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Faculty of Agriculture, Trakia University
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Bulgaria
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Screening of plant protection products against downy mildew on cucumbers (*Pseudoperonospora Cubensis* (Berkeley & M. A. Curtis) Rostovzev) in cultivation facilities

S. Masheva[1]*, N. Velkov[1], N. Valchev[2], V. Yankova[1]

[1]Maritsa Vegetable Crops Research Institute, 4003 Plovdiv, Bulgaria
[2]Department of Plant Production, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria

Abstract. *Screening of plant protection products (PPP) against downy mildew in cucumbers Pseudoperonospora cubensis (Berkeley & M. A. Curtis) Rostovzev was carried out during the period 2006-2011 in the "Maritsa" Vegetable Crops Research Institute, Plovdiv. Their toxicity to the imago of the bioagent Encarsia formosa Gah. were studied. High effectiveness (over 85.00 %) of the PPP with active ingredients: dimethomorph (Zampro SC, Acrobat paplus SC); symoxanil (Korsate Pro WG, Korsate R DF), strobilurins (Eclair 49 WG, Quadris 25 SC) was established. With the lowest effectiveness to the agent of downy mildew is Timorex 66 EC. All studied products are suitable for including in the systems for control of this disease. A non-toxic product to E. formosa is the botanical fungicide Timorex 66 EC, medium toxic products are those containing a.i. strobilurin and propamocarb-hydrochloride. The remaining PPP are slightly or medium toxic to the bioagent. Therefore E. formosa could be applied parallel with slightly toxic fungicides against P. cubensis.*

Keywords: effectiveness, toxicity, index of damage, downy mildew, fungicides, Encarsia formosa

Introduction

Downy mildew on cucumbers caused by the oomycetes fungi *Pseudoperonospora cubensis* (Berkeley & M. A. Curtis) Rostovzev is economically the most important disease in this crop. It is spread all over the world (Holmes et al., 2004). The most radical method for control of this disease is development and growing of new varieties (Olczak-Woltman et al., 2011). Regardless of the introduction of good agricultural practices (GAP) and growing of resistant varieties the chemical control is still a very significant instrument for the management of the disease. During the last decades many fungicides with active ingredient from different chemical groups and combinations from them have been developed. The fungus causing this disease is strongly variable and it gains easily the resistance to the disease and therefore the results from this method are not sufficient (Urban and Lebeda, 2006). Therefore in conventional systems for control it is necessary to include chemical products for plant protection (PPP) and to alternate them. A great number of studies of new PPP have been performed in the world for establishment of their effectiveness and possibilities for gaining resistance to them by the downy mildew agent.

Phenyl amides are fungicides that ensure good control of this disease but their effectiveness was strongly decreased during the past years as a result of appearance of *P. cubensis* populations resistant to them (Reuveni et al., 1980; Shi et al., 2002; Urban and Lebeda, 2006). Great part of amides of carboxylic acids like dimethomorph, flumorph, benthiavalicarb, benthiavalicarb-izopropil, iprovalicarb and mandipropamid are developed for control of mildews (Albert et al., 1988; Stübler et al., 1999; Liu et al., 2000; Reuveni, 2003; Miyake et al., 2005; Lamberth et al., 2006; Zhu et al., 2007). They show strong protective and curative action. The large-scale use resulted in decrease of their effectiveness that is due to the acquired resistance to them. It is considered that for the downy mildew in cucumbers there is a great risk of acquisition of resistance. In order to restrict this process Zhu et al. (2007) recommend application of flumorph in mixtures with fungicides for prevention such as mancozeb or alternation with other fungicides with a different mechanism of action. According to authors it is recommended to perform maximum three treatments with flumorph based fungicides during vegetation of cucumbers. Amoucha and Cohen (1988) established that mixtures of cymoxanil and other active ingredients are suitable for control of this pathogen. In evaluation of the resistance of 63 isolates from *P. cubensis* towards PPP it was established that metalaxyl, metalaxyl-M, cymoxanyl and dimethomorph are ineffective while propamocarb and fozetyl-Al are effective in the registered concentrations (Hübschová and Lebeda, 2010).

