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Vegetative Propagation of *Nepeta cataria* and the Inhibitory Effects of Its Essential Oil on the Adventitious Rooting of Cultivated Lamiaceae Species

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ABSTRACT

Catnip (*Nepeta cataria* L.) has traditionally been used in herbal teas and in cat toy products as a cat attractant. Recent attention has been given to its essential oil as a natural insect repellant effective against mosquitoes, ticks, and bed bugs due to its primary bioactive compound, nepetalactone. As an emerging crop in the medicinal plant industry, techniques to improve and accelerate its clonal propagation are necessary to aid in catnip domestication and breeding programs, which can also provide cultivation support and novel growing methods to farmers. In the present study, we assessed the application of IBA and various organic solvents as supplements to promote adventitious rooting in stem cuttings of catnip. Though significant differences between the control treatments and treated plants were not observed, the rooting variables investigated provide a baseline for ideal root growth and development of catnip cuttings. Because the utility of catnip essential oil (EO) is also applicable to other areas of agronomy, we also report on the allelochemical effects of 'CR9' catnip EO on three different Lamiaceae species known to produce adventitious roots easily: catnip (*Nepeta cataria* L.), basil (*Ocimum basilicum* L.) and oregano (*Origanum vulgare* L.). Nepetalactone-rich catnip EO exerted a strong inhibitory effect on the formation of roots in each of the three species in a concentration-dependent manner.

INTRODUCTION

As one of the largest plant families, Lamiaceae, commonly known as the mint family, contains approximately 236 genera and more than 7,000 species, many lauded for their medicinal and therapeutic value (Hamed et al., 2021). Catnip, scientifically known as *Nepeta cataria* L., is a perennial herbaceous plant within the mint family (Duda et al., 2015; Gomes et al., 2020). Comparable to other plants in the mint family, catnip produces essential oils of commercial importance. Catnip essential oil contains volatile iridoid terpenes, predominantly nepetalactones, which exert strong biological activities (Gomes et al., 2023). Though nepetalactone has economic value as a feliphile compound in cat toys, it has also been cited as an herbicide, insect repellent, and sedative (Duke and Beckstrom-Sternberg, 2000). Recent work has shown catnip essential oils to be more effective than DEET (chemical name, N,N-diethyl-meta-toluamide) at repelling mosquitoes achieving over 95% repellency (Reichert et al., 2019). Additionally, N*epeta cataria* extracts have exhibited antioxidant, hepatoprotective, antidiabetic, antidepressant, spasmolytic, anti-nociceptive, anti-inflammatory,

anticancer, and antimicrobial activities (Aćimović et al., 2021).

Due to the bioactive properties of catnip, interest in its cultivation for commercial harvesting has increased, though its populations are still largely undomesticated. 'CR9,' is the first catnip cultivar in North America bred specially for its higher biomass accumulation, essential oil and *(Z,E)*-nepetalactone yield in addition to its elite field performance owing to its erect growth habit (Reichert et al., 2016). Though catnip is most commonly propagated by seeds, cloning via vegetative cuttings can be a useful and desirable cultivation technique that limits the degree of genetic variation amongst the germplasm and standardizes the quality of plant materials produced (Gomes et al., 2020). A catnip hybrid cultivar, 'CR3,' (Simon et al., 2021) was developed from a clonal population after undergoing selection for best field performance with clones evaluated for ideal conditions including upright growth, winter survival, above-ground biomass, essential oil and *(E,Z)*-nepetalactone yield. The literature is scarce regarding clonal propagation of catnip, though recent work has evaluated how the perennial nature of catnip can influence its aromatic profile in successive harvests as a result of genotype-specific interactions (Gomes et al., 2023).

Indole-3-butyric acid (IBA) is a synthetic auxin frequently utilized for vegetative propagation in horticulture to stimulate the formation of adventitious roots from stem cuttings. It is marketed as a rooting hormone due to its ability to initiate roots and increase the number of roots upon application (Kochhar and Gujral, 2020). Reichert et al. (2019) propagated two distinct catnip populations via vegetative cuttings by dipping their terminal nodes in 0.3% IBA and allowing the roots to develop under misthouse conditions. Another study evaluated the propagation of catnip by terminal and single-node cuttings. St. Hilaire (2003) found terminal cuttings treated with 3 g/kg IBA and propagated for four weeks had the highest root dry weight. The presence of IBA increased rooting in catnip, but the concentration did not significantly impact root formation. A subsequent study, found terminal catnip cuttings propagated for 2 or 3 weeks had higher dry weight shoot biomass than root biomass compared to cuttings propagated for a 4 week period (St. Hilaire et al., 2004).

IBA in combination with talc or an aqueous solvent such as ethanol is a common way to prepare auxin solutions (Chong et al., 1992). One study involving mung bean demonstrated that etiolated hypocotyl cuttings rooted in methanol, acetone, and ethanol when used alone or in combination with the naturally occurring auxin, indole-3-acetic acid (IAA) suggesting that the solvents could potentially support root formation by acting as carbohydrate sources (Bhattacharya et al., 1985). Conversely, other findings suggest that ethanol may exhibit inhibitory effects on adventitious root initiation (Middleton et al., 1978).

