J. Patuakhali Sci. and Tech. Uni. 2017, 8 (1): 15-30 ISSN 1996-4501

INSECTICIDAL EFFICACY OF CULTURE MEDIA OF SYMBIOTIC BACTERIA OF ENTOMOPATHOGENIC NEMATODES ON *BEMISIA TABACI*

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Abstract

Insecticidal activities of culture media of three symbiotic bacteria, *Photorhabdus temperata*, *P. luminescens* and *Xenorhabdus nematophilus,* of entomopathogenic nematodes were tested against *Bemisia tabaci*. Adult *B. tabaci* showed significant mortality when rearing on the leaves which were dipped into 10% culture meidia of symbiotic bacteria. Complete mortality was observed at 60 h in 10% media of *P. temperata* and *X. nematophilus*, but mortality was less than 50% at 72 h in 10% *P. luminescens*. Otherwise, complete mortality was observed in 50% supernatants of three culture media with artificial diet within 60-66 h. In addition, complete mortality was observed in mixtures of 2 or 3 bacterial media of Pt+Pl, Pt+Xn, Pl+Xn, Pt+Pl+Xn within 54, 48, 48 and 42 h, respectively.

Key words: Symbiotic bacteria, *B. tabaci,* mortality.

Introduction

Sweetpotato whitefly is one of the most damaging attractable, cosmopolitan, polyphagous, agronomic and horticultural pests distributed all over the world excluding Antarctica (Boykin et al., 2007; De barro, 2011), causing damage over 600 different plant species (Mound and Halsey, 1978; Greathead, 1986; Secker et al., 1998) either through direct feeding (Oliveira et al., 2001; Al-Deghairi, 2009) or transmitting over 120 plant viruses (Jones, 2003; Hogenhout et al., 2008) or providing the suitable environment for the growth and development of sooty mold fungus by excreting honeydews (Nomikou et al., 2001; Naranjo et al., 2002). Due to global losses of economic crops it is considered one of the top 100 most damaging pests (Touhidul and Shunxiang, 2007; Abdel-Baki and Al-Deghairi, 2008). However the management of whiteflies is always a challenge because of its abaxial surface habitat, high reproductive potential, intercrop movement (Gerling et al., 2001; Al-Deghairi, 2009; Fouly et al., 2011), presence of different life stages at the same time, (Prabhaker et al., 1989), wax secretion, ability to develop resistance rapidly (Cahill et al., 1994; 1996; Ahmad et al., 2002; Cannon et al., 2005) and polyphagous in nature. It is complex species comprising at least 24 morphologically identical but geneically and reproductively isolated species (Boykin et al., 2007; Wan et al., 2009; De barro and Bourne, 2010). The most common practice of whitefly

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management is the use of insecticides alone or mixtures (Castle et al., 1996; Dennehy et al., 1996; Ellsworth et al. 1996; Prabhaker et al., 1998, Palumbo et al., 2001) but these insecticides are partially effective due to waxy layer formed by the pests which resist the penetration of chemicals (James, 2003). Moreover, chemical insecticides are hazardous for environment, mammals and natural enemies. The population of natural enemies suffered due to repeated application of chemical pesticides (Gonzalez-Zamora et al., 2004) which may lead to pest outbreak or pest resurgence. That's why present situation demand alternative pest control strategies. Symbiotic bacteria of entomopathogenic nematode can be used as a valuable tool for whitefly management.

Entomopathogenic nematodes are known to be effective over 200 insect species (Georgis et al., 2006). These nematodes belonging to the genera Heterorhabtid and Steinernematid harbor symbiotic bacteria *Photorhabdus* spp. and *Xenorhabdus* spp., respectively. The bacterium, *Photorhabdus* spp. in the intestinal tract of nematodes while *Xenorhabdus* spp. inhabits in the frontal part of the intestine (Boemare, 2002). Upon entering through spiracles, mouth or anus to host insects, the entomopathgenic nematodes release these bacteria into hemocoel (Forst et al., 2001). *Photorhabdus* are egested through mouthparts whereas *Xenorhabdus* released through anus, moving to hemolypmh then bacteria produce secondary metabolites having antibiotic properties against a series of microorganism (Webstar et al, 2002). In blood stream these bacteria release a range of toxic which is responsible for insect mortality. The actions of all toxin produced by *Photorhabdus* in inducing infection and their biological role still not clear (ffrench-Constant et al, 2003). Among these toxins, toxin complexes (Tcs) consists of high molecular weight multisubunit that are the key factor for virulence (Rodou et al., 2010), showed highly lethal both oral and injectable activity (Blackburn et al., 1998, 2005), In addition, another toxin, the *Photorhabdus* insect related protein (Pir) also has lethal activity via oral ingestion (ffrench-Constant et al., 2007), furthermore, the bacteria produce hydrolytic enzymes (Boemare and Akhurst, 1988; Bowen et al., 2000; Schmidit et al., 1988; Wang and Dowds, 1993). Like *Photorhabdus, Xenorhabdus* showed both oral and injectable toxicity and exert its effect releasing various toxins. *Xenorhabdus* produces three toxic proteins such as Xpt complex (Morgan et al., 2001), cytotoxic pilin subunit (Khandelwal et al., 2004) and XnGroEL associated with oral toxicity (Joshi et al., 2008). These toxins target hemocytes, interfere with the immunity by reducing its vitality (Cowles and Goodrich-Blair, 2005), agglutination (Khandelwal et al., 2004), apostosis or cytosis (Riberio et al., 2003; Vigneux et al., 2007). Recently, rhabduscin is discovered as a potent inhibitor of phenoloxidase, a key component of insect's immune system (Crawford et al., 2012).

Entomopathogenic nematodes are good source for the biological control of pest insects. However, direct application of entomopathogenic nematodes showed varying degree of success because of its stability in different environmental conditions. Otherwise, symbiotic bacteria can be isolated from insect cadaver and massively culture on artificial

media (Forst et al., 1997; Daborn et al., 2001). These culture media is a rich source of insecticidal toxins and can be used as biopesticides (ffrench-Constant et al., 2007). Here, the insecticidal activities of culture media were investigated from three symbiotic bacteria of entomopathogenic nematodes. Mortality was determined using three different culture media, individually or their combination.

