Title:

Effect of Azadirachtin Applied as Seed Coating on the Larval Density of and Root Injury Caused by the Western Corn Rootworm/ *Diabrotica virgifera virgifera*/

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Abstract: The Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte) is one of the most important pests of maize in Hungary. As both larvae and imagoes are capable of causing major economic losses, their control in continuous maize cropping systems is essential. The control of larvae is costly and the related use of large doses of soil disinfectants places an increased burden on the environment. In recent years, several chemical products used as soil insecticides and seed dressings have been phased out, thus increasing the value of environmentally friendly biological products that provide effective protection against the pest. The active ingredient azadirachtin, the extract of the seeds of *Azadirachta indica* is one of such biological agents. In our experiments, we studied the efficiency of two azadirachtin products, Neemazal T/S (1% azadirachtin A;10 g/l) and Neemazal F (5% azadirachtin A+B; 50 g/l) used as seed dressing against corn rootworm larvae. The products were used in different concentrations (10 to 150%) in different regions and on various soil-types in Hungary. The active ingredient could efficiently control the pest in its larval stage. Treatments with concentrations exceeding 50% were efficient in all the replications.

Keywords: *Diabrotica virgifera virgifera*; maize pests; azadirachtin; neem tree; biological control methods

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The datasets generated and analysed during the current study are available at **http://real.mtak.hu/153184/**

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1. Introduction

Supplying food of appropriate quality and quantity while minimizing environmental damage is increasingly challenging in our world characterized by a steadily growing population and diminishing agricultural areas (Campos et al. 2016)

In addition to wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.), maize (*Zea mays* L.) is one of the most important cereals, with a global sown area of 140 to 160 million hectares. Maize has multiple uses in animal nutrition both as roughage and fodder, while it also plays a prominent role in human nutrition; furthermore, it is a versatile industrial plant (Vörös 2019). Today, one of the key pests of maize is the Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte 1868), an insect native to Central America (Krysan 1986) and capable of causing major economic losses both in its larval and imago stages. Larvae feed on the roots (Pálfay 2001; Gyeraj et al. 2021; Ferracini et al. 2021), while the feeding of mature individuals on the pistils results in fertility issues (Culey et al. 1992). The injuries caused by adult beetles reduce photosynthetic productivity as leaf veins are damaged (Čamprag et al. 1994; Moeser 2003; Ludwig et al. 1975; Krysan 1986; Tuska et al. 2002). The pest also serves as vector of the bacterium *Pantoea ananatis* (Krawczyk et al. 2021). Pest control efforts should focus on reducing larval damage, as controlling the larvae will significantly reduce the adult population, too. Crop rotation is one of the most efficient control methods to reduce larval damage (Gilette 1912; Szalai et al 2013; Spencer et al. 2009), the western corn rootworm being a univoltine species with its females laying their eggs into the soil of maize fields (Chiang 1973; Miller et al. 2009). Therefore, crop rotation may be used as an environmentally friendly and cost-effective control measure (Levine et al. 2002).

The quantity of insecticides used for controlling western corn rootworm larvae has increased with the spreading of the pest. With the recent phasing out of numerous soil active chemicals, tefluthrin products have become the most widely used soil disinfectants (Rice 2004; Tímár 2003). In the U.S., multi-year studies confirmed that the regular use of pyrethroids as soil insecticides had contributed to the western corn rootworm developing a resistance to this active ingredient (Pereira et al. 2015; Souza et al. 2020). A further disadvantage of soil treatments is that chemicals applied in large volumes have a detrimental effect on the environment and are harmful to useful organisms. Due to both the phasing out of active ingredients and environmental considerations, the focus should be shifted from chemical control to the application of environmentally friendly yet effective biological products which do not pose a threat to beneficial organisms while also comply with the requirements of occupational health and safety (Lengai et al. 2020).

Azadirachtin, an active ingredient extracted from the seeds of the Neem tree (*Azadirachta indica*) meets the above criteria (Chaudhary et al. 2017). Neem is a fast-growing, evergreen tree native to India which has spread to several Asian and African countries (Nortel et al. 1999; Forim et al. 2014). In addition to azadirachtin, which is the main active ingredient of the extract, need seed oil contains more than 100 biologically active ingredients that greatly reduce the chance of pests developing resistance to the main active ingredient (Mordue et al. 1997; Feng et al. 1995; Nicoletti et al. 2012). Azadirachtin has a broad spectrum of activity, acting as a feeding inhibitor, repellent, and growth regulator. It also blocks the moulting cycle of larvae by disrupting the production of ecdysone and has an effect on reproduction by inhibiting egg laying and causing fertility disorders and even sterility (Brahmachari 2004; Gonzalez-Coloma et al. 2013; Schmutterer 1988; Immaraju 1998; Mordue et al. 1997; Feng 1995; Morgan 2009; Mulla et al. 1999). Similar to colchicine, azadirachtin may also interfere with mitosis, and has a direct histopathological effect on intestinal epithelial cells, muscles, and adipose tissues, resulting in reduced locomotory and flight activity (Wilps et al. 1992; Mordue et al. 1993; Oiao et al. 2014).

Owing to its systemic effect and translocating properties, azadirachtin provides a long-lasting action (Cox 2002). Several studies have been published on its successful application against a number of leaf pests in various crops. For example, it proved to be effective against Hymenoptera (Li et al 2003), Lepidoptera (Mancebo et al. 2002; Michereff-Filho et al. 2008; Tavares et al. 2010), and Hemiptera (Weathersbee et al. 2005; Senthil et al. 2006; Formentini et al. 2016) pests.

