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PRELIMINARY INVESTIGATION ON THE EFFECTS OF BIOLOGICAL AND SYNTHETIC INSECTICIDES ON LARGE WHITE BUTTERFLY (*PIERIS BRASSICAE* L.) LARVAE

ABSTRACT: Control of cabbage pests is oriented towards the use of efficient but high-risk insecticides, some of them being endocrine disruptors. Biopesticides are more environment-friendly, operator- and consumers-safe, but they have low initial toxicity, low efficacy to advanced larval stages, and they require certain knowledge of pest and host biology. In our laboratory experiments we have investigated the effects of formulated synthetic pyrethroid cypermethrin (0.3 l/ha) and biological products — formulations based on *Bacillus thuringiensis* subsp. *kurstaki* (2 and 3/ha) and Spinosad (0.1 l/ha) — on large white butterfly (*Pieris brassicae* L.) larvae-instars 2, 3, 4 and 5. The effect of insecticides was inversely proportional to larval instars. Btk effect could be improved if tank-mixed with cypermethrin. The mixing of ready-made products allows a reduction 3 and 6 times compared with the recommended dose, still obtaining satisfactory results. Rate of leaf damage was reduced when tank mixtures were used. Use of two products in mixture would be of significance especially for control of advanced late instars late in season, when Btk action alone is insufficient. Spinosad was effective in inducing mortality and reducing leaf damage by all larval instars, therefore we assume that the dose could be reduced. Feeding rate and mortality are equally important parameters when assessing biopesticide efficacy. This strategy should also reduce the possibility of inducing resistance in pest population. It also tends to reduce the residues in commodities and is good solution in production of hygienic and health safe food.

KEY WORDS: *Pieris brassicae* L., biopesticides, *Bacillus thuringiensis* subsp. *kurstaki*, spinosad, cypermethrin, tank mix

INTRODUCTION

Cruciferae pests are a limiting factor in cabbage production. The monitoring of cabbage fields at Rimski Šančevi in 2005 showed high densities of large white butterfly (*Pieris brassicae* L.) populations, low densities of small white butterfly (*P. rapae* L.) and diamond cabbage moth (*Plutella xylostella* L.), while cotton bollworm (*Helicoverpa armigera* Hbn.) and *Mamestra* and other noctuids were not registered. This situation was opposite to those registered in 2003 and 2004.

Bioinsecticides are distinguished for their favorable ecotoxicological traits and low initial toxicity. In organic insect management, formulations based on *Bacillus thuringiensis* and spinosad are officially permitted in Europe and USA (A n o n y m o u s, 2004 a; and 2004 b). Synthetic pyrethroids have high initial toxicity, but are less attractive from ecological point. Some pyrethroids have recently been suspected of being endocrine disruptors — ED.

The efficacy of biopesticides depends considerably on application timing. Aim of this investigation were (1) to assess the effect of insecticides from three different classes on mortality and leaf consumption rates of different caterpillar instars; (2) to determine which parameter is more important — mortality or leaf damage — when assessing the effectiveness of the three classes of insecticides.

MATERIAL AND METHODS

Pieris brassicae caterpillars were collected in a cabbage field at Rimski Šančevi. Laboratory experiments were conducted separately for larval instars 2 to 5. D-Stop, a *B. thuringiensis* var. *kurstaki* (Btk) — based insecticide, was applied at doses of 2 and 3 l/ha. Cipkord 20-EC, a cypermethrin formulation representing pyrethroids, was applied at the dose of 0.3 l/ha. The biological insecticide Laser KS (spinosad), formulated as SC with 24% of a.i. (spinosyns A and D), was used at the dose corresponding to 0.1 l/ha. Tank mixtures with reduced quantity of both formulations — D-Stop + Cipkord 20-EC — were tested at the rates 2+0.1, respectively, and 1+0.05 l/ha, respectively. Experimental solutions were based on water consumption of 400 l/ha.

Because of a heavy waxy cuticle of cabbage leaves, Sillwet L-77, a surfactant containing organosilicone trisiloxane, was added to each insecticide at the dose of 0.3 l/ha. Cabbage leaves were immersed in spray liquids and offered to larvae. The experiment was set up in four replications. Different numbers of caterpillars (12, 20, 15 and 5) depending on the instar (2, 3, 4 and 5, respectively) were used. During the experiment, temperature varied from 22 to 24°C, day/night light period was 16/8 h and relative air humidity was 80—85%. The larval mortality was determined after 24 and 48h, and leaf consumption after 48h.

