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RESEARCH CONCERNING POSSIBLE ALTERNATIVES AT SEED TREATMENT WITH NEONICOTINOIDS FOR CONTROLLING THE *Tanymecus dilaticollis* Gyll ATTACK AT SUNFLOWER CROPS

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Abstract

Maize leaf weevil (Tanymecus dilaticollis Gyll) is one of the main pests of the sunflower in Romania. This pest can destroy sunflower seedlings. Seed treatment with systemic insecticides was the most effective measure for maize leaf weevil control. After the ban of the neonicotinoids in the EU, no alternative for sunflower seed treatment remained available in Romania. This study has tested possible alternatives for replacing the neonicotinoids, for controlling the maize leaf weevil. The experience was carried out at the NARDI Fundulea, in the southeast of Romania, in 2021 and 2022. This study has tested seed treatment with cypermethrin active ingredient, seed treatment with cypermethrin, followed by foliar treatment with cu deltamethrin or acetamiprid, seed treatment with neem oil, and treatment with a biological insecticide on the base of Beauveria bassiana entomopathogen fungus, in two doses. This study, in both years, hasn't registered significant statistical differences concerning weevil density between treated sunflower plots and control (untreated) plots. Regarding maize leaf weevil attack on sunflower plants, in both years it hasn't registered significant statistical differences between the control and treated plots.

Key words: *sunflower, weevils, damages, early stages.*

INTRODUCTION

Maize leaf weevil is one of the most dangerous pests for Romania's maize and sunflower crops, especially in the country's south and southeast areas (Popov et Bărbulescu, 2007; Antonie et al., 2012; Georgescu et al., 2014, 2021; Badiu et al., 2019; Trotuș et al., 2015; 2019; Toader et al., 2020; Fătu et al., 2023; Pintilie et al., 2023). The weevils attack crops in early vegetation stages, from the plant's emergence (BBCH 00) to the four-leaf stage (BBCH 14) (Paulian, 1972). In case of a high attack, crops can be destroyed, and farmers must sow again (Bărbulescu et al., 2001b; Čamprag, et al., 2007; Roșca et Istrate, 2009; Vasilescu et al., 2005; Georgescu et al., 2018). At sunflower seedlings, the adult insect can cut the stem close to the soil level. In that case, the plant perishes (Roșca et al., 2011). Data from the literature prove that in Romania, this pest attacks one million hectares of maize and one-half million hectares of sunflowers yearly (Bărbulescu et al., 2001a; Popov, 2002; 2003; Popov et al., 2005, 2007a). The main area damaged by this pest from the European Union is Romania (Meissle et al., 2010). There are possible explanations for this fact, such as maize or sunflower monoculture, short rotation (the crop is sowed on this plot after two years), global warming, and lack of chemical treatments at seeds with systemic insecticides, after the ban of the neonicotinoids in EU (Păcureanu et al., 2007; Rîșnoveanu et al., 2016; Lup et al., 2017; Official Journal of the European Union, 2018a, 2018b, 2018c; Anton et al., 2023; Popescu et al., 2023). According to data from the Ministry of Agriculture, the area cultivated with sunflowers increased in the last decades in Romania (Romanian Statistical

Yearbook, 2023; MADR data, 2024). Over 1.1 million hectares of this crop will be sown in some years, even 1.2 million hectares in 2019 (MADR data, 2024). A possible reason for this is that the price of sunflower oil is higher because of the increasing demand for it in international markets (Popescu, 2020; Panzaru et al., 2023). However, most areas cultivated with this crop are the most favorable for this pest in the south and southeast of Romania (Popov et al., 2006; Brumă et al., 2021). At the same time, these areas are cultivated in high areas with maize (Dinca et al., 2020; Dragomir et al., 2022). Because of this fact, sunflowers cultivated in the south and southeast of Romania are in constant threat every spring because of the higher biological reserve of the maize leaf weevil (Popov et al., 2007b; Badiu et al., 2019; Georgescu et al., 2021). As a result of the lack of alternatives to sunflower seed treatment for controlling this pest, in the last decade, Romania's temporary authorization for neonicotinoid insecticides used in sunflower seed treatment was to protect the plants in early vegetation stages against weevil attacks (Trotuș et al., 2019; Amuza et al., 2021; Leone, 2022). However, it is uncertain if there will be future authorizations for seed treatment with neonicotinoids in Romania (Zaharia et al., 2023). A possible consequence of this is that sunflower, an important crop for this country, will be threatened by the maize leaf weevil without effective control measures, which will have negative consequences for Romanian agriculture and the economy (Ionel, 2014; Kathage et al., 2018; Stoicea et al., 2022). It is necessary to find alternatives to seed treatment with neonicotinoids for controlling maize leaf weevil attacks on the sunflowers. This paper presented the results of a study from the southeast of Romania on the different maize leaf weevil control measures in the absence of neonicotinoid seed treatment.

