Investigations to biological and organic treatments against pests in vegetable cultivation

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Abstract. In organic farming investigations regarding environmental friendly methods of ‘Pests Management’ are of high importance. In order to replace synthetic pesticides, antagonistic insects were tested against White Fly (Trialeurodes vaporariorum Westwood) as well as plant oils. For enhancement of plant growth two different biostimulators based on humic acid (‘Humintech’ and ‘Humentos’) were used, the interaction with the antagonistic insects and the White Fly were investigated. As antagonistic insects, the parasitic wasp (Encarsia formosa Gahan) and the predatory assassin bug (Macrolophus pygmaeus Rambur) were applied apart as well as in combination. In addition against the White Fly two plant oil compounds the Pine oil and the Castor oil were used and their effect on various stages of the White Fly’s development, the adult insects, the parasitic activity and the predator performance were examined in both greenhouse and lab conditions. Under optimal growing conditions of the tomato plants, humic acid preparations ‘Humintech’ and ‘Humentos’ in concentration 0.005% as leaf application had no influence on the yield. However, ‘Humentos’ (0.05%) showed yield increasing effect on tomato plants under suboptimal cultivation conditions and three times application. Both predator insects E. formosa and M. pygmaeus are capable to control population of the White Fly if duly applied. The best results were obtained in case of combination of both antagonists. Both oil preparations (the Pine oil and the Castor oil) had in concentration of 1% an insecticide effect on both larvae as well as adult insects of the White Fly. Best results were achieved with Pine oil a mortality rate of 80% was obtained. The humic acid preparation ‘Humintech’ showed repellent effect on the White Fly and its antagonists. As a result of these research can be confirmed, that investigated methods of biological control can be applied in environment friendly cultivation of tomatoes in protected cultivation.

Keywords: Encarsia formosa, Macrolophus pygmaeus, humic acid, pine oil, castor oil, Trialeurodes vaporariorum

1. Introduction
There are many criteria influencing the yield and quality of vegetables. Very important are suitable growing conditions as soil quality, water and nutrient supply, light and temperature for the different vegetable species. In particular in tropical regions another criteria is of high importance in this regard the protection against various pests. In Europe one of the dangerous pests is the greenhouse white fly (Trialeurodes vaporariorum Westwood), this insect (moth) is belong to the family Aleyrodoidea. The origin of T. vaporariorum is in subtropical regions of Central America, in Europa first time realised 1856 in greenhouses. Beside this white flythere is also the sweetpotato or cotton whitely Bemisia tabaci originated in Pakistan, widespread in tropical and subtropical regions but nowadays also a dangerous pest in European greenhouses.

The number of host plants of Trialeurodesis very large about 274 plants from 84 plant-families (Hodde 1994). There are also many vegetable species which can be damaged in greenhouses, as tomato (Lycopersicon esculentum Mill.), cucumber (Cucumis sativus L.), eggplant (Solanum melongena L.), and pepper (Capsicum annuum L.) (Byrne and Bellows 1991). Lenteren van and
Noldus (1990) determined, *T. vaporariorum* has the best conditions for development on eggplants. The white fly is damaging the crops through direct feeding, inserting their stylet into leaf veins and extracting nourishment from the phloem sap. As a by-product of feeding, honeydew is excreted and that alone can be also a source of damage. The third and potentially most harmful characteristic is the ability of adults to transmit several plant viruses (Cohen and Antignus 1982).

**Antagonistic insects**

As antagonistic insects, the parasitic wasp (*Encarsia formosa* Gahan) was described by Gahan first time 1924 in Ohio (USA) as parasitoid of *T. vaporariorum* in citrus plantations. Nowadays, *E. formosa* is used regular in biological control of white fly in greenhouses where are cultivated vegetables or ornamentals (Lenteren van and Roermund 1997). Many companies are providing this antagonistic insect.

The predatory assassin bug *Macrolophus pygmaeus* first time described by Herrich and Schäffer 1835 as *Capsusnubilus*, today the name *Macrolophus pygmaeus* by Rambur is used (Hillert et al. 2002).

