

## RESEARCHES CONCERNING CONTROLLING OF THE MAIZE LEAF WEEVIL (*TANYMECUS DILATICOLLIS* GYLL) IN LABORATORY CONDITIONS, USING HIGH PEST PRESSURE, AT NARDI FUNDULEA

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### ABSTRACT

Maize leaf weevil (*Tanymecus dilaticollis*) is main pest of the maize crops in south and south-east of Romania. Every year there were attacked approximate one million of hectares cultivated with maize, with different attack intensity degrees. In many cases pest attack is high, as result maize plants were destroyed and farmers must sow again. The attack is dangerous when plants are in first vegetation stages (BBCH 10-14). In this paper, there were presented results of insecticides testing, used for maize seeds treatment against *T. dilaticollis* attack, in laboratory conditions, using high pest pressure (4 insects/plant). It has used imidacloprid, clothianidin and thiamethoxam active ingredients from neonicotinoid insecticides class. Maize plants were sowed in plastic pots and insects were

added in pots, after plants emergence (BBCH 10). It has recorded daily weevils mortality until 8 days from the starting of the experiment. Also it has recorded attack intensity of the weevils at maize plants, using a scale from 1 (plants not attacked) to 9 (total damages). In laboratory conditions, in case of high pest pressure, at control (untreated) variant the attack intensity of *T. dilaticollis* weevils at maize plants, on a scale from 1 to 9 was 8.63. At the end of the experiment, all plants from untreated variant were destroyed. In case of seeds treated with imidacloprid, clothianidin or thiamethoxam active ingredients pest attack ranged from 4.58 to 4.65. At these variants, weevils mortality ranged from 67.50 to 71.25 %. All maize plants from treated variants survive of the attack.

### INTRODUCTION

According MADR data (2017) and INS data (2018), in the last years, Romanian maize area was higher then 2.4 million hectares, with a grains production higher then 10 million tones (except year 2015), even higher then 14 million tones, in 2017. That means maize is one of the main crops from our country. Data from Eurostat (2017) ascertained that in the last years, Romania occupy first place in EU on maize area, but average production per hectare is still low, comparative with countries from West Europe. Lup et al. (2013) mentioned that high numbers of small farmers that practice subsistence agriculture is a

possible reason for average low maize yield per hectare in Romania. Pests attack represents another cause for maize yield losses (Popov et al., 2001; Popov et Barbulescu, 2007; Rosca et Istrate, 2009; Goga et Rosca., 2011; Trotus et al., 2011, 2013). In south and south east of the Romania, maize leaf weevil (*Tanymecus dilaticollis* Gyll) represent the most important pest for the maize crops (Paulian et al., 1969, Voinescu, 1985; Barbulescu et al., 1993, 2001; Cristea et al., 2004; Popov et al., 2007a). Data from the literature demonstrate that, every year, in Romania there were attacked approximate one

million hectares with maize by this pest (Barbulescu, 2001; Popov, 2002; Popov *et al.*, 2003; 2005; 2006a; 2007a). According Paulian *et al.* (1972) the attack of *T. dilaticollis* is dangerous when maize plants are in first vegetation stages, from plants emergence (BBCH 10) until four leaves stage (BBCH 14). In case of higher attack, maize plants can be destroyed and farmers must sow again (Barbulescu *et al.*, 2001). Same author mentioned that in some cases the attack of the weevils can occurred even before plants emergence. In the advanced stages of the maize, weevils attack is less economically important, they consume only leaf margins and plants survive (Rosca *et Istrate*, 2009). Researches made in Romania and neighborhood countries demonstrate that seed treatment with systemic insecticides represents the most effective method to protect maize plants during first vegetation stages (BBCH 10-14) against *Tanymecus dilaticollis* attack (Paulian *et al.*, 1980; Voinescu, 1985; Barbulescu *et al.*, 2001; Vasilescu *et al.*, 2005; Krusteva *et al.*, 2006; Popov *et Barbulescu*, 2007; Popov *et al.*, 2007b; Keszthelyi *et al.*, 2008; Čamprag, 2011; Trotus *et al.*, 2011; Georgescu *et al.*, 2014; 2015). From beginning of the 2014 the use of neonicotinoid insecticides for seed treatment of the spring crops, including maize, was restricted in EU countries (Official Journal of the European Union, 2013). As result of the EU directive 485/2013 no insecticides remain available for maize seed treatment against *Tanymecus dilaticollis* in Romania. However, between 2014 and 2018, Romania obtained temporary authorizations for use of the neonicotinoid insecticides for seed treatment at maize and sunflower crops during spring. As result of the European Commission Regulations 218/783, 218/784 and 218/785, the use of imidacloprid, clothianidin and thiamethoxam active ingredients for all field crops, both like seed treatment and foliar application will be total banned in EU countries, starting

