

Evaluating natural product-based herbicides for effective control of invasive water lettuce (*Pistia stratiotes* L.)

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Abstract: **Background:** *Pistia stratiotes* L., is an invasive aquatic plant that forms dense mats, disrupting ecosystems and affecting waterway navigation. Although various control methods have been studied, there is no clear consensus on the best approach. **Objective:** The goal of this study is to evaluate the efficacy of various control methods in managing *P. stratiotes* infestations. **Methods:** Laboratory trials tested the efficacy of diquat, d-limonene, mineral oil, and potassium salts of fatty acids on *P. stratiotes*. Treatments were applied at concentrations from 10–30%, with weekly monitoring for five weeks to assess treated plants visual quality (injury symptoms), and dry biomass. Greenhouse trials replicated the most effective laboratory treatments. **Results:** In laboratory trials, diquat at 0.89% achieved > 90% reduction in visual quality and dry biomass of *P. stratiotes*, making it the most effective treatment. D-limonene at

30% showed similar efficacy, while mineral oil also significantly reduced *P. stratiotes* populations. Combinations of d-limonene with mineral oil or potassium salts of fatty acids exhibited synergistic effects; some combinations achieved >90% reduction in visual quality and dry biomass. Treatments prepared with seawater with diquat (0.89%) and d-limonene (20% and 30%) were the most effective treatments. Greenhouse trials confirmed these results, suggesting these treatments could be effective.

Conclusions: In conclusion, this study underscores the effectiveness of diverse strategies in controlling *P. stratiotes* infestations. Further research is warranted to evaluate long-term efficacy, cost-effectiveness, and environmental impact. Integrated approaches offer a multifaceted and sustainable solution for managing *P. stratiotes*, mitigating its ecological and economic impacts.

Keywords: Invasive Species Control; Aquatic Plant Management; Herbicidal Treatments; Chemical Control Methods; Plant Population Reduction; Ecosystem Restoration; Integrated Weed Management; Environmental Impact Assessment

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1. Introduction

Pistia stratiotes L., commonly known as water lettuce, originates from the Americas and has spread globally due to human activities such as the aquarium trade and accidental dispersal (European and Mediterranean Plant Protection Organization, Organisation Européenne et Méditerranéenne pour la Protection des Plantes, 2017; Hussner, 2014). Its rapid proliferation and buoyant nature have enabled its colonization in various water bodies worldwide. Initially recorded in the EPPO (European and Mediterranean Plant Protection Organization) region in the Netherlands in 1973 (Mennema, 1977), it has since become established in multiple EPPO zones and is regulated as an introduced species (European and Mediterranean Plant Protection Organization, Organisation Européenne et Méditerranéenne pour la Protection des Plantes, 2017). In Morocco, it was first observed in 2012 (Hill, 2013), leading to its classification as a quarantined organism by the Ministry of Agriculture.

Managing the growth and spread of *P. stratiotes* is essential for mitigating its negative impacts on aquatic ecosystems. The overgrowth of floating vegetation poses several challenges, including reduced oxygen and light penetration into the water, creation of monocultures by outcompeting native plants, and interference with flood control operations through the formation of dense mats that obstruct canals and water flow structures (Gettys, Moore, 2019).

The management of *P. stratiotes* in aquatic ecosystems involves a comprehensive approach incorporating chemical, mechanical, physical, and biological methods. Aquatic herbicides, regulated by the U.S. Environmental Protection Agency (USEPA) to minimize environmental impact, are cautiously employed, taking into account economic and social factors (USEPA, 1996). However, public concerns about herbicide use have led to temporary suspensions of chemical weed management activities by organizations like the Florida Fish and Wildlife Conservation Commission (FWC) (2019). Stakeholder feedback emphasizes the need to reduce chemical usage in favor of environmentally friendly alternatives. Mechanical harvesting and physical removal are available options but are often costlier and less effective than chemical methods (Queensland Government, 2017). The persistence of *P. stratiotes*, with its ability to reproduce vegetatively and from seeds, requires ongoing management efforts over several years (Millane, Caffrey, 2014). Chemical control, utilizing herbicides such as

glyphosate and diquat, can achieve significant biomass reduction (Martins et al., 2002; Emerine, 2010), but eradication of large populations necessitates repeated applications, with the risk of re-infestation from untreated plants and seed (Glomski, Mudge, 2013). Biological control options, involving phytophagous insects, show promise but face challenges due to the predominance of generalist species (Cordo, Sosa, 2000). Despite these challenges, significant financial resources are allocated to *P. stratiotes* management worldwide, reflecting its substantial ecological and economic impact (Center, 1994). In Florida, annual costs for controlling *P. stratiotes* exceed 2 million USD, with over 100,000 USD spent by other eastern US states annually (Center, 1994). Over the past four decades, the combined yearly expenditure for managing both *P. stratiotes* and *Eichhornia crassipes* (Mart.) in Florida amounts to approximately 4.5 million USD (Center, 1994).

