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BIOHERBICIDES: ESSENTIAL OIL AND WEED MANAGEMENT

Presentata da: **Dott. Mattia Alpi**

Coordinatore Dottorato
Prof.ssa Diana Di Gioia

Supervisore
Prof. Giovanni Dinelli

Co-supervisore
Prof. ssa Ilaria Marotti

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Sommario

ABSTRACT

Weeds pose a significant challenge to agriculture, causing yield losses and increasing management costs. Traditional synthetic herbicides have long been the primary solution due to their cost-effectiveness and high efficacy. However, their overuse has raised environmental and health concerns, driven the development of herbicide-resistant weeds, and prompted stricter regulatory controls, such as those outlined in the European Green Deal. These factors have intensified the search for sustainable alternatives, including bioherbicides derived from natural compounds like organic acids and essential oils. This research evaluates the potential of organic acids and essential oils for weed management under various experimental conditions. Acetic acid emerged as a promising bioherbicide due to its ability to disrupt plant cell membranes, leading to rapid desiccation and effective weed suppression. Its low environmental persistence and potential for cost-effective production as an industrial byproduct enhance its appeal. Similarly, pelargonic acid demonstrated high efficacy, though its widespread use is hindered by production costs. Essential oils, while effective in some combinations, require further exploration due to their variable performance and high production costs. The experiments revealed interspecies differences in sensitivity, with dicotyledonous weeds generally more susceptible than monocotyledons. Application timing and weed growth stages significantly influenced efficacy, as did environmental factors like temperature and evaporation rates. Higher application volumes improved effectiveness, but rapid evaporation limited the field-scale applicability of treatments. Adjuvants such as Camelina oil, chitosan, and essential oils showed potential in enhancing acetic acid’s performance, particularly at lower concentrations. However, they did not consistently outperform acetic acid alone, emphasizing the need for further optimization. Despite its potential, organic acids and essential oils require multiple applications and careful timing to maximize its impact while minimizing crop damage. Its role as a bioherbicide could be particularly valuable in organic and small-scale farming systems. Future research should focus on optimizing formulations, refining application strategies, and integrating acetic acid into broader sustainable weed management programs to enhance its viability as an eco-friendly alternative to synthetic herbicides.

1 Introduction

Climate change (CC) refers to alterations in the Earth's climate caused by both anthropogenic and natural factors, including ozone depletion and the greenhouse effect. These changes can result from solar emission fluctuations, long-term variations in Earth's orbital elements (such as eccentricity, obliquity, and precession), natural processes, and human influences on the planet. As a consequence, the 20th century saw the most significant warming trend in the last millennium, with average global temperatures rising by approximately 0.6°C. Future temperature increases are expected to surpass this, with a projected rise of 0.1 to 2°C per decade (Muluneh, 2021). Climate change is anticipated to profoundly affect biodiversity, agricultural production, and food security on a global scale. Additionally, human activities such as changes in land-use patterns, ecosystem degradation, modification and fragmentation, species exploitation, and the introduction of invasive species have intensified the impacts of climate change. However, the effects of CC are likely to vary regionally, depending on factors such as land use, biotic invasions, pollution, human activities, fire, and ecosystem types.

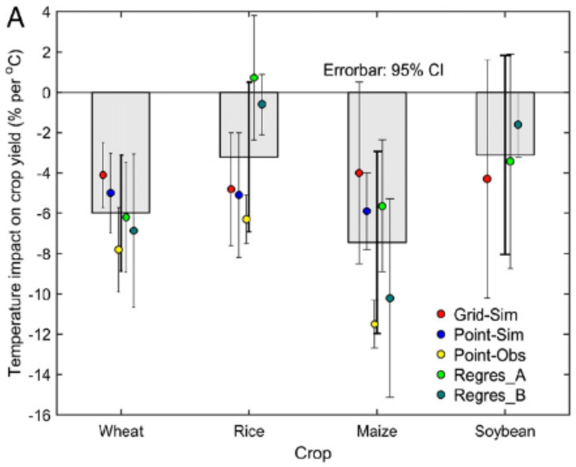
CC is expected to cause long-term shifts in weather patterns, with severe consequences for agricultural production, food security, and the availability, accessibility, and utilization of food (Vincent et al., 2008). Projections show a decrease in crop yields across major food crops and geographical areas under all climate scenarios (Wiebe et al., 2015; Zhao et al., 2017).

The World Food Summit in 1996 defined food security as "existing when all people, at all times, have physical and economic access to sufficient, safe, and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (FAO, 2008). The global population is currently growing by about 1.1% per year, and if these trends continue, the population is expected to reach 9.7 billion by 2050 according to medium-variant projections (UN, 2019). Despite some uncertainty in population forecasts, it is estimated with 95% certainty that the global population will range from 9.4 to 10.1 billion by 2050 (UN, 2019). At the same time, global hunger has been on the rise since 2014, after years of decline (FAO, 2018). The proportion of undernourished people worldwide increased from 10.6% in 2015 to 11% in 2016 (UN, 2018), and the number of undernourished people reached approximately 821 million in 2017—about one in nine people (FAO , 2018).

This increase in food insecurity poses a significant challenge to achieving the Sustainable Development Goal (SDG) of hunger eradication by 2030 (FAO, 2018). Projections based on a 2005 baseline indicate that global crop demand will rise by 100%–110% by 2050, driven by population growth and increased per capita income. However, land-use changes, particularly cropland expansion, are not expected to significantly contribute to food security. Studies show that, globally and in most regions, the risk of undernourishment is expected to increase under all scenarios considered (Molotoks et al., 2020).

Therefore, the links between climate change and food security have been examined in terms of their impact on crop productivity and food production. While sustainable food production is crucial for sustainable development, climate change threatens the achievements of sustainable development strategies and the 2030 Agenda. In light of this, the agricultural sector must enhance its sustainability and adapt to the impacts of climate change in ways that do not undermine global efforts to ensure food security.

1.1 Relation between climate change and food security



B

	Yield changes (%) due to temperature changes by the end of century				
Scenario	Wheat	Rice	Maize	Soybean	Mean
RCP2.6	-6.9 [-15.0, -1.4]	-3.3 [-9.2, 0.8]	-8.6 [-18.6, -1.8]	-3.6 [-11.2,1.7]	-5.6 [-14.4, -0.1]
RCP4.5	-11.4 [-21.7, -3.9]	-5.5 [-13.8, 1.0]	-14.2 [-27.9, -4.9]	-5.9 [-17.0, 3.1]	-9.2 [-21.2, -0.3]
RCP6.0	-14.0 [-25.7, -5.1]	-6.8 [-16.8, 1.3]	-17.4 [-33.1, -5.8]	-7.2 [-20.2, 3.6]	-11.3 [-25.6, 0.1]
RCP8.5	-22.4 [-40.2, -8.5]	-10.8 [-25.3, 2.4]	-27.8 [-50.4, -9.7]	-11.6 [-31.0, 6.0]	-18.2 [-38.6, -0.7]

Figure 1. Multimethod estimates of global cropyield changes in response to temperature increase(Zhao et al., 2017). (A) Impacts on crop yields of a1 °C increase in global temperature in grid-based simulations (Grid-Sim), point-based simulations (Point-Sim), field-warming experiments (Point-Obs), and statistical regressions at the countrylevel (Regres_A) and the global level (Regres_B), (B) Projected changes in yield due to temperature changes by the end of the 21st century (Alain Vidal 2024).

There is substantial evidence showing that global crop yields are decreasing as temperatures rise. For example, using climate data to generate daily weather data model for 2055, predicted average crop yield reductions are estimated at -6% per °C for wheat, -3% per °C for rice and soybeans, and -7 to -10% per °C for maize, with regional variations (Jones & Thornton, 2003; Vidal 2024). Another simulation of maize production in Africa and Latin America, foretold an overall yield reduction of -10% (Gregory et al 2005). Figure 1 illustrates the estimated temperature impact on average crop yields, under four scenarios defined by the Intergovernmental Panel on Climate Change (IPCC). Several studies (Amthor, 2001; Fuhrer,

2003) have assessed how climate change affects crop growth and yields, concluding that the initially anticipated benefits of CO_2 fertilization would likely be offset by nutrient limitations, pollutants, and other interactions with climatic factors (Long et al., 2005; Cramer et al., 2014). Specifically, considering CO_2 fertilization, global maize and wheat yields were estimated to have declined by 3.8% and 2.5%, respectively, from 1980 to 2008, compared to a non-warming baseline (Lobell and Field, 2007; Lobell et al., 2011). The mixture distribution showed a -4.1% decline in global maize yields ($p < 0.1$), with a 90% probability interval ranging from -8.5% to +0.5%. For maize, the impact varied by country, ranging from a -8.6% reduction in India to +13.5% in France. For soybeans, the global mean yield decreased by -4.5% ($p < 0.05$), with a 90% probability interval of -8.4% to -0.5%, reflecting negative impacts across major producing regions. The estimated ensemble mean impact for wheat was −4.7% when no CO2 fertilization was assumed, whereas it was insignificant when CO2 fertilization was considered (Iizumi T. et al., 2018). The mixture distribution for wheat showed a -1.8% global yield decline ($p < 0.3$), with a 90% probability interval ranging from -7.5% to +4.3%. For rice, there was no significant impact at the global (+0.9%; -9.6% to +12.4%) or country levels (Iizumi et al., 2018).

Gregory et al. (1999) summarized experimental results indicating that warming reduces wheat crop duration (and thus yield) and decreases rice yields by about 5% per °C for temperatures above 32°C, with rice being less affected than other crops. Using estimated yield impacts, the global production losses in 2005–2009 were calculated at -22.3 billion USD for maize, -6.5 billion USD for soybeans, -0.8 billion USD for rice, and -13.6 billion USD for wheat (Table 2). The 90% probability intervals of production impacts were -49.3 billion USD to -2.0 billion USD for maize, -21.5 billion USD to +3.5 billion USD for soybeans, -21.8 billion USD to +11.1 billion USD for rice, and -36.6 billion USD to +5.1 billion USD for wheat (negative values indicate losses, positive values indicate benefits). Despite uncertainty in these estimates, climate change is likely to have caused production losses for maize, soybeans, and wheat compared to preindustrial levels, while the impact on rice production is expected to be small or nonexistent (Iizumi et al., 2018).

The spatial variation in climate change effects on potential crop yields was explored by Fischer et al. (2001) using climate predictions for 2080 from various global circulation models (GCMs). The analysis indicated that cereal-producing regions in Canada, northern Europe, and Russia may see increased production due to climate change, while many other regions, including the western U.S. prairies, eastern Brazil, and western Australia, are expected to experience losses.

Therefore, it is critical to assess production strategies that contribute to sustainability goals, such as reducing energy consumption and greenhouse gas emissions, preserving biodiversity and soil health, protecting water resources, and minimizing toxic substance use (and eliminating particularly harmful substances). Low-energy, low-impact agriculture that respects the environment and human health is essential to ensuring the resilience of agricultural systems and meeting global food security needs.

In addition to the effects of climate change, global staple crop yields have stagnated or declined since the mid-1990s, with some exceptions. This trend, particularly evident in Europe, was first analyzed and documented in the early 2010s, showing clear breakpoints in wheat, maize, and barley yields in most European countries during the 1990s. It was challenging to detect these breakpoints earlier, as up to 18 years of stagnation were required to identify a statistically significant yield plateau (Moore and Lobell, 2015; Vidal 2024). The causes of this stagnation remain poorly understood, but recent literature suggests six key factors, ranked by their likely causal impact: limited crop yield increases due to inadequate adaptation to climate change (most likely for all crops); decreased crop diversity (increasing monoculture) and soil carbon content (likely); marginal costs of management interventions leading to limited further investment in production (likely); the physiological yield potential of crops possibly reaching its limit (likely for wheat); political decisions (e.g., European Common Agricultural Policy) contributing to lower investment in breeding or reduced input use (unlikely); and increased relative area of crops grown under organic or regenerative agriculture, leading to stagnation due to generally lower yields than conventional agriculture (unlikely) (Vidal 2023). Research from the Institut du Développement Durable et des Relations Internationales (IDDRI) argues that crop yield stagnation may be largely due to the loss of ecosystem services in monoculture systems under conventional agriculture (Poux & Aubert, 2018; Kurth et al., 2023). The factors involved are both the result of human activities and climate change. In a 2010 study of wheat yield stagnation in Europe, the replacement of 10% of leguminous crops with rape in crop rotations was shown to depress subsequent wheat yields by an average of 0.035 t/ha/year (Brisson et al., 2010). In contrast, a large modeling study of annual yields for 176 crop species across 91 countries found that greater crop diversity at the national level improved year-to-year harvest stability (Renard and Tilman, 2019). A more recent review of 98 meta-analyses from 6167 studies (published between 2010 and 2018) explored the impacts of agricultural practices on crop yields and biodiversity. The review found that while the impact on crop yields was neutral overall, crop diversification had many positive effects, particularly when compared to monoculture (Tamburini et al., 2020). These findings support the idea that increased crop diversity sustains yields more effectively than monoculture, although the exact impact remains unclear. Furthermore, evidence suggests that stagnating crop yields are linked to declining soil organic content (SOC), a proxy for belowground biodiversity, with climate change, deeper tillage, land use changes, and reduced organic fertilization contributing to SOC loss (Wiesmeier et al., 2015). Climate change thus acts indirectly through the loss of ecosystem services that sustain crop yields, but further research is needed to distinguish its specific impact from other ecosystem service losses. The IPBES estimates that climate change will account for one-sixth of the loss of terrestrial ecosystem services (Díaz et al., 2019). Studies have shown that increasing semi-natural habitats (SNH) and agricultural landscape complexity can improve essential ecosystem services, such as pest control, pollination, and nutrient cycling, which may support higher yields (DeClerck et al., 2023; Garibaldi et al., 2018, 2020). However, the contribution of these services to reversing yield stagnation remains context-specific. A meta-analysis covering 49 studies across Europe found that higher SNH and edge density improved crop yields by enhancing pest control and pollination, suggesting that the lack of these services could underlie ongoing yield stagnation despite high agricultural inputs (Martin et al., 2019). A broader study from 43 global studies found that biodiversity and yield improvements occurred in only 23% of cases, more frequently in temperate climates with crop and landscape diversification and no agrochemicals (Jones et al., 2023). Overall, there is limited evidence that ecosystem services provided by SNH and landscape complexity can help overcome yield stagnation, but more research is needed to clarify their role in breaking this trend.

It is clear that today we face one of the biggest challenges of this century, ensure food security in a period of increasing cost of agronomical inputs, lack of resources, crops yield stagnation and climate change impacts. These challenges bring us to a new food production paradigma, where we need to bring back biodiversity and reduce the use of fossil fuels and chemicals, in order to reduce pressure of human activities on ecosystem, meanwhile trying to increase crops yield for a continuously growing world population

1.2 Green Deal EU

In December 2019, the European Commission introduced the European Green Deal (EGD) at the United Nations Climate Change Conference (COP25). For the first time, the Green Deal set the ambitious goal of making the EU the world’s first carbon-neutral continent by 2050. This will be achieved through the development of clean energy and promoting sustainable economic growth within the EU (Wang Yitao, 2022). From an agricultural perspective, the Green Deal not only aims to reduce greenhouse gas emissions but also envisions transforming agriculture into a key player in combating climate change. This includes enhancing farming sustainability, preserving biodiversity, and promoting more efficient use of natural resources. Key aspects of the European Green Deal related to agriculture are outlined below:

The central goal of the Green Deal is climate neutrality by 2050, meaning a reduction of net greenhouse gas emissions to zero. To reach this target, the EU has raised its greenhouse gas reduction goal from 40% to 50%, with an aspiration of 55% by 2030. The European Climate Law, Europe's first legally binding climate law, enforces political commitments to reduce emissions. Supported by this law, the EU has proposed the European Climate Convention and the "Zero Pollution Action Plan" to strengthen climate policies and support emission reduction targets (Wang Yitao, 2022). Agriculture, responsible for about 10% of total EU greenhouse gas emissions, is vital in reaching these goals. Transitioning to low-emission farming practices involves reducing chemical fertilizer and pesticide use, promoting renewable energy in agriculture, and implementing soil management techniques to increase carbon storage.

The Farm to Fork Strategy, a key element of the Green Deal, addresses the sustainability of the entire European food system, from agricultural production to food distribution. This strategy aims to reduce agriculture's ecological footprint, enhance food quality and safety, cut pesticide and fertilizer use, promote organic and sustainable farming, and encourage the consumption of local, healthier food. The strategy targets a 50% reduction in pesticide and fertilizer use, promotes organic farming, integrated pest management, and sustainable nutrient management, and aims to ensure at least 25% of agricultural land is dedicated to organic farming by 2030. These measures will reduce dependence on chemical inputs and promote biodiversity.

The Common Agricultural Policy (CAP) plays a key role in implementing the Green Deal's agricultural objectives. The CAP has evolved to include sustainability-driven policies, linking direct payments to farmers to environmental criteria. The EU has decided to allocate part of CAP funds to practices that align with environmental protection, natural resource conservation, and greenhouse gas emission reduction. CAP guidelines encourage farmers to adopt precision farming, crop rotation, agroecology, conservation agriculture techniques, and sustainable water and soil management. The introduction of climate and environmental payments, such as eco-schemes, further incentivizes farmers to reduce their environmental impact.

Beyond emission reductions, the European Green Deal also emphasizes the conservation of agricultural biodiversity. Intensive farming practices, which rely heavily on pesticides, fertilizers, and monocultures, have severely impacted biodiversity, leading to the decline of various plant and animal species in agricultural landscapes. European policies under the Green Deal aim to restore ecosystems, with goals like protecting 30% of EU land and seas and planting

3 billion trees by 2030. These policies also encourage the creation of natural habitats, including the conservation of pollinator and other wildlife habitats.

In terms of technology, the Green Deal promotes agricultural efficiency and reduces environmental impact through innovation. Precision farming, utilizing technologies like sensors, drones, artificial intelligence, and forecasting models, allows farmers to optimize resource use (e.g., water, fertilizers, and pesticides) while improving productivity and minimizing environmental costs. Transitioning to smart, digital agriculture is crucial to achieving the Green Deal's objectives.

In addition to environmental considerations, the European Green Deal strives for a more equitable and socially inclusive agricultural sector. It aims to support vulnerable farmers, promote practices that improve the quality of life in rural areas, and bolster farmers' resilience to climate change. A "just transition," ensuring support for communities that may be adversely affected by the ecological shift, is a key component of the Green Deal.

1.3 Chemicals' usage in agriculture

As previously mentioned, the use of synthetic chemicals in crop productions is a significant environmental concern, particularly in relation to soil and water pollution. This makes it essential to implement global measures to limit their use and mitigate their negative impacts. Herbicides are one of the primary tools for weed control in agriculture and represent the most economically significant category among pesticides. The amount of herbicides used worldwide, in Europe, and in Italy varies depending on factors such as agricultural policies, practices, dominant crops, environmental regulations, and the awareness of both farmers and the general public. The global use of herbicides has increased notably in recent decades, especially after the introduction of products like glyphosate. According to the Food and Agriculture Organization (FAO) and the Organisation for Economic Co-operation and Development (OECD, 2020), the global herbicide market was estimated at approximately 2.5 million tons in 2020, with herbicides making up around 40-50% of this total. Globally, approximately 2 million tons of pesticides are used annually, with 47.5% being herbicides, 29.5% insecticides, 17.5% fungicides, and 5.5% other pesticide categories (Sharma et al., 2019; Małgorzata et al., 2024). This equates to about 1.04 million tons of herbicides applied each year.

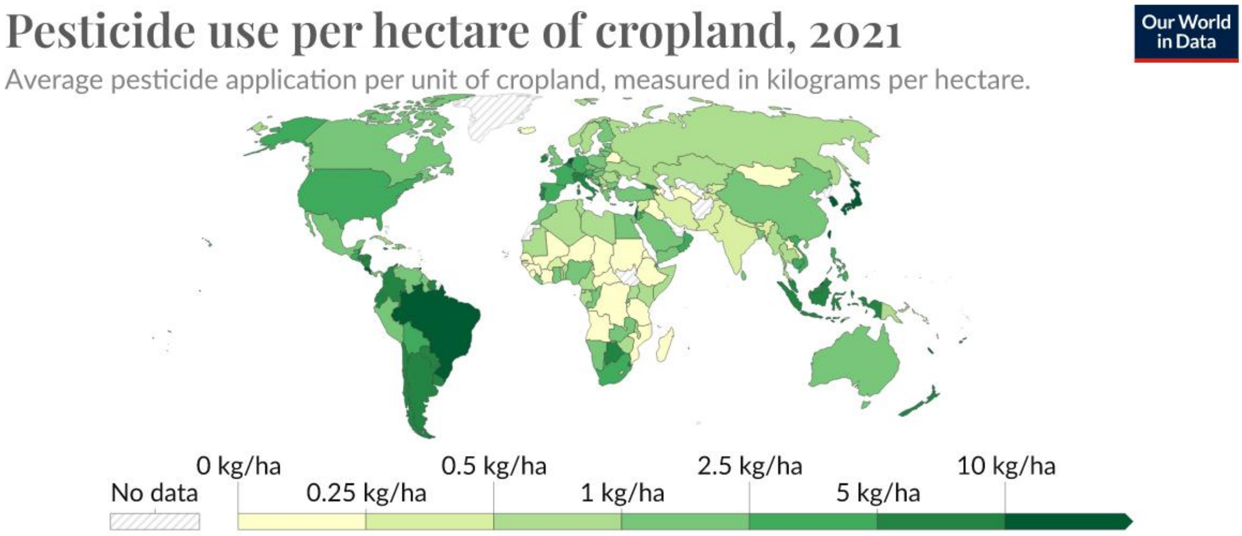


Figure 2: Pesticide use per hectare of cropland in 2021 (Food and Agriculture Organization of the United Nations 2024)

Despite efforts to regulate their use, the demand for herbicides is expected to continue to rise, especially in developing countries where intensive agriculture is expanding. In industrialized nations, where this agricultural model is already established, transitioning away from chemical use will be more challenging without increased awareness and action. Furthermore, the effects of climate change are contributing to a growing need for agronomic inputs to maintain economically viable production, pushing farmers toward cost-effective, chemical-based solutions. Consequently, moving away from chemicals remains difficult.

