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Susceptibility levels of Hungarian Pollen Beetles (*Brassicogethes aeneus*) To Thiacloprid

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CHAPTER 1.0 INTRODUCTION

1.1 Background study

Oilseed rape (*Brassica napus*) is the second most significant oilseed crop and belongs to the Brassicaceae family, which is made up of 338 genera, with diverse species cultivated in different ecological conditions, especially in temperate and mountainous regions (WARWICK et al., 2010). The crop is grown due to its oil-rich seed obtained from crushing it, which is highly valued for culinary and industrial use (LAMB, 1989). The by-product of crushed seeds, called Rapeseed meal, is utilized as animal feed. Europe, Canada, China, India, and Australia are the leading producers of oilseed rape globally.

Brassica napus offers an appropriate alternative for cereal-based agricultural systems since the broad-leaved plant can be produced as a break crop for cereals. Growing oilseed rape has many agronomical advantages; it improves soil structure due to its deep rooting tap roots, which break up the subsurface layers of the soil, and nutrient residues after cropping improves the fertility of the soil for successive crops. The output for wheat crops can increase by 35% in a continuous cereal sequence following Oilseed rape (WIBBERLEY, 1996). Glucosinolate breakdown from Brassica residue left in the soil may have a biocidal effect, helping control soilborne pests and diseases. Most oilseed rape cultivation procedures can be conducted with existing cereal equipment hence minimizing the need for new machinery(BOOTH et al., 2004).

Growth in oilseed rape yields since the 1980s in the main production regions such as Canada, China, Europe, India, and Australia has been majorly linked to the introduction of high-yielding cultivars and an increase in the number of hybrids. Development of double-low varieties (< 2% erucic and < 25 % mol/g glucosinolates) of rapeseed has led to an increase in demand for rapeseed oil for human consumption and as an animal feed due to its improved palatability (MEENA et al., 2022). All of these have consequently led to a notable increase in the area of oilseed rape cultivation. However, since the 1990s, the oilseed rape yields average growth rate has registered a decline in Australia and Europe which has been attributed to ineffective management of biotic stressors to the crop.

Apart from pests and disease infestation, which led to a decline in the annual growth rate of the average yields of Rapeseed in Europe, e.g., in Germany from 2.1% in 1961-1990 to 0.5% in 1991-2018, its decline also linked to various abiotic factors such as low precipitation, warm temperatures(KUTCHER et al., 2010) and reduced availability of registered active ingredients for chemical control e.g., ban on neonicotinoids (PENG et al., 2011).

Management options for Oilseed rape pests majorly employ both the integrated pest management (IPM) strategies and the use of insecticides but relies more on insecticides for insect control. Increasing restrictions on insecticide use in Europe and reduced insecticide efficacy have threatened the profitability of oilseed rape production and its role as an essential break crop in cereal-dominated cropping systems.

1.2 Problem statement of the research

Oilseed rape holds a significant role in Europe's commercial and political sphere (VINNICHEK et al., 2019). It is one of the major crops in European arable farming. Despite its critical significance, European oilseed rape production is threatened by pollen beetle (*Brassicogethes aenue*s), which is considered one of the major pests of the crop (SKELLERN et al., 2018). When the pollen beetles are present in large numbers, they cause a great deal of devastation in oilseed rape plants and decrease the crop strength in response to favourable environmental and agricultural conditions, which cumulatively results in a significant reduction in oilseed rape yields, with oilseed rape farmers experiencing an entire harvest loss in Northern Germany in 2006 due pollen beetle menace (SLATER et al., 2011).

Managing pollen beetles in Europe has been done historically by applying Broad-based insecticides, often applied prophylactically (LUNDIN et al., 2020). After the ban of Organophosphates, pyrethroid became a reliable class for pollen beetle control, but due to selection pressure, which led to enormous resistance by the pollen beetles to pyrethroids, consequently leading to the use of neonicotinoids (a.i thiacloprid) as an alternative to reduce the level of resistance or to slow down the development of resistance (EPPO workshop, 2006).

The first management strategy of pollen beetle resistance, especially in Germany in 2007, was the alternation of pyrethroid with thiacloprid belonging to the class of neonicotinoids (ZIMMER-NAUEN, (2011), which targets insect post-synaptic nicotinic acetylcholine receptors. Application of the neonicotinoids is either through seed-dressing (prophylactic) or as a foliar spray. Since then, neonicotinoids have been an alternative tool for pollen beetle management strategy to pyrethroid resistance; therefore, its performance should be carefully monitored to detect early shifts in pollen beetle susceptibility to thiacloprid.

Monitoring of resistance is necessary on a regional level to detect changes in susceptibility and to develop an appropriate control measure based on a locally available insecticide portfolio (HEAD-SAVINELLI (2008). Therefore, the Adult Vial Test on a thiacloprid 240g L^{-1}OD formulation (Biscaya®) was developed and validated using pollen beetle populations collected in several European countries in 2009 and 2010 to aid in monitoring the susceptibility of pollen beetles to the active ingredient.

Thiacloprid was fully registered in 2007 for *Brassicogethes aenues* control in oilseed rape fields, and diverse baseline susceptibility studies were done in Europe (KAISER et al., 2018a), which allowed comparison of data obtained in subsequent resistance monitoring studies. Some of the neonicotinoid active ingredients In Europe are restricted due to the harm linked to the bee population, with some member states allowing its active ingredients under emergency cases/derogation. However, other active ingredients are still in use, such as acetamiprid (SCHROEDER et al., 2009) , necessitating the need to carry out frequent monitoring of the susceptibility status of these active ingredients.

1.3 Aim of the study

This study aimed to investigate whether there is a susceptibility decline to thiacloprid, which can be used against pyrethroid-resistant pollen beetles, which might have already developed and could be a precursor to resistance.

1.4 Objective

To assess the susceptibility level of pollen beetles to thiacloprid in Hungarian rape fields, a case study in Sorkifalud, Vas county, Hungary.

CHAPTER 2. LITERATURE REVIEW

2.1 Oilseed rape

Oilseed rape (*Brassica napus L.*) is considered the third most crucial oil crop after maize and palm oil in the world (BESZTERDA et al., 2019). Oilseed rape (genome AACC, 2n=38) belongs to Brassicaceae (Mustard) family, which makes up 3000 other species and arises from spontaneous interspecific hybridization between *Brassica rapa* (AA, 2n=20) and *Brassica oleracea* (CC, 2n=18) (RAHAMAN, 2016), resulting in an amphidiploid genome. The Brassicaceae family comprises many economically significant crop species used as sources of oil, food and ornamental (AL-SHEHBAZ et al., 2006). *Brassica napus* is the dominant species in the family of Brassicaceae in Europe. In Asian countries, the dominant rape crop is *Brassica campestris* (ALFORD, 2003).

Oilseed rape is widely grown outside Europe, especially in Canada, and it is majorly referred to as canola. Was coined the name canola during the sixties and seventies after Breeders developed a double low variety in both glucosinolates and erucic acid, hence the name' canola' to enhance its possible use for animal and human nutrition since it had high levels of glucosinolates in all parts of the plant (ORLOVIUS et al., 2003) .

Rapeseed is widely produced in many regions of the world. India has been cultivating oilseed rape since 4000 BC, and it extended to China and Japan about 2000 years ago (SNOWDON et al., 2010) in which the wild form of the relatives was also found (SHAHZADI et al.,2015). Brassica *napus* is the world's most essential and prolific oilseed crop (ZHANG-FLOTTMANN (2016). In 2020, rapeseed was produced at 35.49 hectares worldwide (FAOSTAT, 2020), and approximately 5 million hectares were grown in Europe (ALFORD et al.,2003). Oilseed rape yield is about 70 million metric tons per yield in the world, involving 34 countries in Europe, 15 countries in Asia, nine countries in America, six countries in Africa, and two countries in Oceania (DAUN, 2011) (FAOSTAT 2021).

Oilseed rape is an annual species (GULDEN et al., 2008a); there are different varieties of oilseed rape; winter, semi-winter and spring types which differ in abiotic tolerance; hence the growing condition of each variety differs. Winter Oilseed rape thrives well in high humidity and cooler temperatures.

All parts of an oilseed rape plant are helpful; the flower, seeds, leaves, stem, and root have diverse uses such as food, cosmetics or for industrial applications. Seeds are the most pertinent part of an oilseed rape, as it is a significant source of proteins and oil. Seeds are usually crushed to extract oil (MARTIN et al., 2015). The oil produced after extraction is used for culinary purposes, as fuel and as a lubricant on machines in the food industry (ЕГОРОВА et al., 2015). It is also used in synthetic rubber and soap production. After extraction, the residue of the cake (seed cake) is used as fodder for livestock (MIRPOOR et al., 2021). The uncrushed part is used for feeding birds. The oilseed crop is sometimes grown; green salads and fodder are used for livestock.

2.1.1 Growing conditions and Agronomic management of Oilseed Rape

Oilseed rape is a magnificent season crop in subtropical regions and a winter crop in temperate regions (ZHANG et al., 2017) since it has adapted to have a reasonable growth rate in cool, moist climates with a temperature range of 2-10 degrees Celsius; Moreover, temperatures closer to 10 degrees Celsius accelerate growth (HÅKANSSON et al., 2011). Temperature above 30 degrees Celsius negatively impacts pollination and shortens the pod and seed development phase. In winter, higher temperatures hasten plant growth, shortening the growing season and reducing the yield potential (JANNAT et al., 2022). Rainfall distribution is a mean factor in yield determination of oilseed rape; a long rainy season with enough rains and lower temperatures during earlier development stages of rapeseed is ideal, and after maturity, rainfall is undesirable.

