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BIOASSAYS RESISTANCE OF POLLEN BEETLE (*Meligethes aeneus* F.) TO SYNTHETIC PYRETHROID CYPERMETHRIN

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Abstract

Pollen beetle (Meligethes aeneus F.) as a key rape pest is controlled by use of a wide range of insecticides, to maintain low density. The downside of this method is destruction of the beneficial entomofauna, pollinators the possibility of developing resistance. Massive use in the synthetic pyrethroids to control the pollen beetle led to reduction their efficacy. Therefore, it is determined insecticidal resistance of pollen beetle (Meligethes aeneus F.) to synthetic pyrethroids. Resistance is refining by Bioassays, which includes a study of the reaction of an organism to a given substance, taking into account the concentration changing influences. To this end, they gathered pollen beetles for analysis. The results of the research proved presence of 10% resistance of pollen beetle (M. aeneus) to cypermethrin after 24 hours of action at the highest concentration of 0.250 ppm/cm^2.It was found, that the concentration and duration of cypermethrin action are inversely proportional of the resistance of the elderly.

Key words*: pollen beetle, resistance, synthetic pyrethroids.*

INTRODUCTION

The pollen beetle (*Meligethes aeneus* F.) is the key pest of oilseed rape across Europe (Alford et al., 2003, Williams, 2004, 2010). In separate years the loss of it can reach 60-100% (Hansen, 1996; Coll et al., 1998; Johnen, 2000; Krostitz, 2000; Cook et al., 2004; Kazachkova, 2007; Milovanović, 2007; Ahmanl et al., 2009; Breitenmoser, 2012).

Receiving high yields depends exclusively on the successful fight against the pest. Most often the fight with the control of pollen beetle in practice is taking place using synthetic pyrethroids and neonicotinoids (Wegorek and Zamojaska, 2006; Slater et al., 2011). In the past years is observed reducing efficacy of synthetic pyrethroids, as a result of which this pest is has developed very fast resistance to most active substances. Speed of development of resistance depends of several risky ones factors and they can be divided into two groups. The first group includes agronomic risk: the number, the rate and time of applications of plant protection products for one generation, as well as the specificity of the plant protection product. The second group is risk, which includes migration circles of plants - Hosts of the enemy, its reproductive potential and speed presence in proximity of sensitive populations (Russell, 2001; IRAC, 2013).

The genetic background of resistance to insecticides can be from monogenic or polygeneous character (Groeters and Tabashnik, 2000), but most cases are caused by a single allele of resistance (McKenzie, 2000; Hollingworth and Dong, 2008). Changes at the genetic level are varied include: single nucleotide polymorphisms (SNPs) (Williamson et al., 1996), Genetic Amplification (Bass and Field, 2011), DNA methylation (Field, 2000), microsatellites/tandem repeats STR (Bass et al., 2013), alternative and or improper bindings (Sonoda et al., 2006) and RNA editing (Xu et al., 2006). Changes in DNA/RNA lead to complicated by chain physiological changes in arthropods and they can be extra classified in four main ones the mechanism of resistance. The reason for the development of the resistance of beetles to synthetic pyrethroids is frequent their use in recent years.

If only one is used and also active substance with the same mechanism of action, pests, which are less receptive, survive. As the percentage of insensitive individuals in the population, the efficacy of the active substances decrease

(Hoffmann et al., 1994). In the whole Europe pyrethroid insecticides are with long history in control of pollen beetle (Slater et al., 2011).

The earliest report on *M. aeneus* resistance to pyretroids in France and Scandinavia (Decoin, 2002; Hansen 2003, 2004, 2008; Milanović et al., 2019), its spreading in recent years across Europe has caused some majour pest control problem of oil seed raps in many countries in Europe - France (Ballanger et al., 2007), UK (Richardson, 2008), Denmark (Hansen, 2003, 2004, 2008; Kaiser et al., 2018), Poland (Wegorek et al., 2009; Philippou et al., 2011), Germany (Heimbach et al., 2006; Thieme et al., 2008; Heimbach & Müller, 2013), Swizerland (Philippou et al., 2011), Austria (Slater et al. 2011), Sweden (Kazachkova, 2007) and Czech Rebublic (Stara & Kocourek, 2017).

