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DYNAMICS OF ACETAMIPRID DISAPPEARANCE IN OILSEED RAPE PLANT TISSUES IN CONNECTION WITH ITS TOXIC ACTION AGAINST POLLEN BEETLE (*Meligethes seneus* F.) AND ITS INFLUENCE ON ECOLOGICAL ASPECT OF OILSEED RAPE CHEMICAL PROTECTION

DYNAMIKA ZANIKANIA ACETAMIPRYDU W TKANKACH ROŚLIN RZEPAKU OZIMEGO ORAZ JEGO TOKSYCZNOŚĆ DLA SŁODYSZKA RZEPAKOWEGO (*Meligethes aeneus* F.). ASPEKT EKOLOGICZNY OMAWIANYCH ZJAWISK

Abstract: Acetamiprid is a neurotoxine belonging to the neonicotinoid group. This active substance has contact and stomach action and acts on the insect central nervous system as agonist of the acetylocholine in nicotinic acetylocholine receptors. Acetamiprid has translaminar and systemic proprieties in plants and nowadays is one of the most widely used insecticides against pollen beetle (*Meligethes aeneus* F.) in Poland as a component of a commercial product Mospilan 20 SP. Constant and strong selective pressure of insecticides resulted in resistance of pollen beetle (PB) to many active substances used in Poland. It also influences beneficial fauna especially pollinators. The aim of the study was to determine the effectiveness of acetamiprid against pollen beetle in the period of 15 days that is characterized by rapid elongation of oilseed rape plants. The dynamics of mortal action of acetamiprid against pollen beetle and disappearance of acetamiprid in plant tissues were determined. Short analysis of acetamiprid potential toxic action against pollinators, especially against honeybee (*Apis mellifera* L.), predators and parasitoids was made.

Keywords: acetamiprid, pollen beetle, oilseed rape, systemic mode of action, pollinators' protection

Pollen beetle (PB) is the most dangerous pest of oilseed rape in Poland and in Europe. The pest has developed resistance against most synthetic pyrethroid and organophosphorous insecticides [1–6]. Acetamiprid was introduced against pollen

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beetle in Poland in 1996 as a new active substance belonging to the neonicotinoid group [7], and has become a component of PB insecticide resistance management strategy. In last years a lot of signals on the decrease of acetamiprid effectiveness in PB control were reported from some regions of oilseed rape cultivations in Poland. Bioassays of acetamiprid for resistance monitoring have been performed in the Institute of Plant Protection in Poznań since 2004. The research demonstrated some level of resistance of PB to this active substance [4, 8, 9]. One of important factors influencing survival of insects is active substance concentration and penetration in plant and insect organism. The question: "what happens to acetamiprid molecules after their application in the oilseed plants", is worth of interest because of several aspects. The main one is beneficial fauna protection, that is, in case of oilseed rape, very important. It is also important to work out new effective strategies of pest control based on integrated resistance management programs to prevent PB resistance. And prevention of resistance phenomenon is closely connected with environment protection.

Material and methods

Chemicals and reagents

Acetamiprid – commercial product – Mospilan 20 SP – (200 g of acetamiprid/kg of product) was used. Recommended field concentration (120 ppm) of active substance was calculated assuming that the highest field dose in Poland is 0.12 kg of Mospilan 20 SP/ha/200 dm^3 of water .

Acetonitrile (gradient grade) was purchased from Labscan (Dublin, Ireland). Anhydrous magnesium sulphate (reagent grade), sodium citrate tribasic dihydrate (ACS reagent), and disodium hydrogen citrate sesquihydrate (pure) were all purchased from Sigma–Aldrich (Steinheim, Germany). Sodium chloride was purchased from POCH (Gliwice, Poland), Bondesil PSA bulk sorbent was purchased from Candela (Warsaw, Poland) and Supelclean ENVI-Carb from Supelco (Bellefonte, USA). Formic acid (ACS grade) was purchased from Merck (Darmstadt, Germany). Distilled deionized water was obtained using a Millipore Elix 3 system (Millipore, Billerica, USA). Nitrogen (purity 99.995 %) and argon (purity 99.9998 %) were supplied by Air Products Company, Poland. Certified pesticide standards of acetamiprid and simazine-D10 were purchased from Dr. Ehrenstorfer (Ausburg, Germany) and were of purity above 98.5 %.