The beneficial properties listed above served as our motivation to start studying the use of azadirachtin as a seed dressing agent against the larvae of the western corn rootworm, as this active ingredient also facilitates the transition to sustainable cultivation. Our hypothesis was this botanical insecticide could be used to control the larvae of the western corn rootworm.

2. Materials and Methods

We studied the effect of two seed dressing products, Neemazal T/S and Neemazal F, in 2020 and 2021. Both products are used against the larvae of the western corn rootworm and contain azadirachtin as their active ingredient. Neemazal T/S (Trifolio-M GmBH, Germany) is marketed in Europe and contains 1% (10 g/l) of azadirachtin A. Neemazal F (Coromandel International Limited Bio Products Division Thyagavalli, India), a more concentrated product authorised in India, contains 5% azadirachtin A+B (50g/l). According to the Safety Data Sheet of the product, the active ingredient content of Neemazal F is composed of Azadirachtin A and Azadirachtin B in 80% and 20%, respectively. In addition to the main active ingredient, both products contain smaller amounts of other components of the Neem extract.

Treatments were repeated 4 times, using 18 m^2 plots in a randomised setup (Figures 1 and 2). A row spacing of 0.75 m was applied, combined with a 0.19 m distance between plants. Planting depth was 0.08 m and the seeding rate was 70000 seeds ha^{-1}. Experiments plots were sown with the hybrid DKC-5141 in both years. This hybrid has a 1000 seed weight of 360 g. Seed dressing was applied in a dose of 12 l per 1000 kg of seeds i.e. 4.36 ml per 360 g of seeds.

2.1. 2020 experiments

In 2020, the experimental plots were created in a field in Gyömöre (Western Transdanubia, Hungary) where maize had been grown for 3 consecutive years, on a slightly alkaline (pH 7.43), medium heavy soil with a humus content of 3.21%.

In the various treatments, different concentrations of Neemazal T/S (10 g/L Azadirachtin A) were applied (10% to 100%). The product was diluted with water whenever a dose below 100% was required (Table 1). Percentages refer to the concentrations of Neemazal T/S; thus, 100% means the application of undiluted Neemazal T/S as seed dressing.

Table 1: Concentrations of Neemazal T/S used as seed dressing on DKC-5141 hybrid seeds – 2020

Dose	1000 seed weight	Neemazal T/S (ml)	Water (ml)	Azadirachtin A (mg/seed)	Azadirachtin A (g/ha)
100%	360 g	4.32	-	0.043	3.01
75%	360 g	3.24	1.08	0.033	2.31
50%	360 g	2.16	2.16	0.022	1.54
25%	360 g	1.08	3.24	0.011	0.77
10%	360 g	0.432	3.89	0.0043	0.30

Plots receiving no treatment whatsoever were used as negative control. The plots used as positive control were treated with Force 1.5 G (active ingredient: tefluthrin) at a dose of 15 kg/ha.

As the quantity of seeds to be provided with seed dressing was very low, we opted for manual application. Doses of the necessary quantity and concentration were prepared from Neemazal T/S, by adding water where necessary. Seeds were mixed with the dressing liquid in batches of 360 g. Uniform dressing was ensured by stirring.

25%	75%	F	50%	10%	K	100%
10%	F	100%	K	25%	75%	50%
K	50%	10%	25%	100%	F	75%
100%	K	75%	F	50%	10%	25%

Fig. 1. Layout of the experimental plots in 2020 (k: negative control; F: positive control; values given as percentages refer to the treatments i.e. the concentrations of the liquid solution used for seed dressing)

2.2. 2021 experiments

In 2021, the experiments were conducted at 2 locations, in Röjtökmuzsaj (Western Transdanubia, Hungary) and in Hajdúvid (eastern Hungary). In Röjtökmuzsaj, experimental plots were created in a field where maize had been grown for 60 consecutive years. Various crop protection experiments had already been conducted at this site and we reasonably assumed to find exceptionally large corn rootworm populations in such an old continuous crop. Hajdúvid is located on the Great Plain, the region most severely affected by western cornworm larvae in Hungary. The Hajdúvid site has a medium heavy and almost neutral (pH 6.9) soil with a humus content of 1.6%. In Röjtökmuzsaj, the plots were created on a heavy, weakly acidic soil (pH 6.8) with a humus content of 1.8%.

As the density of larvae was expected to be higher at the site used in 2021, we opted for higher concentrations of the active ingredients, not applying the lowest doses (10% and 25%) from the previous year. Only Neemazal F (5% azadirachtin, composed of azadirachtin A and azadirachtin B in 80% and 20%, respectively) was suitable for preparing solutions of such high concentrations. In the 2021 experiments, the combined concentration of azadirachtin A+B in the 50%, 75% and 100% treatments corresponded to the concentration of azadirachtin A in the 50%, 75% and 100% treatments of the 2020 experiments, respectively (Table 2).

