Volume 24(3), 53 - 57, 2020
JOURNAL of Horticulture, Forestry and Biotechnology
www.journal-hfb.usab-tm.ro

Testing insecticides efficacy on pollen beetles (*Meligethes aeneus* F.)

CSORBA Artúr-Botond[1,2]*, PUTNOKY-CSICSÓ Barna[2,3], NYÁRÁDI Imre-István[2], BÁLINT János[2]*

[1] Doctoral School in Biology and Sport Biology, University of Pécs, Faculty of Sciences, Ifjúság útja 6.
[2] Department of Horticulture, Faculty of Technical and Human Science, Sapientia Hungarian University of Transylvania, Sighisoara Str. 1C. Tirgu-Mures, Romania.
[3]Doctoral School in Plant Science, Szent István University of Gödöllő, Faculty of Agricultural and Environmental Science, Páter Károly 1.

* Corresponding authors email: csorbaartur@yahoo.com, balintjanos@ms.sapientia.ro

Abstract The total cultivation area of the oilseed rape has been considered as 25 million hectares worldwide, from which 20% belongs to Europe. The productivity is severely damaged by several pathogens and pests species causing high economic losses. During the present study, one of the most important rape pest, the pollen beetle (*Meligethes aeneus* F.) was examined. The beetle's sensibility was tested under lab conditions using different insecticides, and mortality rate were assessed. The resistance to pyrethroids, neonicotinoids and organophospates were specifically investigated; these being the most frequently used under normal field conditions against pollen beetles. Altogether two sampling period were followed and a total number of 500 individuals on each period were collected from rape fields near Tirgu Mures, carried in laboratory and insecticide resistance tested. Two experimental insecticides resistance method were used one was a standard method proposed by IRAC, and the one were developed by us. Altogether, we can conclude that no resistant pollen beetle population were found during the experiment. In contrast, several insecticides resistant population of pollen beetles had been reported from other European countries.

Key words

oilseed rape, pest, IRAC, insecticides, pollen beetle

Oilseed rape (*Brassica napus* subsp. *napus*) is a bright-yellow flowering member of the family *Brassicaceae*, cultivated mainly for its oil-rich seed. The total cultivation area of the oilseed rape has been around 25 million hectares worldwide, from which 52% belongs to Asia, 23% to USA, 20% to Europe and 5% belongs to Australia. Altogether there are two varieties: winter and spring rape. [1]. The total production of vegetable oil is around 320 million tons worldwide, from which 50% belongs to soybean, the oilseed rape with 37-39 million tons (11-13%) is holding the third place [1]. The productivity is severely damaged by several pathogens and pest's species causing high economic losses. During the present study one of the most important rape pest, pollen beetle (*Meligethes aeneus* F.) was examined. The pollen beetle can cause high damage if the weather is cold for a longer time, because in this case the bud and flowering phase lasts longer [2]. The number of individuals per plant shows a very different picture across Europe. The average density of pollen beetle's per plant is one individual in Denmark, Switzerland and Scotland, 2-3 individuals in France and up to 15 per plant in England. The adults are capable to cause high economic damage in favorable conditions and years. Up to 80% yield loss can be expected under favorable conditions [3]. The pollen beetle is one of the most representative examples of species that have developed a strong resistance mechanism to many insecticides. The results show that in Denmark, 99% of individuals survived when they were treated with standard dose of *pyrehtroid* and 36% survived when treated with standard dose of *dimethoate* [4]. A questionnaire was sent to European countries to gain some background information on rape growing and on pollen beetle (*Meligethes aeneus*). Information was sought on the use of thresholds, the availability of active substances and on the year in which resistance, if present, had been observed. In total 20 countries responded and resistance in pollen beetle was reported in 12 of those countries [5]. Susceptibility *of Meligethes sp*. (mainly *M. aeneus*) from the Czech Republic was tested with lambda-cyhalothrin from 2009 to 2011, by IRAC method No. 011. Resistant samples were the most frequent in all three years and their proportions increased from 33% to 62% between 2009 and 2011 [6]. In an experiment in Poland susceptibility of *Meligethes aeneus* was tested with acetamiprid