MATERIALS AND METHODS

Field experience was made at the Plant Protection Collective from Agrotechnics Laboratory, National Agricultural Research and Development Institute Fundulea, Călărași County, Romania (latitude: 44°46' N; longitude: 26°32' E; alt.: 68 m a.s.l.), in the spring of 2021 and 2022.

This trial, it has assessed sunflower seed treatment with cypermethrin active ingredient (300 g/l), belonging to the pyrethroid insecticide class, seed treatment with cypermethrin active ingredient (300 g/l) followed by foliar treatment with deltamethrin active ingredient (25 g/l), belonging to the pyrethroid class and cypermethrin active ingredient (300 g/l), followed by foliar treatment with acetamiprid active ingredient, belonging to the neonicotinoid classes. At the same time, it assessed seed treatment with neem oil or soil treatment with *Beauveria bassiana* entomopathogenic fungus in two quantities (Table 1).

Table 1. Active ingredients used in this study

Variant no.	Commercial product name	Active ingredient	Insecticide class	Rate[1]	Type of application
1	Control (untreated)	—	—	—	—
2	Langis+ Faster Delta	Cypermethrin (300 g/l) + deltamethrin (25 g/l)	Pyrethroid+ Pyrethroid	2.0 l/t + 0.3 l/ha	ST+FT[2]
3	Langis+ Mospilan 20 SG	Cypermethrin (300 g/l) + acetamiprid (20%)	Pyrethroid+ Neonicoti-noid	2.0 l/t + 0.1 kg/ha	ST+FT[2]
4	Biosem	neem oil	–	10.0 l/to	ST[3]
5	Langis	Cypermethrin (300 g/l)	Pyrethroid	2.0 l/t	ST[3]
6	–	*Beauveria bassiana*	–	150 kg/ha	GT[4]
7	–	*Beauveria bassiana*	–	300 kg/ha	GT[4]

1 - commercial product
2 - seed treatment (before sowing, BBCH 00) + foliar application (when sunflower was in BBCH 11-12 stage)
3 - seed treatment (before sowing, BBCH 00)
4 - ground application before sowing (BBCH 00)

In 2021, sunflower plants were sown on 10 May. Full plant emergence (BBCH 10) occurred on 19 May, while the four-leaf stage occurred on 27 May. In 2022, sunflower plants were sown on 3 May. Full plant emergence (BBCH 10) occurred on 10 May, while the four-leaf stage occurred on 17 May. In both years, the sowing density was 62000 seeds per hectare, and the sowing depth was 5 cm. For this research, it has sowed Performer sunflower hybrid, created at NARDI Fundulea (Partal, 2022). For this trial, the area of each experimental plot was 1500 m^2.

Assessments:

- According to the EPPO PP1/135 standard, **phytotoxicity** was evaluated on the whole plot when sunflower plants were in the four-leaf stage (EPPO standards, 2014).
- **Pest density** was assessed twice, after plant emergence (BBCH 10) and for the leaves stage (BBCH 14). At each variant, four assessment points were established. It evaluated 100 plants from five rows (20 plants/row) at each assessment point. Before the assessment, plants from each row were marked with sticks in the stair system. It counted weevils from plants or near plants. The pest density was calculated according to a formula elaborated by Paulian (1972), where **E(m^2)** is calculated pest density; **1,2** is a coefficient representing the weevils from plans that are not registered during the assessments; **N** represents the number of the weevils counted on (or near) total plants number from assessments while **d** is number of sunflower plants on row meter.