RESULTS AND DISCUSSION

The results of mortality bioassay are presented in Figures 1 and 2, those of feeding bioassay in Figure 3.

Understanding caterpillars' field biology may provide some insight regarding the use of active ingredients in a cost-effective manner. The speed at which a particular active substance causes mortality through contact or inge-

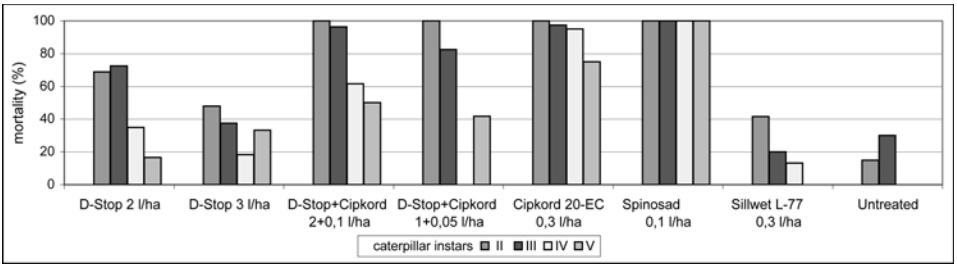

Fig. 1. — *Pieris brassicae* caterpillar mortality (%) after 24 h exposition

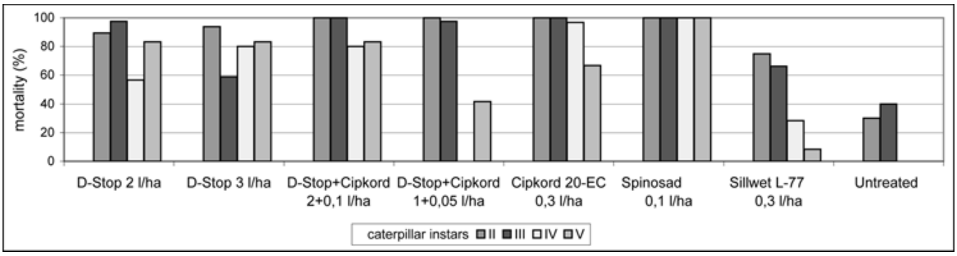

Fig. 2. — *Pieris brassicae* caterpillar mortality (%) after 48 h exposition

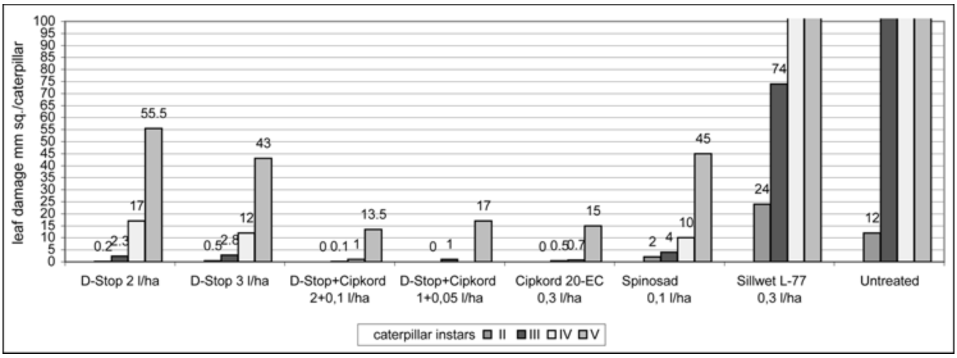

Fig. 3. — *Pieris brassicae* caterpillar cabbage leaf consumption [mm sq./caterpillar/48 h]

stion can be influenced by its mode of action and route of activity. The primary routes of activity differ among the products used in this study. Organophosphate (OP), carbamate and pyrethroids are primarily effective against 2nd larval instar due to numerous chemical and biological factors.

The mortality of caterpillars feeding on leaves treated with cypermethrin, *Btk.* and their mixtures depended on larval instar. Mortality in the experimental units with D-Stop exceeded 80% after 48h in contrast to cypermethrin, which exceeded 90% already after 24 h for the larvae in instars 2nd and 4th. In 5th instar, however, the mortality achieved with the two formulations hardly exceeded 60%. Even the reduced field recommended dose of cypermethrin is capable of providing satisfactory mortality when it was tank mixed with

D-Stop. As cypermethrin is an increased risk insecticide, suspected of being an ED, it is important that its dose in a mixture may be reduced without reducing its efficacy. In mixture with *Btk*, it was possible to reduce cypermethrin dose 3 and 6 times (100% mortality in 2nd instar and 80% mortality in 3rd instar, respectively). The effects on caterpillars in 4th and 5th instars varied from 60 to 80% and from 40 to 80%, respectively. Therefore, repeated treatments will be required for 4t^{h} and 5th instars for better control. Leaf damage (Figure 3) was reduced in all experimental units after application of tank mixed products (0—55.5 mm^2/caterpillar) compared with the untreated leaves (12—3100 mm^2/caterpillar). When used alone, Sillwet L-77 reduced the leaf consumption of instars 2, 3 and 5.