The parasite *Encarsia formosa* Gah. (*Hymenoptera: Aphelinidae*) is one of the known and frequently used bioagents in control of greenhouse whitefly (*Trialeurodes vaporariorum* Westw.) in cultivation facilities (De Vis and Lenteren, 2008). A great part of the PPP has a negative effect on that useful species. The successful combination of chemical products and biological method in the integrated plant protection systems requires knowledge on their toxicity towards the useful species (Zchori-Fein et al., 1994; Oomen et al., 1994; Sterk at al., 2002). The production and implementation of new PPP in vegetable production requires studying of their side-effect to bioagents with a view to maximal protection of them.

The purpose of the investigation was to study newly synthesized and new formed PPP for effectiveness to the agent of downy mildew *Pseudoperonospora cubensis* (Berkeley & M. A. Curtis) Rostovzev and their toxicity to the imago of *Encarsia formosa* Gah.

Material and methods

The study was carried out in the "Maritsa" Vegetable Crops Research Institute, Plovdiv in 2006–2011. Eighteen plant protection products were tested for effectiveness in control of mildew in

* e-mail: smasheva@abv.bg

cucumbers (Table 1). The study was performed in unheated cultivation facilities with cucumber variety Vihra. Three treatments were conducted in an interval of 7–10 days.

Recorded indices:

- Degree of infestation by downy mildew before each treatment and 5 days after the last treatment by a five rating scale (0-4; Mitani et al., 2003): 0 – without spot caused by the disease, 1 – attacked 1–5% of the leaf surface; 2 – attacked 6-25% of the leaf surface; 3 – attacked 26-49 % of the leaf surface; 4 – attacked over 50% of the leaf surface;
- The index of infestation (%) was calculated by McKinney;
- Effectiveness (%) of PPP by Abbott.

Pot experiments for establishment of toxicity effect of the studied PPP to the imago of the endoparasite *Encarsia formosa* Gah. were conducted in laboratory conditions. Specimens that have been newly flied away 24 hours were used. The contact of the parasite adults with PPP is indirect as the tomato leaves are treated by the method of dipping. The mortality of the specimen was recorded after 24 hours. The toxicity was calculated by the Abbott formula. The classification of PPP in 5 groups was made on the basis of adopted parameters by the Working Group to Eastern-Palearctic section of International organization for biological control (Hassan et al., 1983): 1) Non-toxic, toxicity up to 20% (-); 2) Slightly toxic, toxicity up to 37% (+); 3) Medium toxic, toxicity up to 63% (++); 4) Toxic, toxicity up to 80% (+++); 5) Strong toxic, toxicity over 80% (++++). Each variant was recorded in three replications with 10 plants in each replication.

Data were processed by a two-way analysis of variance. Duncan's multiple range test and Cluster analysis by a programme product SPSS 12 for Windows were applied.

Table 1. Studied plant protection products

Commercial product	Active ingredient	Applied dose/concentration
Acrobat MC	Dimethomorph 90 g/kg + mancozeb 600 g/kg	0.2%
Acrobat plus WG	Dimethomorph 90 g/kg + mancozeb 600 g/kg	0.15%
Acrobat paplus SC	Dimethomorph 225 g/l +initium 300 g/l	0.07%
Aliette flash	Aluminium fozetyl 800 g/kg	0.3%
Eclair 49 WG	Trifloxistrobin 25 g/kg + cymoxanil 24 g/kg	0.05%
Zampro SC	Dimethomorph 225 g/l + initium 300 g/l	0.07%
Equation Pro	Famoxadone 225 g/kg + cymoxanil 300 g/kg	0.04%
Infinito SC	Propamocarb-hydrochlorlide 625 g/l +fluopicolide 62,5 g/l	120 ml/da
Korsate Pro WG	Cymoxanil 60 g/kg + copper hydroxide 436 g/l	0.2%
Korsate R DF	Cymoxanil 4,2% +copper oxychloride 39,75%	0.25%
Quadris 25 SC	Azoxystrobin 250 g/l	0.075%
Pergado C 440 SC	Mandipropamid 40 g/l + chlorotalonil 400 g/l	0.25%
Pergado Cu 27 WG	Mandipropamid 25 g/kg + copper 139.5 g/kg	0.5%
Previcur Energy	Propamocarb-hydrochloride 530 g/l+aluminium fozetyl 310 g/l	0.2%
Rival 607 SL	Propamocarb-hydroxychloride 722 g/l	0.25%
Ridomil gold MZ 68 WG	Mefenoxam 4% + mancozeb 64%	0.25%
Timorex 66 EK	Oil from Melaleuca alternifolia 66%	1.0%
Flint multi	Trifloxystrobin 63 g/l + tolylfluanil 625 g/l	0.14%