In many countries common practice is the execution of more than one vegetative treatment with insecticides against the pollen beetle (Richardson, 2008a, 2008b). Hansen (2003) studies resistance the pollen beetle (*M. aeneus*) agains active substances: tauflumivalate, lambda-cyhalothrin, esfenvalerate and dimethoate. Later in a row countries from West and West Central Europa (Heimbach et al., 2006), Finland (Tiilikainen and Hokkanen, 2008), Poland (Wegorek and Zamoyska, 2006), Czech Republic and Slovakia (Seidenglanz et al., 2015), United Kingdom (Richardson, 2008) there is established resistance the pollen beetle (*M. aeneus*) to synthetic pyrethroids. The results of conducted surveys show, that the beetles survive 99% standard doses of synthetic pyroethroids and up to 36% of dimethoate. The first case of show of resistance of rapeseed flower (*M. aeneus*) to the synthetic ones pyrethroids was announced in 1999 in the Champagne region in the Northeast France.

Resistance to pyrethroids the pollen beetle (*M. aeneus*) is not limited only to the individual compounds, but affects the entire chemical group of synthetic pyrethroid, though some of show them higher efficacy at recommended doses of administration, than others (Nauen, 2007). In 2007, in Europe the first strategy was adopted to manage resistance of pollen beetle (*M. aeneus*) in winter oilseed rape and is mainly based of treatment with thiacloprid, belonging to neonicotinoids, directed to nicotinic acetylcholine receptors of insects (Jeschke and Nauen, 2008). Thiacloprid is registered for control of pollen beetle, but since it was introduced tested insecticides, with different modes of action, their effect on pollen beetle, for improvement management of resistance (Longhurst et al., 2007; Schröder et al., 2009).

The limited set of insecticides to control pollen beetle make it necessary to look for new tools to fight a different mechanism of action, which means to provide long-lasting protection of rapeseed from enemies, conservation of useful entomofauna and crop pollinators.

The purpose of this study was to obtain information by testing the pyrethroid resistance of cypermethrin to answer the problems with the effectiveness of the insecticides used in the region of Northeastern Bulgaria and to obtain basic data that can be used in future monitoring actions to detect early changes in the susceptibility of pollen beetle to insecticides.

MATERIALS AND METHODS

Resistance was determined by the quantitative test BIOASSAY; this means the study of the reaction of a living being to a particular substance, taking into account the concentration and changing influences.

For the purpose of laboratory testing, the pollen beetles were collected from winter oilseed rape fields near Shoumen (43°23'25.8"N 26°48'13.8"E). The adult individuals were collected in sunny weather during phenological growth stages BBCH 52-55. The collection was performed according to the linear assessment method, which ensures that the beetles were collected from as many different points in the field (Kupfer and Schröder, 2015), which ensures that the beetles are collected from as many different points in the field as possible. The area was divided into 5 control points and, if necessary, the number of consecutive control point plants increased from 10 to 15 to capture the required number of beetles (520 in total). The beetles ware removed by shaking the plants and collected in plastic bags containing absorbent paper to absorb moisture. There were also 4 flowers for the food. The predatory insects caught in the collection of pollen beetles were removed.

In the laboratory, the beetles were counted in groups of 10 animals and placed in small plastic containers. The test was carried out by three test kits, each with four concentration levels. The concentrations went from 0.002 ppm to 0.250 ppm. Each group of 10 beetles was placed in each test tube and capped. This was done under a room temperature of 21°C.

RESULTS AND DISCUSSIONS

Synthetic pyrethroids are contact insecticides, which block addicts from pressure sodium channels in nerve membranes, as a result of which they follow opening cannot be closed again. Synthetic pyrethroids have a rapid initial effect against almost all insects. The cypermethrin enter the insects through the body cover, after which they spread throughout the body.

Cypermethrin is a neurotoxin, wherein the Na^+ -channels of nerve cells stop closing, so Na + ions flowing unhindered in the interior of the cage.

As a result, they appear uncontrollable nerve impulses, which lead first to agitation convulsions, then to violations of its coordination finally to paralysis.

