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INSECTICIDES IMPACT ON SEED GERMINATION AND EARLY SEEDLINGS GROWTH IN MAIZE (*Zea mays* L.)

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Abstract

The germination is the initial step of plant life cycle and seedling establishment and it is the crucial physiological periods for plant development. On the one hand, an appropriate seed treatment may help seeds to overcome biotic stress during storage and on the other it has beneficial effects on physiological seed quality. In this study were investigated under laboratory conditions the germination and early seedlings growth response of maize to different types of insecticides (antranilamids: HGW86 and E2Y45; neonicotinoids: Poncho 600 FS) applied 90 days before the laboratory trial. Untreated seed was used as control variant. The treatments for maize seeds in order to protect against biotic stress agents generally had no negative effects on germination and early seedlings growth. Nevertheless, choosing the right combination of products to apply as a seed treatment is very important. Future research needs to clarify the impact of insecticides treatment on maize seeds physiological quality.

Key words*: maize, seeds germination, insecticides.*

INTRODUCTION

Maize (*Zea mays* L.) is the most important cereal crop in the world after wheat and rice, with regard to cultivation areas and total production (Verheye, 2010), so it is a topic designed for a multidisciplinary approach (Staller et al., 2006).
Maize is a species with a great capacity to adapt to environmental conditions, but diseases and pest incidence may become the causes leading to decrease production and yield quality. On the other hand, during prolonged storage seeds are exposed to qualitative and quantitative losses caused by pests, as for instance weevils (Antonello et al., 2009).
Germination and emergence of seeds are primordial stages of ontogenetic cycle of the plant influenced by environmental factors, especially temperature and humidity (Baskin and Baskin, 2014). Physiological qualities of maize seeds affect plant behavior to stress factors in the juvenile stage, when incidence of diseases and/or pests can become fatal. Therefore, besides the interest of breeders for obtaining a biological material resistant to diseases and pests, seeds treatments is applied by means that can prevent or reduce the incidence of certain pathogens or pests of interest.
For instance, the agronomic performance of transgenic *Bacillus thuringiensis* (Bt) hybrid versus chemical and seed coated insecticides to control the rootworm pest were studied by Ma et al. (2009). It was showed that Bt rootworm seed technology was effective to control the rootworm larvae and protected grain yield under severe infestation.
The chemical treatments must also consider the possible accumulation of residues in soil. In this context, application of clothianidin did not induced a significantly accumulation of its residues in soil, as well as plant bioavailability of residues in soil was limited, next to that exposure to pollinators was not increase over time in fields receiving multiple applications of this product (Xu et al., 2016).
Therefore, in this study there were evaluated in the laboratory conditions the effects of the new seed treatments submitted for authorization applied to control the soil pests such *Agriotes* spp. (wireworm) and *Tanymecus dilaticollis* Gyll (maize leaf weevil) on maize seed germination and seedlings vigor.

MATERIALS AND METHODS

Biological material
Zea mays L. (*cv Pioneer* PR33A46) seeds purchased from DuPont were used to evaluate

influence of different seeds treatments on germination and seedlings vigor.

Seed Treatments

Prior to treating with insecticides, the maize seeds were treated with the fungicide Maxim XL 2.7 FS, then seven variants were prepared using different insecticides treatments as we can see in *Table 1*. The check plot included fungicide-only (V8).

Table 1.Insecticide treatments applied to corn seeds before germination test

Number	Treatments*	Rate of active ingredient/kernel (UAT)
1.	DPX-HGW86 625 FS	750 µg
2.	DPX-HGW86 625 FS	1000 µg
3.	DPX-HGW86 625 FS	1250 µg
4.	DPX-E2Y45 625 FS	750 µg
5.	DPX-HGW86 625 FS + PONCHO 600 FS	750 µg +500 µg
6.	PONCHO 600 FS	500 µg
7.	PONCHO 600 FS	1250 µg
8.	Untreated with insecticide	Control

*DPX-HGW86 625 FS= cyantraniliprole 625 g L^{-1}, FS
DPX-E2Y45 625 FS= chlorontraniliprole 625 g L^{-1}, FS
PONCHO 600 FS = chlothianidin 400 g L^{-1} + 53.3 g L^{-1} beta-cyfluthrin, FS

The study of germination was performed in laboratory conditions with the seeds stored 90 days after treatment. There were used Petri glass dishes (10 x 30 cm), as described by standardized procedures developed by the Association of Official Seed Analysts (AOSA, 1993). The temperature was daily registered under day/night natural room conditions. There were prepared four replications each consisted of 25 seeds and the double Whatman no.2 filter paper was humidify with 15 ml sterile distillated water. The seedlings were watered as needed.

