



BIOCONTROL FUNGI IN REDUCING THE POPULATION DENSITY OF THE COTTON WHITE FLY ON COWPEAS

*Hadi Alwan Mohammed Alsaïdy¹, Nuhaḍ Asis Alumairy², Firyal Bahjet³, Hussain Ali Alanbugy⁴

^{1,2},Departement of Animal Resources. Colloge of Agriculture. University of Diyala. Baquba. IRAQ, ³Dep.of plant protection. Colloge of Agric.Univ. of Baghdad., ⁴Dep. Of Soil. Colloge of Agric. Univ. of Diyala.

*Corresponding Author

ABSTRACT

This study was conducted for the period from April to June 2013 in the village of Mouradia - Diyala Province / Iraq, to study the effect of isolation fungus *Beauveria bassiana* (BSA3) Carried on the Millet seeds in concentration 1×10^8 and the commercial product Mycotal's fungus *Licanecillium muscarium* concentration of 1×10^7 And bio-pesticide Spinosad utilization rate 0.25 ml / l and the pesticide chemical Hatchi hatchi in reducing the population density of nymphs and adults whitefly *Bemisia tabaci*, the commercial product Mycotal Excelled significant differences on treatments in other biological treatments reduce the population density of whitefly nymphs with 63%, followed by treatment Spinosad and pathogenic fungus *B. bassiana* 52% and 44.67%, respectively. And the results show the effect of the treatments in the number of whitefly adults of *B. tabaci*, where all treatments excelled significantly on the comparison treatment. Also the treatment of the commercial product Mycotal affected in reducing the density of whitefly adults 53%, which significantly excelled on the Spinosad treatment 48.73%, followed by fungus *B. bassiana* (BSA3) treatment 32.1%. The study proved that the spraying of these pesticides to once may be enough in reducing the community to the extent of pest ambition.

Keywords: Hatchi hatchi, Spinosad, Mycotal, *Bemisia tabaci*, (BSA3), cowpeas plant

1. INTRODUCTION

You can not counting the economic damage caused by Whitefly *Bemisia tabaci* for agriculture in most regions of the world because of the wide agricultural areas that are affected by this pest in the continents of the world and the many families who infect. The cotton white fly caused for more than four decades in financial losses for considerable yearly production plant in the different countries of the world. The damage caused by direct feeding of this pest and secretion of the horny symposium are that encourages the growth of mold on the parts of the plant in addition to the ability to move a lot of viral diseases to plants of importance in reducing the output of the qualitative and quantitative terms. The high cost of control, and reduce the value of marketing and lowering profits are important factors (Ellsworth et al, 1999). The using of insecticides leads in most cases, to development of the phenomenon of insect resistance against chemical compounds and thus lowering efficiency of the control. The insect resistance of *B. tabaci* may be attributed to the susceptibility to chemical pesticides members fly on a white cotton insecticide resistance with increasing rates of laying eggs at a pressure of pesticides and this phenomenon is known Hormoligosis (Dittrich et al, 1990). Abdullah et al (2006) noted that females of *B. tabaci* prefer the plants treated in chemical pesticide such as the chemical insecticide Fenvalerate in laying eggs compared to non-treated plants. Some researchers have attributed the reason for increasing populations of whitefly cotton to the inhibition of parasite activity caused by excessive use of insecticides with a wide range and the killer (Eveleens, 1983). The pathogens of insects can be used as agents of anti-biological control against arthropod pests because of their efficiency in the inhibition of the population of many of these pests and non affected on the human and other organisms. And thus exceeded the resistance operations problems arising from the use of pesticides, particularly that caused contamination of human food, animal and means of resistance lead to the maintenance of natural enemies and contributed to supporting diversity in the environmental management system (Lacey et al, 2001), It can be used pathogenic fungi for insect in biological control programs, where there are about 700 species of pathogenic fungi (Hajek, 2004). These fungi are divided into two groups on the basis of familial spectrum, namely: - specialized fungi, and general fungi (multiple families). Among the most important specialized fungi what follows order Entomophthorales class (Zygomycetes) and the general fungi were belonging to the class Deuteromyces subclass Hyphomycetidae. Entomophthorales fungi characterized by its high specialization in parasitism and obligatory parasites on the insects and other arthropods, so exploited in bio resistance programs to avoid damage to other living organisms. Their using Leads to events and severe epidemiological of the population of hosts (De Faria and Wraight, 2007). fungi Hyphomycetidae Includes about 20 genera of pathogenic fungi to Insect (Aoki, 2003). And fungi types considers the most promising in the production of pesticides of Mycoinsecticides origin including Beauveria, Metarhizium, Lecanicillium, Isaria, Aschersonia, Nomuraea and Hirsutella. Also, 98% of the pesticides of Mycoinsecticides origin commercially manufactured under the sub class Hyphomycetidae (De Faria and Wraight,, 2007). These fungi can be development in industry medium and saved for a long time at a relatively low temperature conditions. The progress that has been in the production and preparation of pathogenic fungi to insects and especially products that contain *Licanicillium muscarium* to encourage and support the use of biological control of these factors that have proved good efficiency in curbing the population of whiteflies. As recorded and developed many fungal products containing fungus *Beauveria bassiana*, tested those products for several years and in different regions. In Iraq, there have been many studies that depended on local isolates of the fungus and proved highly efficient against a number of Economic Entomology (Al-Jubouri, et al, 2006). And in many cases led to the achievement of a good biological control in convertibles agriculture and protected.