Globally, herbicide use is particularly concentrated in North America, Latin America, and Asia, where they are applied to control weeds in major crops such as corn, soybeans, wheat, and rice, as well as other industrial crops. This concentration is likely due to the rapid development of these regions, where natural resource depletion and deforestation are leading to increased agricultural land use, resulting in heavy reliance on chemical treatments.

In Europe, herbicide use is more tightly regulated compared to other regions, yet remains high, particularly in crops such as wheat, corn, sugar beets, and soy. According to Eurostat data from 2019, around 175,000 tons of herbicides were used in Europe, accounting for 50% of the total chemicals used. These figures encompass both chemical herbicides and other substances for weed control. Despite some progress in reducing herbicide use, the overall usage remains significant, and public awareness of the direct and indirect negative effects remains low. France is one of the largest consumers of herbicides in Europe, followed by Germany and Italy. However, the European Union has taken steps to curb pesticide use, such as the Farm to Fork

Strategy under the European Green Deal, which aims to reduce chemical pesticide use by 50% by 2030.
In Italy, herbicide use is widespread due to the country's large agricultural area but is also facing increasing scrutiny, especially regarding the environmental and health risks associated with products like glyphosate. Glyphosate has become particularly prevalent in certain sectors, including baked goods and flour, with some brands now offering glyphosate-free products. According to the Italian National Institute for Environmental Protection and Research (ISPRA, 2020), Italy used approximately 150,000 tons of pesticides in 2019, with about 50-60% of this being herbicides. This translates to an estimated 75,000-90,000 tons of herbicides applied annually. These herbicides are used in various crops, including maize, wheat, vegetables, and other intensive farming practices. Herbicide use is particularly concentrated in agricultural regions where there is higher anthropogenic pressure, such as those producing intensive crops.

1.4 Effects of weeds on crops yield

In 1967, the Weed Science Society of America defined weeds as "plants growing where they are not wanted." This definition was updated in 1989 to "any plant that is objectionable or interferes with human activities or well-being," and in 2016, it was further refined to "a weed is a plant that causes economic losses and ecological damage, affecting human and animal health, or growing in places where it is not wanted." This definition reflects an anthropocentric perspective, as it categorizes any plant that disrupts human activities or grows in undesired locations as a weed. In contrast, plants growing in natural, non-anthropogenic environments would simply be considered spontaneous plants.
Weeds have a significant negative impact on crop productivity, and their control represents one of the greatest challenges in agriculture. Of all the biotic and abiotic factors that affect production, weeds account for the largest share of losses, exceeding 30%, followed by insects (18%) and pathogenic organisms (16%) (Głąb et al., 2017). Weeds cause various types of damage, including competition for resources, allelopathic interactions, and parasitism, all of which contribute to yield loss. In addition to reducing quantity, weeds can degrade product quality by contaminating food, leading to increased costs for cleaning and sorting. Some weed species in forage crops can even cause poisoning, which, while not fatal, reduces growth and lowers the quality of milk and meat. Additionally, the presence of certain perennial species or herbicide-resistant weeds may prevent the cultivation of specific crops.

Various studies have estimated the productivity losses in agricultural crops caused by insects, weeds, and pathogenic fungi. A recent study by Scheitza (2006) on major crops worldwide, including rice, wheat, barley, potatoes, soybeans, cotton, and coffee, found that productivity losses could reach up to 52% without treatments. Further research indicates that, on average, 15% of damage is due to insects, 14% to weeds, 13% to pathogenic fungi, and 10% to post-harvest losses (Oerke and Dehne, 1994; Yudelman, 1998).

The extent of yield loss from weed interference depends on various factors, including the type of crop, the weeds present, and growing conditions. Heavy weed infestations throughout the growing season can lead to total crop loss (Singh et al., 2021; Farooq et al., 2022). Weeds reduce crop yields by competing for vital resources such as light, water, and nutrients, impacting crop growth and development. Several factors influence the magnitude of yield losses, such as the timing of weed emergence relative to the crop, weed density, and the pattern of weed growth. In addition to direct competition, weeds also affect crops indirectly through allelopathy (Thesiya et al., 2024; Farooq et al., 2022). For example, allelochemicals produced by weeds can inhibit the growth of competitors, alter developmental processes such as shifting growth and reproduction to occur before resource limitations, and modify resource foraging strategies. Weeds may also increase shoot length to gain a height advantage, or influence the microbiome in ways that can either benefit or harm plant growth. Additionally, epigenetic changes can occur due to plant–plant interactions, affecting a seedling's response to competitors in future generations (Horvath et al., 2023). On average, weeds cause a 5% loss in agricultural production in developed countries, while losses are 10% in developing countries and 25% in least-developed countries (Oerke, 2006).

1.5 Herbicides

Herbicides are one of the primary tools for weed control in agriculture and represent the most economically significant category among pesticides. The use of inorganic chemical compounds for weed management dates back to ancient times, with early references found in writings by Theophrastus, Democritus, and Cato. These texts mention substances like vegetable water from olive pressing, salt, and copper sulfate as early methods of weeding. In the early 1900s, arsenic salts were introduced, although their use was limited. At the same time, sulfuric acid became more widely used in major Western countries, often in a 5-10% aqueous solution for wheat weeding, as it had a selective effect on dicotyledons.

The development of organic herbicides began in 1932 with the introduction of DNOC, a compound that, although highly toxic to mammals, was more selective than sulfuric acid. A significant advancement occurred in 1941, when compounds derived from the distillation of tar were used to synthesize 2,4-D, MCPA, and later MCPP. The effectiveness and cost-efficiency of these herbicides led to their rapid adoption, especially in France.

From the 1950s onward, research focused on developing herbicides with residual activity, which could prevent the emergence of weeds. The introduction of Triazines in the 1960s revolutionized maize cultivation. These herbicides gained popularity due to their high physiological selectivity and broad-spectrum activity. However, their overuse led to significant groundwater pollution issues, culminating in a ban by the late 1980s. By the mid-1970s, the emergence of "compensatory flora" and growing concerns about the environmental reliability of herbicides prompted a shift toward more specific compounds with improved ecotoxicological profiles. This shift resulted in the development of new families of herbicides by the late 1970s, including aryloxyphenoxypropionates, which featured compounds like diclofop-methyl (introduced in 1980) and clodinafop-propargyl in more recent years. These herbicides were valued for their effective leaf absorption, low soil persistence, and selective graminicidal properties.

During the 1970s, glyphosate, a non-selective herbicide, was introduced. This herbicide was particularly notable from an ecotoxicological standpoint and continues to be widely used for weeding in tree crops, fallow lands, and certain transgenic crops. In the 1980s, two new innovative herbicide families emerged: Sulfonylureas (Beyer et al., 1987) and Imidazolinones (Los, 1991). These herbicides were characterized by low toxicity to humans and animals, and their significant biological activity allowed for usage at very low application rates per hectare. This new generation of herbicides further heightened environmental awareness, though it also led to new challenges related to the selection and spread of herbicide-resistant weeds. These developments, combined with reduced chemical pressure on the environment, set the stage for the continued evolution of the herbicide market and its future advancements (Orlando et al., 1997).

Trough development of new synthetic compounds, herbicides were evolved to destroy weeds while not damaging crop plants. They readily penetrate plants through the root system or leaves, as well as through the shoots. It has been noted that herbicides can be taken up by swelling seeds. The mechanism of action of herbicides on weeds involves the disruption of biochemical and biophysical processes in the plant, which are closely related to the metabolism and life processes of plant cells. Herbicides are divided according to their mode of action and weed control spectrum. As reported by Małgorzata B. et al., (2024) there are two main groups of herbicides: selective and non-selective herbicides. Selective herbicides control specific species of weeds, do not damage crop plants, and act onweeds through specific mechanisms that are effective against specific weeds. Examples ofselective herbicides are acetochlor, alachlor, amidosulfuron, atrazine, benazolin, bentazone, bromoxynil, chlorpropham, clopyralid, clomazone, chlorosulfuron, chloro-toluron, 2,4-D,desmediphan, di-flufenican, dicamba, diuron, etofumesat, flufenacet, isoproturon, andmezotrion. Non-selective herbicides, on the other hand, are potent substances used mainlyin areas overgrown with undesirable plants and have a very broad spectrum of action,eliminating most of the weeds they come into contact with. Non-selective herbicidesinclude glyphosate, glufosinate, paraquat, and dichloride.

In particular, the most specific classification system for herbicides developed by the Global Herbicide Resistance Action Committee (HRAC) is their division in terms of chemical properties and mechanism of action. The most commonly used herbicide groups for cropweed control include: 1) Acetyl–CoA carboxylase (ACC) inhibitors—inhibit fatty acid synthesis and destroycell membrane structure; aryloxy-phenoxy propionate group compounds; 2) Acetyl–lactate synthase (ALS) inhibitors—inhibit the production of the branched-chainamino acids leucine, valine and isoleucine; compounds from the cyclohexanedione,imidazolinone, pyrimidinylthio-benzoate, sulfonyl-amino-carbonyl-triazolinone andsulfonylurea group of compounds; 3) Inhibitors of the microtubule system—inhibit cell division in plant roots; triazolo-pyrimidine and dinitroaniline group compounds; 4) Synthetic auxins—inhibit cell growth in newly forming leaves and stem and the nucleicacid metabolism, and interfere with cell wall plasticity; compounds in the pyridine,phenoxy, benzoic acid, carboxylic acid, and quinaline carboxcylic acid groups; 5) Photosynthesis inhibitors at photsystem II level – cause blockage of electron transport in the second stable electron acceptor of photosystem II, which interrupts photosynthesis and energy production; compounds in the group phenyl-carbamate, pyridazinone, triazine, triazinone, triazolinone, uracil, benzothiadiazoles, nitriles, phenyl-pyridazine, amide, and urea; 6) Lipid synthesis inhibitors—inhibit the production of cuticular wax and inhibit shootgrowth; compounds from the thiocarbamates group; 7) 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) inhibitors—inhibit the synthesis of aromatic amino acids; compounds of the organophosphates group; 8) Glutamine synthetase inhibitors—cause accumulation of huge amounts of ammonia,so that plant cells are destroyed; 9) Carotenoid biosynthesis inhibitors/inhibitors of pigment synthesis—damage chlorophyll pigments; compounds from the triazoles, pyridazinone, and isoxazolidinnes group; 10) Cell membrane inhibitors (PPOs)—uses disruption of cell membrane function; compounds in the diphenyl-methyl ester group, bipyridylium, N-phenyl-phthalimides, and oxadiazoles.

1.6 Negative effects of herbicides on environment and human health

Herbicides, while essential for increasing crop yields and ensuring food security, pose significant risks to human and environmental health due to their widespread presence in various environmental settings. When applied in agricultural fields, urban areas, or forests, herbicides can have both short- and long-term harmful effects on humans, animals, insects, wildlife, and fish. Improper or excessive use of herbicides can lead to residues accumulating in plants, soils, and the food chain, as well as the development of herbicide-resistant weeds. These chemicals can spread from agricultural areas to other parts of the environment through processes such as spray drift, volatilization, runoff, and leaching, which can harm non-target organisms and negatively affect both human and environmental health. Research shows that only 45% of sprayed herbicides reach their intended target plants, while 30% are lost through drift, 10% via runoff, leaching, and volatilization, and 15% reach the soil (Schreiber et al., 2018). Herbicides that remain in the soil long after application can enter plants, leading to environmental, soil, and food toxicity. Furthermore, the repeated use of the same herbicides in agricultural management contributes to their persistence due to slow degradation.

The impact of herbicides on human health depends on several factors, including the type of herbicide, dosage, exposure routes, and duration of exposure. Humans can be exposed to herbicides through direct contact, inhalation in agricultural areas, or indirectly through the consumption of contaminated food and water, which can result in both acute and chronic health effects. Prolonged exposure to herbicides has been linked to various health issues, including cancer, neurodegenerative disorders, developmental and reproductive changes, and respiratory problems (Marin-Morales et al., 2013; Gómez-Ramos et al., 2020).

A study by Parven et al. (2024) reviewed research from various sources and identified several factors that influence the environmental toxicity of herbicides, including their physicochemical properties, soil conditions, meteorological factors, application methods, frequency and quantity of use, and the biotic and abiotic characteristics of the environment. The environmental impact of herbicides extends beyond target crops, affecting non-target organisms such as soil microorganisms, aquatic species, insects, birds, and others. Glyphosate, for example, has been found to harm a wide range of organisms, including arthropods, fish, reptiles, mollusks, amphibians, and birds. Glyphosate concentrations above 10 mg/L have been shown to inhibit the growth of beneficial mycorrhizal fungi and negatively affect earthworms, reducing reproduction rates, causing DNA damage, decreasing biomass, and lowering casting activity. Bees, which are essential for pollination in both agricultural and wild plants, are also adversely affected by glyphosate. Long-term use of atrazine and 2,4-D has been linked to negative effects on soil microorganisms, nitrogen-fixing bacteria, and soil enzyme production. In addition, 2,4-D negatively impacts the reproduction and development of earthworms and reduces nitrogenase, phosphatase, and hydrogen photo production activities in Rhizobium species and purple non-sulfur bacteria. Fish exposed to 2,4-D have exhibited behavioral changes such as lethargy and erratic swimming patterns. Dicamba, 2,4-D, and paraquat are particularly toxic to beneficial insects, with ladybird beetle larvae being especially vulnerable to the commercial formulation of 2,4-D, while dicamba has been shown to reduce body weight and increase mortality.

Indirect effects of herbicides pose a higher risk to neighboring habitats than direct effects. By reducing food sources such as insects and grains, herbicides contribute to the decline of bird populations in agricultural areas. A recent study by Deepika et al. (2024) highlights the broad range of unintended effects on the environment, animals, and human health. Herbicides like chloroacetanilides induce abnormalities across various groups, affecting bacteria to animals, with aquatic species particularly at risk. Morphological changes, clastogenic, genotoxic, and cytotoxic effects, as well as disruptions to the endocrine and reproductive systems, are commonly observed in aquatic species, amphibians, and terrestrial organisms. Certain herbicides, such as alachlor, acetochlor, and metolachlor, pose cancer risks to humans due to their persistence in the environment and their entry into the food chain. Imidazolinones and sulfonylurea herbicides, which are highly mobile in the environment, alter microbial and soil enzymatic activities, causing phytotoxicity in sensitive crops and sublethal toxicity in aquatic species and amphibians, including damage to vital organs and DNA. Enantioselective imidazolinones also disrupt metabolic pathways and amino acid synthesis in humans and other animals. Pyrimidinylcarboxy herbicides primarily affect soil microbes, exhibiting synergistic toxic effects, including mortality, neurotoxicity, genotoxicity, and DNA damage, particularly in aquatic species and rodents. Another study specific on soil microorganisms reveal that the soil acts as a repository for agricultural contaminants such as herbicides and serves as a major habitat for microbial communities, including bacteria, fungi, and actinomycetes.T hese microorganisms play a critical role in soil fertility through organic material degradation, organic matter decomposition, and nutrient cycling.

Herbicide application impacts not only target organisms but also soil microorganisms, often adversely affecting key soil functions. A significant side effect of herbicides is their disruption of soil biochemical processes. These chemicals can hinder biochemical reactions by interfering with soil enzymatic activity and microbial growth. Herbicides can be toxic to microbial populations, leading to reduced microbial biomass, soil heterotrophic respiration, and the activity of organic matter-decomposing and nutrient-cycling microbes.

The study by Ikioukenigha et al. (2024) emphasizes the significant impact of herbicides like Chlorimuron-ethyl on the diversity and abundance of nitrogen-fixing bacteria in soil. This is a critical concern, as these bacteria play an essential role in the nitrogen cycle, directly influencing soil fertility. A reduction in nitrogen-fixing bacteria could disrupt the natural nitrogen supply to crops, leading to a heightened dependence on synthetic fertilizers. The effects of herbicides on microbial respiration and enzyme activity vary depending on the specific herbicide used. For example, at low concentrations, 2,4-dichlorophenoxyacetic acid (2,4-D) has only mild, temporary effects, while at higher concentrations, it inhibits important hydrolase activities and stimulates dehydrogenase activities. These changes in enzymatic activity could alter microbial degradation processes in the soil, affecting nutrient cycling and organic matter decomposition. Exposure to herbicides like Atrazine has been shown to induce adaptive changes in the gut microbiomes of animals, especially earthworms. Atrazine reduces the diversity of gut microbiota in these organisms, which could affect ecological functions like soil aeration and nutrient cycling. Similarly, herbicides exert selective pressure on microbial populations, leading to shifts in microbial community composition and potentially decreasing soil biodiversity. Butachlor, a commonly used herbicide, affects phosphatase activity in the soil. At low concentrations, it decreases the activity of both acid and alkaline phosphatases, enzymes vital for the breakdown of organic phosphorus compounds. However, at higher concentrations, alkaline phosphatase activity increases. This biphasic response suggests that Butachlor's effect on soil phosphorus cycling is both concentration-dependent and complex. Herbicides like Butachlor, Metolachlor, and Alachlor reduce nitrogen fixation by cyanobacterial mats, which are essential for soil nitrogen enrichment in aerobic environments. These herbicides decrease the diversity of cyanobacteria and the number of diazotrophs, organisms responsible for fixing atmospheric nitrogen, resulting in lower soil fertility and less nitrogen available for plants. Glyphosate, one of the most widely used herbicides, disrupts ecological interactions in the soil. Research has shown that Glyphosate reduces mycorrhizal spore counts, interferes with the relationships between earthworms, mycorrhizal fungi, and plants, and induces avoidance behavior in *Eisenia fetida* (a common earthworm species). These disruptions can negatively impact plant growth and soil structure by damaging critical symbiotic relationships. Paraquat, another widely used herbicide, significantly reduces bacterial populations, particularly those from the genera Luteimonas and Pseudo-propionibacterium, within a short time (14 days). These bacteria are involved in organic matter decomposition and nutrient cycling, and their decline could impair overall microbial functions in the soil.

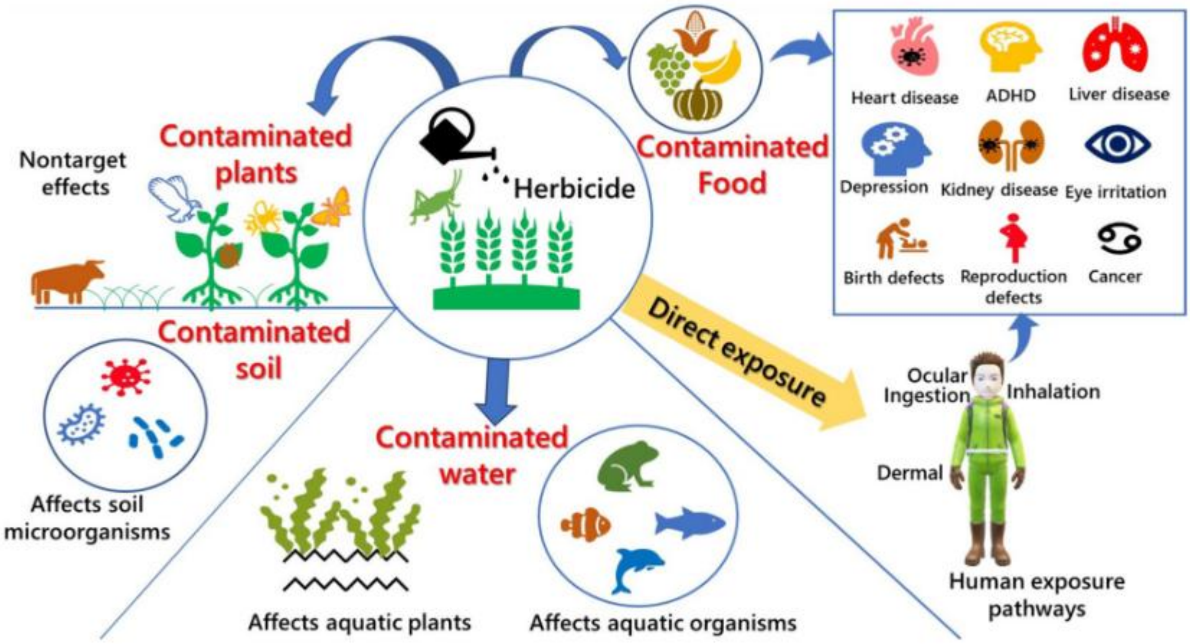


Figure 3: Potential dentrimental effects associated with herbicide exposure in humans and other nontarget organisms (Parven et al., 2024)

In addition to different negative impacts caused by herbicide on environment, the new spread of resistant weed species brings new challenges in the use of chemicals in agriculture. As reported by Riechers et al. (2024), globally, there are 530 unique cases (species by site of action) of herbicide-resistant weeds, encompassing 272 weed species (155 dicots and 117 monocots) that have evolved resistance to168 different herbicides from 21 of the 31 known herbicide sites of action (Heap 2024). Herbicide-resistance mechanisms are generally divided into target-site resistance (TSR) and non–target site resistance (NTSR) mechanisms (Jugulam and Shyam 2019; Murphy and Tranel 2019; Powles and Yu 2010). TSR is conferred by an altered target site or amplification of the target gene, resulting in over expression of the target enzyme that limits herbicide phytotoxicity (Powles and Yu 2010). NTSR includes mechanisms that reduce the amount of active herbicide reaching the target site and may involve reduced retention, decreased absorption, impaired translocation, enhanced metabolism, and/or subcellular sequestration of the herbicide (Devine and Eberlein 1997; Gaines et al. 2020; Nandula et al. 2019; Yu and Powles 2014).

