Egyptian Journal of Biological Pest Control, 14(1), 2004, 225-230
Proceeding of 1st Arab Conference for Applied Biological Pest Control, Cairo, Egypt, 5-7 April 2004

Integration of Three Biocontrol Agents for the Control of the Cabbage Worm, *Artogia* (*Pieris*) *rapae* (L.).

Badr-Elsabah, A. Fetoh and Ahmed M. Azazy
Plant Protection Research Institute (PPRI), ARC, Dokki, Giza.

ABSTRACT

Laboratory and field experiments were conducted to evaluate three biocontrol agents for the control of the cabbage worm, *Artogia* (*Pieris*) *rapae* (L.) as new approaches of integrated pest management (IPM).Under laboratory conditions, larvae and pupae of *A. rapae* were highly susceptible to two nematode species; *Heterorahabditis bacteriophora* (TWF strain) and *Steinernema carpocapsae* (All strain). Dipel 2X (The commercial formulation of the bacteruim *Bacillus thuringiensis* var. *krustaki*) exhibited highly toxic effect against *A. rapae* larvae (93.3% mortality); while Biofly (the commercial formulation of the fungus *Beauveria bassiana*) was less pathogenic (39.2% mortality) and the nematodes alone gave mortality between 73.3 - 100 and 53.3 – 100 % among larvae and pupae, respectively. When both nematodes were combined with Dipel 2X, 100% larval mortality was achieved and when combined with Biofly, mortality ranged between 88.9-93.3%. Under field conditions, insect mortality 7 days after application was 76.1 and 74.1% for *S. carpocapsae*, TWF, after spraying two times. The combination between the nematodes (*S. carpocapsae* and TWF) and Dipel 2X or Biofly, respectively gave 87.5 and 76.1 % for *S. carpocapsae*. The corresponding values for TWF were 98.3 and 76.4 % when combined with the two pathogens, respectively. Dipel 2X and Biofly when used alone, 82.7, 17.9 % insect mortality was recorded for the two pathogens, respectively.

Key Words: IPM, Entomopathogenic nematode, *Steinernema carpocapsae, Heterorhabditis bacteriophora, Bacillus thuringensis, Beauveria bassiana, Artogia* (*Pieris*) *rapae.*

INTRODUCTION

Beneficial nematodes, particularly; Steinernematids and Heterorhabditids, are biological control agents that provide the basis of some bio-insecticides through mixing them with pathogens (including bacteria and fungi) or alone. Vegetable crops, especially cruciferous plants are attacked by several insect pests. The cabbage worm, *Artogia* (*Pieris*) *rapae L.* causes large damage affecting the quantity and quality of cabbage plants, (*Brassica oleraceae* var. *capitata* L.) *A. rapae* is found in the most Egyptian governorates where cabbage plants are cultivated (Hammad *et al.*, 1965), also cabbage is an important vegetable crop in many countries of the world (Thompson and Killy, 1957).Therefore, the present work deals with control of *A. rapae* by entomopathogenic nematodes, bacteria and fungi separated or mixed in laboratory tests as well as in field experiments.

MATERIALS AND METHODS

Laboratory bioassays:

The nematode, *Steinernema ribovars* (Sr) strain was obtained from USA, while *Hetreorhabditis sp.* (TWF) was isolated from Egypt by Abed-El Rhaman (2001). The nematodes produced in vivo in larvae of *Galleria mellonella* L. (Lep.: Pyralidae) maintained in the laboratory using the methods of Woodring and Kaya (1988).Larval instars and pupal stages of *A. rapae* were treated in Petri-dishes (5 larvae or pupae /dish) contained moistened filter paper with EPN, above it circle of cabbage leaves. Three concentrations of the nematodes, *S carpocapsae* and TWF 25, 50 and 100 IJs/ml were prepared and added. Treatments were replicated three times. Mortality of the larvae and pupae were calculated for 48 hours. Groups of ten larvae and pupae dead from each concentration were transferred to extracting dishes that placed on white traps to collect nematodes exiting from infested hosts and counted daily for a month (Dutky *et al.*1964). The trial took place under the laboratory conditions of 25±2°C and 65±2% RH. Larval and pupal mortality were recorded daily and nematode development in cadavers of cabbage worm was ensured after seven days.

