



Concurrent weed growth suppression with essential oils and species-specific response to fractionated coconut oil

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ABSTRACT

Plant-derived oils are phytotoxic and thus are potential non-synthetic herbicides. The present study investigated the effectiveness of three essential oils (red thyme, clove bud, cinnamon bark) and a vegetable oil, fractionated coconut oil (FCO), used alone or in 2-way essential/FCO mixes for controlling some troublesome weeds at seedling stage. The tested weeds were mugwort (*Artemisia vulgaris* L.), crown daisy (*Glebionis coronaria* (L.) Spach), milk thistle (*Sylibum marianum* (L.) Gaertn.), dense-flowered mullein (*Verbascum densiflorum* Bertol.), goosegrass (*Eleusine indica* L. (Gaertn.)), entireleaf morningglory (*Ipomoea hederacea* (L.) Jacq.), small-flower morningglory (*Jacquemontia taminifolia* (L.) Griseb), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), hemp sesbania (*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill).

Greenhouse trials included a negative control (non-treated) and a positive control (glyphosate) and were conducted in Sassari (IT) and Auburn (USA). In both locations, all the essential oils used severely injured weeds by 5 days after treatment (DAT), with least or no recovery by plants, with no harvestable plant biomass 20 DAT. FCO provoked more diverse species-specific responses, reducing biomass of crown daisy, large crabgrass, entireleaf morningglory and hemp sesbania compared to the non-treated control while it stimulated the growth of mugwort, dense-flowered mullein, goosegrass, and small-flower morningglory. Milk thistle was the only plant not influenced by FCO compared to the non-treated control. The essential oils mixed with FCO confirmed high phytotoxic effects with a significant improved action when red thyme oil + FCO was applied to goosegrass. Our research confirms the potential herbicidal effect of some phytotoxic essential oils and the potential beneficial growth effect of FCO to some species.

1. Introduction

Some plant-derived oils are potential non-synthetic herbicides. Essential oils are the volatile secondary metabolites found in plants that provide documented insect repellent, allelopathic, and fungistatic properties (Baker and Grant, 2018). Some essential oils have showed phytotoxic activity against different target plants mainly due to the presence of bioactive compounds such as monoterpenes and sesquiterpenes (Batish et al., 2004), but may include aliphatic and aromatic esters (Baker and Grant, 2018), that can vary considerably depending on several factors such as growth conditions of the plant and the genetics of the variety of the plant species from which they are extracted (Korres et al., 2019). Research indicates that essential oils disrupt mitosis (Duke et al., 2003; Issa et al., 2020) and photosynthesis (Pouresmaeil et al., 2020), but are non-selective and should be managed carefully to avoid

crop injury (De Mastro et al., 2021). These compounds are approved in organic farming, but their effectiveness is limited by their quick volatilization and lack of systemic activity (Korres et al., 2019).

Middle length chain fatty acids (C6 to C10), such as caprylic acid (C8, also known as octanoic acid) and pelargonic acid (C9, also known as nonanoic acid) are utilized as contact herbicides (Mason and Uchanski, 2019; Muñoz et al., 2020). They cause strong and rapid electrolyte leakage and severe damage to cell membranes and thylakoid membranes of treated leaves (Fukuda et al., 2004). Pelargonic acid is considered a compound of low toxicity and low environmental impact but has no residual activity or translocation potential (Dayan et al., 2009) thus most treated weeds tend to recover. Coconut oil is a natural source of middle length chain fatty acids which contains 6–9% caprylic acid (C8) and 6–10% capric acid (C10) along with long-chain fatty acids (Gervajio, 2005). Long-chain fatty acids are solid at room temperature which

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would complicate the use of raw coconut oil as a herbicide. Conversely, FCO obtained from distillation removes long-chain fatty acids and remains liquid at room temperature and largely contains caprylic and capric fatty acids (Gervajio, 2005).

Given that some commercial products containing both caprylic and capric fatty acids are already available on the market (e.g. Suppress® and Homeplate®) as non-synthetic herbicides, we theorized that FCO could be utilized for the same purpose. Flessner et al. (2010) reported that caprylic acid or combinations of caprylic acid and clove oil provided better control of winter broadleaf and grassy weeds than pelargonic acid alone.

Considering their origin and their activity, all these above-mentioned plant-derived oils could be profitably used in combination with other tactics and tools to create effective strategies for weed control especially against those species that under the selection pressure of excessive herbicide use have evolved to resist herbicides modes of action, thus reducing the number of available herbicides for farmers at global scale (Giannini et al., 2021; Loddo et al., 2021).

Therefore, the objective of our research was to determine the phytotoxicity of the selected oils on the chosen weeds (mugwort, crown daisy, milk thistle, dense-flowered mullein, goosegrass, entireleaf morningglory, small-flower morningglory, large crabgrass, hemp sesbania) and whether the addition of FCO to three different essential oils (red thyme, clove bud, cinnamon bark) would improve their efficacy in controlling selected weeds.

2. Materials and methods

2.1. Greenhouse experiments

Greenhouse experiments were conducted in Autumn 2020 in both Sassari, Sardinia, Italy (IT) at the Ottava experimental station of the University of Sassari (40°46'47" N, 8°29'45" E) and in Auburn, Alabama, United States of America (USA) at Auburn University (32.609127° N, -85.48199° E). In Sassari, the greenhouse was unheated and the average minimum air temperature during the trial was 10 °C while the average maximum air temperature was 20 °C. The average elio-phany was ~ 380 min/day. In Auburn, the greenhouse was temperature controlled with an average temperature of 30 (± 2) °C and an average elio-phany of 440 min/day.

Species evaluated in Sassari included mugwort, crown daisy, milk thistle, and dense-flowered mullein. Species evaluated in Auburn were goosegrass, entireleaf morningglory, small-flower morningglory, large crabgrass, and hemp sesbania. Species were propagated from seeds from naturalized, local populations at each location with the only exception of mugwort and dense-flowered mullein that were purchased from Wolfgang Meier (Fürstenwalde, DE, www.exotic-samen.de).

For each experimental run, species were sown in seedbeds with locally sourced potting media and were automatically irrigated three times daily with overhead irrigation to achieve the total of 5 mm of water per day.

After two weeks, single seedling was transplanted in individual pot at both locations. Pots used in US were cubic (volume: 250 ml) and were filled with potting media (Miracle-Gro Moisture Control Potting Mix, Scotts Miracle-Gro, Marysville, OH, USA). Pots used in Italy were truncated cone (volume: 200 ml) and were filled with Radicom substrate (Vigorplant, Fombio, LO, IT). Treatments were performed once plants reached the phenological stage of 3–4 true leaves for dicot plants and one to two tillers for monocot plants.