1.7 Glyphosate effects on environment and human health

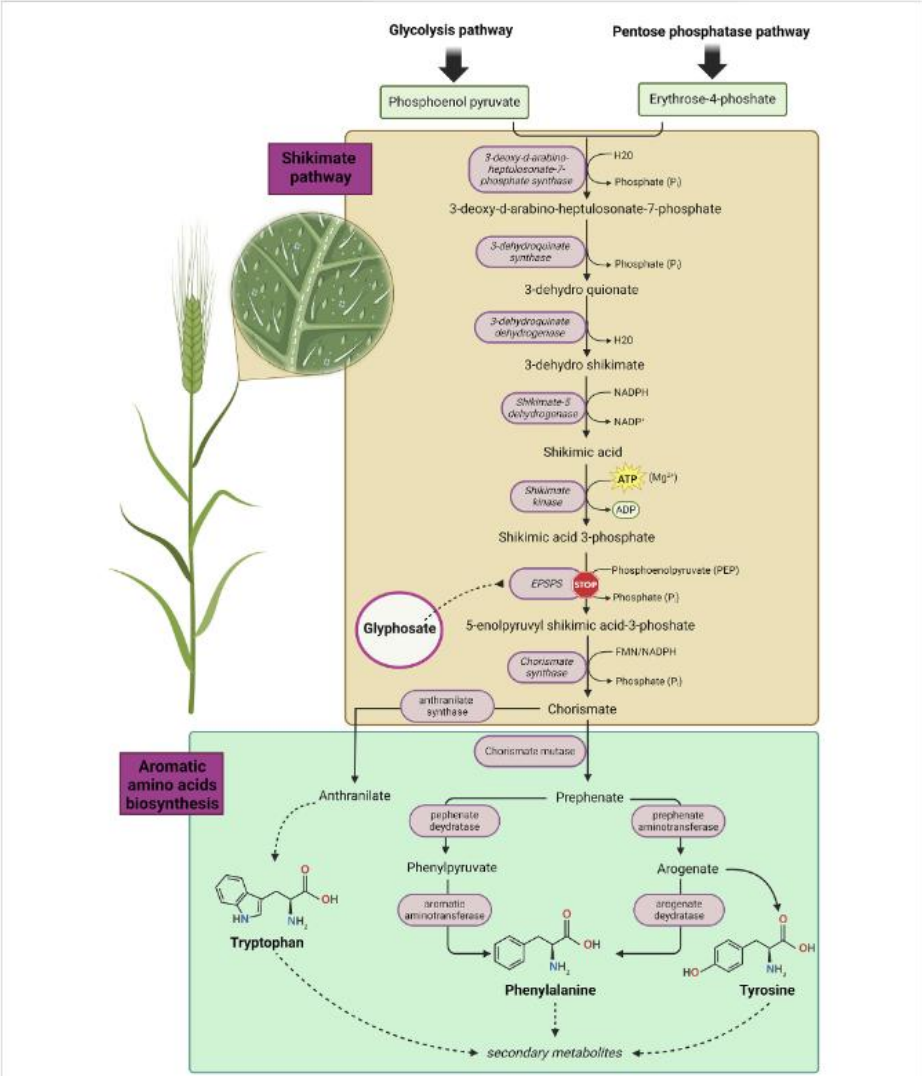


Figure 4: the shikimate pathway in plants (Galli et al., 2024)

Glyphosate (GLY), also known as N-(phosphonomethyl) glycine, is an organophosphorus compound widely used as a broad-spectrum, non-selective herbicide, making it the most commonly used herbicide globally. Its herbicidal effect occurs by inhibiting the enzyme 5-enolpyruvyl shikimate-3-phosphate synthase (EPSP), which is found in plants and microorganisms. This enzyme is essential for catalyzing the condensation of phosphoenolpyruvate and shikimate-3-phosphate, key components in the biosynthesis of vital aromatic amino acids (AAAs) such as phenylalanine, tyrosine, and tryptophan in chloroplasts.

By blocking EPSP, glyphosate disrupts the production of these amino acids, ultimately leading to plant death. Glyphosate was first synthesized in 1950 by Swiss chemist Henry Martin and was approved for use in the United States in 1974, when Monsanto received authorization from the Environmental Protection Agency (EPA) to market it under the brand name Roundup®.

Today, glyphosate is the active ingredient in numerous formulations of glyphosate-based herbicides (GBHs), used to control over 100 weed species and 60 perennial weed types in both industrial and residential settings (Munoz et al., 2021; Ferrante et al., 2023). Between 1996 and 2012, glyphosate use in the U.S. reached a record 12 million tons, a figure projected to rise in the coming years (Benbrook, 2016; Ferrante et al., 2023). As a result, glyphosate-based herbicides have become one of the most widely used classes of plant protection products (PPPs) worldwide, with use in approximately 140 countries (Munoz et al., 2021).

For many years, glyphosate was thought to be harmless, as its target, the EPSP enzyme, is not naturally found in mammals. It was also assumed that glyphosate would degrade into carbon dioxide, with its formulations containing chemicals mistakenly labeled as "inert." However, due to its widespread use, glyphosate has been detected in various environmental matrices, including air, water, and food, and is now commonly found in biological fluids such as urine, blood, and breast milk (Chaufan et al., 2014).

Glyphosate's effects on the environment and non-target organisms have been highlighted in recent reviews, including one by Villanueva et al. (2024). In soil, glyphosate is strongly adsorbed, limiting its movement and reducing the risks of leaching, although its potential for leaching increases when combined with phosphorus fertilizers or under high pH conditions. Glyphosate has also been detected in surface and groundwater, linked to agricultural activities, and can persist in these environments for extended periods, ranging from 2 to 251 days. This persistence raises concerns about bioaccumulation and long-term exposure risks. Ecologically, glyphosate can negatively impact plant communities, reducing species richness and delaying flowering, and has been directly linked to herbicide resistance in weeds, particularly in crops such as soy and corn. Animal studies show that glyphosate's toxicity varies across species. It can harm aquatic life, inhibiting the growth of fish and algae, and causing histopathological changes. In terrestrial animals like pigs, zebrafish, and honeybees, glyphosate leads to sublethal effects on gut microbiota and behavior. Long-term exposure in rats has been associated with increased tumor incidence and liver damage, and concerns about neurotoxic effects are emerging.

Research on the effects of glyphosate on human health, as reported by Villanueva et al. (2024) and Chaufan et al. (2014), shows that glyphosate can enter the human body through dermal absorption, inhalation, ingestion, and consumption of contaminated food and water. This exposure presents significant health risks, including cancer, respiratory issues, and neurological disorders. Once inside the body, glyphosate accumulates in organs such as the kidneys, liver, and colon, and is primarily eliminated through urine and feces. Glyphosate has been detected in the urine, blood, and breast milk of both agricultural workers and the general population, with exposure rates ranging from 60% to 80%. Some studies emphasize the risks of skin exposure, particularly when the skin is damaged. Inhalation, especially among agricultural workers, can lead to respiratory problems such as asthma and declines in lung function. Food contamination, especially in crops treated with herbicides or drying agents, also raises concerns about chronic exposure. Glyphosate exposure has been linked to dysbiosis and gut diseases like inflammatory bowel disease (IBD), potentially disrupting gut microbiota and impairing amino acid production. Animal studies suggest that glyphosate can alter gut composition, cause inflammation, and contribute to conditions like irritable bowel syndrome. Additionally, glyphosate has been associated with changes in DNA methylation and potential cancer development due to its genotoxic effects. While agencies such as the IARC and EFSA have debated its carcinogenicity, some studies link glyphosate to non-Hodgkin's lymphoma, though more research is needed. Glyphosate and its formulations have also been identified as endocrine disruptors, showing estrogenic effects in breast cancer and endometrial cell lines. Animal studies suggest potential reproductive harm, including embryo loss and congenital abnormalities. Despite these concerns, regulatory bodies have renewed glyphosate's approval, fueling ongoing debates about its safety. Future studies should focus on understanding glyphosate's human health impacts, particularly in isolation and under low-dose exposure scenarios.

1.8 Organic farming vs conventional farming

Global agriculture feeds over 7 billion people, but it is also a major contributor to various forms of environmental degradation. Agricultural activities account for 25%–33% of global greenhouse gas emissions, occupy 40% of Earth's land surface, withdraw over 70% of freshwater, drive deforestation and habitat fragmentation, and cause biodiversity loss. Additionally, agriculture contributes to the eutrophication and acidification of aquatic and terrestrial ecosystems due to the use of agrochemicals. These environmental impacts are expected to rise in the coming decades, driven by population growth and shifts toward more meat-based diets linked to increased income (IUCN 2016; Clark & Tilman 2017).

Organic agriculture offers an alternative production management system that promotes agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. It focuses on using natural inputs, such as minerals and plant-based products, while avoiding synthetic fertilizers and pesticides (FAO 2023b). As a result, organic farming generally has a lower environmental impact, and its produce is considered safer and healthier due to the absence of synthetic chemicals (Aulakhet al., 2022). However, agronomic factors such as soil type and fertility, climate, plant management, and cultivars also significantly affect the nutritional and functional value of crops (Madrera et al., 2024). Organic management offers several environmental benefits, including enhanced soil fertility and conservation, improved chemical properties of the soil, better water use efficiency, and increased drought resistance. For example, organic farming typically results in thicker surface horizons, deeper topsoil, reduced soil erosion, higher organic matter content, and greater water retention capacity. These benefits, in turn, contribute to higher resilience to climatic variability and enhanced biodiversity, both on farms and in surrounding ecosystems.

By 2020, nearly 75 million hectares of land worldwide were dedicated to organic farming. However, this represents only 1.6% of global agricultural land. In Europe, the area of organic land reached 17.1 million hectares, with 14.9 million hectares in the European Union. This means that just 9.2% of agricultural land in the EU was used for organic farming, still far from the targeted 25% (Aldott-Sipos et al., 2023). Studies have shown that farming practices that enhance soil health can also boost yields. A 2014 global review found that healthy earthworm populations increased crop yields by an average of 25%, which helped narrow the yield gap between organic and conventional farming systems (Van Groenigen et al., 2014). Another study found that while organic yields were generally 20% lower, diversified organic crop rotations reduced the yield gap to under 10% (Ponisio et al., 2015). A 1990 study showed that organic yields were almost 10% lower on average compared to conventional yields based on 205 comparisons across 26 crops, but in half of the direct comparisons, organic yields were equal to or exceeded conventional yields (Stanhill, 1990). This indicates that the yield gap is not consistent across studies. A more recent review observed that the yield differences between organic and conventional systems decreased significantly over time, reducing or even eliminating the gap after several years of organic production (Fess and Benedito, 2018).

The potential of organic farming and regenerative agriculture to enhance ecosystem services is also confirmed by various studies. Organic farming typically requires 25%–110% more land than conventional systems but uses 15% less energy and has 37% higher eutrophication potential per unit of food. Organic and conventional systems do not significantly differ in greenhouse gas emissions or acidification potential, although organic systems have slightly lower emissions and higher acidification potential (Seufert et al., 2012; Cassman et al., 2002). These environmental differences are primarily due to differences in nutrient management, as organic systems rely on manure as a nitrogen input, unlike conventional agriculture, which uses synthetic fertilizers. Manure application can often result in mismatches between nutrient availability and crop demand, leading to lower crop growth and increased land use. Organic systems tend to offer higher on-farm and near-farm biodiversity due to the reduced use of fertilizers, herbicides, and pesticides. Additionally, organic practices help increase soil organic carbon by promoting carbon storage in agricultural soils (Gattinger et al., 2012). However, organic farming may have a net negative impact on biodiversity and soil organic carbon at larger scales due to the greater land area required and the loss of biodiversity and carbon stocks associated with land conversion from natural habitats (Balmford et al., 2005; Phalan et al., 2011; Gilroy et al., 2014). According to Mansoure et al. (2024) the pool of weed species, species richness, number of unique species, and Shannon's diversity index were greater in the low-input system than that of the conventional system. Despite these concerns, organic systems still offer significant environmental and health benefits compared to conventional farming, which relies heavily on energy, synthetic fertilizers, and pesticides.

Organic foods have been shown to have higher concentrations of micronutrients and lower pesticide residues than conventionally grown foods. Organic farming also offers health benefits by suppressing pathogens and enhancing crop nutrient uptake, thereby improving the nutritional value of food (Wall et al., 2015). Numerous studies have found that farming practices that affect soil life influence mineral uptake and phytochemical production. Organic farming tends to result in higher levels of beneficial phytochemicals, particularly antioxidants and anti-inflammatory compounds, compared to conventional farming. Studies have also shown that dietary differences can affect human health. For example, a study of Swedish children found a significantly higher risk of allergic conditions among those attending public schools compared to those at Steiner schools, where families emphasized a biodynamic, vegetable-rich organic diet (Alm, 1999). Another study involving almost 15,000 European children found that those from public schools who ate conventional diets had higher incidences of asthma and food allergies (Alfven et al., 2006). A pair of Danish studies showed that men who consumed the most organic produce had the highest sperm counts, while those who consumed only conventional produce had the lowest (Jensen et al., 1996; Juhler et al., 1999). Although there is little evidence of significant differences in macronutrient composition, organic crops consistently have higher levels of phytochemicals that contribute to human health. Organic crops often contain several times the levels of beneficial compounds, including antioxidants, compared to conventional crops.

Another study (Anandkumar N. et al., 2023) on energy balance in organic and conventional farming, concluded that there are different outcomes in this kind of comparison, probably due to the many factors that influence agriculture production. Some authors show a net returns per unit area were 38% higher on the conventional farms than the organics, in front of the 22% higher net income in organic farms proposed by another study. With an average price premium of 111–138% and lower production costs and yields, organic systems achieve 2.4 times greater net returns at lower risk. It is to underline that organic farms are more reliant on ecosystem services for the production of high crop yields, whereas conventional farms rely more on external inputs. According to Gomiero et al., (2008), in general organic production needs lower energy inputs in relations to area unit (from 10% to 70% less) and to biomass production (from to 15% to 45% less). The lower energy consumption is due to the lack of synthetic products: synthetic fertilizers (which, between production and transport, can reach 50% of the energy consumption of an agricultural company), pesticides and herbicides

In conclusion, while organic farming may require more land and have higher eutrophication potential, it offers significant environmental, health, and nutritional benefits, particularly in terms of reduced pesticide residues and higher levels of beneficial phytochemicals. Organic and regenerative farming systems provide promising solutions for enhancing ecosystem services, improving human health, and reducing the environmental impact of agriculture.

Unlike conventional farming systems that mostly rely on use of systemic and specific herbicides to control weed, organic farming systems rely on a combination of management practices to control weeds. Differences between practices used in conventional farming compared to organic farming often vary widely in their implementation and relative importance. Reliance on non-chemical weed control methods in organic farming systems make it difficult to fully manage weeds. Organic farming needs to work in a preventive way in order to reduce pressure of weeds and weed seed bank in soil. It is always assumed that fields under organic farming systems have high weed density compared to those under conventional farming systems where there is use of herbicide that can almost clear any type of weed. Approaches to weed management within an organic system revolve around implementing a range of techniques, such as tillage, crop rotation, mulching, flaming and biological or mechanical weeding, often consecutively over the course of the cropping season. These management practices contribute to the variation of weed seeds in the soil which determines composition and density of the weeds. It is clear that the higher request of working hours to manage weeds in organic agriculture poses significant cost impacts on the farm economy. According to Seufert et al. (2012), labor costs in organic farming can be 25-30% higher compared to conventional systems, primarily due to the need for intensive manual labor to remove weeds. The higher use of mechanical management use in organic farming bring certainly to higher cost in short-term period prevision, but the long-term benefits on soil and environmental health could bring to a major competitiveness of organic farming as compared to conventional farming.

1.9 Bioherbicides

The increasing public demand for eco-friendly products has led to the development of a wide range of environmentally safe alternatives for pest control, including bioherbicides. Derived from plant extracts, phytopathogenic microorganisms, or microbial phytotoxins (i.e., mycoherbicides), bioherbicides are a promising approach to sustainable weed management. These products are typically less persistent, reducing the risk of soil and water contamination, and they have minimal negative impacts on non-target organisms.

As noted by Hasan et al. (2021), the development of bioherbicides began in the mid-1970s with the introduction of mycoherbicides. Since then, numerous bioherbicides have been registered and made available on the global market. The earliest bioherbicide project involved the use of the fungus *Fusarium oxysporum* to control *Opuntia ficus-indica* (L.) Mill. In the 1950s, *Cuscuta* spp. was controlled using *Alternaria cuscutacidae* Rudakov. In the late 1960s, a major program focused on identifying pathogens from *Rumex* spp. in the United States and *Rubus* spp. in Chile for weed control purposes. Over time, both registered and unregistered bioherbicides have seen significant increases in usage.

Despite the advantages of bioherbicides for more sustainable weed control, they present challenges compared to traditional synthetic herbicides, particularly at the field scale. One key issue is their relatively short environmental half-life, which, while beneficial in reducing environmental toxicity, also means that they may not persist long enough to effectively control weeds. Bioherbicides were first introduced to the market in 1980, and since then, a variety of biopesticides, including bioinsecticides, biobactericides, biofungicides, and bionematicides, have been introduced globally. However, bioherbicides still represent less than 10% of the total biopesticide market share (Hasan et al., 2021). The study highlights several types of bioherbicides available worldwide, including those derived from fungal microorganisms, bacterial microorganisms, and plant extracts such as essential oils and allelochemicals.

Plant extracts, traditionally used for medicinal and nutritional purposes, have shown potential as bioherbicides for sustainable weed management in agriculture. These bioherbicides are effective in controlling weeds without harming crops. Compounds like alcohols, fatty acids, phenolics, flavonoids, terpenoids, and steroids act as allelochemicals with herbicidal properties. For example, a phenolic extract from *C. cardunculus* L. exhibits phytotoxicity that damages weed plasma membranes, causing oxidative stress. Similarly, water extracts from *Sorghum bicolor* L. have been shown to reduce biomass in *Echinochloa crus-galli L.*, leading to an 18% increase in rice yield. Extracts from *Brassica nigra* inhibit the germination and growth of weeds like *Avena fatua* L. through high glucosinolate content, which is converted to bioactive compounds like isothiocyanates that damage weed cells. Other plants, such as *Pisum sativum L.* and *Medicago sativa L.,* also exhibit strong phytotoxic effects on weeds like *Polygonum persicaria* L. and *Artemisia vulgaris L*.

Essential oils, derived from plant parts such as leaves, flowers, and roots, contain terpenoids (mainly mono- and sesquiterpenes), which have potent phytotoxic effects. These oils cause leaf chlorosis, growth inhibition, and oxidative damage and have been shown to inhibit the germination and growth of several weed species (Rios, 2016; Raveau et al., 2020; Verdeguer et al., 2012; Lotha et al., 2024). For example, pelargonic acid (n-nonanoic acid), a saturated nine-carbon fatty acid, is found in the essential oil of *Pelargonium* species and can be produced from various plant oils. It has been proven to control a variety of weed species effectively (Loddo et al., 2023). Allelochemicals, natural compounds produced by plants, offer significant potential as bioherbicides compared to synthetic herbicides. These chemicals, which come in various chemical structures, are often water-soluble, non-halogenated, and have shorter half-lives, making them environmentally safe. Allelochemicals can inhibit seed germination and seedling growth in weeds and serve as promising candidates for herbicide development (Dayan and Duke, 2014). Examples of herbicides derived from plant allelochemicals include Cinmethylin, AAL toxins, Mesotrione, and Dicamba (Xiao et al., 2017).

The use of plant-based phytotoxic extracts is increasingly recognized as a valuable tool in integrated weed management. Additionally, by-products from natural sources have shown potential in suppressing weed growth. Acetic acid, produced naturally through anaerobic fermentation (vinegar) or synthesized through various industrial methods, has been identified as a natural herbicide for organic weed control (Webber et al., 2018). Distillers' dried grains with solubles (DDGS), a byproduct of ethanol production, effectively control weeds like *Stellaria media* and *Poa annua* and inhibit *Oxalis corniculata* germination (Boydston, et al., 2008). Corn gluten meal (CGM), a byproduct of corn wet milling, has long been known for its herbicidal properties, showing physiological effects on various weeds (Liu and Christians 1997). Mustard seed meal (MSM), derived from mustard oil pressing, contains glucosinolates that exhibit strong phytotoxic effects on weeds (Boydston et al., 2011). Moreover, Brassicaceae seed meals (BSMs) not only inhibit weed growth but also improve soil nitrogen content, enhancing crop yields (Snyder et al., 2009). Seed meal from *Limnanthes alba* has been shown to increase leaf nitrogen content and boost yield while controlling weeds (Intanon et al., 2015). It is clear that today synthetic herbicides are preferred to bioherbicides mainly for economic reasons which seriously influence the economy of the farms. Synthetic herbicides are able to suppress weeds at lower cost as opposed to sustainable practices generally used in organic farming. In addition, the selectivity of some herbicides against certain specific weeds while not damaging the crops, results in high volume treatment application. The environmental and human health problem caused by chemicals bring us today to make a choice on short term and long term benefits of agriculture model and productivity. The use of bioherbicides is an attractive option for targeting Green Deal goals. Bioherbicides may be an effective component of an overall integrated weed management program, offering several opportunities for weed control at different critical stages: as seeds in the soil, as growing and competing plants and during seed production (Aldrich and Kremer,1997)

2 Aim of the thesis

Climate change refers to long-term alterations in Earth's climate, driven by both natural and human factors, including ozone depletion and the greenhouse effect. Projections indicate that future temperature increases will surpass previous trends, with a rise of 0.1 to 2°C per decade. This warming is expected to have significant impacts on biodiversity, agriculture, and food security, with regional variations influenced by land use, pollution, and ecosystem degradation. Rising temperatures are reducing global crop yields, especially for wheat, rice, soybeans, and maize, while also shortening crop durations, particularly for wheat and rice. This is expected to result in considerable production losses, threatening sustainable food production essential for global food security.