Natural herbicides, favored by home gardeners, organic farmers, and others seeking alternatives to synthetic herbicides, encompass a range of products including acids (such as acetic acid or vinegar and citric acid), oils (like clove, pine, peppermint, and citronella), soaps, iron- or salt-based herbicides, corn gluten, and combinations thereof (Smith-Fiola, Gill, 2017). These substances disrupt cell membranes, leading to plant desiccation and death (Baker, 1970; Webber III et al., 2018). Among commercially available natural herbicides, acetic acid is commonly listed as the active ingredient, typically at a concentration of 20% (Bradfield Industries, 2019). However, not all acetic acid products are designated for weed control, with horticultural vinegar containing 30% acetic acid being available but not explicitly labeled as an herbicide (Bradfield Industries, 2019). While citric acid is less prevalent, some products contain d-limonene, a citrus oil, as the active component (Cutting Edge Formulations). The scientific literature regarding the efficacy of natural products like acetic acid and d-limonene as weed control agents remains limited, both alone and in combination. Research suggests that acetic acid at concentrations of 20% or 30% may offer a viable alternative to glyphosate, though multiple applications may be necessary for sustained weed control (Domenghini, 2020). Acetic acid acts as a contact herbicide, damaging only the parts of the plant it directly touches. In contrast, glyphosate is a systemic herbicide that is absorbed and transported throughout the entire plant, leading to complete plant death. Additionally, there is limited data on the effectiveness of natural products like acetic acid in aquatic ecosystems. While acetic acid and d-limonene have shown some efficacy in weed control in certain contexts (Shrestha et al., 2012; Quarles, 2010; Gettys et al., 2021; Gettys et al., 2022; Gettys et al., 2023), their impacts on aquatic fauna and ecological toxicity are not well understood (Stubbs, Layne, 2020). These products are non-selective and may cause damage to off-target flora and fauna (Anderson, 2007). Therefore, their application in aquatic environments

warrants careful consideration and further investigation to evaluate potential environmental risks (Saha et al., 2006).

The primary objectives of this project included evaluating the effects of several natural products, such as acetic acid, d-limonene, mineral oil, and potassium salts of fatty acid, both individually and in combination, on *P. stratiotes*. Diquat dibromide, a nonselective herbicide, was the most commonly used herbicide for managing floating weeds, with a total application of >7,800 gallons of formulation (37.3% diquat dibromide) (Clark, Dew, 2019). Therefore, it was used as the positive control treatment.

2. Material and Methods

2.1 Plant Material and Growth Conditions

Pistia stratiotes L. plants were collected in 2023 from Barrage Koreima in the Rabat-Sale-Kenitra region (33°49'17"N 6°49'29"W), Morocco. The plants were harvested by manually removing them from the water, ensuring minimal damage to the roots and foliage. They were then transferred to plastic containers (60 cm in length, 45 cm in width, and 40 cm in height) (Goodluck products, China). The containers were filled with water and moved to the greenhouse at the Experiment Domain of the National Institute of Agricultural Research (INRA) located in the Zemamra locality (32° 37' N, 8° 42' W). The plants were acclimatized and cultured under semi-controlled environmental conditions, including a temperature range of 25–27 °C, and natural light. A soluble fertilizer with a composition of 3N-3P-2K (Timac Agro, France) was added to every 10 liters of water present in the containers. The water in the containers was changed every two weeks to maintain appropriate nutrient levels, and minimize the accumulation of undesirable substances. Twenty plants were placed in each container and were grown for 4 to 6 weeks to achieve more than 80% surface coverage. Subsequently, the young plants were transferred to approximately 100 plastic containers of the same dimensions and acclimated under similar conditions to complete their development and ensure sufficient vegetative material for the experiments.

2.2 Laboratory trials

The study investigated the impact of three natural products on *P. stratiotes* L under controlled laboratory conditions (25 ± 2 °C, 66 ± 5% RH, and a 12:12 h (L:D) photoperiod). The products evaluated were Limocide (60 g d-limonene/L) (Vivagro, Martillac, France), Insecticide 101 (780 g mineral oil/L) (UPL, Ankleshwar, India), and Hamper (500 g potassium salts of fatty acid/L) (Gowan Crop Protection, Faenza, Italy). Each product was applied individually at concentrations of 10%, 15%, 20%, and 30%. Furthermore, all treatments were also applied in combination with each other. These products were chosen based on their distinct modes of action (Gettys et al., 2021). As a positive control, 37.3% diquat dibromide (Tribune

