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Efficacy of Bio-Additives Combination with Entomopathogen-Beauveria bassiana against Insects

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Abstract: **Entomopathogenic fungi are the most important natural biocontrol agents of many insects. The awareness of harmful effects of chemical pesticides on man and environment indicates the need of biopesticide. Manyentomopathogens have been found to play an important role in insect pest management and efforts have been stepped up by researcher's world over to exploit their potential for large-scale field use. It is being increasingly recognized and accepted that effective formulations and appropriate delivery systems are keys to the success of microbial control. The different treatment for additive study were Beauveria bassiana + Sunflower oil , Beauveria bassiana + Ground nut oil, Beauveria bassiana + Glycerol, Beauveria bassiana + Ginger oil, Beauveria bassiana alone. The efficiency of the additives was sorted out using drop technique with 4 reps of 10 Corcyra cephalonica larvae in each replication. The efficacy of B. bassiana on various additives showed efficacy on B. bassiana and B. bassiana with glycerol, Sunflower oil, groundnut oil, and Ginger oil efficacy were compared. The mortality LT 50 were observed, lethal time for 50% larvae to mortality was Beauveria bassiana + Sunflower oil.**

Keywords: **Entomopathogenic, Biocontrol, Beauveria bassiana, Pesticides, Additives.**

1. INTRODUCTION

Entomopathogenic fungi are the most important natural biocontrol agents of many insects (Carruthers & Hural, 1990). Biological control, in particular when accomplished by entomopathogens, is a technique that should be considered as an important pest population density reduction factor in Integrated Pest Management (IPM) programs. Therefore, the conservation of such entomopathogens, whether they occur in nature, entomopathogenic fungi have the advantages of production & contact action which allows direct penetration of the lost cuticle without injection. The awareness of harmful effects of chemical pesticides on man and environment indicates the need of biopesticide. Manyentomopathogens have been found to play an important role in insect pest management and efforts have been stepped up by researchers' world over to exploit their potential for large-scale field use. It is being increasingly recognized and accepted that effective formulations and appropriate delivery systems are keys to the success of microbial control.

The origins of microbial pest control date back to the early nineteenth century,The Italian scientist Agostino Bassi works about 30 years studying white muscardine disease in silkworms (Bombyx mori L.) and identified Beauveria bassiana (Bals.-Criv). Vuill., named in his honour, as the cause of the disease. His discovery not only laid the foundation for microbial pest control, but also significantly influenced the work Louis Pasteur, Robert Koch and other pioneers of microbiology (Porter, 1973; Van Driesche & Bellows, 1996). Today, over 100 years later, there are no known reports of significant adverse effects that can be attributed to the use of these organisms in biocontrol. Beauveria bassiana kills the pest by infection as a result of the insect coming into contact with fungal spores. After the attachment fungal spores germinate and sending out structures (hyphae) that penetrate the insect's body and proliferate. It kills insect within 3-5 days, but the pest may serve as a source of spores. It spread Insects through mating (Long *et al.* 2000).

There are 8 reports that harmful to human that has been known by the genus Beauveria Vuill. which has alleged cause of fungal infections in humans were identified, from only 4 of them could be concluded to the species of the genus Beauveria. Like any micro-organism, Beauveria bassiana has the potential to act as an opportunistic pathogen, but as the literature study demonstrates, Beauveria infections are extremely rare events. A detailed analysis of case reports allegedly involving Beauveria bassiana reveals that extraordinary circumstances, such as a severely compromised immune system or a history of surgery/injury, are required for a B. bassiana infection to occur. Only two cases of Beauveria infections reports of disseminated mycoses (Henke et al., 2002; Tucker et al., 2004). Both have occurred in immunocompromised patients with acute leukemia. Out of four reports to Beauveria, only two (Sachs et al., 1985; Kisla et al., 2000) can be concluded to Beauveria bassiana. The affected eye had undergone surgery following traumatic injury to the eye, and in all reported cases, the therapy of the injured eye involved corticosteroids and antibiotics, which, according to Sachs *et al.* (1985) can predispose the eye to fungal infections by otherwise non-pathogenic fungi. Eye irritation studies (US EPA, 2006) and literature reports (Ishibashi et al., 1987; Begley & Waggoner, 1992) show no unacceptable effects related B. bassiana exposure to the healthy eye. Furthermore, it has been used in biocontrol for over 100 years with no reports of illness related to exposure to B. bassiana strains used in biocontrol.

