**-48- Summary of**

# BIOLOGICAL CONTROL OF SOME SUGAR BEET INSECT PESTS USING ENTOMOPATHOGENIC NEMATODES

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**By**

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## SUMMARY

**Sugar beet, Beta vulgaris L. is a strategic crop of sugar industry in Egypt. It is threatend by several insect pests among most important of them are the beet fly P. mixta and the beet beetle C. vittata. This work deals with the biological control of these insect pests using entomopathogenic nematodes (EPN). Results of laboratory and field .studies summary of the are given in the fallowing summary**

### :A- Laboratory studies

**These studies included assessment of the virulence of four local and imported EPN to different developmental stages of the two sugar beet pests. The nematodes included the local Steinernema carpocapsae S2, the local Heterorhabditis bactriophora S1, the imported Steinernema feltiae and the imported Heterorhabditis bacteriophora (1-3). Daily mortality of young larvae (1st and 2nd instars), grown larvae, pupae and adults of Cassida vittata and young larvae (1st and 2nd instars), grown larvae (3rd instars), new formed pupae and old pupae of Pegomyia mixta were recorded after treatment with serial .concentrations (from 500 to 4000IJs/ml) of each of four studied EPN**

### ١. Virulence of entomopathogenic nematodes to the beet beetle Cassida .vittata

#### :Virulence to insect larvae .١.١

#### :Young larvae .١.١.١

**After 24 hours of treatment with entomopathpgenic nematodes mortality in young larvae was recorded in levels from 0.0 to 73.3 %.The ranges of mortality percentages were (0.0 : 26.6), (6.6 : 53.3), (20 : 73.3) and (0.0 : 20) for treatment with S. carpocaosae (2), S. feltiae, H. bacteriophora (1-3) and H. bacteriophora (S1), respectively. After 48 hours, the respective mortality percentages were (13.3: 73.3), (66.6: 100), (33.3: 93.3) and (6.6: 46.6) %.While, after 72 hours of treatment by the same concentrations caused (20: 86.6), (86.6: 100), (46.6: 100) and (6.6: 53.3) % mortalities, respectively. Finally, after 96**

**hours, the respective mortality percentages were (26.6: 86.6), (86.6: .% (100), (46.6: 100) and (6.6: 53.3**

**As for the LC50 values of S. carpocaosae (2), S. feltiae, H. bacteriophora (1-3) and H. bacteriophora (S1), for the young larvae of C. vittata, those were found to be 1515, 292, 890 and 4632 IJs/ml, respectively. While, LT50 values were 37.37, 27.71, 20.07 and 248 hours, respectively. The most virulent nematode was S. feltiae while .(the fastest killing nematode was H. bacteriophora (1-3**

**:Full grown larvae .١.١.٢**

**After 24 hours of treatment with entomopathpgenic nematodes, mortality in grown larvae was recorded in levels from 0.0 to 46.6 %.The ranges of mortality percentages were (13.3 : 40), (13.3 : 40), (20 : 46.6) and (0.0 : 26.6) for treatment with S. carpocaosae (2), S. feltiae, H. bacteriophora (1-3) and H. bacteriophora (S1), respectively. After 48 hours, the respective mortality percentages were (33.3: 60), (46.6: 100), (26.6: 60) and (6.6: 40) %.While, after 72 hours of treatment the same concentrations caused (40: 73.3), (60: 100), (26.6: 73.3) and (13.3: 46.6) % mortalities, respectively. Finally, after 96 hours, the respective mortality percentages were (46.6: 86.6), (60: 100), (26.6: 73.3) and .% ((13.3: 60**

**As for the LC50 values of S. carpocaosae (2), S. feltiae, H. bacteriophora (1-3) and H. bacteriophora (S1) for the grown larvae of C. vittata, those were estimated by 1238, 544, 2249 and 5903 IJs/ml, respectively. While, LT50 values were 37.53, 26.24, 31.9 and 334 hours, respectively. The most virulent and fastest killing nematode against the .grown larvae of C. vittata was S. feltiae**