Treatments included cinnamon bark oil, clove bud oil, red thyme oil, FCO, cinnamon bark oil + FCO, clove bud oil + FCO, and red thyme oil + FCO. For the Sassari trial, cinnamon oil, thyme oil and clove oil were purchased from Mystic Moments (Fordinbridge, Hants, UK; www.mysticmomentsuk.com), while FCO was purchased from Naissance (Neath, Wales, UK; it.naissance.com). For the Auburn trial, essential oils and FCO were purchased from J. Edwards International, Inc. (Braintree, MA,

USA; www.Bulknaturaloils.com). The essential oils used in both experiments were 100% pure essential oils, while the fatty acid profile of FCO was composed by caprylic acid (56.9%), capric acid (42.4%), lauric acid (0.39%) and myristic acid (0.11%).

All the oils were applied as a 5% v/v solution and in the case of mixtures, both oils were applied at 5% v/v. A positive control of glyphosate (Auburn: Accord XRT II, Corteva AgriScience, Indianapolis, IN, USA; Sassari: Roundup®Platinum, Bayer CropScience Italia, Milan, IT) at 1 kg ha⁻¹ and a non-treated check were included. Herbicide treatments were applied with 0.5% v/v non-ionic surfactant (for both locations: Polysorbate 80, Naissance, Neath, UK).

In both locations, a similar CO₂-powered backpack sprayer (JR-201S) from BellSpray Inc. dba R&D Sprayers (Opelousas, LA, USA) was used with a spray volume of 460 L ha⁻¹. In Sassari, the backpack sprayer was equipped with 4 nozzles boom XR11003 VS (TeeJet, Spraying Systems Co., Wheaton, IL, USA) spaced 49 cm each. In Auburn, the backpack sprayer was equipped with 4 standard flat fan nozzles XR8002 VS (TeeJet, Spraying Systems Co., Wheaton, IL, USA) spaced 25 cm each. Following treatments, plants were allowed to dry, and then irrigation was resumed after the minimum of 4 h.

Data collected included a visual assessment of percent control based on injury on a 0–100% scale where 0 is no visual phytotoxicity (e.g. 0% of leaf surface is covered by necrosis or chlorosis) and 100% being complete desiccation of all above-ground plant tissue (plant death). For example, 30% injury was translated to 30% phytotoxicity of above-ground biomass compared to the non-treated. Assessments of percent control were made at 5, 10, and 20 days after treatment (DAT). Destructive harvest of above-ground biomass was performed at 20 DAT to determine fresh weight.

The trials were laid out using factorial design with herbicide treatment and weed species as the factors. Treatments were arranged as completely randomized design with five replicates at Sassari and three replicates at Auburn. Trials were repeated twice at each location (IT- 1st spraying run: 20 October 2020, 2nd spraying run: 25 October 2020; US – 1st spraying run: 29 September 2020, 2nd spraying run: 30 October 2020).

2.2. Data analysis

Data were subjected to ANOVA (alpha = 0.05) using the GLM procedure in SAS v. 9.4 (SAS, RTP, NC, USA; https://www.sas.com/en_us/home.html). Data were analyzed by location. Model evaluated main effects of replication, herbicide treatment, species and interactions of treatment by species and treatment by run by species. Means were separated using the Waller-Duncan multiple comparison procedure in the MEANS statement of PROC GLM (Waller and Duncan, 1969). Significant interaction effects were presented over main effects in the analysis.

3. Results

For both locations, Sassari and Auburn, a herbicide treatment by species interaction was observed, therefore multiple comparison procedure was applied to the interaction over main effects. There were two basic responses across all species and treatments: species that responded negatively to essential oils and FCO and species that responded negatively to essential oils and positively or neutrally to FCO. Presentation of results will focus on this distinction. For ease of presentation, results for the different location will be presented separately.

3.1. Italy location

In Sassari, essential oils, red thyme, clove bud, and cinnamon bark, applied alone or in combination with FCO injured all species from 86.5% to 100% at 5 DAT (Table 1), indicating rapid phytotoxic effects. In both locations across all species, glyphosate was slower acting and induced

Table 1

Response of mugwort, crown daisy, milk thistle, and dense-flowered mullein to essential oils and fractionated coconut oil at Sassari (IT).

| Treatment | mugwort | | | | crowndaisy | | | | milk thistle | | | | dense-flowered mullein | | | |
|---------------------------------|------------|--------|--------|--------|------------|--------|--------|--------|--------------|--------|--------|--------|------------------------|--------|--------|--------|
| | 5 DAT | 10 DAT | 20 DAT | 20 DAT | 5 DAT | 10 DAT | 20 DAT | 20 DAT | 5 DAT | 10 DAT | 20 DAT | 20 DAT | 5 DAT | 10 DAT | 20 DAT | 20 DAT |
| | Injury (%) | | | FW (g) | Injury (%) | | | FW (g) | Injury (%) | | | FW (g) | Injury (%) | | | FW (g) |
| Cinnamon bark | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C | 86.5 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C |
| Red thyme | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C | 86.5 A | 95 A | 90 A | 0.10 C | 100 A | 100 A | 100 A | 0.00 C |
| Clove bud | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C |
| FCO | 0 C | 0 C | 0 B | 0.40 A | 0 C | 0 C | 0 C | 0.72 B | 10.5 BCE | 5 C | 0 C | 6.94 A | 0 C | 0 C | 0 B | 1.03 A |
| Cinnamon bark + FCO | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C |
| Red thyme + FCO | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C | 89.5 A | 99.5 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C |
| Clove bud + FCO | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C | 90 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C |
| Glyphosate | 41 B | 70 B | 100 A | 0.00 C | 8 B | 87 B | 100 A | 0.02 C | 26 BCE | 65 B | 70 B | 2.56 B | 13 B | 36 B | 100 A | 0.02 C |
| Non-treated | 0 C | 0 C | 0 B | 0.22 B | 0 C | 0 C | 0 B | 1.17 A | 5.5 C | 0 C | 0 C | 6.98 A | 0 C | 0 C | 0 B | 0.41 B |
| Waller- Duncan MSD ^a | 6.5 | 6.5 | 1.8 | 0.07 | 7.8 | 8.1 | 0 | 0.31 | 16.2 | 7.81 | 11.1 | 1.18 | 5.2 | 8.3 | 0 | 0.14 |

^a Abbreviations: FCO, fractionated coconut oil; DAT, days after treatment; MSD, minimum significant difference; FW, fresh weight

^a Waller-Duncan Minimum Significant Difference test conducted at alpha = 0.05. In each column, means with the same letter are not significantly different.

maximum observed control at 20 DAT. For the majority of the cases, injury was 100% from 5 to 20 DAT and no plant biomass production was recorded at the final harvest (20 DAT) (Table 1). The only minor exception (not statistically relevant) was red thyme oil that injured milk thistle 90% at 20 DAT.