Bacteria and Fungi:

The same technique was repeated using *Bacillus thuringensis* sub sp. *kurstaki* in formulation (Dipel 2X) with concentration of 1 gm /L. and fungi which represented by *Beauveria bassiana* formulation (Biofly) with concentration of 2.5cm^3 / L. replicated three times.

Field application:

Field trials were carried out in a cabbage field at EL-Safe region, Giza governorate during October, 2003, contained nearly 5000 cabbage heads, two months old , cultivated in 0.5 feddan (1feddan=4200m²), using portable sprayer, two liters capacity. The spray was directed towards the hearts of the cabbage plants. *A. rapae* larvae and pupae counts were taken from random samples of 25 plants each (5 replicates x 5 plants) before spraying and 7 days after spraying with 2 liters of each nematode suspension (2000IJs/ml) for each TWF or Sc, respectively, and with 2gm/2 L. for Dipel and 5cm³/2L.for Biofly, separately or mixture from them with each of the nematodes (TWF or Sc). Control was treated with water only. Samples were taken from control and treated plots were counted before applications and one week post application. The percent of reduction in *A. rapae* population in relation to the sprayed nematodes, bacterium and fungus suspensions were calculated according to Henderson and Tiliton equation (19`55):

% reduction=100(1-Ta / Tb X Cb / Ca) where:

Ta =number of insects in treated plots after application.
Tb= number of insects in treated plots before application.
Ca=number of insects in control plots after application.
Cb= number of insects in control plots before application.

Statistical analysis

The data presented in percentage values in the present study were normalized using arcsine transformation. The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Dunncan's multiple range test ($P< 0.05$). Statistical analysis of data was carried out using a computer software package "Costat", a product of Cohort Software Inc., Berkeley, California, USA for laboratory studies but a computer software package "SAS" Institute (1988) was used for analyzed data of the field study.

RESULTS AND DISSCETION

1- Laboratory studies:

a) Infectivity against *A. rapae*:

Laboratory bioassay proved that the entomopathogenic nematodes were highly virulent to *A. rapae*. Mean mortality by the entomopathogenic nematodes *Heterorhabditis* sp. (TWF) and *S. carpocapsae* (Sc) showed no significant difference among all the treatments ($p<0.05$) ranging form (73.3-100%) and (53.3-100%) for pupae and larvae, respectively. The TWF was more effective (73.3-100%) than Sc (Fig.1&2). Generally; there are no significant differences among tested consternations (100, 50 and 25 IJs) or larval and pupal stages. The pupae of cabbage worm were less susceptible to nematode infections than larvae because of the reduced number of portals of entry (Poinar and Deschamps1981; Ahmed, 1982; Kaya 1984 and Azazy 1996 and 2001).

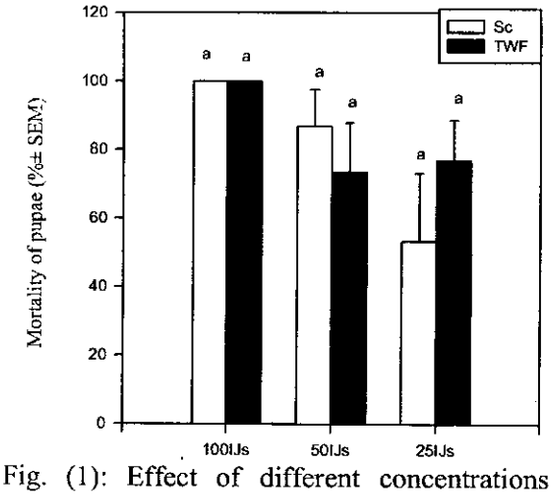

Fig. (1): Effect of different concentrations of entomopathogenic nematodes on pupae of *A. rapae*.

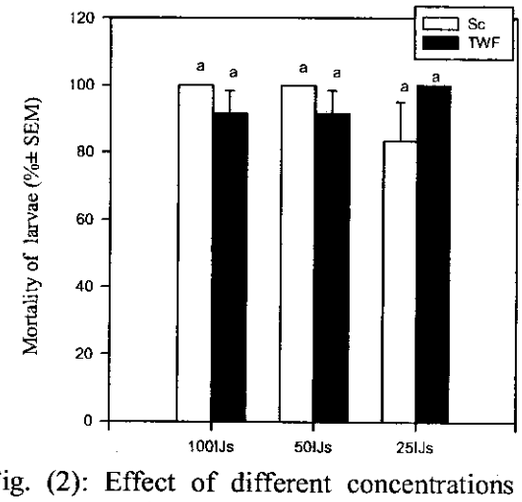

Fig. (2): Effect of different concentrations of entomopathogenic nematodes on larvae of *A. rapae*.