Moreover, the types of genus *Lecanicillium* ability to resist the fungal pathogens of plants through the phenomenon of antagonism and fungal parasitism (Kiss, 2003). Benhamou and Brodeur (2001) mentioned to the method of effect of *Lecanicillium* is the growth in the tissues of the host plant, leading to the events of changes in the cells of the host plant and effectiveness in defense. Many of the research indicated also to the importance of using Spinosad in the insect pests resistance because of the material resulting from the Fermenting antenna of bacteria Actinomycetes for the initial organic materials in the soil type produced by *Saccharopolyspora spinosa*. This rare kind is found in the models of soil obtained of the Caribbean islands in 1982. The commercial Spinosad product characterized that he fast effect against many species of insect through the digestive processes or by contact, where he works to stimulate the nervous system for insect and lead to loss of muscle control and continuous stimulation for nerves leads to the death of the insect within 1-2 days (Thompson et al. 1997), Spinosad also affects on the nerve receptors directives on chemical carrier G- Amino Butric Acid (GABA), and has a toxic effect is similar to the effect of chemical pesticides of neonicotinoid group (Salgado, 1997, 1998). Where this biocides used on many insect pests has given satisfactory results on field crops and fruit trees, especially sucking insects such as cotton insects (Banerjee et al, 2000) and wheat (Fang et al, 2002) and tobacco (Blanc et al, 2004) as well as the whitefly *Bemisia tabaci* (Kumar and Poehling, 2007). Therefore, this study aimed at evaluating the effectiveness of fungi *B. bassiana*, *L. muscarium* and commercial product Spinosad in the form of biocides against some phases of the whitefly and comparing pesticide chemical Hatchi hatchi.

2.MATERIAL AND METHODS

2.1.Preparation of field

The seeds of cowpea (*Vigna unguiculata*) were planted in the field after tilling the soil and preparation an area of 2 acres in the pits and on the lines of a length of 12 meters at the end of April in the village of Muradia 04/25/2013 - Diyala - Iraq. The distance between the line and the last 2 meters and the distance between the plant and another 40 cm. After the plants reach the age of 10 days from seedling emergence. Shoot system were spraying in following pesticides.