Herbicide; Syngenta Crop Protection, Greensboro, NC) at a concentration of 0.89% was included due to its known efficacy against floating weeds as indicated in the FWC National Pollution Discharge Elimination System report for the calendar year 2018 (Gettys et al., 2021). All treatments, including the controls, were prepared using both tap water and seawater. Thus, each treatment underwent two preparations: one using tap water (Trial 1) and the other using seawater (Trial 2) (Table 1). The experiments were conducted on 30 22-day-old *P. stratiotes* plants (seed dispersal stage), each measuring 32.8 cm in height and 17.8 cm in width, which was ideal for observing the effects and symptoms of the tested products. These plants were grown in plastic containers (40 cm length × 30 cm width × 30 cm height) filled with water containing a soluble fertilizer with a composition of 3N-3P-2K (Timac Agro, France) for every 10 liters of water. The water and fertilizer were replaced monthly to maintain optimal growing conditions. The treatments were applied as a mist over the above-water foliage at a rate of 30 mL per plant using a professional 2L manual PE garden pressure hand sprayer (Taizhou Huangyan Jiaji Mould Plastic Co.,Ltd., China) with a pressure of 2 bar to ensure complete coverage. Ten replicates (containers) were used for each tested treatment, utilizing a completely randomized experimental block design. All experiments were conducted twice.

Table 1 - Treatments and concentrations used in *Pistia stratiotes* L. control trials

Trial	Treatment	Concentrations (%)
1	D-limonene (60 g/L)	10, 15, 20, and 30
	Mineral oil (780 g/L)	10, 15, 20, and 30
	Potassium salts of fatty acid (500 g/L)	10, 15, 20, and 30
	D-limonene (60 g/L) + Mineral oil (780 g/L)	10+10, 15+15, 20+20, and 30+30
	D-limonene (60 g/L) + Potassium salts of fatty acids (500 g/L)	10+10, 15+15, 20+20, and 30+30
	Mineral oil (780 g/L) + Potassium salts of fatty acids (500 g/L)	10+10, 15+15, 20+20, and 30+30
	37.3% diquat dibromide	0.89
2	Tap water	-
	D-limonene (60 g/L)	10, 15, 20, and 30
	Mineral oil (780 g/L)	10, 15, 20, and 30
	Potassium salts of fatty acid (500 g/L)	10, 15, 20, and 30
	D-limonene (60 g/L) + Mineral oil (780 g/L)	10+10, 15+15, 20+20, and 30+30
	D-limonene (60 g/L) + Potassium salts of fatty acids (500 g/L)	10+10, 15+15, 20+20, and 30+30
	Mineral oil (780 g/L) + Potassium salts of fatty acids (500 g/L)	10+10, 15+15, 20+20, and 30+30
	37.3% diquat dibromide	0.89
	Seawater	-

Plants were monitored weekly for 5 weeks post-treatment, and a numerical scale ranging from 0 to 10 was used to describe visual quality, as reported by Gettys et al. (2021) (0 = dead; 5 = fair quality, acceptable, somewhat desirable form and color, little to no chlorosis or necrosis; 10 = excellent quality, perfect condition, healthy and robust, excellent color and form). While previous studies have reported visual injury caused by herbicide treatments (Cutelle et al., 2013; Koschnick et al., 2005; Mudge et al., 2007), our research focused on evaluating visual quality. This parameter (treated-plant visual quality) has been utilized in assessing plant responses to various conditions (e.g., Gettys, Moore, 2018; 2019), herbicides (e.g., Gettys, Haller; 2012; Smith et al., 2014), salt stress (e.g., Tootoonchi et al., 2020), and other experimental factors. Following visual scoring, a destructive harvest was conducted to collect live biomass by carefully removing the plants from the containers. The collected materials were placed in paper bags, dried in a forced-air oven at 65 °C for 2 weeks, and then weighed. Visual data underwent arcsine transformation to ensure a normalized distribution. The tested treatment means of visual values and dried biomass were compared with untreated controls (tap water) using one-way ANOVA at a significance level of P = 0.05. Tukey's LSD test was utilized for post-hoc comparisons. Analysis was performed using IBM SPSS Statistics version 23.0 software. Additionally, as proposed by Haller and Gettys (2013), an efficacy benchmark of >90% reduction in visual values and dried biomass parameters of target weeds compared to the untreated control was used.

2.3 Greenhouse trial

Following the results obtained in laboratory trials, the three treatments that displayed the highest efficacy (combinations of d-limonene and mineral oil, combinations of d-limonene and potassium salts of fatty acid, and combinations of mineral oil and potassium salts of fatty acid at 30% prepared using sea water) were chosen for greenhouse trials at 25 to 27 °C under natural light. Thirty 22-day-old *P. stratiotes* plants were grown in plastic containers measuring 60 cm in length, 45 cm in width, and 40 cm in height within a greenhouse. Treatments were applied at a rate of 30 mL per plant as a spray to ensure complete coverage of the leaves. Control plants were sprayed with either seawater or tap water. The experimental design was a completely randomized block design with 10 replicates (containers) for each tested treatment, and the experiment was conducted twice. Plants were monitored weekly for 5 weeks using the same numerical scale, destructive measures, and statistical analysis as in laboratory trials.

