RESEARCH

Effect of selected essential oils on the efficacy of volunteer oilseed rape control and phytotoxicity in maize plants

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ABSTRACT

The presence of weeds in cultivated fields can significantly reduce the yield of crops. In recent years, however, more and more attention has been paid to limiting the amount of chemical plant protection products used in agriculture. Numerous studies are carried out using substances of natural origin, like essential oils, as herbicides, fungicides and insecticides. In the conducted experiment, the use of clove (*Eugenia caryophyllus* (Spreng.) Bullock & S.G. Harrison) and pine (*Pinus sylvestris* L.) essential oils applied pre- and post-emergence as herbicides was investigated. Commercial source materials were selected. Emulsified essential oils were applied at 5, 10, 15, and 20 L ha^{-1}. The test plants were winter oilseed rape (*Brassica napus* L. subsp. *oleifera* (Delile) Sinskaya) and maize (*Zea mays* L.) The comparative herbicides were mesotrione and terbuthylazine (applied in doses registered in maize cultivation). Pre-emergence treatment was performed 1 d after sowing, and post-emergence essential oils and herbicide were applied when oilseed rape was in the 2-3 leaf stage and the maize was in the 3-4 leaf stage. Clove essential oil applied post-emergence contributed to the damage to both plant species (3 d after treatment: 7.5%-46.3% damage of volunteer oilseed rape; 2.5%-25.0% damage of maize, depending on dose). It also influenced the maximum photochemical efficiency of photosystem II. However, its effect was transient, unlike the synthetic herbicide. Applied pre-emergence, it did not affect the development of maize and rape (0% plant damage). Pine essential oil did not damage the test plants in soil and foliar application (0% plant damage).

Key words: *Brassica napus* subsp. *oleifera*, clove, *Eugenia caryophyllus*, mesotrione, phytotoxicity, pine, *Pinus sylvestris*, terbuthylazine, weeds.

INTRODUCTION

There are many methods of weed control, including preventive, cultivating, mechanical (physical), biological and chemical methods (Hamill et al., 2004). The use of various available methods is consistent with the assumptions of integrated pest management (Young et al., 2017). The chemical method has the greatest share in weed control (Moss, 2018). It is considered a relatively cheap way to protect crops from competition from weeds (Upadhyay et al., 2012). Its significant role is indicated in countries where there is a shortage of workers in agriculture (Gianessi, 2013). However, this method carries certain dangers (Kudsk and Streibig, 2003). Currently, society expects a reduction in the amount of chemical plant protection products that end up in the environment (Bakker et al., 2021). Additionally, the problem of weed resistance to herbicides is progressing (Westwood et al., 2018). Therefore, it is necessary to pay more attention to non-chemical methods of plant protection (Sanbagavalli et al., 2016).

Essential oils are an example of potential bioherbicides (De Mastro et al., 2021). The biological method of plant protection has advantages, among which the greatest attention is paid to their safety in relation to the environment and non-target organisms (Liu et al., 2021). Difficulties related to biopesticides are also mentioned, including complicated registration procedures, more difficult application and sometimes lower efficacy compared to chemical plant protection products (Damalas and Koutroubas, 2018; Constantine et al., 2020). Some of the substances of natural origin provide a new mechanism of action for the later developed synthetic plant protection products (Loiseleur, 2017). An example is leptospermone, which led to the introduction of herbicides from the triketones group (Ndikuryayo et al., 2017).

Essential oils are volatile substances (Božik et al., 2017). They are characterized by an intense fragrance (Anupama et al., 2019). They contain a mixture of terpenes - sesquiterpenes and monoterpenes (de las Heras et al., 2003). They dissolve into fixed oils, ether, and alcohol (Dhifi et al., 2016). Essential oils can be obtained from different parts of many plant species (Campolo et al., 2018).

Among the substances used to control weeds in maize cultivation, mesotrione and terbuthylazine are distinguished (Garko et al., 2020). Mesotrione is classified as a triketones (Le Person et al., 2016), the mechanism of action of this group is based on the inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) (Dumas et al., 2017). Terbuthylazine is a substance that affects the function of photosystem II (PSII) (Simić et al., 2012).