**-:Virulence to insect pupae .١.٢**

**After 24 hours of treatment with entomopathpgenic nematodes, no mortality in pupae was recorded. After 48 hours, the ranges of mortality percentages were (0.0 : 33.3), (46.6 : 80), (13.3: 33.3) and (0.0 : 13.3) for treatment with S. carpocaosae (2), S. feltiae, H. bacteriophora (1-3) and H. bacteriophora (S1), respectively.After72 hours, the respective mortality percentages were (20: 60), (60: 80), (20: 53.3) and (0.0: 40) %.While, after 96 hours of treatment by mentioned species by the same concentrations caused (40: 80), (60: 86.6), (26.6: .80) and (20: 73.3) % mortalities, respectively**

**As for the LC50 values of S. carpocaosae (2), S. feltiae and H. bacteriophora (1-3)), for the pupae of C. vittata, they calculated 9891, 577 and 10719 IJs/ml, respectively. The nematode H. bacteriophora (S1) looked the lowest efficient nematode to C. vittata pupae through such bioassay studies. While, LT50 values of S. carpocaosae (2), S. feltiae, H. bacteriophora (1-3) and H. bacteriophora (S1), be 81.21, 7.08, 74.602 and 82.97 hours, respectively. The most virulent and the .fastest in its effect against the pest pupae was S. feltiae**

**: Virulence to adult of C. vittata .١.٣**

**No effect on adults C.vittata appeared during the first 24 hours after treatment with entomopathpgenic nematodes. After 48 hours, no mortality was observed from exposure with entomopathpgenic nematodes in adults C.vittata, except the imported nematode S. feltiae which caused (13.3 : 33.3) % mortalities . After 72 hours, the ranges of mortality percentages were (0.0), (26.6 : 46.6), (13.3: 46.6) and (0.0) for treatment with S. carpocaosae (2), S. feltiae, H. bacteriophora (1-3) and H. bacteriophora (S1), respectively.After 96 hours, the respective mortality percentages were (53.3: 100), (40: 66.6), (40: 66.6) and (13.3: 46.6) %.While, after 5 days of treatment by mentioned species by the same concentrations caused (53.3: 100), (40: 86.6), (40: 73.3) and (26.6: .73.3) % mortalities, respectively**

**As for the LC50 values of S. carpocaosae (2), S. feltiae, H. bacteriophora (1-3) and H. bacteriophora (S1), for the adults of C. vittata, they were found to be 496, 859, 945 and 4771 IJs/ml, respectively. While, LT50 values were 72.94, 87.70, 84.52 and 110.68 hours, respectively. The most efficient and fastest killing nematode .(against the adults of C. vittata was S. carpocaosae (2**

**.Development of entomopathogenic nematodes in cadavers of C .٢**

**:vittata**

**Entomopathogenic nematodes could successfully develop and propagate in larvae, pupae, and adults of the beet beetle C. vittata so that the nematode offspring could be obtained from insect cadavers. The results show the percentages of insects that support development and propagation of nematodes inside their bodies after being infected .with these nematodes**

**Comparing the nematode development and propagation in different stages of C. vittata, it was found that the two steinernematids S. carpocapsae (S2) and S. feltiae were superior over the two heterorhabditids H. bacteriophora (1-3) and H. bacteriophora (S1). S. carpocapsae (S2) was the most successful in development and propagation in adults of pest giving 100 % development and propagation. S. carpocapsae (S2) also developed and propagated in high rates (91.8 and 89.5 %) in grown larvae and pupae of the pest . S. feltiae was the most successful in development and propagation in young larvae of the pest achieving 89.7 %. S. feltiae developed and propagated in rates over 60 % in grown larvae, pupae and adults of the pest. Grown larvae and adults of the pest were found to be more suitable for nematode development and propagation than young larvae or pupae regardless the nematode species or concentration. Grown larvae supported nematode development and propagation at rates from 68.7 at 91.8, while adults of the pest supported nematode .% development and propagation at rates of 32.5 – 100**

