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Utilisation of repellents for the control of Western corn rootworm (*Diabrotica virgifera virgifera* LeConte, 1868)

Využitie repelentov na ochranu proti kukuričiarovi koreňovému (*Diabrotica virgifera virgifera* LeConte, 1868)

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ABSTRACT

The aim of the study was to find the level of repellent effect of two chemical substances (DEET and 2-Undecanone) and two essential oils (from *Allium sativum* and *Eucalyptus oil*) on the Western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, 1868 in field conditions. Repellents were used in the area of 5 x 4 meters with four replications. They were applied as a solution of 700 µl chemical/essential oil in one litter of water. TWEEN 80 was used as a surfactant. After 24 and 48 hours, the repellent effect was checked by visual observation. After 24 hours, yellow sticky traps were installed in each plot and checked after the next 24 hours for the detection of the WCR adult beetles. The experiment was carried out during two years (2019 and 2020) in a maize field in Mojmírovce (48.199284°N, 18.038637°E), Slovakia. The results showed significant repellent effect of chemicals DEET and 2-Undecanone on WCR adults. After 48 hours the number of beetles per plant was significantly higher compared to the control for both DEET and 2-Undecamone. This was confirmed by yellow sticky traps in 2020, but not in 2019, probably due to the lower catching of WCR adults. The results indicate that repellent substances can influence the number of the WCR adults in maize fields.

Keywords: insect repellents, DEET, 2-Undecanone, *Allium sativum*, eucalyptus oil, field

ABSTRAKT

Cieľom práce bolo zistiť hladinu repelentného účinku dvoch chemických látok DEET, 2-Undecanone a esenciálnych olejov z *Allium sativum* a eukalyptového oleja na kukurici siatej proti kukuričiarovi koreňovému (WCR), *Diabrotica virgiera virgifera LeConte*, 1868 v poľných podmienkach. Repelenty boli použité na ploche 5 x 4 metre so štyrmi opakovaniami. Aplikovali sa ako roztok 700 µl chemického/esenciálneho oleja v jednom diely vody. Ako rozpúšťadlo sa použil TWEEN 80. Po 24 a 48 hodinách sa repelentný účinok skontroloval vizuálnym pozorovaním. Po 48 hodinách sa na detekciu výskytu dospelých chrobákov použili žlté lapače. Pokus sa uskutočnil v dvoch termínoch na kukuričnom poli v Mojmírovciach (48.199284°N, 18.038637°E) na Slovensku. Naše výsledky ukázali, že preukazný účinok chemikálií DEET a 2-Undekanone na imága kukuričiara koreňového. Po 48 hodinách bol počet imág v obidvoch prípadoch preukazne nižší ako v kontrolnom variante. Toto sa potvrdilo pri použití žltých lepových lapačov v roku 2020, ale nie v roku 2019 pravdepodobne z dôvodu nižšieho počtu nachytaných imág. Výsledky naznačujú, že repelentné látky môžu ovplyvniť početnosť imág kukuričiara koreňového na kukuričnom poli.

Kľúčové slová: insekticídny repelent, DEET, 2-Undecanone, *Allium sativum*, Eucalyptový olej, pole

INTRODUCTION

Western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, 1868 is one of the most destructive pests of maize. Through the years, populations developed resistance against genetically modified maize cultivars, crop rotation strategies, and insecticides. The majority of yield loss is due to larval feeding on the roots of maize (Gassmann et al., 2012, Lemic et al., 2015, Gyeraj et al., 2021). There are many ways on how to control the pest without the use of synthetic insecticides, which underlines the desirability of further efforts to develop new technologies to control WCR. They include the use of entomopathogenic nematodes (Toth et al., 2020, Modic et al, 2020), entomopathogenic fungi (Mulock and Chandler, 2001), RNAi-based insecticides (Ražná and Cagáň, 2019), etc.

Repellent derives from the Latin verb repellere, meaning "to reject". Generally speaking, the substance should only be considered as a repellent when it causes an organism to make oriented movements away from its source (Deletre et al., 2016). The repellent efficacy is measured by how long it protects at a certain level, under specified minimum condition of arthropod pressure. Repellents are mostly based on chemical products with an offensive smell or taste (Carroll, 2008).