Weeds cause significant agricultural losses, accounting for over 30% of crop productivity losses worldwide, exceeding damage from insects and pathogens. These losses vary regionally, with developing countries suffering higher losses compared to developed nations. For these reasons, herbicides are crucial for managing weeds and increasing crop yields, account for 40-50% of the global pesticide market. The widespread use of herbicides, particularly glyphosate, has grown in regions where intensive agriculture is prevalent. Glyphosate, a widely used non-selective herbicide, works by inhibiting a crucial enzyme in plants, 5-enolpyruvylshikimate-3-phosphate synthase, responsible for the synthesis of an intermediate in the biosynthesis of various amino acids (Komives and Schröder 2016). First synthesized in 1950 and marketed by Monsanto in 1974, glyphosate is effective against more than 100 weed species and remains one of the most used herbicides globally. While herbicides are essential for weed management in conventional agriculture, they present significant environmental and health risks. Herbicide use can lead to contamination of soil, water, and food chains, as well as resistance in weeds (Schreiber et al., 2018; Marin-Morales et al., 2013; Gómez-Ramos et al., 2020; Parven et al. 2024; Deepika et al. 2024; Chaufan et al., 2014; Villanueva et al. 2024).

In response to these challenges, the European Green Deal was launched in December 2019 with the goal of making the EU the world's first carbon-neutral continent by 2050. The European Green Deal emphasizes transforming agriculture to mitigate climate change, preserve crops yield and biodiversity, and reduce use of chemicals in agriculture. Agricultural initiatives, such as the Farm to Fork Strategy, aim to reduce pesticide and fertilizer use, promote organic farming, and decrease the ecological footprint of the food system. By 2030, the EU targets a 50% reduction in pesticide and fertilizer use, with 25% of agricultural land dedicated to organic farming. Agriculture is a significant contributor to environmental degradation, accounting for 25-33% of global greenhouse gas emissions, occupying 40% of Earth’s land, and driving biodiversity loss. Organic farming offers a promising alternative by using natural inputs instead of synthetic chemicals, providing benefits such as improved soil fertility, better water efficiency, and higher biodiversity. However, organic farming requires more land and has higher management costs, particularly for weed control. Bioherbicides, derived from plant extracts and microorganisms, offer a more sustainable alternative to synthetic herbicides. These natural substances, such as organic acids and essential oils, are less persistent and have a minimal environmental impact. While scaling bioherbicides remains challenging, they show potential as a solution for sustainable farming practices and could contribute to the goals of the EU Green Deal.

The aim of the study was to evaluate the potential effects of different natural compounds in weed management and cover crops termination.

In particular the specific aim was to assess the efficacy of acetic acid, lactic acid, pelargonic acid, citric acid and acetic acid mixed with essential oils, at different doses, against weed and crop species of agronomic interest for the European cropping systems. To this aim different trials were conducted under controlled environmental (Experiment 1,2 and 3) and semi-field conditions (Experiment 4).

The outputs of the study will be important to emphasize the potential of natural compounds use in agriculture in order to develop more sustainable agricultural practices and to achieve European Green Deal goals.

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Chapter I - Effect of different natural compound on seed germination

Mattia Alpi[1], Elettra Frassineti [1], Camilla Tibaldi[1], Eros D'Amen[1], Giovanni Dinelli[1], Ilaria Marotti[1*]

1.Department of Agriculture and Food Science and Technologies, University of Bologna

* Corresponding author: ilaria.marotti@unibo.it

ABSTRACT

Seed banks in cultivated soils pose a persistent challenge to agriculture by sustaining invasive weed infestations, which reduce crop yields and increase production costs. Effective weed management requires understanding the weed seed bank's composition and dynamics, influenced by cropping systems and herbicide use. While synthetic herbicides dominate due to their efficacy and cost-effectiveness, their overuse has led to environmental contamination, human health concerns, and herbicide-resistant weeds. This has prompted the search for sustainable alternatives, particularly in the context of the European Green Deal's goals to reduce chemical inputs and expand organic farming.

Organic weed management employs cultural practices, crop rotation, and mechanical methods, often supplemented by organic herbicides such as acetic acid, pelargonic acid, and essential oils. These natural compounds are biodegradable and environmentally friendly but face limitations, including higher costs and slower action compared to synthetic herbicides. This study evaluated the efficacy of organic acids and essential oils as pre-emergent herbicides, focusing on their effects on seed germination and early seedling growth. Acetic acid and pelargonic acid demonstrated significant herbicidal activity, with selective effects on weed species compared to crop species like *Triticum aestivum* L. Essential oils, however, reduced the efficacy of acetic acid at all tested doses.

The results highlight the potential of organic acids, particularly due to their lower production costs as natural byproducts, to offer a sustainable alternative to synthetic herbicides. Further research is needed to optimize application timing and assess selectivity across weed and crop species. These findings contribute to the development of cost-effective and environmentally

sustainable weed management strategies, bridging the gap between organic and conventional farming practices.

4 INTRODUCTION

Seed banks in cultivated soils can pose significant challenges to agricultural activities by ensuring the persistence of invasive plant infestations over long periods, even when new seeds are prevented from entering the area. This results in reduced crop yields and higher production costs (Webber et al., 2024). Understanding the weed seed bank, which serves as a reservoir for future annual weed infestations, is essential for improving weed management strategies (Gallandt, 2006). The composition and density of seed bank species reflect the historical success or failure of cropping systems in preventing weed seed production or promoting seed predation and decay (Buhler et al., 1997). Modern pre-emergent herbicides, with long-lasting residual effects in the soil, can disrupt the dynamics of seed entry and exit by controlling germination. This, in turn, affects the species composition and abundance of weeds over the medium to long term (Amim et al., 2016). In pre-emergence chemical weed management, herbicides with the most persistent effects are generally chosen.

However, improper use of synthetic herbicides—such as excessive application or the repeated use of the same herbicide—can lead to environmental contamination (soil, water, and air) (Morales et al., 2013; Velki et al., 2019), adverse human health effects (Huovinen et al., 2015), the development of weed resistance, herbicide residues in plants (Soltys et al., 2013), and negative impacts on non-target organisms (Filimon et al., 2021). The active ingredients in synthetic herbicides are often difficult to degrade in the soil, and their chemical structures are not environmentally friendly. For instance, diuron can remain in the soil for up to 60 days (Muhamad et al., 2013; Soltys et al., 2013).

To reduce chemical use in agriculture, as outlined in the Farm to Fork strategy, there is an increasing need for new solutions in weed management, especially in organic farming. Organic farmers typically rely on cropping systems that combine tillage with cultural practices, such as crop rotation, the use of competitive crop varieties, cover crops, and reduced row spacing to minimize weed competition and prevent infestations (Liebman & Davis, 2009). In organic weed management, practices like mechanical tillage, physical weed control, crop fertilization strategies, crop rotation, intercropping, irrigation, and cover crops are used together, with herbicides rarely involved (Bàrberi, P. 2001). While these methods may be less effective than chemical herbicides, they can be combined to suppress weeds and reduce seed banks over time (Gallandt, 2014).

Although research on the effectiveness of organic herbicides in suppressing weed populations is limited, the literature often highlights a holistic agroecosystem approach to weed management in organic agriculture. Currently, commercially available organic herbicides are mainly marketed for residential and small-scale operations (DeNuxet al., 2024).

Bioherbicides present an alternative to synthetic herbicides for weed control. These biological agents target key plant processes such as metabolism, photosynthesis, and growth hormone production (Triolet et al., 2020). Bioherbicides offer targeted, environmentally friendly weed control with fewer risks to non-target organisms and ecosystems. Their reduced environmental impact makes them suitable for sensitive areas like organic farms, gardens, parks, and water bodies. Bioherbicides also have shorter environmental persistence and are biodegradable, minimizing soil, water, and air pollution. However, they may act more slowly and require multiple applications, unlike chemical herbicides (Islam, et al., 2024).

Organic herbicides, typically non-selective, use a range of natural active ingredients in their formulations. These include natural chemicals like organic acids, essential oils, and allelopathic compounds, as well as natural pest control agents such as insects, nematodes, or predatory animals (DeNuxet al., 2024). Several products are available for domestic use, such as organic acids (acetic acid and pelargonic acid) and various essential oils, which work by destroying plant tissue through direct contact. The acids penetrate cellular membranes, causing leakage and the breakdown of membrane lipids (peroxidative activity), facilitated by singlet oxygen produced when sunlight interacts with displaced chlorophyll in the thylakoid membranes of chloroplasts. The resulting peroxidation causes necrosis and rapid drying of weeds, driven by potent oxidative radicals (Ciriminna, et al., 2019).

The goal of this study was to evaluate the effectiveness of different natural compounds (organic acids and essential oils) on seed germination, identifying the optimal concentrations and application volumes. The objective was to develop a pre-emergent herbicide for weed control in the soil seed bank, providing a sustainable alternative to chemical herbicides with a reduced environmental impact.

5 Materials and Methods

5.1 Plant material

Seeds of weeds and crops were evaluated. Seeds from weed species such as *Lolium perenne* L., *Setaria viridis* L. and *Cichorium intybus* L. were obtained from the germplasm collection available at the Seed Research and Test Laboratory (LaRAS) of the Department of Agricultural and Food Sciences, University of Bologna.

Crop seeds such as *Triticum aestivum* L., *Panicum miliaceum* L., *Vicia sativa* L. were purchased from Arcoiris (Modena, Italy), whereas seeds of *Medicago sativa* L. were obtained from LaRAS.

5.2 Experimental design

To investigate the herbicide effects of different natural compounds on seed germination, two different trials were set up. Both experiments were conducted in growing chambers at the Department of Agricultural and Food Sciences (DISTAL-University of Bologna) and were designed as a completely randomized trial with 3 replications.

Treatments (acetic acid, pelargonic acid, citric acid, lactic acid), except essential oils (eugenol, geraniol), were sprayed at the corresponding volume of 1000 L/ha at four different compound concentrations 0%, 5% (v/v), 10% (v/v) and 20% (v/v), except for citric acid which had 0%, 5% (w/v), 10% (w/v) and 20% (w/v). Conversely, essential oils were added in the formulations as adjuvants and mixed at the concentration of 1.5% (v/v) to acetic acid at 5% (v/v), 10% (v/v) and 20% (v/v).

Treatments were prepared adding distilled water to a.i obtain final solutions at concentrations of 0%, 5% (v/v), 10% (v/v) and 20% (v/v), except for citric acid which had 0%, 5% (w/v), 10% (w/v) and 20% (w/v), respectively. For each treatment a control with tap water was included in the experimental layout.

Acetic acid was provided formulated at 20% by the company Flortis (Italy), whereas pelargonic acid, citric acid, lactic acid, eugenol and geraniol were purchased from Merck (Germany).

The effect of treatments on seed germinability of weeds and crops sown in both agar substrate (first trial) and soil-like substrate (second trial) was evaluated.

In the first trial the germination tests were performed in 90-mm diameter glass petri dishes with 15 ml of 1.5% (w/v) agar (Merck, Germany).

Seeds were soaked in tap water and distributed on Petri plates. For each replicate, 20 seeds for each plant species were sown on top of the substrate at day 1 (t0). Treatments were applied by spraying onto the plate with a corresponding volume of 1000 L/ha in order to wet all the seeds. Petri plates were randomly placed in the growth chamber set at 20°C with 16 h light/8 h dark photoperiod. After 7 days (t1) germinated seeds were counted and treatments efficacy was evaluated.

In the second trial seeds were sown in pots of 6 cm diameter and covered with a commercial peat/sand substrate (VigorPlant, Tappeti erbosi, Lodi, Italy). Treatments were sprayed at day 1 (t0) at the volume of 1000 L/ha directly on the surface of the soil. Pots were incubated at 20°C in growth chamber and 16/8 h photoperiod. After 10 days (t1) germinated seeds were counted, and efficacy of the treatments was evaluated as number of germinated seeds in treated replicates over germinated seeds in control replicates. Each seed was deemed 'germinated' once the tegument was torn by the growth of the apical tip (Bewley, 1997).

5.3 Statistical analysis

Statistical analyses were conducted using the Statistica 6.0 software (2001, StatSoft, Tulsa, OK, USA). Two-way analysis of variance (ANOVA) in conjunction with Tukey's honest significant difference was performed. Significant differences between means were determined by least significant difference values for $p < 0.05$. Pearson's correlation coefficient (r) was calculated at significance level of $p < 0.01$.

6 Results and discussion

In order to evaluate the interaction between factors (species, treatments and concentration) 3-way Anova analysis was established, and the interaction between factors for $p < 0{,}001$ was significatively high. For this reason, results were reported in function of the different concentration of p.a. (5, 10 and 20%), assuming that higher concentrations lead to higher efficacy. For this reason, results were evaluated as control-based percentage, to evaluate which p.a. the higher activity at each concentration.

Concerning 5% concentration (fig. 5), citric acid and lactic acid showed higher germination inhibition control for all the analyzed weed and cover crops species. *Triticum aestivum* L.

germinability was mildly influenced by citric and lactic acid treatments. The effect of pelargonic acid treatment was slightly higher for weeds than for cover crops and wheat seeds.

Acetic acid and pelargonic acid resulted in lower control efficacy at 5% concentration compared to citric and lactic acid. Acetic acid treatment obtained higher effect on cover crop species than on weeds. This effect was probably due to the higher acidity of acetic acid in front of other acids (pelargonic acid has the lower acidity), as previously reported acids 4% treatments stimulate seed imbibition due to positive effect of acids on teguments (Manyi F. et al., 2024)). Additionally, the positive effect of acetic acid 5% treatment on germinability was also linked to specie-specific tegument characteristics and dormancy mechanisms. Otherwise, the addition of essential oil to acetic acid 5% reduced the efficacy of acetic acid, especially for some weeds (*Cichorium intybus* L. and *Lolium perenne* L.) and cover crops species (*Medicago sativa* L.).

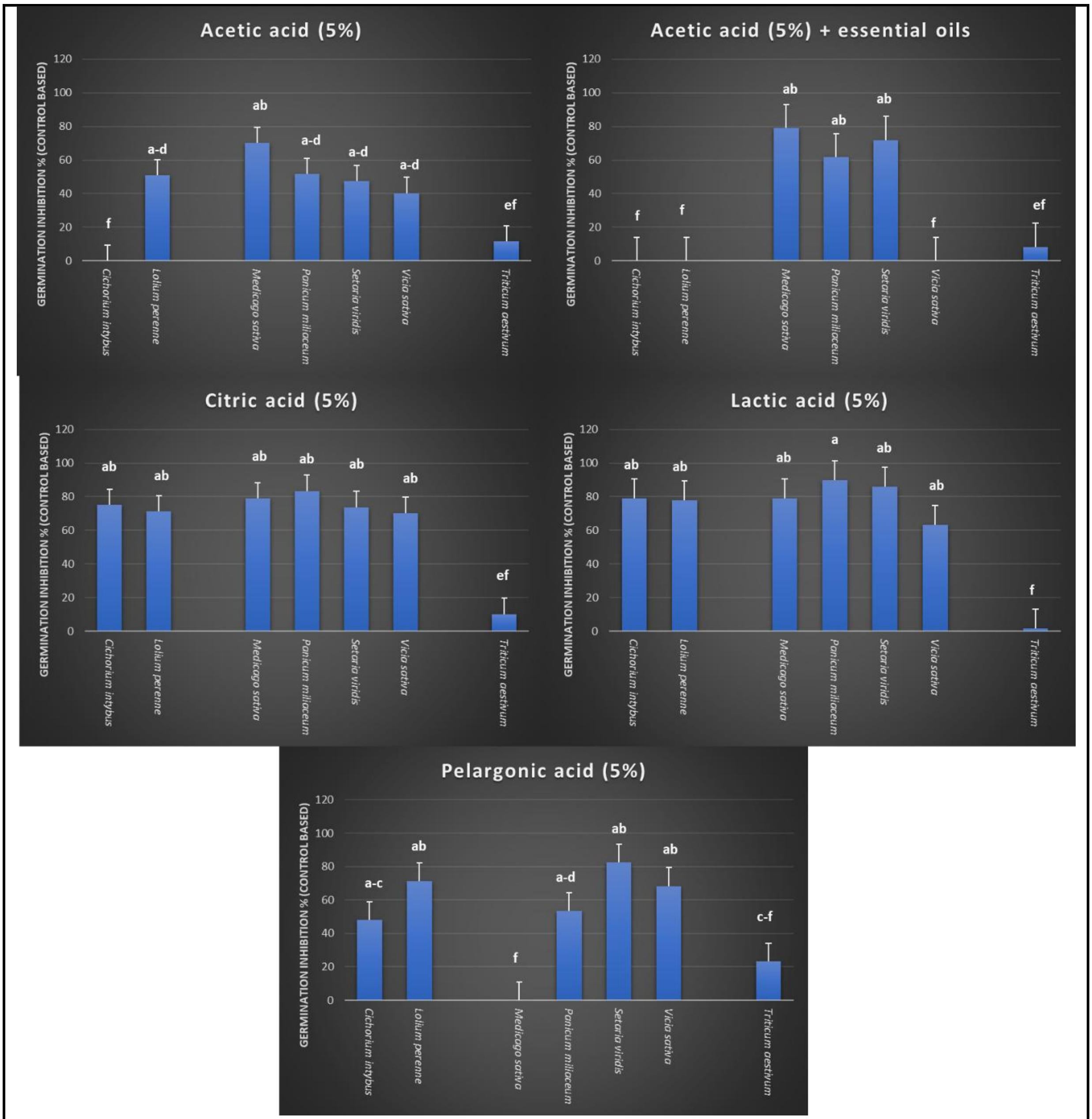

Fig. 5: Germination inhibition in % (control based) for different treatments

Similar scenarios were observed for 10% concentration (fig. 5). Citric acid and lactic acid had similar activity to 5% concentration results. Citric acid affected negatively germinability for evaluated weeds and cover crops, and the same trend was observed also for lactic acid treatment. Otherwise, for both lactic and citric acid treatments a negative control effect was obtained in *Triticum aestivum* L. seeds, that resulted in higher germinability, as previously observed in 5% treatments. Acetic acid and pelargonic acid at 10% affected weeds and cover crops germinability more compared to lower percentage treatments. The major effects of acetic acid were observed mainly for *Cycorium intybus* L., *Setaria viridis* L. and *Medicago sativa* L.; on the other hand, pelargonic acid had a high impact on germinability for all species except for *Cycorium intybus* L. At 10% concentration the activity of the treatments showed a more similar trend compared to 5% concentration for cover crops and weeds. Otherwise, acetic acid and pelargonic acid impacted *Triticum aestivum* L. germinability, notably the highest activity was observed for pelargonic acid. This could be explained certainly by the higher activity of acetic acid and pelargonic acid at 10% concentration or higher. As for the 5% concentration, adding essential oils to acetic acid lowered the effect of treatment activity on seed germination inhibition for all the species, except for *Cichorium intybus* L.