In addition, FCO did not injure mugwort, crown daisy, and dense-flowered mullein at any observation time. Conversely, FCO injured milk thistle 10% and 5% at 5 and 10 DAT, respectively. At 20 DAT, milk thistle recovered completely. FCO caused mugwort biomass to double and dense-flowered mullein biomass to more than double compared to non-treated plants (Fig. 1, Fig. 2). Conversely, crown daisy yielded more biomass when non-treated than under treatment with FCO (Fig. 3). No biomass growth difference for milk thistle was observed between FCO and non-treated control (Table 1, Fig. 4).

The positive control, glyphosate, injured crown daisy, dense-flowered mullein and mugwort 100% 20 DAT; thus no biomass was harvestable. However, injury of milk thistle by glyphosate was only 70% at 20 DAT, thus biomass was harvestable but less than non-treated control and FCO treatment (Table 1).

3.2. . US location

In Auburn, the responses of the tested weeds to treatments were more diverse with less injury and more recovery occurring compared to species evaluated in Italy. However, species followed the same dichotomous pattern by negatively responding to essential oils with and without FCO and resulting in diverse responses to applications of FCO alone. As the data will show, large crabgrass, entireleaf morningglory, and hemp sesbania responded negatively to essential oils and FCO alone, while goosegrass and small flower morning glory responded negatively to essential oils and positively to FCO alone.

Essential oils, except for red thyme oil alone, applied alone or mixed with FCO injured large crabgrass > 99% at 5 DAT and 100% at 20 DAT (Table 2). Red thyme oil was an exception since it injured large crabgrass ~91% at 5 DAT, but large crabgrass recovered to ~87% injury at 10 DAT and ~61% injury at 20 DAT. At 10 DAT, FCO significantly injured large crabgrass compared with non-treated control, while at 20 DAT there were not differences. However, a negative growth response was observed at 20 DAT compared to non-treated control (Table 2, Fig. 5). Glyphosate injured large crabgrass less than the essential oils sprayed alone or mixed at 5 DAT. From 10 DAT, glyphosate injury was similar to the other essential oils alone (except for red thyme oil) or

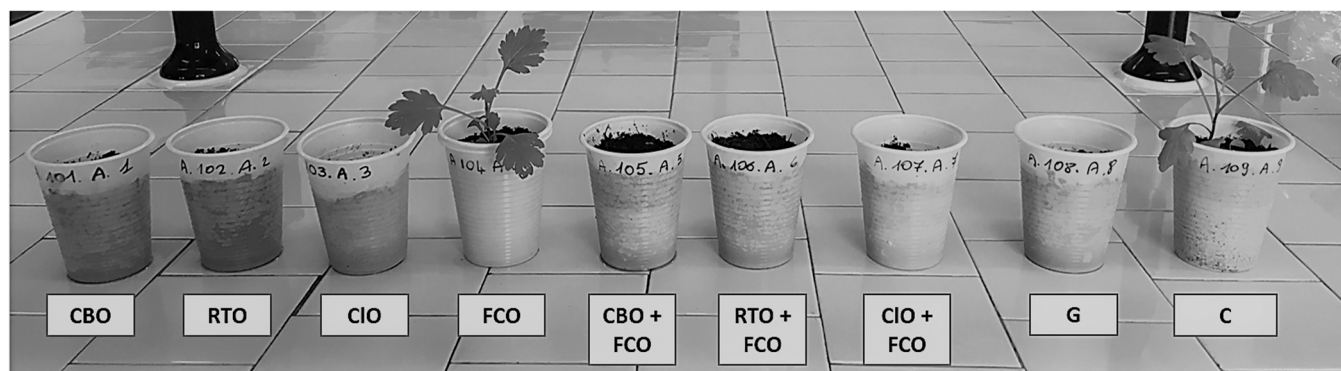


Fig. 1. Mugwort plants 21 days after treatment with cinnamon bark oil (CBO), red thyme oil (RTO), clove bud oil (CIO), fractionated coconut oil (FCO), cinnamon bark oil + fractionated coconut oil (CBO+FCO), red thyme oil + fractionated coconut oil (RTO + FCO), clove bud oil + fractionated coconut oil (CIO + FCO), glyphosate (G), non-treated control (C).

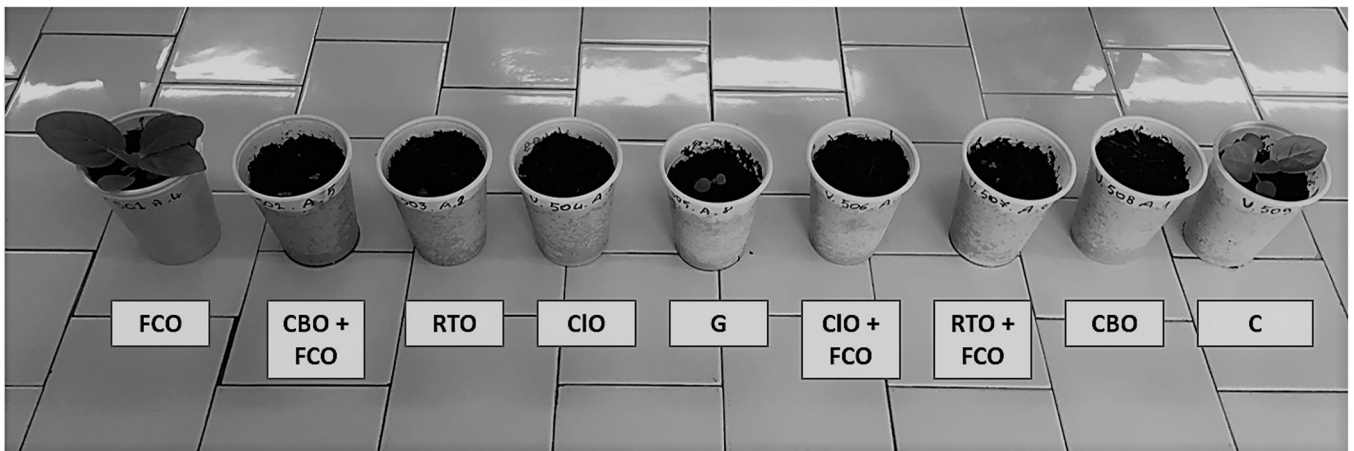


Fig. 2. Dense flowered mullein plants 21 days after treatment with cinnamon bark oil (CBO), red thyme oil (RTO), clove bud oil (CIO), fractionated coconut oil (FCO), cinnamon bark oil + fractionated coconut oil (CBO+FCO), red thyme oil + fractionated coconut oil (RTO + FCO), clove bud oil + fractionated coconut oil (CIO + FCO), glyphosate (G), non-treated control (C).