Materials and Methods

Insect rearing

Bemisia tabaci was initially collected from the Korean melon (*Cucumis melo* var. *makuwa*) greenhouse in Seongju, Korea in 2009 and maintained in the laboratory as a colony on tomato plants (*Lycopersicon esculentum* Mill.) within insect-proof cages (45 cm × 60 cm × 90 cm). The colony was reared in separate insect-rearing rooms under conditions of 25 ± 2°C, 60 ± 5% relative humidity, and a 16 h light/8 h dark (16L:8D) photoperiodic cycle.

Collection, identification, and culture of symbiotic bacteria of entomopathogenic nematodes

Symbiotic bacteria were isolated from entomopathogenic nematodes, *Heterorhabditis* spp. and these were collected from soil samples of various regions in Korea. Isolated bacteria were streaked onto NBTA medium agar supplemented with 0.025% (wt./vol.) bromothymol blue and 0.004% (wt./vol.) triphenyl tetrazolium chloride. Broth cultures were grown from a single primary phase colony in 5YS medium (5% yeast extract, 0.5% NaCl, 0.05% K_2HPO_4, 0.05% $NH_4H_2PO_4$, 0.02% $MgSO_4 . 7H_2O$) on a shaker (180 rpm) at 28 °C for 72 hours. Isolated symbiotic bacteria were identified by the nucleotide sequence analysis of the 16S ribosomal DNA (rDNA).

Extraction of supernatants from the bacterial culture broths

The isolated bacterial strains of *Photorhabdus temperata* sp., *P. luminescens* and *Xenorhabdus nematophilus* were cultured in NBTA medium for 72 hours. Then, the bacterial culture broths were centrifuged at 10,000 g for 20 min at room temperature (modified from Uma et al., 2010). Supernatants were collected in new containers and stored at 4 °C until used.

Bioassays

To determine insecticidal activity of culture media of symbiotic bacteria, two different assays were conducted. Firstly, freshly plucked tomato leaves as a natural diet dipped into diluted culture media solutions for 20 seconds, air dried for 10 min at room temperature. Whiteflies released into the leaves within the glass chamber and mortality was measured for every 6 h. Leaves dipped in water were used as a control (Figure 1).

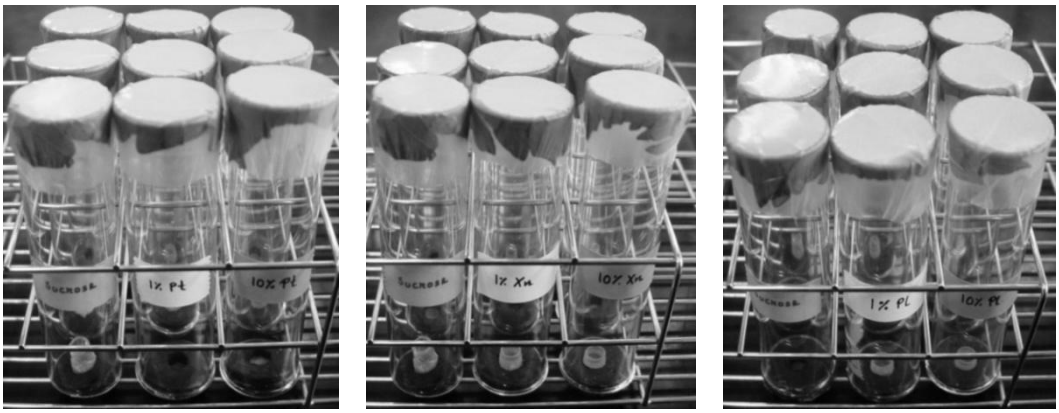

Figure 1. Experimental set up for mortality test of *B. tabaci* using natural diet. Fresh tomato leaves were dipped in 0, 1 and 10% each of three symbiotic culture media for 20 secs followed by dried 10 min and fixed in upper portion of glass tubes with the help of parafilm and 25 adult *B. tabaci* were allowed to feed. Number of dead whitefly was counted in every six hours.

Secondly, culture media diluted into a sucrose solution (20%) as an artificial diet and allowed whiteflies to ingest within the glass cage with parafilm. Glass tubes (length 12 cm × diameter 3 cm) were prepared for the feeding chambers of the whiteflies. One end of the chamber was covered with 2 layers of parafilm containing a sucrose solution between them. The bottom end of the chamber was covered with a thick black paper sheet with a hole in the center. The hole was plugged by a homemade ventilator using a pipette tip and a fine meshed net pasted onto it. Concentrations of each of the three bacterial supernatants in sucrose solution were 10, 30, 50% and their different combination used for mortality assay. Mortality was determined by counting dead or alive adults at every 6 hours at 25 ± 2 °C. Each set of experiments were done 3 times with similar environmental conditions (Figure 2).

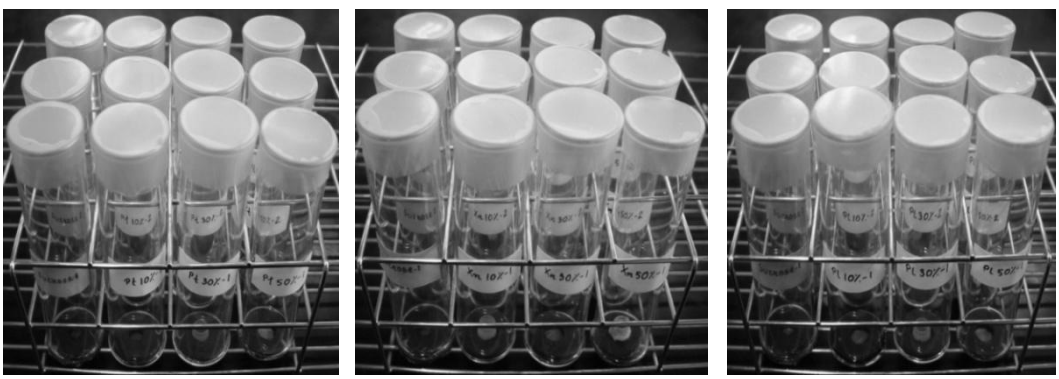

Figure 2. Experimental set up for mortality test of *B. tabaci* using artificial diet. 0%, 10%, 30% and 50% each of three symbiotic culture media with artificial diet sucrose were fixed in upper portion of glass tubes with the help of parafilm and 25 adult *B. tabaci* were allowed to ingest. Number of dead whitefly was counted in every six hours.