Although not traditionally used in horticulture for this purpose, essential oils have also been shown to exert phytotoxic effects on adventitious rooting. A study conducted with *Heterothalamus psiadioides* demonstrated that its essential oil strongly inhibited the adventitious rooting of *Arabidopsis thaliana* and induced high levels of H_2O_2, a reactive oxygen species, altering its development (Lazarotto et al., 2014). An *in vitro* study conducted in petri plates assessed the growth inhibition activity of two *Nepeta* essential oils, from *N. rtanjensis* Diklić and Milojević, and *N. cataria* L., on the invasive species, *Ambrosia artemisiifolia* L., (ragweed). After exposure for 2 weeks to the *Nepeta* essential oils, the *in vitro* morphogenesis of ragweed was significantly affected including inhibition of its rooting and reduction of its fresh shoot and root weights due to oxidative stress (Dmitrovic et al., 2015). Another *in vitro* study assayed the allelochemical effects of the essential oil isolated from *Nepeta nuda* subsp. *Albiflora* on five plant species: *Triticum aestivum*, *Raphanus sativus*, *Lactuca sativa*, *Lepidium sativum* and *Portulaca oleracea* (Bozok et al., 2017). The essential oil inhibited seed germination as well as radicle and plumule elongation for all tested species.

To date catnip essential oil distilled from *Nepeta cataria* L. has not been reported to inhibit adventitious rooting in stem cuttings. This bioactive property could expand the utility of this natural

product in the agriculture industry as a plant-derived herbicide. Therefore, the aims of this study were: 1) to assess the effects of different IBA doses and aqueous solvents on the adventitious rooting of catnip stem cuttings and 2) to investigate the concentration-dependent inhibitory effects of catnip essential oil on the rooting of catnip, basil, and oregano stem cuttings.

MATERIALS AND METHODS

Mother plants.

Catnip (*Nepeta cataria* L.) cultivar 'CR9' was selected from the Rutgers University and New Jersey Agricultural Experiment station germplasm collection and breeding program to conduct this work (Reichert et al., 2016). Oregano cuttings were taken from the cultivar, *Origanum vulgare* cv. Pierre, developed through the Rutgers Oregano Breeding Project (Reichert et al., 2021). Basil cuttings were taken from the downy mildew resistant variety, 'Rutgers Obsession-DMR' (Simon et al., 2018). The mother plants of all three species were kept under greenhouse conditions in plastic pots (8.8 in x 8.25 in) with monthly fertigation (15-15-15 N-P-K) for 1 year. Stem cuttings were collected from the mid-portion of the mother plants during the vegetative stage of their growth.

Cutting preparation.

The stem cuttings were collected in April of 2022. Catnip and basil stem cuttings averaged 10 cm in length and were made with a beveled cut at the base of each cutting. The two leaves at the apical end of each cutting were cleaved in half to reduce the leaf area. Oregano cuttings were made about 4 cm long with two whole leaves kept at the apical end. During the period of cutting preparation, the bases of each were retained in water until the time of treatment to deter wilting. Each base of the cuttings was immersed for 2 minutes in one of the selected rooting treatments to assess their potential elicitor or inhibitor effects. After this period, the cuttings were placed in polypropylene 128-cell plug trays filled with commercial soil media and kept in a mist chamber, with intermittent misting of 5 seconds every 30 minutes. The cuttings had their rooting-associated variables assessed 30 days after planting.

Experiment 1

Selection of rooting treatments.

The rooting treatments investigated during 'Experiment 1' included: distilled water (control), 0.1% indolebutyric acid (IBA) in talc powder (Hormodin 1, Olympic Horticultural Products [OHP], Kalamazoo, MI), 0.3% IBA (Hormodin 2, OHP), 0.8% IBA (Hormodin 3, OHP), ethanol (99.5% purity, Fisher Chemical, Hampton, NH), acetone (99.5% purity, Fisher Chemical), and methanol (99.8% purity, Fisher Chemical).

Treatment of cuttings.

Each catnip stem cutting was treated with talc powder containing auxin (Hormodin) at different concentrations (0.1% IBA, 0.3% IBA, or 0.8% IBA) or one of the liquid organic solvents (ethanol, acetone, or methanol) and subsequently planted in commercial soil. After 30 days, the following variables were assessed: rooting percentage (percentage of cuttings with roots of at least 1 mm in length), number of roots per cutting; average length of the three longest roots per cutting (cm); survival percentage (cuttings that survived the time course of the study), sprouting percentage (percentage of cuttings that developed new leaves); and leaf retention percentage (cuttings that maintained their original leaves during the rooting period). The experiment was organized in a completely randomized design with 4 repetitions and 16 cuttings per experimental unit.

