Indian Journal of Entomology, **75**(2): 95-98 (2013)

EFFECT OF ENTOMOPATHOGENIC FUNGI *BEAUVERIA BASSIANA* (BALS.) AND *LECANICILLIUM MUSCARIUM* (PETCH) ON *TRIALEURODES VAPORARIORUM* WESTWOOD

NASER MALEKAN,* BIJAN HATAMI, RAHIM EBADI,* ALIREZA AKHAVAN,* AZIDAH BINTI ABDUL AZIZ*** AND ROUHOLLAH RADJABI**

**Plant Protection Department, Isfahan University of Technology, Isfahan, Iran*
***Plant Protection Department, Islamic Azad University, Khorasgan Branch, Isfahan, Iran*
****Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia*
*****Plant Protection Department, Islamic Azad University, Dezful Branch, Dezful, Iran.*
E-mail: rohollah_rajabi@yahoo.com

ABSTRACT

The advantage of a combination treatment with two entomopathogenic fungi *Beauveria bassiana* and *Lecanicillium muscarium* was assessed in laboratory bioassays on different nymphal stages of *Trialeurodes vaporariorum.* Among the fungal isolates, two isolates of each fungus differing in virulence from the highly virulent against *T. vaporariorum* were chosen. The results showed that there was a significant difference in mortality between the 10^3conidia/ml and 10^6 conidia/ml for both *L. muscarium* and *B. bassiana.* The mortality due to the combination were generally higher than that caused by each in 10^6 conidia/ml. on young nymphs. The pathogenicity in the combination treatments 10^6 conidia/ml of *L. muscarium* with *B. bassiana,* no synergistic effect was observed. The mortality was higher in 10^3 conidia/ml of *L muscarium* plus 10^3 conidia/ml of *B. bassiana* compared to 10^3 conidia/ml of *B. bassiana* alone while there was no significant difference between 10^3conidia/ml of *L. muscarium* and 10^3 conidia/ml of *L muscarium* plus 10^3 conidia/ml of *B. bassiana.* From these experiments, beneficial effect was observed in using the two fungi together compared compared to the separate application of *B. bassiana.*

Key words: Entomopathogenic fungi, *Beauveria bassiana, Lecanicillium muscarium, Trialeurodes vaporariorum,* Combination treatment

Several entomopathogenic fungal pathogens of whiteflies such as *Beauveria bassiana, Lecanicillium spp.* and *Metarhizium anisopliae* with good potential for control had been isolated from a variety of insects (Wraight et al. 1998; 2000, Faria and Wraight, 2007). When the conidia contact the host, they germinate, penetrate the cuticle, and grow inside, killing it (Joseph *et al*. 2010). Mycoinsecticides are a wide range of fungi which invade actively the cuticle of insects which is, an advantage for the management of aphids, thrips and whiteflies. For the successful biological control the biology of the control agent but also it's compatibility with other agents is important (Cuthbertson and Murchie, 2007). Appropriate co-application of two or more fungi or fungal strains with different host ranges and climatic tolerances can be considered a strategy to achieve a reasonable control. It has been shown that there is a need for coformulation studies to examine whether fungi will act synergistically (Wang *et al.* 2002). Very few previous studies had evaluated the efficacy of combined application of two entomopathogenic fungi. Inglis et al. (1999) tested the efficacy on the grasshopper, *Melanoplus sanguinipes* with *B. bassiana* and *Metarhizium anisopliae* and to determine if efficacy over different temperatures could be increased. The objective of our study was to investigate the compatibility effect of the entomopathogenic fungi, *B. bassiana* and *L. muscarium* against nymphal stages of *T. vaporariorum* under laboratory conditions.

MATERIALS AND METHODS

The adults of greenhouse whitefly, *Trialeurodes vaporariorum* collected from a colony maintained in a greenhouse in campus of the Isfahan University and Technology (Isfahan, Iran) was reared on tomato variety CH. Falat at 24±2°C, and 16:8 h photo period following the method of Cuthbertson *et al*. (2005). Nymphal instars were obtained from this colony. The colony was established from an approximately of 1000 male and female adults kept in insect-free cages (80H-80W-80D cm). Each tomato leaflet was covered with

a clip cage. Approximately 40 adults (female and male), randomly collected from a single population, were transferred into each clip cage. Then allowed to lay eggs on the leaflet, for 72h at 24±1°C, 70±10 R.H under 16:8 (L:D) photoperiod. After a period of 11 days, the first and second instar nymphs appeared and 20 days after the laying eggs, the third and fourth instars appeared.