3. Results and Discussion

3.1 Laboratory trials

3.1.1 Impact of applying d-limonene, mineral oil, and potassium salts of fatty acids [prepared with tap water] on the population density of *Pistia stratiotes*

ANOVA results indicated significant variations among treatments on their effect on treated plant visual quality ($F_{13, 266} = 510.390$, $P < 0.0001$) and dry biomass weight ($F_{13, 266} = 331.736$, $P < 0.0001$). Diquat (0.89%) proved most effective, with the lowest visual quality recorded (0.2500). D-limonene at a 30% concentration achieved comparable results to diquat (0.89%) (>90% reduction compared to the control), with an average visual quality score of 0.45. Mineral oil and potassium salts of fatty acids at 20% and above showed significant reductions in dry biomass compared to the control. Additionally, the treatments d-limonene (20%) and mineral oil at 20 and 30% reduced *P. stratiotes* treated plants visual quality to >50%, five days after treatment compared to the control (Figure 1). The dry biomass weight of *P. stratiotes* treated plants was reduced by most single-product treatments compared to the untreated control. Diquat (0.89%) and d-limonene at 30% and 20% achieved a 90% reduction, while d-limonene (15%), mineral oil (30% and 20%), and potassium salts of fatty acids (30%) reduced dry biomass weight by more than 50% (Figure 2). These findings suggest varying levels of effectiveness among treatments, with diquat and d-limonene demonstrating the greatest efficacy.

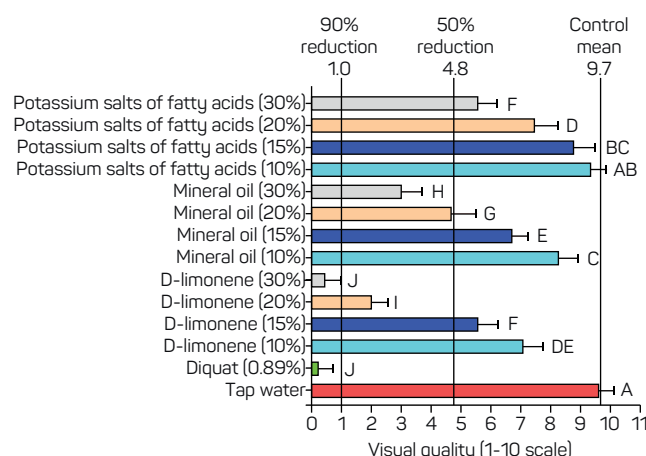


Figure 1 - Visual quality of *Pistia stratiotes* plants 5 weeks after single-product treatment. A numerical scale of 0 through 10 is used to describe visual quality, where 0 represents dead; 5 denotes fair quality, acceptable, somewhat desirable form and color, with little to no chlorosis or necrosis; and 10 indicates excellent quality, perfect condition, healthy and robust, with excellent color and form. Bars represent the mean of 20 replicates. Treatments labeled with the same letter are not different at $P = 0.05$. The right bold vertical line indicates the mean of control plants (tap water), whereas the central and left bold vertical lines indicate 50% and 90% reductions compared with control plants

3.1.2 Impact of applying d-limonene, mineral oil, and potassium salts of fatty acids [prepared with sea water] on the population density of *Pistia stratiotes*

Statistical analyses revealed significant variations among treatments on their effect on treated plant visual quality ($F_{14, 285} = 422.612$, $P < 0.0001$) and dry biomass weight ($F_{14, 285} = 385.941$, $P < 0.0001$). Based on visual quality results, the only single-product treatments that provided more than 90% reduction compared to the control were diquat (0.89%) and d-limonene at 20 and 30% concentration. D-limonene (15%), mineral oil at 20 and 30%, and potassium salts of fatty acids at 20 and 30% reduced water lettuce visual quality by > 50%, five days after treatment. All the other tested treatments had an effect on water lettuce visual quality compared to control, but did not reach 50% (Figure 3). *Pistia stratiotes* plant dry biomass weight was reduced by all single-product treatments compared with untreated control plants, with diquat (0.89%) and d-limonene at 20 and 30%, mineral oil at 30%, and potassium salts of fatty acids at 30% reducing dry biomass weight by >90% (Figure 4). All tested treatments, except seawater and mineral oil plus potassium salts of fatty acids at 10% concentration, reduced dry biomass weight by >50% (Figure 4).