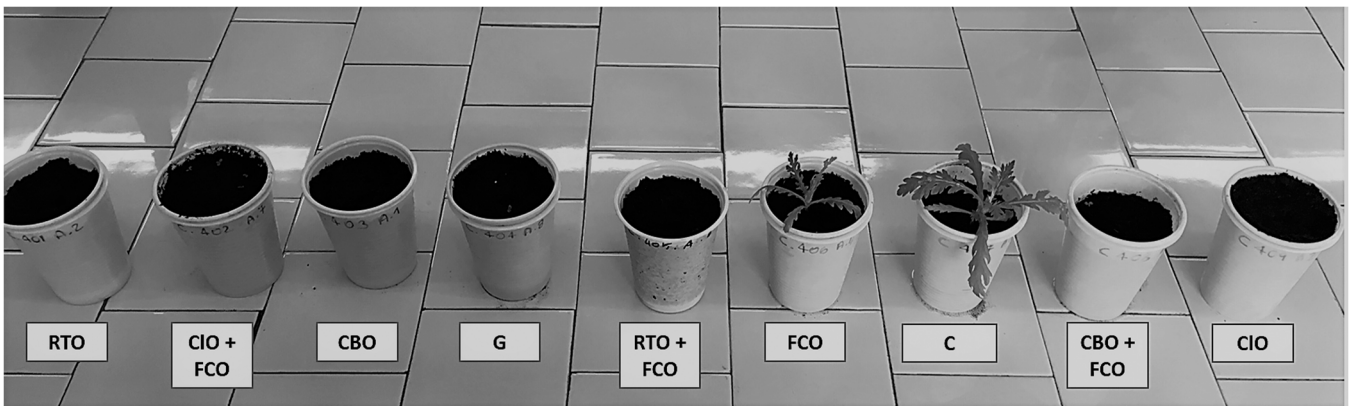


Fig. 3. Crown daisy plants 21 days after treatment with cinnamon bark oil (CBO), red thyme oil (RTO), clove bud oil (CIO), fractionated coconut oil (FCO), cinnamon bark oil + fractionated coconut oil (CBO+FCO), red thyme oil + fractionated coconut oil (RTO + FCO), clove bud oil + fractionated coconut oil (CIO + FCO), glyphosate (G), non-treated control (C).

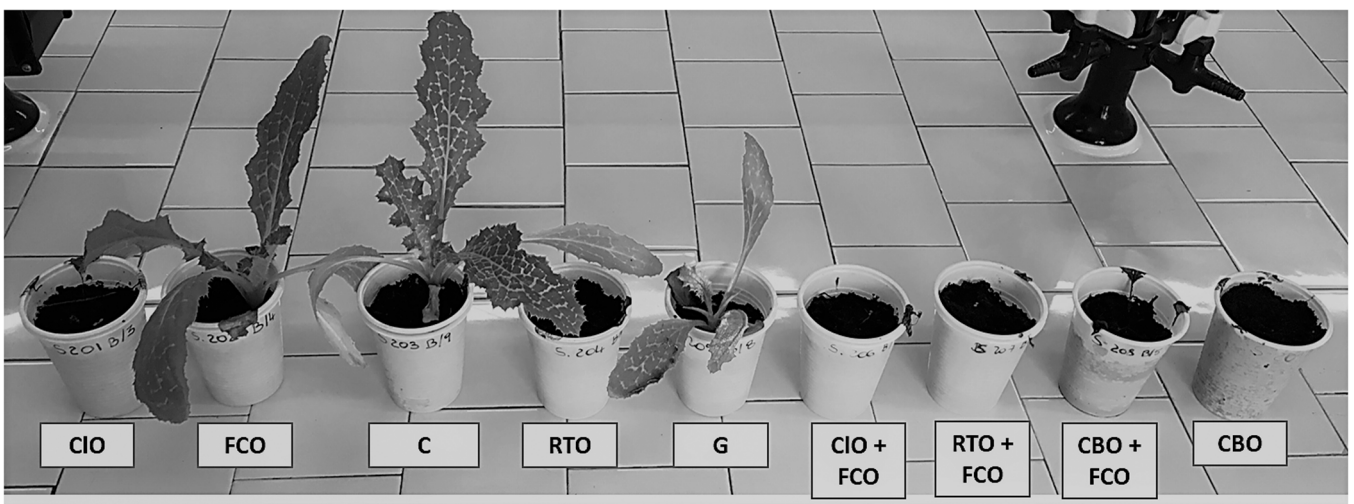


Fig. 4. Milk thistle plants 21 days after treatment with cinnamon bark oil (CBO), red thyme oil (RTO), clove bud oil (CIO), fractionated coconut oil (FCO), cinnamon bark oil + fractionated coconut oil (CBO+FCO), red thyme oil + fractionated coconut oil (RTO + FCO), clove bud oil + fractionated coconut oil (CIO + FCO), glyphosate (G), non-treated control (C).