There is no negative effects were found in Beauveria ecotoxicology studies with mammals, birds or fish (US EPA, 2006). Further, B. bassiana is not known to cause adverse effects to plants or earthworms. Compared to the second species of the genus, Beauveria basssiana has a wider host range and a, therefore, in theory, a somewhat higher potential to affect non-target arthropods.

The present study focuses on improving the efficacy of the activity and viability of B. bassiana. In this study, the efficacy of various additives to the fungal culture was been studied out, so they can help in increased affinity to the pest.

2. MATERIALS AND METHODS

The materials used in the experiments are mainly the microbial bioagent – Beauveria bassiana and Corcyra cephalonica which was used for finding the efficacy of the new formulation and additive bioassay.

Laboratory conditions:

These experiments were conducted at room temperature of 32 ± 2º C.

CULTURING OF CORCYRA CEPHALONICA FOR ASSAY:

Corcyra cephalonica was cultured in half ground Pearl millet (*Pennisetum typhoides*) grains. Heat-sterilized broken grains were taken at the rate of 2.5 kg per plastic basin to which groundnut kernel powder was added at 100 gm/basin and yeast powder at 5g/basin. To prevent bacterial infection in the food medium, streptomycin sulphate at 0.05% spray was given at 10 – 20 ml/basin, using a hand-operated sprayer or atomizer. Sulphur (WP) was added at 5 g/basin to prevent storage mite. Inoculums culture of *Corcyra cephalonica* eggs was added at 0.5 ml (about 5000 eggs) to a basin containing 2.5 kg of grain medium. After a period of 45 days larvae were obtained which were used for bio-assay.

STOCK PREPARATION FOR ADDITIVE STUDIES:

Glycerol, Sunflower oil, Groundnut oil and ginger oil were mixed with B. bassiana so that the percentage of the additives is 20% in the solution.

STOCK PREPARATION FOR COMPATIBILITY STUDIES:

Preparation of neem oil (every 1000ml):

Neem Oil	-	905.7ml
Aza	-	0.30ml
Aromax	-	39ml
Solvent	-	5ml
E-Acetate buffer	-	50ml

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Product for compatibility test	Recommended (ml)/L
Metarizium anizophila	10
Sun agro300ppm (Neem)	5
Malanthion	2

Efficiency of Beauveria bassiana with additives:

Standard culture of B. bassiana with CFU unit of 106- 108 /ml will be used. The mixing of the culture with the additives @ 20% volume to be done first before the experiment. Add a drop of Tween 20 as spreader. Take for each treatment, all the four reps and expose 10 larvae each per rep. Keep the larva back after topical application in hundred Petri dishes (wet filter paper on top and bottom. Handle the larvae in the sterilized forceps and avoid using the same forceps to other treatment without sterilizing. Keep them for observations incubated under room temperature. Observe and Record the number of larvae [death] and also the numbers showing fungal growth on larvae bodies' daily observation for 8 days continuously.

3. RESULT

EFFICIENCY OF BEAUVERIA BASSIANA WITH ADDITIVES:

The mortality of larvae with different additives were observed for a period of 8 days continuously and tabulated in table.

Mortality effect of various additives with B. bassiana:

The efficacy of B. bassiana on various additives has been evaluated in the present study showed efficacy on B. bassiana and B. bassiana with glycerol showed effective mortality. B. bassiana with glycerol showed complete mortality on 168 hrs. The effective killing by B. bassiana was enhanced with glycerol, but even tho the initial effectiveness was observed in the oil additives ground nut oil and sunflower oil showing 3.8 and 4.5 on within 24 hrs. Sunflower oil started the effective mortality rate and ended with a little low mortality compared to others. The next effective treatment was with groundnut oil and then was ginger oil. Mortality due to sunflower oil showed a periodically increasing activity. Ginger oil additive showed a delayed mortality response starting on 72 hrs, but had a good efficacy comparing others oil additives. (Table: 1).