Table 2: Liquid solutions used for seed-dressing in 2021

Dose	Mass of 1000 seeds (g)		Neemazal F (ml)	Water (ml)	Azadirachtin A+B (mg/seed)	Azadirachtin A+B (g/ha)
150%	360		1.29	3.03	0.065	4.55
125%	360		1.08	3.24	0.053	3.71
100%	360		0.86	3.46	0.043	3.01
75%	360		0.65	3.67	0.033	2.31
50%	360		0.43	3.98	0.022	1.54

As in the previous year, plots receiving no treatment whatsoever were used as negative control. The plots used as positive control were treated with Force 1.5 G (active ingredient: tefluthrin) at a dose of 15 kg/ha. The seed-dressing process and the experimental procedure were identical to those applied in 2020. The layout of the experimental plots is presented in Fig. 2.

100%	**125%**	**50%**	**150%**	**K**	**F**	**75%**
K	**100%**	**F**	**75%**	**125%**	**50%**	**150%**
50%	**75%**	**150%**	**K**	**100%**	**125%**	**F**
150%	**K**	**125%**	**50%**	**F**	**75%**	**100%**

Fig. 2. Layout of the experimental plots in 2021 (k: negative control; F: positive control; values given as percentages refer to the concentration of liquid solutions used for seed-dressing)

The method of evaluating the results was the same in both years. The larvae present in the root zone were surveyed and roots excavated. From the survey data, average larval count per plant and root injury levels were established using the Modified Iowa Scale. This is a scale of 1 to 6 with an increment of 0.5. The severity of root injury increases with the numbers. On the Modified Iowa Scale, the economic damage threshold is 3.5.
To be able to properly schedule the survey of larvae, we relied on forecasts using yellow traps with sex pheromones. The survey was started as soon as the first imagoes appeared as their emergence indicated the development of larvae had reached the final stages (L3 and pupa). From each experimental plot, 5 plants were removed with root balls of 20×20 cm. Live larvae were counted both in the soil removed from root balls and in the pits left behind (pupae, L3 larvae and imagoes freshly emerged were not distinguished). In 2021, the first site to be surveyed was Hajdúvid, followed immediately by Röjtökmuzsaj. This schedule was justified by the advanced larval development here, with adults emerging 1 to 2 weeks earlier in Hajdúvid (Great Plain, Hungary) compared to Röjtökmuzsaj (Transdanubia, Hungary). The roots excavated from experimental plots were collected in labelled bags and transferred to the Gyömöre Station, where root injury was assessed after soaking, using the Modified Iowa Scale. Larval counts and root injury data were recorded continuously and then analysed using mathematical and statistical methods.

One-way ANOVA (SPSS 16.0) was used to compare the averages of several independent groups of data. The Tukey HSD Post hoc test was used for pairwise comparisons. P values of 0.05 or less were considered statistically significant.

3. Results

3.1. Effect of treatments on larval count

At all three experimental sites, the highest number of larvae was recorded for the untreated control plots (negative control). Larval density was the lowest at the Gyömöre site (2.7±3.05 larvae/plant). Densities almost twice as high were found in Röjtökmuzsaj and Hajdúvid (4.95±2.68 and 4.20±2.91 larvae/plant, respectively). As Figures 3 to 5 illustrate, the density of larvae decreased with the increasing concentration of azadirachtin at all three sites.

At the Gyömöre site, the 100% treatment resulted in low larval counts (0.40±0.50); the density of larvae in these treatments was similar to those observed in the positive control (Fig. 3).

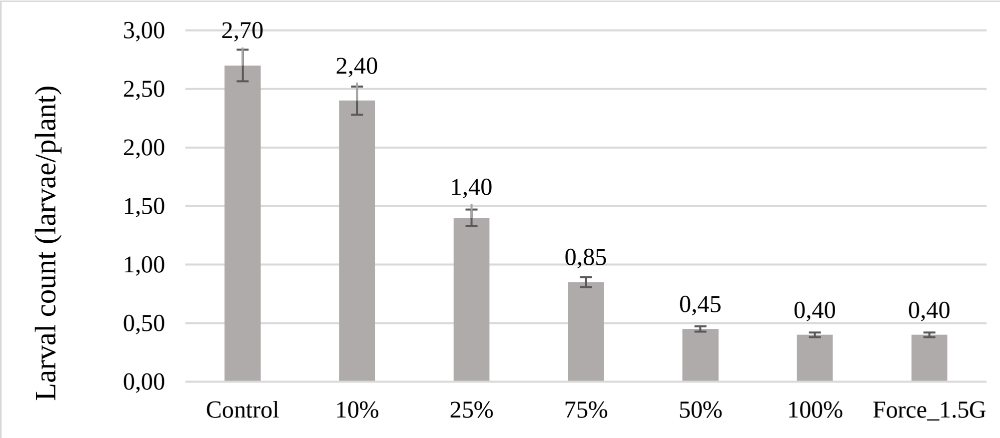

Fig. 3. Average larval counts per plant in the various treatments at the Gyömöre site in 2020. Error bars stand for confidence intervals.

Larval counts recorded for the plots in Gyömöre differed significantly (p=0.000; F=6.109). Results of the post hoc test indicated a significant difference between the negative control and the 100% (SE=0.557 p=0.001), 75% (SE=0.557 p=0.019) and 50% (SE=0.557 p=0.002) treatments. Results from the negative and positive control plots were also significantly different (SE=0.557 p=0.001). Since results from the plots treated with lowest azadirachtin concentrations (10%, 25%) were not significantly different from those of the negative control in 2020 (p>0.05), these concentrations were not used again in 2021. Instead, higher doses (125% and 150%) of azadirachtin were tested.