(Mospilan 20 SP). The results show that non-resistant individuals were found only in one case, weak resistance were found in six cases and moderate resistance in five additional cases [7]. After the examination of individuals which survived the treatment they were unable to fly, had uncoordinated movement and were unable to eat [8]. Paweł Węgorek with his colleagues used a chemical compound in their experiment that can slow down the activity of enzymes responsible for breakdown of active compounds and may increase the effect of the insecticides. The substance known to block the activity of mixed function oxidase is *piperonyl butoxide* (PBO) [9]. The compound, which creates a stable complex with cytochrome P-450, blocks enzymes responsible for insecticide detoxification in insects [10]. The addition of PBO to the insecticides distinctly increased mortality of tested individuals. This compound most strongly increased the activity of *gamma-cyhalothrin* increasing the mean mortality to 97.62% [9]. Several studies have sought to find a correlation between the number of adults per plant and yield loss. Experiments from England have shown that 3 to 6 adults per plants have already caused economic damage [2]. In a similar experiment in Sweden the number of individuals which can cause severely damage was determined in six adults per plant [11]. In view of the economic importance, the researchers also carried out experiments in which the relationship between the nitrogen supply of rapeseed, the density of pollen beetle's and the yield loss was investigated. They established that under favorable conditions for plant and with a balanced nitrogen supply, the pollen beetle was not able to cause high or significant damage [12] [13].

Guidelines for the control of pollen beetle: most insecticides attack the nervous system of insects, inhibit its function, nerve impulses are transmitted as electrical signals [14]. The impulse reaches the gaps (synapses) between the nerv cells with the help of chemical mediators, like acetylcholine [14]. The above-mentioned process can be inhibited in several places and can be exploited by appropriate insecticidal interventions:

1. By inhibition of the enzyme acetylcholinesterase (organophosphates, carbamates)
2. By inhibiting the binding of acetylcholine to the receptor (nicotine, neonicotinoids)
3. By inhibiting the permeability of Na+- ion channels (pyrethroids) [14].

Material and Method

The pollen beetle adults were collected a total of two times from an oilseed rape field located 6 km far from Targu Mures. The first individuals were collected on April 28, 2018, and the date of the second collection was May 6, 2018. A total number of 500 individuals on each period were collected from rape fields near Târgu-Mureș. The insects were carried in to the Sapientia University's laboratory where we set up the experiment.

Insecticides used in the experiment:

1. *lambda-cyhalothrin* (Karate Zeon 50 CS)
2. *deltamethrin* (Decis Mega 50 EW)
3. *thiacloprid* (Calypso 480 SC) and
4. *acetamiprid* (Mospilan 20 SG)
5. *dimethoate* (Novadim Progress)

Two experimental insecticides resistance method were used. One was a standard method proposed by IRAC (Insecticide Resistance Action Committee, Method nr. 11, Method nr. 21, Method nr.25 and Method nr.27), and the second method were developed by us. The above-mentioned methods are currently being widely used in Western Europe for monitoring sensitivity of *Meligethes spp*. populations in oilseed rape to synthetic pyrethroids, neonicotinoids and organophosphates.

Description of the first method (IRAC):

During a collection approx. 500 adults (*Meligethes aeneus* F.) were collected from different points of the oilseed rape field. After collecting necessary number of individuals, we put them into a holding cage for 24 hours and put some oil seed rape leaves plus two or three rape inflorescences as food source. After the collection we carried them to the laboratory and could rest for 12 hours. Standard IRAC tools were used according to descriptions. The planned concentrations of each insecticides were measured in $\mu g/cm^2$, and then the necessary amount was carefully considered. Two replicates were followed for each experiment and concentrations. We used a total of 26 vials, two of them were used as control. 1.5 ml of distilled water were used for each vial and the measured insecticides were added to the water. For the control vials, we used 1.5 ml of acetone. At room temperature the vials were rotated in horizontal position under laminar box until the water and the acetone had evaporated. Once 10-10 adults were used for every vial. Then under microscope the survival rate was assessed for each individual after 2, and then after 12 and 24 hours.