Table 1: Mean total mortality of larvae for different additives

Hours	B. bassiana	B. bassiana + glycerol	B. bassiana + ground nut oil	B. bassiana + sunflower oil	B. bassiana + ginger oil	Control
24	0.5	1.3	3.8	4.5	0.0	0.0
48	1.0	2.8	5.5	6.5	0.0	0.0
72	1.8	3.0	6.0	7.5	4.5	0.5
96	2.5	4.5	7.3	8.0	5.0	0.5
120	9.8	9.0	9.5	9.0	8.8	0.8
144	9.8	9.8	9.8	9.3	9.0	1.0
168	9.8	10.0	9.8	9.5	9.3	1.0
192	10.0	10.0	9.8	9.5	9.8	1.0

The product additive showed the best activity by 120 hrs on which they reached the showed maximum mortality rate. The additive ground nut oil, sunflower oil, ginger oil and glycerol would have made the adherence of the spore on the larvae by which it may enhance the activity of Beauveria bassiana. The mortality peaks at 120 hrs (Fig: 1) which shows that the effect of Beauveria bassiana with additive and along is somewhat similar for the kill down ability.

The additive study showed between the six treatments in (Fig 2) pictorially is been represented which shows that on day 1-108 the best treatment on individual days was showed by Beauveria bassiana + Sunflower oil upto day 4. By which all the other treatment reaches high mortality for day 5 (9.8, 9.0, 9.5, 9.0, 8.8) to make the same with Beauveria bassiana + Sunflower oil.

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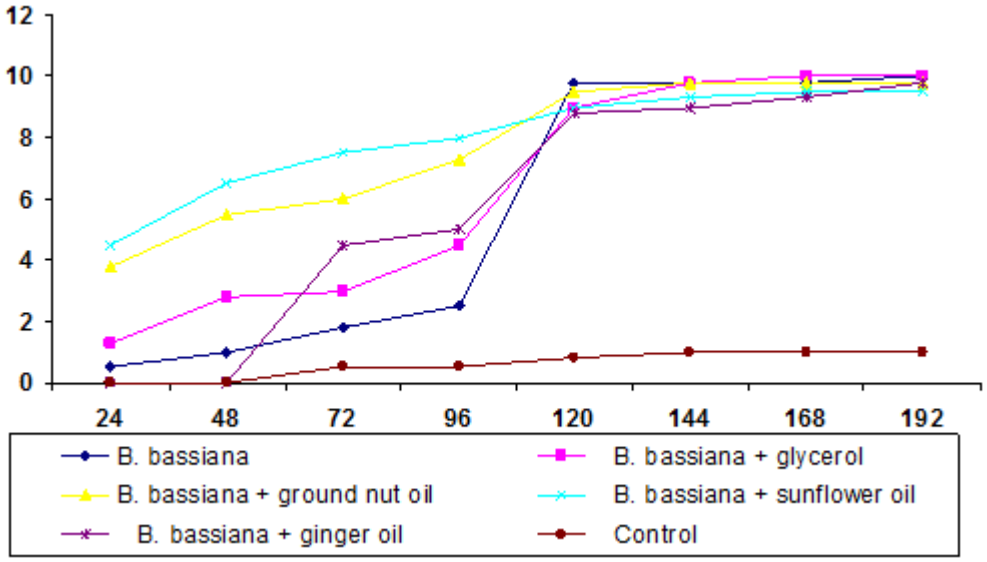

Fig 1: Day wise pattern of mortality for different additives with Beauveria bassiana (in hrs).