The followings physiological indicators were registered: the degree of seeds imbibition (%); germination percentage (GP) (%) on the 4th day; mean germination time (MGT) (days); length of coleoptile and radicle were recorded on the 5th day after the start of the trial (expressed in cm $plant^{-1}$); fresh and dry weight of radicle and coleoptiles (g seedling $^{-1}$). As usually the criterion for germination is emergence of the root and some researchers consider that a certain length must be reached to be a valuable data, seeds were considered germinated when radicle length exceeded the length of the seed.

Electrolyte leakage (EL) was determined by immersing seedlings in 20 ml distilled water and reading after 3.5 hours, and 24 hours respectively, electrical conductivity of that solution, using a model OK-101 conductivity meter. The result was expressed in µS $cm^{-1}g^{-1}$.

The objective of the study was to determine seed germination traits of maize at different types and levels of seed treatment, specifically aimed at improving seeds and seedlings behavior under the incidence of some biotic stress factors.

RESULTS AND DISCUSSIONS

As recent highlight Baskin and Baskin (2014), before carrying out studies on seeds germination it is recommended to test if seeds will imbibe water. Germination rate depends not only to seeds vigor and/or the treatments applied, but also to its capacity to absorb water, especially during the imbibition phase, as the first process that assure seeds hidratation and subsequently permits storage substances breakdown.

Data in *Figure 1* show that there were not significantly differences between the seeds treatments, from the view point of imbibition degree. The insecticides seeds treatment applied did not affect water supply in the first step of water acquisition by the desiccated seed.

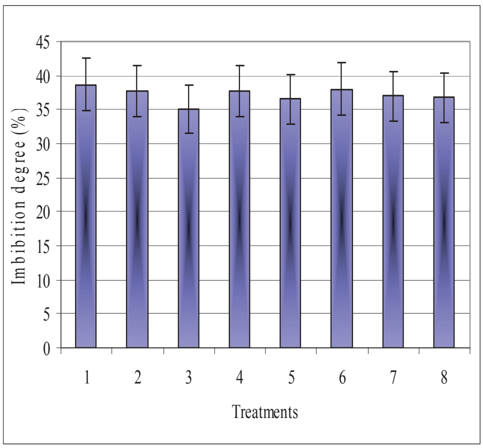

Figure 1. Maize seeds imbibition degree after insecticides treatment

Imbibition importance for the proper development of subsequent physiological processes was evidenced in the case of maize by Tnani et al. (2012). They identified after maize seeds imbibition 30 genes expressed in the scutellar epithelium and many of them activated in different metabolic processes, especially having a digestive function during germination. Moreover, according Law and Gallagher (2015) due to the release of volatile substances during imbibition, it can also lead to seed detection by invertebrate and seed consumption respectively. Therefore, it may also represent one way of activating the incidence of biotic stress, while DNA repair mechanisms activated during imbibitions enhance seed vigor (Ventura et al., 2012).

Germination percentage (GP) was evaluated at the fourth day and data presented in *Table 2,* to further clarify the effect of seeds treatment on germination. As we can see, the obtained results indicate that GP was not significantly changed under the insecticide applied treatments. However, the mixture of DPX-HGW86 625 FS + PONCHO 600 FS (750 µg +500 µg) (V5) negatively affected the seeds germination and the differences against the control were very significantly on the fourth day after the start of the trial.

Our results indicate that maize seeds treated with insecticides had a capacity to promote seed germination and early seedling growth. At least in part, these capacities may be first explained by the non influence on seed imbibition, then no influence on storage nutrients mobilization. On the other hand, the second phase of germination (germination in the strict sense) was not affected by the active ingredient used. It is well known that the early protection of seeds as against some stress factors may be crucial to germination performance, as well as to early seedlings growth.