Insect immobilized in within a few minutes, the so-called "knockdown" effect is %.

Survival of beetles reported in each tube at 1, 5 and 24 hours after application of test concentration. The insects they were not observed coordinated movements within 30 s, were classified as dead.

At a test concentration of 0.002 ppm/cm^2, after contact action in 24 hours, 97% survival in which after 1 and 5 hours they were not observed signs of poisoning in the pollen beetle.

Taking into account the levels of a concentration of 0.010 and 0.050 ppm/cm^2, after 1 and 5 hours, 3% or 7% of the beetles with signs of poisoning. After contact action in for 24 hours the number of beetles, with signs of poisoning was rising both levels of concentration reached 13%, which means survival rate of 87%. The highest concentration in this one experimental group was 0.250 ppm/cm^2. After contact time of 1 hour, of said concentration, 37% of beetles showed signs poisoning, after 5 hours the degree of disability increased to 43%. At the latter reporting after 24 hours survival was 10%. This means that at the highest concentration, used in biological research, resilience of Pollen beetle was 10%. From everyone results it can be said, that its concentration duration of action of cypermethrin are inversely proportional the resistance of the beetles (Table 1).

Table 1. Bioassays of cypermethrin against pollen beetle

Test concentration, µg/cm^3	Mean number of vital beetles	Mean number of beetles with intoxication symptoms	Survival rate [%]	Efficiency [%]
	after 1 hours			
untreated	10.0	0.0	100.0	0.0
0.002	10.0	0.0	100.0	0.0
0.010	9.7	1.3	97.0	3.0
0.050	9.3	1.7	93.0	7.0
0.250	6.3	3.7	63.0	37.0
	after 5 hours			
untreated	10.0	10.0	100.0	0.0
0.002	10.0	10.0	100.0	0.0
0.010	9.7	1.3	97.0	3.0
0.050	9.3	0.7	83.0	7.0
0.250	5.7	4.3	57.0	43.0
	after 24 hours			
untreated	10.0	10.0	10.0	0.0
0.002	9.7	0.3	97.0	3.0
0.010	9.0	1.0	90.0	10.0
0.050	8.7	1.3	87.0	13.0
0.250	0.1	9.0	10.0	90.0

The pollen beetle is known to be a species in which, in addition to its natural resistance, there is a strong resistance to some synthetic active substances (Tillikainen and Hokkanen 2008; Węgorek et al. 2009; Nauen et al, 2012; Seidenglanz et al, 2015). The results of our studies have shown that the pest exhibits high-level resistance to the test concentration of 0.002 ppm/cm^2. Changes in the level of sensitivity were observed at concentrations of 0.010 and 0.050 ppm/cm^2, but they were not significant. The reported high survival rate of individuals 8 of 0.250 ppm/cm^2 suggests mechanisms of detoxification in the test population that also determine the low efficacy of cypermethrin against pollen beetle. The pollen beetle resistance to pyrethroids has been reported to be due to a mechanism of physiological resistance based on oxidative enzymes (Slater and Nauen, 2007; Węgorek et al., 2011).

The experiments presented were carried out in connection with many signals from farmers for the low efficiency of chemical control of pollen beetle, especially when using pyrethroids. The results showed low toxicity of cypermethrin to pollen beetle. With this study, we report for the first time the existence of a resistant population of pollen beetle on oilseed rape in the area near the town of Shumen, Northeastern Bulgaria, but this claim requires lengthy observations and investigations. In view of the results obtained from our studies, literature and experience we can strongly recommend that alternative control of pollen beetle be sought. Strict adherence to the Sustainability Management Guidelines issued by local experts or published annually by the Insecticide Resistance Action Committee (IRAC) will exclude unnecessary combinations of insecticides and treatments and the emergence of new resistant individuals or populations.

CONCLUSIONS

As a result of research can draw the following conclusions:

-Established is 10% resistance of the pollen beetle to cypermethrin after 24 hours impact on the highest a concentration of 0.250 g/cm^2.

-The results have proven, that concentration and duration of action of cypermethrin are inversely proportional the resistance of the elderly individuals.

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