Statistical analysis

Mortality was reported as the mean ± standard error. Analysis of variance (ANOVA) was done to analyze the means using PROC General Linear Model (GLM) by the Statistical Analysis System program (SAS, 2003) version 9.1 to identify significant effects of the dose specific treatments. The separation of treatment means was done using Duncan's multiple range tests at a 95% confidence level. Data were analyzed by completely randomized design with three replications.

Results and Discussion

Effect of bacterial cells and supernatant of *P. temperata* on the mortality of *B. tabaci*

In this study it was observed that cell and cell free culture of symbiotic bacteria had significant effects on the mortality of whitefly using both natural and artificial diets. In a leaf dipping assay, mortality of whiteflies was less than 6% by feeding of water-dipped leaves (control) for 72 h. However, mortalities were gradually increased by the ingestion of leaves which dipped in 1% and 10% concentrations of bacterial culture media of *Photorhabdus temperata* (Figure 3). Mortalities were higher in high concentrations of bacterial cuilture media. Complete mortality was observed after 60 h, feeding with 10% culture medium of *P. temperata*. Similarly, using bacterial supernatant in leaves very less mortality was observed (data not shown here).

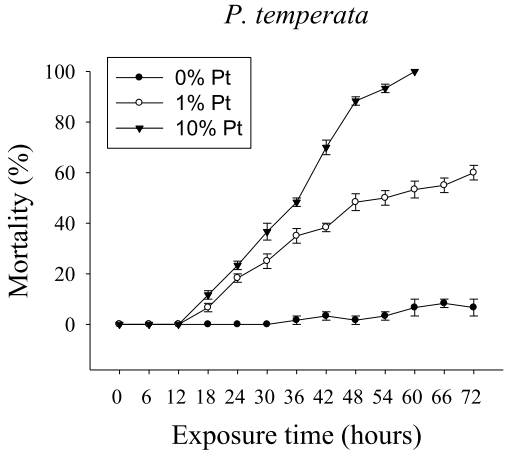

Figure 3. Effect of culture medium of symbiotic bacteria, *P. temperata* on the mortality of whitefly using natural diet. Leaves of tomato plants dipped in 0%, 1% and 10% symbiotic culture media directly for 20 sec followed by dried with 10 min at room temperature. After fixed with glass chamber using parafilm 25 whiteflies were released and allowed to feed. The mortality was observed in every 6 hours. Each point represents mean±SE of three replications.

In another study, parafilm sandwiched assay, mortality of whitefly, *B. tabaci* tested against supernatant of *P. temperata.* Using 10% supernatant of *P. temperata* after 60 hours 36% mortality was observed while, 86.33% mortality was obtained using 30% Pt. Complete mortality was observed at 60 hours using 50% supernatant (Figure 4).

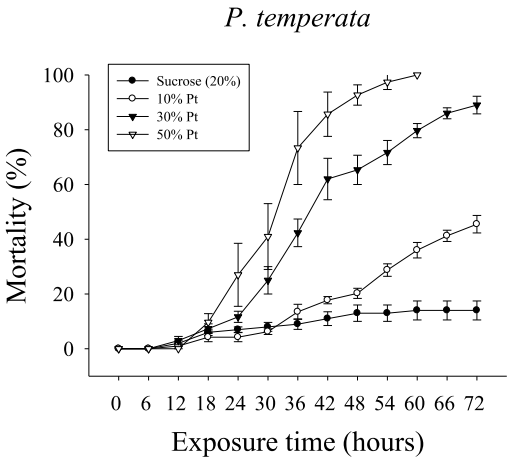

Figure 4. Effect of supernatant of symbiotic bacteria, *P. temperata* on the mortality of whitefly using natural diet. Leaves of tomato plants dipped in 0%, 1% and 10% symbiotic culture media directly for 20 sec followed by dried with 10 min at room temperature. After fixed with glass chamber using parafilm 25 whiteflies were released and allowed to feed. The mortality was observed in every 6 hours. Each point represents mean±SE of three replications.

In addition, when *P. temperata* used in combination with supernatant of another two bacteria *P. luminescens and Xenorhabdus nematophilus,* mortality effects was stronger compared to their individual effect, required less time to cause the death of 100% insects. Using 50% supernatant of *P. temperata*100% mortality was achieved after 60 hours of ingestion while in combination with *P. luminescence* (Pt+Pl) ; *X. neematophilus* (Pt+Xn) and (Pt+Xn+Pl) required exposure time for complete mortality was 54, 48 and 42 hours respectively (Figure 5).

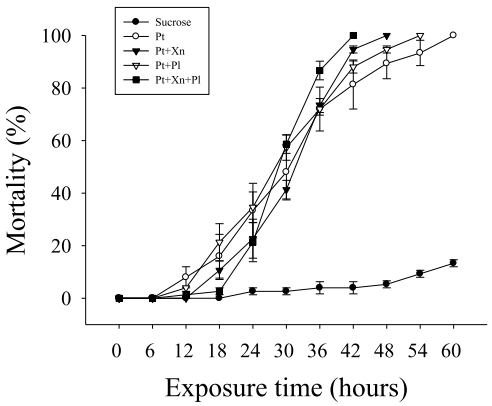

Figure 5. Combine effect of the supernatant of *P. temperata* with *X. nematophilus* and *P. luminescens* bacteria on the mortality of whitefly, *B. tabaci.* (n=25) adult *B. tabaci* exposed to different combinations of supernatant of symbiotic bacteria with artificial diet sucrose (1:1:1:1 v/v) and mortality was observed in every six hours. Each point represents mean±SE of three replications.