1-Spores of pathogenic fungus *B. bassiana* (BSA3) was obtained from the National Center for Organic Agriculture where the spores fungus loaded with millet seeds, and is used at a rate of 4 g / liter of water, where the millet seeds loaded in fungus spores were soaked in sterile glass baker (1 liter) and for one hour at room temperature and then separated from the seeds of millet suspense mediated cloth of medical gauze. The concentration was prepared by 1×10^8 spore / ml of fungus spores through the account number of fungal spores suspended in Hemacytometer and by the equation:

The number of spores per ml = total number of spores in the four peripheral cells $\times 2500 \times 610$ (Hansen, 2009)

And then added a drop of 0.01 Tween-20 for fungus suspension, a substance that helps to spread spores in suspense (Ameri, 2009).

2- A commercial product of the fungus *Lecanicillium muscarium* (Mycotal) used at a rate of 4 g / L for concentration 1×10^6 Spore / mL according to the manufacturer's recommendations and is produced by the Netherlands company Koppert.

3- Bio-pesticide Spinosad (*Saccharopolyspora spinosa*), a production company Dow AgroSciences and used according to the manufacturer's recommendations and at a rate of 0.25 ml / L.

4- Chemical pesticide Hatchi hatchi 15% EC with active substance (Tolfenpyrad). Production company Nihon Nohyaku Japanese concentration of 20 ml / L, according to the recommendations of the company.

The number of adults and nymphs of white fly in this experiment were estimated in direct counting method and calculated the numbers after 2, 5, 10, 14, 21 and 30 days of treatment and use equation Abbott (1925). The relative efficiency of each pesticide were estimated according to the following equation:

$$\text{corrected ratio of death} = 1 - \frac{\text{The average number of pest in treatment after control}}{\text{The average number of pest in comparison after control}} \times 100$$

2.2.Sample collection

The samples were collected from leaf cowpea (*Vigna unguiculata*) during the stage of plant growth and harvest even for the period from 5/17 - 06/24/2013, in the morning, where I took 3 leaves / replicates and by 3 replicates / treatment and placed in a bag of polyethylene, and then point mark. And a total of 45 leaves at each reading, the samples were transferred to the laboratory were examined by a magnifying glass or use an optical microscope when needed, and that after placing the samples in the refrigerator for 1-2 hours to inhibition of the movement of insects.

2.3.Statistical Analysis

The data were analyzed by a pratical experiment using RCBD and the significant differences LSD with level 0.05 and the SAS (2001) program was used to analyze data.

3.RESULTS AND DISCUSSION

3.1.The effect of biogenic fungi and pesticide Hatchi hatchi in adult whitefly

The results of Table 1 indicates to fungus *B. bassiana* (BSA3) and product Mycotal treatment excelled on product Spinosad treatment in the two days after spraying 37.45% and 25.09%, respectively. The isolation of fungus *B. bassiana* (BSA3) and Mycotal excelled for the treatment of Spinosad 59.88% and 40.12% after five days of spraying, respectively, and continued effectiveness of the fungus *B. bassiana* (BSA3) in reducing community pests (nymphs) for two weeks where the value of the relative efficiency of its amounted to 68.21%, the reason is attributed to the nature of the plant cowpeas and direct spraying method on the vegetative parts causing fungus spores adhesion on cuticle nymphs and high density, which led to easy penetrate (Long et al, 2000). And the value of the relative efficiency of isolation (BSA3) decreased after three weeks of spraying to 59.88% and the reason for this is to a decline the level of moisture in the air because of the high temperatures in the field or to the lower numbers of whitefly nymphs on the plant in general, As

shown in Figure 1 where observed the decreasing number of nymphs dramatically after 3 weeks of spraying and the reason may be due to the aging of the plant and the lack of palatability his leaves by the insect nymphs.