Opposite to the quick knock down effects of organophosphate and pyrethroids, *Bt* product first has to be ingested. This results in low initial toxicity. Because *Bt* is UV light and heat sensitive some growers find spraying at night will give the longest period of efficacy and best control and of cutworms (A n o n y m o u s, 2001).

When using *Bt*, one cannot expect fast, 100% control and must accept a certain level of crop damage. For enhancing the efficacy of *Bt* to lepidopterous larvae of older instars, some authors suggest adding pyrethroids. In our field experiments (I n đ i ć et al., 2005) we proved that *Btk*. could be equally effective in larvae control as synthetic pyrethroids if used in two narrow-spaced application. The efficacy of tank mixture of this biopesticide and cypermethrin at reduced rate was lower, but still at the same significance level as when cypermethrin is used alone.

Products containing endotoxin *Btk*. should be used as alternative to chemical insecticides. This would reduce the danger of resistance appearance (R o u s h and T i n g e y, 1994). In our country, resistance towards this type of insecticides has not been registered. Their current use in agricultural production is small; therefore, we do not expect the appearance of resistance under field conditions soon. However, there are already numerous data in scientific literature on *H. armigera* sensitivity changes (A r m e s et al., 1994), as well as *Plutella xylotella* resistance to *Btk* since 1990 (A n o n y m o u s, 1999; J o i a and C h a w l a, 1995).

Btk products are rather safe for operators and consumers. In our country, the *Btk* post-harvest interval (PHI) for cabbage for pickling is restricted to 21 days (M i t i ć, 2004). A similar period has been set in Croatia (M a c e l j s k i, 2002). In our country, the re-entry interval has not been determined. In Russia, PHI for cabbage is 5 days (A n o n i m u s, 2004), in Hungary one day (O c s k ó, 2006). In spite of environmental compatibility, *Btk* bioinsecticides are rarely used in cabbage production. K a n d i b i n (cit. G l e z et al., 2002) explains the low use of biopesticides in general with scant information of experts and agriculture producers about ecological advantages, poor marketing strategy and low production volume — insufficient to meet the needs of cabbage growers.

V o l o v i k and G l e z (cit. G l e z and Č e r k a š i n, 2002) proved the possibility of tank mixing the biological product *Bt* with synthetic pesticides. They controlled a high-density population of Colorado potato beetle with tank mixtures of *Bt* and Fastac EC (alpha-cypermethrin) or *Bt* and esfenvalerate (97% mortality). *Pieris rapae* was more efficiently controlled when *Btk* was used tank mixed with lambda-cyhalothrin (I n đ i ć et al., 2003). Biopesticide use in tank mix is restricted, due to the sensitivity of living organisms comprising *Bt* to active substances or solvent content in partner product. *Btk* is compatible with a number of insecticides, acaricides, fungicides and growth regulators. *Bt* + pyrethrin are formulated as ready mix (T o m l i n, 1997). *Bt* products should not be mixed with alkali products, captan, folpet, oils (M i t i ć, 2004), or with copper-based products. Formulated product is not compatible with azinfos-ethyl, captafol, demeton-S-methyl, dimethoat, dinocap, isoprocarb, phenthoate, phosalon, propoxur, tetrachlorvinphos, alkali compounds (Bordeaux mixture), or under certain conditions with foliar fertilizers (T o m l i n, 2000).

At the dose used, Spinosad induced high mortality of all larval instars already after 24 hrs and it probably could be used at a lower rate for the control of this pest. To enhance the efficacy on 5th instar, some authors suggest adding pyrethroids to Spinosad for late-season control. Adding cypermethrin to biological products is justified when older instars predominate in a treated population.

Spinosad acts as a neurotoxicant and its route of activity is translaminar. Some authors have estimated that it takes 1—2 days to achieve > 90% mortality of small larvae (< 5 mm) and 2 days for large larvae (> 10 mm) (P a l u m b o, 1999). In our experiment, the initial toxicity and mortality achieved were high probably due to the high rates used.