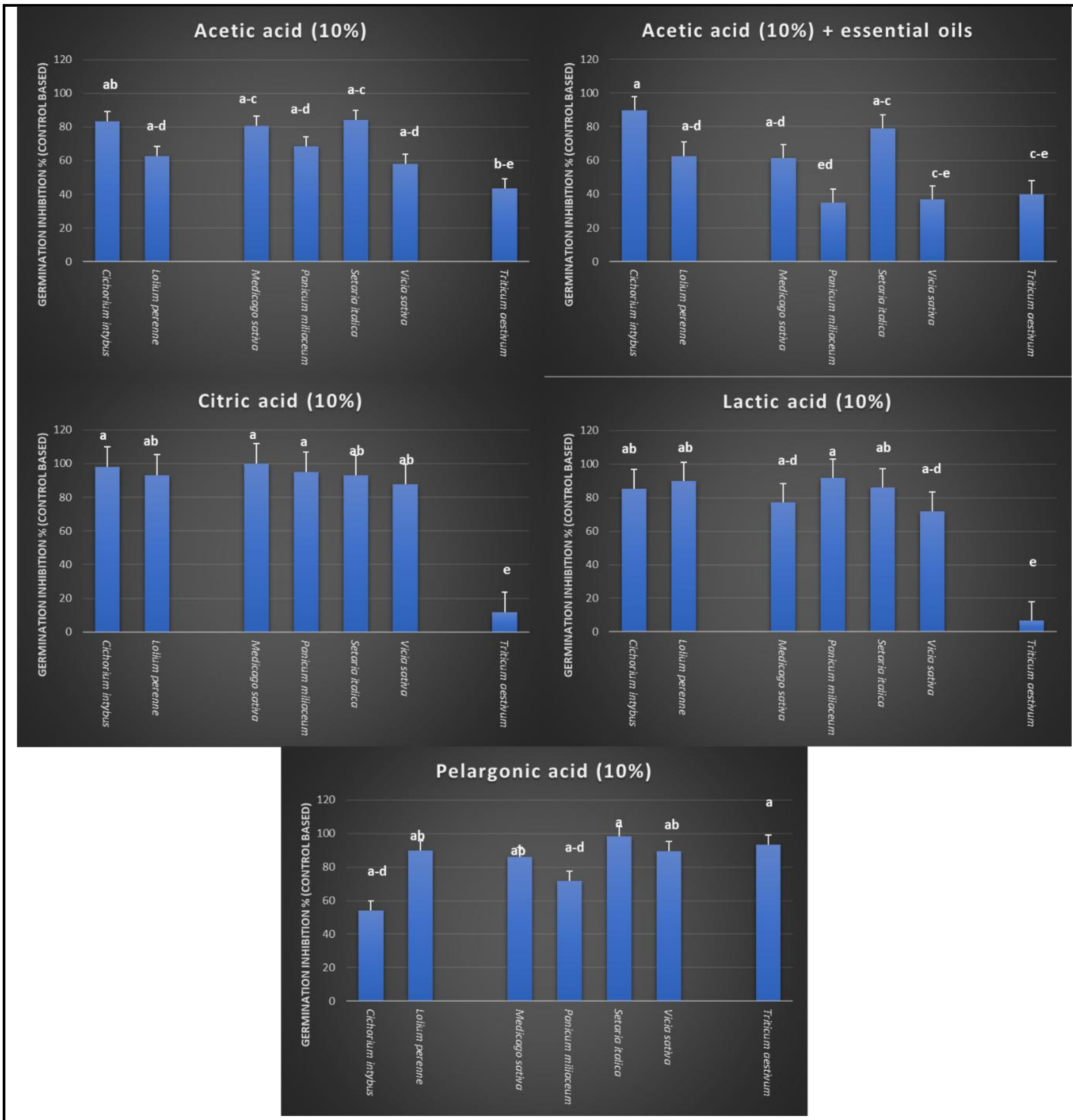

Fig. 6: Germination inhibition in % (control based) for different treatments

Different results were shows for 20% concentration activity (fig. 6). It can be observed that all the treatments reached higher activity (above 90% efficacy) compared to lower concentrations.

In general, all the treatments had significantly high seed germination inhibition activity for weed and cover crop species with 20% dose. Likewise, for essential oils added to acetic acid at 20% concentration a similar activity to other treatments was observed, except for *Medicago sativa* L seeds. Concerning the effect on *Triticum aestivum* L. seeds, citric and lactic acid treatments did not impact seed germinability even at higher concentration, otherwise acetic and pelargonic acid had at higher concentration negatively impacted cover crops, weeds as well as wheat germinability. Specifically, in wheat (*Triticum aestivum* L.), exposure to acetic acid has been observed to influence early growth stages. Seedlings grown containing acetic acid exhibited a more rapid formation of the first stem tiller compared to those grown without acetic acid (Madruga de Tunes et al. 2011). Studies on the impact of acetic acid on crop seeds as carrot and maize were previously reported. Carrot seeds treated with 5% acetic acid improved germination rates, while a 20% concentration significantly decreased germination (Othmen et al. 2019). Similarly, pre-planting applications of 10% and 20% acetic acid did not inhibit maize seed germination but reduced shoot growth (Pujisiswnto et al. 2015). In Petri germinability trials, the effect of citric and lactic acid seemed to be partially linked to the concentration of the active ingredient, and it did not affect wheat germinability; on the other hand the effect of pelargonic acid and acetic acid was strongly related to their concentration in the solution, and it had no selectivity.

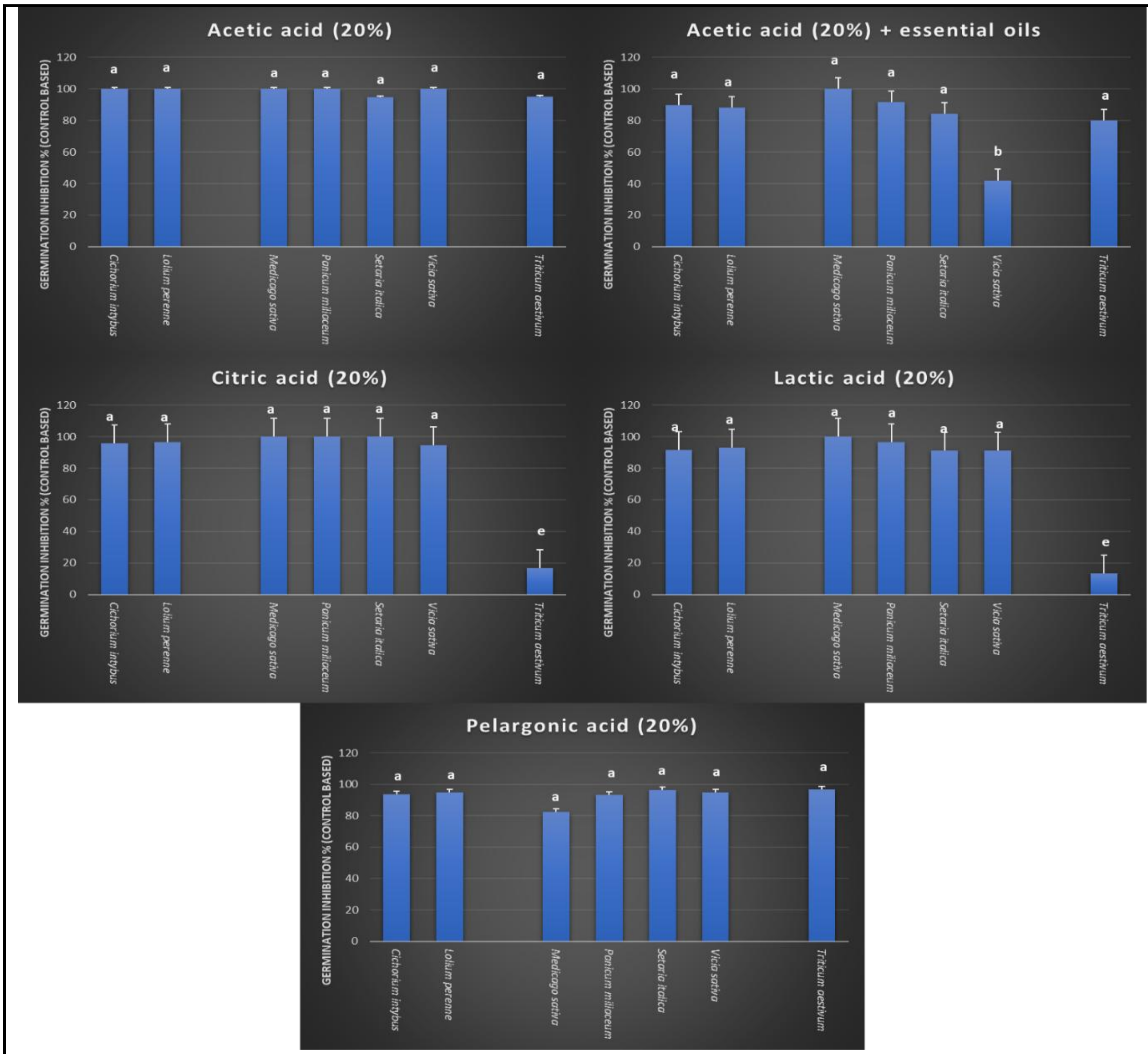

Fig. 7: Germination inhibition in % (control based) for different treatments

The following table (Tab. 1) shows the values of I50 that correspond to the doses (concentration) able to reduce the seeds germination of 50%. Interaction between factors resulted highly significant, for this reason data were shown as interaction. I50 results demonstrated what previously reported for different concentrations analysis in Fig. 5; 6; 7, concerning the lower activity on *Triticum aestivum* L. In more details, the analysis showed that 34% concentration might be necessary to reduce seeds germination of *Triticum aestivum* L. by the 50%. In particular, for citric and lactic acid a concentration of 71.8% might be necessary to obtain a significant reduction in wheat germinability. Similar results were obtained for weed and cover crop species, ranging between 3.8% and 8.7% concentration to reach the 50% seed germination inhibition. In more details, pelargonic acid had higher activity (5.8%), followed by acetic acid (7.1%), acetic acid + essential oils (10.5%) citric acid (13.3%) and lactic acid

(13.4%). It should be noticed that citric acid and lactic acid results were highly influenced by their low activity on *Triticum aestivum* L., instead similar results to pelargonic acid and acetic acid were found for weed and cover crop species.

Tab. 1: I50 results as interaction between species and treatments

	Acetic acid	Citric acid	Lactic acid	Aceti acid + essential oils	Pelargonic acid	
Cichorium intybus	8.1 (bc)	3.6 (c)	3.7 (c)	8.4 (bc)	8.3 (bc)	**6.4 (b)**
Lolium perenne	6.6 (c)	3.5 (c)	3.5 (c)	12.2 (c)	3.8 (c)	**5.9 (b)**
Medicago sativa	4.5 (c)	4.2 (c)	4.2 (c)	4.8 (c)	10.3 (bc)	**5.4 (b)**
Panicum miliaceum	6.2 (c)	3.1 (c)	3.1 (c)	8.2 (bc)	7.2 (bc)	**5.6 (b)**
Setaria viridis	5.1 (bc)	3.4 (c)	3.4 (c)	4.1 (c)	3.2 (c)	**3.8 (b)**
Vicia sativa	7.9 (bc)	5.0 (c)	5.0 (c)	22.5 (b)	3.9 (c)	**8.7 (b)**
Triticum aestivum	11.5 (bc)	71.8 (a)	71.8 (a)	13.2 (bc)	4.1 (c)	**34.9 (a)**
	7.1 (ab)	**13.3 (a)**	**13.4 (a)**	**10.5 (ab)**	**5.8 (b)**	

It should be considered that seeds were imbibed during the experiment allowing the start of the primary phases of the germination process and the radicle emission, but it was noticed that organic acid treatments instantly burn the radicle. This might lead to a misunderstanding in efficacy data interpretation for different organic acids. In addition, in Petri dishes trials the seeds were directly exposed to the treatments, conversely to field conditions, where soil does not allow the direct action of the treatment on the seed. For these reasons a second trial was set up in the growth chambers of the University of Bologna, where seeds of the different species were sown in commercial substrate, in order to better reproduce field conditions. In this case, sprout emergence reduction activity of the different treatments was evaluated.

For what concerns the second trial in phytotron, third level interaction between factors resulted highly significant for $p < 0,001$. For this reason, results were reported as a function of the different concentration of a.i (5, 10 and 20%), as it can be assumed that their higher concentrations lead to higher efficacy. Results were also shown as control-based percentage.

Data reported lower activity of all products compared to germination trial in Petri dishes at 5% (fig. 8), 10% (fig. 9) and 20% concentration (fig. 10), probably due to the direct exposure of the seeds to the treatments in petri trials. A similar trend was observed in the activity of compounds related to their concentration in this trial, compared to the petri tests. As expected, higher concentrations lead to higher inhibition activity on sprouts emergence. At 5% concentration (fig. 8) different efficacy was observed between species and treatments. Acetic acid at all the concentrations had higher activity on *Cichorium intybus* L. Further, *Lolium perenne* L. was particularly affected by citric acid and lactic acid at 5%, 10% and 20% concentration. Citric acid and lactic acid had similar efficacy on *Triticum aestivum* L. as previously observed in the first trial. Very little information is available in literature about plant tolerance to high lactic acid concentration, but it was observed that leguminous seeds exposure to 2 to 6% lactic acid concentration affects shoots length and fresh weight of the plant (Sumalapao et al. 2018). In the present study, an evident effect of lactic acid on *Medicago sativa* L. shoots was not observed. Otherwise, acetic acid, acetic acid + essential oils and pelargonic acid demonstrated lower activity on *Triticum aestivum* L. in the second trial compared to the previous one. In more detail, *Triticum aestivum* L. was affected only by acetic acid, with and without essential oil, at 10% (fig. 9) and 20% concentration (fig. 10). At 20% concentration, acetic acid and acetic acid + essential oils resulted in higher efficacy on weed and cover crop sprout emergence compared to previous analysis, except for *Lolium perenne* L. case study where acetic acid with and without essential oil, showed lower activity. In summary, acetic acid at varying concentrations has demonstrated potential as a natural herbicide, particularly effective against young, small weeds. However, its efficacy diminishes with larger weeds, and higher concentrations may adversely affect crop seed germination and growth (Pujisiswnto et al. 2015; Othmen et al. 2019). It's important to note that while acetic acid can act as an herbicide, its non-selective nature means it may also affect non-target plants, including crops like wheat. Therefore, caution is advised when considering its use in agricultural settings to avoid unintended damage to desired plants.

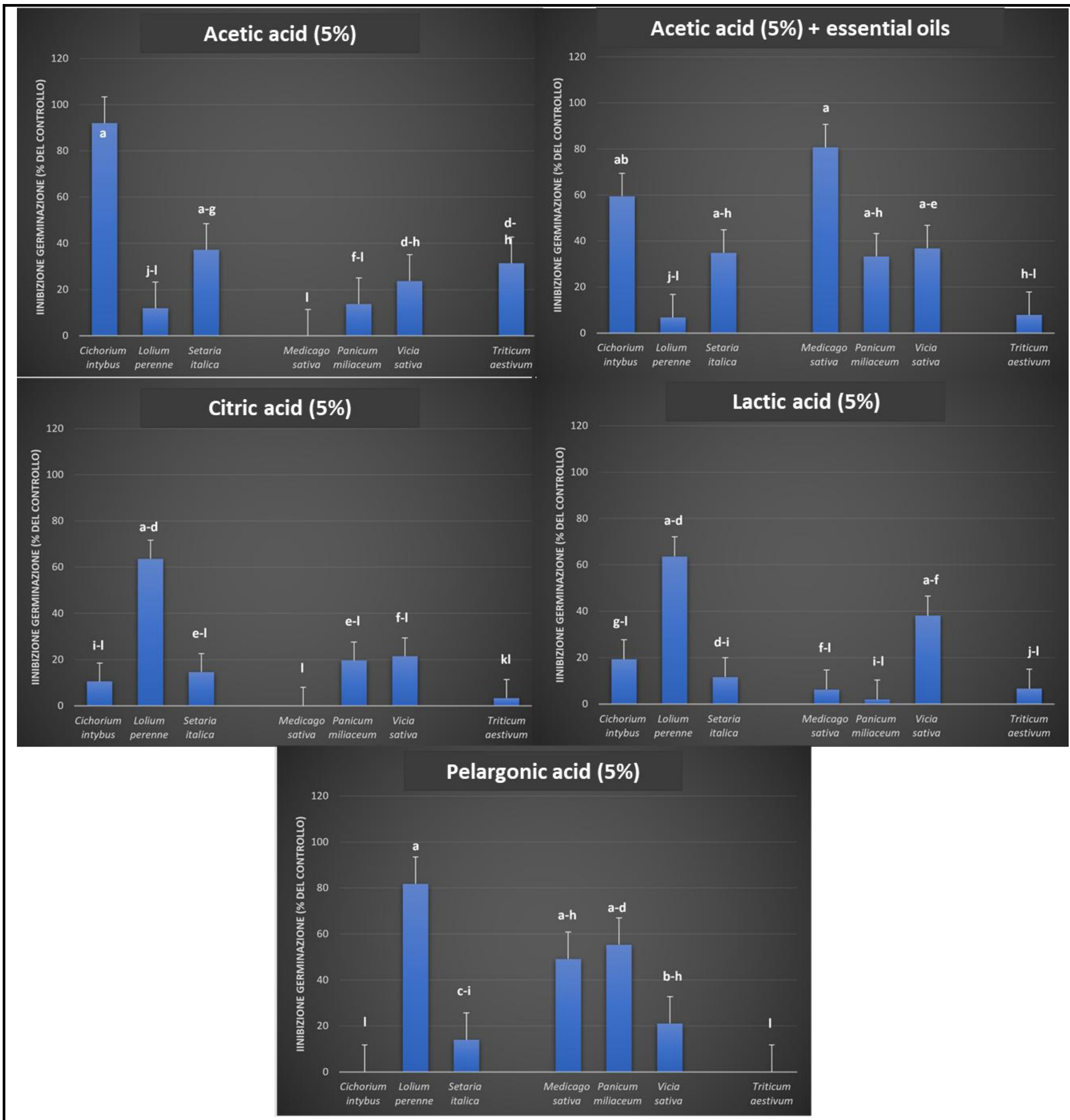

Fig. 8: Sprout emergence inhibition in % (control based) for different treatments

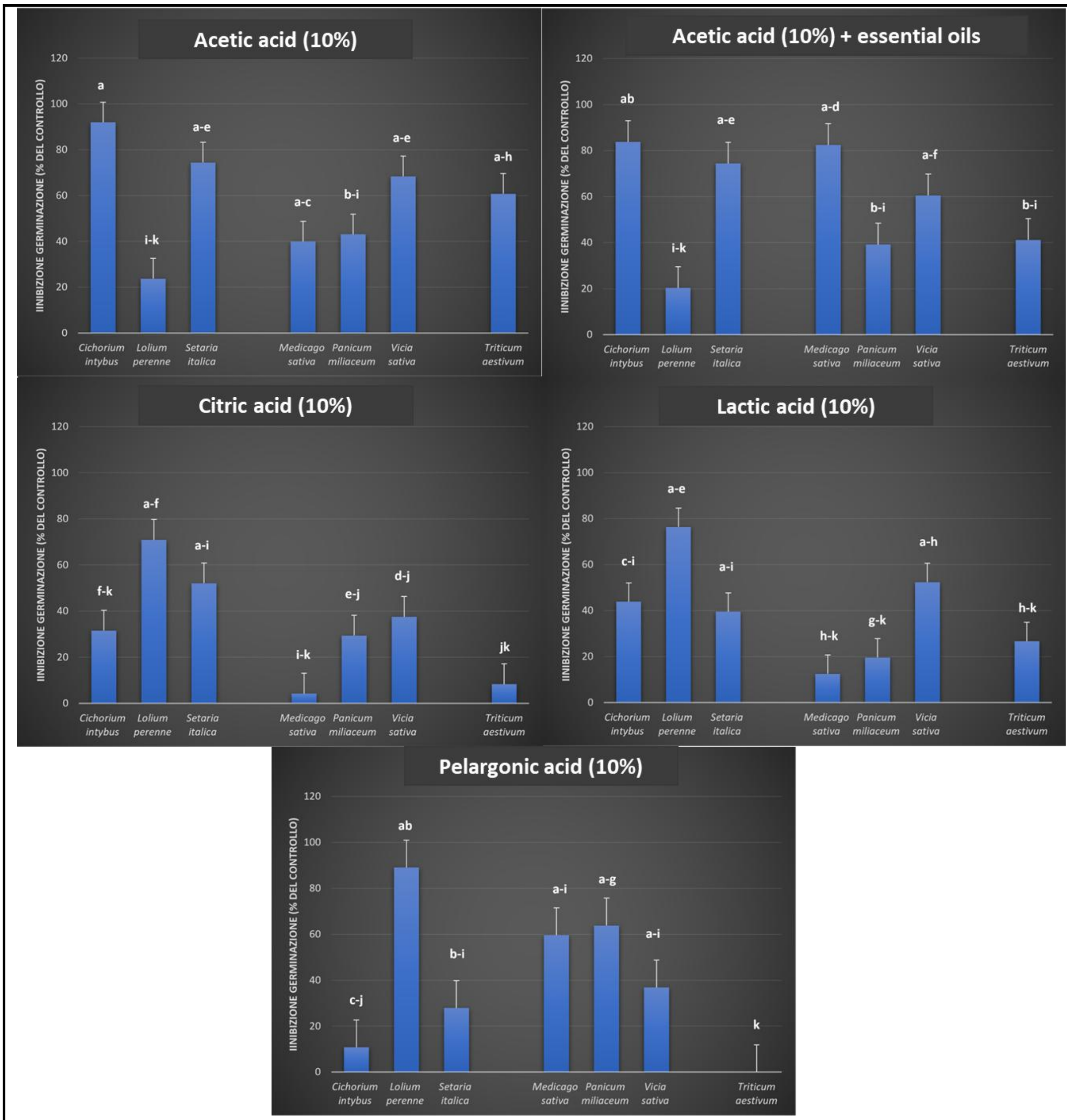

Fig. 9: Sprout emergence inhibition in % (control based) for different treatments