The analysis of data from 2021 indicated the lowest larval density (1.40 larvae per plant) for the 100% treatment in Röjtökmuzsaj. The larval count recorded for these plots was even lower than that found in the positive control (2.40 larvae per plant; Fig. 4)**.**

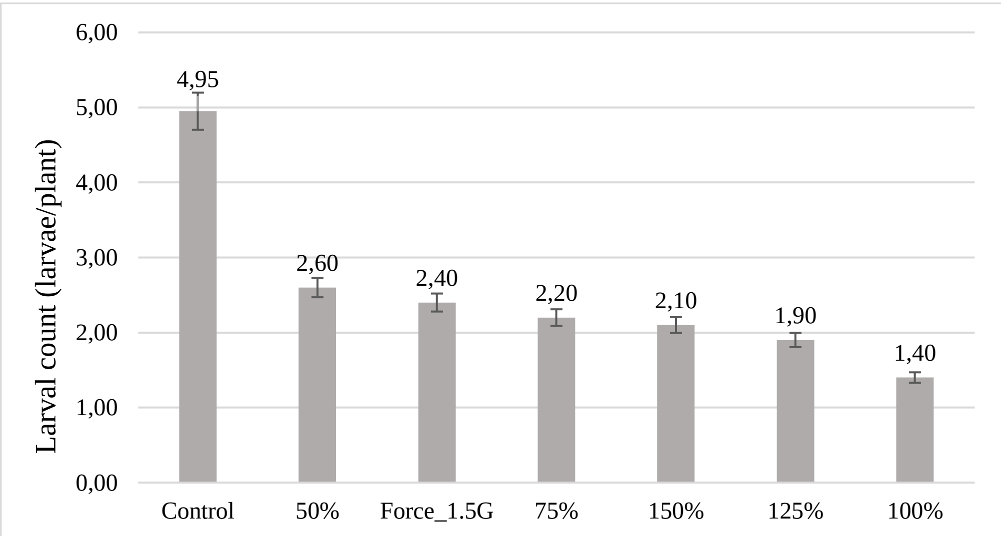

Fig. 4. Average larval counts per plant in the various treatments at the Röjtökmuzsaj site in 2021. Error bars stand for confidence intervals.

One-way ANOVA indicated a significant difference between the plots in Röjtökmuzsaj in terms of larval counts (p=0.000; F=7.151). According to the pairwise comparisons of the Tukey HSD Post hoc test, each treatment differed significantly from the negative control i.e. treatments had detectable effects. The difference between treatments on the other hand was not significant in any of the comparisons (p>0.05).

At the Hajdúvid site, the lowest average larval counts per plant were observed for the 150% and 125% treatments; with density values of 1.50 (±1.61) and 1.2 (±1.40), respectively. When these treatments were compared with the positive control, results were obviously different, with numbers being about twice as high in the latter case (Fig. 5)

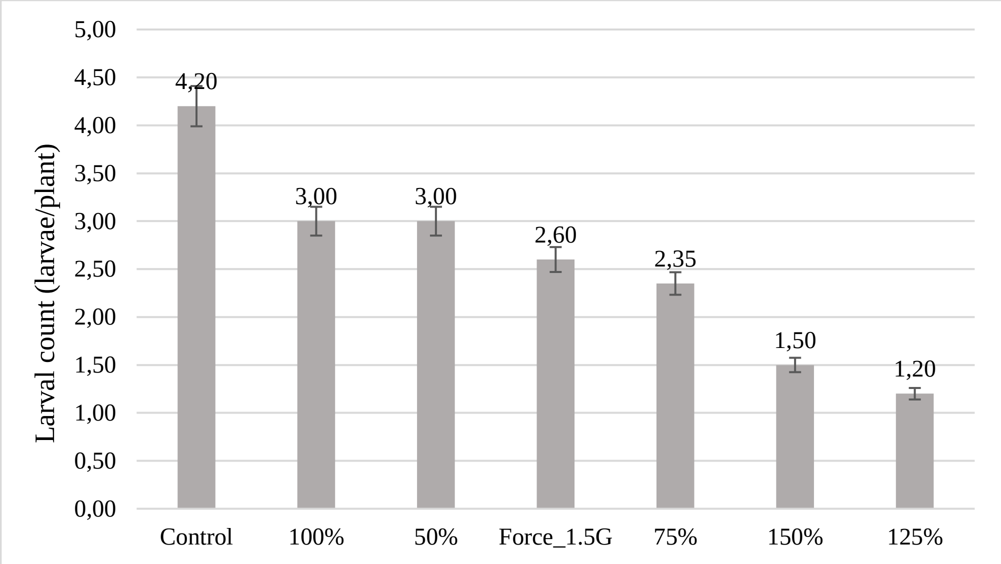

Fig. 5 Average larval counts per plant in the various treatments at the Hajdúvid site in 2021. Error bars stand for confidence intervals.

According to the results of the Tukey HSD Post hoc test, the negative control only differed significantly from the 150% and 125% treatments (SE=0.663, p=0.002 and SE=0.663, p=0.000, respectively).

3.2. Effect of treatments on root injury levels

As shown in Figures 6 to 8, the lowest root injury levels were measured in Gyömöre (m.Iowa: 3.23). This result is also supported by the larval count data. Root injury levels in both the Röjtökmuzsaj and the Hajdúvid negative controls exceeded the economic threshold (m.Iowa 3.55±1.07 and 4.42±1.16, respectively). Azadirachtin seed dressing reduced root injury levels in all the applied concentrations.