3.1.3 Impact of simultaneous application of d-limonene, mineral oil, and potassium salts of fatty acids [prepared with tap water] on the population density of *Pistia stratiotes*

Statistical analyses indicated significant variations among treatments on their effect on treated plant visual quality ($F_{13, 266} = 459.065$, $P < 0.0001$) and dry biomass weight ($F_{13, 266} = 402.084$, $P < 0.0001$). The most promising combinations to control *P. stratiotes* were diquat (0.89%), d-limonene + mineral oil (20%), d-limonene + mineral oil

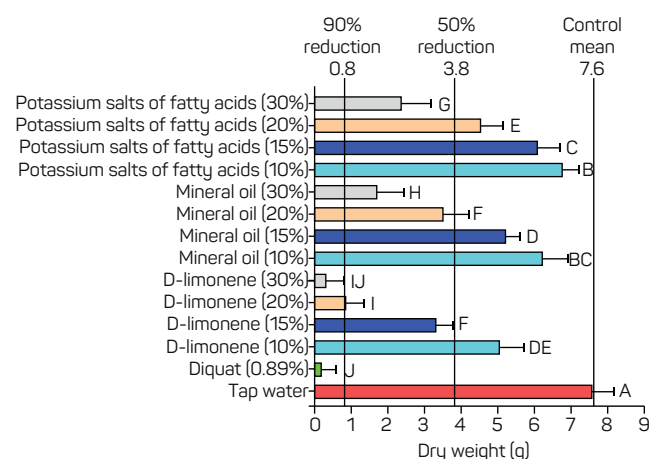


Figure 2 - Biomass of *Pistia stratiotes* plants 5 weeks after single-product treatment. Bars are the mean of 20 replicates. Treatments coded with the same letter are not different at $P = 0.05$. The right bold vertical line indicates the mean of control plants (tap water), whereas the central and left bold vertical lines indicate 50% and 90% reductions compared with control plants

(30%), d-limonene + potassium salts of fatty acids (30%), and mineral oil + potassium salts of fatty acids (30%); these treatments reduced visual quality (Figure 5), and dry biomass weight by >90% (Figure 6). The d-limonene + potassium salts of fatty acids (30%) treatment also reduced dry biomass weight by >90% compared with untreated control plants. Only d-limonene + potassium salts of fatty acids (10%) and mineral oil + potassium salts of fatty acids (10%) failed to reduce dry biomass weight by >50%

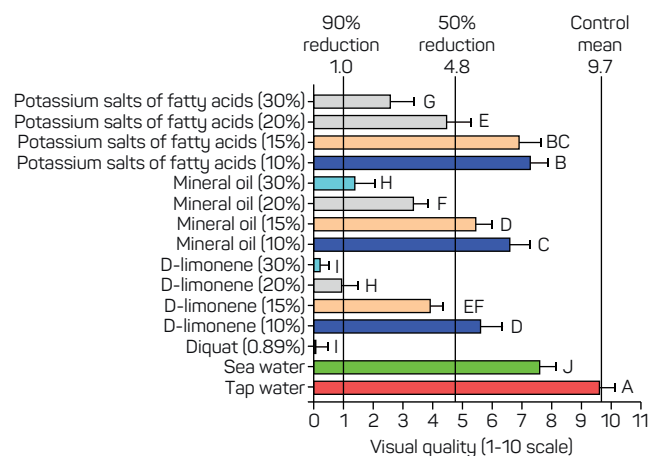


Figure 3 - Visual quality of *Pistia stratiotes* plants 5 weeks after single-product treatment. A numerical scale of 0 through 10 is used to describe visual quality, where 0 represents dead; 5 denotes fair quality, acceptable, somewhat desirable form and color, with little to no chlorosis or necrosis; and 10 indicates excellent quality, perfect condition, healthy and robust, with excellent color and form. Bars represent the mean of 20 replicates. Treatments labeled with the same letter are not different at $P = 0.05$. The right bold vertical line indicates the mean of control plants (tap water), whereas the central and left bold vertical lines indicate 50% and 90% reductions compared with control plants

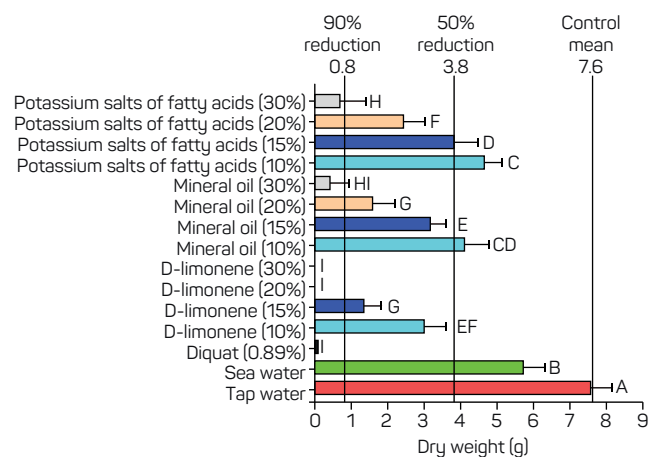


Figure 4 - Biomass of *Pistia stratiotes* plants 5 weeks after single-product treatment. Bars are the mean of 20 replicates. Treatments coded with the same letter are not different at $P = 0.05$. The right bold vertical line indicates the mean of control plants (tap water), whereas the central and left bold vertical lines indicate 50% and 90% reductions compared with control plants

compared with untreated control plants. Similarly, the latter treatments with mineral oil + potassium salts of fatty acids (10%) treatment failed to reduce visual quality by >50%.