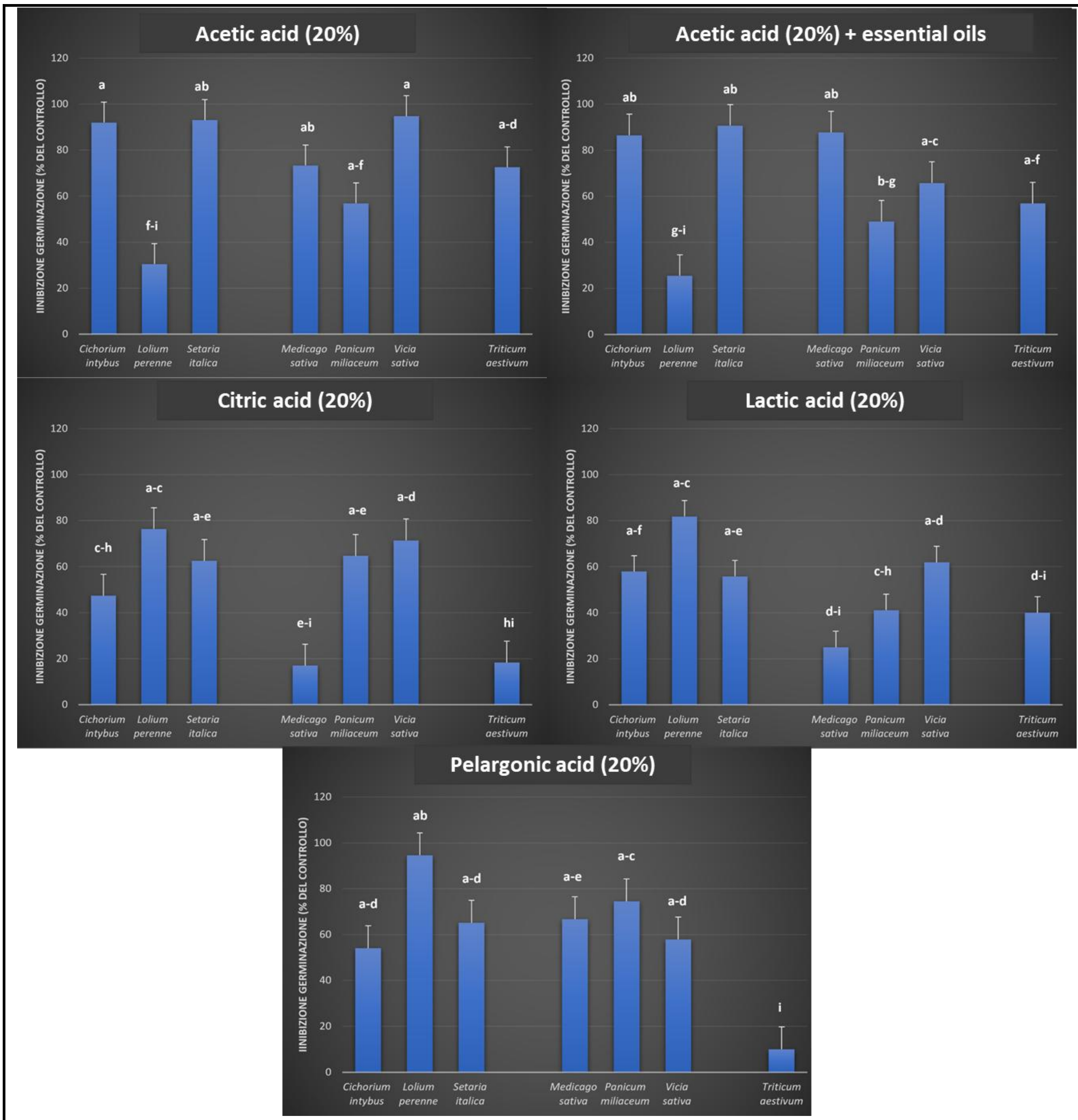

Fig. 10: Sprout emergence inhibition in % (control based) for different treatments

I50 values, that correspond to the doses (concentration) able to reduce the sprout emergence of the 50%, are reported in the following table (Tab. 2). Interaction between factors resulted highly significamt, for this reason data were shown as interaction. The reduced damage on *Triticum aestivum* L. was observed for all the treatments (tab. 2), resulting in a 43% concentration necessary to lower the sprout emergence of the 50%. For other treatments similar values of I50 compared to previous germination trials were observed, with 10,6% values for *Setaria viridis* L. and *Vicia sativa* L., and 24.5% for *Medicago sativa* L. *Medicago sativa* L. is primary used as crop, similarly to *Triticum aestivum* L. Thus, it could be assumed that treatments had higher activity on weeds and cover crops species, compared to crop species (*Medicago sativa* L. and

Triticum aestivum L.). Concerning the difference between each treatment (tab. 2), acetic acid and acetic acid + essential oils resulted in higher inhibition of sprout emergence, compared to citric acid, lactic acid and pelargonic acid.

Tab. 2: I50 results as interaction between species and treatments

	Acetic acid	**Citric acid**	**Lactic acid**	**Acetic acid + essential oils**	**Pelargonic acid**	
Cichorium intybus	3.7 (f)	19.3 (d-f)	4.6 (f)	14.4 (d-f)	13.1 (d-f)	**11.0 (c)**
Lolium perenne	35.9 (c-e)	5.1 (f)	4.7 (f)	38.1 (cd)	3.4 (f)	**17.4 (c)**
Medicago sativa	16.1 (d-f)	28.5 (c-f)	44.1 (c)	14.9 (d-f)	19.1 (d-f)	**24.5 (b)**
Panicum miliaceum	6.4 (f)	12.8 (d-f)	6.9 (f)	15.5 (d-f)	11.5 (ef)	**16.4 (c)**
Setaria viridis	5.1 (d-f)	3.4 (d-f)	3.4 (c-f)	4.1 (d-f)	3.2 (f)	**10.6 (c)**
Vicia sativa	8.3(f)	14.3 (d-f)	8.8 (f)	7.6 (f)	14.3 (d-f)	**10.6 (c)**
Triticum aestivum	10.1 (ef)	62.5 (b)	26.5 (c-f)	15.8 (d-f)	100.0 (a)	**43.0 (a)**
	13.9 (b)	**22.9 (a)**	**19.8 (ab)**	**14.9 (b)**	**23.8 (a)**	

7 Conclusion

Synthetic chemical herbicides have played a crucial role in modern weed management, providing significant economic benefits. However, growing concerns about their toxic effects on the environment and human health, along with the development of herbicide-resistant weeds, have led to stricter regulations regarding herbicide registration and usage. As a result, the European Green Deal sets ambitious goals for the coming decades, including reducing chemical usage and increasing organic farming. This has driven the need to find non-synthetic alternatives for weed control, and considerable research efforts have been dedicated to evaluating natural products, such as organic acids and essential oils, as potential solutions for weed management. Despite ongoing scientific research, only a few natural products have successfully reached the market for professional use within the EU. Acetic acid and pelargonic acid are likely among the most successful examples, with several commercial products registered for use on various crops and urban areas. Recent studies also indicate growing interest in the effectiveness of essential oils for weed management (Lotha et al., 2024; Nikolova et al., 2021; Somala et al., 2023; Moura et al., 2025; Awojide et al., 2022; Ben Kaab et al., 2024; Souihi et al 2024; Benvenuti et al., 2017). However, the high cost of pelargonic acid, synthetically produced acetic acid, and essential oils currently limits their widespread adoption. Therefore, testing the efficacy of reduced doses at different plant growth stages and evaluating their impact on both cover crops and main crops is of great interest to establish specific usage guidelines and application periods. In this study, essential oils results show to reduce the efficacy of acetic acid at all the doses, in contrast to what was expected. Otherwise, good herbicidal efficacy has been obtained with organic acids, especially acetic acid and pelargonic acid on seeds germination and sprouts emergence of the tested species, but with large inter-specific differences of sensitivity have been showed when used under controlled environment conditions. This underlines a certain higher selectivity of some compounds for weed species in front of crops species. For example, acetic acid without essential oil shows higher injury on weed seeds in front of *Triticum aestivum* L. seeds and sprouts. Citric acid and lactic acid show the lower activity in front of *Triticum aestivum* L. seeds and sprouts. These results could open to a large potential of practical use for natural compounds. In conclusion, is important to better evaluate the potential and the selectivity of the different compounds on different weed and crop species at different growth stage to understand the best application timing to amplify the tools of organic like weed management. In addition, organic acids show higher potential due to the lower cost of production. In fact, acetic acid and lactic acid can be naturally produced, and founded as waste or byproducts, reducing the economic input for weed management. As mentioned, chemicals are still preferred today for their high efficacy, selectivity and in particular for the low price. Weed management in organic farming can reach the same results of weed control in conventional farming but at higher cost due to the lack of chemicals. The potential of the reduced price for some of the tested organic acids shows the potential of natural compounds to be implemented as a more sustainable tool for weed management.

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Chapter II – Evaluation of herbicide efficacy of different natural compounds for weed management and cover crops termination under greenhouse conditions

Mattia Alpi[1], Elettra Frassineti [1], Enrico Toschi[1], Eros D'amen[1], Giovanni Dinelli[1], Ilaria Marotti[1*]

1.Department of Agricoltural and Food Science and Technologies, University of Bologna

* Corresponding author: Ilaria.marotti@unibo.it

ABSTRACT

Weed management is a critical aspect of modern agriculture to mitigate crop yield losses caused by crop-weed interference. Herbicides, particularly chemical-based solutions, have traditionally been indispensable for efficient weed control, reducing labor costs and increasing agricultural productivity. However, concerns regarding herbicide resistance, environmental contamination, and human health risks have spurred interest in sustainable alternatives, including bioherbicides derived from natural compounds. This study evaluated the effectiveness of various natural compounds, including organic acids (acetic, citric, lactic, and pelargonic acids) and essential oils, in controlling weeds, cover crops, and crops at three concentrations (5%, 10%, and 20%). The results demonstrated that all tested species were effectively controlled by these compounds, with efficacy influenced by concentration and compound type. Acetic acid combined with essential oils showed synergistic effects, achieving higher green cover area reductions than acetic acid alone. Pelargonic acid emerged as the most effective treatment, achieving fresh biomass reductions of up to 77.6% at 10% concentration. I50 values indicated that concentrations between 8.2% and 15.4% were sufficient to achieve a 50% reduction in green cover area across species. Differences in treatment efficacy were linked to species morphology and phenological stage, with dicotyledons generally more responsive than monocotyledons due to greater leaf surface area exposure.

These findings highlight the potential of natural compounds as broad-spectrum, low-concentration alternatives for weed and cover crop management. Despite their higher cost relative to synthetic herbicides, these bioherbicides align with sustainable agriculture goals and EU Green Deal targets, offering a viable pathway to reduce dependence on conventional chemical herbicides while minimizing environmental and health impacts. Further research is recommended to optimize application strategies and address cost barriers.

9 Introduction

The use of herbicides in agriculture is a long-established practice for controlling weeds, which compete with crops for vital resources such as water, light, and nutrients, ultimately reducing both yield and crop quality. Weeds are a major cause of crop yield loss due to crop-weed interference, making effective weed control a crucial component of agricultural production. Chemical herbicides are designed to destroy or suppress weed growth and are essential in modern farming. Herbicides reduce the need for hand labor or mechanization, which results in higher costs for farmers. The economic losses faced by European Union (EU) farmers in the absence of herbicides for key crops such as wheat (*Triticum aestivum* L.), potatoes (*Solanum tuberosum* L.), and grapes (*Vitis vinifera* L.) are estimated at 24 billion kg (worth €10.5 billion), 10.4 billion kg (worth €2 billion), and 4.7 billion kg (worth €4.2 billion), respectively (Wynn and Webb, 2022).

Herbicides are also essential for terminating cover crops, a crucial factor in cover crop cultivation. Cover crops have gained importance in sustainable agricultural systems due to their numerous benefits, particularly in weed management. These benefits include competition with weeds for light, nutrients, and resources, reduced soil erosion, and improved nutrient cycling, water quality, and soil conservation (Reicosky et al., 1998; Teasdale et al., 2007; Edward et al., 2005; Cornelius et al., 2017; Yeo et al., 2014). The effectiveness of cover crops depends on the species (grasses, legumes, and brassicas) and the biomass produced. However, growing cover crops presents challenges, such as difficulty in effectively terminating them at later growth stages, which can hinder the emergence and yield of cash crops (Cornelius et al., 2017). Poor termination can also affect the growth and vigor of cash crops, especially early in the season, due to the release of allelochemicals and the depletion of soil moisture and nutrients. For instance, incomplete termination of cereal rye (*Secale cereale* L.) and a cereal rye-legume mixture has been linked to a 12% yield reduction in corn (*Zea mays* L.) and soybean (*Glycine* *max* L.) (Eckert 1988; Mitchell et al., 1977). Likewise, ineffective termination of hairy vetch, wheat (*Triticum aestivum* L.), annual ryegrass (*Lolium perenne* L. ssp multiflorum (Lam.) Husnot), and crimson clover (*Trifolium incarnatum* L.) has been shown to reduce soybean yields by 7–29% (Whalen et al., 2019; Reddy et al., 2001; Thelen et al., 2004).

A nationwide survey of 1,691 cover crop growers, conducted in 2012 and 2013, revealed that 48% use herbicides, 21% employ mechanical methods, and 20% rely on winter kill for terminating cover crops (SARE, 2014). In certain regions, as much as 95% of cover crop growers use herbicides for termination (Oliveira et al., 2019). Chemical termination is achieved using various selective and non-selective herbicides before or after cash crop planting. Glyphosate (often combined with other chemicals) has proven effective in terminating a variety of grassy cover crop species, including cereal rye, winter wheat, and annual ryegrass (Cornelius et al., 2017).

Glyphosate [IUPAC chemical name N-(phosphonomethyl) glycine] is the most widely used pesticide globally and the active ingredient in all glyphosate-based herbicides (GBHs), including the formulation "Roundup" (Myers et al., 2016; Benbrook 2016; Smith & Oehme, 1992). It is marketed primarily as a broad-spectrum systemic herbicide and crop desiccant. The production and use of glyphosate have surged since the introduction of genetically modified (GM) glyphosate-tolerant crops in 1996. In the United States, glyphosate is found in over 750 products, particularly herbicides for GM crops, but also in products for agriculture, forestry, urban, and home use (IARC, 2015). The widespread and growing use of GBHs has resulted in global occupational exposure for manufacturing workers and applicators (farmers), as well as increasing exposure for the general population. Glyphosate residues have been found in air (Majewski MS et al., 2014), groundwater (ISPRA, 2016; Battaglin et al., 2014), drinking water (Rendón-von Osten et al., 2016), crops (Cuhra, 2016; USDA, 2013), food (EFSA, 2016; PRIF, 2016), and animal feed (Mesnage et al., 2015). The potential health effects of GBHs, including both carcinogenic and non-carcinogenic concerns such as endocrine disruption, neurotoxicity, and developmental and reproductive toxicity, especially during sensitive periods like fetal development, are a topic of intense public debate (IARC, 2015; EFSA, 2015; ECHA, 2017; Shehata et al., 2013). Moreover, around 267 weed species worldwide have developed resistance to herbicides, which underscores the need for new, effective herbicide alternatives (Heap, 2023).

As societal and EU policies push for reduced pesticide use, stricter approval criteria for new herbicides, and lower maximum residue thresholds, bioherbicides are becoming increasingly important in weed management (Kristoffersen et al., 2008; Hillocks, 2012).

The goal of this study was to evaluate the effectiveness of different natural compounds (organic acids and essential oils) on weed termination and cover crops termination, identifying the optimal concentrations and application volumes for the purpose.

10 Materials and Methods

10.1 Plant material

Seeds of weeds and crops were evaluated. Seeds from weed species such as *Lolium perenne* L., *Setaria viridis* L. and *Cichorium intybus* L. were obtained from the germplasm collection available at the Seed Research and Test Laboratory (LaRAS) of the Department of Agricultural and Food Sciences, University of Bologna.

Crop seeds such as *Triticum aestivum* L., *Panicum miliaceum* L., and *Vicia sativa* L. were purchased from Arcoiris (Modena, Italy), whereas seeds of *Medicago sativa* L. were obtained from LaRAS.

10.2 Experimental design

Different natural compounds on weeds and cover crops were evaluated. In particular, the effect of organic acids as acetic acid, pelargonic acid, citric acid and lactic acid, and essential oils as eugenol and geraniol, on seeds germination were evaluated. Treatments except for essential oils were sprayed at the corresponding volume of 300 lt/ha at four different compound concentrations 0, 5, 10 and 20 % (v/v) and 5, 10 and 20% (w/v) for citric acid. Whereas essential oils were mixed at the concentration of 1,5% (v/v) to acetic acid at 5, 10 and 20% (v/v); in this case acetic acid was simply used as adjuvant.

Acetic acid was provided formulated at 20% by the company Flortis (Italy), whereas pelargonic acid, citric acid, lactic acid, eugenol and geraniol were purchased from Merck (Germany)

The experimental trials were conducted in the greenhouse of the Department of Agriculture and Food Science e Technology of the University of Bologna. A randomized block scheme with three replicates, for all examinate factors was adopted. Control was provided for each species and treatment. Different species were sowed in rectangular pots of 15 lt volume at T0, and sowing density was adapted to each species covering capacity, in order to obtain a uniform cover of the pots surface. Each plant density was following reported: *Lolium perenne* L. (50 kg/ha), *Vicia sativa* L. (100 kg/ha), *Setaria viridis* L. (50 kg/ha), *Cichorium intybus* L. (5 kg/ha), *Medicago sativa* L. (50 kg/ha), *Triticum aestivum* L. (150 kg/ha), *Panicum miliaceum* L. (50 kg/ha). Substrate was prepared mixing peat (50%), turf soil (40%), vermiculite (5%) and agriperlite (5%). Plants were irrigated with tap water until the treatments. Plants were grown for 21 days (t1) under greenhouse conditions and treated with acetic acid, pelargonic acid, citric acid and lactic acid with three different concentrations (5, 10, 20% p.a.) and additionally a mixture of acetic acid (5, 10, 20%) and essential oils (eugenol 1,5% and geraniol 1,5%) was also tested. Treatments were sprayed with commercial sprayer at the volume of 300 lt/ha. Treatments will be indicated as p.a. + concentration as: acetic acid 5% (a5), acetic acid 10% (a10), acetic acid 20% (a20), pelargonic acid 5% (p5), pelargonic acid 10% (p10), pelargonic acid 20% (p20), citric acid 5% (c5), citric acid 10% (c10), citric acid 20% (c20), lactic acid 5% (l5), lactic acid 10% (l10), lactic acid 20% (l20), mix of acetic acid and essential oils 5% (m5), mix of acetic acid and essential oils 10% (m10) and of acetic acid and essential oils mix 20% (m20). Herbicide efficacy was evaluated as injury on green part and green biomass at the end of the trial for all investigated treatments. Efficacy was evaluated as a green part area change (GCA) and calculated through the analysis of plants photos at different time, before (t0, pre-treatment) and 7 days after treatment (T1) with a commercial camera Nikon D5100. The green cover area (leaf cover) of weeds was estimated by counting the number of green pixels. Each image was taken at a height of 100 cm, covering an area of 100 x 100 cm2. All pictures were processed with a public domain java-based image processing software "ImageJ". The image was split into hue, saturation and brightness by using "Color Threshold" function. Color thresholds were adjusted: hue 40-110, saturation 0-255, brightness 0-255. Green leaf cover and background were segmented. Then adjustation of hue values in colour threshold, all background pixels were eliminated. The filtering process reduced noise and improved the segmentation result of the image in binary format. The binary format of the processed image contained only the vegetation pixels of the weeds. These pixels were counted to estimate percentage leaf cover from each plot. Efficacy was also evaluated by biomass index. At the end of the trial plants were harvested and fresh weight (FW) from each pot was measured in gram (g). Dry weight (DW) in g were obtained by dehydratation of plats throught BIOSEC PRO purchased from Tauro Essicatori srl.

10.3 Efficacy evaluation

Herbicide efficacy on weeds and cover crops were evaluated 7 days after treatment (t1). Efficacy as green cover area reduction (gca reduction) in % was obtained as a difference of green part at t1 and t0. Green cover area reduction was presented as % on control. Efficacy was also evaluated as reduction in fresh weight reduction of the plant's biomass, as % on control value.

10.4 Statistical analysis

Statistical analyses were conducted using the Statistica 6.0 software (2001, StatSoft, Tulsa, OK, USA). Two way and one-way analysis of variance (ANOVA) in conjunction with Tukey's honest significant difference was performed. Significant differences between means were determined by least significant difference values for $p < 0.05$. Pearson's correlation coefficient (r) was calculated at significance level of $p < 0.01$.

11 Results and discussion

In order to evaluate the interaction between factors (species, treatments and concentration) 3-way ANOVA analysis was established, and the interaction between factors for p <0,001 was significatively high. For this reason, results were reported in function of the different concentration of p.a. (5, 10 and 20%), assuming that higher concentrations lead to higher efficacy. For this reason, results were evaluated as control-based percentage, to evaluate which p.a. the higher activity at each concentration.

Concerning green cover area reduction in 5% concentration treatments (fig. 11), interaction between species and treatments was significatively high for p<0,001. In contrast with previous trials (chapter 1), essential oils added to acetic acid led to better results in terms of reduction of the green cover area of the different species, compared to acetic acid without essential oils and other treatments. In particular 5% acetic acid + essential oils treatment resulted in a green cover area reduction of above 60% compared to control for all the analyzed species (weeds, cover crops and crops), except for *Triticum aestivum* L. that resulted in 55%. This result could be explained by the synergic effects of the two compounds: essential oil mixture allowed a longer persistence of acetic acid on the leaf surface. Acetic acid is a very volatile compound which require specific conditions and timing for irroration in open field conditions, its use in sunny and hot days may lead to higher evaporation of the product, and consequently to less persistence on the plants surface.

Acetic acid alone and pelargonic acid treatments had lower efficacy compared to acetic acid + essential oil, even if a sufficient effect on plant green area development was maintained for all analyzed species. The lowest efficacy was observed by Citric acid and lactic acid treatments. In particular, citric acid reached values below 50% for all the species, except for *Lolium perenne* L. Similar values were obtained for Lactic acid treatment for all the species, except for *Cicorium intybus* L. and *Panicum miliaceum* L. that had a green cover reduction of the 67 and 62%, respectively.

As previously reported, acetic acid has been explored as a potential herbicide, suggesting concentrations ranging from 5% to 20% can effectively control small, young weeds, particularly broadleaf species. However, its efficacy decreased as weeds mature, and higher concentrations should be required for larger weeds (Smith-Fiola and Stanton, 2024).