Biological material

Pollen beetles from untreated oilseed rape field were collected for testing from the population of Winna Góra. Insects were placed into insulators made of airy material and filled with rape plants and transported to the laboratory. In the laboratory insects were kept for 24 hours at the temperature 10 ^oC (climatic chamber).

Oilseed plants were grown in pots of 25 cm diameter and 5 dm^3 volume in natural conditions. In the vegetative phase BBCH 45 (Bundesanstalt Budessortenamt und

Chemical Industry) oilseed plants were sprayed with demineralized water dilution of Mospilan 20 SP using hand sprayer (acetamiprid concentration was 120 ppm). Control plants were sprayed with demineralized water. Sprayed plants were collected 24, 120, 240 and 360 hours after treatement and used for PB mortality tests and for acetamiprid residue determination.

Laboratory tests

For determination of insect mortality 24, 120, 240 and 360 hours after treatment and lethal concentrations LC50 and LC95, a standard method recommended by Insecticide Resistance Action Committee (IRAC method no. 7) was used. The initial concentration of acetamiprid in demineralized water in laboratory tests was 240 ppm. The following 5 concentrations: 144, 86.4, 51.84, 31.1 and 18.66 ppm were used. Oilseed rape plants were dipped in each concentration of acetamiprid for about 5 seconds, then placed on a paper towel to dry for 3 hours in laboratory conditions (20–22 ^{o}C, photoperiod of 16:8 (L:D). Control plants were dipped in demineralized water. Dry treated plant material was placed into 0.9 dm^3 jars of 10 cm diameter filter paper lining their bottom. Length of plant material was of height of a jar (15 cm) and weight was 20 g. One hundred of PB beetles were placed in each jar. The jars were closed with airy material for ensuring a good evaporation and ventilation. Each dose was represented by three replications and a single control. A final assessment (lethal effect of acetamiprid) was determined after 24 hours and expressed as per cent mortality of insects at each dose in relation to untreated control.

For LC 50 and LC 95 calculations (expressed in ppm of acetamiprid) – computer program POLO PC was used. The program is based on Finney probit analysis method [10].

Sample preparation procedure

A portion of finely ground subsample was placed in a polypropylene centrifuge tube and water was added. Later, an internal standard solution (IS simazine-D10) and acetonitrile were added and the sample was extracted using Ultra-Turrax homogenizer. Further, disodium hydrogen citrate sesquehydrate, trisodium citrate dihydrate, anhydrous magnesium sulphate and sodium chloride were added, and the mixture was immediately hand-shaken, then centrifuged. Afterwards, aliquot portion of the supernatant was transferred to a polypropylene centrifuge tube containing of cleanup mixture (anhydrous magnesium sulphate, ENVI-Carb and PSA). The tube was vortexed and centrifuged. An aliquot portion of the supernatant was transferred into a glass test tube and the extract was evaporated to dryness under a stream of nitrogen and the residue was re-dissolved in mixture of 0.1 % formic acid in methanol and 0.1 % formic acid in water prior to its injection into the LC–MS/MS system.

Chromatographic conditions

An ACQUITY UPLC ultra-performance liquid chromatograph system (Waters, USA) with a column and autosampler thermostats equipped with a tandem quadrupole

mass spectrometer (Waters Quattro PremierXE) was operated using MassLynx software [11]. Waters ACQUITY UPLC column (BEH C18 2.1 × 100 mm, 1.7 μm) was used for final LC analysis. Temperature of the column and autosampler thermostats was 30 ^{o}C. 5 mm^3 samples were injected into the system. Then, the column was eluted with the mobile phase: water with 0.1 % formic acid (A) and methanol with 0.1 % formic acid (B) at the flow rate of 0.3 cm^3/min using gradient mode.

MS/MS conditions: Typical interface conditions for maximum intensity of the precursor ions were optimized as follows: nebulizer and desolvation (drying gas) N2 flows were set at 100 and 700 dm^3/h, respectively, source block and desolvation temperatures were 120 and 350 ^{o}C, respectively.

Argon was used as a collision gas at the pressure of 6.9 × 10.3 mbar. Selection and tuning of MRM transitions were performed individually for acetamiprid and simazine--D10. Both compounds were analyzed using a positive electrospray ionization mode (ESI+).