Table 2

Response of large crabgrass, goosegrass, entireleaf morningglory, small flower morningglory and hemp sesbania to essential oils and fractionated coconut oil at Auburn (USA).

| Treatment | large crabgrass | | | | goosegrass | | | | entireleaf morningglory | | | |
|---------------------------------|-----------------|--------|--------|--------|------------|--------|--------|----------|-------------------------|----------|---------|----------|
| | 5 DAT | 10 DAT | 20 DAT | 20 DAT | 5 DAT | 10 DAT | 20 DAT | 20 DAT | 5 DAT | 10 DAT | 20 DAT | 20 DAT |
| | Injury (%) | | | FW (g) | Injury (%) | | | FW (g) | Injury (%) | | | FW (g) |
| Cinnamon bark | 100 A | 100 A | 100 A | 0.00 C | 97.5 A | 99.2 A | 100 A | 0.00 C | 81.7 AB | 86.7 AB | 81.7 B | 0.93 CD |
| Red thyme | 90.8 A | 86.7 B | 60.8 B | 0.17 C | 88.3 A | 84.2 A | 51.7 B | 0.35 BCE | 80.8 ABC | 95.8 A | 91.7 AB | 0.45 C |
| Clove bud | 100 A | 99.2 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C | 94.2 A | 96.7 A | 100 A | 0.00 D |
| FCO | 10 C | 25.8 C | 16.7 C | 0.72 B | 5 C | 11.7 C | 11.7 C | 2.88 A | 5 D | 33.3 D | 48.3 C | 3.15 B |
| Cinnamon bark + FCO | 99.2 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C | 64.2 C | 79.2 BCE | 83.3 AB | 0.89 CD |
| Red thyme + FCO | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C | 71.7 BCE | 79.2 BCE | 64.2 C | 2.17 BCE |
| Clove bud + FCO | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C | 95.8 A | 96.7 A | 100 A | 0.00 C |
| Glyphosate | 58.3 B | 99.2 A | 100 A | 0.00 C | 16.7 B | 98.3 A | 100 A | 0.00 C | 15.8 D | 75.8 C | 88.3 AB | 0.79 CD |
| Non-treated | 0 C | 0 D | 15 C | 1.34 A | 0 C | 0 C | 0 D | 1.30 B | 0 D | 0 E | 0 D | 9.5 A |
| Waller- Duncan MSD ^a | 12.3 | 8.4 | 18 | 0.41 | 14.6 | 13.2 | 15.3 | 0.52 | 17.1 | 10.3 | 16.9 | 1.51 |

| Treatment | small-flower morningglory | | | hemp sesbania | | | | |
|---------------------------------|---------------------------|--------|--------|---------------|------------|---------|--------|--------|
| | 5 DAT | 10 DAT | 20 DAT | 20 DAT | 5 DAT | 10 DAT | 20 DAT | 20 DAT |
| | Injury (%) | | | FW (g) | Injury (%) | | | FW (g) |
| Cinnamon bark | 82.2 A | 100 A | 100 A | 0.00 C | 95.8 A | 96.7 AB | 100 A | 0.00 C |
| Red thyme | 99.2 A | 100 A | 83.3 A | 0.00 C | 97.5 A | 96.7 AB | 99.2 A | 0.00 C |
| Clove bud | 100 A | 100 A | 100 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C |
| FCO | 2.5 B | 0 C | 0 B | 3.74 A | 13.3 C | 10 C | 13.3 C | 0.47 B |
| Cinnamon bark + FCO | 96.7 A | 99.2 A | 83.3 A | 0.00 C | 95 A | 97.5 AB | 97.5 A | 0.05 C |
| Red thyme + FCO | 99.2 A | 99.2 A | 83.3 A | 0.00 C | 92.5 A | 95 AB | 95.8 A | 0.07 C |
| Clove bud + FCO | 100 A | 100 A | 83.3 A | 0.00 C | 100 A | 100 A | 100 A | 0.00 C |
| Glyphosate | 5 B | 35 B | 75.8 A | 0.32 C | 45 B | 66.7 B | 88.3 B | 0.13 C |
| Non-treated | 0 B | 0 C | 0 B | 1.48 B | 0 C | 0 C | 0 C | 1.06 A |
| Waller- Duncan MSD ^a | 15 | 2.3 | 31.1 | 0.55 | 14.3 | 11.2 | 6.5 | 0.18 |

^a Abbreviations: FCO, fractionated coconut oil; DAT, days after treatment; MSD, minimum significant difference; FW, fresh weight.

^a Waller-Duncan Minimum Significant Difference test conducted at alpha = 0.05. In each column, means with the same letter are not significantly different

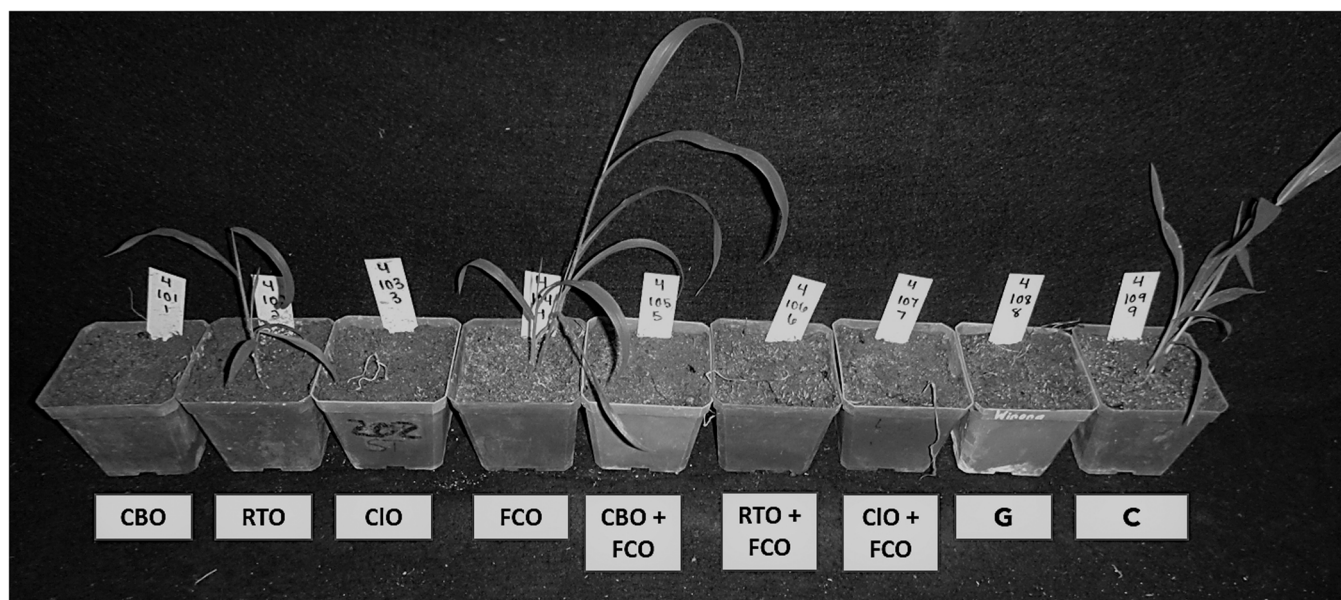


Fig. 5. Large crabgrass plants 21 days after treatment with cinnamon bark oil (CBO), red thyme oil (RTO), clove bud oil (CIO), fractionated coconut oil (FCO), cinnamon bark oil + fractionated coconut oil (CBO+FCO), red thyme oil + fractionated coconut oil (RTO + FCO), clove bud oil + fractionated coconut oil (CIO + FCO), glyphosate (G), non-treated control (C).

mixed.