## ٣. Virulence of entomopathogenic nematodes to the beet flies Pegomyia mixta

### ٣.١. Virulence to insect larvae:

#### ٣.١.١. Young larvae:

After 24 hours of treatment with entomopathpgenic nematodes mortality in young larvae was recorded in levels from 0.0 to 73.3 %.The ranges of mortality percentages were (0.0 : 60), (0.0 : 73.3), (0.0 : 13.3) and (0.0 : 400) for treatment with S. carpocaosae (2), S. feltiae, H. bacteriophora (1-3) and H. bacteriophora (S1), respectively. After 48 hours, the respective mortality percentages were (46.6: 73.3), (20: 80), (0.0: 20) and (0.0: 40) %.While, after 72 hours of treatment by mentioned species at the same concentrations caused (53.3: 86.6), (33.3: .93.3), (0.0: 26.6) and (0.0: 46.6) % mortalities, respectively

The result also shows mortality in the insect pupae that was treated as young larvae. Total percentage of mortality in young larvae of P. mixta and resulted formed pupae due to the nematode S. carpocapsae (S2) were 86.6, 93.3, 93.3 and 100 % when treated with the nematode concentrations of 500, 1000, 2000 and 4000IJs/ml. respectively. Total mortality percentage due to the same concentration of S. feltiae was 73.3, 86.6, 93.3 and 100 %, respectively. The heteroehabditid, H. bacteriophora (1-3) induced total mortality percentage of 26.6, 33.3,40 and 86.6 %.The local heteroehabditid H. bacteriophora (S1), induced total mortality percentage of 46.7,80,80 and 93.3 %. Most of mortality was induced during the pupal stage due to their relative less efficiency .on the young larvae

As for the LC50 values of S. carpocaosae (2), S. feltiae, H. bacteriophora (1-3) and H. bacteriophora (S1), for the young larvae of P. mixta, those were found to be 491, 930, 17684 and 4572 IJs/ml, respectively. While, LT50 values were 26.4, 20.21, 174.9 and 97.10 hours, respectively. The most virulent nematode against the young larvae of P. mixta S. carpocaosae (2) while the fastest in its effect was .S. feltiae

**:Full grown larvae .٣.١.٢**

**Mortality perecentages of these larvae ranged from 0 to 20 % due to S. corpocapsae, H. bacteriophora (1-3) and H. bacteriophora (S1) but it .reached 46.7 % due to S. feltiae**

**The results also shows mortality in the insect pupae that treated as grown larvae. Total percentage of mortality in grown larvae of P. mixta and resulted formed pupae due to the nematode S. carpocapsae (S2) were 26.6, 53.3,73.3 and 93.3 % when treated with the nematode concentrations of 500,1000,2000 and 4000IJs/ml. respectively. Total mortality percentages due to the same concentration of S. feltiae were 66.6, 73.3, 86.6 and 93.3 %, respectively. The heteroehabditid, H. bacteriophora (1-3) induced total mortality percentages of 6.6, 13.3,40 and 93.3 %.The local heteroehabditid H. bacteriophora (S1), induced total mortality percentages of 26.6, 40, 53.3 and 73.3 %. Most of mortality was induced during the pupal stage because of their relative .less efficacy on the grown larvae**

**As for the LC50 values of S. feltiae and H. bacteriophora (S1), for the grown larvae of P. mixta, those were found to be 5562 and 46083 IJs/ml. While, LT50 values were 186.29 and 155.48 hours. Other tested .nematodes were lower in their virulence**

**:Virulence to insect pupae .٣.٢**

**:New formed pupae .٣.٢.١**

**These studies showed mortality percentages in new pupae of P. mixta after treatment with local and imported entomopathogenic nematodes. It also showed mortality in the adults that were treated as new pupae.**