The word effective and best-known insect repellents currently on the market are DEET (N, N-diethyl-3-methylbenzamide) and 2-Undecanone (Maia and Moore, 2011). DEET, an active ingredient that has been used worldwide since 1946, is a highly efficient repellent for a wide range of insect species (Maia and Moore, 2011). 2-Undecanone (methyl nonyl ketone), a natural non-toxic insect repellent compound, has been found in palm kernel oil and soybean oil (Sanghong et al., 2015).

Essential oils (EOs) or their chemical compounds derived from them can attract beneficial insects while repelling undesirable insects. Essential oils are produced in 17,500 aromatic species of higher plants belonging mostly to families of Myrtaceae, Lauraceae, Lamiaceae, and Asteraceae (Regnault, 1997). Eucalyptus leaves are a rich source of bioactive constituents possessing fungicidal, insecticidal, and herbicidal activities (Sousa et al., 2013). Garlic extracts had a toxic effect on an important agricultural pest (Deb-Kirtaniya et al., 1980). Lee et al. (1997) demonstrated the toxicity of several EOs constituents against WCR. In laboratory experiments, the extracts of *Allium sativum* Linnaeus, 1793 showed insecticidal properties on several coleopteran pests (Abdalla et al., 2017). Garlic emulsion had effective repellent activity towards *Tribolium castaneum* (Herbst, 1797) (Jahromi et al., 2012, Mobki et al., 2014).

Major substances of *Eucalyptus* EOs are 1,8-cineole and α-pinene (Sebei et al., 2015) or 1,8-cineole and linalool (Boukhatem et al., 2020). Essential oil of *Allium sativum* Linnaeus, 1793 includes organic molecules that contain sulphur, mainly diallyl disulfide, allyl propyl disulfide, allyl sulfoxide, and allicin. The activity of diallyl disulfide as a fumigant against many insects and pathogens has been reported by Chiam et al. (1999). Especially in developed countries, several EOs are used in registered commercial formulations. Among these products, the most frequent is garlic oil (Regnault et al., 2012).

Previous laboratory studies indicated repellent or lethal effects of some compounds on WCR larvae (Brandl et al., 2016; Bernklau et al., 2016), or *Diabrotica speciosa* Germar, 1824 adults (Barbosa et al., 2013), but similar studies were not done under field conditions. This idea is not new. Hammack et al. (1999) mentioned that the practicality of using repellents to manipulate the behaviour of gravid corn rootworm beetles deserves further evaluation. Hence the primary objective of this study was to determine the level of repellency of selected substances to WCR adults in a maize field.

MATERIAL AND METHODS

In 2019 and 2020, the repellent effect of four substances on Western corn rootworm (WCR) was tested in a maize field in Mojmírovce in Slovakia (48.199284°N, 18.038637°E; Agricultural Cooperative Farm Mojmírovce). The maize field was a monoculture for more than ten years without any chemical control. It was used as a food for wild animals. Maize was harvested in very late autumn or in winter. In the new season, the field was cultivated using the same method as it was in the other maize fields of the farm.

At the locality, the flight of the WCR adults started at the beginning of July and adults were usually present until early October. The experiments with repellents started on August 7, 2019, and on August 12, 2020. According to our former results (Cagáň and Rosca, 2012), WCR adults increase their movement in the maize field in August. Yellow sticky traps were also checked for the presence of beneficial insects from the families Syrphidae, Coccinellidae, and Anthocoridae.

The experiment included control and four “repellent” variants. Repellent variants included chemical substances DEET, 2-Undecanone, and essential oils from *Allium sativum* and *Eucalyptus*. Chemical substances were obtained from Sigma-Aldrich Company, DEET (N, N - diethyl - 3 methylbenzamide) in 97% concentration and 2-Undecanone (methyl nonyl ketone) in 99% concentration. Essential oils were obtained from Mystic Moments Inc. as commercial essential oils. According to the supplier, the main constituents of eucalyptus oil were 1, 8 cineole (80.51%), 5P-cymene (4.06%), dipentene (8.60%), beta pinene (0.29%), and alpha pinene (2.57%). Principle peaks (typical GC analysis) of garlic oil product were methylallyl sulfide 2.394, dimethyl disulfide 0.543, diallyl sulfide 5.283, methyl allyl disulfide 11.293, dimethyl trisulfide 0.628, diallyl disulfide 37.231, methyl allyl trisulfide 8.338, diallyl trisulfide 18.287, and diallyl tetrasulfide 3.819. TWEEN 80 was used as a surfactant (Abidi et al., 2018, Łyczko et al., 2020). Repellents were used in the area of 5 x 4 meters with four replications randomly allocated in the field. They were applied as a solution of 700 μl chemical/ essential oil in one litre of water directly onto plants. All plants in the plots were treated. TWEEN 80 was used as a solvent. We applied the substances only on eatable plant parts as the health risk is lower than with ethanol, acetone, etc. After 24 and 48 hours, the repellent effect was checked by visual observation. Twenty randomly selected plants were watched in each plot. After 24 hours, two yellow sticky traps were installed in each plot and then checked after the next 24 hours for detecting WCR adult beetles. Data were subjected to ANOVA (Tukey HSD test).