Entireleaf morningglory and hemp sesbania responded in a similar fashion as large crabgrass. Essential oils alone and clove bud oil +FCO injured entireleaf morningglory greater than all other treatments at 10 DAT, while glyphosate injured ~76% and FCO ~33% (Table 2). At 20 DAT, all essential oils alone injured entireleaf morningglory > 80%, while FCO, sprayed alone, injured entireleaf morningglory ~48%. The least effective among all treatments containing essential oils was red thyme oil + FCO (64.2%). For the vast majority of the treatments, the

recorded biomass was lower than 1 g. The only exception was registered for plants sprayed with FCO, red thyme oil + FCO whose biomass was around 2–3 g and consistently lower than non-treated control (Fig. 7).

All essential oils with or without FCO injured hemp sesbania from 92.5% to 100% at 5 DAT, while glyphosate injured 45% (Fig. 9). At 10 and 20 DAT, all essential oils both alone and mixed with FCO highly injured hemp sesbania (95–100%), while glyphosate injury reached ~88% at 20 DAT. At 20 DAT, FCO injured only 13%, however this seemingly minor injury resulted in > 50% reduction in hemp sesbania

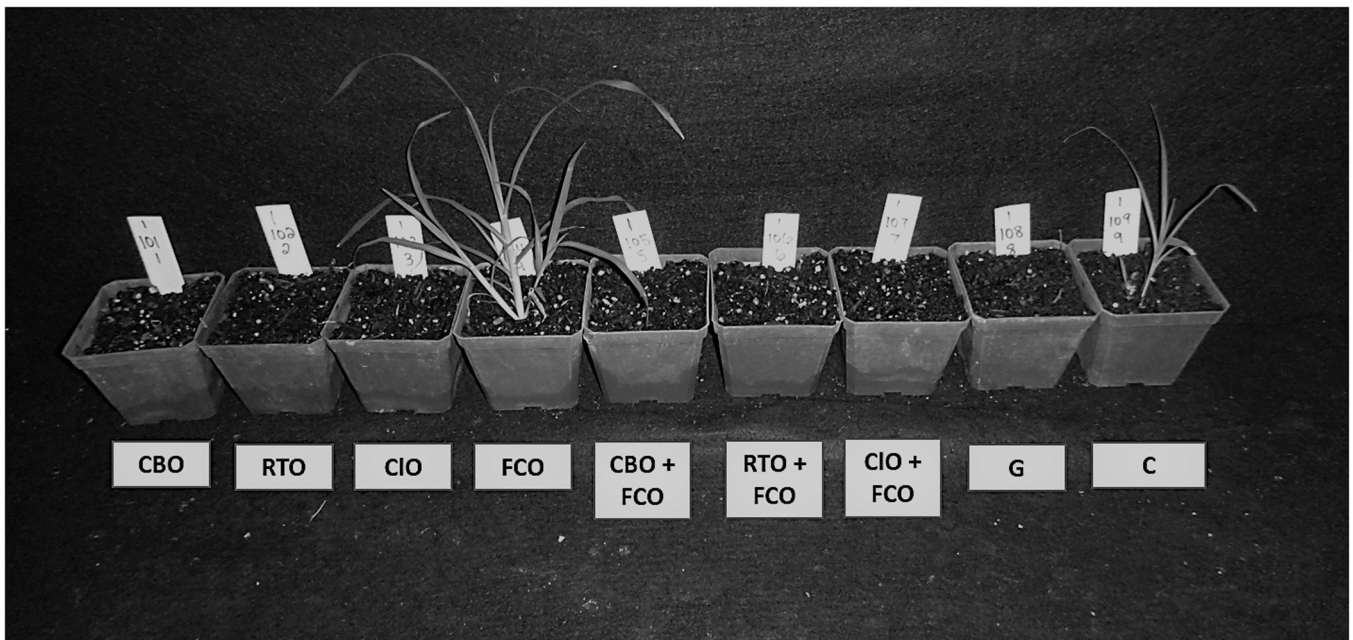


Fig. 6. Goosegrass plants 21 days after treatment with cinnamon bark oil (CBO), red thyme oil (RTO), clove bud oil (CIO), fractionated coconut oil (FCO), cinnamon bark oil + fractionated coconut oil (CBO+FCO), red thyme oil + fractionated coconut oil (RTO + FCO), clove bud oil + fractionated coconut oil (CIO + FCO), glyphosate (G), non-treated control (C).