Brassica napus thrive well in various soil types; the plant prefers soils of medium texture and well-drained soils. Oilseed rape should never be seeded in soils where other plants of the same family have been produced previously within 3-4 years to avoid accumulation and potential attack of the pest and diseases. Numerous studies on fertilizer showed that oilseed rape responds to fertilizers. Especially nitrogen, phosphorous and potassium are a few of the major elements needed to increase the yield of oilseed rape significantly. However, fertilizer dosage differs in oilseed rape production due to varying levels of NPK in the soils (SÜZER, 2015).

2.1.2 Propagation of Oilseed rape.

Oilseed rape is propagated by seeds drilled in rows of a well-prepared field, which is a fine, firm, moist and well-structured seedbed which aims to encourage faster and uniform germination and establishment in the field (BOOTH-GUNSTONE (2004b). The tiny seeds are sown shallowly at about 2-3cm depth. Spring sown type is drilled in rows 18-23cm apart, while winter sown type oilseed rape variety requires more space and is recommended to be drilled in rows approximately 40cm apart (IRETON, 2019). The optimum sowing date differs according to the latitude and the onset of winter. In northern Europe, early sowing of winter variety is critical for satisfactory development, and the sowing date is from half August; in southern Europe, sowing dates can be extended until early September (BOOTH et al., 2004). The aim is to prepare the plant to withstand the challenges of winter sufficiently.

2.1.3 Management practices

Control of weeds is vital in oilseed rape cultivation as weeds have a high potential to affect crop growth and yield negatively (SKELLERN et al., 2018b). One of the main aspects of weed control is the establishment of weed-free fields. To achieve this, better seedbed preparation is needed to reduce the growth of weeds. Thus, to reduce weed infestation in a rape field, winter-type oilseed rape should be sown immediately after the harvest, while spring-type should be sown when the soil moisture is sufficient for the entry of machinery. Moreover, herbicide use effectively controls weeds

(JANKOVSKA et al., 2023). However, herbicide effectiveness has declined in some herbicides due to a notable increase in herbicide resistance in some weeds, which has led to legislative changes.

Oilseed rape has a high nutrient requirement, and a soil test must be conducted before seeding to prevent nutrient deficiencies (BARRACLOUGH, 1989). Fertilizer is either applied by row placement at planting or a combination of row and broadcast placement (FORSTER, 1977). The level of primary nutrients should be maintained. Depending on the soil fertility status, Nitrogen requirements range from 45 to 70 kgs per acre, phosphorous rate of application is 0 to 30 kgs per acre, and potassium is 0 to 65 kgs (JANKOVSKA-BORTKEVIČ et al., 2023). Humidity levels in the soil are fundamental for oilseed rape high yielding, especially during the phase of flowering, yield formation and ripening. Therefore, irrigation water needs to be applied during water scarcity conditions to avoid a reduction in the yields, especially at the beginning of flowering, which tends to improve nitrogen assimilation and oil content (ISTANBULLUOGLU et al., 2010).

Oilseed rape is prone to several pests and diseases, which reduces yields drastically. The most common pests that attack rapeseed include cabbage steam flea beetles, Brassica pod midge, rape stem weevil, Cabbage seed and stem weevil and pollen beetles (*Brassicogethes aenues*). Management of oilseed rape pests entails complex sets of alternative solutions (ALFORD, 2003), but it has become challenging due to a reduction in effective chemical control options, which is resistance to pyrethroids and ban of neonicotinoids seed treatments (SLATER et al., 2011).

2.1.4 Harvesting oilseed rape

Oilseed rape reaches the ripening stage fast, and it is ready for harvesting when the seeds change from green to black (OSMASTON, 2001). The pods bearing the seeds quickly dehisce as the seeds ripen. Harvesting in oilseed rape is done mechanically using a combine harvester or swathing (PRICE et al., 1996). The stage of maturity during harvest is a crucial factor which can affect the quality of seeds (DEMIR et al., 2008). Harvesting, when done too early, may result in low yield and quality due to improper development of vital structures of seed. When done too late, it may result in shattering and reduce the quality of seeds due to ageing (MENDHAM et al., 1981). Therefore, detection and implication of the correct time of harvesting is a pre-requisite for high-yield realization in oilseed rape (GHASSEMI et al., 2011). Seed losses both from natural shedding and mechanical harvesting are significant. Bad harvesting conditions may result in around 50% total yield loss (PARI et al., 2012).

2.1.5 Health Benefits of Oilseed Rape

Brassica oilseeds are mainly produced for their oil (MATTHAUS et al., 2016). Rapeseed oil is used for culinary and industrial purposes, among many other purposes (Gunston, 2004). Culinary oil is used in kitchen cooking, while the latter is used for chemical and automotive industries (PAHL, 2008), canola has defined cut-off levels of erucic acid and glucosinolates for both human and animal consumption (MAG, 1983) and low erucic acid oilseed rape plant was introduced in Europe (SWERN, 1982).

Canola oil has low levels of saturated fatty acids (7%) and considerable levels of monounsaturated fatty acids and polyunsaturated acids, oleic acids, linoleic acids and alpha-linoleic acids (DUPONT et al., 1989), sterols and tocopherols (GUNSTONE, 2011). Regarding canola oil properties, it is regarded as a cardiovascular protective. In addition, tocopherols, which form part of vitamin E, can reduce the risk of diseases linked to the nervous system and muscles (EFSA Panel on Dietetic Products and Allergies (NDA), 2015) (MATHUR et al., 2015)

Carotenoids are significant antioxidants which specifically neutralize superoxide anions. Thus, rapeseed oil supplies substantial antioxidants (LIN et al., 2020). An intake of Omega-3 fatty acids of 1g/day contained in canola oil-based products is recommended, which aids in treating existing cardiovascular diseases (GEBAUER et al., 2006).

2.1.6 Taxonomy and Morphology of Oilseed Rape

The Brassicaceae family is composed of 338-360 genera and about 3709 species (HASANUZZAMAN, 2020). The Brassicaceae family consists mainly of species from the genus

Brassica. Many of the members of this family are economically and agronomically essential, and it has some of the earliest domesticated plants (RAZA et al., 2020). Brassica, the largest genus in the family of Brassicaceae, is used as fodder, oil for culinary or industrial use, vegetables, and condiments (SALEHI et al., 2021). Prominent crop plants are domesticated wild forms of wild cabbage (Brassica oleracea), including white and red cabbage, kohlrabi, brussels sprouts, and broccoli. Other significant crops members of the genus Brassica are pak choi, Chinese cabbage (*B. Rapa subsp. Chinesis*), white beet (*B.rapa subsp. rapa*), turnip rape (*B.rapa subsp. oleifera*), swede (*B. napus subsp. rapifera*) and rapeseed (*B. napus subsp. napus*). Other members that can be named are *B. nigra* (black mustard) and *Sinapis alba* (white mustard). Plants representing the genus Raphanus are radish types (R. sativus), horseradish (*Armoracia rusticana*) and Cresse (Lepidium) (LIU et al., 2018). *Arabidopsis thaliana*, a well-known model plant, also belongs to the family of Brassicaceae and weeds such as *Sinapsis arvensis.*

Brassica napus have diverse common names in English, which include; oilseed rape, rape, rapeseed, rutabaga, annual rape, Argentine canola, canola, colza, Hannover salad, Siberian kale, summer rape, Swede, winter rape, swede rape, and Swedish turnip (GULDEN et al., 2008b), (MARJANOVIĆ et al., 2018). Rapeseed and canola do not originate from a single species and do not necessarily mean the same species. Rapeseed is an oilseed from the species *Brassica napus and B. rapa. In contrast,* canola is a specific variety of rapeseed produced after intensive breeding and selection to produce edible products for both humans and animals, which has 2% erucic acid and 30 μg/mol glucosinolates (PRZYBYLSKI, 2011).

Brassica rapa is one of the first species in the family of Brassicaceae to be domesticated (GUPTA et al., 2007) , and *Brassica napus* is the economically most significant species in the genus Brassica (SOENGAS et al., 2008). Oilseed rape has an immense history of breeding and selection, leading to its high levels of morphological characteristics (FRIEDT et al., 2010). *Brassica napus* is an amphidiploid resulting from interspecific hybridization between *B. oleracea* and *B. rapa* (KAMIŃSKI et al., 2020).

Brassica napus is an annual biennial plant species (INIGUEZ, 2011). The stems are erect, simple to freely branched, smooth or sparsely hairy and can grow up to a height of 1.5 m. leaves are waxy with a smooth underside and an enlarged base that clasps the stem. It has a raceme inflorescence formed on the main and axillary branches. Flowering starts at the base of the raceme, and the buds are borne above the open flowers. The flowers are pale yellow, with four sepals and four obovate petals diagonally opposite and arranged as a cross when viewed from above. It has six stamens (tetradynamous), with four long and two short stamens in each flower. *Brassica napus* ovary is superior and has a globose stigma.

Oilseed rape fruit is linear cylindrical silique with a slight constriction at regular intervals and dehiscent valves in the lower 4-10 cm segment of the fruit. Seeds are in a single row in the fruit, and the upper 3.5 to 5.0 mm thick of the silique is narrow and typically seedless. Seed formation begins with about one-third of the branches of the main stem, and the seed in each silique can be 15 or more. Seeds are initially translucent, but they become green in colour upon attaining their full size. The seed coat at maturity is reddish brown, brown or black with a diameter of 1.8 to 2.7 mm with a net-veined surface. Yellow-coloured seeds for *Brassica napus* are a desired trait since they are associated with seeds that have higher oil and protein content and lower fibre content. Plant breeders have been attempting to develop yellow-seeded *B. napus* genotypes using naturally yellow-seeded Brassica species because there is no naturally occurring yellow-seeded *Brassica napus* (RAHMAN et al., 2011).