Acetamiprid MS/MS parameters: Cone: 35 V, Ion Transition 1: 223 > 126, Collision Energy 1: 20 eV, Ion Transition 2: 223 > 90, Collision Energy 2: 30 eV

Simazine-D10 MS/MS parameters: Cone: 35 V, Ion Transition: 212 > 137, Collision Energy: 20 eV

The second acetamiprid transition was used for qualitative determination of target compound. Retention time of acetamiprid was 1.68 min. The limit of quantification of acetamiprid was determined at 0.01 mg/kg.

Validation of the method

All validation procedures were performed using control samples of oilseed rape plant. Recoveries were determined for five replicate determinations at two spiking concentrations: 0.01 and 0.1 mg/kg. Precision was expressed as relative standard deviation (RSD) at each spiking level (5 samples spiked at each level). Linearity of the method for determination of acetamiprid was assessed by multilevel standard calibration curves (from 0.01 μg/cm^3 to 0.5 μg/cm^3, R^2 = 0.9948). The samples of oilseed rape plant spiked with acetamiprid were extracted applying the method described above. Analytical standard signal was compared with the signal of the control sample extract spiked with the target compound before the whole procedure. Average residues of acetamiprid were 90.16 ± 15.55 % at the level 0.01 mg/kg and 93.32 ± 11.73 % at the level 0.1 mg/kg.

Results and discussion

Results shown in Table 1 indicate quick decline of PB mortality with the increase of time after spraying oilseed rape plants with Mospilan 20 SP. Survival of 80 % of beetles 120 hours after treatment with the recommended concentration of acetamiprid indicates some level of resistance to acetamiprid of examined pest population.

Table 1

Mortality of pollen beetle after contact with oilseed rape plants treated with Mospilan 20 SP

Hours after treatment	Mortality [%]
24	92
120	20
240	15
360	5

Results presented in Table 2 show that one day after treatment mean LC50 concentration for the tested PB population was 29.6 ppm and mean LC95 value was 340.6 ppm.

Table 2

Lethal concentrations of acetamiprid for pollen beetle expressed as LC50 and LC95, in ppm

Lethal concentration	Date of testing			Mean
	25.04.2008	30.05.2008	17.06.2008	
LC50 (confidence intervals)	24 (9.32–38.04)	37 (21.8–50.4)	28 (17.2–38.9)	29.6
LC95	342	258	422	340.6

Results shown in Table 3 and Figure 1 confirm the conclusion that satisfactory effect of acetamiprid systemic action against pollen beetle is short and, in practical conditions, may be too weak to control resistant populations of this pest during the period longer than a few (2–3) days.

Table 3

Acetamiprid contents in oilseed plant tissues after treatment with Mospilan 20 SP, in ppm

Hours after treatment	Sample 1	Sample 2	Sample 3	Mean
	[ppm]			
24	44.26	41.60	39.81	41.89
120	4.46	4.28	4.31	4.35
240	1.77	1.91	1.89	1.86
360	0.49	0.55	0.59	0.54

Dependently on population density, in field conditions, the satisfactory effect of insecticide action should be on the level of 70–85 % mortality of beetles. The research show that the level of active substance concentration enabling the achievement of this level, is too low and unsatisfactory 2–3 days after oilseed rape plants treatment. So, systemic action of acetamiprid in oilseed rape plants seems to be too weak. It can be caused by rapid plant elongation. This phenomenon probably causes diluting of the active substance in plant tissues and decreases of acetamiprid content on the leaves' surface to the level, which is tolerated by PB. In Polish climatic conditions PB attack is

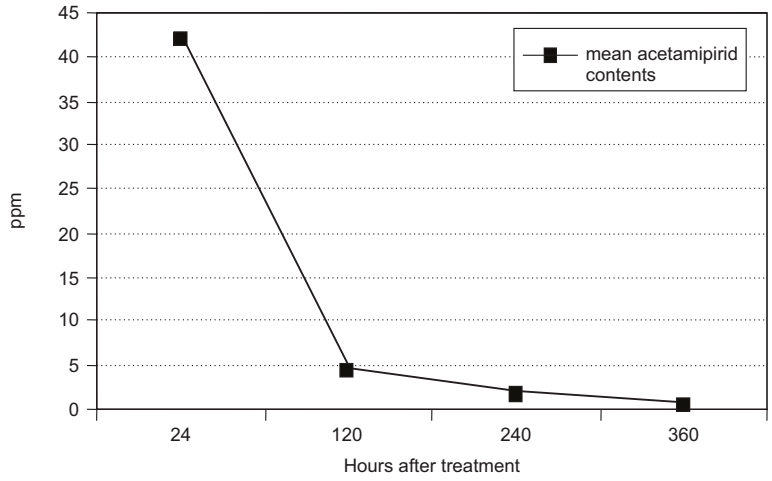

Fig. 1. Dynamics of acetamiprid disappearance in oilseed rape plant tissues treated with Mospilan 20 SP