Description of the new method:

Standard Petry dishes were used during the second experiment. We used total of 26 Petry dishes similarly with the first method, two of them formed the control. The pre-measured insecticides were filled into a 0.5L hand sprayer, an adhesion promoter was added due to character of Petry dishes and then we sprayed twice from the same distance on both side of the Petry dishes. After drying we put 10-10 adult in each Petry dish. Under microscope the survival rate was assessed for each individual after 2, and then after 12 and 24 hours.

Data analyses

The IRAC (*Insecticide Resistance Action Committee*) susceptibility rating scheme was used to evaluate the results for the treatments of pyrethroids (*lambda-*

cyhalothrin - Karate Zeon, *deltametrin* - Decis Mega 50 EW) (Table. 1) and organophospates (*dimethoate*-Novadim Progress) (Table. 2).

Table 1. Susceptibility rating scheme-*pyrethroids*

Concentration (% of label rate)	Affected	Classification	Code
100% 20%	100% 100%	Highly Susceptible	1
100% 20%	100% < 100%	Susceptible	2
100%	<100% to ≥ 90%	Moderatley Resistant	3
100%	< 90% to ≥ 50%	Resistant	4
100%	< 50%	Highly Resistant	5

Table 2. Susceptibility rating scheme-*organophosphates*

Rate	%affected	Classification	Code
0.3µq/cm^2	≤100 to 90	Susceptible	**1**
0.3µq/cm^2	<90	Potential to be tolerant	**2**

For neonicotinoid treatments, IRAC uses a similar system to examine the proportion of live and dead individuals and the mortality rate after 24 hours.

Results and Discussions

During the processing of the results, we did not find any significant differences after performing the pesticide resistance tests recommended by the Insecticide Resistance Action Committee (IRAC) and the tests we developed and performed.

The pyrethroid treatments with *lambda-cyhalothrin* (Karate Zeon 50 CS) *and deltamethrin* (Decis Mega 50 EW) caused 35% mortality rate after 2 hours with 100% concentration and 60% after 12 hours. For the results measured after 24 hours, the mortality was close to 100% (Fig. 1). No significant differences were found between the two methods.

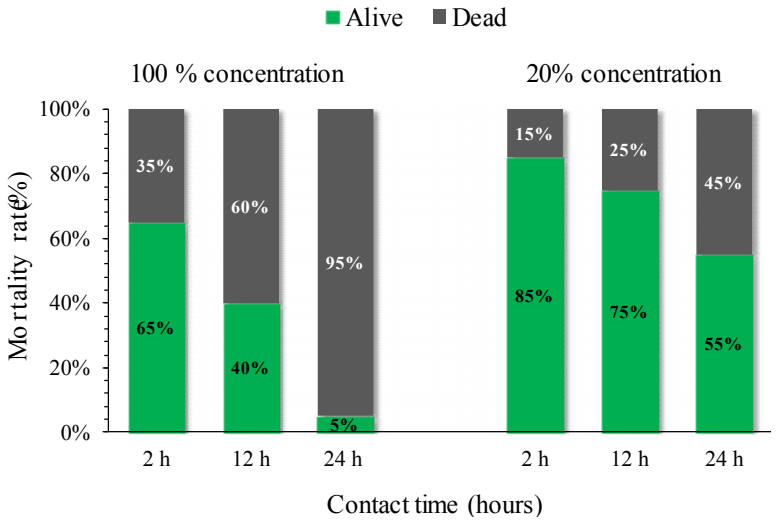

Fig. 1. Efficacy of *lambda-cyhalothrin* on pollen beetle adults (second method)

The neonicotinoid treatments with *thiacloprid* (Calypso 480 SC) and *acetamiprid* (Mospilan 20 SG) caused 40% mortality rate after 2 hours at 200% concentration treatment, and above 50% after 12 hours. After 24 hours the mortality rate was higher than 90%. At 100% concentration, the survival rate was higher after 2 hours, 75% of the individuals were alive. After 12 hours the proportion changed to 50%. After 24 hours only one alive individual was found. The treatment at 20% concentration had the highest survival rate. After

2 hours 80% of the individuals were alive, after 12 hours it showed stagnation and after 24 hours more than 60% of individuals died (Fig. 2). No significant differences were found between the two methods.