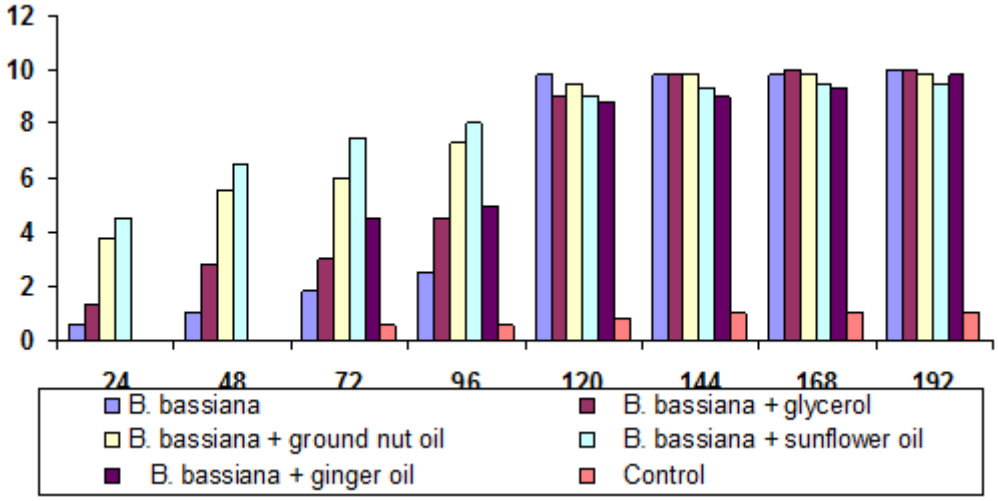

Fig 2: Comparative study on mortality rate for different additives with Beauveria bassiana based on different periods (in hrs).

Correlation results:

The respective trend graph for individual treatments correlating the time period and mortality rate has been shown in (Fig: 3-7). They showed good correlation with the R2 which were all above 0.8 ranging from 0.8342 to 0.8983.

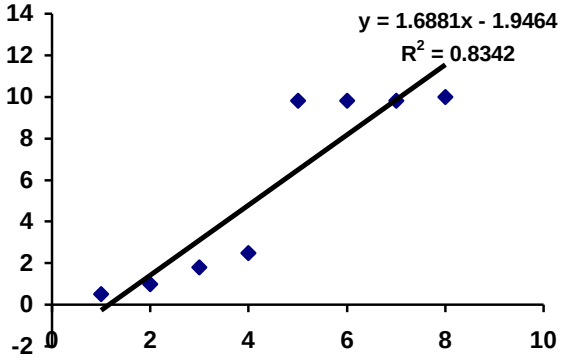

Fig 3: B. bassiana

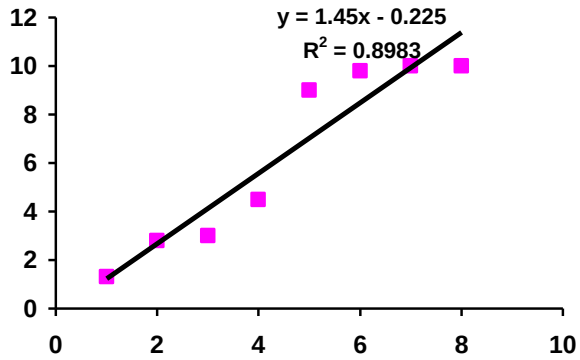

Fig 4: B. bassiana + glycerol

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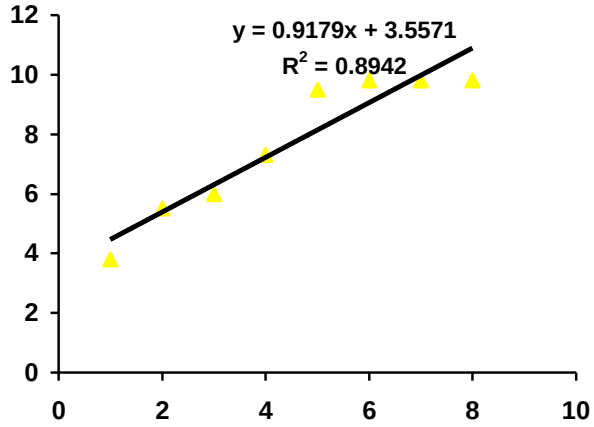