Experiment 2

Essential oil extraction and chemical composition.

The essential oil utilized in 'Experiment 2' was extracted via hydrodistillation using a Clevenger-type apparatus. The 'CR9' mother plants were distilled by the method previously described by Juliani et al. (2008). Catnip plant material was oven dried at 40°C for 4 days and ground; then 100 g samples of aboveground biomass were placed in 2 L round-bottom glass flasks in addition to 1 L of

deionized water. The flasks were placed over heating mantles for 2 hours and the essential oil was collected in a Clevenger trap. Approximately 10 mL of catnip essential oil were obtained from the hydrodistillation. Regarding the chemical analysis, the essential oil was characterized by Gas Chromatography-Mass Spectrometry (GC-MS) as described by Patel et al. (2023). Compound separation and identification were performed on a Shimadzu 2010 Plus gas chromatograph equipped with a TQ8040 Mass Spectrometer (Shimadzu Scientific, Somerset, NJ).

Treatment of cuttings.

To determine the rooting inhibition effects of catnip essential oil at various concentrations, stem cuttings of basil, oregano and catnip were treated. The cuttings were taken from the mother plants of each species and prepared as previously described. The 'CR9' catnip essential oil was serially diluted using 99.5% ethanol: 100% EO, 50% EO, 25% EO, 12.5% EO, 6.25% EO, and 0% EO (ethanol control). Stem cuttings for 'Experiment 2' were assessed by the following variables: mortality percentage, rooting percentage, sprouting percentage, and leaf retention percentage.

Experimental design and statistical analyses.

For experiment 1, one-way ANOVA was performed, and no statistical differences were observed for any of the assessed variables (Table 1). Given the lack of statistical difference, no post-hoc tests were applied. The results are presented as bar graphs with standard errors. For experiment 2, the results are plotted as averages with confidence intervals (α=0.05). The statistical analyses were performed using GraphPad Prism version 6.04 for Windows, GraphPad Software (www.graphpad.com).

RESULTS

Experiment 1

Regarding the 'Experiment 1' results, the one-way ANOVA (Table 1) showed that there were no significant differences among the treatments for the percentages of survival and rooting. The survival percentages averaged 98.4% for all treatments while the overall rooting average was 91.7% (Figure 1A and 1B). The percentage of survival ranged from 95.3% in the control and methanol treatments to 100% survivability in all the IBA treatments as well as the ethanol treatment. Comparatively, the percentage of rooting varied from 89.1% in the control plants to 100% in the 0.1% IBA treatment although the values did not result in statistically significant differences at the $p > 0.05$ level.

Table 1. Analysis of variance (ANOVA) table for percentages of survival (S), rooting (R), alive without roots (A), sprouting (Sp), and leaf retention (LR) and values for root number (RN), average root length (RL), root dry mass (RM) and shoot dry mass (SM) of catnip (*Nepeta cataria* L.) stem cuttings treated with indole-butyric acid and organic solvents.

Source of variation	DF	Sum of squares								
		S (%)	R (%)	A (%)	Sp (%)	LR (%)	RN	RL (cm)	RM (g)	SM (g)
Treatments	6	117.2^{ns}	502.2^{ns}	329.2^{ns}	1635^{ns}	438.1^{ns}	380.8^{ns}	11.22^{ns}	0.225^{ns}	0.207^{ns}
Residuals	21	400.4	114.0	791.0	10947	2324	675.3	16.5	0.628	0.751
Total	27	517.6	1645.0	1120	12582	2762	1056	27.7	0.853	0.959
Average		98.4	91.7	3.8	85.5	92.4	18	7.0	0.42	0.32

^{ns} Not statistically different ($p > 0.05$); DF: degrees of freedom.

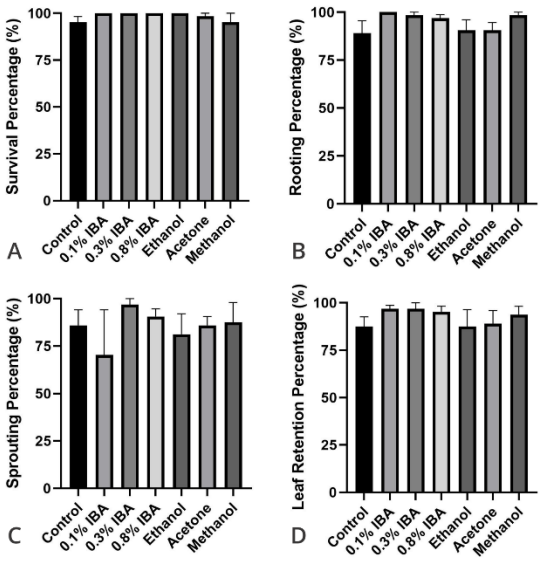

Figure 1. Averages of survival percentage (A), rooting percentage (B), sprouting percentage (C), and leaf retention percentage (D) of catnip (*Nepeta cataria* L.) stem cuttings submitted to basal treatments with Indole butyric acid (IBA) and organic solvents. Error bars represent standard error of the mean.