**Organic plant protection and growth regulators**

**Humic acids** can accelerate the plant-growth, stimulates the process of the formation of plant-organs, increases the unspecific resistance of the plants against stress-conditions like to high temperature, frost, drought, strong radiation (Faust, 1999, Boehme et al. 2008). Humates have an influence on the nutrient-uptake and the respiration-process, the amount of sugar and amino acids, further reduce the accumulation of nitrate and make the plants resistant against diseases and viruses (Levinsky 1996; Boehme et al. 2005).

**Castor oil** from the seeds of the castor plants (*Ricinus communis*) contain 90 % ricinoleic acid, also 6.8-9 % oleic acid and 1.4-3 % linoleic acid. Furthermore this oil contains the highly toxic ricin and the insecticide ricin. The oil of castor seeds is belong to the so called 'dry' oils, therefore it can be used also in medicine and for technical purposes.

**Pine oil** (*Pinus sylvestris*) means essential oil distilled using pine needles, and branches with a diameter until 2.5cm. For steam distillation the material will be chopped and then pressed in a specific vessel with a steam pressure of 0.5 to 0.7 MPa the distillations is finished after 5 to 6 hours. In the needles the oil concentration is about 75%, in the thin branches 17% and in thicker branches ca. 10%. The composition of the pine oil is in average as follow- α- Pinen 46%, Camphen 3%, β- Pinen; β- Myrcen 28%, Limonen 8.6 and Ocimen 3.5% (Georgiev 1995).

The aim of the research was in substrate culture of tomatoes (*Lycopersicon esculentum* Mill.) in greenhouse (i) to investigate the effect of the antagonistic insets as the parasitic wasp (*Encarsia formosa*) and predatory assassin bug (*Macrolophus pygmaeus*) against white fly (*Trialeurodes vaporariorum*), (ii) to investigate the effect of organic plant regulators and plant oils, and (iii) the interaction of some of the treatments against white fly.

2. **Materials and methods**

1\textsuperscript{st} experiment antagonistic insects and humates against white fly

In-determinant tomato plants cv. Ferrari RZ were cultivated under greenhouse conditions. A hydroponic substrate culture system was used with Peat Slabs ‘GroBoard’ (width 20 cm, length 100 cm, height 9 cm) wrapped with plastic delivered by KEKKILÄ Oyj Company Finland. Pore volume was 94% v/v, the water holding capacity was 62% v/v, and the air capacity was 31%. The slabs were placed in channels for drainwater collection. Drip irrigation was used for watering.

The humates Humintech ® (85% humic acid) and Humentos ® (3.5% humic acid) were applied as leaf application 3 times, 2 weeks after development of the first inflorescence, with a concentration of 0.005% and 0.05% respectively. Between 40 and 50ml of the treatments were applied per plant. The first experiment using *Encarsiaformosa* was carried out late spring early summer season (02.04 until 01.07) whereas the experiment with treatments of *Macrolophus pygmaeus* was carried out in late summer early autumn (05.09 until 6.11). The air temperature was in average 23-24°C and the humidity 55-60% in the experiment with *Encarsia* and with *Macrolophus* the air temperature was in average 19-21°C and the humidity 45-60%.
The antagonistic insects against white fly *E. formosa* and *M. pygmaeus* by the company Katz Biotech Ltd., were applicate in the greenhouse following the calculation of Adam (1988), starting the application after 20 adult white flies per 100m² were be found. The level of parasitization (LP) was calculated with following equation:

\[
LP(\%) = \frac{\text{no. of parasitized puparia}}{\text{no. of parasitized puparia} + \text{no. of not parasitized puparia}} \times 100
\]

2nd experiment pine oil, castor oil and humate against white fly

The tomato plants cv. Ferrari RZ, were cultivated in pots with 6L perlite, average grain size between 0.06 mm and 1.5 mm, 45% of all grains with a grain diameter of 1 mm. Pore volume was 84% v/v, the water holding capacity was 45% v/v and the air capacity was 39%. Drip irrigation was used for watering. The pine and the castor oil were applied in concentrations of 1% and Humintech ® 0.05% as leaf application 3 times in an interval of 1 week starting after larva population density of 22-28.