from 2019. Because of high pest pressure, especially in favorable areas of the maize leaf weevil from south and south-east of the Romania, lack of seed treatment alternatives of the spring crops can have negative impact on Romanian agriculture, in next years (Ionel I.I., 2014).

Seed treatment effectiveness it can be assessed in field conditions after a methodology developed by Paulian (1972). The experiments were placed in monoculture system (maize sowed after maize) to have high pest pressure. However climate conditions from the spring period were different from one year to another. According Popov *et al.* (2006b) *Tanymecus dilaticollis* weevils are very active at high air temperatures and low humidity registered in period when maize plants are the most susceptible for the pest attack (BBCH 10-BBCH 14). Same author mentioned that low air temperatures and high rainfall amount from spring period represent unfavorable conditions for weevils activity. Researches made in last 10 years make in evidence that climate changes from Central and South-East of the Europe countries can have negative impact on local agriculture and, in the same time, can favor pests attack (Olesen *et al.*, 2011; Bebbber *et al.*, 2014). In last years, in south-east of the Romania it has observed atypically weather during spring period (Georgescu *et al.*, 2015). Because of atypically evaluation of the climatic conditions from spring period in the favorable area of *T. dilaticollis* the attack of this pest was different from one year to another. As result, at NARDI Fundulea, Paulian (1972) has developed a laboratory method for testing of the maize seed treatment effectiveness against maize leaf weevil. The advantage of this method is that experiment is carried out in controlled conditions using high pest pressure for simulating real field situations. Using this method it has tested cloro-derivate insecticides (DDT, HCH) effectiveness concerning maize leaf weevil control (Paulian, 1972), carbofuran

insecticides effectiveness (Paulian et al., 1980) or neonicotinoid insecticides effectiveness used for maize and sunflower seed treatment (Barbulescu et al., 2001; Vasilescu et al., 2005). In laboratory conditions it has studying insects behavior (Paulian et Popov, 1973) or role of the nutrition in maize leaf weevil life cycle (Paulian et al., 1979). After 2010, at Plant Protection Institute, Manole et al. (2013) has studied mass rearing of *Tanymecus dilaticollis* weevils, successive generations, in controlled conditions. According this author, after a few generations, at insects reared in laboratory controlled conditions can appear physiological disturbances such as female sterility.

Data from the literature make in evidence that laboratory experiments it has made in neighborhood countries, too. In Bulgaria, Keszthelyi et al. (2008) studied effectiveness of seed treatment and soil disinfection, on three soils type,

using isolators previously sown with corn (1-2 leaf stage). In Bulgaria, Draganova et al. (2012) makes bioassays of *Tanymecus dilaticollis* weevils with some *Beauveria bassiana* isolates, for biological control of this pest.

Most of the cited authors use for laboratory assessments insects collected from the open field, from places where maize plants or soil was untreated.

The laboratory testing of different active ingredients used for maize seed treatment continues at NARDI Fundulea too (Georgescu et al., 2014; 2016). In this paper there were presented results of laboratory experiment concerning maize seed treatment effectiveness for controlling of the *Tanymecus dilaticollis* attack using high pest pressure (4 insects/plant). It has tested three active ingredients from neonicotinoid class (imidacloprid, clothianidin and thiamethoxam), used in Romanian agriculture for maize seed treatment.