When assessing the plants that survived without producing roots (data not shown), the control plants averaged 7.8% while the ethanol treated plants averaged 9.4% and the acetone treated plants averaged 4.7% for this variable. Given the plants were evaluated four weeks post-treatment, it is possible this variable would decrease over time if the plants were allowed to continue rooting.

The sprouting variable refers to the production of new shoots over the time-course. The plants treated with 0.3% IBA exhibited a sprouting percentage of 96.9% in contrast to the plants treated with ethanol averaging 81.3% and the control plants averaging 85.9%; however, since these values are not significantly different the treatments did not hinder or improve sprouting (Figure 1C). Sprouting percentage averaged 85.5% across all treatments.

Another variable assessed was the leaf retention post-treatment. This reflected the percentage of stem cuttings that retained either 1 or 2 of their original leaves throughout the duration of the study. The average percentage of leaf retention was 92.4% ranging from 87.5% in the control plants and the ethanol-treated plants to 96.9% in both the 0.1% IBA and 0.3% IBA-treated plants, not differing statistically among the treatments (Figure 1D).

The average number of roots across all treatments was 18 roots. The control plants averaged 18 roots per cutting while the plants treated with 0.3% IBA averaged 26 roots per cutting (Figure 2A). The average root length was approximately 7 cm, with no statistical differences among treatments (Figure 2B). The dried root weight ranged from 0.28 grams from the ethanol treated plants to 0.58 grams from the 0.3% IBA-treated plants. The overall average of dried belowground material was 0.42 grams with the roots from the control plants weighing 0.48 grams (Figure 2C). The dried shoot weight followed a similar pattern to the dried root weight with the ethanol-treated plants averaging 0.21 grams and the control plants averaging 0.49 grams (Figure 2D). However, these variables did not show statistically significant differences among the treatments at the $p > 0.05$ level.

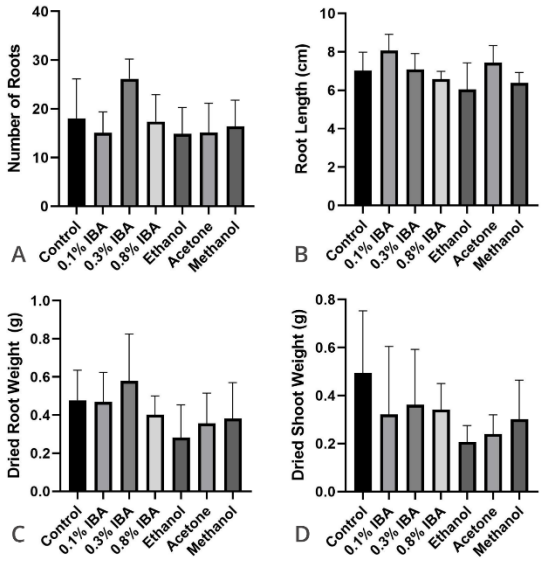

Figure 2. Averages of number of roots (A), root length (B), dried root weight (C), and dried shoot weight (D) of catnip (*Nepeta cataria* L.) stem cuttings submitted to basal treatments with Indole butyric acid (IBA) and organic solvents. Error bars represent standard error of the mean.

Experiment 2

After observing that catnip stem cuttings root easily and efficiently without the addition of rooting aides, we conducted a follow-up study to assess if the essential oil of catnip cv. 'CR9' would exert inhibitory effects on the adventitious rooting of the

same catnip species as well as two other Lamiaceae species known to root readily: basil (*Ocimum basilicum* L.) and oregano (*Origanum vulgare* L.). The hydrodistilled essential oil obtained from catnip cv. 'CR9' was majorly composed of (*Z,E*)-nepetalactone (84.2%), followed by β-caryophyllene (9.43%) and β-pinene (2.78%) (Table 2). Each essential oil (EO) preparation was serially diluted (v/v) using 99.5% ethanol and the following catnip EO concentrations were tested: 100% EO, 50% EO, 25% EO, 12.5% EO, 6.25% EO, and 0% EO.

Table 2. Chemical profile (in terms of relative percent peak area) of the essential oil of *Nepeta cataria* cv. CR9 obtained by GC-MS.

ID #	RT	Compound	% Peak Area ± SD
1	7.789	*α*-pinene	t
2	8.248	Unidentified 1	t
3	8.316	*β*-pinene	2.78 ± 0.19
4	11.649	(*Z,E*)-nepetalactone	84.2 ± 0.69
5	12.066	*β*-caryophyllene	9.43 ± 0.42
6	12.153	Unidentified 2	0.52 ± 0.32
7	12.308	*α*-humulene	0.77 ± 0.03
8	13.141	Caryophyllene oxide	0.76 ± 0.09
9	14.340	Unidentified 3	0.71 ± 0.28
		Total Identified Peaks	98.3
		Total Unidentified Peaks	1.7

The relative peak percentage areas of the compounds are presented as the mean of three replicates ± standard deviation (SD). R_T: Retention Time; t: trace amounts (less than 0.5%).