Conversely, treatments efficacy on *Triticum aestivum* L. was higher at stage 10 to 12 BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale compared to germination and sprout stages, as previously observed (chapter I). However, in this trial wheat resulted as the less impacted species for all the analyzed treatment, with lowest inhibition obtained by citric and pelargonic acid treatments, with values below 40%.

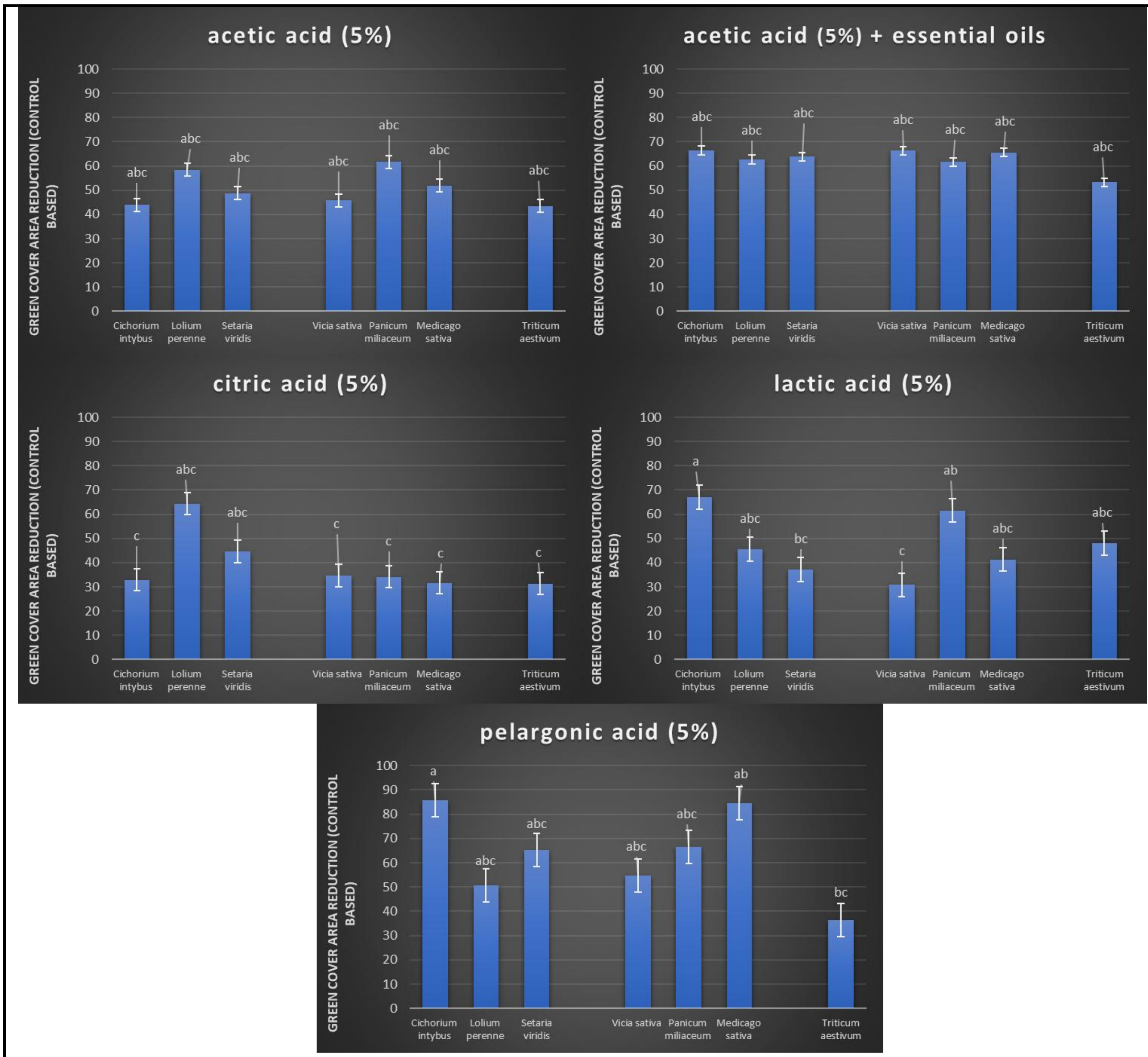

Fig. 11: Green cover reduction in % (control based) in different treatments

Concerning fresh and dry weight in 5% concentration treatments (fig. 12), no significative interaction between species and treatments was observed.

Concerning fresh weight of aboveground biomass in 5% concentration treatments (fig. 12), Factorial ANOVA identified no significant effect on interaction of dose and species on fresh weight biomass reduction. Data were analyzed as % on control (not treated plants). For fresh weight, the species response was different from one species to the other, with a higher general effect of the treatments on *Cichorium intybus* L., *Medicago sativa* L., and *Lolium perenne* L., with obtained values of 60.52%, 55.16%, and 52.39%, respectively, and lower effect for *Triticum aestivum* L. (47.52%), *Panicum miliaceum* L. (40.13%), *Vicia sativa* L. (38.78%) and *Setaria viridis* L. (26.43%).

These responses might be linked to differences in leaves surface areas exposed to treatments between dicotyledons (*Cichorium intybus* L. and *Medicago sativa* L.) and monocotyledons species. In addition, between dicotyledons the action of the treatment was impacted also by the habitus of the species. Indeed, treatment efficacy on *Vicia sativa* L. had lower results compared to the other two analyzed dicotyledon species, due to its leaves morphology and plant habitus, resulting in a lower leaf surface area exposed to treatments. Concerning the effect of natural compounds on different species statistic differences were observed. Pelargonic acid resulted as the treatment with the highest efficacy with a reduction in fresh biomass of 75.45%, followed by acetic acid + essential oils (49.21%), lactic acid (45.08%), acetic acid (39.34%) and citric acid (20.72%). The positive herbicide effect of pelargonic acid against different weed species in controlled environment was previously evaluated (Munoz et al. 2020, Travlos et al. 2020, Kanatas et al. 2022). Further, considering fresh weight biomass the efficacy of pelargonic acid was in 80% range (Loddo et al. 2023), and in the present study comparable values were obtained for *Cicorium*, due to habitus and leaves exposed area. Indeed, contrasting results of pelargonic acid efficacy have been reported in various crops. Loddo et al. (2023) reported that pelargonic acid, even at higher doses, caused minimal and transient injuries. Conversely, as previously reported (Webber et al. (2014a, 2014b) broadleaf weeds were more sensitive to pelargonic acid and inconsistent desiccation was found for grass and broadleaf weeds with pelargonic acid application. Similarly inconsistent weed desiccation efficacy was reported with pelargonic acid in several crops (Cabrera-Pérez et al., 2022; Kanatas et al., 2021; Martelloni et al, 2020; Rowley et al., 2011; Travlos et al., 2020).

Otherwise, lower values were obtained compared to the reported data for what concern acetic acid at 5% concentration. As previously reported by Webber et al. (2024) and Abouziena et al. (2009) the efficacy of acetic acid 5% ranged between 45% and 60% up to 90% in controlled environment conditions Webber et al. (2024) and Abouziena et al. (2009).

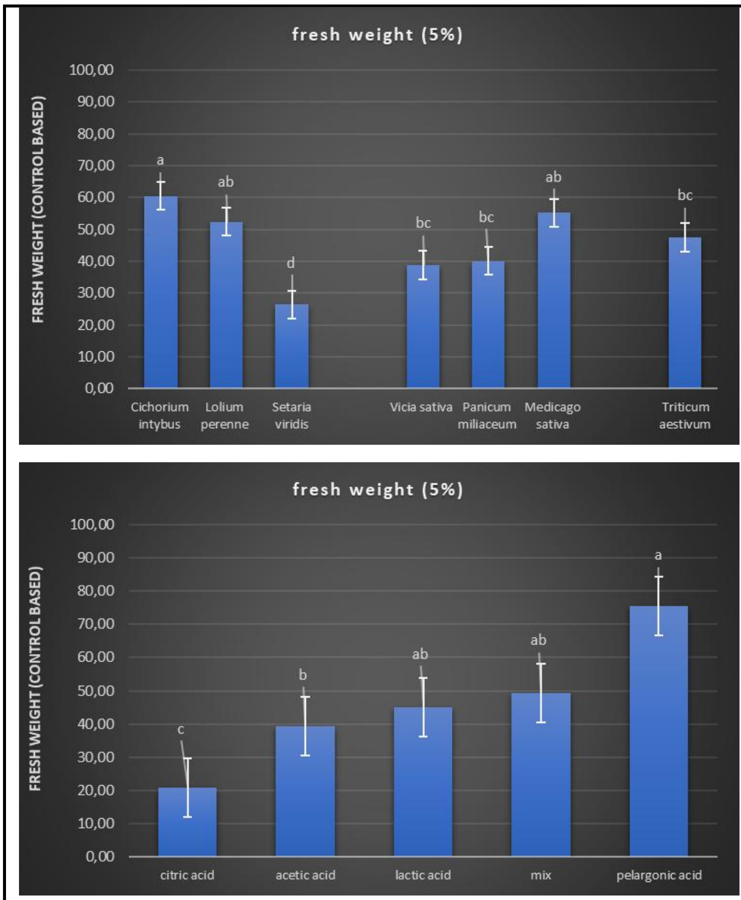

Fig. 12: fresh weight in % (control based) in different species and treatments

Concerning green area reduction in 10% concentration treatments (fig. 13), no significative interaction between species and treatments was observed. In general, all the species were controlled by natural compounds. Into specific, *Cichorium intybus* L. green cover area was reduced of the 70% by the treatments, followed by *Lolium perenne* L., *Setaria viridis* L., *Panicum miliaceum* L. and *Medicago sativa* L. *Vicia sativa* L., and *Triticum aestivum* L. showing the lowest area reduction with values above 50%. Pelargonic acid and acetic acid + essential oils, showed the highest control for all the species, followed by acetic acid. The higher efficacy of the acetic acid mixed with essential oils was obtained in the 5% concentration too, as a consequence of the synergic effect of the two active principles. Adding oils to the treatment may reduce the evaporation of acetic acid, allowing a higher persistence on leaf surface. The same effect was observed for pelargonic acid (nonaoic acid) treatment with 10% concentration, since the lipophilic nature of this molecule may allow a higher persistence on the irrorated surface. Thus, higher concentrations of nonaoic acid in solution improved the activation of its properties, compared to 5%. Finally, the lowest efficacy was observed for Lactic acid and citric acid with values ranged 50% of green cover area reduction.

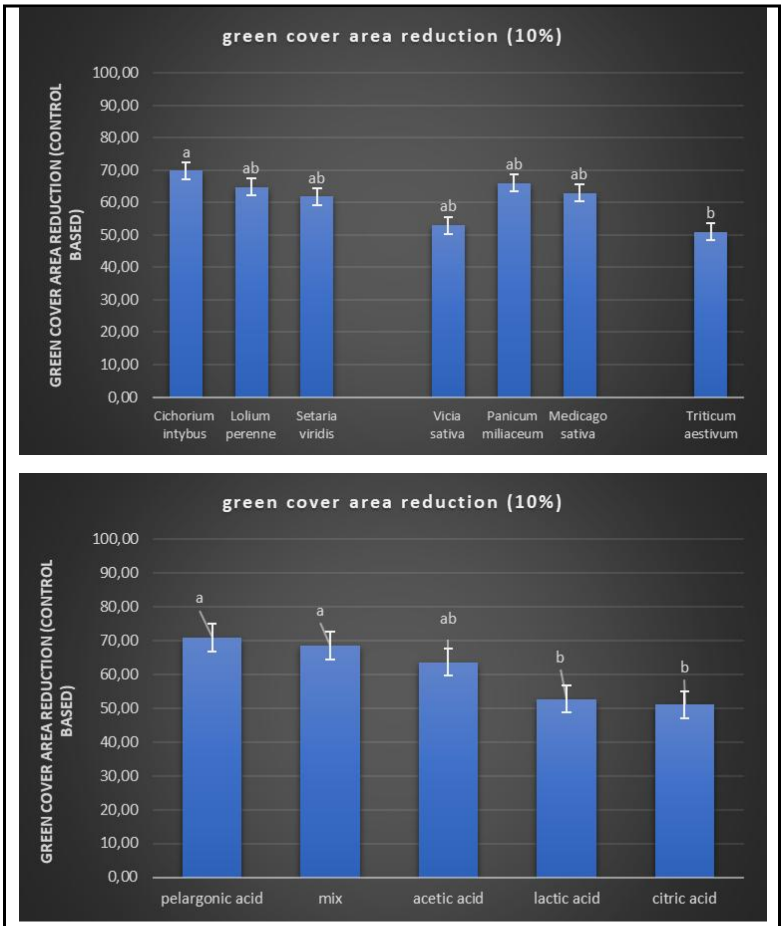

Fig. 13: green cover reduction in % (control based) in different species and treatments

Concerning fresh weight of aboveground biomass in 10% concentration treatments (fig. 14), Factorial ANOVA identified no significant effect on interaction of dose and species on fresh weight biomass reduction. High values of biomass reduction were observed for weed species, in particular for *Cichorium intybus* L. and *Lolium perenne* L. both with 70% fresh biomass reduction. No difference was observed between monocotyledons and dicotyledons species. Significant statistical differences among treatments were observed. As expected, fresh weight reduction in 10% concentration treatments was higher compared to 5% concentration treatments, even if the same trend for 5% concentration results was observed. Pelargonic acid (77.60%) treatment obtained the highest efficacy considering fresh biomass reduction, followed by acetic acid + essential oils (61.71%), lactic acid (59.10%), acetic acid (52.74%) and citric acid (36.62%). Pelargonic acid at 10% concentration had the same trend reported for the 5%, mainly linked to the same reasons previously described (habitus and leaves area). Similar results were obtained by Rahayuningsih and Supriadi (2016) in field trials on weeds, where citric acid 10% and acetic acid at 10% reached similar efficacy. Conversely, data obtained by Abouziena, et al. (2009) reported high control efficacy of citric acid at 10% with high intra-specific effects related to the single characteristics of each species.

However, information on lactic acid herbicides is still missing in literature, since this molecule is not commercially distributed, unlike pelargonic and acetic acid.

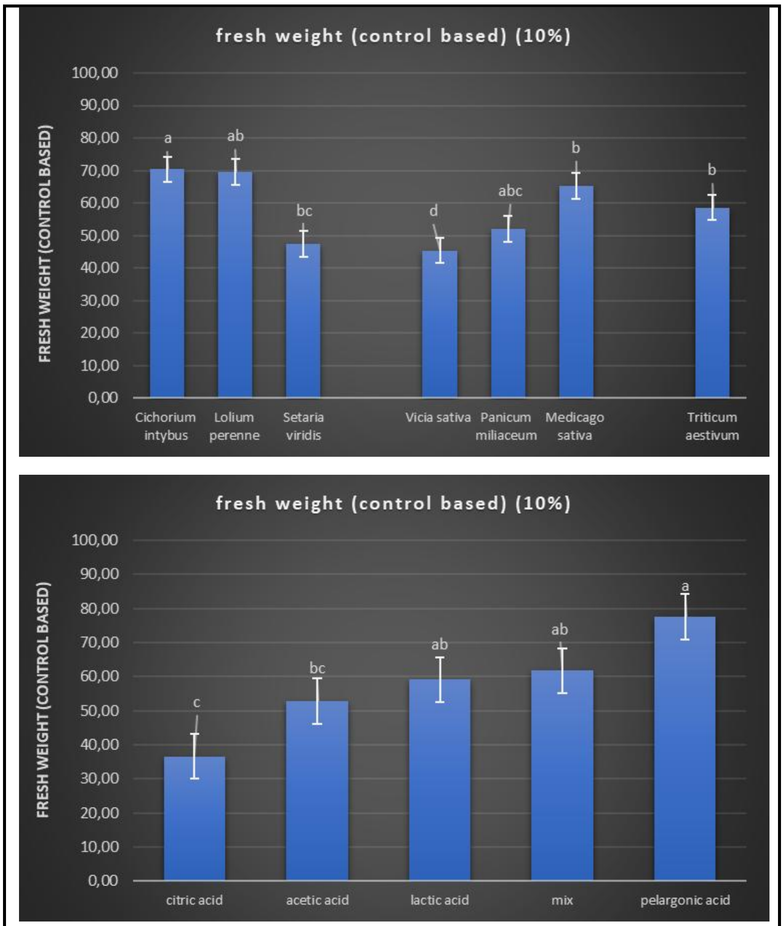

Fig. 14: fresh weight in % (control based) in different species and treatments

Concerning green reduction area in 20% concentration treatments (fig. 15), no significative interaction between species and treatments was observed. Green cover area of all the analyzed species was highly reduced by the different treatments. Values of green cover area reduction ranged between 62.85% (*Vicia sativa* L.) and 74.65% (*Panicum miliaceum* L.) but no statistic difference was observed among species. Acetic acid was the treatment resulting in the highest efficacy (a), followed by pelargonic acid (ab) and acetic acid + essential oils (ab), lactic acid (bc) and citric acid (c), and all the treatments showed high green cover area reduction effect, above 58%.

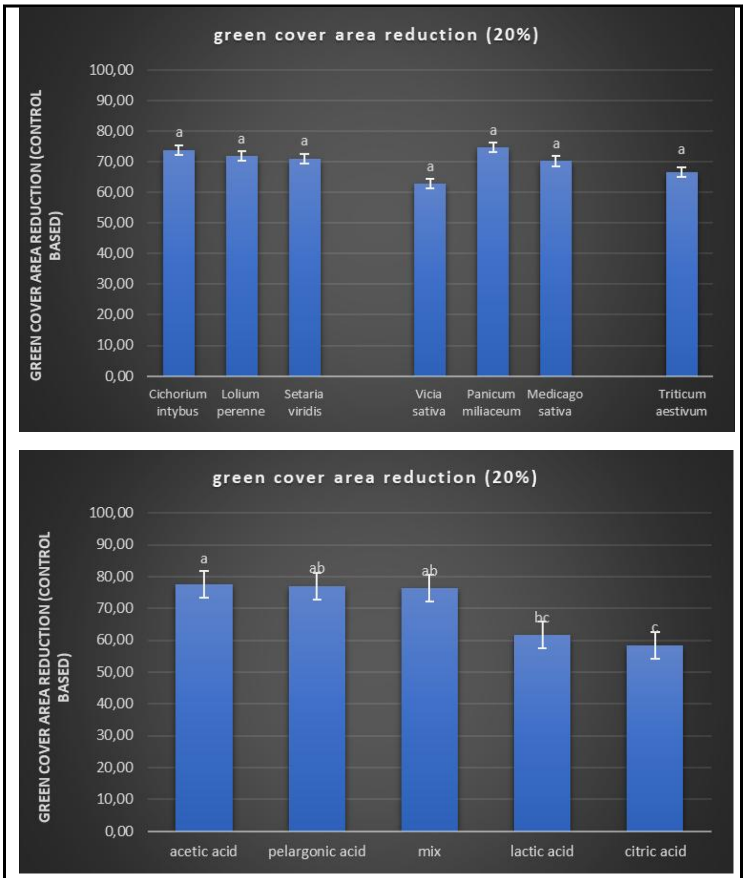

Fig. 15: green cover reduction in % (control based) in different species and treatments

Concerning fresh weight of aboveground biomass in 20% concentration treatments (fig. 16), Factorial ANOVA identified no significant effect on interaction of dose and species. For all the analyzed species the effect of treatments rate at 20% concentration treatment above the 50% of fresh weight reduction. As previously observed for 5% and 10% concentration treatments trials, *Cichorium intybus* L. (77.33%), *Lolium perenne* L. (82.02%) and *Medicago sativa* L. (77.17%) obtained the higher efficacy. *Vicia sativa* L. resulted in the lowest controlled species, as for 5% and 10% concentration treatments. Different results were also obtained for what concerns the efficacy of the different treatments. In general, all the treatments reached the same efficacy on tested species, except for citric acid that showed a fresh weight reduction efficacy under 50% (45.56%). As previously observed for 5 and 10% treatments, pelargonic acid was the most effective treatment in reducing fresh weight.