RESULTS

Table 1 shows the number of adults WCR in 2019 counted by visual observation and the number of adults caught by two yellow sticky traps (YST) installed 24 hours after repellent application and checked after the next 24 hours. The average number of adults per one plant counted after 24 hours depended on the treatment, and it was as follows: 2-Undecanone 5.03; DEET 3.45; garlic oil 7.13; eucalyptus oil 6.18; and 6.20 in the control. A significant difference was found only between the control and the area, where DEET was applied. After 48 hours, the average number of beetles per plant was 4.6 (2-Undecanone), 5.275 (eucalyptus oil), 3.025 (DEET), 7.125 (garlic oil), and 6.975 (control). A significant difference was found between the control and treatments by DEET or 2-Undecanone. The number of WCR adults on the yellow sticky traps in control variant was higher than in other variants, but the difference was not significant.

Table 2 shows the results from the experiment in 2020. Average number of adults per one plant counted after 24 hours depended on the treatment, and it was as follows: 2-Undecanone 1.925; DEET 2.425; garlic oil 4.425; eucalyptus oil 4.650, and the control 4.725. Significant difference was found between the control and treatments by DEET and 2-Undecanone. 48 hours after application of repellents, the average number of beetles per plant was 2.900 (2-Undecanone), 4.225 (Eucalyptus oil), 1.925 (DEET), 3.775 (Garlic oil), and 6.050 (control). There were significant differences between the control and the other four treatments by repellents. Captures on yellow sticky traps achieved 50.25 adults in the control, followed by garlic oil (46.50), eucalyptus oil (46.00), 2-Undecanone (27.50), and DEET (17.50) treatments. There was also a significant difference between the 2-Undecanone/DEET treatments and the other treatments.

The numbers of beneficial insects from the families Syrphidae, Coccinellidae, and Anthocoridae in yellow sticky traps were very low (averages achieved 0.25-1.75

Table 1. The number of adult WCR counted by visual observation and the number of adults captured with yellow sticky traps (YST) in a maize field in 2019 (Mojmírovce, Slovakia, 48.199284°N, 18.038637°E). EO = essential oil, SD = standard deviation

Repellent	Number of adults per plant after 24 hours (visual observation)		Number of adults per plant after 48 hours (visual observation)		Number of adults per YST	
	Average	SD	Average	SD	Average	SD
Garlic (EO)	7.125b	3.244	7.125bc	3.729	18.25a	4.272
Eucalyptus (EO)	6.175b	3.789	5.275bc	3.012	17.25a	3.403
2-Undecanone	5.025ab	3.662	4.600ab	3.161	16.50a	5.446
DEET	3.450a	2.855	3.025a	2.526	16.50a	4.795
Control	6.200b	3.709	6.975c	3.826	22.25a	3.304

* Numbers in columns marked with the same letters are not significantly different (Tukey HSD test; P = 0.05)

Table 2. The number of adult WCR counted by visual observation and the number of adults captured with yellow sticky traps (YST) in a maize field in 2020-2019 (Mojmírovce, Slovakia, 48.199284°N, 18.038637°E). EO = essential oil, SD = standard deviation

Repellent	Number of adults per plant after 24 hours (visual observation)		Number of adults per plant after 48 hours (visual observation)		Number of adults per YST	
	Average	SD	Average	SD	Average	SD
Garlic (EO)	4.425b	2.427	3.775bc	2.336	46.50b	7.549
Eucalyptus (EO)	4.650b	2.069	4.225c	2.336	46.00b	7.023
2-Undecanone	1.925a	1.508	2.900ab	1.316	27.50a	6.027
DEET	2.425a	1.567	1.925a	2.042	17.50a	3.109
Control	4.725b	2.640	6.050d	3.895	50.25b	8.981

* Numbers in columns marked with the same letters are not significantly different (Tukey HSD test; P<0.05)

adults per one yellow sticky trap) and there were no significant differences among variants (Tukey HSD test; P = 0.05).