**Total percentage of mortality in new pupae of P. mixta and formed adults due to the nematode S. carpocapsae (S2) were 46.6, 73.3, 93.3and 100 % when treated with the nematode concentrations of 500, 1000,2000 and 4000IJs/ml. respectively. Total mortality percentage due to the same concentration of S. feltiae was 73.3, 73.3,80 and 93.3 %, respectively. The heteroehabditid, H. bacteriophora (1-3) induced total mortality percentage of 53.3, 60, 73.3 and 93.3 %.The local heteroehabditid H. bacteriophora (S1) induced total mortality .% percentage of 53.3, 60, 73.3 and 93.3**

**As for the LC50 values of S. carpocaosae (2), S. feltiae, H. bacteriophora (1-3) and H. bacteriophora (S1), for the new pupae of P. mixta, they were 2160, 128, 2529 and 1726 IJs/ml, respectively. S. feltiae. was the most efficient nematode against new formed pupae of .P.mixta**

**:Old pupae .٣.٢.٢**

**Mortality percentages in old pupae of P. mixta showed mortality in the adults that treated as old pupae. Total percentage of mortality in old pupae of P. mixta and emerged adults due to the nematode S. carpocapsae (S2) were 40, 66.6, 86.6 and 100 % when treated with the nematode concentrations of 500, 1000, 2000 and 4000IJs/ml. respectively. Total mortality percentage due to the same concentration of S. feltiae was 40, 46.6,60 and 66.6 %, respectively. The heteroehabditid, H. bacteriophora (1-3) induced total mortality percentage of 66.6,80,80 and 86.6 %.The local heteroehabditid H. bacteriophora (S1), induced total mortality percentage of 40 and 73.3 % when treated with the nematode concentrations of 2000 and .4000IJs/ml**

**As for the LC50 values of S. carpocaosae (2), S. feltiae and H. bacteriophora (1-3) for the old pupae of P. mixta, those were found to be 41585, 1100 and 505 IJs/ml, respectively. Other tested nematodes .were lower in their virulence against old pupae**

**٤- Development of entomopathogenic nematodes in cadavers of P. mixta:**

**Entomopathogenic nematodes could successfully develop and propagate in larvae, pupae, and adults of the beet fly P. mixta so that the nematode offspring could be obtained from insect cadavers. The results showed percentages of insects that support development and propagation of nematodes inside their bodies after being infected with these nematodes. Sometimes nematode offspring were noticed emerging from the next stage of treated insect. When the grown larvae of P. mixta were treated with the nematodes S. carpocapsae and H. bacteriophora (S1), these nematodes were noticed emerging from adults of the insect instead treated of pupae. This phenomenon was noticed frequently in the present studies; as much as 85% of propagated nematodes emerged from the insect stage following the stage of treatment. The S. carpocapsae offspring emerged from the pupae as a result of treating the insect grown larvae. The interpretation of the action is that the nematode remained alive until pupal or adult formation and attacked the formed pupae or adults so that they can develop and propagate. S. feltiae was the sole nematode that developed and propagated in the same insect stages that received the nematode treatment. This may reflect the high virulence and specificity of this nematode to dipterous insects.**

**Comparing the development levels of different nematodes in cadavers of P. mixta it could be concluded that all tested nematode succeeded in propagation among all insect stages especially. S feltiae which developed at 57.9-84.3 %. The most suitable stage for nematode development was the grown larvae of the insect supporting 57.9-81.4% of nematode development and propagation.**

**B – FIELD STUDIES:**

**١- Control of P.mixta and C. vittata in sugar beet fields using S. feltiae and H. bacteriophora (1-3):**

**:Beet fly,P. mixta .١.١**

**:Effect of S. feltiae .١.١.١**

**Percent of reduction in population of larvae of P. mixta due to S. feltiae ranged form 16 to 81.3 % according to concentration and time after treatment. At the concentration of 1000 IJs/ml, percentages of reduction in larval population were 16.0, 23.4 and 28.5 % after 2, 5 and 7 days of treatments, respectively. At 3000 IJs/ml, larval population was reduced by 45.7, 52.4 and 58.5 % after 2, 5 and 7 days of treatments, respectively. When the nematode concentration was increased to 6000 IJs/ml, the population of the insect larvae was reduced by 48.9, 75.3 and 81.3 % after 2, 5 and 7 days of treatments, .respectively**