In Gyömöre, 75% and 100% treatments reduced injury levels by half compared to the negative control (Fig. 6).

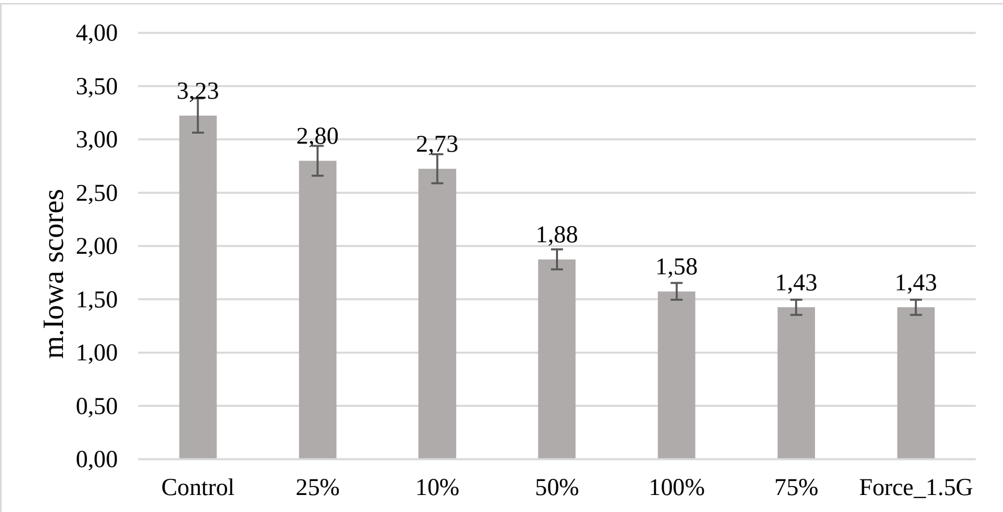

Fig. 6 Average root injury scores measured on the m.Iowa scale at the Gyömöre site in 2020. Error bars stand for confidence intervals.

The statistical analysis indicated a significant difference between the m.Iowa scores of the plots (p=0.000; F=30.441). According to the Tukey HSD Post hoc test, results for the negative control significantly differed from the 100%, 75% and 50% treatments and the positive control (SE=0.1920 and p=0.000 for all comparisons; Fig. 6). The 10% and 25% treatments did not show significant differences when compared to the negative control; however, significant differences could be observed when they were compared to other treatments or the positive control (p>0.05; Fig.6).

In Röjtökmuzsaj, only the negative control scored higher than 3.5 on the m.Iowa scale. This clearly demonstrates that root injury levels could be reduced below the economic threshold by applying azadirachtin seed dressing (Fig. 7).

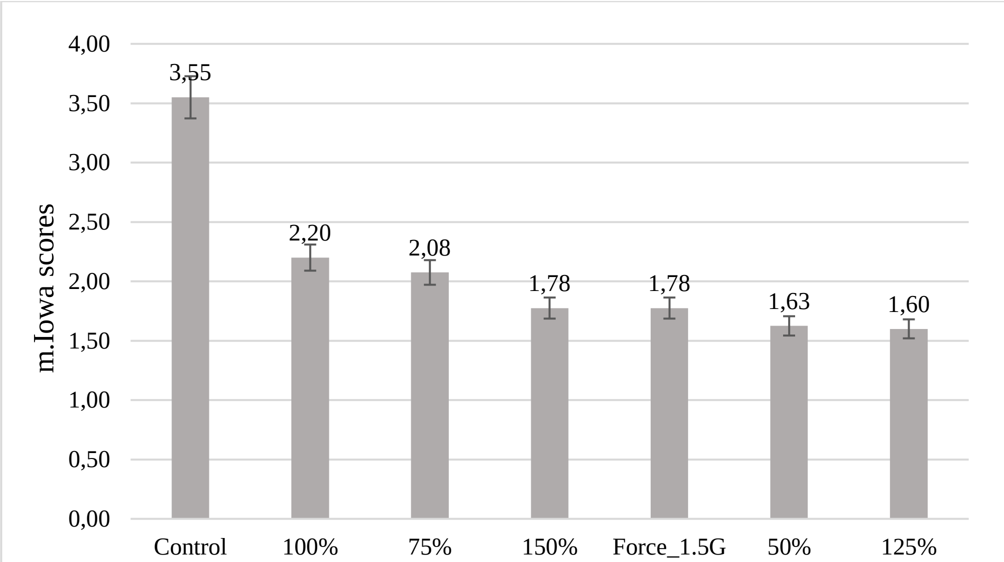

Fig. 7. Average root injury scores measured on the m.Iowa scale at the Röjtökmuzsaj site in 2021. Error bars stand for confidence intervals.

One-way ANOVA indicated a significant difference between the m.Iowa scores of the plots (p=0.000; F=19.088). The results of the Tukey HSD Post hoc test demonstrated that the negative control plot differed significantly from all the treatments. Treatments did not differ significantly in the pairwise comparisons.

In Hajdúvid, the highest root injury level was measured for the negative control (m.Iowa: 4.42±1.16). This score is far above the economic damage threshold score of 3.5. Regarding the treatments, the highest root injury level (2.68±0.75) was detected for the plots treated with 50% azadirachtin, followed by the 100% and 75% treatments and the positive control (2.28±0.92 and 2.13±0.67 and 2.03±0.60, respectively). The least severe damage was observed in the case of the 125% and 150% treatments (1.85±0.49 and 1.75±0.62, respectively; Fig. 8).