## MATERIALS AND METHODS

The researches have been carried out in 2018 at Plant and Environmental Protection Collective in frame of National Agricultural Research Development Institute (NARDI) Fundulea, Calarasi County, Romania.

The insects were collected from the maize untreated crops, located both at the Plant and Environmental Protection Collective and Ecological Agriculture Laboratory experimental fields (44°30' N,

24°10' E). Insects were collected at the end of April or beginning of May. This period coincides with maximum activity of these weevils at the soil surface. Optimum time for collecting insects from the open field is between 11:00 AM and 15:00 PM. Also it is recommended to collect the insects in warm days with clear sky and low wind. These conditions are favorable for weevils activity at the soil surface (Popov et al., 2006b).



Fig. 1-Plastic pots, sowed with maize, used at laboratory experiment, for evaluating seed treatment effectiveness against *T. dilaticollis* Gyll

Until starting of the laboratory experiment, weevils collected from the field were maintaining in climatic chambers, for a few days, at  $15 \pm 2$  °C air temperature and 80-85 % relative air humidity. At this temperature the insects remain inactive.

For laboratory experiment it has used plastic pots (12x12x10 cm). Before sowing, pots were filling  $\frac{3}{4}$  with soil, harvested from the areas without chemical treatments (preferably from the edge of the forest). In each plastic pot it has sowed five maize seeds (Fig. 1).

For this experiment it has used Olt maize hybrid. After sowing, pots were complete filled with soil, then soil from each pot was slight compressed and soaking with water for ensure uniform emergence of maize plants. All pots receive same water quantity. It is very important to have uniform emergence of

After beginning of the plants emergence, when maize seedlings arrive above soil surface (BBCH 10), the insects collected from the open field were added in plastic pots. For ensure higher pest pressure, in each pot it has added 20 insects to have a pest density of 4 adults per plant (Barbulescu et al., 2001). Insects must manipulated carefully for not hurt then. After insects were added, the pots were covered with isolators, bonnet with bolter (Fig. 2). Each variant have four

the maize plants from all plastic pots. For this reason the experiment was carried out in laboratory room with uniform light for all pots.



Fig. 2-Pots covered with isolators, bonnet with bolter (NARDI Fundulea)

replications, each pot represent one replication. Active ingredients used in this experiment were imidacloprid, clothianidin and thiamethoxam (Tab. 1).

**Insects mortality** was assessed daily after pots infestations. After each assessment, dead insects from all pots were removed. Results of these assessments were evaluated as average insects mortality percent, at the end of the assessments period (8 days from pots infestations).

Tab. 1

Active ingredients used in the laboratory experiment concerning maize seed treatment effectiveness against *Tanymecus diladicollis* Gyll

Variant	Active ingredient	Commercial product	Dose (mg a.i./grain)
1	control (untreated)	—	—
2	imidacloprid (600 g/l)	Nuprid 600 FS	1.30
3	clothianidin (600 g/l)	Poncho 600 FS	0.50
4	thiamethoxam (350 g/l)	Cruiser 350 FS	0.83

**Attack intensity** of *Tanymecus dilaticollis* adults was rated when maize plants from plastic pots arrive at four leaf stage (BBCH 14). Attacked plants were rated on a scale from 1 to 9, elaborated

and improved by Paulian (1972), as follows:

**note 1**-plant not attacked;

**note 2**-plant with 2-3 simple bites on the leaf edge;



**note 3**-plants with bites or clips on all four leaf's edge;

**note 4**-plants with leaf's chaffed in proportion of 25 %;

**note 5**-plants with leaf's chaffed in proportion of 50 %;

**note 6**-plants with leaf's chaffed in proportion of 75 %;

**note 7**-plants with leaf's chaffed almost at the level of the stem;

**note 8**-plants with leaf's completely chaffed and beginning of the stem destroyed;

**note 9**-plants destroyed, with stem chaffed close to soil level.

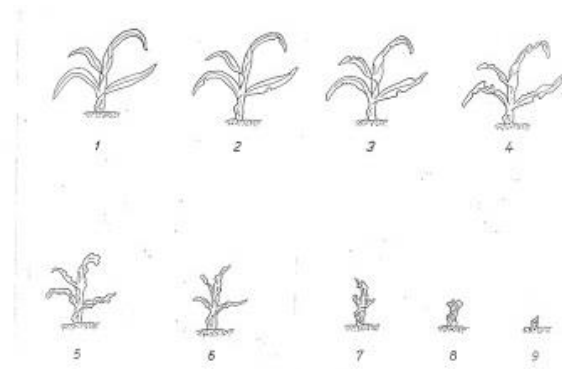


Fig. 3-Attack intensity scale (1-9), elaborated and improved by Paulian (1972), NARDI Fundulea

Plants with higher attack intensity (note 8 or 9) have all leaf's damaged and stem partial or total destroyed (Fig. 3). These attacked plants couldn't recovery and will die (Rosca *et* Istrate, 2009). Same authors mentioned that in case of lower attack of the pest (plants with damaged leaf's but stem less affected) maize seedlings will not be destroyed but the yield can be reduced.

During the laboratory experiment air temperature and relative air humidity were recorded with Klimalogg.pro data logger. This climatic data were recorded one time at each 15 minutes.

The data from laboratory assessments were **statistical analyzed** through Student Newman-Keuls test.

## RESULTS AND DISCUSSIONS

Air temperature registered in laboratory room where experiment was placed ranged between 20.8 and 28.5 °C. Paulian (1972) mentioned that when average air temperature registered during day time is higher than 20 °C insects have maximum activity on soil surface. In this experiment, temperatures registered during day time, in laboratory room, were higher than 24.0 °C (Fig. 4). Relative air humidity registered during this experiment ranged between 37.1 and 65.8 % (Fig. 5). Overall climatic conditions from the laboratory, during experiment, were favorable for both *T. dilaticollis* weevils mating and feeding process.

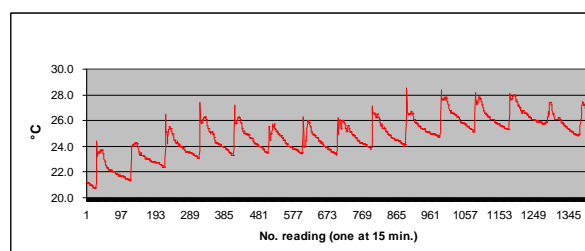


Fig. 4-Air temperature (°C) registered during laboratory experiment

After insects were added in plastic pots, immediately they start feeding with maize young plants. Also it has observed starting of weevils mating process. This observations concerning insects behavior in laboratory conditions was in accordance with those mentioned by Paulian *et* Popov (1973) and Paulian *et* al. (1979).

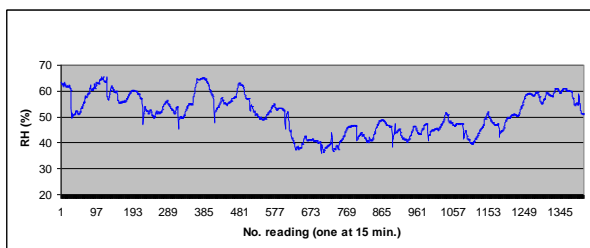


Fig. 5-Relative air humidity (%) registered during laboratory experiment

At treated variants the effects of the active ingredients it was starting to be observed after 10-15 minutes from the moment when insects start feeding. Weevils start to have uncontrolled movements and couldn't continue feeding and mating processes.