One of the important species of weeds is volunteer oilseed rape (Weber et al., 2014). In research on herbicides, it is also important to determine their phytotoxicity to the crop (de Almeida et al., 2018). A way to test the stress that herbicides exert on treated plants is to measure the plant chlorophyll fluorescence. The parameters determined in this study include maximum photochemical efficiency of photosystem II (F_v/F_m), a decrease in the value of this parameter indicates plant stress (Hazrati et al., 2016).

The aim of the research was to determine the composition of selected essential oils from a commercial source, their effect on the development of winter oilseed rape and maize, and the impact on the maximum photochemical efficiency of PSII.

MATERIALS AND METHODS

Analysis of chemical components

The conducted research used essential oils from a commercial source, Etja, Elbląg, Poland. Two essential oils were tested, clove (*Eugenia caryophyllus* (Spreng.) Bullock & S.G. Harrison) essential oil and pine (*Pinus sylvestris* L.) essential oil. Chemical components were extracted from essential oils using the solid-phase microextraction (SPME) method. Essential oils samples (8 mL) were placed in 20 mL vials and extracted by means of headspace SPME for 30 min at 50 ºC with 200 mm -53/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber StableFlex (Merck KGaA, Darmstadt, Germany) (Buśko et al., 2016; Perczak et al., 2019). The analyses were run on a gas chromatograph (7890A, Agilent, Santa Clara, California, USA) hyphenated to a mass spectrometer (TruTOF HT, LECO, St. Joseph, Michigan, USA), using an RTX-5 (0.20 mm × 10 m) capillary column. The injection port temperature was 260 ºC, transfer line temperature was 280 ºC and the analyses were performed with programmed temperature: initial 40 ºC held for 1 min, from 40 to 180 ºC at 10 ºC min^{-1}, 180 to 260 ºC at 40 ºC min^{-1}. The helium flow rate was held constant at 0.8 mL min^{-1}. Spectra were acquired at 50 spectra s^{-1} within a range of 30-380 Da. The detector voltage was 2500 V, electron energy 70 V. The content of chemical components was estimated by comparing the area of their total ion current (TIC) peaks with the internal standard (tridecane, 25 ng in pentane) and expressed as their ratio (RU). Compounds were identified by comparing their mass spectra with spectra from the National Institute of Standards and Technology (NIST)/U.S. Environmental Protection Agency (EPA)/National Institutes of Health (NIH) Mass Spectral Library (NIST Standard Reference Data Program, Gaithersburg, Maryland, USA) and retention indices were compared to data available in the literature.

Greenhouse research

Under greenhouse conditions, two series of experiments were performed in a completely randomized design, with four replicates for each combination in each series. The conditions in the greenhouse were controlled. Air humidity was maintained at the level of 50%-80%. The light level was supplemented by irradiation with sodium lamps (HPS) with

103 a capacity of 400 W (Elektro-Valo Oy, Uusikaupunki, Finland). The photoperiod was kept at the level of 16:8 h. The temperature in the greenhouse was 25 ± 2 °C during the day and 20 ± 2 °C during the night.