Using cell and cell free culture of *X. nematophilus* another study was conducted similar to P. temperate. 100% mortality of adult B. *tabaci* was achieved using 10% bacterial cells of *Xenorhabdus nematophilus* at 60 hours of exposure time (Figure 6), while using supernatant of X. *nematophilus* 100% mortality was observed at 50% concentration after 60 hours (Figure 7).

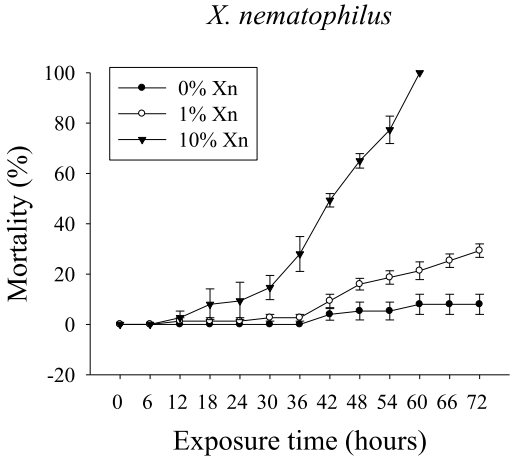

Figure 6. Effect of bacterial cell of *X. nematophilus* on the mortality of whitefly using natural diet. Leaves of tomato plants dipped in 0%, 1% and 10% symbiotic culture media directly for 20 sec followed by dried with 10 min at room temperature. After fixed with glass chamber using parafilm 25 whiteflies were released and allowed to feed. The mortality was observed in every 6 hours. Each point represents mean±SE of three replications.

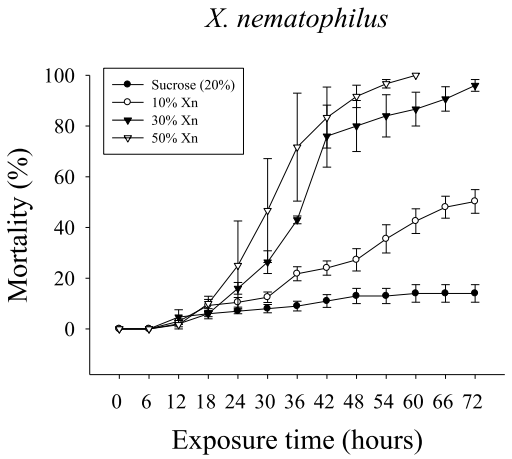

Figure 7. Effect of supernatants of *X. nematophilus* on the mortality of whitefly using natural diet. Leaves of tomato plants dipped in 0%, 1% and 10% symbiotic culture media directly for 20 sec followed by dried with 10 min at room temperature. After fixed with glass chamber using parafilm 25 whiteflies were released and allowed to feed. The mortality was observed in every 6 hours. Each point represents mean±SE of three replications.

In addition, *X. nematophilus* when used with PT and PL the effects was stronger comared to its ints individual effects. 100% mortality was achieved using supernatant of *X. nematophilus* with *P. temperatea* (Xn+Pt) and *P. luminescence* (Pl) at 48 hours of exposure time in both cases (Figure 8). Using three bacterial supernatant (Xn+Pt+Pl) 100% mortality was achieved after 42 hours (Figure 8).

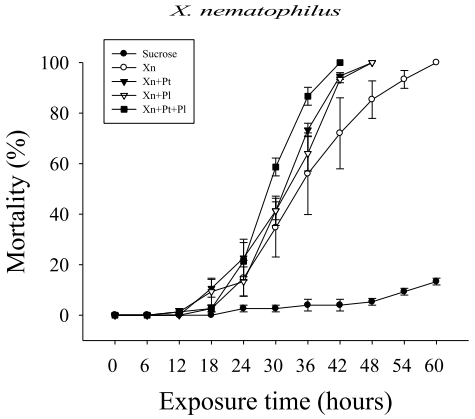

Figure 8. Combine effect of the supernatant of *X. nematophilus* with *P. temperata* and *P. luminescens* bacteria on the mortality of whitefly, *B. tabaci.* (n=25) adult *B. tabaci* exposed to different combinations of supernatant of symbiotic bacteria with artificial diet sucrose (1:1:1:1 v/v) and mortality was observed in every six hours. Each point represents mean±SE of three replications.

Using cell and cell free culture of *P. luminescence* another study was conducted similar to P. temperate. 45.33% mortality of adult *B. tabaci* was achieved using 10% bacterial cells of *P. luminescence* at 72 hours of exposure time (Figure 9), while using supernatant of *P. luminescence*

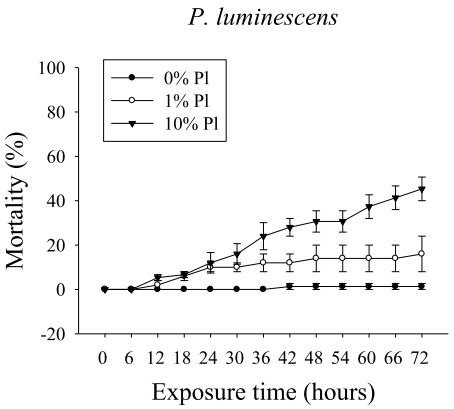

Figure 9. Effect of bacterial cell of *P. luminescens* on the mortality of whitefly using natural diet. Leaves of tomato plants dipped in 0%, 1% and 10% symbiotic culture media directly for 20 sec followed by dried with 10 min at room temperature. After fixed with glass chamber using parafilm 25 whiteflies were released and allowed to feed. The mortality was observed in every 6 hours. Each point represents mean±SE of three replications.

100% mortality was observed at 50% concentration after 66 hours (Figure 10). In addition, *P. luminescens* when used with Pt and Xn the effects was stronger comared to its individual effects. 100% mortality was achieved using supernatant of P. *luminescens with X. nematophilus* (Pl+Xn) and *P. temperatea* (Pl+Pt) at 48 and 54 hours respectively (Figure 11). Using three bacterial supernatant (Xn+Pt+Pl) 100% mortality was achieved after 42 hours (Figure11).