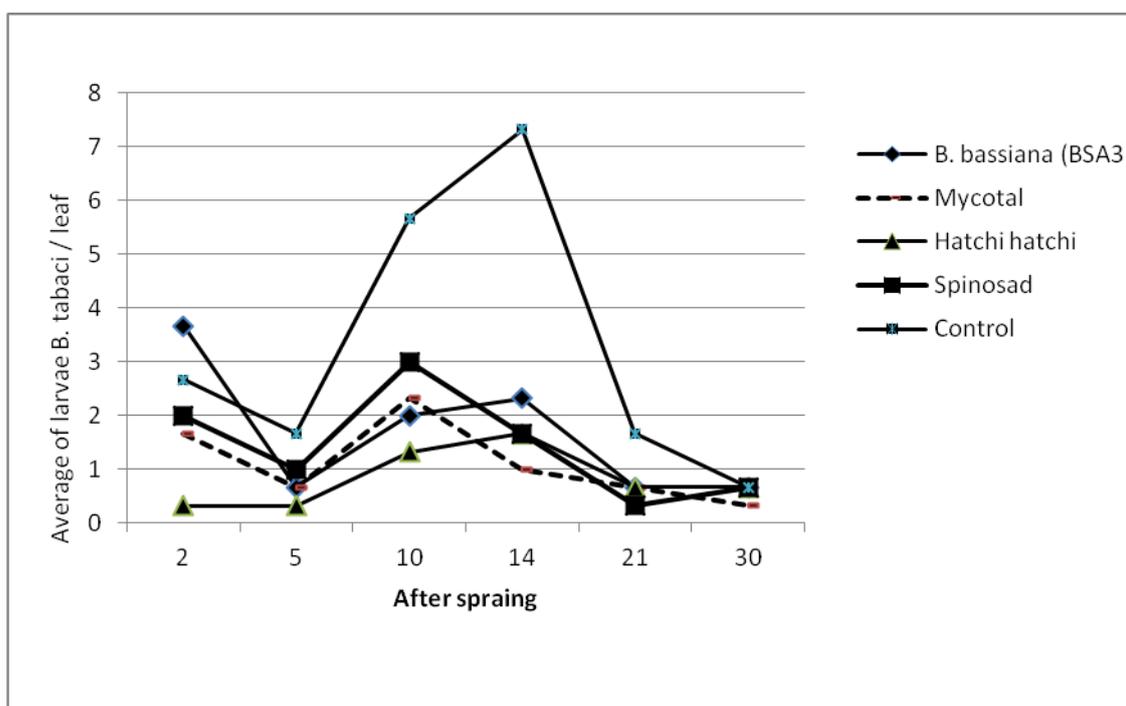


Fig.1. average number of larve *B. tabaci*/leaf of cowpeas on field

Long et al (2000) indicates that the speed of the death of insects treatment depends on several factors, including the number of spores that conjoined in the insect, and the age of the insect and environmental conditions, especially temperature and humidity, and explained as well as methods of contact the insect with fungus spores, whether the fall of the fungus spores directly on the insect during spraying, or when the movement of the insect on surfaces treated with fungus, and also fed on food treated with fungus. When the fungus spores contact the cuticle insect, they germinate and send fungal hyphae penetrates the body of the insect.

Table 1. The relative efficiency of different treatments to reduce the population density of whitefly nymphs on the cowpeas in the field

Treatments	Values of the relative efficiency of transactions / day						Average
	2	5	10	14	21	30	
<i>B. bassiana</i> (BSA3)	37.45	59.88	64.73	68.21	59.88	0.00	44.67
Mycotal	37.45	59.88	58.91	86.36	59.88	50.75	63
Spinosad	25.09	40.12	47.09	77.22	80.24	0.00	52
Hatchi hatchi	87.64	80.24	76.54	77.22	59.88	0.00	70.67
LSD 0.05	4.560	1.594	1.914	1.819	1.479	1.649	0.913