In the experiment winter oilseed rape (*Brassica napus* L. subsp. *oleifera* (Delile) Sinskaya) and maize (*Zea mays* L.) were tested. For foliar treatment, seeds were sown in 1 L pots filled with Kronen peat soil. After germination, eight winter oilseed rape plants or four maize plants were left in each pot. When the oilseed rape was in the 2-3 leaf stage and the maize was in the 3-4 leaf stage, a treatment was performed. In the case of soil treatment, pots with a capacity of 1 L were filled with soil from the field (loamy sand: 64% sand; 13% clay; 23% silt). Fifteen rapeseed or five maize seeds were sown in individual pots. The treatment was performed 1 d after sowing. The individual substances were applied using a greenhouse sprayer with a TeeJet 1102 nozzles (TeeJet Technologies GmbH, Schorndorf, Germany). The outflow of the liquid was 200 L ha^{-1}, and the pressure was 0.2 MPa. The control sample was not sprayed. In one of the combinations, the herbicide containing mesotrione was applied at a dose of 1 L ha^{-1} (2-(4-methylsulfonyl-2-nitrobenzoyl)cyclohexane-1,3-dione; 100 g ai L^{-1}, Kideka 100 SC, Nufarm GmbH & Co KG, Linz, Austria) for foliar application or the herbicide containing terbuthylazine at a dose of 1 L ha^{-1} (2-*N*-*tert*-butyl-6-chloro-4-*N*-ethyl-1,3,5-triazine-2,4-diamine; 500 g ai L^{-1}, Tezosar 500 SC, Ciech Sarzyna SA, Nowa Sarzyna, Poland) in the case of soil application. In subsequent combinations, clove essential oil or pine essential oil mixed with ethoxylated rapeseed oil (Rokacet RZ17, PCC group, Brzeg Dolny, Poland) were applied in a 4:1 ratio. Individual substances were applied in four doses (5, 10, 15, and 20 L ha^{-1}).

Plant chlorophyll fluorescence measurements were made using a multi-mode chlorophyll fluorometer (OS5p, Opti-Sciences, Hudson, New Hampshire, USA) and photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) was used. The study was performed 1 and 6 d after application (DAA). For each of the measurements, 12 replicates were made for each combination. For 30 min before the measurement, the leaves were dark-adapted using white clips to silence photosynthesis. The maximum efficiency of photosystem II (PSII) (F_v/F_m) protocol was chosen for measurement. Before the measurement, the parameters of the device were set so that the set fluorescence signal was in the range of 150-250 counts and was stable. They were compatible with the OS5p user's guide. Measurements were made on the youngest, fully developed leaves.

Visual assessment of the efficacy and phytotoxicity of individual substances was made at 3, 7, 14 and 21 DAA for foliar application and 14 and 21 DAA for soil application. The results were expressed on a scale of 0%-100% (0: no effect, 100: complete destruction of plants). The data were subjected to ANOVA and then to Tukey's protected LSD test with a probability level of 0.05.

RESULTS

Composition of the studied essential oils

In the composition of the clove essential oil, the chemical compound present in the highest concentration was eugenol, then eugenyl acetate and β-caryophyllene. In the case of pine essential oil, the dominant substance was α-pinene, and more than 10% of β-pinene, camphene, limonene and β-phellandrene were also detected. More chemical components were found in the composition of pine essential oil, and in the case of clove essential oil, the dominant substance was present in a higher concentration (Table 1).

Results of plant chlorophyll fluorescence measurements

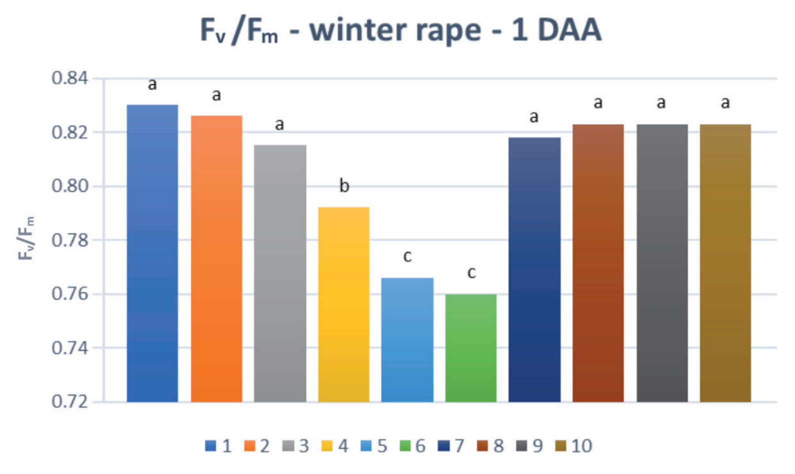
Clove essential oil applied in doses of 15 and 20 L ha^{-1} contributed to the greatest extent to the decrease in the value of the F_v/F_m parameter tested on rape plants 1 d after the treatment. Mesotrione, clove essential oil applied at the lowest dose and all doses of pine essential oil did not cause a significant decrease in F_v/F_m compared to the untreated control (Figure 1). During the measurement carried out 6 d after the treatment, only in the combination in which mesotrione was applied, a significant decrease in F_v/F_m value was observed compared to the oilseed rape control (Figure 2).