Results and discussion

A two-way analysis of variance was performed for establishment of the effect of the studied products on downy mildew development in cucumbers for the corresponding schemes of treatment (Table 2). The results from the analysis demonstrate the proven effect of the factors Product (A), Year (B) and the interaction between them (AxB). The power of effect of the first factor is the most considerable that explains 71% of the total variation of the results. The interaction of the Product and the Year (AxB) have the same great effect – 24,51%, and the factor Year (B) has the lowest effect – 1,74%. These results clearly show that the applied product has determining effect in control of mildew in cucumbers. The second significant conclusion is that the effectiveness of the products varies depending on the year. In some years a certain product could be with better effectiveness compared to another but in next years this effectiveness can be slighter.

The results show that the analysis of the data should be made as a comparison of products in the separate years. In order to make more detailed analysis of the results the products were classified in groups depending on the active ingredient (Table 3). The products with a. i. dimethomorph have good effectiveness to mildew. In the two years of study the product Zampro SC is described with the highest effectiveness (91,45% and 87,20%). The product Acrobat paplus SC is proven with lower effectiveness (87,51% and 85,63%). The difference in the effectiveness of Acrobat MC and Acrobat plus WG is not proven as these products belong to one group of significance. High effectiveness of dimethomorph to *P. cubensis* has been established by Cohen et al. (1995) in a comparative study of this product with metalaxyl.

The second group of products containing cymoxanil also demonstrates good effectiveness towards mildew in cucumbers.

Table 2. A two-way analysis of variance and effect of the factors on the attack of downy mildew in cucumbers.

Source of Variation	SS	df	MS	F	Power of influence η%	F crit
Product (A)	2764.573	18	153.5874	108.97	71.00***	1.741189
Year (B)	67.88352	1	67.88352	48.16324	1.74***	3.96676
Interaction (AxB)	954.2291	18	53.01273	37.61244	24.51***	1.741189
Within	107.1179	76	1.409446			
Total	3893.804	113				

Table 3. Index of infestation and effectiveness of PPP against downy mildew in cucumber Pseudoperonospora cubensis (Berkeley & M. A. Curtis) Rostovzev