The type of germination for oilseed rape is epigeal, with a stout taproot and lateral roots; hypocotyl and epicotyl lengths of about 5 and 4 mm, respectively. Cotyledons are folded lengthwise around the radicle in the seed, and in the germination phase, seedlings emerge with the growing point located between the cotyledons. Cotyledons are 6-12 mm wide, kidney-shaped, with a deep, wide, rounded notch at the tip. Seedlings develop to form a rosette of 5-6 leaves, with older leaves situated at the base and the smaller younger leaves at the Centre of the rosette. Basal rosette leaves are short-petiolate, indented, green, ovate to elongate, entire to lobed, containing 1 to 5 pairs of lateral lobes and a large terminal lobe.

Differentiating between *Brassica napus* and its' close relatives can be difficult as they share similar morphological features, but there are few differences between *Brassica napus* and *Brassica rapa;* the leaves of *B. napus* are hairless, smooth, fleshy, and bluish-green while those of *B. rapa* are yellowish green. *B. napus* leaves partially clasp the stem at the base, whereas those of *B. rapa* fully clasp the stem. Buds are formed above the opened florets in *B. napus,* while in *B. rapa,* the buds are borne below the open florets in the raceme, and *B. rapa* flowers are smaller and darker yellow compared to flowers of *B. napus.*

Figure 1 Oilseed rape botanical illustration by Lizzie Harper

Source: https://www.google.com/urlsa=i&url=https%3A%2F%2Flizzieharper.co.uk%2F2013%2F01%2Foil-seedrapeandquackingducks%2F&psig=AOvVaw1OZOWigBW2wM2svUPL5VZ&ust=1712941095046000&source=images&cd=vfe&opi=89978449&ved=0CBIQjRxqFwoTCNiA3_7QuoUDFQAAAAAdAAAAAABAJ

2.1.7 Production of Oilseed Rape

The EU chiefly cultivates three types of oilseed crops; the central two rape and turnip rape, and sunflower, but also some soya is produced. Significant producers of oilseed rape in the European union are France, Germany (POPESCU et al., 2019). In 2018, the European union cultivated 11.8

hectares of land with oilseed crops, which entailed 58% rapeseed, 33.8% of the sunflower and 8% of soybean (POPESCU et al., 2019a). At the global level in 2018, the production of soybean seeds was 360.08 million metric tonnes, 70.91 million metric tonnes of rapeseeds and 51.46 million metric tonnes of sunflower seeds (STATISTA).

In 2022, the EU harvested an estimated 32.5 million tonnes of oilseeds, representing an increase of 1.2 million tonnes compared to 2021. 19.4 million tonnes of rape and turnip rape seeds in the EU were harvested in 2022, which was an increase from the harvest of 2021 by 2.5 million tonnes, an equivalent to a +14.6 % rise. The rise in production in 2022 was attributed to an expansion in the area for cultivation of rape and turnip rape. The production area in 2022 rose to 5.9 million hectares, representing an increase in area of 10.5% in 2021.

However, unfavourable weather affected the yields of sunflower seeds across the EU in 2022. 9.3 million tonnes of sunflower seeds were harvested, which was 1.1 million tonnes less than in 2021 (a decline of 10.1 %), despite the immense increase (+12.9 %) in the area harvested compared to the area harvested in 2021. Soybean production also decreased (-7.6 %) in 2022 compared to the yield of 2021 due to adverse weather conditions affecting the apparent yield, regardless of the expansion in the production area.

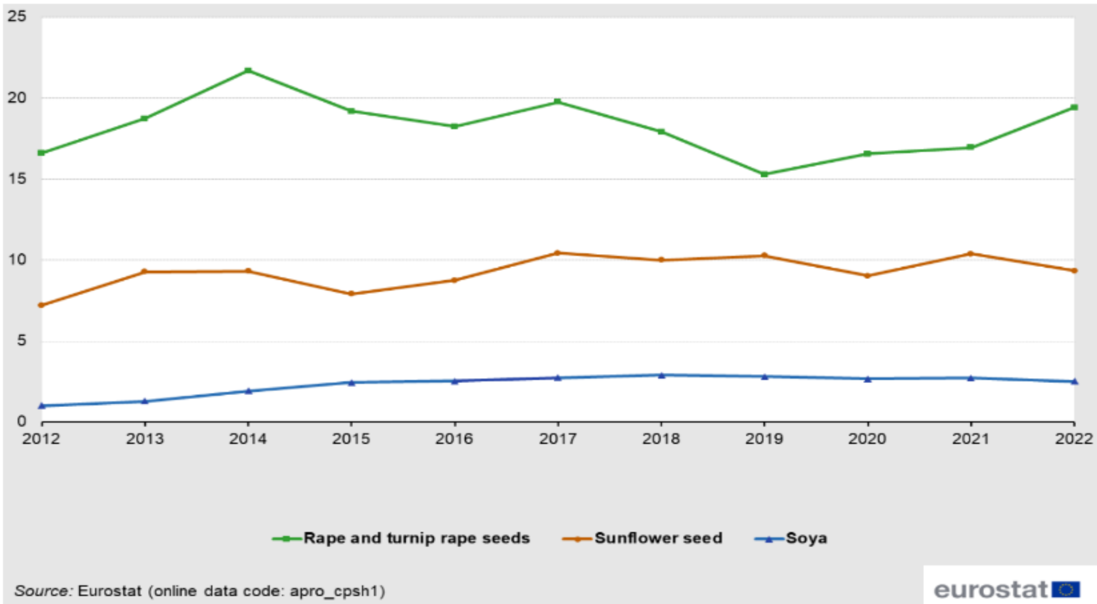

Figure 2: Production of rape and turnip rape seed, sunflower seed, and Soybean in million tonnes in the European Union from 2012 to 2022.

Source; https://ec.europa.eu/eurostat/databrowser/view/apro_cpsh1/default/table?lang=en

Sunflower seeds are majorly produced in the Eastern and southern parts of Europe (SOARE-CHIURCIU,(2018). In 2015, Romania's sunflower production accounted for 22.6 % of the world's total sunflower production and was considered the leading producer and exporter of sunflower (POPESCU, 2018). Romania, Bulgaria, Hungary, and France collectively produced 78.8% of the European union-28 sunflower seed crop, making up over 75% of its surface area in 2015.

Winter oilseed rape is Europe's primary oilseed crop and is cultivated in most areas of Europe (TUCK et al., 2006) for the production of biofuel and edible oil (DUREN et al., 2015). For the past 20 years, rapeseed has registered significant growth in its production globally. The production of oilseed rape transcended cottonseed in the early 2000s, making it the second oilseed crop after soybeans (CARRÉ-POUZET, 2014). Currently (2023/2024), production of oilseed rape stands at 87.44 million metric tons, which is in second place after soybeans which stand at 398.21 million metric tonnes, while the sunflower seeds are at 55.08 million metric tons (STATISTA) and cottonseed at the fifth place.

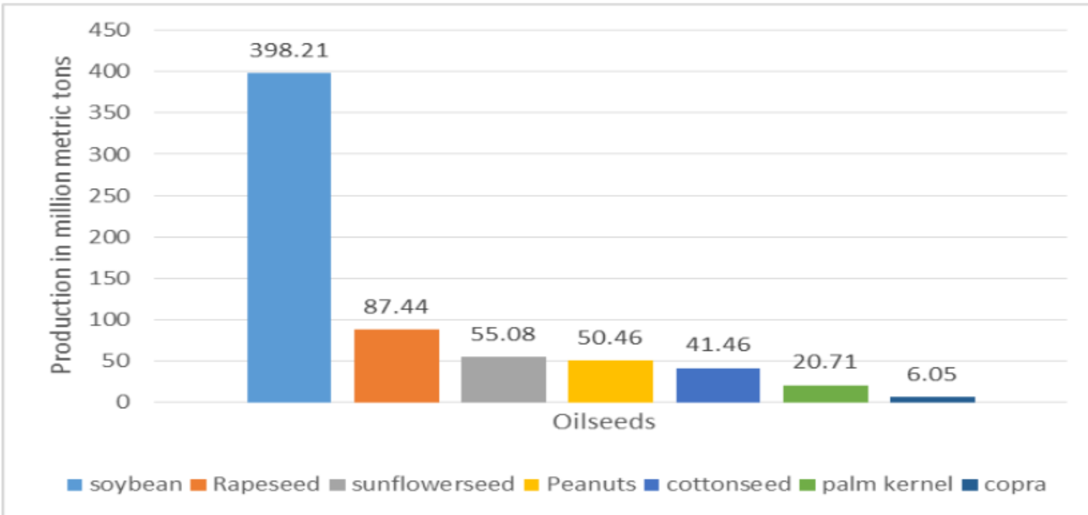

Figure 3: Worldwide production of oilseeds by type in the year 2023/2024 (Own design)

Source of data: https://www.statista.com/statistics/report-content/statistic/267271

In the rapeseed world trade of seeds, countries are categorized either those that produce more than they process, like Ukraine, Australia, and Canada, those whose production of rapeseed is the same as their processing, such as Europe, China, Russia, and India or countries that produce less than they process like Germany and Mexico (CARRÉ-POUZET, 2014).