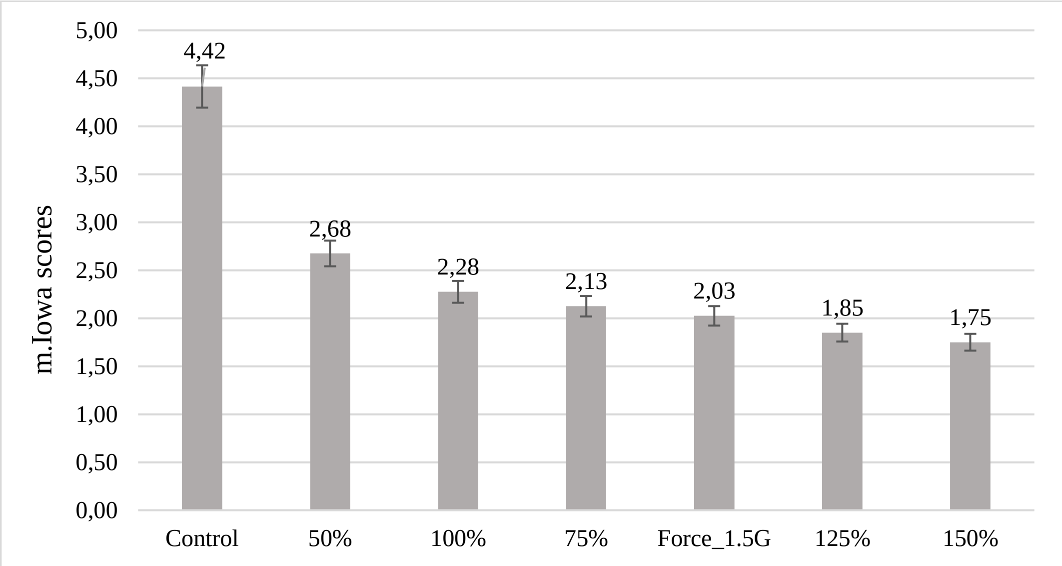

Fig. 8. Root injury levels as measured on the m.Iowa scale (Hajdúvid, 2021). Error bars stand for confidence intervals.

The results of the Tukey HSD Post hoc test demonstrated a significant difference between the negative control and the treatments in terms of m.Iowa scores in all the pairwise comparisons. (ANOVA: $p=0.000$; $F=28.322$). Treatments did not differ significantly in the pairwise comparisons.

4. Discussion

Controlling the western corn rootworm has become increasingly difficult in the European Union as a result of phasing out a growing number of active ingredients. Indeed, the list of available active ingredients has become so limited that the pest is very likely to develop a resistance to the authorised insecticides, currently considered effective, in the near future. Novel, effective active ingredients are needed that can be incorporated into existing integrated agricultural practices.

In our experiments, the active ingredient azadirachtin used as seed dressing was tested against the larvae of the western corn rootworm. Azadirachtin proved to be highly effective, reducing larval counts and hence root injury levels.

Our experiments confirmed that azadirachtin could be successfully used against the larvae of the western corn rootworm. Above a concentration of 50%, positive effects were achieved at all the sites. At the sites with extremely high larval density (Hajdúvid and Röjtökmuzsaj), the highest concentrations (125% and 150%) proved to be the most effective. Our studies demonstrated identical and, in some cases, superior effectiveness compared to the positive control (Force 1.5 G containing tefluthrin).

In comparison with chemical pest control products, azadirachtin as an active ingredient of biological origin has the advantage that it does not pose a threat to beneficial organisms nor to the staff performing the control measures. Furthermore, it does not have a negative impact on the environment, and as such it may also be used in ecological farming. The product used in our experiments can be applied easily as seed dressing, and the associated costs are not higher than those of the other insecticides available on the market.

Azadirachtin seed dressing renders the control of the most dangerous pest of maize, a crop with one of the largest global sown areas, simple, efficient, and sustainable.