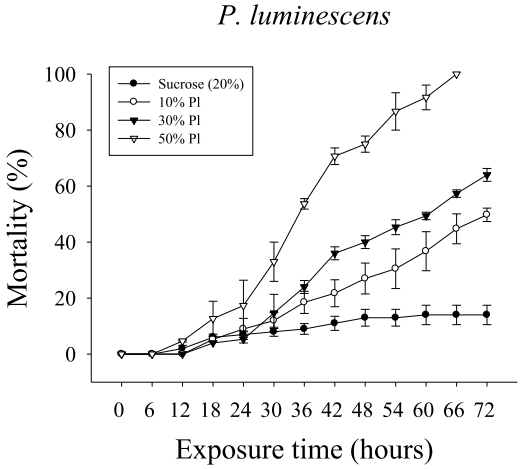

Figure 10. Effect of supernatants of *P. luminescens* on the mortality of whitefly using natural diet. Leaves of tomato plants dipped in 0%, 1% and 10% symbiotic culture media directly for 20 sec followed by dried with 10 min at room temperature. After fixed with glass chamber using parafilm 25 whiteflies were released and allowed to feed. The mortality was observed in every 6 hours. Each point represents mean±SE of three replications.

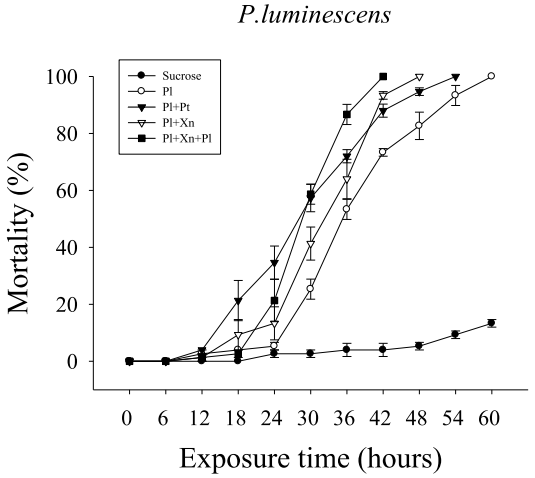

Figure 11. Combine effect of the supernatant of *P. luminescens* with *X. nematophilus* and *P. temperata* and bacteria on the mortality of whitefly, *B. tabaci.* (n=25) adult *B. tabaci* exposed to different combinations of supernatant of symbiotic bacteria with artificial diet sucrose (1:1:1:1 v/v) and mortality was observed in every six hours. Each point represents mean±SE of three replications.

In this study, it was found that symbiotic bacteria *P. temperata*, *P. luminescens* and *X. nematophilus* caused mortality of whiteflies effectively either using natural or artificial diet. Dipping with bacterial cells in natural diet required relatively lower concentration compared to cell free culture. In natural diet 100% mortality was observed using 10% symbiotic culture medium of *Photorhabdus temperata* and *Xenorhabdus nematophilus* while using only supernatant require three fold higher to cause 100% mortality of whiteflies. This is due to combine action of both cells and supernatant. Forst et al. (1997) reported that bacteria secreted several extracellular products directly in the culture medium that are the pathogenic factors and believed to be secreted into insect hemolymph at the stationary phase of the bacteria. This study suggests that supernatant of three symbiotic bacteria could be used mixing with one another. The mortality of *B. tabaci* was higher in additive concentration of supernatant even in combination. Mixture of small portion of each of the supernatant was almost equally effective in their individual effects of the same volume. Part of my results shared with the previous findings using supernatant of *P. temperata* against whitefly through oral ingestion (Shrestha and Lee, 2012). Both oral feeding and hemocoel injection of toxin complex produced by *P. luminescens* was highly lethal to the larvae of *Manduca sexta* (Blackburn et al, 1998). *X. nematophilus* cell and secreted protein orally toxic against neonate larvae of *Helicoverpa armigera* (Khandelwal and Banerjee-Bhat, 2003). Cell and cell free supernatant of primary and secondary forms of *P. luminesecens* were equally virulent and pathogenic to thrips nymphs (Uma et al., 2010), Oral ingestion of cell and cell free supernatant of *P. temperata* showed highly lethal several immature insects and against whitefly (Shrestha et al., 2011; Shrestha and Lee, 2012). *Photorhabdus* releases a variety of toxins that kill the host within 48 hours (Billard et al., 2002; Burnell and Stock, 2000). Rodou et al (2010) reported that several virulent factors play the principal role in insect pathogenesis, such as toxin complexes (Tcs), makes caterpillar floppy toxins (Mcf), *Photorhabdus* insect related proteins (Pir), and *Photorhabdus* virulence cassettes (PVC). Among these toxins, toxin complex (Tcs) is the most abundance toxin and both oral ingestion and hemolymph injection showed highly lethal to *Manduca sexta, Leptinotarsa decemlineata, Bemisia tabaci* (Blakburn et al., 1998; 2005). Tcs high molecular weight protein consisting with four subunit complexes, Tca, Tcb, Tcc and Tcd (Bowen et al., 1998; Rodou et al, 2010). Tca works on insect midgut, causing blebbing of the endothelium and eventual cell lysis (Blackburn et al., 1998). Among RTX-like toxin identified in *Photorhabdus*, the metalloprotease PrtA, may be involved in host immunosupresion (ffrench-Constant et al., 2000; Duchaud et al., 2003) and host bioconversion (Bowen et al., 2003). Two Mcf toxins reported by Rodou et al. (2010) cause the death of insects inducing apoptosis of both midgut epethelial and phagocytic cells (Dowling et al., 2007). Insect related toxins PirA and PirB demonstrate injectable activity (ffrench-Constant et al., 2007) and Pir toxins can be used as a alternate biological control toxins of Bt (Ahantarig et al., 2009). The PVCs are encoded by multiple copies of genes that are involved in antifeeding (Yang et al., 2006; Rodou et al., 2010), these gene products work on hemocytes which lead to insect killing. However, *Xenorhabdus* toxin complex 1 is composed of three proteins (XptA2, XptB1 and XptC1),