3.1.4 Impact of simultaneous application of d-limonene, mineral oil, and potassium salts of fatty acids [prepared with sea water] on the population density of *Pistia stratiotes*

Statistical analyses revealed significant effects of the treatments on both visual quality ($F_{14, 285} = 726.191$, $P < 0.0001$) and dry biomass weight ($F_{14, 285} = 779.849$,

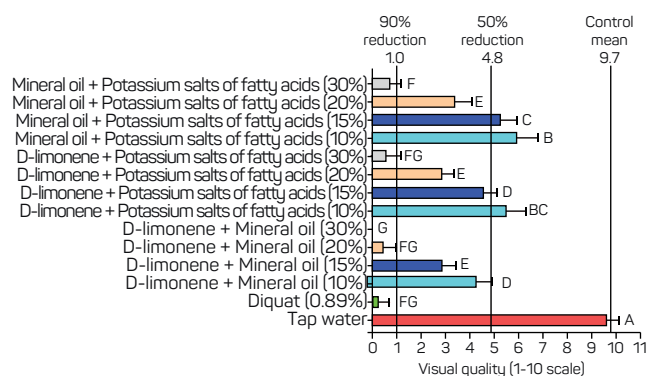


Figure 5 - Visual quality of *Pistia stratiotes* plants 5 weeks after treatment with combinations of D-limonene, mineral oil, and potassium salts of fatty acids. A numerical scale of 0 through 10 is used to describe visual quality, where 0 represents dead; 5 denotes fair quality, acceptable, somewhat desirable form and color, with little to no chlorosis or necrosis; and 10 indicates excellent quality, perfect condition, healthy and robust, with excellent color and form. Bars represent the mean of 20 replicates. Treatments labeled with the same letter are not different at $P = 0.05$. The right bold vertical line indicates the mean of control plants (tap water), whereas the central and left bold vertical lines indicate 50% and 90% reductions compared with control plants

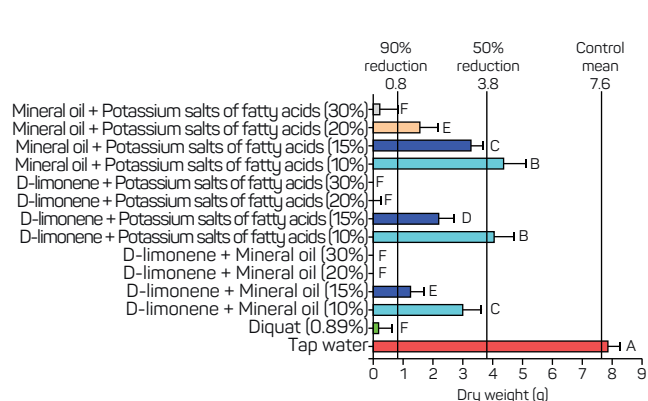


Figure 6 - Biomass of *Pistia stratiotes* plants 5 weeks after treatment with combinations of D-limonene, mineral oil, and potassium salts of fatty acids. Bars are the mean of 20 replicates. Treatments coded with the same letter are not different at $P = 0.05$. The right bold vertical line indicates the mean of control plants (tap water), whereas the central and left bold vertical lines indicate 50% and 90% reductions compared with control plants

$P < 0.0001$) of treated *P. stratiotes* plants. All the tested treatments successfully reduced visual quality (Figure 7), and dry biomass weight by >50% (Figure 8). Only 4 of the 15 treatment combinations failed to reduce dry biomass weight by >90% compared with untreated control plants, and only 6 combinations failed to reduce visual quality by >90% compared to the untreated plants.

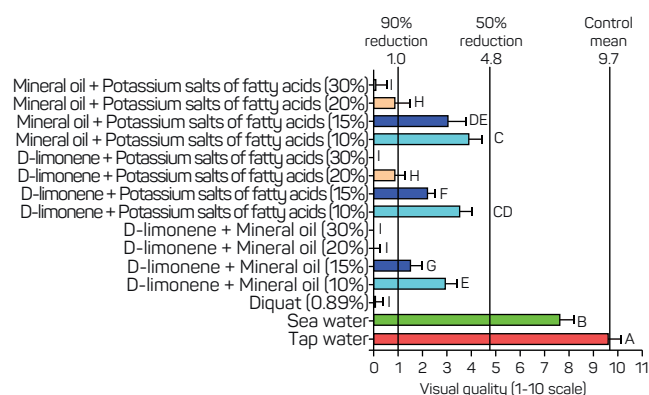


Figure 7 - Visual quality of *Pistia stratiotes* plants 5 weeks after treatment with combinations of D-limonene, mineral oil, and potassium salts of fatty acids. A numerical scale of 0 through 10 is used to describe visual quality, where 0 represents dead; 5 denotes fair quality, acceptable, somewhat desirable form and color, with little to no chlorosis or necrosis; and 10 indicates excellent quality, perfect condition, healthy and robust, with excellent color and form. Bars represent the mean of 20 replicates. Treatments labeled with the same letter are not different at $P = 0.05$. The right bold vertical line indicates the mean of control plants (tap water), whereas the central and left bold vertical lines indicate 50% and 90% reductions compared with control plants