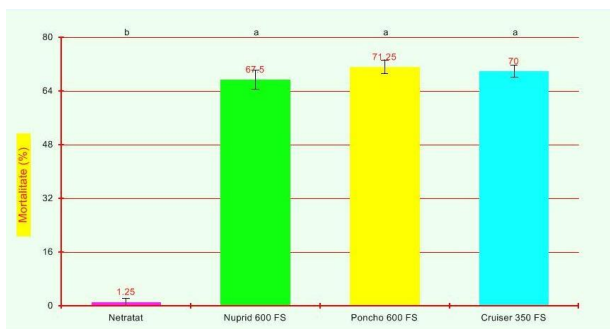


Fig. 6-T. dilaticollis adults mortality (%), in laboratory conditions, at NARDI Fundulea

However weevils don't die immediately. A possible explication for this is because of different ways of insecticides action on target pests including maize leaf weevil (*Tanymecus dilaticollis* Gyll). The insecticides from neonicotinoid class affect nervous system of the insects (Chao *et al.*, 1997; Jeschke *et al.*, 2011; Adak *et al.*, 2012). At the end of the observations period, at treated variants, insects mortality ranged from 67.50 %, in case of variant treated with imidacloprid active ingredient and 71.25 %, in case of variant treated with clothianidin (Tab. 2). At variant treated with thiamethoxam active ingredient it has registered a mortality of 70.00 %. There weren't statistical differences between treated variants (Fig. 6). However, higher statistical differences it has registered

between all treated variants and control (untreated) variant ( $P < .05$ ).

Tab. 2

The effectiveness of seeds treatment at maize crop against *Tanymecus dilaticollis* Gyll, in laboratory conditions, at NARDI Fundulea

Nr. crt.	Active ingredient	Insects mortality (%)	Attack incidence (%)	Attack intensity (1-9)
1	control	1.25b	100a	8.63a
2	Imidacloprid	67.50a	100a	4.65a
3	clothianidin	71.25a	100a	4.60a
4	thiamethoxam	70.00a	100a	4.58a
LSD (P=.05)		7.05	0	0.41
Standard Deviation		4.41	0	0.26
CV		8.40	0	4.57

Means followed by same letter do not significantly differ (P=.05, Student-Newman-Keuls)

Analyzing data from table 2 it has ascertained that attack intensity of *T. dilaticollis* at maize untreated plants, in conditions of high pest pressure (4 insects/plant), on a scale from 1 to 9, were almost maximum ( $I=8.63$ ). At eight days after pots infestation, maize plants were destroyed because of high insects attack and can't recover (Fig. 7). At treated variants, attack intensity of *T. dilaticollis* at maize plants ranged from 4.58 in case of variant treated with thiamethoxam and 4.65 in case of variant treated with imidacloprid. At variant treated with clothianidin, on a scale from 1 to 9, attack intensity was 4.60. In this laboratory experiment, in conditions of high pest pressure (4 insects/plant), at treated variants, plants have leaves chaffed in proportion of 25-50 %. However maize plants survive of the attack and have normal later development.

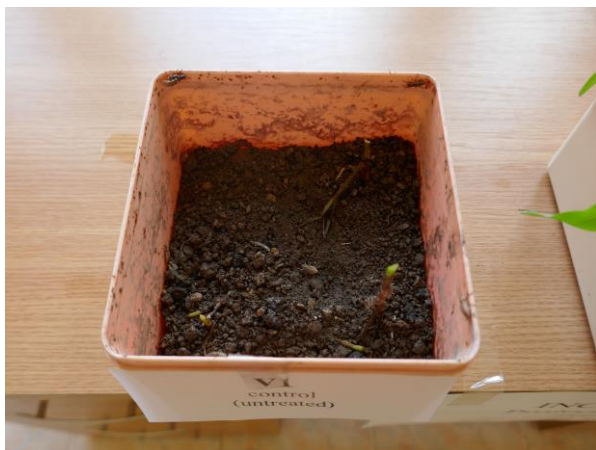


Fig. 7-Untreated maize plants destroyed by *T. dilaticollis* weevils at 8 days from the start of the experiment

Regard as attack intensity there weren't statistical differences between treated variants ( $P < .05$ ). However higher statistical differences it was registered between untreated (control) variant and all treated variants (Fig. 8).