This was confirmed by Walstod et al (1970) as the optimum temperature for the growth of fungus *B. bassiana* ranging between 22-26 °C and maximum limits for growth ranging from 6-44 °C . We note through the general rates of the values of the relative efficiency superior significantly to the commercial product (Mycotal) for the bio-fungus *L. muscarium* amounting to 63% on all biogenic treatments except for the treatment of chemical pesticide amounting to 70.67%, several researchers Ekbohm (1979), Hall (1981) to the efficiency of the fungus *L. muscarium* being a bio-control factor against the aphids and thrips and scale insects and whiteflies. The Hall (1982), Fransen (1987) indicates to the role of bio-pesticide Vertalec with active substance (fungus *Verticillium lecanii*) in the control of whitefly (*Trialeurodes vaporariorum*) and cotton aphids (*Aphis gossypii*) on vegetable crops cultivated in greenhouses, and Deacan (1983) indicated to the effectiveness of Vertalec in glasshouses to control the aphids on chrysanthemum flowers, while Heyler (1993) described the fungus as a bio-pesticide has proven successful in controlling the types of aphids and thrips in the fields, Gindin et al (1996) recommended to the importance of the use of pathogenic fungus *V. lecanii* as an important element in biological control programs against the types of aphids and whiteflies and thrips. While Sharma et al (1999) indicated to the importance of timing when spraying fungus where the effect of fungus *V. lecanii* increasing quickly and effectively against the larval stages that are sensitive to infection, and Mor et al (1996) confirmed the sensitivity of larval stages of infection in fungus spores *V.lecanii* especially whitefly, and Ameri (2011) indicated to the effectiveness of the commercial product Mycotal in the control of all stages of whitefly *Bemisia tabaci* when used spraying on plant eggplant.

3.2.Effect of biogenic fungal and chemical pesticide Hatchi hatchi spraying in adult whitefly

The results in table 2 indicates to the commercial product Mycotal treatment significantly excelled on other treatments after 5 days of spraying in reducing the number of whitefly adults on plant cowpeas to 71.24%, this may be attributable to the infection caused by the fungus spores and mecilia *Lecanicillium muscarium* and spread rapidly in the field and the incidence of adult whitefly during direct spray on vegetative parts that exist mostly under

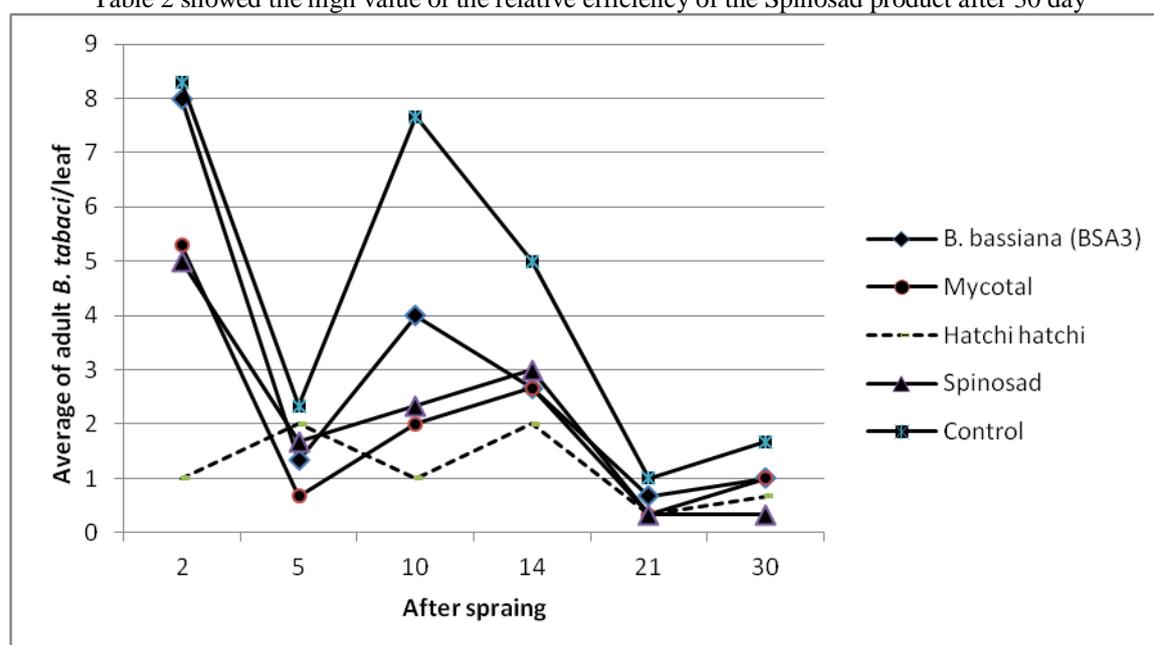
Table 2. The relative efficiency of the biogenic fungal and Hatchi hatchi pesticide in reducing the population density of the adult whitefly on cowpeas