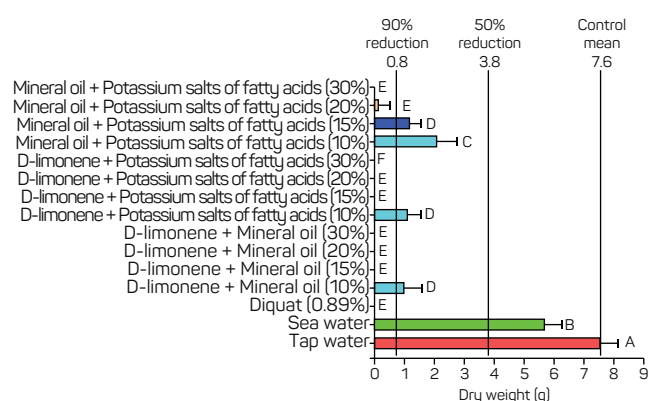


Figure 8 - Biomass of *Pistia stratiotes* plants 5 weeks after treatment with combinations of D-limonene, mineral oil, and potassium salts of fatty acids. Bars are the mean of 20 replicates. Treatments coded with the same letter are not different at $P = 0.05$. The right bold vertical line indicates the mean of control plants (tap water), whereas the central and left bold vertical lines indicate 50% and 90% reductions compared with control plants

3.2 Greenhouse trials

ANOVA results indicated significant variations among treatments in their effects on treated plant visual quality ($F_{5, 114} = 717.525$, $P < 0.0001$) and dry biomass weight ($F_{5, 114} = 473.383$, $P < 0.0001$). All the tested treatments except the seawater treatment reduced visual quality (Figure 9), and dry biomass weight by >90% (Figure 10)

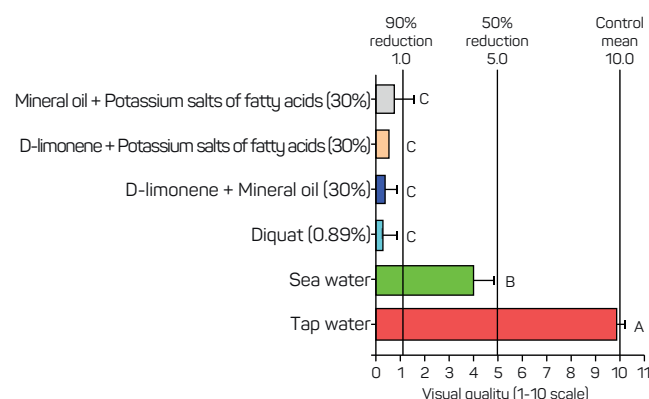


Figure 9 - Visual quality of *Pistia stratiotes* plants 5 weeks after treatment with combinations of D-limonene, mineral oil, and potassium salts of fatty acids under greenhouse conditions. A numerical scale of 0 through 10 is used to describe visual quality, where 0 represents dead; 5 denotes fair quality, acceptable, somewhat desirable form and color, with little to no chlorosis or necrosis; and 10 indicates excellent quality, perfect condition, healthy and robust, with excellent color and form. Bars represent the mean of 20 replicates. Treatments labeled with the same letter are not different at $P = 0.05$. The right bold vertical line indicates the mean of control plants (tap water), whereas the central and left bold vertical lines indicate 50% and 90% reductions compared with control plants

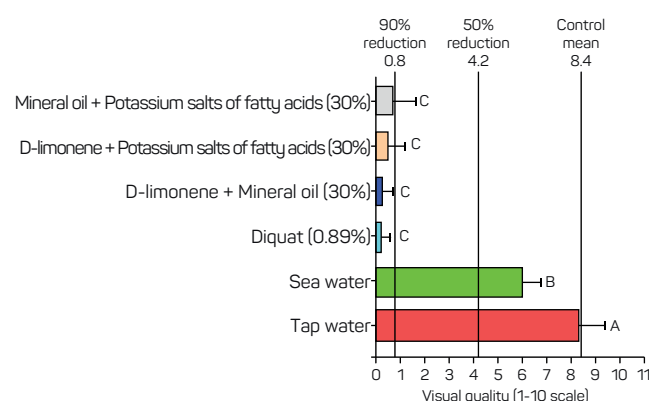


Figure 10 - Biomass of *Pistia stratiotes* plants 5 weeks after treatment with combinations of D-limonene, mineral oil, and potassium salts of fatty acids under greenhouse conditions. Bars are the mean of 20 replicates. Treatments coded with the same letter are not different at $P = 0.05$. The right bold vertical line indicates the mean of control plants (tap water), whereas the central and left bold vertical lines indicate 50% and 90% reductions compared with control plants

compared to the tap water control. Visual quality was reduced by >50% in plants treated with seawater only.