Statistics

The experiment comprised in both experiments 10 plants per treatment randomly distributed. Data were evaluated by ANOVA (SPSS) and the Tukey-test.

3. Results and discussion

Antagonistic insects and humates against white fly

- *Encarsia formosa* application

The population of the white fly increased end of April after increasing of the temperature. In this time 500 pieces of *Encarsia formosa* (parasitized larvae) were placed in the greenhouse. Beginning in middle of May the parasitization increased slowly and was middle of June satisfying. In the time between 30th Mai and 15th Junethe parasitization was a little inhibited probably also because the decreasing of the temperature (Fig. 1 A and B) (Dimitrov 2005). However, in case of treatments with the humate Humintech the inhibition of the parasitization was stronger (Fig. 1A), it seems this humate had a little repellent effect because the specific smell against the adults of the parasitic wasps. In general no significant differences in the parasitization by *E. formosa* between the control and the two treatments.

![Figure 1](image_url)

**Figure 1.** Development of the parasitization of larvae/puparia von *T. vaporariorum* by *E. formosa* in tomato cultivation in greenhouse after leaf treatments with Humintech (0.05 %) (A) and Humentos (0.05 %) (B) (Hatching = not parasitized Larvae)

The parasitization by *E. formosawas* delayed in the beginning of the tomato cultivation in particular in the Humintech variant. Whereas, in the variant with Humentos treatment the parasitization was in the beginning even higher than in the Control (Fig. 2). In a later stage after 21th May no significant differences between all variants could be observed. The parasitization of the *T. vaporariorum* by the parasitic wasp *E. formosawas* successful in the end of this experiment a parasitization of 92-93% was achieved. Further investigations should clarify whether the Humentos treatments had a repellent effect and what kind of factors are influencing this effect.
Figure 2. Level of parasitization (LP) of larvae/puparia of *T. vaporariorum* by *E. formosa* in tomato cultivation in greenhouse after leaf treatments with humates

- **Macrolophus pygmaeus** application

This experiment was carried out in late summer/autumn the temperature were sometimes below 20°C, therefore the population of the white fly was establishing slowly. Nevertheless, the predatory activity of *M. pygmaeus* was comparable high. In beginning of October the population of the white fly increased very fast, parallel increased the predatory activity of *M. pygmaeus* (Fig. 3 A).

Figure 3. Predatory activity of *M. pygmaeus* against the larvae of *T. vaporariorum* in tomato cultivation in greenhouse, control (A) and after leaf treatments with Humintech (0.05%) (B) (Hatching = not sucked Larvae)

After the leaf treatments with Humintech (Fig. 3 B) the predatory activity decreased very intensive und was on a low level until the end of the cultivation. The obviously repellent effect by the humate Humintech was much stronger pronounced in case of *M. pygmaeus* than in case of *E. formosa*. Similar results were not described in the literature, maybe because humates are mostly used as treatment to the roots and not to the leaves (Böhme and Hoang 1996). However, Hillert et al. (2002) referred, that *M. pygmaeus* was very sensitive against chemical agents as Imadocloprida systemic insecticide which acts as an insect neurotoxin.

Effect of a simultaneously application of *E. formosa* and *M. pygmaeus* against *T. vaporariorum*

The simultaneously application of the antagonistic insects (*E. formosa* and *M. pygmaeus*) against the white fly (*T. vaporariorum*) was very successful. In particular in the case of very high population density of the white fly in the greenhouse in the summer time these combination was very effective (Fig. 4). It can be assumed also because the different adaptation to the greenhouse temperature, *E. formosa* is more active by temperatures between 18 and 24°C, whereas, *M. pygmaeus* is active as predator in a temperature range between 9 and 36°C (Perdikis and Lykouressis 2004). In the literature, few investigations about simultaneously application of both antagonistic insectscould be...
found. Wiethoff and Meyhöfer (2002) reported about similar investigations with the same positive effects, they used the parasitic wasp (Aphidius colemani) or together with green lacewings (Chrysoperla carnea) or with the aphid midge (Aphidoletes aphidimyza) against the green peach aphid (Myzus persicae).

