PRELIMINARY RESULTS CONCERNING TESTING OF THE NEW ACTIVE INGREDIENTS USED LIKE MAIZE SEED TREATMENT FOR CONTROLLING OF THE *TANYMECUS DILATICOLLIS* GYLL IN LABORATORY CONDITIONS, AT NARDI FUNDULEA

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INTRODUCTION

Maize leaf weevil (*Tanymecus dilaticollis* Gyll) is an economically important pest of the maize crop in south and south-east of the Romania (Cristea et al., 2004). Recent data from literature suggest that higher attack of this pest occur in south-west of the country too and some counties from Moldova region (Popov et al., 2003, 2005, 2007). Same author mention that every year, 1.000.000 ha with maize from the favorable areas are affected by the insect attack. *T. dilaticollis* is dangerous when maize plants are in first vegetation stages, between plant emergence and four leaf stage (Paulian, 1972). In some cases, maize plants can be destroyed because of the insect attack before plants arrive at soil surface (Barbulescu et al., 2001). At a density between 25 and 30 insects/m^2, average yield losses were 34 % (Paulian, 1979). In last decades, several studies from Romania and other countries from South-East of Europe make in evidence that seed treatment is one of the most effective method for protect maize young plants against pests attack, such as *T. dilaticollis* (Voinescu, 1985; Barbulescu et al., 2001; Krusteva et al., 2006; Keszthelyi et al., 2008; Popov et Barbulescu, 2007; Čamprag, 2011, Trotus et al., 2011). From middle of the years ’90 it has tested insecticides from neonicotinoid class for maize seeds treatment against main pests of this crop, especially when plants are in first vegetation stages (Popov et Barbulescu, 2007). Higher effectiveness of these active ingredients was mentioned in different communications (Barbulescu et al., 2001; Popov, 2002; Vasilescu et al., 2005). As result of EU directive 485/2015, from 1 December the use of neonicotinoid insecticides for seed treatment of the spring crops, including maize, was restricted for two years (Official Journal of the European Union, 2013). After this directive no insecticides remain available for maize seed treatment against *T. dilaticollis* in Romania. Although it has obtained temporary authorizations for use of the neonicotinoid insecticides like seed treatment at maze crop in spring period, both, in 2014 and 2015, it is necessary to testing new insecticides for evaluate there effectiveness for controlling of the maize leaf weevil. These types of assessments were organized in field conditions, in spring period (Paulian 1972; Voinescu 1985; Barbulescu, 2001; Vasilescu, 2005). However climatic conditions from spring were variable from one year to another. In years with reduced rainfall level in the spring, the attack of *T. dilaticollis* on maize untreated plants was maximum or almost maximum, while in years with higher rainfall level the attack of this pest on maize untreated plants was lower (Popov et al., 2006). At NARDI Fundulea, Paulian (1972) develop a laboratory method for evaluate effectiveness of seed treatments, where maize emerged plants are placed in conditions of high attack pressure. Barbulescu A. et al. (2001) tested the efficacy of the maize and sunflower seeds treatment with neonicotinoid insecticides in laboratory conditions, using a pest density from four to seven adult insects per plant. In Hungary, Keszthelyi et al. (2008) study effectiveness of both, seed and soil treatments, on three soils type, using isolators, previously sown with corn. In Bulgaria, Draganova S. (2012) testing effectiveness of some *Beauveria bassiana* isolates for biological control of *T. dilaticollis* adults in laboratory conditions. The aim of the authors

collective is testing different insecticides used like seed treatments, both in laboratory and field conditions, for find alternative at the neonicotinoids treatment in eventuality of permanent ban of these substances in EU. In this paper are presented preliminary results of testing seven active ingredients from three different insecticide classes (pyrethroids, neonicotinoids and ryanoids), in laboratory conditions, using pest high pressure.