The area under cultivation for oilseed rape in Hungary in 2018 was approximately 335,000 hectares which resulted in a yield of an estimate of about 1 million metric tons of oilseed rape in the same year, and the year registered an increase in harvested area, which directly influenced the yield. Since 2018 henceforth, there has been a decline in the area under cultivation of oilseed rape which has significantly reduced the yield compared to 2018. 2012 had the lowest yield and smaller area under cultivation than other years (FAOSTAT). Production levels and land area under cultivation of oilseed rape depend on diverse factors such as government policies, weather conditions and market demand (LILIANE-CHARLES, 2020). Thus, the provided figures should be treated as approximates and may not reflect the actual production status.

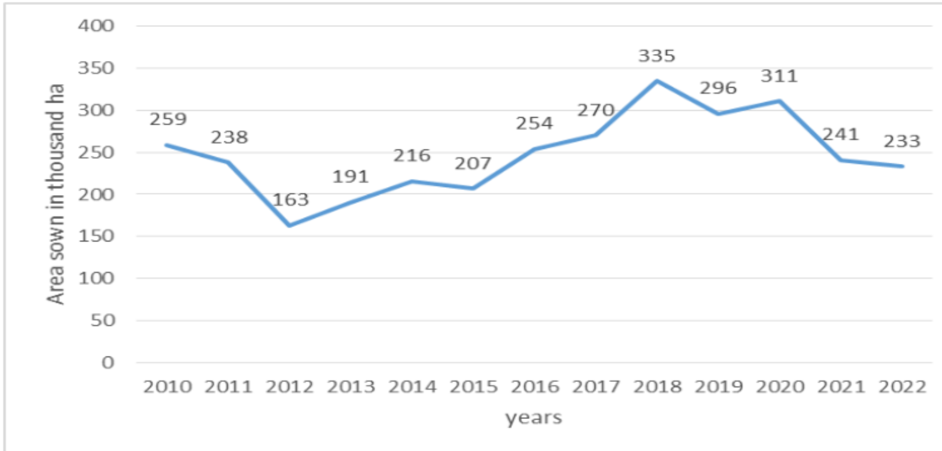

Figure 4: National sown area of oilseed rape in Hungary from 2010 to 2022 in thousand hectares

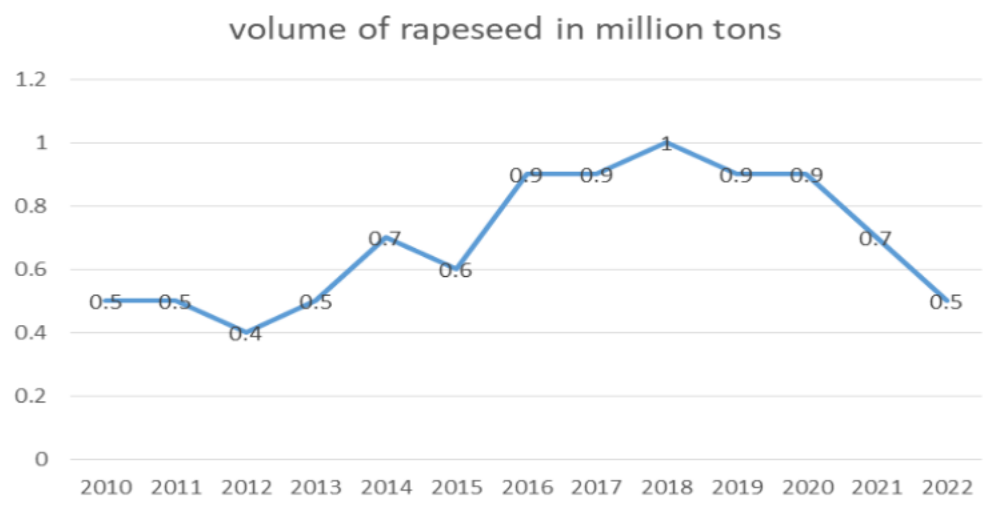

Figure 5: Rapeseed produced in millions of tonnes in Hungary from 2010 to 2022. Source:https://www.statista.com/statistics/1206508/hungary-volume-of-rapeseed-production/plant

The number of plants per unit area is critical to plant production (MA et al., 2014). Plant population concerning the sowing rate of oilseed rape affects the number of pods and seed number per pod. Other several factors such as; soil properties, seed quality, field germination, sowing time, plant morphology, diseases, pest, and seedbed preparation (BALODIS et al., 2016) affect the general yield of the oilseed rape, hence explaining the nature of the curve in the figure of yields and harvested area in Hungary.

2.2 Pollen beetle (*Brassicogethes aeneus*)

One of the predominant pests wreaking havoc in oilseed rape in Europe is the pollen beetle (*Brassicogethes aeneus) (*SKELLERN, 2018). They cause considerable damage to various cruciferous crops throughout Europe (BROMAND, 1990). (HANSEN, 1996), reported that the level of harmfulness of *Brassicogethes aeneus* differs between European countries; in Denmark, Scotland, and Switzerland, one pollen beetle per plant. In France, 2-3 beetles per plant when the buds are green, while in England, 15 pollen beetles per plant can inflict harm on the oilseed rape. In the United Kingdom, the population of pollen beetles have increased by 162% since the 1980s, which is not directly proportional to an increase in oilseed rape.

This kind of pest is univoltine, meaning it feeds on flowers both as a larva and an adult. Currently, insecticides are used to control it, but populations are becoming more resistant to pesticides like pyrethroids. Resistance to pyrethroid insecticides in beetles found in Europe has been observed as early as 1999, and concerns regarding managing these beetles in natural environments have been extensively reported (SLATER et al., 2011). Due to resistance imposed by pollen beetles to pyrethroids (EPPO workshop, 2006), the proposed alternation of pyrethroids with thiacloprid.

The evolution of robust resistance mechanisms in the pollen beetles exemplifies the ability of certain insect species to adapt and withstand the most active ingredients employed for their control in European regions (ZAMOJSKA et al., 2009). In substantial quantities, the beetle can inflict considerable harm to the plant. Severe infestations by these beetles led to significant crop losses in northern Germany in 2006, with some growers experiencing a complete yield loss (HILLOCKS, 2012).

Pollen beetles are considered active flyers, and adults overwinter away from Oilseed rape fields (WILLIAMS, 2010). Frequent insecticide applications are commonly employed to safeguard against potential harm caused by pollen beetles (*Brassicogethes aeneus*). (STARA et al.,2018). However, prolonged use of broad-based-spectrum insecticides leads to resistance (BASS et al., 2015)

2.2.1 Main Host Plants

Brassica juncea var. juncea (Indian mustard), *Brassica napus var. napobrassica* (swede), *Brassica napus var. napus* (rape), *Brassica rapa subsp. oleifera* (turnip rape), *Brassica rapa subsp. rapa* (turnip) and *Sinapis arvensis* (wild mustard).

2.2.2 damage caused by pollen beetles

Oilseed rape (*Brassica napus L.*) Stands out as a crucial oilseed crop within the agricultural landscape of Europe(ZAJAC et al., 2016), and *Brassicogethes aeneus* (Fabricius 1775) (Coleoptera: Nitidulidae), commonly known as the pollen beetle, is recognized as a highly destructive pest affecting Brassica oilseed crops in Europe(AUSTEL et al., 2021). Both winter and spring oilseed rape crops are susceptible to a high yield loss caused by the beetle, with the potential for a yield reduction exceeding 80% in spring oilseed rape (HANSEN, 2004)

Adult pollen beetles consume pollen from closed flower buds, causing damage that results in bud abscission, leading to podless stalks and a later reduction in yield (JUHEL et al., 2017). They feed on the flower's pollen, nectar and petals, destroying an entire flower. The eggs laid by female pollen beetles are found in the buds and flowers of oilseed rape plants. 2-3 eggs (sometimes up to 10) are deposited in each floral bud, and each female can lay up to 250 eggs in its lifetime. The eggs hatch into larvae. The resulting larvae consume the flowers and harm developing pods more, causing damage to the crop and leading to a significant yield loss. Adult pollen beetles can harm flowering structures from the green to yellow bud stages and feed on non-cruciferous taxa, which contrasts with major herbivorous insects feeding on Brassica, considered specialists on crucifers.

Their feeding activity decreases the number of buds capable of maturing into pods, leading to the absence of pods on the stalks due to damage to the ovary (HANSEN, 2004). *Brassicogethes aeneus* eggs, once deposited in oilseed rape buds, will hatch into larvae, and the larvae develop within buds and drop to the ground to undergo pupation. The new generation of pollen beetle appears a few weeks later, in the early summer, and seeks overwintering sites in woodlands. In the early spring (March-April), the adults migrate from the woodlands to Oilseed Rape fields to feed on pollen and oviposit. If they arrive before flowering, they destroy the buds to feed and can inflict severe yield losses (JUHEL et al., 2017).