Means with the same letter are significantly different ($P<0.05$, Duncan's multiply range test).

b) Effect of combination between the nematodes and biopesticides on larvae of *A. rapae*:

In the laboratory, data showed that the using entomopathogenic nematodes alone or in mixtures with *Bacillus thuringensis* achieved 100% mortality (Fig.3). But its mixtures with *B. bassiana* resulted mortality of 93.3 and 89.9% for *S. carpocapsae* (Sc) and *Hetrerhabditis* sp.(TWF), respectively. The data revealed that there were high significant differences between the two nematode species and the nematode+*B.t.* and *B. bassiana* (ANOVA P = 170.3, F = 0.0000). It could be mentioned that using *B. t.* and *B. bassiana* alone achieved mortality rates of 93.3% and 39.2%, respectively.

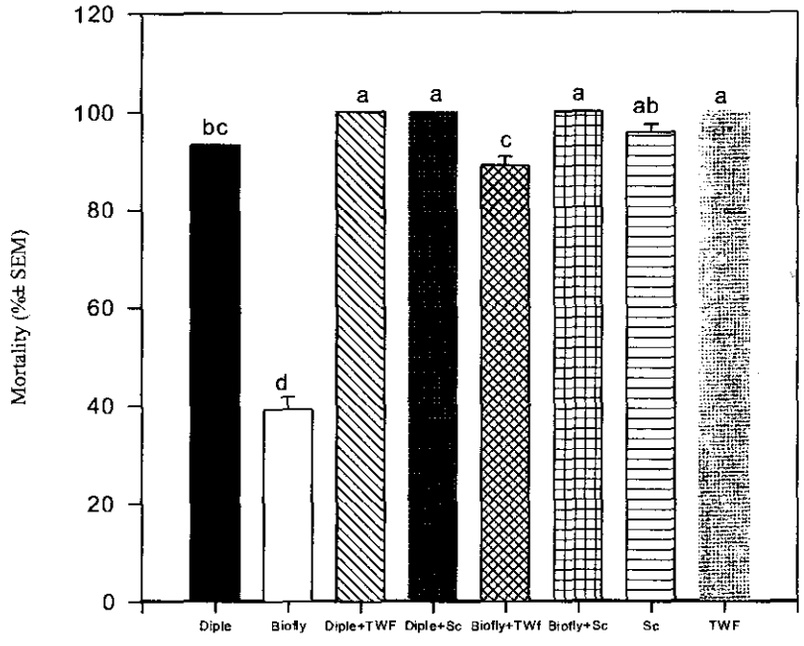

Fig. (3): Effect of combination between the entomopathogenic nematodes and *B. thuringensis* or *B. bassiana* on *A. rapae.*

Means with the same letter are not significantly different (P<0.05, Duncan's multiple range test)

c) Nematode reproduction in larvae, pupae and adult moth of *A. rapae*:

The strain of *S. carpocapsae* gave the highest yield of infective dauer stages approximately in all tested concentrations (Fig.4). However, it could be concluded that the pupae and larvae of *A. rapae* gave the highest yield of Sc and TWF. This may be to lipid content abundance in these stages (Kondo and Ishibashi, 1984 and Kondo, 1989). Suitability for successful infection and nematode reproduction is known to be a function of host size, age and stage (Gaugler and Molloy, 1981; Azazy, 1996 and Kaya, 1985).

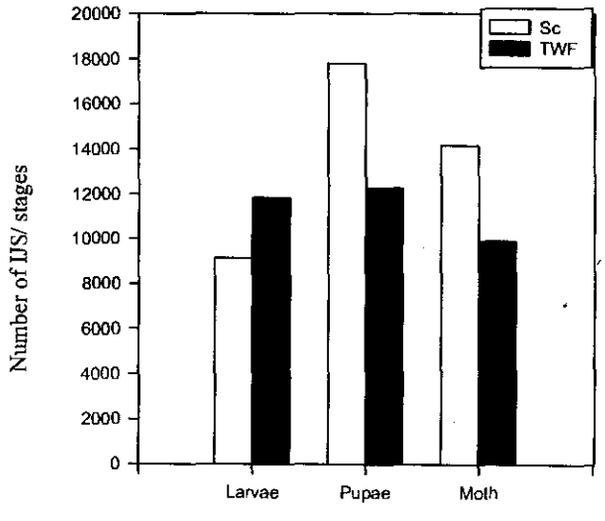

Fig. (4): Reproduction of *S. carpocapsae* and *H. bacteriophora* in larvae, pupa and moth of *A. rapae.*