Clove essential oil applied at the highest dose contributed to the greatest extent to the reduction of F_v/F_m of the tested 1 DAA on maize plants. Clove essential oil applied at the lowest dose and all doses of pine essential oil did not cause a significant decrease in F_v/F_m compared to the maize control (Figure 3). During the measurement performed 6 DAA of all substances to maize plants, nonsignificant differences were observed in F_v/F_m (Figure 4).

Table 1. Composition of essential oils used in the experiment.

Clove essential oil		Pine essential oil	
Chemical compounds	Concentration	Chemical compounds	Concentration
	%		%
Eugenol	87.10	α-Pinene	21.13
Eugenyl acetate	8.03	β-Pinene	12.50
β-Caryophyllene	3.52	Limonene	10.38
α-Humulene	0.40	Camphene	10.35
(*E*)-β-Ocimene	0.31	β-Phellandrene	10.12
2-Nonanone	0.20	Germacrene D	7.32
p-Allyl phenol	0.18	Unknown	4.73
α-Copaene	0.10	Bornyl acetale	4.11
Caryophyllene oxide	0.10	α-Terpineol	3.31
2-Heptanone	0.03	Aristolochene	2.20
α-Pinene	0.01	Aromadendrene	2.14
Limonene+1,8-Cineole	0.01	α-Guaiene	1.99
Linalool	0.01	Tricyclene	1.84
		β-Selinene	1.70
		(*E*)-β-Farnesene	1.44
		Phenylethyl isovalerate	0.74
		Myrcene	0.65
		γ-Terpinene	0.48
		α-Amorphene	0.42
		Phenylethyl-3 methyl butanoate	0.42
		δ-3-Carene	0.33
		α-Terpinolene	0.26
		α-Phellandrene	0.23
		β-Ocimene	0.22
		α-Rlangene	0.22
		α-Cubebene	0.21
		Sabinene	0.15
		Terpinen-4-ol	0.15
		α-Thujene	0.12
		α-Terpinene	0.11

Figure 1. Effect of mesotrione and essential oils of maximum photochemical efficiency of photosystem II (F_v/F_m; non-nominated units) of winter rape 1 d after foliar application (DAA).



Different letters indicate significantly different mean LSD ($p < 0.05$) = 0.021.
1: Untreated; 2: mesotrione; 3-6: clove essential oil (doses: 5, 10, 15, 20 L ha^{-1} respectively); 7-10: pine essential oil (doses: 5, 10, 15, 20 L ha^{-1} respectively).

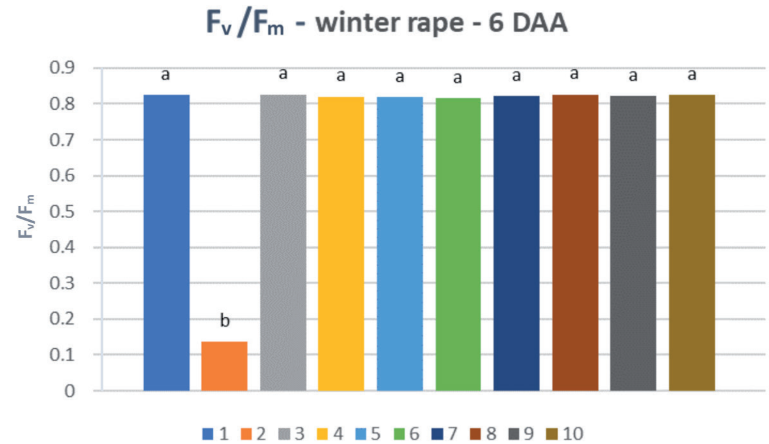
Visual assessment of effectiveness and phytotoxicity

Clove essential oil contributed most to the damage of winter oilseed rape, which was observed in the highest degree of 3 and 7 DAA (Table 2). In the course of subsequent assessments, it was observed that mesotrione caused greater damage to the plants. In the combinations where clove oil was applied, the young leaves showed no signs of damage, while the plants treated with the herbicide showed bleaching of the leaves. Pine oil did not cause any visually visible damage to the tested plants.