often strong, constant and prolonged in time. Results indicate that during the elongation period of oilseed rape plants and strong PB attack the chemical treatment with acetamiprid should be repeated 3–4 days after the first one.

In relation to beneficial fauna (*Apis mellifera, Coccinella septempunctata, Poecilus cupreus, Typhlodromus pyri* and *Aphidius rhopalosihpi),* when comparing results of experiments presented above (Table 1–3, Fig. 1) with data from the European Commission – Acetamiprid [12, 13] it can be stated that recommended concentration of acetamiprid in Poland (120 ppm or 0.24 μg/cm^2) can cause high initial mortality of *Aphidius rhopalosiphi,* because even 90 ppm of acetamiprid results in 53.1 % mortality of this species. It can be also toxic for *Typhlodromus pyri,* because 90 ppm of acetamiprid results in 51.7 % mortality of this species. In relation to *Poecilus cupreus* even such a high concentration as 1000–2000 ppm results in 3.3 % mortality of this species. The concentration 450 ppm causes 100 % mortality of *Coccinella septempunctata* larvae. The dose of 0.24 μg/cm^2 recommended in Poland, in relation to honeybee *Apis mellifera,* for which acute oral toxicity LC50 is 8.85 μg/bee and acute contact toxicity LC50 9.26 μg/bee, seems to be moderately toxic up to 24 hours after treatment. The dynamics of acetamiprid disappearance in oilseed plants' tissues is quick and this active substance does not cause much risk for the pollinators and other beneficial fauna of oilseed rape fields.

Conclusions

1. Acetamiprid is relatively save for honeybee and some insect species of oilseed rape fields in concentration recommended in Poland.

2. Pollen beetle is a pest insect species of high natural resistance to many natural and synthetic active substances. Tested population from Winna Góra demonstrated some level of tolerance to acetamiprid.

3. Rapid oilseed plant elongation reduces the systemic action of acetamiprid.

4. A widespread use of Mospilan 20 SP can lead to PB resistance level increase and control failure in practical conditions.

5. Migration and prolonged attack of PB on oilseed rape plants in connection with its resistance requires repeated plant protection chemical treatment against this pest.

References

[1] Hansen L.M.: *Occurrence of insecticide resistant pollen beetles (Meligethes aeneus F) in Danish oilseed rape (Brassica napus L.) crops*. EPPO Bulletin, 2008, **38**(1), 95–98.

[2] Slater R. and Nauen R.: *The development and nature of pyrethroid resistance in the pollen beetle (Meligethes aeneus) in Europe.* Presentation Abstract, EPPO Workshop on insecticide resistance of *Meligethes* spp. (pollen beetle) on oilseed rape. Berlin 2007, 3–5.

[3] Heimbach U., Müller A. and Thieme T.: *First steps to analyse pyrethroid resistance of different oilseed rape pests in Germany: An extended abstract.* IOBC Bulletin, 2006, **29**(7), 131–134.

[4] Węgorek P.: *Preliminary data on resistance appearance of Pollen beetle PB (Meligethes aeneus F.) to selected pyrethroids, organophosphorous and chloronicotinyls insecticides, in 2004 year, in Poland*. Resist. Pest Manage. Newslett., 2005, **14**(2).

[5] Hansen L.M.: *Insecticide – resistant pollen beetles in Danish oilseed rape.* Pest Manage. Sci., 2003, **59**, 1057–1059.

[6] Mrówczyński M.: *Studium nad doskonaleniem ochrony rzepaku ozimego przed szkodnikami.* Rozprawy Naukowe Instytutu Ochrony Roślin, 2003, 61 pp.