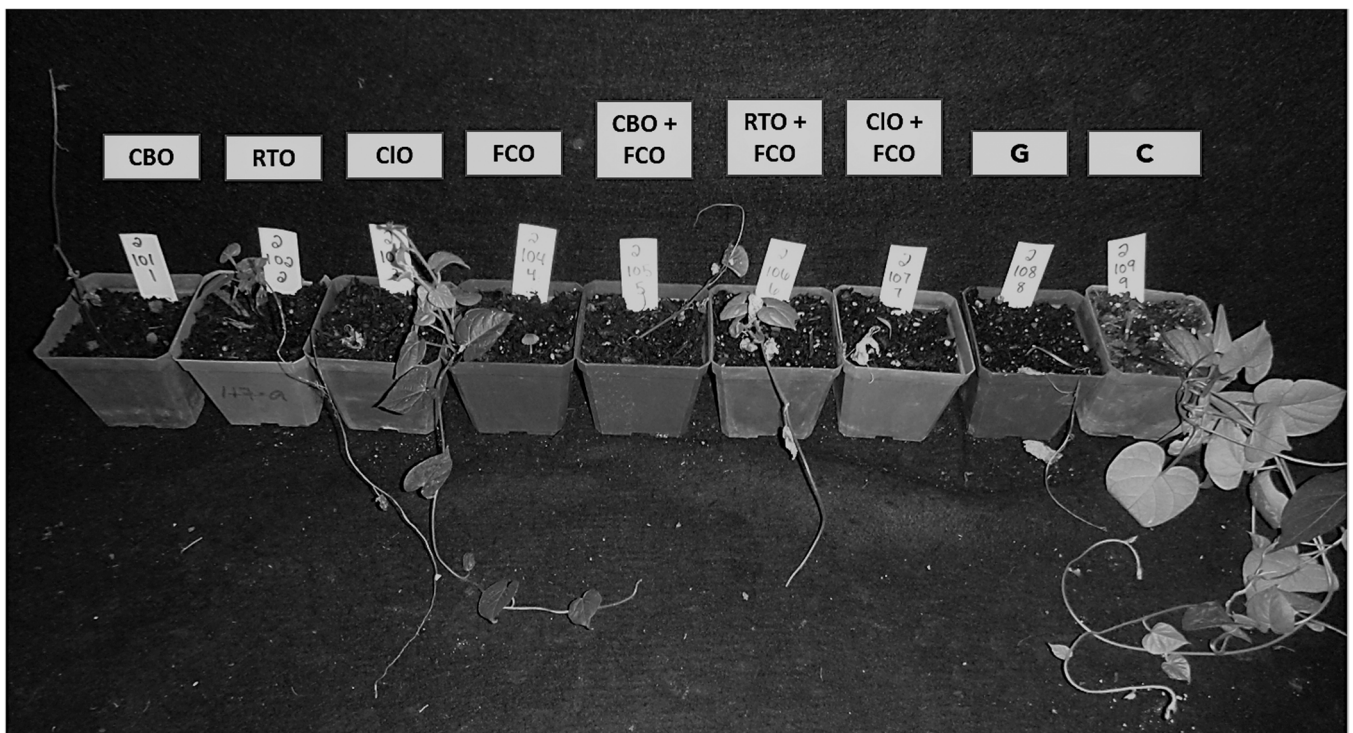


Fig. 7. Entireleaf morningglory plants 21 days after treatment with cinnamon bark oil (CBO), red thyme oil (RTO), clove bud oil (CIO), fractionated coconut oil (FCO), cinnamon bark oil + fractionated coconut oil (CBO+FCO), red thyme oil + fractionated coconut oil (RTO + FCO), clove bud oil + fractionated coconut oil (CIO + FCO), glyphosate (G), non-treated control (C).

biomass.

Conversely, essential oils induced a growth reduction in goosegrass and small flower morningglory, while FCO stimulated their growth. All essential oils with or without FCO injured goosegrass from 88% to 100% at 5 DAT (Table 2), while glyphosate injured goosegrass 17% at 5 DAT and ~98% at 10 DAT. In goosegrass, beyond non-treated condition, the

least injury was observed for FCO sprayed alone. Similarly, all essential oils with or without FCO injured small-flower morningglory from 82% to 100% at 5 DAT and 99–100% at 10 DAT, while less than 3% injury was caused by FCO and non-treated control at any time. At 20 DAT, all essential oils with or without FCO and glyphosate injured goosegrass 100%, with the exception of red thyme oil alone, which injured

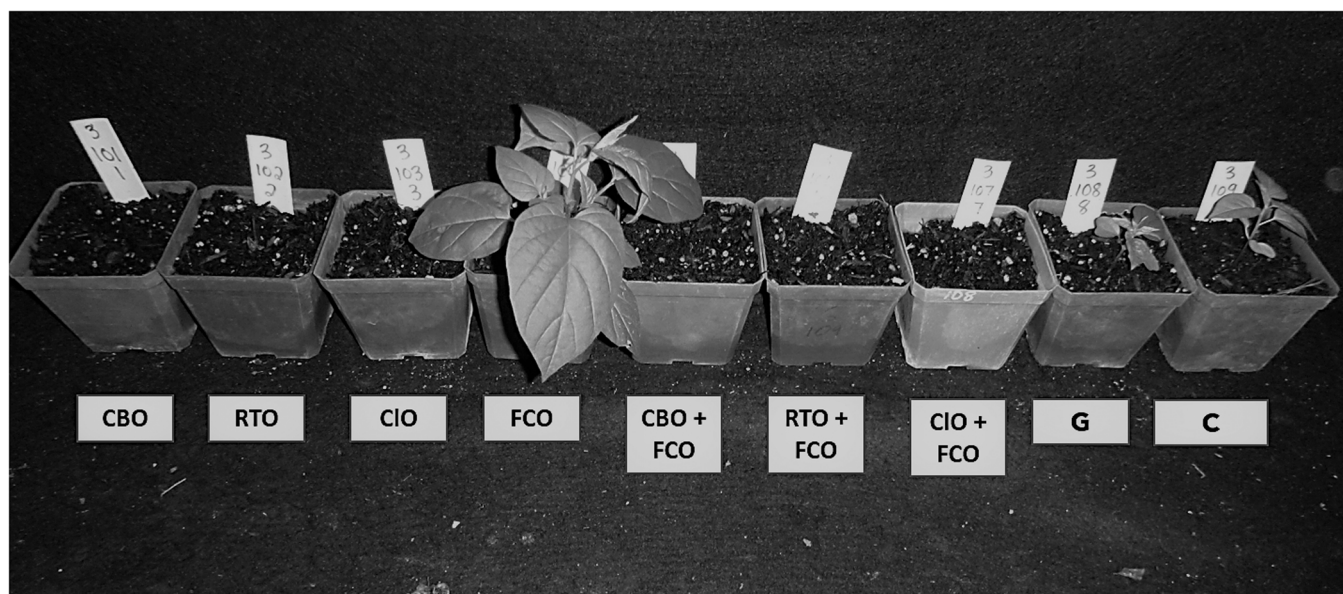


Fig. 8. Small flower morningglory plants 21 days after treatment with cinnamon bark oil (CBO), red thyme oil (RTO), clove bud oil (CIO), fractionated coconut oil (FCO), cinnamon bark oil + fractionated coconut oil (CBO+FCO), red thyme oil + fractionated coconut oil (RTO + FCO), clove bud oil + fractionated coconut oil (CIO + FCO), glyphosate (G), non-treated control (C).