Clove essential oil was the only substance that contributed to the visible damage to maize, which was statistically confirmed. The phytotoxicity effect was transient. During the assessments performed with 14 and 21 DAA on the plants with the lowest dose of the substance, no damage was observed. Chemical plant protection agent and pine essential oil were selective in relation to the discussed plant (Table 3).

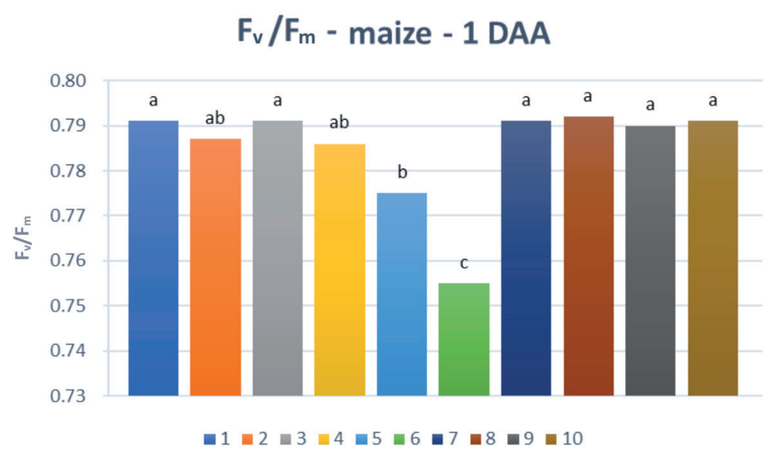
In the case of preemergence application of individual substances, damage to the test plants was observed only in the case of the combination in which terbuthylazine was applied to the treatments where winter oilseed rape was sown. In the case of combinations in which the test plant was maize, no damage was observed on any of the variants tested (Table 4).

Figure 2. Effect of mesotrione and essential oils of maximum photochemical efficiency of photosystem II (F_v/F_m; non-nominated units) of winter rape 6 d after foliar application (DAA).



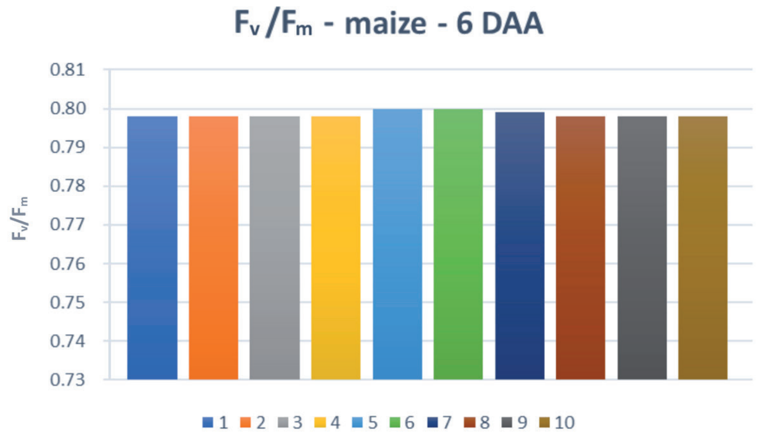
Different letters indicate significantly different mean LSD ($p < 0.05$) = 0.042.
1: Untreated; 2: mesotrione; 3-6: clove essential oil (doses: 5, 10, 15, 20 L ha^{-1} respectively); 7-10: pine essential oil (doses: 5, 10, 15, 20 L ha^{-1} respectively).

Figure 3. Effect of mesotrione and essential oils of maximum photochemical efficiency of photosystem II (F_v/F_m; non-nominated units) of maize 1 d after foliar application (DAA).