The results of the laboratory and greenhouse trials provide comprehensive insights into the efficacy of various treatments for managing *P. stratiotes*. This invasive aquatic plant poses significant ecological and economic threats, making effective control measures essential.

Diquat (0.89%) emerged as to was the most effective single-product treatment in both laboratory and greenhouse trials, consistently achieving over 90% reduction in both visual quality and dry biomass weight of *P. stratiotes*. This finding aligns with previous studies that highlight the potency of diquat in controlling aquatic weeds (Martins et al., 2002; Mudge, Haller, 2012). Notably, d-limonene, a natural product, demonstrated promising efficacy, particularly at concentrations of 20% and 30%, where it achieved comparable results to diquat. These concentrations and their effectiveness on waterlettuce are consistent with the findings of Gettys et al. (2021). This finding underscores the potential of natural alternatives for weed control, offering environmentally friendly options for management practices while potentially reducing reliance on synthetic herbicides (Invasive Species Specialist Group, 2005; Rojas-Sandoval et al., 2013). Previous studies have highlighted d-limonene's potential for selective weed management without harming desirable native plants (Emerine, 2010; Mudge, Haller, 2012; Glonski, Mudge, 2013). However, its high application cost compared to traditional herbicides may limit broad-scale deployment (Gettys et al., 2021). The reduction in visual quality and dry weight is likely due to the impact of the treatments on the plant's growth and reproductive processes, as observed in similar studies (Ajuonu et al., 2003; Gettys et al., 2021). The simultaneous application of different products yielded promising results, especially in greenhouse trials. Combinations such as d-limonene + mineral oil and d-limonene + potassium salts of fatty acids demonstrated substantial reductions in both visual quality and dry biomass weight of *P. stratiotes*. These findings highlight the synergistic effects that can be achieved by combining multiple control methods (Cilliers et al., 1996; Gettys et al., 2021). The use of potassium salts of fatty acids can help reduce plant growth by increasing the salinity of the aquatic environment; however, this increase in salinity may not be desirable in all contexts, as it can adversely affect non-target organisms and the overall ecosystem, making it essential to consider the specific environmental conditions and potential impacts before applying these treatments, while mineral oil can create a film on the water's surface, limiting photosynthesis and thus suffocating the plant, and it is not recommended if there is a possibility of the water being used for drinking purposes (Service, 1962). By leveraging the strengths of different products, enhanced efficacy can be achieved while potentially reducing the risk of resistance development and environmental impact.

Variations in temperature and humidity throughout the study period reflect typical weather patterns, which may affect the growth and reproduction of *P. stratiotes* as well as the efficacy of the treatments. These environmental factors could affect the performance of the natural products tested and should be taken into account in future research and practical applications (Queensland Government, 2017; Millane, Caffrey, 2014). Despite the promising results, several challenges and considerations need to be addressed. The persistence of *P. stratiotes*, facilitated by vegetative reproduction and seed longevity, underscores the need for sustained and integrated management efforts (Millane, Caffrey, 2014). Additionally, the cost of adopting alternative control methods, such as natural products, must be carefully evaluated. While these products may offer environmentally friendly alternatives, their higher costs compared to conventional herbicides could pose challenges for widespread adoption, particularly in large-scale management programs (Center, 1994). Gettys et al. (2021) reported that using a combination of 20% d-limonene + 10% acetic acid (the most cost-effective treatment) instead of diquat for waterlettuce management would result in a 22-fold increase in material costs. Thus, a cost-benefit analysis is crucial to determine the feasibility and sustainability of implementing these control strategies on a broader scale.

4. Conclusions

In conclusion, the findings of this study highlight the effectiveness of diverse strategies in controlling *P. stratiotes* infestations. Both single-product treatments and combinations of products have shown promise in reducing the population density and biomass of *P. stratiotes*. Single-product treatments, such as specific herbicides or natural compounds like d-limonene, offer targeted control measures, while combinations of products, such as d-limonene with mineral oil or potassium salts of fatty acids, provide synergistic effects that enhance overall efficacy. However, while these findings are encouraging, further research is warranted to comprehensively assess the efficacy, cost-effectiveness, and environmental impact of these control methods. Integrated approaches that combine chemical, physical, and biological control methods have emerged as particularly effective in addressing *P. stratiotes* infestations. By harnessing the strengths of each approach, these integrated strategies offer a multifaceted and sustainable solution for managing *P. stratiotes*, thereby mitigating its ecological and economic impacts.

Author's contributions

All authors read and agreed to the published version of the manuscript. ME and MS: conceptualization of the manuscript and development of the methodology. ME: data collection and curation. ME and MS: data

analysis. ME and MS: data interpretation. ME and MS: funding acquisition and resources. ME and MS: project administration. MS: supervision. ME: writing the original draft of the manuscript. ME and MS: writing, review and editing.

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