Isolates of *B. bassiana* and *L. muscarium* (DAOM198499) were obtained from the Research Institute of Forest and Rangelands (Tehran, Iran). These fungi were grown in the petri dishes on potato dextrose agar (PDA) in the incubator at 24 ± 2 °C under a 16h photoperiod.

A mixture of conidia and hyphae were harvested from 13-15 days-old cultures by scraping with a sterile glass bar and suspended in 10 ml of sterile distilled water supplemented with 0.01% (vol/vol) Tween 80 surfactant. Conidia were separated into 2 layers of sterile tissue paper shaken by horizontal shaker for 5 min at 3000 rpm for producing a homogenous conidial suspension. The concentration of conidial suspension was determined by using a Neubauer haemocytometer. According to the method of Hall (1976), the viability of the conidia was assessed ensure it is more than 95.5%. For each treatment, conidial suspensions of 10^3-10^4-10^5-10^6 conidia/ml were applied to the each leaves using a sterlized hand spray with fine droplet spray nozzle from a distance of 20 cm. The experiment was performed in a complete randomized block design with six replicates. The leaves with insects were carefully transferred to petri dishes (90 mm diameter) that was lined with two 90 mm wetted filter papers. The leaf petiole which was cut and covered with a piece of sterile cotton containing 1% NPK (20-20-20) fertilizer. A piece of plastic was placed on the filter papers so that the leaves did not touch the wet surface. The control leaves were treated only with sterile distilled water that contained 0.01% (vol/vol) Tween 80 and were left to dry at room temperature for a few minutes. The dishes were incubated at 24±1°C,70±10, R.H. under 16:8 (L:D). The pathogenicity on *T. vaporariorum* nymphs was monitored 7 days after incubation using a binocular. Nymphs that were either covered by dense mycelia mass or whose color changed from transparent greenish to opaque white were considered dead.

The percentage of mortality was transformed by Abbott's (1952) formula and the final proportion of dead nymphs at each concentration were analyzed by a one-way ANOVA followed by Least Significant Difference (LSD) test at 5% level of significance. All statistical analyses were carried out using SAS software version 8 (SAS Institute 1999).

RESULTS AND DISCUSSION

In laboratory tests, *T. vaporariorum* nymphs were susceptible to the fungi, and there was a significant reduction in survival of the young nymphs as the fungal concentration increased. However, there was a significant difference in mortality between the lower (10^3 conidia/ml) and higher fungal concentrations (10^6 conidia/ml) for both *L muscarium* and *B. bassiana* ($F = 133.95$, $df = 12$, $P < 0.001$). The data revealed that the mortality due to the combination of fungi was higher compared to separate fungus treatments in 10^6 conidia/ml (Table 1). By contrast, no significant difference between mortality caused by combination of two fungi in 10^6 conidia/ml and 10^6 conidia/ml of *L. muscarium* alone has been observed. The mortality was

Table 1. Mean mortality percentage of young instars of *Trialeurodes vaporariorum* caused by *Beauveria bassiana* and *Lecanicillium muscarium*

Treatments	Percentage mortality ±SE
10^6 conidia/ml of *L muscarium*+10^6 conidia/ml of *B. bassiana*	71.12±0.11a*
10^6 conidia/ml of *L. muscarium*+10^3 conidia/ml of *B. bassiana*	66.76±0.46ab
10^6 conidia/ml of *L. muscarium*	66.66±1.82ab
10^6 conidia/ml of *B. bassiana*	62.74±0.53b
10^3 conidia/ml of *L muscarium*+10^6 conidia/ml of *B. bassiana*	49.78±0.68c
10^3 conidia/ml of *L muscarium*+10^6 conidia/ml of *B. bassiana*	18.75±0.38d
10^3 conidia/ml of *L muscarium*	14.12±1.13d
10^3 conidia/ml of *B. bassiana*	3.44±0.52e
control	2.22±0.1e

*Means with the same letter are not significantly different as determined by LSD Test.