As shows in *Figure 2*, mean germination time was closely to 2.1 days. The germination rates of maize seeds were increased on different ranges under different insecticides treatments, but generally there were no significant differences compared to control (untreated with insecticide). It is reported that combining the DPX-HGW86 625 FS + PONCHO 600 FS (750 µg a.i./kernel) with Poncho (500 µg a.i./kernel) caused an increase in average time of germination, as against the control and the other experimental treatments.

Cyantraniliprole contained by HGW 86 represents a new class of insecticides with systemic action against cutworm infestation. The insecticide is safe for beneficial insects as well as for humans, and studies conducted by the DuPont have also revealed that it increases plant vigor. Poncho is a new chloronicotinyl (CNI) seed treatment insecticide under development for use on corn in the United States, with Environmental Protection Agency (EPA) registration in the 2[rd] quarter of 2003. It is effective against corn pests such as: wireworm, cutworm, grape colapsis, billbug and corn rootwarm (Pitts, 2003).

Table 2. The dinamic of the germination percentage (%) of maize seeds after insecticides treatment

	After 48 h			After 72 h			After 96 h		
Treatment	Mean value	Diference	Semnification	Mean value	Diference	Semnification	Mean value	Diference	Semnification
T1	75	0.00	-	89	-6.00	-	90	-6.00	-
T2	74	-1.00	-	88	-7.00	-	88	-8.00	0
T3	69	-6.00	-	90	-5.00	-	92	-4.00	-
T4	83	8.00	-	92	-3.00	-	96.50	-0.50	-
T5	70	-5.00	-	81	-14.00	00	81	-15.00	000
T6	82	7.00	-	94	-1.00	-	96	0.00	-
T7	87	12.00	-	96	1.00	-	97	1.00	-
T8	75	-	Control	95	-	Control	96	-	Control
	DL 5%	DL 1%	DL 0.1%	DL 5%	DL 1%	DL 0.1%	DL 5%	DL 1%	DL 0.1%
	12.44	16.93	22.85	8.11	11.03	14.89	7.02	9.55	12.90
	16%	22%	30%	9%	12%	16%	8%	10%	14%

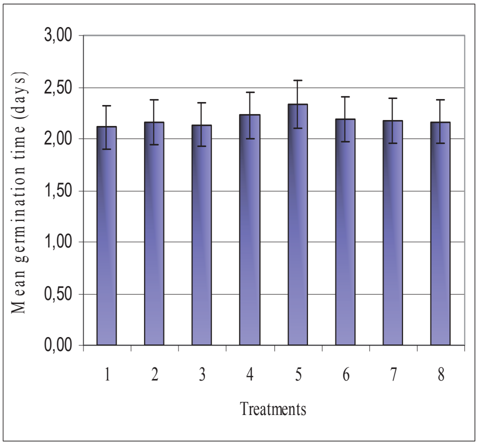

Figure 2. Mean germination time of maize seeds after insecticides treatments

The diamine insecticides: chlorantraniliprole and cyantraniliprole could be useful to reduce the negative effect of the neonicotinoides used long time for seed treatment and not only in reducing the number of foliar applications required for lepidopteran pests on soybean, as recently was reported by Thrash et al. (2013).
However, reduced seed germination and vigor caused by applying fungicide and insecticides treatments is variable in relation to the product used and also depends on the duration over which the seeds remain stored (Deuner et al., 2014).
Radicle and coleoptile lengths on the five day of germination are presented in Figure 3.

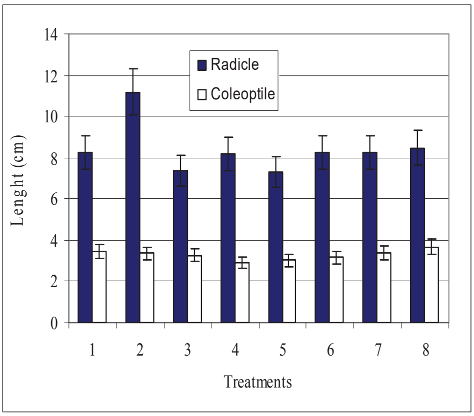

Figure 3. The radicle and coleoptile lengths on the five day

As we can observe, the radicle length was significantly higher in the case of T2 as against the Poncho treatment or control, while in rest not pronounced differences were registered. However, the coleoptile length was not influenced by insecticides treatments.
Single or combined insecticide treatments influenced radicle and coleoptile fresh weight (Figure 4), also their dry weight/fresh weight ratio (Figure 5). As a general rule, values were lower in the case of applied insecticides.