DISCUSSION

In both years of our experiment, 2-Undecamone and DEET had a significant repellent effect on WCR adults in the maize field. The repellent effect of essential oils was usually not significant, however the number of WCR in plots was lower compared to the control. Currently, repellents are being used more widely in plant protection as an alternative to conventional pesticides, and our results support these statements (Koutsaviti et al., 2018). DEET and 2-Undecanone are primarily used as insect repellents due to their strong odour (Witting-Bissinger et al., 2008).

The situation with EOs was different. In the experiment, EOs of garlic and eucalyptus oil had significantly low repellence at very high concentration even though EOs are promising alternatives to insecticides for controlling insect pests (Koutsaviti et al., 2018). Another study showed a strong repellent activity of garlic towards *Tribolium castaneum* (Herbst, 1797) (Jahromi et al., 2012, Mobki et al., 2014, Mangang et al., 2019), and the extracts of *Allium rotundum* Linnaeus., 1762 showed a moderate level of insecticidal, antifeedant and repellent activity against the larvae of lepidopterans (Elisovetcaia et al., 2018). Plata-Rueda (2017) tested garlic EOs against storage pest *Tenebrio molitor* Linnaeus, 1758, and their results showed that the essential oil of garlic was the most repellent. On the other hand, Bedini et al. (2020) and several other authors reported the toxic effect of garlic

EOs on pests in case of high concentrations. Essential oils extracted from selected eucalyptus species against *Sitophilus zeamais* (Motschulsky, 1855) also showed a very good repellent effect (Karimi et al., 2013). This study did not confirm these data in maize field conditions where the target pest was WCR.

There are many reasons why essential oils from the same plant can show variable effects. The composition and impact of the EOs depend on plant type, geographical location, and collection season (Milos et al., 2000). Results may be unpredictable due to varying concentration in the used plant material (Shiferaw et al., 2011), as well as differences in the preparation method. Different plant parts are highly variable in active ingredient content and concentration. The plant's genotype, a wide range of environmental factors, and the development stage of the plant can strongly affect both content and concentration (Carter et al., 2013). In this experiment, EOs were from the same company and the extraction method was steam distillation. Maybe some other formulation or source would show different results.

The next question is how long the effect of repellents persists in the field after application. The results show that the numbers of WCR adults decreased in the first 24 hours after the application of repellents, but this decrease did not continue in the following 24 hours. This suggests that repellents were effective only in a relatively short time. In any case, it was found that also essential oils from plants should have "some" repellents to WCR adults in the maize field. This effect could probably be increased if the essential oils were applied in other forms, not just as a liquid solution.

Another matter is how the repellents can influence the behaviour of WCR. In the field, *Spodoptera frugiperda* Smith, 1797 reduced WCR abundance, but only when it arrives on the plant first, i.e., WCR rejects *S. frugiperda*-infested plants only when arriving second (Huang at al., 2017). For this, additional studies will be necessary, especially on how plant repellents can disturb the "dialogue" between *Spodoptera* and *Diabrotica*.

Because of its harmfulness, the multi-colored Asian lady beetle, *Harmonia axyridis* (Pallas, 1773) is interesting for the development of appropriate repellents. Garlic powder is a part of such repellent (Glemser et al., 2021). It was found that DEET has good potential for repelling *H. axyridis* adults and should be field-tested on urban structures (Riddick et al, 2004). But most studies were oriented to the attraction of beneficial insects (e.g., Lövei et al., 1992). In this experiment, the numbers of beneficial insects from the families Syrphidae, Coccinellidae, and Anthocoridae in yellow sticky traps were very low and there were no significant differences among variants. It seems that the repellents did not crucially influence their populations and this topic needs further study. It was confirmed by results from intercropping of aromatic plants that effects vary across aromatic plant species and more study is required to identify optimal plant species (Tang et al., 2012).

CONCLUSIONS

The repellent effect of some substances on the Western corn rootworm, *Diabrotica virgifera virgifera* under field conditions was studied, but there was no repellent effect detected in the use of garlic and eucalyptus oils as repellents. The results mentioned in this paper showed significant repellent effect of chemicals DEET and 2-Undecanone on WCR adults. It means that repellent substances can influence the number of WCR adults in a maize field. These results indicate that there are probably more substances with the same properties. The study also confirmed that application of repellents in field conditions could significantly influence insect pest abundance, however, the abundance of pest usually decreased for less than 50 percent.

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