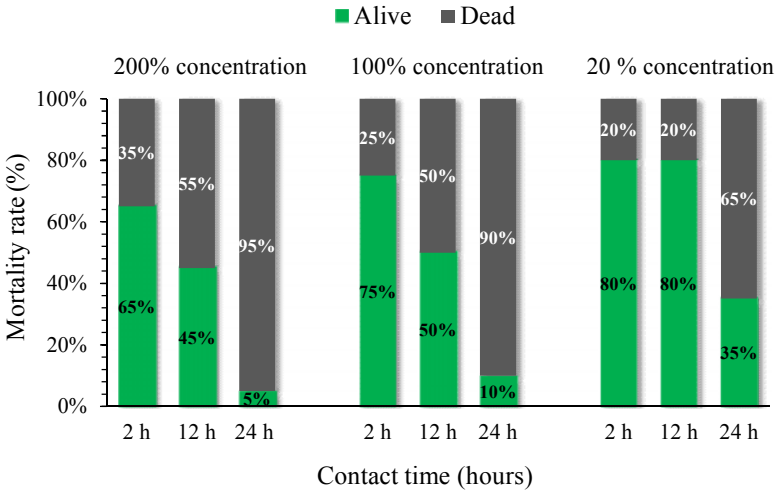

Fig. 2. Efficacy of *acetamiprid* on pollen beetle adult (second method

The organophosphate treatment with *dimethoate* (Novadim Progress) caused an 50% mortality rate after 2 hours at 16% concentration. After 12 hours the survival rate went under 20%. After 24 hours the mortality rate was above 90% (Fig. 3).

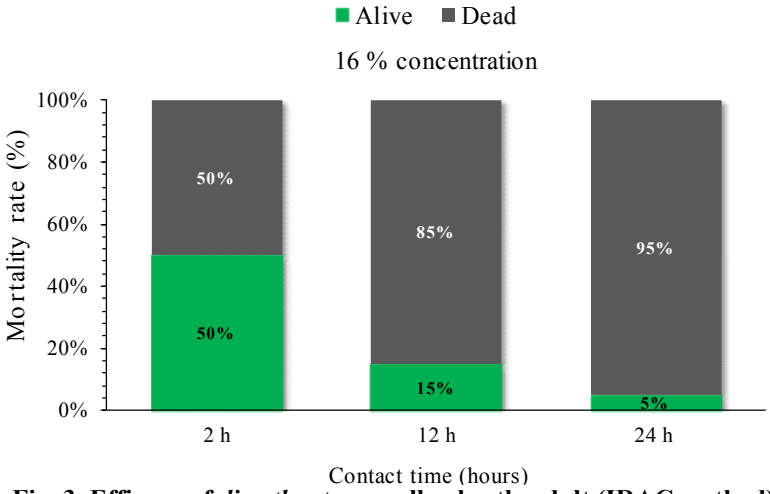

Fig. 3. Efficacy of *dimethoate* on pollen beetle adult (IRAC method)

No significant differences were found between the IRAC method and the one developed by us. Altogether it can be concluded that no susceptible populations of pollen beetle were detected around Târgu-Mureș and the treatments were effective against the adult pollen beetles.

Conclusions

After performing the experiment and processing the data, it can be concluded that no susceptible populations of pollen beetle were detected around Târgu-Mureș and the treatments were effective against the adult pollen beetles. The importance of the present experiment should also be emphasized because it has already been indicated in several Western European countries that insecticides *(pyrethroids, neonicotinoids, organophosphates*) used against pollen beetle have no effect and they developed resistance against insecticides. Susceptibility to *pyrethroids* were high after *lambda-cihalotrin* use, according to IRAC both at 100% and 20% after 24 hours. A same effect after *deltametrin* use were detected and no resistance to these insecticides can be considered nor after 100% and 20% concentrations. Using *neonicotinoides*, high mortality at 200% and 100% were detected, while using 20%, the mortality rate of 50% were only observed after 24 hours. Using *acetamiprides*, high, above 80% mortality rate were detected after 24 hours, while 70% mortality at 20% concentration were observed. By using organophosphate insecticides, the effect of dimethoate was high, because at 16% above

90% mortality was detected after 24 hours. Altogether it can be concluded that no susceptible populations of pollen beetle were detected around Târgu-Mureş. Therefore, the standard concentrations can be used to control pollen beetles but the principal mechanisms in the insecticide's effects must be followed to reduce the possibility of induced resistance of pollen beetles against particular compound.