Table 2. *Trialeurodes vaporariorum* vs. *Beauveria bassiana* and *Lecanicillium muscarium*

Treatments	Mortality % ±SE
10 conidia/ml of *L. muscarium*+10^6 conidia/ml of *B. bassiana*	89.43±1.17a*
10^6 conidia/ml of *L muscarium*	86.63±0.64a
10^6 conidia/ml of *L muscarium*+10^3 conidia/ml of *B. bassiana*	72.56±0.69b
10^3 conidia/ml of *L muscarium*+10^6 conidia/ml of *B. bassiana*	72.02±0.8b
10^6 conidia/ml of *B. bassiana*	70.43±1.09b
10^3 conidia/ml of *L. muscarium*+10^3 conidia/ml of *B. bassiana*	18.83±0.39c
10^3 conidia/ml of *L muscarium*	15.79±0.81c
10^3 conidia/ml of *B. bassiana*	13.26±0.55c
control	2.76±0.15d

*Means with same letter are not significantly different.

higher in 10^6 conidia/ml of *L. muscarium* plus 10^3 conidia/ml of *B. bassiana*, compared to 10^6 conidia/ml of *B. bassiana* with plus 10^3 conidia/ml of *L. muscarium;* also the mortality was higher in 10^3 coniHMml of *L. muscarium* plus 10^3 conidia/ml of *B. bassiana* compared to 10^3 conidia/ml of *B. bassidna* alone. There was no significant difference between 10^3conidia/ml of *L muscarium* and 10^3conidia/ml of *L. muscarium* plus 10^3conidia/ml of *B. bassiana.*

No synergistic effect was evidenced but in contrast, the percentage of mortality in combination treatment of 10^6 conidia/ml was more than that of 10^6 conidia/ml of *B. bassiana* alone.

The results revealed that *L. muscarium* and *B. bassiana* can be effective. The effects of *L. muscarium* and *B. bassiana* on different life stages of whiteflies and aphids are well known (Poprawski and Jones 2000; Gindin et al. 2000; Down *et al*. 2009; Abd El- Salam and El- Hawary 2011). Our study showed that the third and fourth instars were more susceptible (Table 1 and 2). This finding is supported by Poprawski *et al* (2000) who stated that the third instar of *T. vaporariorum* was highly susceptible to the infection of *Paecilomyces fumasoroseus* and *B. bassiana* on cucumber plants. All nymphal stages were more susceptible to *L. muscarium* compared the *B. bassiana.* In treatments with both *B. bassiana* and *L. muscarium,* only one species sporulated on the nymphs' body as reported earlier (Inglis *et al*. 1999; Wang et al. 2002; Rao *et al*. 2006; Islam *et al*. 2010). Our results revealed a significant difference in percentage mortality between *L muscarium* and *B. bassiana* in all instars. Moreover, *L. muscarium* caused more mortality when compared to *B bassiana* in 10^6 conidia/ml. Similar results on the gypsy moth larvae treated with entomopathogenic fungus *Entomophaga maimaiga* and virus, fungal sporulation on infested larvae had been shown (Malakar *et al*. 1999). It was also shown that *B. bassiana* and *L. muscarium* are mutually compatible in the in vitro tests. This study showed that there was a beneficial effect in/ using two fungi together compared to *B. bassiana* alone. However, their mode of action needs to be investigated further, as it seems that the integrated usage of the insect pathogenic fungi *L. muscarium* and *B. bassiana* could be an effective strategy.

ACKNOWLEDGEMENTS

This work was supported by a grant from the graduate school of Isfahan University of Technology, Iran. The authors would also like to thank Dr. H. Askary (Forests and Rangelands Institute, Tehran, Iran) for supplying the isolate DAOM198499 of *L muscarium* and isolate Fashand of *B. bassiana.*

LITERATURE CITED

Abbott W. S. 1952. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, **18**:265-267.

Abdl Salam, A.M.E. and El-Hawary F. M. A. 2011. Lethal and pathogenic effects of *Beauveria bassiana* and *Lecanicillium lecanii* on the adult and nymph of *Aphis craccivora* Koch. *Archives of Phytopathology and Plant Protection*, **44**(1): 57-66.

Cuthbertson, A. G. S., Walters K. F. A and P. Northing. 2005. The susceptibility of immature stages of *Bemisia tabaci* to the entomopathogenic fungus *Lecanicillium muscarium* on tomato and verbena foliage. *Micropathologia*, **159**: 23-29.