Treatments	Values of the relative efficiency of transactions / day						
	2	5	10	14	21	30	Average
<i>B. bassiana</i> (BSA3)	13.61	42.92	47.85	46.60	33.00	40.12	32.10
Mycotal	36.14	71.24	73.92s	46.60	67.00	40.12	53.81
Spinosad	39.76	28.33	69.62	40.00	67.00	80.24	48.73
Hatchi hatchi	87.95	14.16	86.96	60.00	67.00	59.88	79.45
LSD 0.05	7.67	4.81	2.07	1.92	1.28	1.51	1.71

the bottom surface of the young leaves, which are usually high moisture content suitable and this is essential that help in the events of infection and diffusion (Clarkson and Charnley, 1996), and continued effectiveness of the Mycotal product in reducing community whitefly adults after 10 days and 14 days as the value of relative efficiency of 73.92% and 46.6%. We note from the general rates in Table 2 that the Mycotal treatment (fungus *Lecanicillium muscarium*) excelled than other biogenic treatments and excelled significantly also on Spinosad treatment, which in turn excelled on treatment of pathogenic fungus *B. bassiana* (BSA3) and the values of the relative efficiency amounted to 53.81%, 48.73% and 32.1%, respectively. But compared with the treatment of the chemical pesticide Hatchi hatchi, it emerged that the treatment of pathogenic fungi *B. bassiana* (BSA3) has significantly excelled on other biogenic treatments, the relative efficiency amounting to 79.45%, this was attributed to the Hatchi hatchi pesticide of systemic herbicide with strong influence in the sucking insects. And can be observed from table 2 also reduced the value of the relative efficiency of the Hatchi hatchi pesticide after 30 days spraying to 59.88%

And Figure 2 illustrates the high number of whitefly adults in Hatchi hatchi treatment compared with Spinosad treatment where the number of adult less than 0.5 / leaf.

Table 2 showed the high value of the relative efficiency of the Spinosad product after 30 day

**Fig.2. Average number of whitefly adults *B. tabaci*/leaf on cowpeas in fields**

of spraying to 80.24% and attributed to the toxicity of the Spinosad pesticide and systemic nature of the spread and its effect rapidly in whitefly adults through the stomach, or to the nature of the cowpeas plant leaves saturated Spinosad pesticide and the rapidly effect of whitefly adults when you contact between them with the molecules of pesticide (Thompson et al, 1997). And Immaraju et al (1992) indicated to the best way to use the Spinosad pesticide is sprayed on vegetative parts of the infected in the sucking insects, and does not affect the environment, mammals, birds and fish, and recommended to use it on more than 100 crops, including apples, almonds, citrus, eggplant, tomato, cotton and onions. A study was conducted in Italy in 2010 on the integrated control of thrips onions *T. tabaci*, Mautino et al (2011) in which he indicates to the possibility of the use of Spinosad as an alternative to chemical pesticides currently used in the control of onions thrips, such as Deltamethrin, Diazinon, Dichlorovas pesticides and others, and several studies indicated to the emergence of resistant insects for a large number of pesticides (Martin et al, 2003; Diaz-Montano et al, 2010). There is no chemical pesticide can be used in the homogeneity with bio-resistance factors, Mautino et al (2011) suggested the use of bio-pesticide Spinosad as an alternative to chemical pesticides for the protection of natural enemies.