When temperatures rise after overwintering in spring, the adult pollen beetles feed on the pollen of the various plants since they are considered pollinivorous. The movement of the pollen beetles is influenced by climate and the closeness of the overwintering sites (WILLIAMS-COOK, 2010) . As the temperatures rise gradually to above 15 degrees Celsius, *Brassicogethes aenues* adults seek out oil seed rape plants to feed with a combination of its tactile, olfactory and visual cues, which aids the pest in finding the host plant and the site of oviposition. Oilseed rape is a member of the Brassicaceae family; thus, it has a range of glucosinolates (GSLs) (RAHMANPOUR et al., 2010) . Hence, the myrosinase-glucosinolate defence system of Brassica plants is essential in interactions with the beetles. Pollen beetles are attracted by the yellow colour of the flowers (GIAMOUSTARIS-MITHEN, 1996) , and it has been seen to use upwind anemotaxis to navigate towards their host crops (WILLIAMS et al., 2007)

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Significant damage is caused by adult pollen beetles while feeding during the green bud stage, which lasts around 1-3 weeks (BBCH growth stage 51–59) (FERGUSON et al., 2015). Many studies point out that tiny buds suffer most from adult feeding effects, while medium-sized buds are used by the pollen beetles for oviposition (SEIMANDI-CORDA et al., 2021). The adults destroy petals, sepals and carpels when accessing the pollen, leading to immense bud abortion and directly affecting the grain yield. Damaged carpels may lead to the abortion of floral buds during oviposition, and a high density of larvae destroy the flower. However, (WILLIAM et al., 1978) did not find an effect of larval infestation on the setting of oilseed rape pods.

Figure 6: *Brassicogethes aenues* on Oilseed rape bud

Source; https://www.shutterstock.com/image-photo/brassicogethes-formerly-meligethes-aeneus-abundant-pollen-1043405188

Although pollen beetles can be highly damaging, Oilseed rape has a considerable tolerance (PINET et al., 2015). The development of supplementary pods can tolerate damage in oilseed rape. However, an oilseed rape plant facing other abiotic and biotic stresses reduces its ability to tolerate pollen beetle attacks.

Pollen beetle population on oilseed rape plants depends on the growing stage, duration of flowering and production of lateral stems. Variations in the number of pollen beetles, sex ratio and oogenesis are accounted by dissimilarity in phenological stages of rape for feeding and oviposition (ŠEDIVÝ, 1993), this pest is a primary target of insecticides applied on oilseed rape plants in spring, and sometimes, more than one treatment is done to achieve sufficient control (RICHARDSON, 2008)

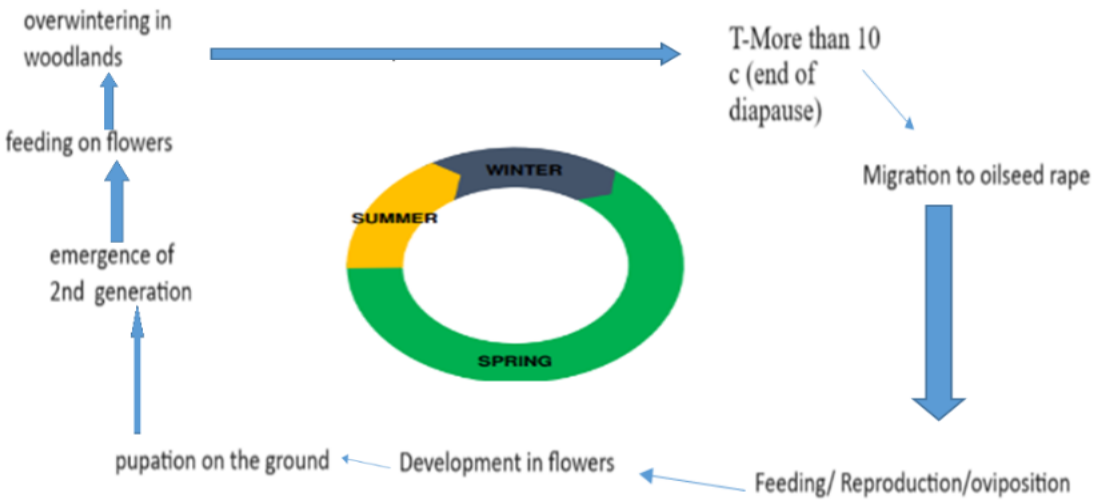

Figure 7: Life cycle of the pollen beetles (*Brassicogethes aenues*)

2.2.3 Factors enhancing the resistance of pollen beetles to a selected insecticide.

Resistance of insects to insecticides results from the increased ability of the individual insect species to survive insecticide treatment (COMINS, 1977). Insecticide resistance of pollen beetles to pyrethroid is believed to be mediated by detoxification carried out by cytochrome P450s and mutation of the pyrethroid target-site, voltage-gated sodium channel (ZIMMER et al., 2014). According to (HECKEL, 2012), (LI et al., 2007) affirm that insecticide resistance progresses by two main mechanisms: increased levels of detoxification enzymes leading to metabolic resistance and target-site mutations resulting in reduced binding affinity of the respective insecticides. Contact and systemic action against both larvae and adults of oilseed rape pollen beetles enhance the field efficacy of thiacloprid when compared with pyrethroid (ZIMMER et al., 2014)

2.2.4 Symptoms of pollen beetle attack

Presence of beetles with the head and body (adult 1.9- 2.7 mm long) being black with metallic shine or green, blue or purple and the legs are lighter up to dark yellow, and its antennae length is nearly long as the width of the head crawling on the oilseed rape flowers affirms the infestation of the *Brassicogetehs aenues* (HRUDOVÁ et al., 2023). Floral and bud abortion resulting in a podless stalk shows severe damage by the pollen beetles (COOK et al., 2002). Holes on the buds due to oviposition by the female pollen beetles are also a sign of pollen beetle damage (ULBER et al., 2010).

Figure 8: Image of *Brassicogethes aenues*

Source;https://bugguide.net/images/cache/DK4/K1K/DK4K1K5KEK1QA06QO06QHS2QRS8KHSWQY0XKBK5KHSXKTK5KAKHKPKGQ2K2Q2K8QWK5QNKKKD0EQLSVQCKNQRS7K30.jpg

CHAPTER 3. MATERIALS AND METHODS

3.1 Materials

For the laboratory, the following materials were used to experiment insect-proof containers, fine pointed brush, beakers for test liquids, syringes/ pipettes for liquids or weighing balance for solids, acetone, syringes/ pipettes for making dilutions, 20ml glass vials, vial roller (or hotdog roller), small funnel to transfer beetles to vial, microscope or hand lens, paper towels, paper towels and a maximum/ minimum thermometer.

3.2 Method

Samples of pollen beetles (*Brassicogethes aeneus*) were collected using a sweep net from an Oilseed rape growing village known as Sorkifalud (coordinates: 47.13342°N 16.73494°E) in Vas County, Hungary, for two consecutive years which was 19.04. 2018 and 12.04.2019. The locations were given based on previous studies to represent the ecologically different rape-growing areas of the Hungarian Trans-Danubian region. About 200 adult beetles were collected across the infested field, and the pollen beetles collected from a particular field were considered a single sample. The beetles were stored in an aerated plastic container. A dry paper towel was placed at the bottom of the container to prevent excess moisture. Some oil seed rape leaves plus three rape inflorescences were added into the container as a food source and to supply shelter during transportation. The beetles were not subjected to excessive temperature, humidity or starvation stress during transportation and physical handling of the beetles was minimized. The containers were taken to the test laboratory as fast as possible, and on arrival, the beetles were released into a ventilated holding cage and left to recover overnight.

Figure 9: Sweep net

Figure 10: Collected adult Beetle

Figure 11: Place of collection, Sorkifalud

Source: https://en.wikipedia.org/wiki/Sorkifalud

3.2.1 IRAC Susceptibility Test Method No: 021 version 3.4

This method is also called the Adult-Vial- Test for neonicotinoids, using thiacloprid as a reference, based on IRAC Method No. 011 for synthetic pyrethroids. It is used for monitoring the sensitivity of the *Brassicogethes aenues* population in oilseed rape to neonicotinoids which support resistance management approaches in oilseed rape, and it is obligatory to carry out sensitivity monitoring.

The standard test neonicotinoid is thiacloprid (Used as the commercially available formulation 'Biscaya®' (240 g thiacloprid/litre in oil dispersion; preliminary trials revealed that technical material is inappropriate). Other neonicotinoids were not tested yet.

Test containers used for this study were 20ml glass vials with 2.27cm diameter, 8.6 cm length and an inner surface area of 58.218 cm^2

The stock solution was prepared by solving Biscaya® in distilled water (2% of the total solvent volume) and subsequently adjusted to 100ml with acetone. All further dilutions were made with acetone. Glass vials (20ml volume, 58.218 cm2 internal surface area) were filled with 500-1500 µl of the solution for coating purposes. For all bioassay, eight thiacloprid concentrations were used: 1.44 $\mu g/cm^2$ (corresponding to 200% of the typical field rate), 1.08 $\mu g/cm^2$ (corresponding to 150% of the typical field rate), 0.72 $\mu g/cm^2$ (corresponding to 100% of the typical field rate), 0.54 $\mu g/cm^2$ (corresponding to 75% of the typical field rate), 0.36$\mu g/cm^2$ (corresponding to 50% of the typical field rate), 0.18 $\mu g/cm^2$ (corresponding to 25% of the typical field rate), 0.029 $\mu g/cm^2$ (corresponding to 4% of the typical field rate) and acetone solution only used for control. At the start of the coating process, the interior walls of glass vials were entirely covered by the test solution and rotated for 2 hours in a fume hood at room temperature until the acetone was utterly evaporated. Because of the oil dispersion formulation and small amounts of water in the solutions, the vials had to be rotated for a minimum of 2 hours and later subjected to a further evaporation phase without rotation for a minimum of 2 hours (or overnight), which is considered as obligatory before locking and storing the vials. The prepared vials could be stored at room temperature (dark) for eight weeks without significantly losing thiacloprid. Three repetitions of each concentration and control were used. A total of 20 adult pollen beetles were transferred to each test glass vial (with the aid of a funnel), capped, and stored upright at 20 ±2ºC.