The catnip stem cuttings exhibited 0% mortality when treated with the 0% EO dilution (Figure 3A), though the 6.25% EO treatment resulted in 70.8% mortality. All dilutions from 12.5% - 100% EO yielded a 100% mortality rate in the catnip stem cuttings (Figure 3C). The mortality percentage of the basil stem cuttings, however, produced variable results demonstrating greater resiliency to the catnip essential oil treatments. In basil (Figure 3D), the 0% EO treatment led to 20.8% mortality, which increased to 58.3% mortality from the 6.25% EO dilution and a 75% mortality rate from both the 12.5% and 25% EO dilutions. The data exhibited a progressive increase in mortality of the basil stem cuttings as the catnip EO concentration increased. Although both the 50% and 100% EO dilutions resulted in 100% mortality of the cuttings (Figure 3F). The oregano stem cuttings appeared to be the most sensitive to the inhibitory effects of the catnip essential oil. The 0% EO dilution averaged a mortality percentage of 4.2% in the oregano stem cuttings (Figure 3G), while the 6.25% EO dilution averaged a mortality rate of 91.7%. All EO dilutions from 12.5% – 100% yielded a 100% mortality rate in the oregano stem cuttings in similar form to the catnip mortality averages (Figure 3I).

Another variable assessed post-treatment with the catnip EO dilutions was the rooting percentage of all stem cuttings. For the 0% EO treatment, catnip exhibited the greatest rooting percentage at 95.8% (Figure 3B) while oregano and basil averaged 91.7% and 62.5% respectively (Figure 3H; Figure 3E). The 6.25% EO dilution resulted in 70.8% rooting in catnip, 25% rooting in basil, and 8.3% rooting in oregano. The catnip and oregano stem cuttings did not yield roots in any of the EO dilutions ranging from 12.5% - 100%. In contrast, the basil stem cuttings averaged a rooting percentage of 8.3% at the 25% EO dilution, though, the cuttings didn't yield roots at the 12.5%, 50% and 100% EO dilution levels.

Variation in the average mortality, rooting, sprouting and leaf retention percentages as well as their confidence intervals for catnip, basil and oregano stem cuttings treated with catnip EO at different concentrations were observed (Figure 4). For the 0% EO dilution, the sprouting percentage was highest in the oregano stem cuttings at 100%, followed by the catnip stem cuttings at 95.8% and 83.3% for basil. The 6.25% EO dilution lowered the sprouting percentage to 37.5% in basil, 29.5% in catnip and 8.3% in oregano. All dilutions from 12.5% - 100% EO yielded a 0% sprouting rate in both the catnip and oregano stem cuttings. This is demonstrative of the necrotic effects of the catnip

essential oil as the concentration increases. The basil stem cuttings, however, demonstrated greater resilience in response to the catnip EO. At both the 12.5% and 25% EO dilution levels, basil averaged a sprouting percentage of 25% then lowered to 0% sprouting at the 50% and 100% EO dilutions levels.

Lastly, the percentage of leaf retention was determined for all stem cuttings. Oregano and catnip retained the highest percentage of leaves at 100% and 95.8% respectively compared to the basil cuttings, which only retained 54.2% of its leaves at the 0% EO dilution level. As the EO concentration increased to 6.25%, both the catnip and basil leaf retention values reduced to 20.8%, while the oregano leaf retention percentage lowered to 4.2%. All dilutions from 12.5% - 100% EO resulted in 0% leaf retention for both catnip and oregano. Comparatively, the basil stem cuttings averaged 8.3% leaf retention at the 12.5% EO dilution level and exhibited 0% leaf retention from the 25% - 100% EO treatments.