References

- Brahmachari, G (2004) Neem—An Omnipotent Plant: A retrospection. 5:408-421 https://doi.org/10.1002/cbic.200300749
- Campos EVR, Lima R, Fraceto FL, Oliveira LJ, Pascoli M (2016) Neem oil and crop protection: From Now to the Future. Front. Plant. Sci, 7:1 https://doi.org/10.3389/fpls.2016.01494
- Čamprag D, Sekulič R, Bača F (1994) A kukorica új kártevője az amerikai gyökérféreg Jugoszláviában ("Western corn rootworm is new maize pest in Yugoslavia"). 4(7):41-42
- Chaudhary S, Kanwar RK, Sehgal A, Cahill D.M, Barrow CJ, Sehgall R, Kanwar JR (2017) Progress on Azadirachta indica Based Biopesticides in Replacing Synthetic Toxic Pesticides. Front. Plant Sci. 8: 610 https://doi.org/10.3389/fpls.2017.00610
- Chiang HC (1973) Bionomics of the northern and western corn rootworms. Annu. Rev. Entomol. 18:47-72
- Cox C (2002) Pyrethrins/pyrethrum Insecticide Factsheet. J. Pestic. 22:14-20
- Culey MD, Edwards CR, Cornelius JR (1992a) Effect of silk feeding by western corn rootworm (Coleoptera, Chrysomelidae) on yield and quality of inbred corn in seed corn production fields. J. Econ. 85:2440-2446
- Feng R, Isman MB (1995) Selection for resistance to azadirachtin in the green peach aphid, Myzus persicae. Experimentia 51:831-833 https://doi.org/10.1007/BF01922438
- Ferracini C, Blandino M, Rigamonti IE, Jucker E, Saladini MA, Reyneri A, Alma A (2021) Chemical-based strategies to control the western corn rootworm, Diabrotica virgifera LeConte. Crop Protection 139. https://doi.org/10.1016/j.cropro.2020.105306
- Forim MR, Fernandes DSMF, Fernandes JB, and Vieira PC (2014) Processo de Obtenção de Nanopartículas Biopoliméricas Contendo Óleo e Extratos de Azadirachta Indica a. Juss (neem). Nanopartículas Biopoliméricas e Micropartículas em pó, WO2014113860A1
- Formentini MA, Alves LFA, and Schapovaloff ME (2016) Insecticidal activity of neem oil against Gyropsylla spegazziniana (Hemiptera: Psyllidae) nymphs on Paraguay tea seedlings. Braz. J. Biol. 76:951-954
- Gilette CP (1912) Diabrotica virgifera as a corn rootworm. J. Econ. Entomol., 5:364-366
- Gonzalez-Coloma A, Reina M, Diaz CE, Fraga BM, Santana-Meridas O (2010) Natural Product-Based Biopesticides for Insect Control https://www.researchgate.net/publication/276271852_Natural_Product-Based_Biopesticides_for_Insect_Control Accessed 12 April 2022
- Gyeraj A, Szalai M, Pálinkás A, Edwards CR, Kiss J (2021) Effects of adult western corn rootworm (Diabrotica virgifera LeConte, Coleoptera: Chrysomelidae) silk feeding on yield parameters of sweet maize. Crop Protection, 140 https://doi.org/10.1016/j.cropro.2020.105447
- Immaraju J (1998) The commercial use of azadirachtin and its integration into viable pest control programmes. Pesticide Science, 54(3):285-289 https://doi.org/10.1002/(SICI)1096-9063(1998110)54:3<285::AID-PS802>3.0.CO;2-E
- Krawczyk K, Forys J, Nakonieczny M, Tranawska M, K. Beres P (2021) Transmission of Pantoea ananatis, the causal agent of leaf spot disease of maize (Zea mays), by western corn rootworm (Diabrotica virgifera LeConte). Crop Protection, 141 https://doi.org/10.1016/j.cropro.2020.105431
- Krysan JL (1986) Introduction: Biology, Distribution, and Identification of Pest Diabrotica. In: Krysan J. and Miller, T. (eds): Methods for the Study of Pest Diabrotica. Springer-Verlag: New York, USA, 1986 pp 1-23 https://doi.org/10.1007/978-1-4612-4868-2_1
- Lengai GMW, Muthomi JW, Mbega ER (2020) Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. Scientific African 7 https://doi.org/10.1016/j.sciaf.2019.e00239
- Levine E, Spencer JL, Onstand DW, Gray ME (2002) Adaptation of the western corn rootworm to crop rotation: Evolution of a new strain in response to a management practice. Am. Entomol., 48:94-107 https://doi.org/10.1093/ae/48.2.94
- Li SY, Skinner AC, Rideout T, Stone DM, Crummey H, and Holloway G (2003) Lethal and sublethal effects of a neem-based insecticide on balsam fir sawfly (Hymenoptera: Diprionidae). J. Econ. Entomol, 96:35-42 https://doi.org/10.1093/jee/96.1.35
- Ludwig KA, Hill RE (1975) Comparisons of gut contents of adult western and northen corn rootworms in northeast Nebraska. Environ. Entomol., 4(3):435-438 https://doi.org/10.1093/ee/4.3.435