2) Field experiments:

Mortality percentages of *A. rapae* due to application with TWF and Sc alone or mixed with *B.t.* and *B. bassiana* are shown in (Fig.5). The data revealed that there were no significant differences between nematodes treatments and the Dipel 2X and Biofly mixtures. But there was significant difference between all treatments and Biofly alone. However, the mixtures between the entomopathogenic nematodes TWF and Sc with *B. t.* gave higher mortality rates (98.3 and 87.5%) and 76.4 and 85.1% *with B. bassiana*, respectively, than the entomopathogenic nematodes alone (74.1 and 76.1 % of TWF, Sc). *B.t.* has been effective in controlling early larval instars within 4-10days. In the same respect Bari and Kaya(1984) indicated that combination of nematode and bacterium did not result in significantly greater control than that achieved by the nematode alone. Deseo *et al* (1984), EL-Bishry (1994), Gill and Micheal (1994), Azazy (1996 and 2001) confirmed this phenomenon. Barbercheck and kaya (1991) reported that when the larval host, *Spodoptera exigua*, is in continual contact with *B. bassiana* conidia, *H. bacteriophora* and *S. carpocapsae*, gave higher mortality than achieved with the pathogen alone.

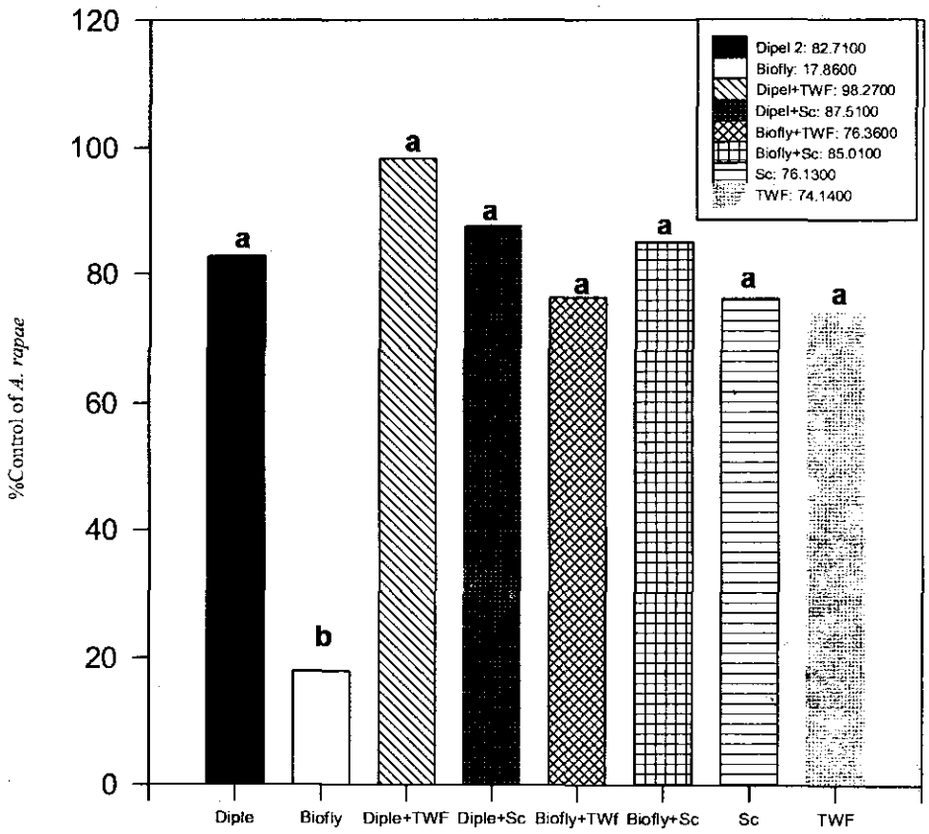

Fig. (5): Field application of the entompathogenic nematodes, *B. thuringensis* and *B. bassinan* against larvae of *A. rapae*.