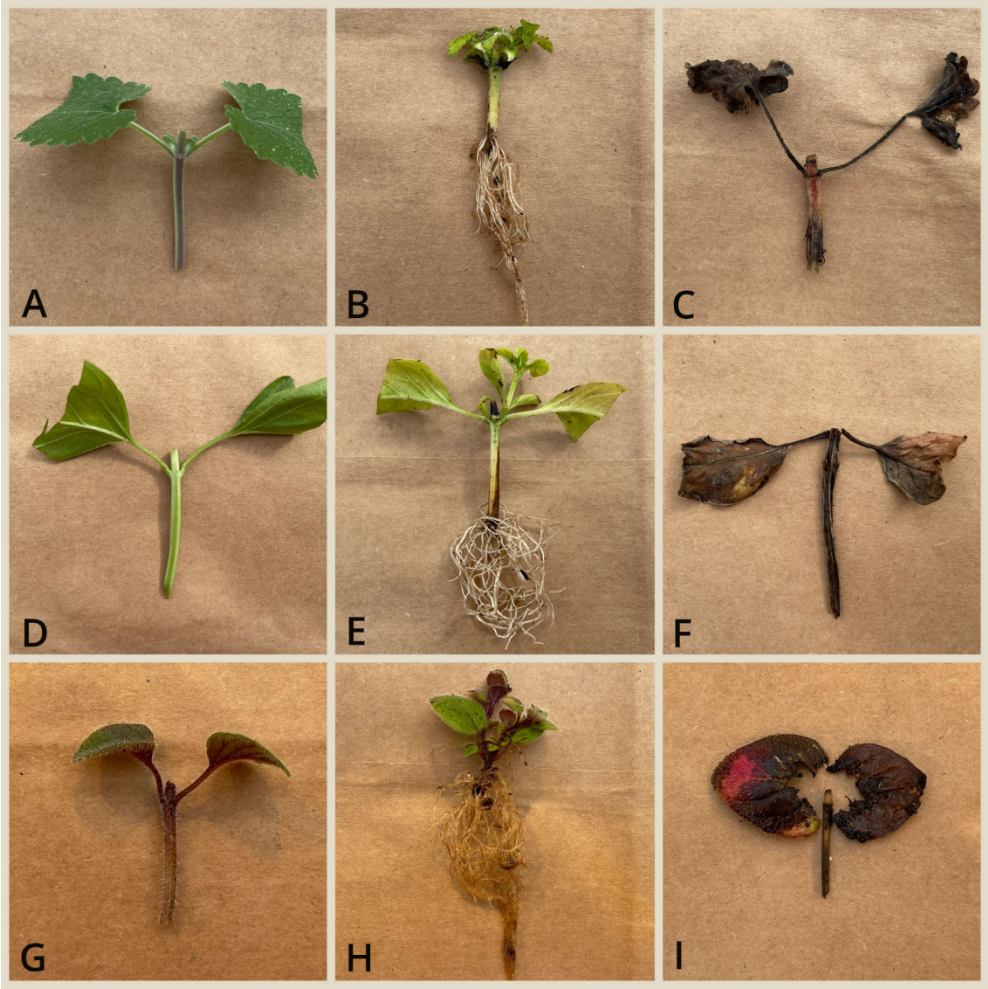

Figure 3: Catnip (*Nepeta cataria* L) stem cutting (A), rooted cutting control (B), necrosed cutting treated with 'CR9' catnip essential oil (EO) (C); basil (*Ocimum basilicum* L.) stem cutting (D), rooted cutting control (E), necrosed cutting treated with 'CR9' catnip EO (F); oregano (*Origanum vulgare* L) stem cutting (G), rooted cutting control (H), necrosed cutting treated with 'CR9' catnip EO (I).