Figure 12: Glass vials with 58.218 cm2 internal surface area.

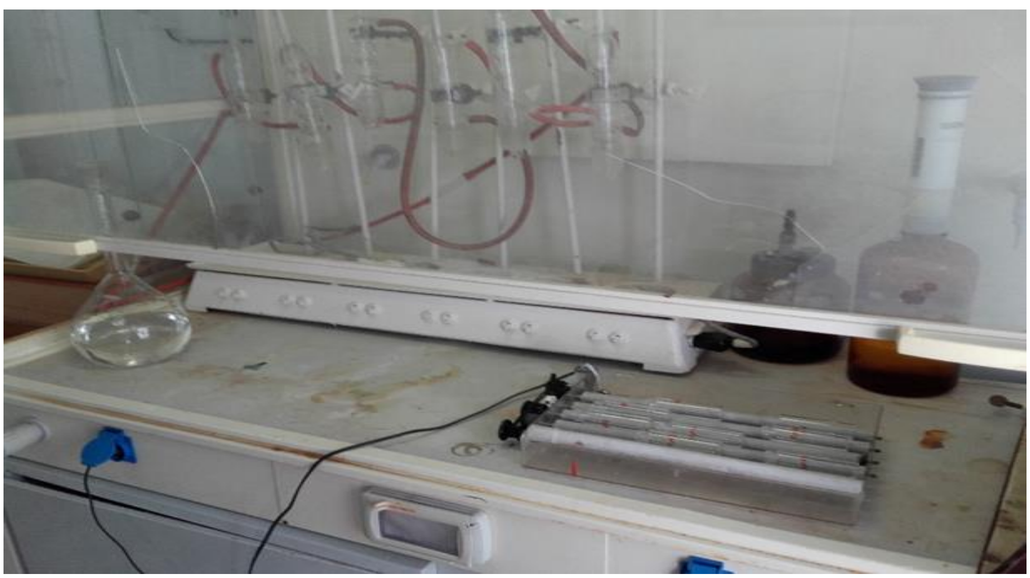

Figure 13: Rotation of the vials in a fume hood to vaporize acetone

The affected beetles (dead and moribund) were assessed after 24 hours using a white paper sheet with a 15 cm diameter circle, and prior to assessment, the vials were briefly shaken to differentiate between the alive and affected beetles quickly. The results were expressed as percentage mortality. If mortality were greater than 20% in the control group, "untreated," then the study would be considered invalid.

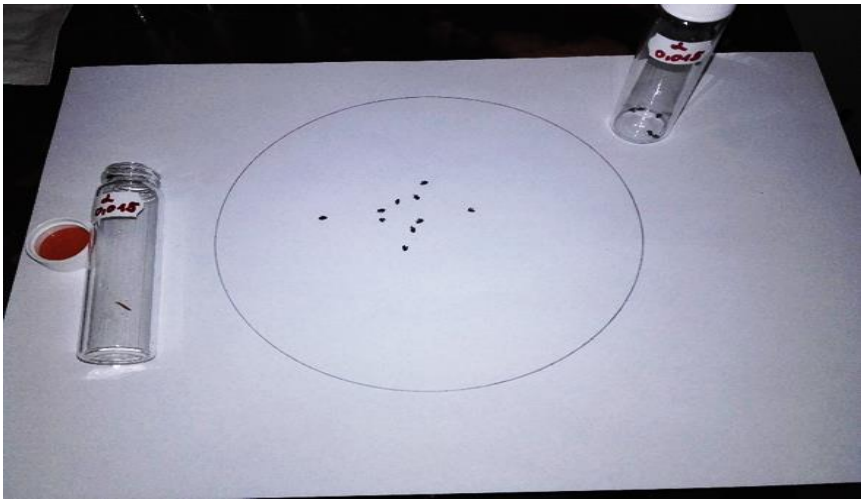

Figure 14: Mortality assessment on a white paper with a 15cm circle

3.3 Statistical Analysis

After being assessed for normality of distribution and homogeneity of variance, collected data did not meet parametric analysis since it was not normally distributed as it is often found in field collection experiments and even after transformation. Therefore, data was analyzed using Kruskal-Wallis, a one-way non-parametric ANOVA. A pair-wise comparison was performed using the Mann-Whitney U test to determine/ recognize significant differences in the treatments used in both years. The significance values have been adjusted by the Bonferroni correction for multiple tests, and mortality graphs against the various concentrations used were done for 2018 and 2019 to visualize the results quickly. All statistical procedures were performed using SPSS Software.

Results were compared with the existing IRAC classification scheme for thiacloprid, which is based on percentage mortality scored at:

200% of field rate: 98% ± 3%

100% of field rate: 93% ± 6%

20% of field rate: 50% ±10%

CHAPTER 4. RESULTS AND DISCUSSION

Approximately 1300 adult pollen beetles (*Brassicogethes aenues*) were collected in Sorkifalud, an oilseed rape cultivating area in Hungary, during the two-year study period (2018 and 2019), and their susceptibility levels to neonicotinoids insecticides (active ingredient thiacloprid) were analyzed. Each pollen beetle collection population was subjected to an adult vial test 021 according to IRAC methods with an extended concentration range for curve generation. It is worth noting that pollen beetles are active flyers, and adults hibernate away from oilseed rape fields and the migration is influenced by climate and proximity to oilseed rape fields (WILLIAMS et al., 2010); thus, the results obtained represent the susceptibility of pollen beetles in a specific geographical position.

After subjecting the data for 2018 and 2019 to non-parametric, Kruskal-Wallis one-way Analysis of Variance (ANOVA), it showed that the distribution of mortality is the same across the dose μg/cm² categories. Each asymptotic significance of each treatment used was displayed, and the significance level was 0.05.

In 2018, there were significant differences in treatments in some of the concentrations of the active ingredient. For instance, when the concentration was 1.08 μg/cm², it had a value of 0.016, and that of 1.44 μg/cm² had a value of 0.01 in the first repetition and the second repetition, the concentration of the active ingredient of 1.08 μg/cm² and 1.44 μg/cm² registered a significant difference with the values being 0.037 and 0.04 respectively (Table 1-marked in yellow), which was less than significant value of 0.05. The same concentrations in 2019 had significant values as high as 1.00 levels, contrasting with 2018's. This indicates that a population of Brassicogethes aenues in Sorkifalud has reduced susceptibility to thiacloprid.

In 2019, concentrations of 0.540 μg/cm² and 0.720 μg/cm² registered a significant difference, with p values being 0.026 and 0.02, respectively (Table 2-marked in yellow), below the significant value of 0.05 while in 2018, the same concentration had significant values of 0.721 and 0.129. This affirms a shift in the pollen beetle's susceptibility level to thiacloprid in both years. However, most of the sample's treatment had values above the significance of 0.05. Significance differences displayed in both years in different sample treatments show that resistance of thiacloprid by oilseed rape pollen beetles' population is building up and spreading in Sorkifalud, Hungary.

Table 1: Kruskal–Wallis mortality test for 2018

Each node shows the sample average rank of Dose µg/cm2.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0.000-0.029	-1.500	6.615	-.227	.821	1.000
0.000-0.180	-6.750	6.615	-1.020	.308	1.000
0.000-0.360	-10.750	6.615	-1.625	.104	1.000
0.000-0.540	-14.750	6.615	-2.230	.026	.721
0.000-0.720	-18.750	6.615	-2.834	.005	.129
0.000-1.080	-22.750	6.615	-3.439	.001	.016
0.000-1.440	-26.750	6.615	-4.044	.000	.001
0.029-0.180	-5.250	6.615	-.794	.427	1.000
0.029-0.360	-9.250	6.615	-1.398	.162	1.000
0.029-0.540	-13.250	6.615	-2.003	.045	1.000
0.029-0.720	-17.250	6.615	-2.608	.009	.255
0.029-1.080	-21.250	6.615	-3.212	.001	.037
0.029-1.440	-25.250	6.615	-3.817	.000	.004
0.180-0.360	-4.000	6.615	-.605	.545	1.000
0.180-0.540	-8.000	6.615	-1.209	.227	1.000
0.180-0.720	-12.000	6.615	-1.814	.070	1.000
0.180-1.080	-16.000	6.615	-2.419	.016	.436
0.180-1.440	-20.000	6.615	-3.023	.002	.070
0.360-0.540	-4.000	6.615	-.605	.545	1.000
0.360-0.720	-8.000	6.615	-1.209	.227	1.000
0.360-1.080	-12.000	6.615	-1.814	.070	1.000

Table 2:Kruskal–Wallis mortality test for 2019

Each node shows the sample average rank of Do...