**:(Effect of H. bacteriophora (1-3 .١.١.٢**

**The population reductions at the concentration of 1000 IJs/ml were 11.2, 14.4 and 15.9 %. Such percentages at the concentration of 3000 IJs/ml were 29.5, 34.9 and 42.8 %. At a concentration of 6000 IJs/ml, the population reduction reached 44.0, 72.7 and 75.9 % after 2, 5 and 7 .days, respectively**

**In a compassion between the two nematode species, S. feltiae seems to be more effective than H. bacteriophora (1-3) against larvae of P. mixta in the field. The half-lethal concentrations of S. feltiae (LC50) values were 5134, 2644 and 2110 IJs/ml after 2, 5 and 7 days, respectively. While, LC50 values for H. bacteriophora (1-3) were 7599, .3630 and 3147 IJs/ml after 2, 5 and 7 days, respectively**

**:Beet beetle, C. vittata .١.٢**

**:Effect of S. feltiae .١.٢.١**

**At the concentration of 1000 IJs/ml, percentages of reduction in larval population, computed according to Henderson and Tilton (1955) equation were 3.3, 12.7 and 12.7 % after 2, 5 and 7 days, respectively. By increasing the concentration to 3000 IJs/ml the reduction in larval**

**population became 8.8, 26.5 and 41.8 % after 2, 5 and 7 days, respectively. At the highest concentration (6000IJs/ml), the reduction in larval population of C. vittata reached 31.4, 55.7 and 63.8 % after 2, .5 and 7 days of treatment, respectively**

**Percentages of population reduction in pupae were 10.2, 17.4 and 22.7 % after 2, 5 and 7 days using the concentration of 1000 IJs/ml. When the concentration increased to 3000 IJs/ml, the reduction in pupal population reached 19.8, 28.4 and 31.3 % after 2, 5 and 7 days, respectively. At the highest concentration (6000IJs/ml), the reduction in pupal population of C. vittata reached 48.6, 51.2 and 53.3 % after 2, .5 and 7 posttreatment, respectively**

**Percentages of reduction in adults' population were 8, 14.5 and 38.7 % after 2, 5 and 7 days using the concentration of 1000 IJs/ml . After increasing the concentration to 3000 IJs/ml, the reduction in adults' population became 14.5, 23.4 and 49.4 % after2, 5 and 7 days, respectively. At the highest concentration (6000IJs/ml), the reduction in adults' population of C. vittata reached 28.7, 39.7 and 61.7% after2, .5 and 7 days posttreatment, respectively**

**LC50 values of S. feltiae for larvae were 11668, 5623 and 3893 IJs/ml after 2, 5 and 7 days of treatment, respectively. LC50 on pupae were 7388, 6622 and 6127IJs/ml after 2, 5 and 7 days of treatment, respectively. LC50 values of S. feltiae on adults were 21920, 12308 and .2632 IJs/ml after 2, 5 and 7 days of treatment, respectively**

**These values show higher susceptibility of pupae and adults of C. .vittata to infection with S. feltiae than the larvae**

**:(Effect of H. bacteriophora (1-3 .١.٢.٢**

**Field treatments showed that spraying sugar beet plants with three concentrations of H. bacteriophora (1-3) suppressed populations of larvae, pupae and adults of C.vittata and reduced percentages of .population after nematode treatments**

**At nematode concentration of 1000 IJs/ml caused percentages of reduction in larval population by 23.1, 23.5 and 25.7 % after 2, 5 and 7 days. By increasing the concentration to 3000 IJs/ml, the percentages reduction in larval population were 30.6, 32.3 and 46.2 % after 2, 5 and 7 days, respectively. At the highest concentration (6000 IJs/ml) the reduction in larval population of C. vittata reached 45.2, 64.9 and 68.2 .% after 2, 5 and 7 days of treatment, respectively**