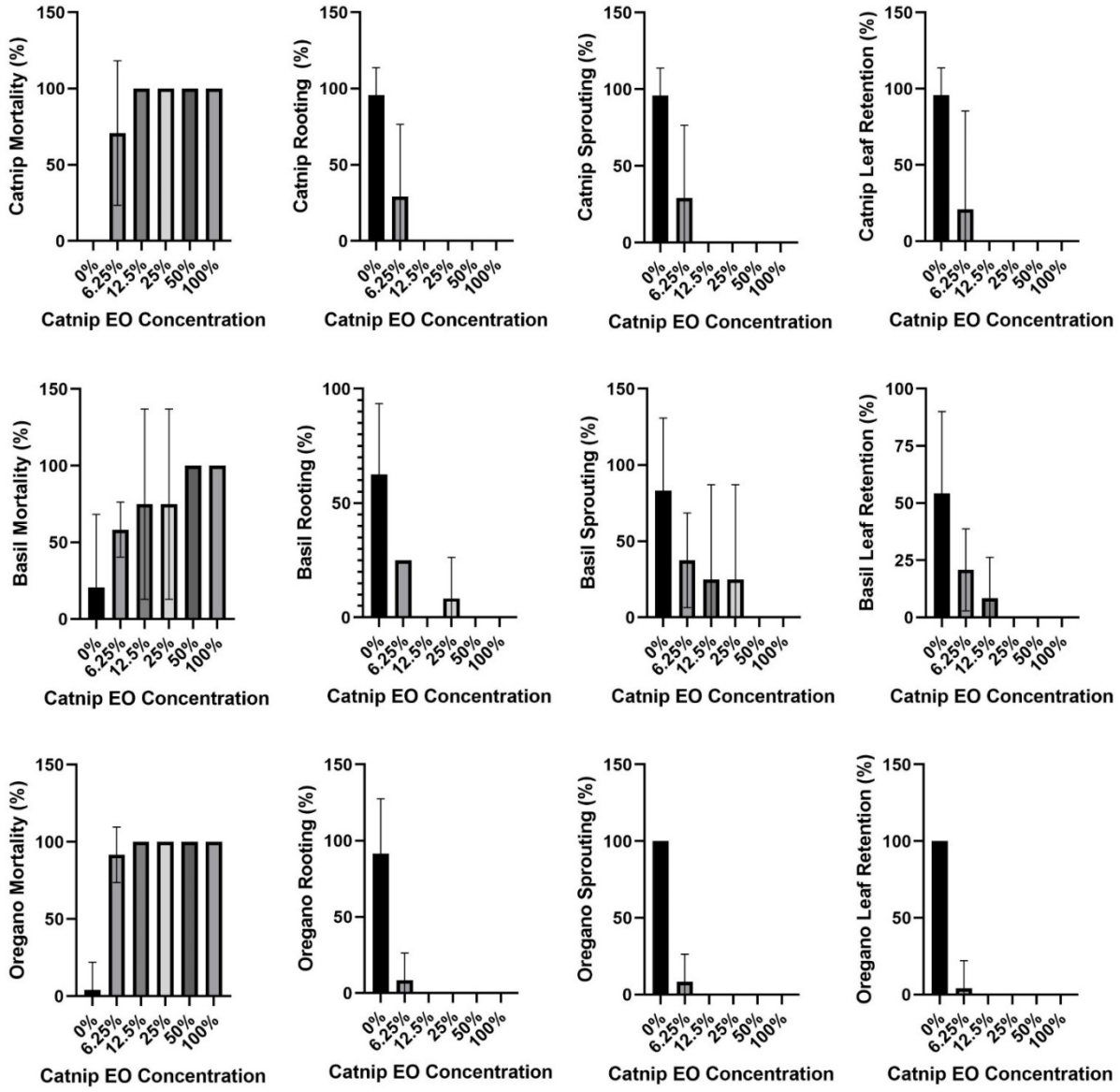

Figure 4. Average mortality percentage, rooting percentage, sprouting percentage and leaf retention percentage of catnip (*Nepeta cataria* L.), basil (*Ocimum basilicum* L.) and oregano (*Origanum vulgare* L.) stem cuttings submitted to different concentrations of catnip essential oil (EO) diluted in ethanol. Error bars represent confidence intervals (α=0.05).

DISCUSSION

The practice of clonally propagating select cultivars to inherit desirable traits and ideal growth performance has been a horticultural strategy for thousands of years (Janick, 2005; Gradziel et al., 2019). Cultivating progeny vegetatively can enhance the profitability of novel germplasm by immortalizing hybrid genotypes whereas seeds from hybrid plants would segregate desirable traits in the self-progeny (Mehandru et al., 2014; Del Valle-Echevarria et al., 2019). This asexual method of propagation can ensure minimal genetic variability of selected genotypes during breeding programs. Among the methods of vegetative propagation, stem cuttings are one of the cheapest and simplest methods, with widespread use in the cultivation of medicinal and aromatic plants (Waman et al., 2019). According to Waman et al., (2019), many factors govern the success of propagation through stem

cuttings including physiological maturity of cuttings, length of cuttings, leaf retention of cuttings, seasonal and environmental conditions, influence of rooting media and growth regulators.

In our first experiment, we assessed the effects of ethanol, methanol, and acetone as possible rooting stimulants. These organic solvents are commonly used to dissolve plant growth regulators, notably auxins, that are applied to the bases of stem cuttings to ensure sufficient rooting during propagation (McCracken, 1987). Another method involves the treatment of cuttings with solvents as prewetting agents followed by the application of an auxin in talc formulation (Howard, 1985). Previous authors have reported that aqueous solutions of these solvents could serve as sources of carbohydrates that can support the induction of adventitious rooting (Bhattacharya et al., 1985). In the present study, however, the solvents alone did not exert any effects on adventitious rooting of the catnip stem cuttings. This result is congruent with data from previous sources citing the use of solvents alone as ineffective at promoting root formation from stem cuttings (Wendling et al., 2013; Pereira et al., 2021).

Auxins are the main hormones involved in adventitious rhizogenesis (Epstein and Ludwig-Müller, 1993; Blythe et al., 2007). Among the auxins utilized commercially in plant propagation, IBA in particular, has been reported to hasten root initiation, increase overall rooting percentage, enhance the number and quality of roots, and improve the uniformity of rooting in several species (Hartmann et al., 1997; Macdonald, 1986; Blythe et al., 2007). The effects of this plant growth regulator can vary according to many factors, including the species being treated, its mode of application and concentration as well as the duration of treatment (Epstein and Ludwig-Müller, 1993; Hartmann et al., 1997). For many species, high concentrations of indole-butyric acid can hinder the rooting process due to phytotoxicity (Gomes and Krinski, 2019). While other studies have demonstrated that low auxin concentrations often contribute to root growth, expansion, and elongation (Evans et al., 1994; Fu and Harberd, 2003; Barbez et al., 2017;). In the present study different concentrations of IBA neither inhibited the formation of roots nor stimulated the production of roots in catnip stem cuttings.