Spinosad's mode of action is invariably toxic for *P. brassicae* at the rates applied. Routes of activity involve both ingestion and contact activity (A n o n y m o u s, 2004). Moving into and across the leaf tissue, toxin reservoirs are formed on and within the treated foliage. This exposes the larvae to much greater amounts of the active ingredient and their chances for intoxication become much higher. The rate of foliage consumption is greater for old, large larvae, which potentially consume larger amounts of the active ingredient.

For effective control, the optimum timing of Spinosad treatment should be directed primarily at the newly hatched larvae, although large larvae are easily controlled too. The 3rd instar larvae consume large amounts of foliage (A n o n y m o u s, 1999). Delay in control increases the rate of plant injury proportionally. As far as enhancement of efficacy is concerned, some authors have suggested adding pyrethroids to Spinosad for late-season control and to *Bt* for midseason- and late-season control.

Wetting agents, spreaders and stickers are typically toxic to biocontrol agents. In our experiments, there was no reduction in mortality or adverse effect on feeding on leaves as a consequence to D-Stop mixing with Sillwet L-77 (0.3 l/ha). This commercially formulated trisiloxane affected 2nd instar larvae, inducing mortality after 24h and 48h (40—70%) and 3rd instar larvae (65%).

Lepidoptera ability to develop resistance to organochlorine, organophosphate, carbamate and pyrethroid classes of insecticides is known (W o l f e n s b a r g e r cit. G o r e & A d a m c z y k, 2004). Therefore, different strategies are applied to postponing the appearance of resistance.

To sustain product efficacy it is of importance for management of lepidopterous larvae during the growing season to avoid an overuse of a single product, rotate chemistries through the season, and not to apply than at the rates below the labeled ones. This strategy will optimize the control of the lepidopterous larval complex and maximize the longevity of new formulations.

CONCLUSION

Leafy and cole vegetables are endangered by a number of harmful insects. Due to frequent insecticide treatments, risk of residues is always present. As raw vegetables are part of regular diet, it is recommended to use bioinsecticides with low risk for consumers and operators.

The mortality induced by *Btk* is inversely proportional to larval instar.

Btk effect could be improved if tank mixed with cypermethrine.

Cypermethrin, potential endocrine disruptor, could be used at reduced rates if tank mixed with *Btk* and still produce satisfactory results.

Spinosad, effective on all instars could be used at rates below 0.1 l/ha, which remains to be proved experimentally.

Feeding rate and mortality are equally important when assessing efficacy of biopesticides.

Btk could resolve the problem of cabbage protection in integrated and organic production.

Organosilicone wetter Sillwett L-77 could be used on cabbage, as it did not affect adversely the effect of biological products.

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ПРЕЛИМИНАРНА ИСПИТИВАЊА ЕФЕКТА БИОЛОШКИХ И СИНТЕТИЧКИХ ИНСЕКТИЦИДА НА ЛАРВЕ ВЕЛИКОГ КУПУСАРА (*PIERIS BRASSICAE* L.)

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Резиме

Сузбијање штеточина купуса оријентисано је на примену ефикасних али високо ризичних инсектицида од којих су неки и ендокрини дисруптори. Биопестициди су мање опасни за животну средину, безбеднији за оператера и конзументе, али ниже иницијалне токсичности и ниске ефикасности на гусенице у касним узрастима и захтевају знање о биологији штеточине и домаћина.

У нашим лабораторијским испитивањима упоредили смо ефекат синтетског пиретроида циперметрина (0,3 l/ha) и биолошких препарата на бази *Bacillus thu-*

ringiensis subsp. *kurstaki* (*Btk*) (2 и 3 l/ha) и препарата на бази спиносада (0,1 l/ha) на гусенице великог купусара *Pieris brassicae* L. у 2, 3, 4. и 5. узрасту. Ефекат препарата је био обрнуто пропорционалан узрасту гусеница. Ефекат *Btk* препарата може бити побољшан мешањем с препаратом на бази циперметрина у смањеној количини 3 и 6 пута у односу на препоручену, а да се притом постигну задовољавајући резултати. Мешање синтетског пиретроида и биолошког препарата је нарочито важно у касним узрасним развојним фазама када је дејство *Btk* недовољно. Биолошки препарат на бази спинпсада је био врло ефикасан за ларве свих узраста, те је претпоставка да се количина примене може и смањити. Примена мешавине интензивира обуставу исхране. Интензитет исхране и морталитет су једнако важни параметри у процени ефиканости биопестицида. Оваква примена треба да спречи рану појаву резистентности штеточина у популацији, да доведе до смањења резидуа у намирницама и добро је решење у производњи здравствено безбедне хране.