MATERIALS AND METHODS

The researches have been carried out at Plant Protection Collective in frame of National Agricultural Research Development Institute, Fundulea, Calarasi County, Romania. The insects were collected from natural populations of the pest from maize untreated crops, cultivated in monoculture system, located on the Plant Protection Laboratory experimental field (44° 30′ N, 24° 1′ E). It is not recommended to collect adults of *T. dilaticollis* from the plots sowed with maize treated seeds. Generally, insects were collected at the end of April or beginning of May, the period that coincide with maximum activity of this pest on the soil surface. The optimum period of collecting insects is between hours 11:00 and 15:00. Until the starting of the laboratory assessments, *T. dilaticollis* adults collected from the field were maintaining in laboratory, for a few days, at 15±2 ºC air temperature and 80-85 % relative air humidity. At this temperature the insects were inactive.

Tab. 1

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

Insecticide class	Active ingredient	Concentration
Neonicotinoids	thiacloprid	400 g/l
	thiacloprid	400 g/l
	flupyradifurone	480 g/l
	clothianidin	600 g/l
Pyrethroids	tefluthrin	200 g/l
	bifenthrin	200 g/l
Ryanoids	cyantraniliprole	625 g/l

For laboratory experiments it has used plastic pots (12x12x10 cm). Before sowing, pots are filing ¾ 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). After sowing, pots are complete filled with soil, then soil from each pot was slight compressed and soaking with water for ensure uniform emergence of maize plants. Each variant have four replications, each pot represent one replication. The active ingredients used in this experiment are presented in table 1. After beginning of the plants emergence, when maize seedlings arrive above soil surface, 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 are added, the pots were covered with isolators, bonnet with bolter (Fig. 2).

Died insects were checked at 1, 2, 3, 5 and 8 days after pots infestation. After each assessment, dead insects from all pots were removed. Results of these assessments were evaluated as average insects mortality percent.

Attack intensity of *T. dilaticollis* adults on maize plants was evaluated at 8 days after pots infestation, when maize plants arrive at four leaf stage (BBCH 14). This laboratory assessment is similar with field evaluation of the pest attack. The 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 leaf edge; note 4, plants with leafs chafed in proportion of 25 %; note 5, plants with leafs chafed in proportion of 50 %; note 6, plants with leafs chafed in proportion of 75 %; note 7, plants with leafs chafed almost at the level of the stem; note 8, plants with leafs completely chafed and beginning of the stem destroyed; note 9, plants destroyed, with stem chafed close to soil level.

Plants height was checked at 8 days after pots infestation, by measuring with a corbel all plants from the pots. The data were statistical analyzed through variance analysis method, using Microsoft Excel, version 2003 and ARM, version 8.5.0, software.

RESULTS AND DISCUSSIONS

The insects start feeding with maize plants, immediately after they were added in plastic pots, sowed with maize. At 24 hours after pots infestation, only in case of seeds treated with clothianidin it has registered adults mortality. Even if the effects of the insecticide it has observed after 10-15 minutes from moment when insects start feeding, as result pest couldn't continue feeding process, however it has registered low values of the mortality. A possible explication is because of different ways of insecticide action on target pests. The insecticides from neonicotinoid class affect nervous system of the insects (Chao et al., 1997; Jeschke et al., 2011; Adak et al., 2012) while insecticides from ryanoid class affect muscular system of the insects (IRAC, 2012; Selby et al., 2013).