Different letters indicate significantly different mean LSD ($p < 0.05$) = 0.011.
1: Untreated; 2: mesotrione; 3-6: clove essential oil (doses: 5, 10, 15, 20 L ha^{-1} respectively); 7-10: pine essential oil (doses: 5, 10, 15, 20 L ha^{-1} respectively).

Figure 4. Effect of mesotrione and essential oils of maximum photochemical efficiency of photosystem II (F_v/F_m; non-nominated units) of maize – 6 d after foliar application (DAA).



All means were nonsignificantly different.
1: Untreated; 2: mesotrione; 3-6: clove essential oil (doses: 5, 10, 15, 20 L ha^{-1} respectively); 7-10: pine essential oil (doses: 5, 10, 15, 20 L ha^{-1} respectively).

Table 2. Visual assessment of the efficacy of the applied postemergence substances in relation to volunteer oilseed rape.

			Visual assessment of the efficacy (volunteer oilseed rape)			
Nr	Treatment	Dose	3 DAA	7 DAA	14 DAA	21 DAA
		L ha^{-1}	%			
1	Untreated	-	0.0e	0.0e	0.0f	0.0e
2	Mesotrione	1	0.0e	25.6b	60.0a	76.3a
3	Clove essential oil	5	7.5d	7.5d	7.5e	6.3d
4		10	20.0c	16.3c	12.5d	11.3c
5		15	32.5b	27.5b	18.8c	13.8bc
6		20	46.3a	35.0a	23.8b	15.0b
7	Pine essential oil	5	0.0e	0.0e	0.0f	0.0e
8		10	0.0e	0.0e	0.0f	0.0e
9		15	0.0e	0.0e	0.0f	0.0e
10		20	0.0e	0.0e	0.0f	0.0e
NIR (0.05)			5.08	5.3	2.94	2.71

DAA: Days after application.

Table 3. Visual assessment of phytotoxicity of the substances applied postemergence.

			Visual assessment of phytotoxicity (maize)			
Nr	Treatment	Dose	3 DAA	7 DAA	14 DAA	21 DAA
		L ha^{-1}	%			
1	Untreated	-	0.0c	0.0d	0.0d	0.0d
2	Mesotrione	1	0.0c	0.0d	0.0d	0.0d
3	Clove essential oil	5	2.5c	2.5d	0.0d	0.0d
4		10	5.0c	6.3c	2.5c	3.1c
5		15	18.1b	15.6b	11.9b	8.8b
6		20	25.0a	21.3a	15.6a	13.8a
7	Pine essential oil	5	0.0c	0.0d	0.0d	0.0d
8		10	0.0c	0.0d	0.0d	0.0d
9		15	0.0c	0.0d	0.0d	0.0d
10		20	0.0c	0.0d	0.0d	0.0d
NIR (0.05)			5.81	3.17	2.25	1.51

DAA: Days after application.

Table 4. Visual evaluation of the efficacy and phytotoxicity of substances applied preemergence.

L.p.	Treatment	Dose	Visual assessment of the efficacy (volunteer oilseed rape)		Visual assessment of phytotoxicity (maize)	
			14 DAA	21 DAA	14 DAA	21 DAA
		L ha^{-1}	%		%	
1	Untreated	-	0.0b	0.0b	0.0	0.0
2	Terbuthylazine	1	67.5a	91.3a	0.0	0.0
3	Clove essential oil	5	0.0b	0.0b	0.0	0.0
4		10	0.0b	0.0b	0.0	0.0
5		15	0.0b	0.0b	0.0	0.0
6		20	0.0b	0.0b	0.0	0.0
7	Pine essential oil	5	0.0b	0.0b	0.0	0.0
8		10	0.0b	0.0b	0.0	0.0
9		15	0.0b	0.0b	0.0	0.0
10		20	0.0b	0.0b	0.0	0.0
NIR (0.05)			2.23	2.02	n.s.	n.s.

DAA: Days after application.

DISCUSSION

Many substances have been detected in the composition of essential oils. In the case of clove essential oil, eugenol dominated, while in pine essential oil the highest compound was α-pinene. These substances are characteristic of the studied oils (Kamatou et al., 2012; Yang et al., 2016). Attempts have been made to use eugenol as a single component to determine their herbicidal effectiveness (Vaid et al., 2010). However, it is noted that the content of many substances in the composition of essential oils can be a good way to reduce the problem of resistance (Pavela and Benelli, 2016).