The absence of effects exerted by different concentrations of IBA and the application of organic solvents to catnip cuttings is likely due to the species producing sufficient endogenous levels of auxin and rooting co-factors. This is evidenced by the high rooting percentages in the control plants. Additionally, various herbaceous genera within the Lamiaceae family such as *Mentha spp.* easily root through vegetative propagation (Rosengarten, 1969; Waman et al., 2019). Kuris et al. (1980) analyzed the rooting patterns and the initial establishment of stem cuttings from oregano (*Origanum vulgare* L.), peppermint (*Mentha piperita* L.) and lemon balm (*Melissa officinalis* L.). Though the untreated stem cuttings formed adventitious roots, hormonal treatment of the cuttings aided and accelerated root formation in all three species.

The essential oil of catnip has been shown to exert different biological activities in a series of organisms such as euphoric effects on cats, repellent effects against arthropods, and antimicrobial effects against several species of bacteria and fungi (Bol et al., 2017; Azizian et al., 2021). Many of those activities have been attributed to the compound nepetalactone (McElvain et al., 1941), which is also the single most abundant compound in the 'CR9' catnip essential oil used in this study. The chemical composition of the essential oil from cultivar 'CR9' aligns with previous studies conducted with this new genotype (Reichert et al., 2019; Gomes et al., 2023) evidencing a stable and consistent production of the (*Z,E*)-nepetalactone isomer in our germplasm.

When investigating the agricultural applications of nepetalactone-rich essential oils derived from *Nepeta* species, reports indicate their inhibitory actions on *in vitro* seed germination and their allelopathic activities on several species (Mutlu and Atici, 2009; Mutlu et al., 2011; Saharkhiz et al., 2016). Therefore, we hypothesized that when applied to the base of stem cuttings, 'CR9' catnip oil would

exert inhibitory effects on adventitious roots even in species that are known to propagate easily such as catnip, basil, and oregano (Kuris et al., 1980; El-Keltawi and Abdel-Rahman, 2006; Rim and Jang, 2017).

The concentration-dependent effects of catnip essential oil were species-specific, with more pronounced effects on catnip and oregano cuttings than basil. Catnip and oregano cuttings showed smaller percentages of mortality (less than 5%) when subjected to the control treatment (99.5% ethanol) but showed a steep increase in mortality with EO concentrations as low as 6.25% and a 100% mortality rate starting at the 12.5% EO concentration level. For basil, despite having a higher mortality average in control cuttings, a small percentage of basil cuttings survived EO concentrations of up to 25%. Rooting percentages followed an inverse pattern, decreasing as the concentration of essential oil increased. Although the mechanism of action is not completely understood, the allelopathic effects of *Nepeta spp.* essential oils have been attributed to oxidative damages in plant tissues (Mutlu et al., 2011).

Catnip essential oil also strongly inhibited the induction of new shoots and the retention of leaves in stem cuttings of catnip, oregano, and basil, with complete inhibition starting at the 12.5% EO concentration level for catnip cuttings and 50% EO for basil. It is possible to infer that the essential oil acts as a stressor on the stem cuttings, causing higher accumulation of ethylene, which in turn causes leaf abscission (Brown, 1997). Leaf retention is an important factor for the rooting of stem cuttings, since it provides auxins and carbohydrates, and is a variable positively correlated with rooting and survival (Belniaki et al., 2018; Vieira et al., 2021).

In this study we show that 'CR9' catnip essential oil not only inhibits rooting, but also reduces the survival of catnip, basil, and oregano stem cuttings. Other studies have proven the effectiveness of essential oils at inhibiting rooting of stem cuttings (Kibbler et al., 2002; Lazarotto et al., 2014). Additionally, nepetalactone-rich essential oil as well as pure nepetalactone were shown to inhibit *in vitro* rooting of ragweed (Dmitrovic et al., 2015). Such results highlight the potential of catnip essential oil as an allelochemical, inhibiting both germination and vegetative propagation, which is an important strategy in supporting weed control (Dudai et al., 1999). Future work involving agricultural field trials would be necessary to assess whether catnip EO or extract products have herbicidal activity. More extensive studies evaluating the reduction of weed growth and the inhibition of weed seed germination, would be required to qualify the potential of catnip as an herbicide. In addition, stability tests in field soil conditions would be needed to discern the degradation of catnip-based products. The current findings add to an already extensive list of biological activities of this versatile aromatic plant and should be further investigated with invasive species and under field conditions.

CONCLUSION

The vegetative propagation of catnip cv. 'CR9' is possible through the rooting of its stem cuttings and does not require the application of additional plant growth regulators. Rooting of herbaceous stem cuttings is not affected by treatment with IBA or organic solvents. Additionally, the essential oil of catnip cv. 'CR9,' rich in (*Z,E*)-nepetalactone has a strong inhibitory effect on the adventitious rooting of cultivated Lamiaceae species when applied to the base of stem cuttings. The rooting inhibition acts in a concentration-dependent manner and shows promising potential to be used as a plant-based herbicide.

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