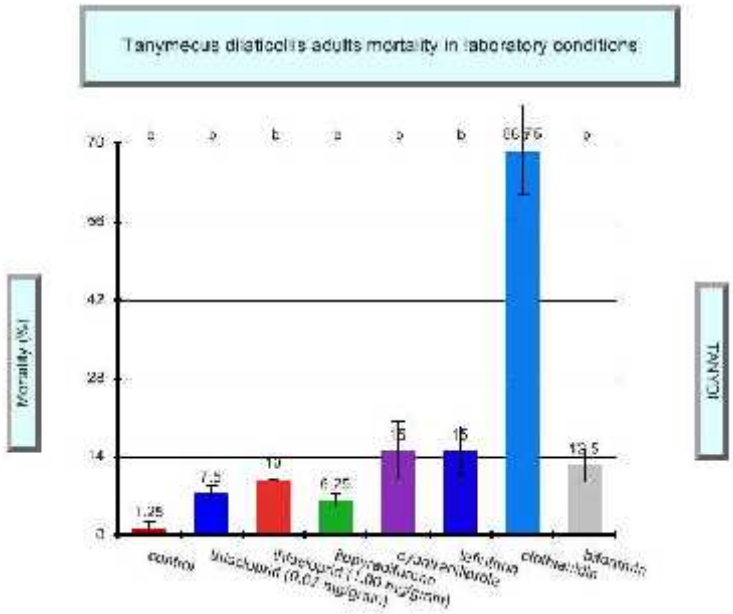

Figure 1*-T. dilaticollis *adults mortality (%), in laboratory conditions

At the end of the observations period, higher mortality percent of the *T. dilaticollis* adults it has registered in case of variant treated with clothianidin active ingredient (Fig. 1). At variants treated with cyantraniliprole and tefluthrin active ingredients, adult mortality, after 8 days from pots infestation, was of 15 %. Lower mortality percent it has registered in case of both doses of thiacloprid. The differences between adults mortality registered at clothianidin variant and the rest of the treated variants were statistical assigned (P<0.001).

Tab. 2

The effectiveness of some insecticides used for seed treatment at maize crop against *Tanymecus dilaticollis* Gyll, at NARDI Fundulea, in laboratory conditions

Nr. crt.	Active ingredient	Rate (mg/grain)	Atack intensity (1-9)	Plant height (cm)
1	control	—	8.88a	1.81a
2	thiacloprid	0.67	8.75a	2.05a
3	thiacloprid	1.00	8.27a	2.35a
4	flupyradifurone	0.40	8.63a	1.94a
5	cyantraniliprole	0.40	6.36b	6.78a
6	tefluthrin	0.40	7.52a	5.51a
7	clothianidin	0.50	3.58c	18.25b
8	bifenthrin	0.06	7.72a	3.99a
			$LSD_{5\%}$= 1.05 $LSD_{1\%}$= 1.42 $LSD_{0,1\%}$= 1.92	$LSD_{5\%}$= 3.24 $LSD_{1\%}$= 4.40 $LSD_{0,1\%}$= 5.94

Analysing data from table 2 it has ascertained that attack intensity of *T. dilaticollis* at maize untreated plants, in conditions of high pest pressure (4 insect/plant), in laboratory conditions, on a scale from 1 to 9, were almost maximum (I=8.88). At eight days after pots infestation, maize plants were almost destroyed because of the insect attack and can't recover. Higher attack of *T. dilaticollis* it has registered in case of variants treated with both doses of thiacloprid and variant treated with flupyradifurone. At variants treated with tefluthrin and bifenthrin the attack intensity of pest on maize plants were lower comparative with control variant, but differences were not statistical assigned ($P<0.05$). In case of variant treated with cyantraniliprole active ingredient, the attack intensity of *T. dilaticollis* on maize plants was of 6.36. Even if the difference was significant comparative with control variant ($P<0.05$) however this active ingredient don't assign effective protection of the maize plants, in first vegetation stages, in conditions of high pest pressure (4 insect/plants). At this variant, after 8 days from pots infestation, most of the plants were chaffed in proportion of 75 % and some plants present leafs complete chaffed. In this experiment, only clothianidin active ingredient in dose of 0.40 mg/grain assigns effective protection of the maize young plants against *T. dilaticollis* attack, in conditions of the high pest pressure (I=3.58). Even if the all plants from this experimental variant were attacked by the insects, however leafs were chaffed in proportion of 5-20 % and plants recover after the attack.

CONCLUSIONS

In the conditions of high pest pressure (4 adults/plant), maize plants from untreated variant were destroyed after eight days from pots infestation, by *T. dilaticollis* adults.

In this assesment only at variant treated with clothianidin active ingredient it has registered higher adults mortality percent. In the rest of the treated variants, mortality percent was low, the differences comparative with untreated variant wasn't statistically assigned.

Laboratory assessments represent a complementary method with field assessments, concerning effectiveness of insecticides used like seed treatments for controlling of the maize leaf weevil pest (*T. dilaticollis*). However, because of different ways of insecticides actions, further studies are necessary in the future. Also is needed to adapt laboratory assessment methodology to the new testing insecticides.

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