- Mancebo F, Hilje L, Mora GA, and Salazar R (2002) Biological activity of two neem (Azadirachta indica A. Juss., Meliaceae) products on Hypsipyla grandella (Lepidoptera: Pyralidae) larvae. Crop Prot., 21(2):107-112 https://doi.org/10.1016/S0261-2194(01)00069-2
- Michereff-Filho M, Torres JB, Andrade LN, and Nunes MUC (2008) Effect of some biorational insecticides on Spodoptera eridania in organic cabbage. Pest Manag. Sci., 64(7):761-767 https://doi.org/10.1002/ps.1554
- Miller NJ, Giordano R, Siegfried BD, Gray ME, Meinke LJ, Sappington TW (2009) Genes, gene flow and adaptation of Diabrotica virgifera virgifera. Agric Forest Entomol, 11:47-60 https://doi.org/10.1111/j.1461-9563.2008.00398.x
- Moeser J (2003) Nutritional ecology of the invasive maize pest Diabrotica v. virgifera LeConte in Europe, PhD, Georg-August-University, Goettingen.
- Mordue (Luntz) AJ, Blackwell A (1993) Azadirachtin: an update. J. Insect Physiol, 39(11):903-924 https://doi.org/10.1016/0022-1910(93)90001-8
- Mordue AJ, Artes RJ (1997) Feeding Deterrence and Toxicity of Neem Triterpenoids. Journal of Chemical Ecology, 23(9):2117-2132 https://doi.org/10.1023/B:JOEC.0000006433.14030.04
- Morgan ED (2009) Azadirachtin, a scientific gold mine. Bioorg. Med. Chem, 17(12):4096-4105 https://doi.org/10.1016/j.bmc.2008.11.081
- Kuhlmann U, Moeser J, Vidal S (2003) Summary of International Symposium Ecology and Management of Western Corn Rootworm, Göttingen, Germany
- Mulla MS, and Su T (1999) Activity and biological effects of neem products against arthropods of medical and veterinary importance. J. Am. Mosq. Control Assoc, 15(2):133-152
- Nicoletti M, Petitto V, Gallo FR, Multari G, Federic E, Palazzino G (2012) The modern analytical determination of botanicals and similar novel natural products by the HPTLC fingerprint approach. Stud. Nat. Prod. Chem, 37:217-258. https://doi.org/10.1016/B978-0-444-59514-0.00008-0
- Norten E, Pütz J (1999) Neem: India’s Miraculous Healing Plant., Inner Traditions/Bear & Co: Rochester, USA
- Pálfay G (2001) Talajfertőtlenítéssel a kukoricabogár ellen (“Soil disinfection to control corn rootworm”). Gyakorlati Agrofórum (“Practical Agroforum”) 12(5):6
- Pereira AE, Wang H, Zukoff SN, Meinke LJ, French BW, Siegfried BD (2015) Evidence of Field-Evolved Resistance to Bifenthrin in Western Corn Rootworm (Diabrotica virgifera virgifera LeConte) Populations in Western Nebraska and Kansas. Plos One, 10(11) https://doi.org/10.1371/journal.pone.0142299
- Qiao J, Zou X, Lai D, Yan Y, Wang Q, Li W, et al. (2014) Azadirachtin blocks the calcium channel and modulates the cholinergic miniature synaptic current in the central nervous system of Drosophila. Pest Manag. Sci, 70:1041-1047 https://doi.org/10.1002/ps.3644
- Rice ME (2004) Transgenic rootworm corn: Assessing potential agronomic, economic, and environmental benefits. Plant Health Progress, 5(1) https://doi.org/10.1094/PHP-2004-0301-01-RV. Published online.
- Schmutterer H (1988) Potential of azadirachtin-containing pesticides for integrated pest control in developing and industrialized countries. Journal of Insect Physiology, 34(7):713-719 https://doi.org/10.1016/0022-1910(88)90082-0
- Senthil NS, Kalaivani K, Sehoon K, and Murugan K (2006) The toxicity and behavioural effects of neem limonoids on Cnaphalocrocis medinalis (Guenée), the rice leaffolder. Chemosphere, 62(8):1381-1387 https://doi.org/10.1016/j.chemosphere.2005.07.051
- Souza D, Peterson JA, Wright RJ, Meinke LJ (2020) Field efficacy of soil insecticides on pyrethroid-resistant western corn rootworms (Diabrotica virgifera virgifera LeConte). Pest Manage Sci., 76(2):827-833 https://doi.org/10.1002/ps.5586
- Spencer JL, Hibbard BE, Moeser J, Onstad DW (2009) Behaviour and ecology of the western corn rootworm (Diabrotica virgifera virgifera LeConte). Agricultural and Forest Entomology, 11(1):9-27 https://doi.org/10.1111/j.1461-9563.2008.00399.x
- Szalai M, Kiss J, Kövér Sz, Toepfer S (2013) Simulating crop rotation strategies with a spatiotemporal lattice model to improve legislation for the management of the maize pest Diabrotica virgifera virgifera. Agricultural Systems, 124:39-50 https://doi.org/10.1016/j.agsy.2013.10.009
- Tavares WS, Costa MA, Cruz I, Silveira RD, Serrão JE, Zanuncio JC (2010) Selective effects of natural and synthetic insecticides on mortality of Spodoptera frugiperda (Lepidoptera: Noctuidae) and its

predator Eriopis connexa (Coleoptera: Coccinellidae). J. Environ. Sci. Health B Pesticides Food Contaminants and Agricultural Wastes, 45:557-561 https://doi.org/10.1080/03601234.2010.493493

- Tímár A (2003) Komoly kihívásra, komoly válasz… ("A serious response to a serious challenge..."). Gyakorlati Agrofórum Extra („Practical Agroforum Extra"), 2:3-89
- Tuska T, Kiss J, Edwards CR, Szabó Z, Ondriusz I, Miskucza P, Garai A (2002) Az amerikai kukoricabogár (Diabrotica virgifera virgifera LeConte) imágóveszélyességi küszöbértékének (biberágás) meghatározása vetőmag-kukoricában ("Determination of the imago hazard threshold (pistil biting) of western corn rootworm (Diabrotica virgifera virgifera LeConte) in seed maize"). Növényvédelem ("Plant Protection"), 38:505-511
- Vörös L (2019) Options for the control of western corn rootworm (Diabrotica virgifera virgifera) larvae. MsC, Széchenyi István University, Mosonmagyaróvár
- Weathersbee AA, McKenzie CL (2005) Effect of a neem biopesticide on repellency, mortality, oviposition, and development of diaphorina citri (homoptera: psyllidae). Fla. Entomol, 88:401-407 https://doi.org/10.1653/0015-4040(2005)88[401:EOANBO]2.0.CO;2
- Wilps H, Kirkilionis E, Muschenich K (1992) The effects of neem oil and azadirachtin on mortality, flight activity, and energy metabolism of Schistocerca gregaria forskal — A comparison between laboratory and field locusts. Comp. Biochem. Physiol. C Comp. Pharmacol, 102(1):67-71 https://doi.org/10.1016/0742-8413(92)90045-9