In summary, the trails demonstrate that biological control agents such as entomopathogenic nematodes and *B. thuringensis* or *B. bassiana* could provide sufficient control of *A. rapae* under field conditions. The isolated strains from Egypt were more effective when sprayed in the field where the majority of the pest larvae found. Therefore, the nematodes were able to kill most of the larvae before developing into pupae (Saleh, 1995).Some pesticides act synergistically or additively with entomopathogenic nematodes and therefore improve nematodes efficacy in inundative applications such as *B. t.* (Koppenhoffer and Kaya, 1997, Koppenhoffer *et al* .1999).

REFERENCES

Abd EL-Rahman, R. M. 2001. Studies on the entomopathogenic nematodes of the family Heterorhabditidae in Egypt. Ph. D. Thesis, Fac. Science, Cairo Univ,125 pp.

Ahmed, N. M. 1982.Comparative studies on the effect of pesticides and entomogenous nematodes in controlling some economic pests. Thesis, Fac. Agric, Cairo Univ.

Azazy, A. M. 1996. Biological control of insects with insect parasitic nematodes. M Sc. Thesis, Fac.Moshtohor Zagazig Univ. Benha Branch.

Azazy, A. M. 2001. Comparative studies between insect pathogenic nematodes and other methods in controlling some soil insects and fruit tree borers .Ph.D. Thesis, Fac. Moshtohor Zagazig Unvi. Benha Branch.

Barbercheck, M. E and Kaya, H. K. 1991. Competitive interactions between entomopathogenic nematodes and *B. bassiana* (Deuteromcotina: Hyphomycetes) in soil borne larvae of *Spodoptera exigua* (Lepidoptera: Noctuidae). Environmental Entomology 20,707-712.

Bari, M. A. and Kaya, H. K. 1984. Evaluation of the entomogenous nematodes *Neoaplectana carpocapsae* (=*Steinernema feltiae*) Weiser (Rhabditida: Steinernematidae) and bacterium *Bacillus thuringensis* Berliner (var.Kurstaki) for suppression of the artichoke plum moth(Lepidoptera: Pterophoridae) .J.Econ.Entomol.,77(1):225-229.

Deseo, K. V., Grassi, S.; Foschi, F. and Rovesti, L. 1984. A system of biological control against the leopard moth *Zeuzera pyrina L.* (Lepidoptera: Cossidae).Atti. Giornata Fitopatol, 2: 403-414.

Duncan, D. B. 1955. Multiple ranges and multiple F-test. Biomterics, 11(1):1-42.

Dutky, S. R., Thompson, J. V. and Cantwell, G.E. 1964. A technique for the mass propagation of the DD-136 nematode. J. Insect. Pathol, 6: 417-422.

El-Bishry, M. H. 1994. Interaction between entomopathogenic nematodes and *Bacillus thuringiensis*: A new approach for biological control of insects. Egypt. J. Biol. Pest Cont., 4: 105-111.

Gaugler, R. and Molloy, D. 1981. Instars susceptibility of *Simuliium vittaum* (Diptera: Simuliidae) to the entomogenous nematode, *Neoaplectana carpocapsae*.J.Nematology., 13:1-5.

Gill, S. A. and Micheal, J. Raupp 1994. Using entomopathogenic nematodes and conventional and biorational pesticides for controlling bagworm .J. of Abriculture. 20(6):318-322.

Hammad, S. H. Arosy, H .and Asem, M. A. 1965. Pests and disease of vegetable crops and their methods of their control, Cairo (In Arabic).

Henderson, C. F. and W.A. Tiliton. 1955. Test with acaricides against the wheat mites. J. of Econ.Entomol.49:157-161.

Ahmed K. S. 1995. Insect pests infesting cabbage and turnip plants and their natural enemies with special reference to *Diaeretiella (Aphidius) rapae* Mcintosh (Hymenoptera: Aphidiidae) in Saudi Arabia. Egypt J. Biol. Pest cont., 5(2), 95-101.

Kaya, H. K. 1985. Entomogenous nematodes for insect control in IPM systems. In: Hoy, M.A. & Herzog, D.C. (Eds.), "Biological Control in Agriculture IPM Systems". Academic Press, New York, pp. 283-302.

Kaya, H. K. 1984. Effect of the entomopathogenous nematode *Neaplectana carpcapase* on the tachinid parasite *Compsilura concinnata* (Diptera: Tachinidae). J.Nematology. 16, 9-13.