PPP	I-st year		II-nd year		Average	
	I (%)	E (%)	I (%)	E (%)	I (%)	E (%)
Dimethomorph						
1. Acrobat MC	8.87 ^{a}	83.52 ^{c}	10.05 ^{ab}	84.19 ^{ab}	9.46 ^{a}	83.85 ^{c}
2. Acrobat plus WG	8.25 ^{ab}	84.56 ^{bc}	11.25 ^{a}	82.33 ^{b}	9.75 ^{a}	83.45 ^{c}
3. Acrobat paplus SC	6.71 ^{b}	87.51 ^{b}	9.80 ^{ab}	85.63 ^{ab}	8.26 ^{b}	86.58 ^{b}
4. Zampro SC	4.54 ^{c}	91.45 ^{a}	8.15 ^{b}	87.20 ^{a}	6.34 ^{c}	89.31 ^{a}
Cymoxanil						
5. Equation Pro	10.00 ^{a}	81.72 ^{b}	15.60 ^{a}	77.32 ^{b}	12.80 ^{a}	79.52 ^{b}
6. Korsate R DF	6.34 ^{b}	88.36 ^{a}	8.04 ^{b}	88.30 ^{a}	7.19 ^{b}	88.35 ^{a}
7. Korsate Pro WG	5.38 ^{b}	90.12 ^{a}	8.72 ^{b}	87.34 ^{a}	7.05 ^{b}	88.73 ^{a}
Propamocarb-hyrdochloride						
8. Infinito SC	10.00 ^{b}	82.10 ^{a}	12.60 ^{a}	77.68 ^{a}	11.30 ^{b}	79.89 ^{a}
9. Previcur Energy	9.09 ^{b}	83.27 ^{a}	11.20 ^{a}	80.04 ^{a}	10.15 ^{b}	81.66 ^{a}
10. Rival 607 SL	20.12 ^{a}	63.00 ^{b}	13.83 ^{a}	75.51 ^{a}	16.97 ^{a}	69.26 ^{b}
Strobilurins						
11. Eklair 49 WG	10.80 ^{b}	86.98 ^{a}	9.37 ^{b}	85.27 ^{a}	10.05 ^{c}	86.13 ^{a}
12. Flint Multi	17.78 ^{a}	78.52 ^{b}	13.13 ^{a}	79.35 ^{b}	15.46 ^{a}	78.94 ^{c}
13. Quadris 25 SC	12.50 ^{b}	85.35 ^{a}	12.33 ^{a}	80.66 ^{b}	12.42 ^{b}	83.78 ^{b}
Other PPP						
14. Aliette Flash	15.00 ^{cd}	78.58 ^{ab}	10.92 ^{c}	77.50 ^{b}	12.96 ^{d}	78.35 ^{bc}
15. Ridomil gold MZ 68 WG	13.13 ^{d}	81.17 ^{a}	8.33 ^{d}	82.83 ^{a}	10.73 ^{e}	82.00 ^{ab}
16. Timorex 66 EC 0.5%	35.33 ^{a}	49.43 ^{d}	17.08 ^{a}	64.78 ^{c}	26.21 ^{a}	57.10 ^{e}
17. Timorex 66 EC 1.0%	27.75 ^{b}	60.37 ^{c}	15.57 ^{ab}	67.93 ^{c}	21.66 ^{b}	64.15 ^{d}
18. Pergado C 440 SC	17.40 ^{c}	75.31 ^{b}	14.90 ^{b}	78.81 ^{b}	16.15 ^{c}	77.06 ^{c}
19. Pergado Cu 27 WG	13.00 ^{d}	81.58 ^{a}	11.78 ^{c}	83.28 ^{a}	12.39 ^{de}	82.43 ^{a}

^{a,b,c} – Duncan's multiple range test ($p < 0.05$)

The products Korsate R DF and Korsate Pro WG are effective towards the pathogen in the same degree – 88,35% and 88,73% on average for the two years. The third product from the group Equation Pro is with lower activity – 79,52%. The difference in the effectiveness is probably due to the second active ingredients that are part of the content of these products. In Korsate R DF and Korsate Pro WG this ingredient is copper hydroxide and in Equation Pro – famoxadone. For the conditions and the pathogen population in the Chech Republic, Hübschová and Lebeda (2010) have established low effectiveness of dimethomorph and cymoxanil.

Among the PPP containing propamocarb-hydrochloride the most effective are Previcur Energy and Infinito SC containing aluminum fozetyl and fluopikolide as a second active ingredient while the slightest activity is recorded in Rival 607 SL containing propamokarb-hydrochloride only. These results do not confirm the good effectiveness of propamocarb established by Hübschová and Lebeda (2010) against the agent of downy mildew in cucumbers.