[7] Yamamoto J. and Cassida J.E.: Nicotinoid Insecticides and Nicotinic Acetylcholine Receptor. Springer--Verlag, Tokyo 1999, pp. 300.

[8] Węgorek P., Stęplowska-Obrępalska A., Zamoyska J. and Nowaczyk K.: *Resistance of Pollen beetle (Meligethes aeneus F.) in Poland.* Resist. Pest Manage. Newslett., 2006, **16**(1), 28–32.

[9] Węgorek P. and Zamojska J.: *Resistance of Pollen beetle (Meligethes aeneus F.) to pyrethroids, chloronicotinyls and organophosphorous insecticides in Poland.* IOBC Bull., 2006, **29**(7), 135–140.

[10] Finney D.J.: Probit analysis (A statistical treatment of the sigmoid response curve). Cambridge Univ. Press, 1952, 236–245.

[11] Drożdżyński D., Folkman W. and Gnusowski B.: *Determination of Clothianidin Residues in Inflorescences of Common Horse Chestnut (Aesculus hippocastanum L.) by Ultra-Performance Liquid Chromatography with Tandem Mass Detection (UPLC-MS/MS).* Chem. Anal. (Warsaw), 2008, **53**, 379–389.

[12] European Commission. *Review report for the active substance acetamiprid.* SANCO/1392/2001 – Final, 2004, 34.

[13] Nippon Soda Co., LTD.: *Mospilan NI 25 common name: Acetamiprid systemic insecticide for foliar and soil application. Technical Information.* Edition 2, 3–23.

[14] Nauen R., Ebbinghaus-Kintscher U., Elbert A. and Jeschke P.: *Acetylocholine receptors as sites for developing neonicotinoid insecticides.* [In:] Biochemical sites of insecticide action and resistance. (I. Ishaaya ed.), Springer, Verlag, Berlin 2001, 77–106.

[15] Tomizawa M. and Cassida J.E.: *Neonicotinoid insecticide toxicology: Mechanisms of selective action.* Ann. Rev. Pharmacol. Toxicol., 2005, **45**, 247–268.

DYNAMIKA ZANIKANIA ACETAMIPRYDU W TKANKACH ROŚLIN RZEPAKU OZIMEGO ORAZ JEGO TOKSYCZNOŚĆ DLA SŁODYSZKA RZEPAKOWEGO (*Meligethes aeneus* F.). ASPEKT EKOLOGICZNY OMAWIANYCH ZJAWISK

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Abstrakt: Acetamipryd jest silną neurotoksyną należącą do chemicznej grupy neonikotynoidów wykorzystywaną szeroko w chemicznej ochronie roślin. Ta substancja aktywna działa na centralny system nerwowy owadów na drodze kontaktowej oraz żołądkowej. Jej molekularne działanie polega na agoni-

stycznym, w stosunku do acetylocholiny, działaniu na postsynaptyczne receptory tego neurotransmitera. Acetamipryd ma właściwości przenikania do tkanek roślin i oddziaływania systemowego. Obecnie w Polsce należy on do najpopularniejszych insektycydów w zwalczaniu słodyszka rzepakowego (*Meligethes aeneus* F.) oraz wielu innych szkodników jako komponent środka ochrony roślin – Mospilan 20 SP. Trwający już ponad 50 lat stały nacisk selekcyjny chemicznymi środkami spowodował u słodyszka rzepakowego w Polsce narastanie zjawiska odporności na wiele substancji aktywnych. Acetamipryd wpływa również na faunę pożyteczną, głównie na owady zapylające. Celem pracy było określenie dynamiki zanikania acetamiprydu w tkankach roślin rzepaku ozimego w okresie 15 dni od zabiegu chemicznego w fazach szybkiego wzrostu roślin oraz równoległe zbadanie jego toksyczności w stosunku do słodyszka rzepakowego, który jest w tym okresie głównym szkodnikiem atakującym rzepak. Na podstawie uzyskanych wyników oraz danych dotyczących acetamiprydu zamieszczonych w badaniach toksykologicznych tej substancji chemicznej przeprowadzono analizę zagrożenia działania tego insektycydu dla owadów zapylających, a szczególnie dla pszczoły miodnej (*Apis melliera* L.), drapieżców i parazytoidów.

Słowa kluczowe: acetamipryd, słodyszek rzepakowy, rzepak ozimy, systemowy mechanizm działania, ochrona zapylaczy