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
0.000-1.440	.000	6.567	.000	1.000	1.000
0.000-0.029	-6.000	6.567	-.914	.361	1.000
0.000-0.180	-10.000	6.567	-1.523	.128	1.000
0.000-0.360	-14.375	6.567	-2.189	.029	.801
0.000-1.080	-17.875	6.567	-2.722	.006	.182
0.000-0.540	-21.750	6.567	-3.312	.001	.026
0.000-0.720	-26.000	6.567	-3.959	.000	.002
1.440-0.029	6.000	6.567	.914	.361	1.000
1.440-0.180	10.000	6.567	1.523	.128	1.000
1.440-0.360	14.375	6.567	2.189	.029	.801
1.440-1.080	17.875	6.567	2.722	.006	.182
1.440-0.540	21.750	6.567	3.312	.001	.026
1.440-0.720	26.000	6.567	3.959	.000	.002
0.029-0.180	-4.000	6.567	-.609	.542	1.000
0.029-0.360	-8.375	6.567	-1.275	.202	1.000
0.029-1.080	-11.875	6.567	-1.808	.071	1.000
0.029-0.540	-15.750	6.567	-2.398	.016	.461
0.029-0.720	-20.000	6.567	-3.046	.002	.065
0.180-0.360	-4.375	6.567	-.666	.505	1.000
0.180-1.080	-7.875	6.567	-1.199	.230	1.000
0.180-0.540	-11.750	6.567	-1.789	.074	1.000
0.180-0.720	-16.000	6.567	-2.437	.015	.415
0.360-1.080	-3.500	6.567	-.533	.594	1.000

Graphs of the mean mortality of pollen beetles against the dosage used were done for 2018 and 2019. These graphs facilitate a more straightforward interpretation of the data and visualize the susceptibility status of the pollen beetles based on the concentration used and the mortality rate. Different concentrations of the active ingredient corresponded to a different mortality rate. A low mortality rate is realized in lower concentrations, while high mortality rates are registered in higher concentrations. Mortality rates increased as the concentration of the active ingredient increased.

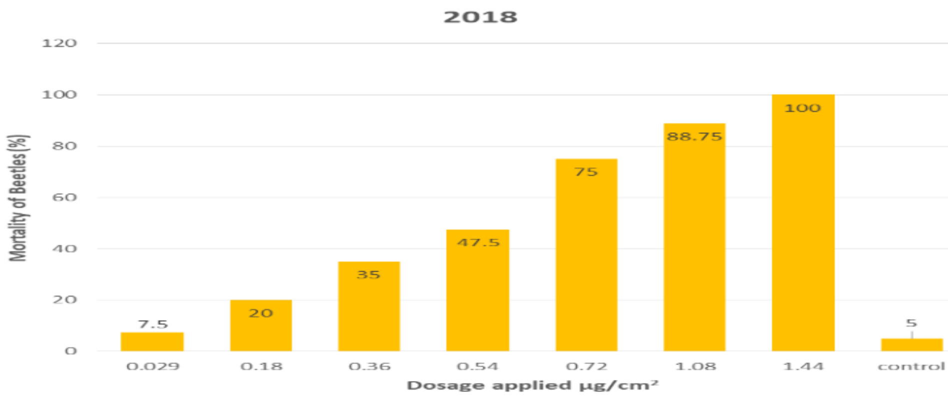

Figure 15: Graph of mortality for 2018

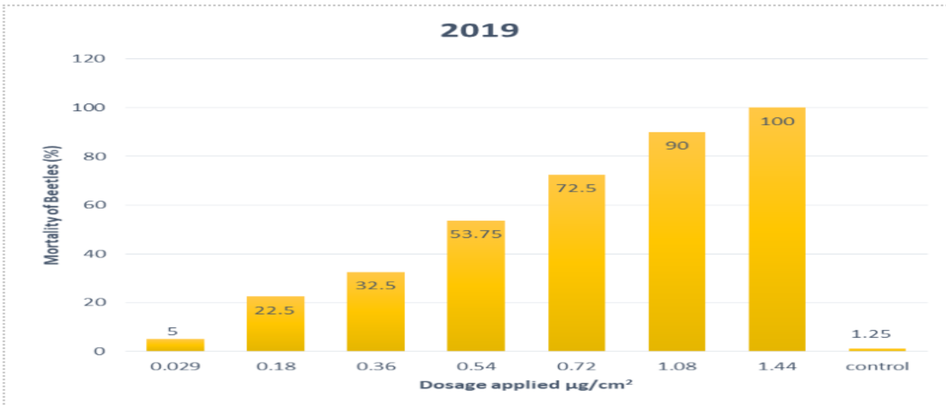

Figure 16: Graph of mortality for 2019, Sorkifalud

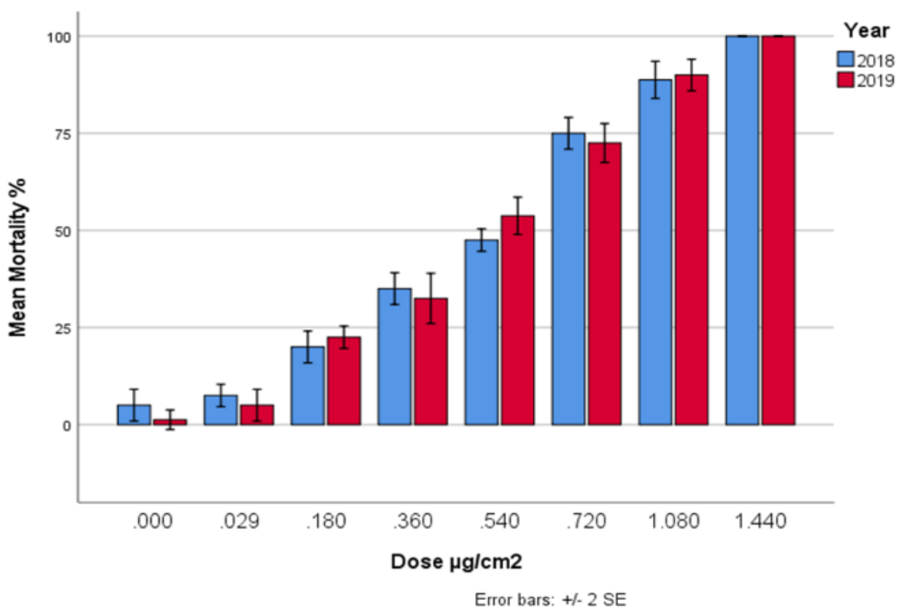

Figure 17: Graph of mortality for 2018 and 2019 combined

Different active ingredients of thiacloprid displayed a varying degree of mortality, ranging from the lowest concentration of 0.029 µg /cm^{2,} corresponding to 4% of the typical field application rate, to 1.44 µg /cm^2 of the active ingredient, corresponding to 200 % of the typical field application rate in both years. The graphs make it easy to visualize and compare the mortality rates between 2018 and 2019. In both years, the concentration of 1.44 µg /cm^2 could register a 100 % average mortality rate of the oilseed rape pollen beetles. A 100 % mortality rate of 1.44 µg /cm^2 of the active ingredient of thiacloprid corresponds to the IRAC expected value, hence affirms that the *Brassicogothes aenues* population in the Sorkifalud area is susceptible to thiacloprid at 1.44 µg /cm^2

1.08 µg /cm^2 of the active ingredient (150 % of the typical field rate) registered a mortality rate in 2018 and 2019 of 88.75 % and 90%, respectively. This mortality rate represents a decline in the susceptibility rate of the pollen beetle to the active ingredient at this application rate. 1.08 µg /cm^2 is supposed to cause a high mortality rate of up to 100 %. This also affirms the significantly different values obtained when the treatments were subjected to the Mann-Whitney U test.

Active ingredients of 0.72 µg/cm² (100% of the typical field rate) of thiacloprid could cause a mortality rate of 75 % and 72.5 % in 2018 and 2019, respectively. The percentage of mortality rate registered at 0.72 µg/cm² is way below the expected value of thiacloprid at 100 % typical field application rate by the Insect Resistance Action Committee (IRAC), which is supposed to be 93% ± 6 %. A concentration of 0.18 µg/cm² (25 % typical field rate) caused a per cent % mortality rate of 20 % and 22.5 % in 2018 and 2019, respectively, in the sample. This is way below the expected percentage value of IRAC that the concentration could cause at a 25 % typical field rate. Reduced susceptibility rate of oilseed rape to thiacloprid at 1.08 µg /cm^2, 0.72 µg/cm² and 0.18 µg/cm² is an indication of the presence and spread of a resistant population of oilseed rape pollen beetles to thiacloprid in Sorkifalud, Hungary.

In 2014, a study carried out to determine susceptibility levels of Danish pollen beetles against thiacloprid presented a finding that the pollen beetle was highly susceptible to the active ingredient, with a dose of 20% application rate of thiacloprid could cause a mortality rate ranging between 55 to 100% (KAISER et al., 2018b). In six Swedish pollen beetle populations, the same dose of 20% could cause a mortality rate of 80-100% (KAISER et al., 2018b). Compared with this study's findings, a 25 % application rate of thiacloprid caused a mortality rate of 20 and 22.5 % in 2018 and 2019, respectively. This affirms that there is a population of Hungarian oilseed rape pollen beetles which is resistant to thiacloprid.

It is worth noting that studies carried out in different regions on susceptibility levels of thiacloprid in Europe presented a diverse lethal concentration level of the active ingredient, which does not directly indicate field resistance or product failure of thiacloprid to oilseed rape pollen beetles but variance in thiacloprid toxicity could be an indicator for development of resistance by the pollen beetle population in different regions necessitating constant monitoring (SPARKS et al., 2015)

In the Czech and Slovak Republics, (SEIDENGLANZ et al., 2015) reported a decreasing trend of common pollen beetle sensitivity to thiacloprid for a population sampled from 2011- 2013. This indicates that a resistant population of pollen beetles to thiacloprid has developed in diverse regions in Europe, and this corresponds well with this study's findings in Sorkifalud-Hungary. (SEIDENGLANZ et al., 2015b) Indicated that there was a statistically significant cross-resistance between lambda-cyhalothrin (pyrethroid) and thiacloprid, but (SPITZER et al., 2020) in their study determined no significant cross-resistance between thiacloprid and other active ingredients such as lambda-cyhalothrin, etofenprox and chlorpyrifos.