The forth group of PPP that have demonstrated high effectiveness towards the downy mildew are with active ingredients strobiluruns – Éclair 49 WG, Quadris 25 SC and Flint multi. In the first

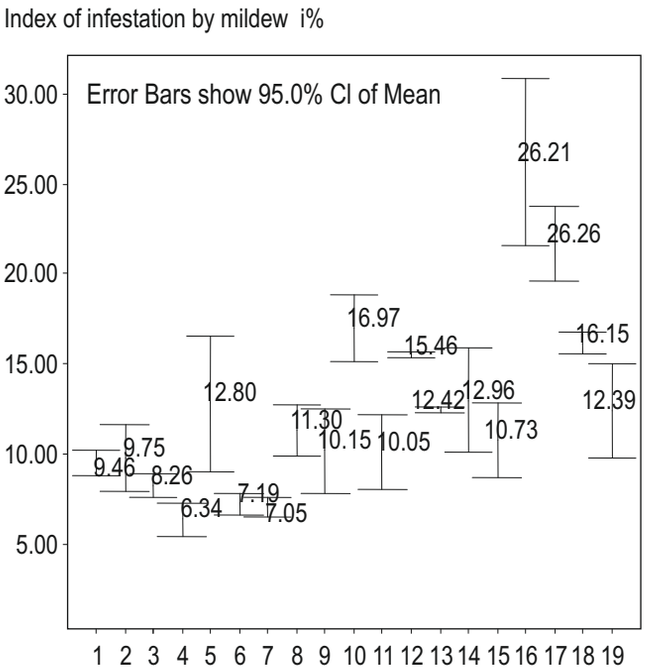

Figure1. Index of infestation by downy mildew (%) in cucumbers average for the period and confidential intervals in level of significance 95%.
*(the names of the products from 1 to 19 are given in Table 3)

year the strongest effect on the mildew attack have exerted the products Eclair 49 WG (86,98%) and Quadris 25 SC (85,35%), but in the second year the product Eclair 49 WG is with proven higher effectiveness (85,27%). These results confirm those established by the two-way analysis of variance that in some cases the year has significant effect on the effectiveness of the products. The third product from the group is more slightly effective that probably is due to the presence of tolilfluanil as a second active substance. Average for the period the products are classified in three groups of significance Éclair 49 WG (86,13%), Quadris 25 SC (83,78%) and Flint multi (78,94%). The recorded results confirm the high effectiveness of azoxistrobin towards the downy mildew and powdery mildew in cucumbers established by Anand et al. (2008).

The combined group of PPP is characterized with higher variability concerning effectiveness. This is logical because of the ingredients used in their composition. The highest effectiveness is proven for the products Pergado Cu 27 WG and Ridomil gold MZ 68 WG, for the two periods of reading. The products Pergado C 440 SC and Aliette Fflash form the second group of significance with lower effectiveness. The products Timorex 66 EC 0,5% and Timorex 66 EC 1,0% have the slightest effect on downy mildew in cucumber.

The comparative evaluation of particular PPP shows that the lowest index of infestation by mildew – up to 10% have the plants treated with the products: Acrobat MC (i=9,46%); Acrobat plus WG (i=9,75%); Acrobat paplus SC (i=8,26%); Zampro SC (i=6,34%); Korsate R DF (i=7,19%); Korsate Pro WG (i=7,05%). The highest index of infestation was recorded in Timorex 66 EC 0,5% (i=26,21%) and Timorex 66 EC 1,0% (i=31,66%) (Figure 1). A greater part of the studied products has strong effect on the development of mildew in cucumbers as the index of infestation is within 10–15%.

Table 4. Toxicity of PPP to the imago of Encarsia formosa Gah.