representing class A, B, and C proteins combined in a respective 4:1:1 stoichiometry (Sheets et al., 2011). Combination of the three class A, B and C proteins form a active toxin complex, and that class B and C proteins from *Photorhabdus* (TcdB2 and TccC3) can substitute for the B and C proteins from *Xenorhabdus* to form an active hybrid toxin complex that has greater insecticidal activity than the native toxin complex (Sheets et al., 2011). XptA2 has only modest oral toxicity against lepidopteran insects, but as a complex with co-produced XptB1 and XptC1 has high levels of insecticidal activity. From earlier studies it has been suggested that class A proteins harbors the cytotoxic effects of the Tc toxins, whereas class B and C proteins rather modulate and enhance the toxicity of class A proteins (Guo et al., 1999).

This study suggests that culture media of symbionts of entomopathogeic nematodes can be used at 10% Pt, Xn culture media using natural diet and supernatant of Pt, Pl, Xn with artificial diet sucrose in 1:1 ratios, as useful microbial pesticides for the control of *B. tabaci*.

References

Abdel-Baki, N.F. and Al-Deghairi, M.A. 2008. Role of host plants on the biological aspects and parasitism levels of *Eretmocerus mundus* Mercet (Hymenoptera: Apheinidae) a parasitoid of *Bemisia tabaci* Gennadius (Aleyrodidae:Hemiptera). J. Entomol. 6: 356-368.

Ahantarig, A., Chantawat, N., Waterfield, N.R., ffrench-Constant R. and Kittayapong, P. 2009. PirAB toxin from *Photorhabdus asymbiotica* as a larvicide against dengue vectors. Appl. Environ. Microbiol. 75: 4627–4629.

Ahmad, M., Arif, M.I., Ahmad, Z. and Denholm, I. 2002. Cotton whitefly (*Bemisia tabaci*) resistance to organophosphate and pyrethroid insecticides in Pakistan. Pest Manage. Sci. 58: 203–208.

Al-Deghairi, M.A. 2009. Combining effect of *Beauveria bassaina* (Bals.) and *Eretmocerus mundus* Mercet (Hymenoptera: Apheinidae) on sweetpotato whitefly, *Bemisia tabaci* Gennadius (Aleyrodidae:Hemiptera). J. Entomol. 6: 72-78.

Blackburn, M., Golubeva, E., Bowen, D., ffrench-Constant, R.H. 1998. A novel insecticidal toxin from *Photorhabdus luminescens*: histopathological affects of Toxin complex A (Tca) on the midgut of *Manduca sexta*. Appl. Environ. Microbiol. 64: 3036–3041.

Blackburn, M.B., Domek, J.M., Gelman, D.B. and Hu, J.S. 2005. The broadly insecticidal *Photorhabdus luminescens* toxin complex a (Tca): activity against the Colorado potato beetle, *Leptinotarsa decemlineata*, and sweet potato whitefly, *Bemisia tabaci*. J. Insect Sci. 5: 1-11.

Boemare, N. 2002. Biology, taxonomy and systematics of *Photorhabdus* and *Xenorhabdus*. In: Gaugler R (ed) Entomopathogenic nematology. CABI Publishing, New York, pp 35–56.

Boemare, N.E. and Akhurst, R.J., 1988. Biochemistry ical and physiological characterization of colony form variants in *Xenorhabdus* spp. J. Gen. Microbiol. 134: 751–761.

Bowen, D., Blackburn, M., Rocheleau, T., Grutzmacher, C. and ffrench- Constant, R.H., 2000. Secreted proteases from *Photorhabdus luminescens*: Separation of the extracellular proteases from the insecticidal Tc toxin complexes. Insect Biochem. Mol. Biol. 30: 69–74.

Bowen, D., Rocheleau, T.A., Blackburn, M., Andreev, O., Golubeva, E., Bhartia, R. and ffrench-Constant, R.H., 1998. Novel insecticidal toxins from the bacterium *Photorhabdus luminescens*. Science. 280: 2129–2132.

Bowen, D.J., Rocheleau, T.A., Grutzmacher, C.K., Meslet, L., Valens, M., Marble, D., Dowling, A., ffrench-Constant, R. and Blight, M.A. 2003. Genetic and Biochemiical characterization of PrtA, an RTX-like metalloprotease from *Photorhabdus*. Microbiol. 149: 1581–1591.

Boykin, L.M., Shatters, R.G., Rosell, R.C., McKenzie, C.L., Bagnall, R.A., De Barro, P.J. and Frohlich, D.R., 2007. Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences. Mol. Phylogenet. Evol. 44: 1306–1319.

Brillard, J., Duchaud, E., Boemare, N., Kunst, F., Givaudan, A., 2002. The PhlA hemolysin from the entomopathogenic bacterium *Photorhabdus luminescens* belongs to the two-partner secretion family of hemolysins. J. Bacteriol. 184: 3871-3878.

Burnell, A.M. and Stock, S.P. 2000. Heterorhabditis, Steinernema and their bacterial symbionts-lethal pathogens of insects. Nematol. 2: 31–42.

Cahill, M., Byrne, F.J., Denholm, I., Devonshire, A.L. and Gorman, K.J. 1994. Insecticide resistance in *Bemisia tabaci.* Pestic. Sci. 42: 137–139.

Cahill, M., Gorman, K. Day, S. and Denholm, I. 1996. Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (Homoptera: Aleyrodidae). Bull. Entomol. Res. 86: 343-349.

Cannon, R.J.C., Eyre, D., MacLeod, A., Matthews, L., Malumphy, C., Cheek, S. and Bartlett, P.W. 2005. Interceptions and outbreaks of *Bemisia tabaci* in the UK. The BCPC International Congress – Crop Science and Technology 2005. Congress Proceedings Vol. 2: 1007- 1012.