Acknowledgement

I would like to thank Ferenc Molnár Fekete for his help in collecting and transporting the beetle's and everyone's contribution to the experiment.

References

[1] J. Antal, Z. Izsáki, K. Pocsai, M. Bacsa, A. Máté, and L. Nagy, *Növénytermesztéstan 2*. Mezőgazda Kiadó, 2008.

[2] H. Gould J., "Surveys of pest incidence on oil-seed rape in south central England," *Annals of Applied Biology*, vol. 79, no. 1, pp. 19–26, 1975.

[3] E. Petraitiené, I. Brazauskiené, R. Smatas, and V. Makunas, "The spread of pollen beetles (Meligethes aeneus) in sprin oilseed rape (Brassica napus) and the efficacy of pyrethroids," *Zemdirbyste-Agriculture*, vol. 95, no. 3, pp. 344–352, 2008.

[4] H. Lars Monrad, "Insecticide-resistance pollen beetles (Meligethes aeneus F) found in Danish oilseed rape (Brassica napus L) fields," *Pest Management Science*, vol. 59, no. 9, pp. 1057–1059, 2003.

[5] R. David Mark, "Summary of findings from a participant country pollen beetle questionnaire," *Bulletin OEPP/EPPO Bulletin*, vol. 38, no. 1, pp. 68–72, 2008.

[6] M. Seidenglanz *et al.*, "Changes in Meligethes aeneus (Coleoptera: Nitidulidae) Susceptibility to Lambda-cyhalotrin in the Czech Republic between 2009 and 2011," *Plant Protection Science*, vol. 51, no. 1, pp. 13–32, 2015.

[7] P. Wegorek, M. Mrówczynski, and J. Zamojska, "Resistance of pollen beetle (Meligethes aeneus F.) to selected active substances of insecticides in Poland," vol. 49, no. 16–2, 2009.

[8] P. Wegorek and J. Zamojska, "Current status of resistance in pollen beetle (Meligethes aeneus F.) to selected active substances of insecticides in Poland," *EPPO Bulletin*, vol. 38, no. 1, pp. 91–94, 2008.

[9] P. Wegorek, J. Zamojska, and M. Mrówczynski, "High resistance to phyretroid insecticides in the Polish pollen beetle (Meligethes aeneus F.): the role of oxidative metabolism," *Phytoparasitica*, vol. 39, no. 1, pp. 43–49, 2011.

[10] C. A. Silcox, G. M. Ghidiu, and A. J. Forgash, "Laboratory and field evaluation of piperonyl butoxide as a pyrethroid synergist against the colorado potato beetle (Coleoptera: Chrysomelidae)," *Journal of Economic Entomology*, vol. 78, no. 6, pp. 1399–1405, 1985.

[11] E. Sylven and G. Svennson, "Effect on yield of damage caused by Meligethes aeneus F. (Col.) to winter rape, as indicated by cage experiments," *Annales agriculturae Fenniae*, vol. 15, pp. 24–33, 1976.

[12] Z. Marczali, "A termesztett keresztesvirágű növényeken élő Meligethes és Ceutorhynchus fajok elterjedése és ökológiája," Veszprémi Egyetem, Keszthely, 2006.

[13] F. Daebeler, K. Röder, B. Hinz, and W. Lücke, "Schadwirkung des Rapsglanzkafers bei unterschiedlisch hohen Stickstoffgaben.," *Nachrichtenblatt für den Pflanzenschutz in der DDR*, vol. 34, no. 1, pp. 13–16, 1980.

[14] R. Ábrahám, T. Érsek, G. Kuroli, L. Németh, and P. Reisinger, *Növényvédelem*. Debreceni Egyetem, Nyugat Magyarországi Egyetem, Pannon Egyetem, 2011.