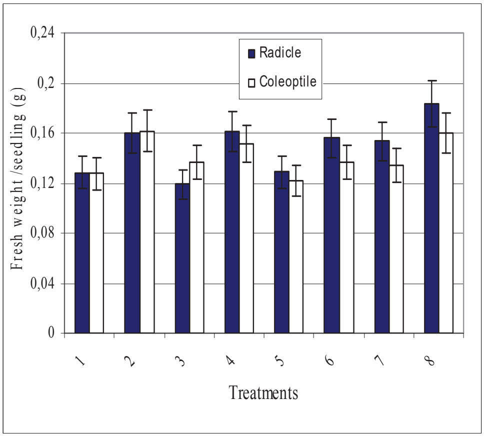

Figure 4. Maize seedlings fresh weight on the seven day

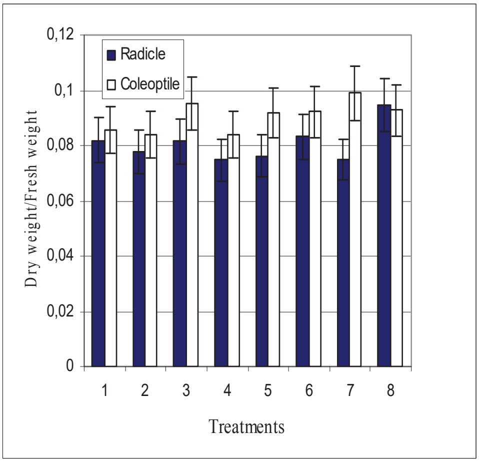

Figure 5. Maize seedlings dry weight/fresh weight ratio

Our results are in line with the findings of Wozniak and Martineau (2004) in the case of maize seeds pre-treated with Poncho™ 1250 when seedlings were visible smaller than those from Poncho™ 250 indicating an inhibitory effect of Poncho™ at the higher rate.

Also, improved germination is related to repairing of cell membrane integrity during seed imbibition. In this context, our data as regard as electrolytes leakage (Figure 6) indicate significantly differences as against the control, especially in the case of T2.

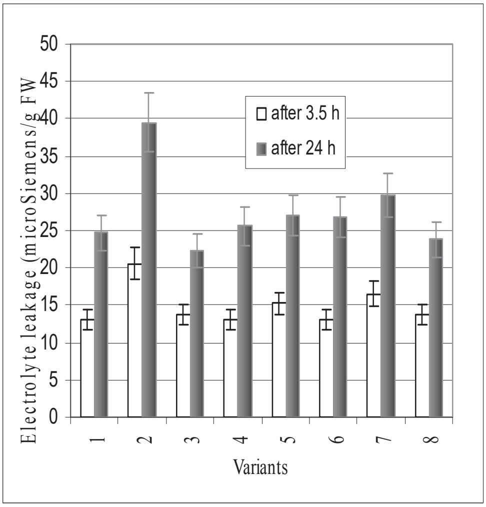

Figure 6. Electrolyte leakage during maize seeds imbibition

The massive out flow of solutes that occurs in the early hours of imbibitions can helps accelerate the germination processes, as it decreases the concentration of inhibitors. This process can be also associated with disturbances caused by membranes operation due to an inadequate rehydration. For instance, maintaining membrane integrity demonstrated by decreasing electrolyte leakage was assured by magnetic treatment of corn, which reduced the effect of aging on seed vigor (Vashisth and Nagarajan, 2009).

Understanding the molecular responses associated with seed rehydration (e.g. characterization of gene expression level, enzyme activity, difference in signal transduction response and regulatory mechanisms in stored seeds during the early period of imbibition) allows obtaining useful information that can be translated into applications in order to obtain high quality seeds (see review Ventura et al., 2012).

CONCLUSIONS

The treatments for maize seeds in order to protect against biotic stress agents during storage (90 days) didn't generally negative effects on physiological seed quality. Nevertheless, choosing the right combination of products to apply as a seed treatment is very important. Future research needs to clarify the impact of maize seed insecticides treatment on seeds physiological quality. Taking into consideration that companies constantly search for new alternatives or improved products, further investigation on their impact on seeds should be recommended.

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