Castle, S.J., Henneberry, T.J., Prabhaker, N. and Toscano, N.C. 1996. Trends in relative susceptibilities of whiteflies to insecticides through the cotton season in the Imperial Valley, CA. In: Dugger, P., Richter, D. (Eds.), Proceedings Beltwide Cotton Conference. National Cotton Council, Memphis, TN. 1032–1035.

Cowles, K.N. and Goodrich-Blair, H. 2005. Expression and activity of a *Xenorhabdus nematophila* haemolysin required for full virulence towards *Manduca sexta* insects. Cell Microbiol. 7: 209–219.

Daborn, P.J., Waterfield, N., Blight, M.A. and ffrench-Constant, R.H. 2001. Measuring virulence factor expression by the pathogenic bacterium *Photorhabdus luminescens* in culture and during insect infection. J. Bacteriol. 183: 5834-5839.

De Barro, P.J. and Bourne, A. 2010. Change in oviposition choice by an invader to avoid competition accelerates the displacement of a competitor. Biol. Invasions 12: 3013–3023.

De Barro, P.J., Liu, S.S., Boykin, L.M. and Dinsdale, A.B. 2011. *Bemisia tabaci*: a statement of species status. Annu. Rev. Entomol. 56: 1–19.

Dennehy, T.J., Williams, III., L., Russell, J.S., Li, X. and Wigert, M. 1996. Monitoring and management of whitefly resistance to insecticides in Arizona. In: Dugger, P., Richter, D. (Eds.), Proceedings Beltwide Cotton Conference. National Cotton Council, Memphis, TN, pp. 743–748.

Dowling, A.J., Waterfield, N.R., Hares, M.C., Le Goff, G., Streuli, C.H. and ffrench-Constant, R.H. 2007. The Mcf1 toxin induces apoptosis via the mitochondrial pathway and apoptosis is attenuated by mutation of the BH3-like domain. Cell Microbiol. 9, 2470-84.

Ellsworth, P.C., Dennehy, T.J. and Nichols, R.L. 1996. Whitefly management in Arizona cotton 1996. University of Arizona Cooperative Extension Publication No. 196004, IPM Series 3, 2pp.

ffrench-Constant, R., Waterfield, N., Daborn, P., Joyce, S., Bennett, H., Au, C., Dowling, A., Boundy, S., Reynolds, S. and Clarke, D. 2003. Photorhabdus: towards a functional genomics analysis of a symbiont and pathogen. FEMS Microbiol. Rev. 26: 433–456.

ffrench-Constant, R.H. and Bowen, D.J., 2000. Novel insecticidal toxins from nematode-symbiotic bacteria. Cell. Mol. Life Sci. 57: 828–833.

ffrench-Constant, R.H., Dowling, A. and Waterfield, N.R. 2007. Insecticidal toxins from *Photorhabdus* bacteria and their potential use in agriculture. Toxicon. 49: 436- 451.

Forst, S., Clarke, D. and Gaugler R. Ed. 2001. Entomopathogenic Nematol. London: CAB International pp. 57-77.

Forst, S., Dowds, B., Boemare, N. and Stackebrandt, E. 1997. Xenorhabdus and Photorhabdus spp.: bugs that kill bugs. Annu. Rev. Microbiol. 51: 47-72.

Fouly, A.H., AL-Deghairi, M.A. and Abdel-Baky, N.F. 2011. Biological aspects and life tables of *Typhodrmips swirskii* (Acari: Phytoseiidae) fed *Bemisia tabaci* (Hemiptera: Alerodidae) J. Entomol. 8: 52-62.

Georgis, R., Koppenhöfer, A.M., Lacey, L.A., Bélair, G., Duncan, L.W., Grewal, P.S., Samish, M., Tan, L., Torr, P. and Van Tol, R.W.H.M. 2006. Successes and failures in the use of parasitic nematodes for pest control. Biol. Control 38: 103-123.

Gerling, D., Alomar, O. and Arno, J. 2001. Biological control of *Bemisia tabaci* using predators and parasitoids. Crop Prot. 20: 779-799.

Gonzalez-Zamora, J.E., Leira, D., Bellido, M.J. and Avilla, C. 2004. Evaluation of the effect of different insecticides on the survival and capacity of *Eretmocerus mundus* Mercet tocontrol *Bemisia tabaci* (Gennadius) populations. Crop Prot. 23: 611-618.

Greathead, A.H. 1986. Host plants. In: Cock, M.J.W. (Ed.), *Bemisia tabaci* FA Literature Survey on the Cotton Whitefly with an Annotated Bibliography. CAB International Institutes, Biological Control. Silwood Park, UK, 17–26.

Guo, L., Fatig, R.O., III, Orr, G.L., Schafer, B.W., Strickland, J.A., Sukhapinda, K., Woodsworth, A.T. and Petell, J.K. 1999. *Photorhabdus luminescens* W-14 insecticidal activity consists of at least two similar but distinct proteins. Purification and characterization of toxin A and toxin B. J. Biol. Chem. 274: 9836-9842.

Hogenhout, S.A., Ammar, E.D., Whitfield, A.E. and Redinbaugh, M.G. 2008. Insect vector interactions with persistently transmitted viruses. Annu. Rev. Phytopathol. 46: 327–359.

James, R.R. 2003. Combining azadirachtin and Paecilomyces fumosoroseus (Deuteromycotina: Hyphomycetes) to control Bemisia argentifolii (Homoptera: aleyrodidae). J. Econ. Entomol. 96: 25-30.

Jones, D.R. 2003. Plant viruses transmitted by whiteflies. Eur. J. Plant Pathol. 109: 195-219.

Joshi, M.C., Sharma, A., Kant, S., Birah, A., Gupta, G.P., Khan, S.R., Bhatnagar, R. and Banerjee, N. 2008. An insecticidal GroEL protein with chitin binding activity from *Xenorhabdus nematophila.* J. Biol. Chem. 283: 28287–28296.