$$E(m^2) = 1,2 * (\frac{N * d}{100})$$

  The assessment concerning weevil density was made when the air temperature was higher than 20°C. At this temperature, insects' activity on the soil ground is higher.
- **Attack incidence** of *T. dilaticollis* weevils at sunflower was assessed when plants were in the four-leaf stage (BBCH 14). Each plant from the assessment points was photographed with a Panasonic G9 photo camera with a Leica DG O.I.S. lens (12-60 mm, f 2.8/4). Then, images were analyzed by a computer to see if bites were produced by the weevils.
- **Attack intensity** of weevils was assessed when sunflower plants were in the four-leaf stage (BBCH 14). Attack incidence and intensity of maize leaf weevils were assessed at the same plants. Weevils attack was rated on a scale from 1 to 9 (Figure 1), as follows:
  - Note 1: plant not attacked;
  - Note 2: a plant with 2-3 simple bites on the leaf edge;
  - Note 3: plants with bites or clips on all leaf edges;
  - Note 4: plants with leaves chafed in the proportion of 25%;
  - Note 5: plants with leaves chafed in the proportion of 50%;
  - Note 6: plants with leaves chafed in the proportion of 75%;
  - Note 7: plants with leaves chafed almost at the level of the stem;
  - Note 8: plants with leaves completely chafed and the beginning of the stem destroyed;
  - Note 9: plants destroyed, with stem chafed close to soil level.

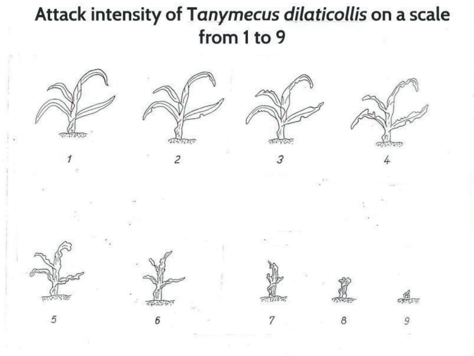

Figure 1. Attack intensity scale (Paulian, 1972, cited by Roșca and Istrate, 2009)

- **Yield** was assessed at the sunflower harvest from this trial on 24 September 2021 and 12 September 2022. Each variant was harvested separately, and the yield was calculated at STAS humidity (14%).

Meteorological data were collected from an automatic weather station 1 km from the experimental site. It has monitored daily air temperature and rainfall amount that occurred in the most sensitive stage of the plants during the weevil attack, from emergence (BBCH 00) to the four-leaf stage (BBCH 14).

Data were **statistically analyzed** using Tukey's honestly significant difference test (HSD) at a significance level of $p \leq 0.05$. For statistical analysis, it has used ARM 2022 software (Gylling Data Management, 2022). The results of the field trial were presented as the absolute and mean values for phytotoxicity, attack incidence and weevil attack intensity, yield, the standard deviation from the average values (SD), the coefficient of variation (CV), replicate F, and treatment F.

RESULTS AND DISCUSSIONS

Data from the meteorological stations reveal that in 2021, the experimental site from NARDI Fundulea registered significant rainfalls in the first 24 hours after plant emergence while temperature decreased (Figure 2). Temperatures increased in the next 48 hours, but the weather remained cloudy. It registered slight rainfall amounts in the last 72 hours of this interval.

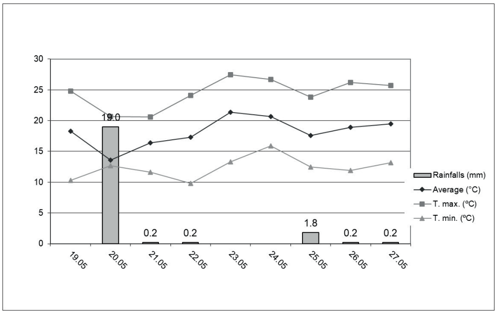

Figure 2. Daily temperatures and rainfalls between the plants' emergence and four-leaf stage in the spring of the year 2021 at the experimental site

In 2022, weather conditions in the early sunflower vegetation stage were less favourable for weevil activity on the ground, including feeding. Maximum temperatures were higher than 20°C on all days, while the average temperature was higher than 15°C in the first two days after plant emergence and higher than 20°C in the next four days (Figure 3).