Kondo, E. and Ishibashi, N. 1984. Infectivity and multiplication of *Steinernema feltiae* (str. Mexican) on the common cutworm, *Spodoptera litura.* Japan. J. Entomol. Zool., 28: 229-238.

Kondo, E. 1989.Studies on the infectivity and propagation of entomopathogenic nematodes, *Steinernema* spp., (Rhabditida: Steinernematidae), in the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). Bull. Fac.Agr. Saga Univ. 67:1-8 .

Koppenhoffer, A. M. and Kaya, H. K. 1997. Additive synergistic interactions between entomopathogenic nematodes and *B. thuringensis* for scarab grub control. Biological Control. 8, 131-137.

Koppenhoffer, A. M., H. Y. Choo and H. K. Kaya, D. W. Lee and W. D. Gelernter 1999. Increased field and green house efficacy against scarab grubs with a combination of an entomopathogenic nematode and *B. thuringensis*. Biological Control 14, 37-44.

Saleh. M. M. E. 1995. Efficiency of the Egyptian entomopathogenic nematode *Heterorhabditis tayserii* (Nematoda: Heterorhabditidae) in controlling the cabbage worm, *Pieris rapae* (L.) (Lepidoptera: Pieridae). Egypt. J. Biol. Pest Cont., 5(2), 103-105.

Pionar, G. O. J. R. and Deschamps, N. 1981. Susceptibility of *Scolytus multistriatus* to neoaplectanid and *Heterorhabditid* nematodes. Environ.Entomology.10, 85-87.

SAS Institute.1988.SAS/STAT user's guide, 6.03 ed. SAS Institute, Cary, Nc.

Thompson, H. S. and W. S. Killy. 1957. Vegetable crops. Edited by Thompson Washington and London (Arabic translated, ed .Modern. Egypt. Press, Cairo, Cyprus).

Woodring, J. L. and Kaya, H. K. 1988. Steinernematid and Heterorhabditid Nematodes: A Handbook of Biology and Techniques. Southern Cooperation Series Bull. 331: 30 pp.

التكامل بين ثلاث وسائل حيوية لمكافحة دودة الكرنب *Artogeia (Pieris) rapae* (L.)

بدر الصباح عبد المنعم فتوح و أحمد محمد عزازى

معهد بحوث وقاية النباتات، مركز البحوث الزراعية، الدقى، الجيزة، مصر.

أجريت تجارب معملية وحقلية لتقويم ثلاث وسائل حيوية (نيماتودا و بكتريا و فطر) لمكافحة دودة الكرنب *Artogeia (Pieris) rapae* (L.) كاتجاه حديث من اتجاهات المكافحة المتكاملة للآفات. أظهرت يرقات وعذارى أبو دقيق الكرنب (دودة الكرنب) حساسية عالية لنوعى النيماتودا *Heterorhabditis bacteriophora* (TWF) , *Steinernema carpocapsae* (All strain). وكذلك أظهر الدايبل (مستحضر تجارى من بكتريا *Bacillus thuringiensis*) كفاءة عالية على دودة الكرنب حيث أعطى نسبة موت ٩٣،٣%، بينما أظهر البيوفلاى (مستحضر تجارى من فطر *Beauveria bassiana*) تأثيرا ممرضا أقل يعادل ٤٤،٢%، وقد أعطت النيماتودا نسب موت تراوحت ١٠٠-٧٣،٣% ، ١٠٠-٥٣،٣ % لكل من اليرقات والعذارى، وعند خلط النيماتودا مع الدايبل كانت نسب موت اليرقات ١٠٠%، وتراوحت مع البيوفلاى بين ٨٨،٩و ٩٥،٥ %. تراوحت نسبة الموت بعد ٧ أيام من الرش فى التجارب التى أجريت فى المعمل بين ٨٣،٤و ٨٥،٨٩ و بين ٧٧،٧ و ٧٩،٩١% لكل من نوعى النيماتودا المستخدمة بعد الرش مرتين. أعطى خلط نوعى النيماتودا كل حدة مع الدايبل أو البيوفلاى نسبة موت ٩٤،٢٩،٩٣،٧٣ % لنوع النيماتودا (All) و ٩٤،٥٨، ٨٧،٦٩% لنوع النيماتودا (TWF)، وعند إستخدام الدايبل والبيوفلاى كل على حدة تراوحت نسب الموت بين ٨٨،٨١، ٤٠،١% لكل من البكتريا والفطر على التوالى.