4. REFERENCES

- Al Amery , S. A. 2009. Determination of variance resource and mechanism of 22 pathogenicity for some *Beauveria bassiana* isolates and evaluation their efficiency in control corn stem borer *Sesamia cretica* under field condition. Ph. University of Baghdad- College of Agriculture .89 p.
- Al- Jabouri, Ibrahim Jaddou, Ismail Ahmed Al-Zobaie and Sndab Sami Dahwi .2006. Evaluate the effectiveness of two isolates of fungus *Beauveria bassiana* in the resistance of some insect pests and acaros and test the efficiency of some breeding medium. Aden University Journal of Natural Sciences and Applied 10 (1): 6.

- Al-Ameri, D. T. 2011. Efficacy of the entomopathogenic fungus *Beauveria bassiana* and the commercial formulation of the fungus *Lecanicillium muscarium* (Mycotal) for the control of the cotton whitefly *Bemisia tabaci*. M. Sc., University of Baghdad- College of Agriculture .66 p.
- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. ; 18 : 265 – 267 .
- Abdulla, N. M. M.; Singh, J. and Sohal, B. S. 2006. Behavioral hormoligosis in oviposition preference of *Bemisia tabaci* on cotton. Pesticide Biochem. and Physiol., 84: 10 – 16.
- Aoki, J. (2003) Keys to the taxa of entomogenous fungi. Pp. 3-29.
- Banerjee, S. K. Turkar and R. Wanjari . 2000. Evaluation of newer insecticides for the control of bollworms in cotton. Pestology; 24: 14-16.
- Benhamou, N. and Brodeur, J. 2001. Pre-inoculation of Ri T-DNA transformed cucumber roots with the mycoparasites, *Verticillium lecanii*, induces host defense reaction against *Pythium ultimum* infection. Physiol. Mol. Plant Pathol., 58: 133-146.
- Blanc, M., C. Panighini, F. Gadani and L. Rossi. 2004. Activity of spinosad on stored- tobacco insects and persistence on cured tobacco strips. Pest Manag; 60: 1091-1098.
- Clarkson, J. M. and A. K. Charnley. 1996. New insights into the mechanisms of fungal pathogenesis in insects. Trends in Microbiol. 4: 197 – 204.
- de Faria, M. R., and S. P. Wraight. 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol. Control, 43: 237-256.
- Deacan, J.W. 1983. Microbial control of pests use of fungi. Microbial control of Plant pests diseases. (VNB) U. K. pp 31-41.
- Diaz-Montano, J., M. Fuchs, B. A. Nault and A. M. Shelton. 2010. Evaluation of onion cultivars for resistance to onion thrips *Thrips tabaci* Linde. (Thysanoptera : Thripidae) and Iris Yello Spot Virus. J. of Economic Entomology 103 (3) : 925- 937. .
- Dittrich, V.; Ernst; G.H.; Ruesh, O. and Uk, S. .1990. Resistance mechanisms in sweet whitefly (Homoptera: Aleyrodidae) populations from Sudan, Turkey, Guatemala, and Nicaragua. J. Econ. Entomol. 83, 1665–1670.
- Ekbom, B. S. 1979. Investigations on the potential of a parasitic fungus (*Verticillium lecanii*) for biological control of the greenhouse whitefly (*Trialeurodes*) Sweden. J. of Agric. Res., 9: 129 – 138.
- Ellsworth, P. C.; Tronstad, R.; Leser, J.; Godfrey, L. D.; Henneberry, T. J.; Hendrix, D.; Brushwood, D.; Naranjo, S. E.; Castle, S. and Nichols, R. L. 1999. Sticky cotton sources and solutions. Univ. Arizona, Coop. Ext. Publ. No. AZ1156, IPM Series 13, 4 Pp. Eveleens, K. G.1983. Cotton-insect control in the Sudan Gezira: analyses of crises. Crop Prot.2, 273–287.
- Fransen, J. J. 1987. Control of greenhouse whitefly T. Vaporarium by the fungus *Aschersonia aleyrodis*. IOBC/WPRS Bulletin 10(2): 57 – 61.