The presence of resistant Oilseed rape pollen beetles to thiacloprid in Sorkifalud has a significant implication to plant protection strategies that are in place for oilseed rape. This highlights the need for continuous monitoring and survey of pest populations to ensure better management strategies are implemented. Most of the active ingredients used in the class of neonicotinoids, such as imidacloprid, clothianidin, and thiamethoxam, were subjected to a partial ban in 2013 and thiacloprid use was not renewed in 2020 due to public and political scrutiny because of the growing concerns raised by beekeepers and bee researchers. Despite this, other active ingredients, such as acetamiprid, are still used in the European Union to manage pests. These regulations present a severe challenge of managing the Oilseed rape pest to both the farmers and researchers since pyrethroid use is faced with a severe resistance issue.

However, contrasting findings related to this study suggest that *Brassicogethes aenues* is still highly susceptible to thiacloprid. Present authors support the view of (ZAMOJSKA et al., 2009) that continuous monitoring for the development of resistance against different classes of insecticides used especially for neonicotinoids should be done since thiacloprid and acetamiprid display lower intrinsic activity and are used in lower rates compared to other active ingredients such as lambda-cyhalothrin. Thus, once resistance sets in, the field performance of thiacloprid and acetamiprid will likely be affected much faster than that of pyrethroids.

The results of this study indicate the presence and spread of resistant oilseed rape pollen beetles (*Brassicogethes aenues*) in the Sorkifalud- western region of Hungary to thiacloprid, With a decline in mortality rate observed in most of the concentrations used in both years of the study. In contrast, the pollen beetle is still susceptible at 1.44$\mu g/cm^2$ (200% of the typical field rate application rate). This study suggests a chance of reduced field performance of thiacloprid if applied in emergency cases. These findings have a significant implication for plant protection strategies and highlight the need for continuous monitoring, enhancement of integrated pest management approaches, and adjustments to pesticide application rates and strategies to manage resistant pest populations effectively.

CHAPTER 5. CONCLUSION AND RECOMMENDATION

Results presented in this susceptibility monitoring study identify that there is reduced sensitivity of thiacloprid to oilseed rape pollen beetles in most of the concentrations. This signals that *Brassicogethes aenues* is developing resistance towards thiacloprid. (SEIDENGLANZ et al., 2015b) Moreover, (SEIDENGLANZ et al.,2014) reported a decreasing trend of Pollen beetle population in the Czech and Slovak Republic sensitivity to thiacloprid and statistically significant cross-resistance between lambda-cyhalothrin and thiacloprid and thus affirms the results presented in this study that there is a developing trend of resistance of pollen beetle to the thiacloprid.

Monitoring of oilseed rape pollen beetles' resistance to insecticides is an essential aspect of insect management strategies, and it is pertinent to understand the development and spread of resistance to assist in developing and implementing appropriate control strategies for the pest (KAISER et al., 2018b). Resistance of pests to insecticides presents a significant challenge in oilseed rape production as the efficacy of the insecticides reduces, increases the cost of production and poses a significant health hazard to the environment and human health. Farmers, when uninformed about the resistance, will tend to increase the concentration of the active ingredient to counter it.

Even though the application of thiacloprid was terminated in 2020 since the approval of thiacloprid and other plant protection products was not renewed (European commission-pesticide database, 2020) due to the impact it poses on bee population and general human health, it is worth monitoring it susceptibility levels. Article 53 of the European union commission of pesticide regulation (EC) NO 1107/ 2009 "the regulation" allows the member states to place in the market plant protection products in exceptional circumstances and derogating from the regular authorization process for a period not exceeding 120 days (about four months) and for limited and controlled use, where such a measure is necessary because of a danger which cannot be contained by any other reasonable means

(Pesticides_aas_guidance_wd_emergency_authorisations_article53_post-210301 (1)) and thus these terminated/ banned plant protection products can still find it way in the market in emergency cases.

A study by the Pesticide Action Network in Europe (PAN), a non-governmental organization, reported that pesticide active substances banned in the EU are still in the market due to emergency derogation measures. For instance, 236 emergency authorizations were granted for 14 substances between 2019 and 2022. Austria was a champion of derogation, and Finland, Romania, Czech Republic, and Greece were among, where these banned products were included in the market. This affirms that susceptibility levels for this active substance should be constantly monitored. Pesticide Action Network (PAN Europe) proposes, in line with EU regulations, that nonchemical strategies should be prioritized before synthetic pesticides, and derogation should be exceptionally granted in emergencies when all other integrated pest management strategies are not applicable.

Since the use of most active substances in the class of neonicotinoids is restricted, and thiacloprid has been considered an excellent substitute for pyrethroid since 2007, its possibility of use was terminated in 2020. Thus, protecting oilseed rape against pollen beetles is becoming a challenging issue day by day for farmers and researchers, and thus, developing insecticides with new modes of action would be of significance in the current resistance of pests to insecticides in oilseed rape farming.

Breeding for plant resistance to insect pests (HERVÉ-CORTESERO, 2016) , in addition to adopting diverse cultural measures (VEROMANN et al., 2013), can assist in combating the menace of insecticide resistance induced by pests. Breeding involves the identification of a resistant gene (from naturally occurring cultivars) and transferring it to the plant through selection and introgression. Using resistant plants will reduce the number of times synthetic pesticides are applied, reducing the selection pressure.

Insecticides, when applied at sub-lethal dosage or without exposing the pest to a sufficient dose of the active ingredient, will create selection pressure, leading to the development of resistance in the population. This arises either due to professional error or omission from the plant protection advisors and the indiscriminate use of pesticides by the farmers. Repeated use of the same class of insecticides without rotation can contribute to resistance, and to overcome the challenge, it is advisable to constantly rotate the plant protection products applied to reduce the chances of resistance development.

Efforts in place in managing the resistance of pests to insecticides need to include a reduction in prophylactic insecticide application through improved pollen beetle forecasting (FERGUSON et al., 2016) and monitoring (SKELLERN et al., 2017) to detect early signs of resistance. It entails tracking the efficacy of insecticides and conducting regular resistance screening tests to identify resistant populations. Resistance bursting agents should be used if resistance is seen to mitigate it. The agents target specific biochemical pathways not targeted by the insecticides previously used. Furthermore, the determination of a more accurate control threshold is needed for the damaging pest in oilseed rape production (RAMSDEN et al. 2017).

Increasing professional competence by imparting essential skills to handle and apply plant protection products to agricultural advisors and farmers is highly significant. It entails sensitizing stakeholders on the need for integrated pest management (IPM) principles, which aim to use diverse pest management strategies and minimize synthetic pesticides. Research is ongoing to develop alternative control strategies, especially in enhancing biological control methods (DORN et al., 2014).

Scientists are trying to find a viable alternative to neonicotinoids, which an alternative can replace with little or no environmental impact. Unfortunately, the chemical alternatives to replace neonicotinoids in the market also have a high environmental impact. Thus, its immediate ban has led to increased losses in yield and extra costs incurred in oilseed rape production.

In conclusion, developing resistance in pest population is a significant challenge in pest management resulting from inappropriate insecticide use leading to selection pressure and thus a raft of measures such as increasing availability of pesticide data in Europe for better analysis of use and environmental impact, enhancing integrated pest management strategies, encouraging arable crop rotations, monitoring of pest occurrence in various regions, use of collective crop insurance and stimulating circumstances in which natural enemies (parasitoids) of pests thrive. These measures will reduce the need for broad-spectrum insecticide use in oilseed rape production, which will minimize the negative impacts caused by insecticides on the environment and to the public health.

CHAPTER 6. SUMMARY

Oilseed rape pollen beetles *Brassicogethes aenues* are one of the predominant pests wreaking havoc in European oilseed rape fields. Managing oilseed rape pollen beetles has been historically through the application of insecticides, such as pyrethroids. Due to resistance imposed by pollen beetles to pyrethroids, European and Mediterranean Plant Protection organisation (EPPO workshop, 2006), proposed alternation of pyrethroids with thiacloprid. As recommended by the insecticide resistance action committee (IRAC), monitoring of insects' resistance should be done to detect early shifts in susceptibility levels. Therefore, an Adult Vial Test on a thiacloprid 240g L^{-1}OD formulation (Biscaya®) was developed and validated to aid in monitoring the susceptibility of pollen beetles to thiacloprid.

Samples of oilseed rape Adult pollen beetles were collected from the oilseed rape growing region known as Sorkifalud in Hungary in 2018 and 2019. The collection was done under the guidelines of IRAC. They were subjected to different concentrations of treatments of thiacloprid and acetone as a control to determine their susceptibility status to thiacloprid.

The method used entailed IRAC Susceptibility Test Method No: 021 version 3.4 (Adult- Vial- Test for neonicotinoids) using thiacloprid as reference. Analysis was performed using the Kruskal-Wallis mortality test. The results for the two growing seasons, 2018 and 2019, portrayed the same distribution of mortality percentages across the dose μg/cm². A lower mortality rate is realized against concentrations used compared with expected mortality rates in the IRAC baseline mortality/susceptibility reference guideline. This indicates the presence of resistant oilseed rape pollen beetle population to thiacloprid in Sorkifalud. A concentration of 1.44 μg/cm (200% typical field application rate) could only cause a 100% mortality rate in both growing seasons. The presence of thiacloprid-resistant *Brassicogethes aenues* population calls attention to effective management strategies for oilseed rape insect pests, such as; enhancing integrated pest management (IPM) strategies.

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