Clove essential oil had the greatest effect on the F_v/F_m of volunteer oilseed rape 1 d after the treatment and also significantly influenced the discussed parameter tested on maize plants. In the course of the next measurement, however, it was observed that F_v/F_m of the combination was significantly equal to the control. In the case of the application of mesotrione, during the measurement performed 1 DAA, the herbicide showed no effect on F_v/F_m, but contributed to a significant decrease in the value of this parameter during the next measurement carried out on volunteer oilseed rape. At the same time, it showed no effect on F_v/F_m determined in maize. Mesotrione is a systemic substance, whereas the clove essential oil had a contact effect. Systemic substances show their action later than contact substances. For contact substances, it is important to cover the plants thoroughly (Qasem, 2011). This was confirmed with the visual assessment. Clove essential oil visually showed its effect shortly after the treatment. In this case, the exact point of contact of the spray drops with the leaf surface of the treated plants was visible. Among the methods that allow for better coverage of plants with spray liquid, the use of adjuvants is distinguished. However, this may lead to a reduction in the evaporation time of the spray liquid (Li et al., 2019). With volatile essential oils, this can be a significant problem.

Clove essential oil was applied at a dose much higher than the synthetic herbicide. Currently, attention is paid to the ease of application of plant protection products and the cost of the treatment. The need for the potential use of high doses of essential oils could be a factor that would reduce the widespread use of these substances in practice (Boyd and Brennan, 2006). For organic farms that prohibit the use of chemicals, this prospect could be realized, but methods need to be developed beforehand to make essential oils more effective.

The foliar application of clove essential oil contributed to the damage of both studied species. These species belong to various groups of plants, which proves the lack of selectivity of the clove essential oil in relation to selected individual species. From the point of view of the method of weed control after emergence of the crop plant, this is not a favorable result. However, it should be remembered that non-selective substances, including glyphosate, are of great importance in weed control. Finding an alternative to glyphosate that is also safe would be an important part of the development of plant protection, but the search for new herbicides is a long process in which many substances have to be tested.

The essential oils applied pre-emergence did not damage the test plants. Studies reported in the literature show that various essential oils inhibit plant germination, but these are laboratory tests. In the case of soil application, it should be remembered that soil properties influence the efficacy of herbicides (Nordmeyer, 2015). On the other hand, the lack of influence on germinating plants may prove the safety of essential oils in relation to successively cultivated plants. Demonstrating a follow-up effect or its absence is an important element of research on the use of herbicides (Pintar et al., 2020).

The limited efficacy of essential oils may be related to the properties of these substances. They are characterized by a high level of volatility and a short half-life. Modification of these parameters could significantly contribute to the improvement of the effectiveness of the test compounds. Scientists are taking up the topic of modifying the properties of essential oils and the need to stabilize them (Pavoni et al., 2020). The potential of the individual substances should be investigated in advance. The application of pine essential oil did not damage the tested plants, which proves that not all essential oils show the same effectiveness. However, information is available in the literature on the herbicidal efficacy of pine extracts (Giepen et al., 2014). The lack of activity in relation to the test plants may indicate the selectivity of pine essential oil in relation to the tested plants or the need to increase the applied dose. It may also be because pine essential oil is obtained from different species of pine.

CONCLUSIONS

The search for new methods of weed control is an important aspect of agricultural research. Currently, much attention is paid to the possibility of using substances of natural origin, including essential oils. In the experiment, clove essential oil applied as foliar application contributed to significant damage to the test plants, which, however, regenerated over time after the treatment. The mentioned substance had an effect on the maximum photochemical efficiency of photosystem II immediately after application. However, the clove essential oil was not effective in the pre-emergence application. Pine essential oil applied both as foliar and soil application did not affect the development of the test plants. Essential oils have the potential to be used as plant protection products, but more research is needed to improve their efficacy and reduce the phytotoxicity effect.

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