Figure 4. Number of the parasitized by E. formosa or sucked by M. pygmaeus Larvae of white fly T. vaporariorum treated with Humintech (A) or Humentos (B) in tomato cultivation in greenhouse (Hatching = not parasitized/not sucked Larvae).

**Effect of pine, castor oil and Humintech against the larva and adults of T. vaporariorum**

In this experiment the population of white fly was increasing very early starting middle of February. The greenhouse temperature rose up to 24°C, therefore, already middle of March were observed more than 20 larvae per leaf.

As treatments against E. formosa the humate ‘Humintech’ and the plant oils pine oil and castor oil were used in a tomato cultivation system using pots under greenhouse conditions. The application of the treatments started after a larvae density was observed with 19 to 22. The number of larvae per leaf increased very fast as well in the Control as after treatments of Humintech (Fig. 5).

Figure 5. Number of living larvae of T. vaporariorum on tomato plants in greenhouse after 3 times application of Humintech (0.05), castor and pine oil (1%) (Different letters indicate statistical differences (P=0.05 Tukey-Test)

It was distinct that humate had no influence on the development of the white fly larvae. In difference castor oil and pine oil had significant toxic effect against the white fly larvae.

Whereas, the effect against the larvae with pine oil treatments was higher than with castor oil. Sengalevich et al. (2002) had similar results in his research using pine and castor oil against the larvae of Colorado beetle with about 70-80% mortality of the larvae.
Furthermore, was analysed the influence of the white fly population density following the quantity of living larvae after treatments with Humintech, castor and pine oil on the yield of tomatoes cultivated in greenhouse (Fig. 6). The tomato yield was reduced because the high white fly population and the damaging activities of the adults and the larvae. Humintech was not disturbing the white fly, therefore no significant difference could be determined in comparison to the Control. The tomato yield in the variant with castor oil application was higher, but the difference to the Control was also not clear significant. The highest tomato yield is visible for the treatment with pine oil, this result is significant different to the other variants. In this experiment was obvious a strong correlation between the white fly population (quantity of living larvae per leaf) and the yield. On the other hand this is showing the strong yield decreasing danger of the white fly in greenhouses.

![Figure 6. Correlation between the effect of Humintech (0.05 %), castor oil and pine oil against the quantity of living larvae of *T. vaporariorum* and the tomato yield, cultivated in greenhouse (different letters indicate significant differences (P=0.05 Tukey- Test).](image)

4. Conclusion

The growth stimulators based on humic acid Humintech (0.005 % or 0.05 %) and Humentos (0.05 %) had no insecticide effect against the white fly (*T. vaporariorum*). Therefore, this preparations cannot be used as treatments against white fly. On the other hand, fortunately the humates are not aggressive against the antagonistic insects *E. formosa* and *M. pygmaeus*. Nevertheless, it can be considered a repellent effect of Humintech against the *M. pygmaeus* which is stronger in comparison to the *E. formosa*.

The parasitic wasp *E. formosa* parasitized the larvae and puparia very good, the level of parasitization (LP) was up to 94%. The predatory activity of *M. pygmaeus* was satisfying up to 80% in a greenhouse experiment with tomato during autumn.

The simultaneous application of *E. formosa* and *M. pygmaeus* had a very good effect in reducing of the white fly larvae. The parasitization and predatory activity of both antagonistic insects come to a level of parasitization (LP) of 97%.

The plant oils from castor and pine (1%) were showing an insecticide effect against the larvae and adults of white fly (*T. vaporariorum*). It could be observed a significant higher effect of pine oil against the larvae of white fly. Whereas, the effect of castor oil was significant lower, but higher than Humintech.
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