Cuthbertson, A. G. S., and Murchie, A. K. 2007. A review of the predatory mite *Anystis baccarum* and its role in apple orchard pest management schemes in Northern Ireland. *Journal Entomology*, 4 (2): 275-278.

Down, R. E., Cuthbertson, A. G. S., Mathers, J. J., and Walters, K. F. A. 2009. Dissemination of the entomopathogenic fungi,

Lecanicillium longisporum and *L muscarium*, by the predatory bug, *Orius laevigous*, to provide concurrent control of *Myzus persicae*, *Frankliniella occidentalis* and *Bemisia tabaci* *Biological Control*, **50** (2): 172-178.

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

Gindin, G, Geschtovt N.U, Raccah, B. and Barash I. 2000. Pathogenicity of *Verticillium lecanii* to different developmental stages of the silverleaf whitefly, *Bemisia argentfolii*. *Phytoparasitica*, **28**: 229-239.

Hall, R.A.1976. A bioassay of the pathogenicity of *Verticillium lecanii* conidiospores on the aphid, *Macrosiphoniella sanborni*. *Journal of Invertebrate Pathology*, **27**: 41-48.

Inglis, G.D, Duke, G.M, Kawchuk L.M. and Goettel, M.S. 1999. Influence of oscillating temperatures on the competitive infection and colonization of the migratory grasshopper by *Beauveria bassiana* and *Metarhizium flavoviride* . *Biological Control*, **14**: 111- 120.

Islam Md. T, Castle, S.J., and Sh. Ren. 2010. Compatibility of the insect pathogenic fungus *Beauveria bassiana* with neem against sweet potato whitefly, *Bemisia tabaci*, on eggplant. *Entomologia Experimentalis et Applicata*, **134**: 28-34.

Jaronski, S.T. 2007. Soil ecology of the entomopathogenic Ascomycetes: a critical examination of what we (think) we know. In: Maniana, K, Ekesi, S. (eds) Use of entomopathogenic fungi in biological pest managment. Research Sign Post, Trivandrum, pp: 91-144

Joseph, I., Edwin Chellaiali D. and Ranjit Singh A. J. A. 2010. Studies on the influence *of Beauveria bassania* on survival and gut flora of groundnut caterpillar, Spodoptera litura Fab. *Journal of Biopesticides*, **3**(3): 553 - 555.

Poprawski, T.J., Greenberg, S.M, and Ciomperlik, M.A. 2000. Effect of host plant on *Beauveria bassiana* and *Paecilomyces fumosoroseus* induced mortality of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *Environmental Entomology*, **29**: 1048-1053.

Poprawski, T.J., and Jones, W.J. 2000. Host plant effects on activity of the mitosporic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* against two populations *of Bemisia* whiteflies (Homoptera: Aleyrodidae). *Mycopathologia*, **151**:11-20.

Rao, C.U.M., Uma Devi. K. and Akbar Ali Khan P. 2006. Effect of combination treatment with entomopathogenic fungi *Beauveria bassiana* and *Nomuraea rileyi* (Hypocreales) on *Spodoptera litura* (Lepioptera: Noctuidaeae). *Biocontrol Science and Technology*, **16**: 221- 232.

SAS Institude, Inc. 1999. SAS Proceeding Guid. Version 8, SAS. Institude, Inc., Gary, NC. p 1643.

Wang, C.S., Li, Z.Z., and Butt, T.M. 2002. Molecular studies of co-formulated strains of the entomopathogenic fungus *Beauveria bassiana* . *Journal of Invertebrate Pathology*, **80**: 29- 34.

Wraight, S.P., Carruthers, R.I., Bradley, C.A., Jaronski, S.T., Lacey, L.A., Wood, P, and Galaini-Wraight, S. 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silver leaf whitefly, *Bemisia argentifolii*. *Journal Invertebrate Pathology*, **71**: 217-226.

Wraight, S.P., Carruthers, R.I., Jaronski, S.T., Bradley, C.A., Garza C.J., and Galaini-Wraight, S. 2000. Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of the silver leaf whitefly, *Bemisia argentifolii*. *Biological Control*, **17**: 203-217.

(Manuscript Received : July, 2012)