Fig 5: B. bassiana + ground nut oil

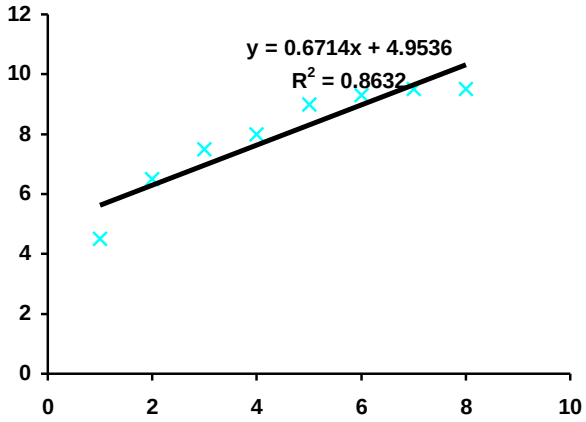

Fig 6: B. bassiana + sunflower oil

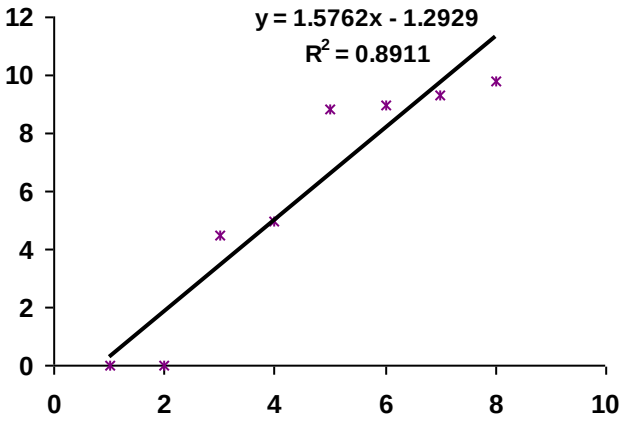

Fig 7: B. bassiana + ginger oil

Lethal Time for 50% mortality of larvae (LT 50):

The LT 50 of different samples was found using the correlation graphs we know the time at which it shows 50% mortality. Finding out the mortality LT 50 of different treatment showed that Beauveria bassiana + Sunflower oil had 50% mortality with 16.12 hrs (Table: 23) followed by Beauveria bassiana + Ground nut oil in 45.09 hrs then Beauveria bassiana + Glycerol with 88.40 hrs. Then the Beauveria bassiana + Ginger oil with 97.09 hrs. Beauveria bassiana alone showed the mortality rate of 50% at 100.17 hrs.the best letal time for 50% larvae to mortality was Beauveria bassiana + Sunflower oil (Fig: 8).

Table 2: Lethal time for mortality of 50% larvae for different additives

TREATMENT	LT 50 (in hrs)
B. bassiana	100.17
B. bassiana + glycerol	88.40
B. bassiana + ground nut oil	45.09
B. bassiana + sunflower oil	16.12
B. bassiana + ginger oil	97.09

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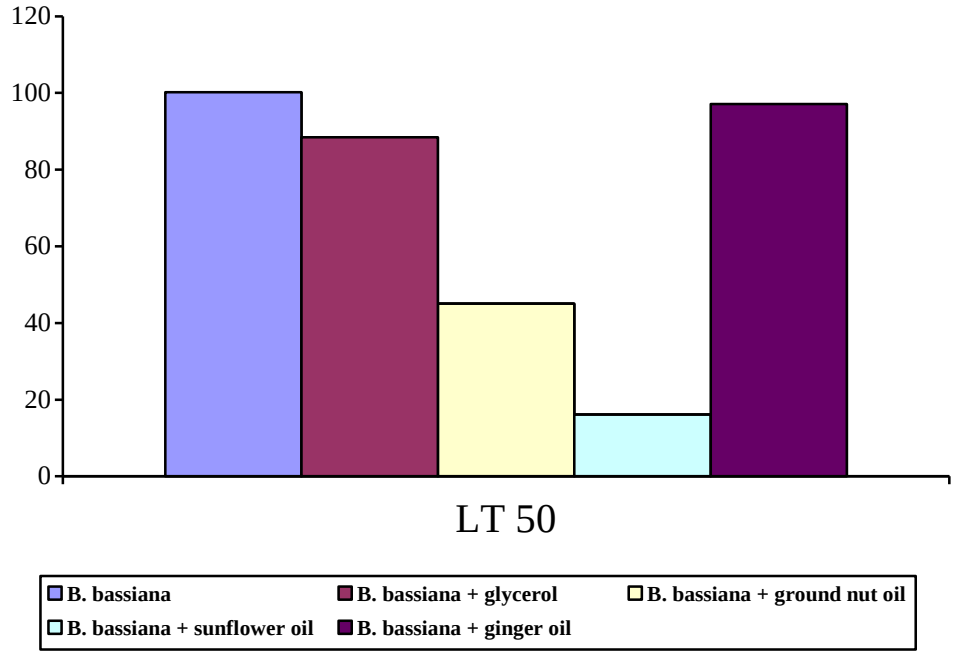

Fig 8: Comparison of LT 50 for different additive treatments.