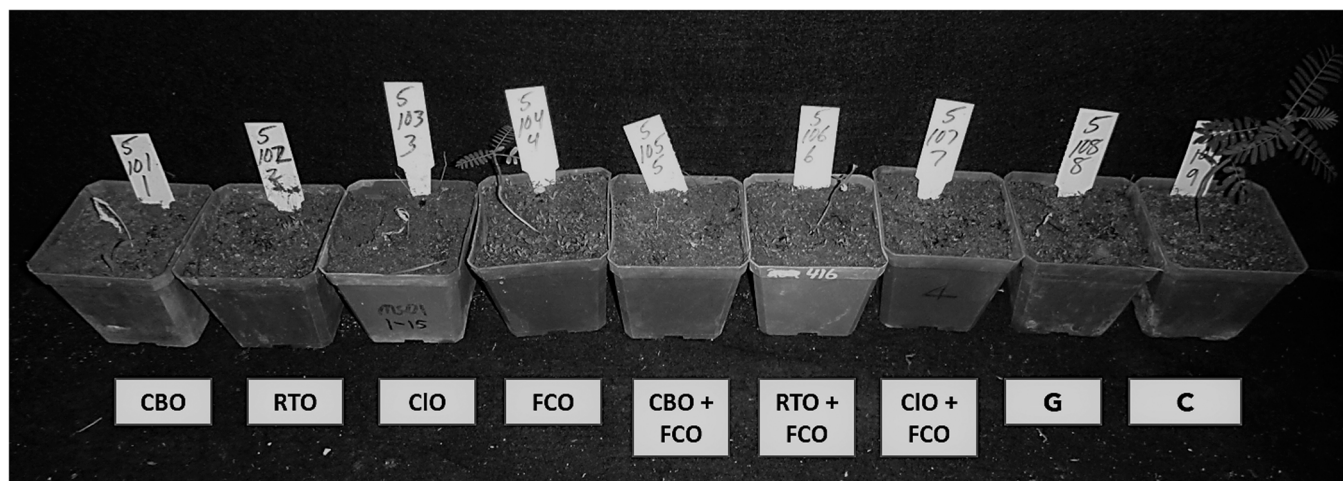


Fig. 9. Hemp sesbania plants 21 days after treatment with cinnamon bark oil (CBO), red thyme oil (RTO), clove bud oil (CIO), fractionated coconut oil (FCO), cinnamon bark oil + fractionated coconut oil (CBO+FCO), red thyme oil + fractionated coconut oil (RTO + FCO), clove bud oil + fractionated coconut oil (CIO + FCO), glyphosate (G), non-treated control (C).

goosegrass ~52%, thus suggesting plant recovery after spraying. Unexpectedly, FCO sprayed alone increased goosegrass biomass compared to non-treated control harvested at 20 DAT (Fig. 6). At 20 DAT, small-flower morningglory sprayed with essential oil + FCO and red thyme oil alone partially recovered, but this was not enough to harvest biomass. Small-flower morningglory biomass treated with FCO was double than under non-treated control at 20 DAT (Fig. 8).

4. Discussion

The experimental results indicate that spraying essential oils alone or mixed with FCO induced phytotoxic effects on the selected species as already observed in some other researches (Tworowski, 2002; Jouini et al., 2020; Travlos et al., 2020). Our results also indicate that in some cases, essential oils and/or their mixtures with FCO were more rapid in controlling weed growth than glyphosate as observed in Italy with milk thistle and in USA with entireleaf morningglory, small-flower

morningglory and hemp sesbania. In some cases, essential oils and/or their mixtures with FCO were even more effective than glyphosate (e.g. milk thistle and hemp sesbania).

Although glyphosate resistance has been not documented for the above-mentioned species, the effectiveness of essential oils in control would be extremely useful in situations where these species are problematic such as hemp sesbania for soybean, cotton and rice fields (Lorenzi and Jeffery, 1987) or entireleaf morningglory and small-flower morningglory in row crops (Bryson et al., 2008).

For all the other cases for which effectiveness of essential oils and/or their mixtures with FCO were comparable to glyphosate, it is noteworthy that these findings would be useful for control of species such as: goosegrass, which is considered one of the five most harmful weeds in the world, showing resistance to 8 sites of action (Heap, 2021); large crabgrass that has evolved resistance to the acetolactate synthase (ALS)-inhibiting herbicide nicosulfuron (Mei et al., 2017) and to ACCase inhibitors (Heap, 2021).

Given that in our case the application of the sole essential oils at 5% v/v was able to kill plants, our hypothesis that the mix with FCO would improve essential oils effectiveness was not confirmed.

The only exception was observed in goosegrass and large crabgrass, that were more injured by the mix of red thyme oil + FCO than by red thyme oil sprayed alone, thus confirming Flessner et al. (2010). Conversely, entireleaf morningglory survived to the treatment combining red thyme oil with FCO, while it was totally injured by the red thyme oil alone. The most variable responses were related to the applications of FCO alone, that was selected since it is composed of 99% by capric oil and caprylic oil which have proved to have herbicidal effects used individually (Fukuda et al., 2004; Coleman and Penner, 2006; Li et al., 2019) and mixed as it happens for some available commercial products (Homeplate®, Suppress®). Our experiment proved that its behavior was more species-specific but in none of the species it caused total necrosis. The biomass reduction observed in our experiment for hemp sesbania, entireleaf morningglory and large crabgrass sprayed with FCO was confirmed by Abugho (2020) that found an overall similar biomass reduction at 21 DAT in plants sprayed with Suppress®.

As far as we know, we are the first to identify the possibly growth promoting effects of fractionated coconut oil. A possible explanation of this unexpected promoting effect on biomass accumulation could be justified by the presence of small percentages of myristic acid (~ 0.11%) and lauric acid (~ 0.39%) that according to Zhang et al. (2012) could have showed promoting effects on biomass. The addition of fatty acids such as myristic acid, lauric acid and palmitic acid in the soil mixture (1.5% w/w) used for the cultivation of *Cucumis sativus* infested by *Meloidogyne incognita*, induced plant biomass increase (Zhang et al., 2012).

5. Conclusion

Using non-synthetic products aids in contributing to meet the goals of 'Farm to Fork' Strategy by 2030 for EU and more generally helping to diversify the tools and tactics used for weed management in the next future (Loddo et al., 2021). The present research identified the effectiveness of red thyme oil, cinnamon bark oil and clove bud oil in controlling the selected species in their seedling stage, when sprayed at 5% v/v. Research on lower doses of essential oils (< 5% v/v) to reduce costs, addition of surfactants to improve absorption, timing of applications, and evaluation of additional weed species are needed. Further research will be useful to explore the effectiveness of essential oils and their mixtures on weeds that have overcome the seedling stage. The most surprising result regarding the species-specific responses induced by FCO spraying need further investigation since it could be possibly used as a selective herbicide based on these results or even as a biostimulant to some species. In this last hypothesis, it would be necessary using FCO on crops that could benefit from it, and possibly infested by weeds that are neutrally or negatively influenced by FCO.

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CRedit authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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