Khandelwal, P. and Banerjee-Bhat nagar, N. 2003. Insecticidal activity associated with the outer membrane vesicles of *Xenorhabdus nematophilus*. Appl. Environ. Microbiol. 69: 2032-7.

Khandelwal, P., Choudhury, D., Birah, A., Reddy, M.K., Gupta, G.P. and Banerjee, N. 2004. Insecticidal pilin subunit from the insect pathogen *Xenorhabdus nematophila*. J. Bacteriol.186: 6465–6476.

Morgan, J.A.W., Sergeant, M., Ellis, D., Ousley, M., and Jarrett, P. 2001. Sequence analysis of insecticidal genes from *Xenorhabdus nematophilus* PMFI296. Appl. Environ. Microbiol. 67: 2062.

Mound, L.A. and Halsey, S.H. 1978. Whitefly of the World: a Systematic Catalogue of the Aleyrodidae (Homoptera) with Host Plant and Natural Enemy Data. Wiley, New York, 340pp.

Naranjo, S.E., Ellsworth, P.C., Chu, C.C. and Henneberry, T.J. 2002. Conservation of predatory arthropods in cotton: Role of action thresholds for *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Econ. Entomol. 95: 682-691.

Nomikou, M., Janssen, A., Schraag, R. and Sabelis, M.W., 2001. Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. Expt. Appl. Acarol. 25: 271-291.

Oliveira, M.R.V., Henneberry, T.J. and Anderson, P. 2001. History, current status, and collaborative research projects for *Bemisia tabaci*. Crop Prot. 20: 709–23.

Palumbo, J.C., Horowitz, R. and Prabhaker, N. 2001. Overview of insecticidal control and resistance management for *Bemisia tabaci.* Crop Prot. 20: 739–765.

Prabhaker, N., Toscano, N.C. and Coudriet, D.L. 1989. Susceptibility of the immature and adult stage of the sweetpotato whitefly (Homoptera: aleyrodidae) to selected insecticides. J. Econ. Entomol. 82, 983-988.

Prabhaker, N., Toscano, N.C. and Henneberry, T.J. 1998. Evaluation of insecticide rotations and mixtures as resistance management strategies for *Bemisia argentifolii* (Homoptera: Aleyrodiae). J. Econ. Entomol. 91: 820–826.

Ribeiro, C., Vignes, M. and Brehe´lin, M. 2003. *Xenorhabdus nematophila* (Enterobacteriacea) secretes a cation-selective calcium- independent porin which causes vacuolation of the rough endoplasmic reticulum and cell lysis. J. Biol. Chem. 278: 3030–3039.

Rodou, A., Ankrah, D.O. and Stathopoulos, C. 2010. Toxins and Secretion Systems of *Photorhabdus luminescens*. Toxins. 2: 1250-1264.

SAS Institute, 2003. SAS Version 9.1, SAS Institute Inc., Cary. NC, USA.

Schmidt, T.M., Bleakley, B. and Nealson, K.H. 1988. Characterization of an extracellular protease from the insect pathogen *Xenorhabdus luminescens*. Appl. Environ. Microbiol. 54: 2793-2797.

Secker, A.E., Bedford, I.A., Markham, P.G., William, M.E.C., 1998. Squash, a reliable field indicator for the presence of B biotype of tobacco whitefly, *Bemisia tabaci*. In: Brighton Crop Protection Conference-Pests and Diseases. British Crop Protection Council, Farnham, UK, pp. 837–842.

Sheets, J.J., Hey, T.D., Fencil, K.J., Burton, S.L., Ni, W., Lang, A.E., Roland Benz, R. and Aktories, K. 2011. Insecticidal toxin complex proteins from *Xenorhabdus Nematophilus* structure and pore formation the J. Biol. Chem. 286: 22742–22749.

Shrestha, Y.K., Jang, E.K. Yu, Y.S., Kwon, M., Shin, J.H. and Lee, K.Y., 2011. Oral toxicity of symbiotic bacteria *Photorhabdus* spp. against immature stages of insects. J. Asia-Pac. Entomol. 14: 127–130.

Shrestha, Y.K., and Lee, K.Y. 2012. Oral toxicity of Photorhabdus culture media on gene expression of the adult sweetpotato whitefly, *Bemisia tabaci*. J. Invert. Pathol. 109: 91–96.

Touhidul, M. and Shunxiang, R. 2007. Development and reproduction of *Bemisia tabaci* on three tomato varieties. J. Entomol. 4: 231-236.

Uma, G.P., Prabhuraj, A. and Vimala. 2010. Bioefficacy of *Photorhadus luminescens*, a symbiotic bacterium against *Thrips palmi* karny (Thripidae: Thysanoptera), J. Biopest. 3: 458-462.

Vigneux, F., Zumbihl, R., Jubelin, G., Ribeiro, C. and Poncet, J., Baghdiguian, S., Givaudan, A., Brehe´lin, M. 2007. The xaxAB genes encoding a new apoptotic toxin from the insect pathogen *Xenorhabdus nematophila* are present in plant and human pathogens. J. Biol.Chem. 282: 9571.

Wan, F.H., Zhang, G.F., Liu, S.S., Luo, C., Chu, D., Zhang, Y.J., Zang, L.S., Jiu, M., Lu, Z.C., Cui, X.H., Zhang, L.P., Zhang, F., Zhang, Q.W., Liu, W.X., Liang, P., Lei, Z.R. and Zhang, Y.J. 2009. Invasive mechanism and management strategy of *Bemisia tabaci* (Gennadius) biotype B: progress report of 973 programs on invasive alien species in China. Sci. in China Series C Life Sci. 52: 88–95.

Wang, H. and Dowds, B.C.A. 1993. Phase variation in *Xenorhabdus luminescens*: cloning and sequencing of the lipase gene and analysis of its expression in primary and secondary phases of the bacterium. J. Bacteriol. 175: 1665-l 673.

Webster, J.M., Chen, G., Hu, K. and Li, J. 2002. Bacterial metabolites. In: Gaugler, R. (Ed.), Entomopathogenic Nematol. CABI, Wallingford, UK, pp. 99–114.