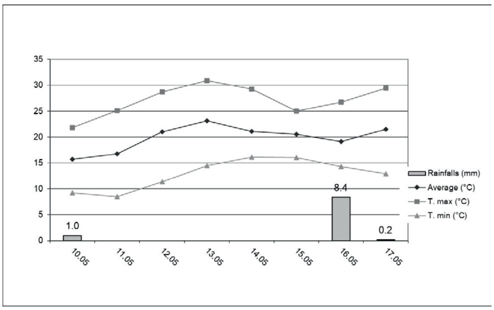

Figure 3. Daily temperatures and rainfalls between the plants' emergence and four-leaf stage in the spring of the year 2022 at the experimental site

At the same time, slight rainfalls amount to 24 hours after plant emergence and higher than 8.0 mm in the last 48 hours before plants arrived at the stage, representing the end of the sunflower critical period for weevil attack.

Roșca and Istrate (2009) mentioned that at a temperature higher than 20°C during the day, weevils activity is higher. Popov et al. (2006) prove that *T. dilaticollis* is a species favored by higher temperatures and drought in spring.

In both years, no phytotoxicity effect was observed after seed treatment with cypermethrin active ingredient or neem oil or after foliar treatment with deltamethrin or acetamiprid active ingredients (Table 2). At the same time, no phototoxicity was recorded in sunflower plants after treatment with *Beauveria bassiana* entomopathogenic fungus on the ground.

Table 2. Phytotoxicity

Variant	2021	2022
Control (untreated)	0a	0a
Langis+ Faster Delta	0a	0a
Langis+ Mospilan 20 SG	0a	0a
Biosem	0a	0a
Langis	0a	0a
Beauveria bassiana (150 kg/ha)	0a	0a
Beauveria bassiana (300 kg/ha)	0a	0a
Tukey's HSD	0	0
SD	0	0
CV	0	0
Replicate F	1.000	1.000
Treatment Prob (F)	1.000	1.000

Means followed by the same letter do not significantly differ ($p \leq 0.05$, Tukey's HSD test)

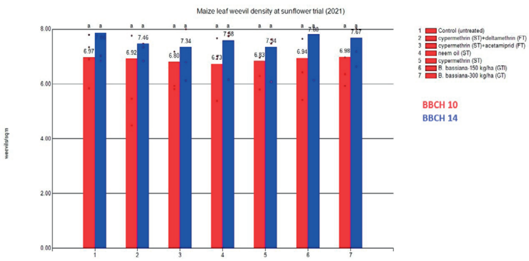

Figure 4. Weevils density at the experimental site, in 2021

Research at NARDI Fundulea by Paulian (1972) concluded that the economic damage threshold for maize leaf weevils (*T. dilaticollis*) species is 5 weevils/m^2. In 2021, at the experimental site, pest density varied slightly from 6.73 to 6.98 weevils/m^2 (Figure 4). The density increased 8 days later when plants were in the four-leaf stage. However, there weren't significant statistical differences between the control variant (untreated) and treated variants ($p \leq 0.05$). This year, the pest density was higher than the economic damage threshold.

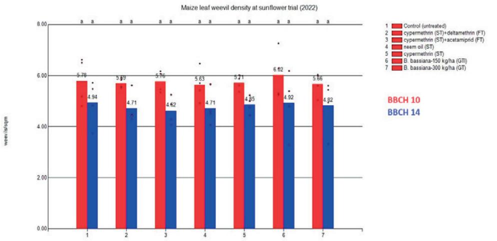

Figure 5. Weevils density at the experimental site, in 2021

In the spring of 2022, after sunflower emergence (BBCH 10) at the experimental site, pest density ranged from 5.63 to 6.02 weevils/m^2 (Figure 5). The pest density decreased when plants were in the four-leaf stage (BBCH 14). This year, the weevil density was higher than the economic damage threshold only at the BBCH 10 stage of the sunflower. Like the previous year, there are significant statistical differences between pest density at the control (untreated) variant and treated variants ($p \leq 0.05$).