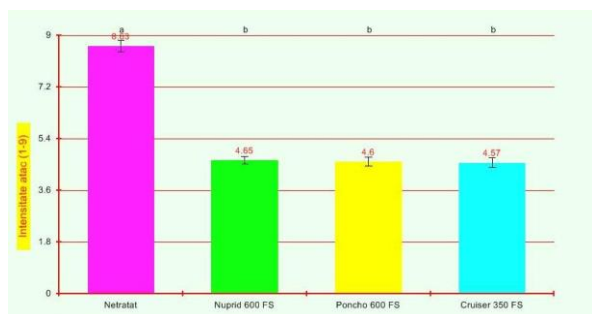


Fig. 8-*T. dilaticollis* attack intensity, at maize plants in laboratory conditions, at NARDI Fundulea

Between insects mortality and attack intensity there were negative correlation (Fig. 9).

In controlled conditions, using high pest pressure (4 insects/plant) seed treatment with imidacloprid, clothianidin and thiamethoxam active ingredients provide effective protection of the maize plants, in first vegetation stages (BBCH 10-14) against maize leaf weevil (*T. dilaticollis* Gyll) attack (Fig. 10). At same conclusion arrive Barbulescu *et al.* (2001), Vasilescu *et al.* (2005), Keszthelyi

*et al.* (2008), Georgescu *et al.* (2014, 2015, 2016).

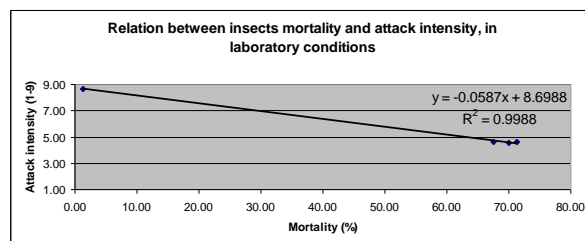


Fig. 9- Relation between insects mortality and attack intensity, in laboratory conditions at NARDI Fundulea

Laboratory experiment, in conditions of high pest pressure is a good method to evaluate the effectiveness of the insecticides used for maize seed treatment. This method couldn't replace field assessments but is a good way to observe insecticides mode of action on target pest. Also this method is a good simulation for field situations with heavy weevils infestation (25-30 insects/m<sup>2</sup> or higher densities).



Fig. 10-Laboratory experiment, at 8 days from the adding of the insects in the plastic pots. Left-untreated variant.

On base of the laboratory assessments results, it can be elaborated mathematic models for better understanding of *T. dilaticollis* weevils attack at maize plants.

## CONCLUSIONS

In the conditions of high pest pressure (4 insects/plant), maize plants from untreated variant were destroyed after eight days from pots infestation, by *T. dilaticollis* weevils. Attack of this pest at maize untreated plants, on a scale from 1 to 9 was 8.63.

In this laboratory experiment at variants treated with imidacloprid, clothianidin and thiamethoxam active ingredients weevils mortality ranged from 67.50 to 71.25 %. There weren't statistical differences between treated variants.

In this laboratory experiment at variants treated with imidacloprid, clothianidin and thiamethoxam active ingredients the attack of *T. dilaticollis* at maize plants, on a scale from 1 to 9, ranged from 4.58 to 4.65. There weren't statistical differences between these variants.

In conditions of high pest pressure (4 insects/plant) seed treatment with

imidacloprid, clothianidin and thiamethoxam active ingredients provide effective protection of the maize plants, in first vegetation stages (BBCH 10-14) against maize leaf weevil attack.

Laboratory assessments represent a complementary method with field assessments concerning maize leaf weevil control.

Further researches are necessary to evaluate different active ingredients effectiveness for controlling of *T. dilaticollis* weevils, in eventuality of neonicotinoids permanent ban.

Also it is necessary new researches concerning rearing of *T. dilaticollis* in laboratory conditions.

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