	12.67a (61.73)	10.44a (67.49)	14.00a (72.67)	13.78a (31.10)	11.56a (67.97)	15.67a (57.90)
Imidacloprid17.8% SL (O.OO5%)	76.22	22.49b (57.48)	17.07a (53.86)	23.14b (30.11)	12.17a (69.10)	21.27ab (58.47)	10.25a (48.75)	16.13a (55.33)	17.27a (53.60)
Control (Mean number)	69.22	52.89c	37.00c	33.11c	32.11b	51.22c	20.00b	36.11c	37.22b
CD @5%	NS	5.53	7.4	5.05	4.1	5.5	3.8	5.54	4.48

The values followed by same alphabet are statistically on par with each other by DMRT

Table 2. Management of whiteflies on gerbera in polyhouse during November- December 2011

Treatment	Mean number and percentage reduction (in parenthesis) over control of whiteflies per leaf after the spray				
	Pre spray	Spray 1		Spray 2	
		3DAS	7DA1	14DAS	3DAS	7DAS
B. bassiana @1x10[9] spores/ml + Sunflower oil	36.2	16.45c (62.13)	7.48b (85.65)	14.25ab (69.63)	8.55d (73.96)	13.56c (65.59)
M. anisopliae @1x10[9] spores/ml + Sunflower oil	36.4	30.33d (30.18)	25.28c (51.51)	16.12bc (65.64)	11.20e (65.90)	10.11b (74.35)
L. lacaenii @1x10[9] spores/ml + Sunflower oil	38.1	17.32c (60.13)	7.87b (84.90)	12.50a (73.36)	6.32c (80.76)	14.23c (63.89)
Imidacloprid17.8% SL (O.OO5%)	35.6	7.74b (82.18)	1.68a (96.78)	16.21bc (65.45)	2.36b (92.81)	0.02a (99.95)
Buprofezin 25% SC (O.OO5%)	30.8	6.40a (85.27)	1.42a (97.28)	16.33bc (65.20)	1.23a (96.25)	0.02a (99.95)
Spirotetramat 150 OD (O.OO5%)	30.9	7.12a (83.61)	1.70a (96.74)	18.21c (61.19)	2.12ab (93.54)	1.21a (96.93)
Control (Mean number)	36.2	43.44e	52.13d	46.92d	32.84f	39.41d
CD @5%	NS	1.14	2.32	2.33	0.98	1.25

The values followed by same alphabet are statistically on par with each other by DMRT

Moreover, overall mortalities significantly increased over time after the second spray and peaked on day 7 or 14 after the second spray.

Entomopathogenic fungi, in general, have a great potential to control whiteflies (Wraight *et al.*, 1998; Meade and Byrne, 1991 and Lacey *et al.*, 1996). Using *L. lecanii, M. anisopliae* and *B. bassiana* conidia suspended in sunflower oil, observed high mortality rates in all developmental stages of *whiteflies*. In the current study, high percent mortality of adults and larvae was also observed when oil formulation was used, indicating that *L. lecanii* has potential for controlling *B. tabaci*. In our experiments, all the treatments led to a sufficient whiteûy control to a level below the threshold which a grower would tolerate in commercial cut gerbera production. Therefore, if whiteûy population density in polyhouse is above 10–20 nymphs and adults per leaf, a prophylactic spray of chemical insecticide can be recommended. Good coverage and long persistence of conidia on foliage are important for effective control of whitefly with fungal pathogens.

The efficiency of the native parasitoid, *Encarsia transvena* Timberlake for the management of greenhouse whitefly, *Bemisia tabaci* (Gennadius) was evaluated in cages and a greenhouse in India (Pillai *et.al.* 2013). Parasitism by *E. transvena* of *B. tabaci* on *Lycopersicon esculentum* L. (tomato), *Solanum melongena* L. (eggplant) and *Nicotiana tabacum* L. (tobacco) were evaluated in polyhouse and suggested them as potential banker plants. Along with the banker plants, three to four rounds of weekly application of mycopathogens *(L. lecanii, B. bassiana*, *M. anisopliae),* could also provide consistent reduction in whitefly population. Among the insecticides, Spirotetramat which belongs to the chemical class of ketoenols, was highly effective throughout the trials.

The data on efficacy of various treatments revealed that the mycopathogens in different formulations varied in their efficacy during the two seasons. *L. lecanii, B. bassiana*, *M. anisopliae* in sunflower oil were found to be effective and were comparable with the efficacy of the chemical (acetamiprid 0.005%). The IGR, Buprofezin was found to be promising for integrating with the control measures as they gave control comparable with the chemical pesticide. The ability of an entomopathogen to persist in the habitat of its host is important for the effectiveness of naturally occurring and introduced pathogens (Jacques, 1983). Our results indicate that enotmopathogens are able to survive well in the polyhouse and to kill whiteflies over a period of at least one month. Oil assists in the process of conidial adhesion to the arthropod cuticle, which is the first step of the infection process, and facilitates conidial germination and penetration through the tegument (Polar *et al.*, 2005, Prior *et al.*, 1988). In addition, oil-based solutions themselves may be toxic to arthropods. In the current study, however, control groups treated with just mineral oil exhibited very low mortality indicating that whitefly mortality in the treatment groups was caused by *entomopathogenic* infection. On gerbera both declined, suggesting a less favourable environment for persistence of conidia. In conclusion, local entomopathogenic fungi strains are effective for whitefly control under polyhouse conditions. For practical application, a high efficacy has to be guaranteed under various environmental conditions. The persistence of fungus on gerbera leaves over long periods of time is a positive aspect of these biocontrol agents. Since newly emerged whitefly adults move upwards from the old canopy to younger leaves for feeding and oviposition (van Lenteren and Noldus, 1990), reapplication of the entomopathogen to new canopy will still be needed. Overall, sufficient evidence is observed in this study that the whiteflies can be successfully suppressed with frequent entomopathogenic fungus applications.

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