- Gindin, G.; I. Barash; B. Racciah, S. Singer; I. Ben – zeev and Klein. 1996. The potential of some entomopathogenic fungi as Biocontrol against onion thrips, *Thrips tabaci* and western flower thrips. *Frankliniella accidentalis*. Folia Entomologica Lvl: 37 – 42 .
- Hajek, A. E. 2004. Natural enemies: an introduction to biological control. Cambridge University Press, UK. Pp. 396.
- Hall, R. A. 1981. The fungus *Verticillium lecanii* as a microbial insecticide against phids and scales, pp. 484 – 498. In: Microbial control of pests and plant diseases, 1970 – 1980. Academic Press, London .
- Hall, R. A. 1982. Control of whitefly, *Trialeurodes vaporariorum* and cotton aphid, *Aphis gossypii* in glasshouse by two isolates of the fungus *Verticillium lecanii*. Annals of Applied Biology, 10: 1 – 11.
- Hansen, P.J. 2009. Use of a hemacytometer. University of florida.
www.animal.ufl.edu/hansen/protocols/hemacytometer.htm.
- Heyler, N. 1993. *Verticillium lecanii* for the control of aphids and thrips on cucumber. IOBC/WPRS bulletin, 16: 63 – 66.
- Kiss, L. 2003. A review of fungal antagonists of powdery mildew and their potential as biocontrol agents. Pest Manag. Sci., 59: 475-483.
- Kumar, P. and H. Poehling. 2007. Effects of azadirachtin, abamectin, and spinosad on sweetpotato whitefly (Homoptera: Aleyrodidae) on tomato plants under laboratory and greenhouse conditions in the humid tropics. J. Econ. Entomol; 100: 411-420.
- Lacey, L. A.; Frutos, R.; Kaya, H. K. and Vail, P. 2001. Insect pathogens as biological control agents: Do they have a future? Biol. Control, 21: 230-248.
- Long, D. W., G. A. Drummond and E. Groden .2000. Horizontal transmission of *Beauveria bassiana*. Agricultural and Forest Entomology, 2: 11-17.
- Martin, N.A., P.J. Workman and R.C. Butler .2003. Insecticide resistance in onion thrips (*Thrips tabaci*) (Thysanoptera:Thripidae) . NZ. J. Crop Hortic. Sci. 31, 99-106.
- Mautino G. C., L. Bosco and L. Tavella .2011. Integrated management *Thrips tabaci* (Thysanoptera: Thripidae) on onion in north-western Italy: basic approaches for supervised control pest management science. 68 (2): 185-193.
- Mor, H., G. Gindin., I.S. Ben-Zeev., N.U. Geschtovt, N. Arrkhozina and I. Barash.1996. Diversity among isolates of *Verticillium lecanii* as expressed by DNA polymorphism and virulence towards *Bemisia tabaci*. Phytoparasitica. 24: 111-118.
- Salgado, V.1997. The mode of action of spinosad and other insect control products. Down to Earth; 52: 35-44.
- Salgado, V.1998. Studies on the mode of action of spinosad: insect symptoms and physiological correlates. Pestic. Biochem. Physiol. ; 60:91-102.
- SAS .,2001. SAS/STAT. User's Guide for personal computers. Release6.12 SAS Institute Inc., Cary . NC, U.S.A .
- Sharma, S.; R. B. L. Gupta and S. P. S Yadava. 1999. Mass multiplication and formulation of entomopathogenic fungi and their efficacy against white grubs. Journal of Mycology and Plant Pathology, 29(3): 299 – 305.

Thompson, G. K. Michel and R. Yao .1997. The discovery of *Saccharopolyspora spinosa* and a new class of insect control products. Own to Earth; 52: 1-5.

Walstod, J. D., R. F. Anderson and W. J. Stanbaush .1970. Effect of environmental Condition on two species of muscardine fungi *Beauveria bassiana* and *Metarhizium anisopliae*. J. Invert pathol.16: 221 – 226 .