Table 3. Weevils attack incidence (%)

Variant	2021	2022
Control (untreated)	100a	100a
Langis+ Faster Delta	100a	100a
Langis+ Mospilan 20 SG	100a	100a
Biosem	100a	100a
Langis	100a	100a
Beauveria bassiana (150 kg/ha)	100a	100a
Beauveria bassiana (300 kg/ha)	100a	100a
Tukey's HSD	0	0
SD	0	0
CV	0	0
Replicate F	1.000	1.000
Treatment Prob(F)	1.000	1.000

Means followed by the same letter do not significantly differ ($p \leq 0.05$, Tukey's HSD test)

Data from Table 3 reveal that in 2021 and 2022, the incidence of maize leaf weevil attacks on sunflower plants was maximum. That means that this pest attacked all plants. This result was similar to those from the previous studies (Georgescu et al., 2014; 2018; 2021; Toader et al., 2020).

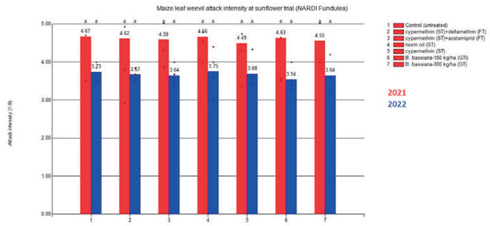

Figure 6. Weevils attack intensity on sunflower plants at experimental site in 2021 and 2022

When sunflower plants were in the four-leaf stage (BBCH 14), it assessed weevils' attack intensity on a scale from 1 (plants not attacked) to 9 (plants destroyed). From NARDI Fundulea's experimental site, higher weevil attack intensity was recorded in the spring of 2021. In the control (untreated) variant, the attack intensity of the weevils at sunflower plants was 4.67 (Figure 6). At the treated variants, only a slightly lower attack intensity was observed from 4.49 to 4.66. It hasn't registered significant statistical differences concerning weevil attack intensity at control (untreated variant) and treated variants ($p \leq 0.05$). The attack intensity of the weevils at sunflower plants in 2022 was lower than in 2021. Like the previous year, there weren't significant statistical differences between variants from this study. Higher attacks from 2021 can be related to higher weevil density but with weather conditions, too. Popov et al. (2006) mentioned that even at higher weevil density, the attack intensity is lower if temperatures are lower and humidity is higher.

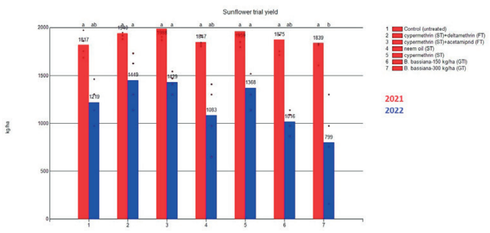

Figure 7. Sunflower yield from experimental site in 2021 and 2022

In 2021, sunflower yield ranged from 1817 kg/ha to 1988 kg ha (Figure 7). There weren't significant statistical differences concerning yield between the control (untreated) variant and the rest of the treated variants ($p \leq 0.05$). In 2022, the yield of the sunflower crop from the experimental site will be lower compared with 2021. The main reason for this fact is the severe drought registered in the spring and summer of 2022 (Partal et al., 2023). Another reason for this is birds attack during early vegetation stages of sunflower.

CONCLUSIONS

The treatment variants tested in this two-year study in one of the most favorable areas for maize leaf weevil weren't effective in controlling this pest during sunflower early vegetation stages.

Seed treatment with cypermethrin active ingredient or neem oil could significantly decrease weevil attacks on young sunflower plants.

A similar situation was in the case of seed treatment with cypermethrin active ingredient followed by foliar treatment with deltamethrin of acetamiprid active ingredients.

In this study, applying the treatment with *B. bassiana* entomopathogenic fungus before sowing the sunflower wasn't effective in controlling the maize leaf weevil.

Further studies are necessary to find effective alternatives to banned neonicotinoid seed treatment to protect sunflower crops in early vegetation stages against maize leaf weevil attacks.

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