Active ingredient	Product	Concentration, %	Toxicity	
			%	Group
Dimethomorph 90 g/kg + mankozeb 600 g/kg	Acrobat MC	0.2	30.00	+
Dimethomorph 90 g/kg + mankozeb 600 g/kg	Acrobat plus WG	0.15	35.00	+
Dimethomorph 225 g/l + initium 300 g/l	Acrobat paplus SC	0.07	33.33	+
Alluminium fozetyl 800 g/kg	Aliette Flash	0.3	35.00	+
Trifloxistrobin 25 g/kg + cymoxanil 24 g/kg	Eclair 49 WG	0.05	45.00	++
Dimethomorph 225 g/l + initium 300 g/l	Zampro SC	0.07	33.33	+
Famoxadone 225 g/kg + cymoxanil 300 g/kg	Equation Pro	0.04	36.00	+
Propamocarb-hydrochlorlide 625 g/l +fluopicolide 62,5 g/l	Infinito SC	0.12	60.00	++
Cymoxanil 60 g/kg + copper hydroxide 436 g/l	Korsate Pro WG	0.2	23.81	+
Cymoxanil 4,2% +copper oxychloride 39,75%	Korsate R DF	0.25	31.06	+
Azoxystrobin 250 g/l	Quadris 25 SC	0.075	62.25	++
Mandipropamid 40 g/l + chlorotalonil 400 g/l	Pergado C 440 SC	0.25	30.00	+
Mandipropamid 25 g/kg + copper 139.5 g/kg	Pergado Cu 27 WG	0.5	23.33	+
Propamocarb-hydrochloride 530 g/l+aluminium fozetyl 310 g/l	Previcur Energy	0.2	40.95	++
Propamocarb-hydroxychloride 722 g/l	Rival 607 SL	0.25	30.00	+
Mefenoxam 4% + mancozeb 64%	Ridomil gold MZ 68 WG	0.25	21.00	+
Oil from Melaleuca alternifolia 66%	Timorex 66 EC	1.0	16.00	-
Trifloxystrobin 63 g/l + tolylfluanil 625 g/l	Flint multi	0.14	40.00	++

Note: Non toxic, toxicity up to 20% (-); slightly toxic, toxicity up to 37% (+); Medium toxic, toxicity up to 63% (++); Toxic, toxicity up to 80% (+++); Strong toxic, toxicity over 80% (++++).

The second investigation concerning the toxicity of the studied fungicides to the imago of *Encarsia formosa* Gah., shows that they are classified in three basic groups (Table 4). The most friendly is the action of the product Timorex 66 EC, the toxicity of which is 16%. It belongs to the group of non-toxic products. The second group is the greatest, slightly toxic, including the products Acrobat MC, Acrobat plus WG, Acrobat paplus SC, Aliette flash, Zampro SC, Equation Pro, Korsate Pro WG, Korsate R DF, Pergado C 440 SC, Pergado Cu 27 WG, Rival 607 SL and Ridomil gold MZ 68 WG. Their effect of toxicity varies from 23% to 35%. The last group is represented by Eclair 49 WG, Infinito SC, Quadris 25 SC, Previkur Energy and Flint multi with toxicity exceeding 40%. They are medium toxic.

The results from the experiments demonstrate that a greater part of the studied fungicides is slightly toxic towards the imago of the parasite. This gives a possibility for *E. formosa* to be used in parallel with its application against *P. cubensis* . In order to determine such similarity in the action of the products to the downy mildew and their toxicity to the imago of *E. formosa* we performed cluster analysis (Figure 2). The results demonstrate that regarding the two indices three basic groups of similarity are formed. The first group is the greatest one and it includes almost all products. It is described with good action against the downy mildew and average toxicity to the imago of *E. formosa*. The second group combines Infinito SC and Quadris 25 SC that are with considerable effect in their action against downy mildew but the toxicity is the highest. In the last group is the phytofungicide Timorex 66 EC which is with the lowest effect on downy mildew as well as with the lowest toxic effect towards the bioagent *E. formosa*.

The results of the performed experiment clearly show that in control of the most dangerous pathogens both in cucumber and all cucurbita plants the approach should be selective. We recommend in downy mildew control to used the products from the first group and in each treatment to apply products with different active ingredient. This will contribute to decrease of the risk for appearance of new races of the pathogen that are resistant to the defined group of PPP.

Figure 2. Hierarchical cluster analysis on the basis of degree of infection and toxicity of the PPP. Dendrogram using Average Linkage (Between Groups)

Conclusion

All studied chemical PPP are effective against downy mildew in cucumbers *Pseudoperonospora cubensis* (Berkeley & M. A. Curtis) Rostovzev and could be included in the systems for control of this disease.

The highest effectiveness was established for PPP Zampro SC (89.31%), Korsate Pro WG (88.73%), Korsate R DF (88.35%) and Acrobat paplus SC (86.58%). Non-toxic to the imago of the parasite *Encarsia formosa* Gah is the botanical product Timorex 66 EC.

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The manuscript should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods,Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

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Thesis:

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