**When the concentration of 1000 IJs/ml was used, percentages of reduction in pupal population were 17.7, 34.3 and 52.2 % after 2, 5 and 7 days, respectively. Increasing the concentration to 3000 IJs/ml, leads to a reduction in pupal population of 40.8, 49.4 and 69.7 % after 2, 5 and 7 days, respectively. At higher concentration (6000 IJs/ml), percentages of reduction in pupal population of C. vittata reached 63.8, .78.5 and 83.2 % after 2, 5 and 7 days of treatment, respectively**

**When the concentration of 1000 IJs/ml was used, percentages of reduction in adults' population were 7.5, 19.0 and 34.0 % after 2, 5 and 7 days, respectively. Increasing the concentration to 3000 IJs/ml, the reduction in adults' population became 20.5, 39.4 and 62.2 % after 2, 5 and 7 days, respectively. At higher concentration (6000 IJs/ml), the reduction in adults' population of C. vittata reached 32.5, 74.8 and 81.1 .% after 2, 5 and 7 days of treatment, respectively**

**LC50 values of H. bacteriophora (1-3) for larvae of C. vittata were 10089, 4136 and 3057 IJs/ml after 2, 5 and 7 days of treatment, respectively. LC50 values for pupae were 3571, 2192 and 933 IJs/ml after 2, 5 and 7 days of treatment, respectively. LC50 values for adults**

**were 13608, 3223 and 1827 IJs/ml after 2, 5 and 7 days of treatment, .respectively**

**Control of C.vittata in sugar beet fields by Steinernema carpocapsae .٢ .( (S2**

**At nematode concentration of 2500 IJs/ml, percentages of reduction in larval population, were 2.8, 16.6 and 37 % after 2, 5 and 7 days, respectively. By increasing the concentration to 5000 IJs/ml, the percentages reduction in larval population became 26.1, 50 and 62.2 % after 2, 5 and 7 days, respectively. At the highest concentration (10000 IJs/ml), the reduction in larval population of C. vittata reached 32.8, .62.1 and 65 % after 2, 5 and 7 days of treatment, respectively**

**When the concentration of 2500 IJs/ml was used, percentages of reduction in pupal population were 50, 63.3 and 75.7 % after 2, 5 and 7 days, respectively. Increasing the concentration to 5000 IJs/ml induced reduction in pupal population to 52, 65.4 and 83.6 % after 2, 5 and 7 days, respectively. At the highest concentration (10000IJs/ml), the recorded reduction in pupal population of C. vittata recorded 79, 86.2 .and 92 % after 2, 5 and 7 days of treatment, respectively**

**When the concentration of 2500 IJs/ml was used, percentages of population reduction in adults' were 14.5, 22.3 and 14.5 % after 2, 5 and 7 days, respectively. By increasing the concentration to 5000 IJs/ml, the reduction in adults' population became 18, 37.1 and 40.3 % after 2, 5 and 7 days, respectively. At higher concentration (10000IJs/ml), the reduction in adults' population of C. vittata reached .37.3, 54.4 and 57.3 % after 2, 5 and 7 days of treatment, respectively**

**LC50 values of S. carpocapsae for larvae were 14472, 6293 and 3935 IJs/ml after 2, 5 and 7 days of treatment, respectively. LC50 values for**

**pupae were 3056, 1550 and 645 IJs/ml after 2, 5 and 7 days of treatment, respectively. LC50 values of S. carpocapsae for adults were 21504, 8403 and 7584 IJs/ml after 2, 5 and 7 days of treatment, .respectively**

**It is observed that values of LC50 for pupae were clearly less than those of larvae and adults of C. vittata which reflect how effectively the nematode can suppress the pupal population in the field.An explanation of such observation may refers to that pupae of C. vittata are yusually present inside the ecodysiol coccon of the last larval instars which full with nematode suspension and give advance of nematode to direct with the pupal body. Another explanation of such decrease of LC50 values of the tested nematodes against the pupal stage of C. vittata is that pupae are immobile stage and nematode spray can easily reach the pupae among the leaf biases of the plants in .the field**