4. DISCUSSION

ADDITIVE STUDY:

Beauveria bassiana, an entomopathogenic has good efficacy on infecting Lepidoptera pest. As per the result shows the additives which are been mixed with Beauveria bassiana also has the ability to quicker response to kill the larvae. Additives like ground nut oil and Sunflower oil increase the efficacy of Beauveria bassiana at the initial period rather than Beauveria bassiana which glycerol or Beauveria bassiana alone. The initial mortality suggest, as soon as the spray was made with the additives it can effectively enhance activity of the fungi. This are results shown similar vith small variation to the studies reported by Dunn and mechalas, 1963; Kaur et al .,1999; Periera and Roberts., 1999; Knudsen *et al.*, 1991. The oily shows would have been acting as an effective adhesive substance and may be a substrate for the growth of Beauveria bassiana and effective mortality within hours,

5. CONCLUSION

The additives with ground nut oil and sunflower oil showed good result and can be helpful in increasing the efficacy of Beauveria bassiana show that initial mortality of larvae can be obtained.

REFERENCES

[1] Begley CG & Waggoner P (1992). Soft contact lens contamination by Beauveria bassiana. International Contact Lens Clinic 19: 247-251. http://www.sciencedirect.com/ science/article/B6T7B-4C2R9M6-4/2/4a88b87bd79f6836 ecbf3ecb464a693c.

[2] Carruthers,R.I.soper,R.S.1987.Fungal diseases.In :Epizootiology of Insect Diseases.(Eds.)J.R. Fuxa and Y.Tanada.john Wiley and sons, New York.pp.357-16

[3] Dunn, P.H. and Mechalas, B.J.1963.the potential of Beuvaria bassiana (Balsamo) vuillemin as a microbial insecticides .J. insect.Pethol.. 5:491-499.

[4] Henke MO, De Hoog GS, Gross U, Zimmermann G, Kraemer D & Weig M (2002). Human deep tissue infection with an entomopathogenic Beauveria species. Journal of clinical microbiology 40: 2698-2702.

[5] Ishibashi Y, Kaufman HE, Ichinoe M & Kagawa S (1987). The Pathogenicity of Beauveria-Bassiana in the Rabbit Cornea. Mykosen 30: 115-126.

[6] Long, D.W., G.A. Drummond, E. Groden. 2000. Horizontal transmission of Beauveria bassiana. Agriculture and Forest Entomology 2:11-17.

[7] Kaur, G.;Padmaja,V. And sasikala ,V.1999. control of insect pests on cotton through mycopesticide formulations. Indian J Microbial., 39:169-173.

[8] Kisla T, Cu-Unjieng A, Sigler L & Sugar J (2000). Medical management of Beauveria bassiana keratitis. Cornea. 19: 405-6.

[9] Knudsen et al , G.R.: Eschen.,G.J:Dandurand, L.M.and wang, Z.G.1991. Method to enhance growth and sporulation of pelletized biocontrol fungi. Appl. Environ .Microbial., 57:149-156

[10] Pereira,R.M and Roberts, D.W .1991.Aiginate and cornstarch mycelia formulations of entomopathogenic fungi ,*Beauvaria bassiana* and Metarhizium anisopliae.J. econ. Entomol., 84 :1657-1661.

[11] Porter JR (1973). Agostino Bassi bicentennial (1773-1973). Bacteriological Reviews 37: 284-288.

[12] Sachs SW, Baum J & Mies C (1985). Beauveria-Bassiana Keratitis. British Journal of Ophthalmology 69: 548-550.

[13] Tucker D, Beresford C, Sigler L & Rogers K (2004). Disseminated Beauveria bassiana infection in a patient with acute lymphoblastic leukemia. Journal of clinical microbiology. 42: 5412-4.

[14] US EPA (2006). Beauveria bassiana Strain GHA (128924) Technical Document. RE. http://www.epa.gov/ pesticides/biopesticides/ingredients/tech_docs/tech_128924.htm, accessed: Sep 12, 2006.

[15] Van Driesche RG & Bellows TS (1996). Biological Control. Chapman & Hall.