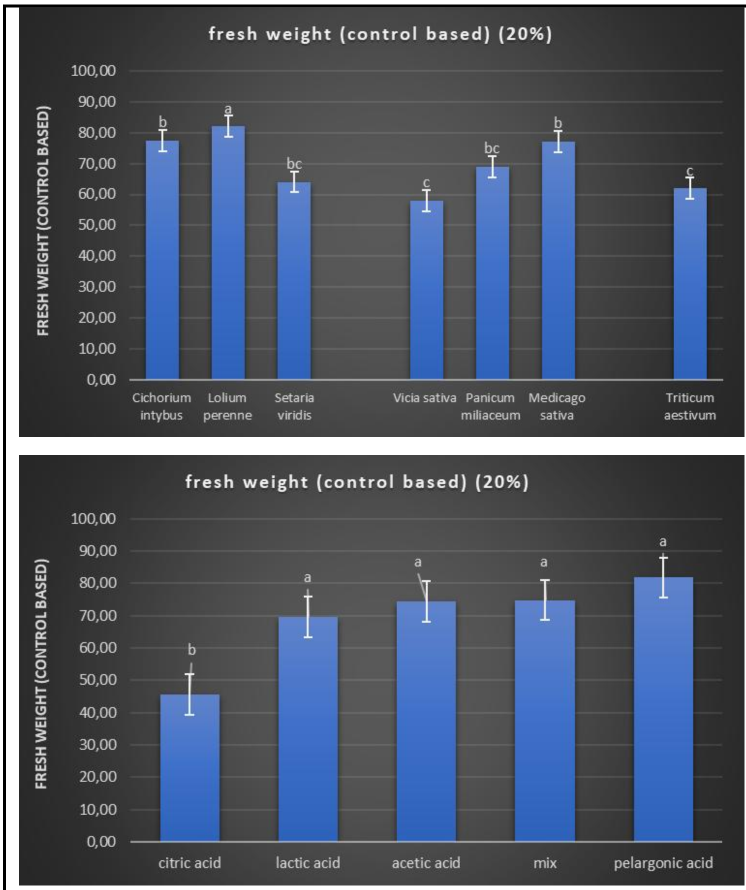

Fig. 16: fresh and dry weight in % (control based) in different species and treatments

In the following table (Tab. 3) I50 values, that correspond to the doses (concentration) able to reduce the green cover area of the tested species of the 50%, were evaluated. Interaction between factors resulted highly significative, for this reason data were showed as interaction. As previously observed for 10% (fig. 13) and 20% (fig. 15) concentrations, all the species had been well controlled by the treatments. Indeed, no statistical differences between species were observed, with I50 values ranging from 9.2% for *Lolium perenne* L. to 11.7% for *Triticum aestivum* L. In contrast, there were statically significant differences between treatments. Therefore, the lowest principal concentration was needed for pelargonic acid (8,2%) to reduce of 50% the green cover area of tested species, followed by acetic acid + essential oils (9.2%), acetic acid (10.0%) and lactic acid (11.6%). Otherwise, citric acid (15.4%) needed the highest concentration to reduce by 50% the green cover area of the tested species, except for *Lolium perenne* L., for which the required concentration was similar to the other treatments (10.2%).

Tab. 3: I50 results as interaction between species and treatments

	Acetic acid	Citric acid	Lactic acid	Acetic acid + essential oils	Pelargonic acid	
Cichorium intybus	10.2 (bcde)	21.5 (a)	8.6 (de)	8.8 (e)	4.8 (a)	**10.8 (a)**
Lolium perenne	12.4 (bcde)	10.2 (bcde)	13.1 (abcde)	9.3 (de)	9.5 (cde)	**9.2 (a)**
Setaria virids	11.1 (de)	14.9 (abcd)	13.1 (abcde)	9.9 (cde)	8.3 (de)	**11.6 (a)**
Panicum miliaceum	9.5 (de)	18.5 (abc)	10.8 (bcde)	11.5 (bcde)	7.6 (de)	**10.7 (a)**
Medicago sativa	6.4 (de)	19.1 (ab)	15.3 (abcd)	7.6 (de)	5.1 (e)	**10.9 (a)**
Vicia sativa	10.8 (bcde)	14.3 (d-f)	8.8 (f)	7.6 (f)	14.3 (d-f)	**11.4 (a)**
Triticum aestivum	10.1 (ef)	14.4 (abcd)	13.4 (abcde)	9.6 (cde)	10.5 (bcde)	**11.7 (a)**
	10.0 (bc)	**15.4 (a)**	**11.6 (b)**	**9.2 (bc)**	**8.2 (c)**	

12 Conclusion

Weeds are a major cause of crop yield loss due to crop-weed interference, making effective weed control a crucial component in agriculture. The application of synthetic herbicides, with particular attention to selective herbicide, for effective weed control has thus become indispensable in modern agriculture, thanks to be time- and cost-efficient. Chemical herbicides are designed to destroy or suppress weeds growth without damaging thr crops (Hasan et al., 2021). However, improper use of synthetic herbicides, such as excessive application or the repeated use of the same herbicide, can lead to environmental contamination (Morales et al., 2013), negative human health effects (Huovinen et al., 2015), the development of weed resistance, and negative impacts on non-target organisms (Filimon et al., 2021). For these reasons, and to cope with the European Green Deal goals, farmers are shifting from harmful chemical-dependent conventional agriculture to more sustainable and greener farm practices. The fundamental philosophy of sustainable weed management is based on preventing the spread of weeds rather than controlling them until they have developed and started to cause crops damage. Sustainable weed management does not rely on any single technique, but in a pool of practices like mechanical tillage, physical weed control, crop fertilization strategies, crop rotation, intercropping, irrigation, and cover crops are used together (Bàrberi 2001). While these methods may be less effective than chemical herbicides, they can be combined to suppress weeds and reduce seed banks over time (Gallandt, 2014). Bioherbicides are naturally originated products which can be used to control weeds. Bioherbicides consist of microorganisms such as pathogens and other microbes or compounds derived from microbes, insects, or plant extracts that act as a natural herbicide for weed control. Bioherbicides that are thought to be safer for human health and to have reduced impact on environment due to their lower persistency, have drawn attention as scientific reports provide increasing evidence of their efficacy (Hasan et al., 2021). Despite considerable research, there are only a few commercially available bioherbicides worldwide due to limitations in their development. In particular, organic acids and essential oils reach the scientific attention due to their potential as tool for weed managent (Evans et al., 2009; Loddo et al., 2023; Lotha et al., 2024; Pannacci et al., 2022; Webber et al., 2024), but their high cost, in front of chemicals, led to their use in agriculture.

The aim of this study was to evaluate the efficacy control and injury of different natural compounds (organic acids and essential oils) at different concentrations (5%, 10% and 20%) on weeds, cover crops and crops. The results showed different response of the different species. No particular effects were observed for weeds or cover crops and crops. All the species were controlled by the tested natural compounds, highlighting the large spectrum of action at low concentrations. In fact I50 results, showed that a concentration between 8.2% and 15.4% is needed to reduce by 50% the green cover area of the tested plants. Difference between treatments showed a higher efficacy of pelargonic acid, acetic acid and acetic acid + essential oils. Adding essential oils to acetic acid can increase the efficacy when used with acetic acid at low concentration (5% and 10%). Adding essential oils to acetic acid at 20% concentration did not increase the efficacy of acetic acid in controlled environment trials. The higher efficacy of pelargonic acid, acetic acid and acetic acid + essential oils, as compared to citric acid and lactic acid is probably due to the chemical structure of the different compounds. In particular among tested organic acids , acetic acid was the more acidic, followed by citric acid, lactic acid and pelargonic acid. For this reason, it is plausible to cause higher damage when sprayed directly on the leaf surface. The activity of pelargonic acid and essential oil in general were also observed in literature. As reported by Ciriminna et al. (2019), the acids penetrate cellular membranes, causing leakage and the breakdown of membrane lipids (peroxidative activity), causes necrosis and rapid drying of weeds, driven by potent oxidative radicals.

In conclusion, acetic acid and essential oils show great potential as weed managment tool. Their low concentration needed to be effective could help to spred this compounds in farming practices. The key to the transistion to more sustainable practices in agriculture is to find a cost-effective tool. Pelargonic acid and essential oils in general require high cost production due to the low yield of essential oils in medicinal and aromatic plants and due to the process of distillitation that reduce further the yield of the final products. Acetic acid contrarly can be naturally produced and founded as by-product at low price, positively affect the economicity of weed managment.

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Chapter III – Evaluation of herbicide efficacy of natural acetic acid for weed management and cover crops termination in controlled environment

Mattia Alpi[1], Elettra Frassineti [1], Enrico Toschi[1], Eros D'amen[1], Giovanni Dinelli[1], Ilaria Marotti[1]

1.Department of Agricultural and Food Sciences, University of Bologna

ABSTRACT

The growing challenges posed by climate change, population growth, and soil degradation demand sustainable strategies to enhance agricultural efficiency and quality. Synthetic herbicides like glyphosate, widely used for weed management, are associated with environmental persistence and potential health risks, necessitating eco-friendly alternatives. This study investigates the efficacy of acetic acid as a natural bioherbicide for weed and cover crop termination, focusing on application strategies and interaction effects among species, application methods, and volumes.

Two application volumes (200 and 300 L/ha) and two methods (wetted and sprayed) were tested. At 200 L/ha, green cover area reduction varied by species, with Triticum aestivum L. achieving 63% reduction at 3 days after treatment (DAT), but regrowth observed at 7 DAT. At 300 L/ha, efficacy improved significantly, with reductions of 95% for *Triticum aestivum* L. and 90% for *Cichorium intybus* L. at 3 DAT, and no regrowth across species at 7 DAT. Fresh weight reductions were similarly species-dependent, with *Triticum aestivum* L. and *Setaria viridis* L. showing higher sensitivity. Application methods showed no statistical differences, though sprayed methods demonstrated slightly better results.

Acetic acid at 20% (v/v) concentration with a 300 L/ha application volume was highly effective but exhibited low selectivity, increasing the risk of crop damage. Its use as a pre-emergence bioherbicide or for cover crop termination is promising but requires further investigation into optimal application timing, method precision, and cost-effectiveness. This study underscores

the potential of acetic acid as a sustainable alternative for weed management in modern agriculture.

14 Introduction

The increasing problematics due to climate change, increase in global population, soil degradation and contamination, and the more intense biotic and abiotic stress conditions (Bernardo et al., 2018; Raza et al., 2019), underscore the necessity of adopting sustainable strategies to enhance crop efficiency and quality in modern agriculture. Soil quality is declining in many regions, which impacts the productivity, resilience, and sustainability of agri-food systems (Ferreira et al., 2022; Rust et al., 2022). The accumulation of contaminants from intensive agricultural practices, pesticides, and their subsequent emission and transformation into other environmental pollutants are significant contributors to the deterioration of soil quality and food production (Chen et al., 2021; Destouni et al., 2021).

Weeds, due to their competitive nature in agroecological systems, interfere with crop efficiency by competing for nutrients, moisture, light, and space (Dew, 1972; Holt & Orcutt, 1991). They are one of the most critical factors influencing crop yield, responsible for about 10% of global financial losses in agricultural production (Ekwealor, et al., 2009). Herbicides, the most widely used group of pesticides, play a major role in weed management and the termination of cover crops (Abbas et al., 2019). However, their use leads to significant environmental problems, such as water contamination, pollution, and risks to human and animal health (Rashid et al., 2010).

Glyphosate (GLY), or N-(phosphonomethyl) glycine, is an organophosphorus compound and the most commonly used broad-spectrum, non-selective herbicide worldwide (Chaufan et al., 2014; Ferrante et al., 2023). It works by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSP), an enzyme crucial for the biosynthesis of aromatic amino acids such as phenylalanine, tyrosine, and tryptophan, ultimately leading to plant death (Peillex and Pelletier, 2020; Leino et al., 2021). Roundup® was the first glyphosate-based herbicide (GBH).

Glyphosate is now the active ingredient in many GBH formulations, used to control over 100 species of weeds and 60 species of perennial plants in both industrial and residential environments (Munoz et al., 2021; Ferrante et al., 2023). Between 1996 and 2012, the U.S. market recorded a total of 12 million tons of GLY used for weed control, with future usage expected to surpass this amount (Benbrook, 2016; Ferrante et al., 2023). Consequently, GBHs have become one of the most widely used classes of plant protection products (PPPs) globally, applied in approximately 140 countries (Munoz et al., 2021). Due to their widespread use, GLY is detectable in various environmental matrices, including air, water, and food, as well as in biological fluids such as urine, blood, and breast milk (Yoshioka et al., 2011; Mária Mörtl et al., 2013; Zouaoui et al., 2013; Mercurio et al., 2014; Simonetti et al., 2015; Steinborn et al., 2016; Demonte et al., 2018; Marino et al., 2021; Munoz et al., 2021; Cellier et al., 2022; Connolly et al., 2022; Iohanna Filippi et al., 2024). Exposure to glyphosate and its metabolite AMPA, found in various crops and food (Vandenberg et al., 2017), may pose health risks, further amplified by their long half-lives and prolonged environmental persistence (Battaglin et al., 2014).

To reduce the dependence on toxic chemical herbicides, the use of eco-friendly bioproducts, such as bioherbicides, offers significant benefits for both farmers and consumers. Bioherbicides are less persistent than synthetic herbicides (Seiber, et al., 2014; Saini, et al., 2018). Secondary metabolites, such as phenolic compounds, short-chain fatty acids, terpenoids, and alkaloids, provide natural sources for new phytotoxic products with multitarget action mechanisms (Croteau, et al., 2000; Dayan,et al., 2009; Dayan, & Duke, 2014; Verdeguer, et al., 2020). Reports from literature highlight the potential of vinegar (acetic acid, CH3COOH) as a natural herbicide for organic weed control (Evans & Bellinder, 2009; Evans et al., 2009; Evans et al., 2011; Johnson et al., 2003; Radhakrishnan et al., 2002, 2003; Webber et al., 2012). Vinegar, produced through the fermentation of plant materials containing sugars, typically contains 5% acetic acid. Acetic acid acts as a contact herbicide, breaking down rapidly in the environment and yielding water as a byproduct. It is believed to kill plants by damaging their cell membranes, leading to rapid desiccation of plant tissues (Owens, 2002). Acetic acid is not absorbed by plants or translocated to other parts, making it less effective on mature plants and perennials, which can regenerate from their roots. Multiple applications and proper timing relative to weed size, maturity, and life cycle can enhance control.

Previous studies have assessed the effectiveness of acetic acid and other natural products for weed control in vegetable crops and other agricultural systems (Boyd et al., 2006; Evans et al., 2009; Radhakrishnan et al., 2002), as well as their effects on crops (Bingaman et al., 2000; Evans and Bellinder, 2009; Evans et al., 2011; Moran and Greenberg, 2008; Patton and Weisenberger, 2012).

For instance, a study by Glenn J. Evans (2009) showed that vinegar's effectiveness in controlling weeds varied significantly depending on weed species and size. The study monitored 23 weed populations, including eight species, and found that control was more

effective on smaller weeds, with performance declining as weed size increased. The effectiveness of natural products is also influenced by factors such as weed pressure, species composition, and weed size at the time of application. Vinegar proved effective in reducing weed pressure when applied to young broadleaf weeds. In 61% of cases, treatments reduced weed biomass, but the effectiveness diminished with larger weeds and regrowth. Timing was key to success, as smaller weeds responded better to the treatment. Vinegar applied at 636 L/ha provided the highest control (91%) and biomass reduction (93%) for smaller weeds. However, the same treatment on larger weeds (more than six leaves) showed reduced control (14% less) and increased biomass (38% more). Larger volumes and higher concentrations of vinegar produced better results, but also caused more crop injury. Although vinegar initially eliminated weeds faster than glyphosate, it did not provide long-term control like commercial herbicides.
The goal of this study was to evaluate the effectiveness of acetic acid on weeds termination and cover crops termination, identifying the optimal application volumes and irroration methods for weed management.

15 Materials and Methods

15.1 Plant material

Seeds of weeds and crops were evaluated. Seeds from weed species such as *Lolium perenne* L., *Setaria viridis* L. and *Cichorium intybus* L. were obtained from the germplasm collection available at the Seed Research and Test Laboratory (LaRAS) of the Department of Agricultural and Food Sciences, University of Bologna.
Crop seeds such as *Triticum aestivum* L., *Panicum miliaceum* L., and *Vicia sativa* L. were purchased from Arcoiris (Modena, Italy), whereas seeds of *Medicago sativa* L. were obtained from LaRAS.

15.2 Experimental design

The experimental trials were conducted in the greenhouse of the Department of Agriculture and Food Science e Technology of the University of Bologna. A randomized block scheme with three replicates, for all the species and treatments was adopted, and a control (not treated) was provided for each species. Different species were sowed in rectangular pots of 15 lt volume at different density *Lolium perenne* L. (50 kg/ha), *Vicia sativa* L. (100 kg/ha), *Setaria viridis* L. (50 kg/ha), , *Cichorium intybus* (5 kg/ha), *Medicago sativa* L. (50 kg/ha), *Triticum aestivum* L.

(150 kg/ha), *Panicum miliaceum* L. (50 kg/ha). Sowed density for different species was chosen in order to obtain a uniform cover on the pots surface. Substrate was prepared mixing peat substrate (50%), turf soil substrate (40%), vermiculite (5%) and agriperlite (5%). Plants were irrigated with tap water until the treatments. Plants were irrigated with tap water until treatments. Plants were growed for 21 days (t0) and treated with a commercial solution composed by natural acetic acid at 20% (v/v) concentration purchased by Flortis. Different volume of solution was applied at 200 lt/ha and 300 lt/ha, and different methods of irroration such as sprayed with a commercial pump sprayer and wetted with a painter roller were exminated. Herbicide efficacy was evaluated on green part and green biomass of different treatments. Growth and injury quantification of plants after treatments were observed with a commercial camera NikonD5100. Photos of plants were taken before treatments at day 21 (t0), and 3 days after treatmets (day 24 - t1) and 7 days after treatments (day 28 - t2). The green cover area (leaf cover) of weeds was estimated by counting the number of green pixels. Each image was obtained at a height of 100 cm, covering an area of 100 x 100 cm2. All pictures were processed with a public domain java-based image processing software "ImageJ". The image was split into hue, saturation and brightness by using "Color Threshold" function. Color thresholds were adjusted: hue 40-110, saturation 0-255, brightness 0-255. Green leaf cover and background were segmented. Then hue values in color threshold, all background pixels were adjusted and eliminated, allowing noise reduction and improving the segmentation result of the image in binary format. On so doing, the binary format of the processed image allowed to examinate only the vegetation pixels of the weeds. Thus, pixels were counted to estimate the percentage of green cover area in each plot. Efficacy was also evaluated through biomass index analysis. At the end of the trials plants material was harvested and fresh weight (FW) from each pot (g) were evaluated.

15.3 Statistical analysis

Statistical analyses were conducted using the Statistica 6.0 software (2001, StatSoft, Tulsa, OK, USA). Two way and one-way analysis of variance (ANOVA) in conjunction with Tukey's honest significant difference was performed. Significant differences between means were determined by least significant difference values for $p < 0.05$. Pearson's correlation coefficient (r) was calculated at significance level of $p < 0.01$.

16 Results and discussion

In order to evaluate the interaction between factors (species, treatment methods and application volume) 3-way Anova analysis was established, and the interaction between factors for $p < 0{,}001$ was significatively high. For this reason, results were reported in function of the different application volume (200 lt/ha and 300 lt/ha), assuming that higher volumes lead to higher efficacy. For this reason, results were evaluated as control-based percentage, to evaluate which application methods had the higher activity on the different species.

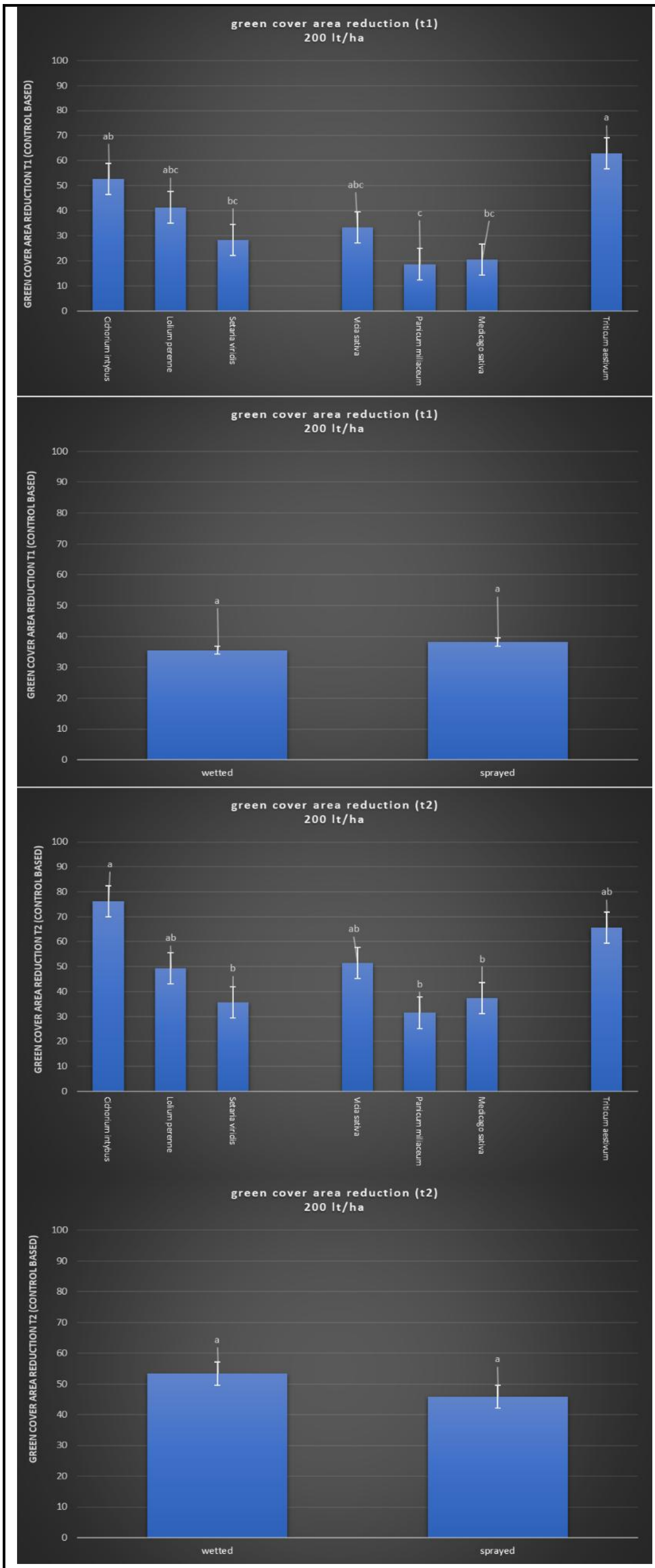

Fig. 17: green cover area reduction in t1 (3 DAT) and t2 (7 DAT) in species and application methods

Considering 200 lt/ha irroration volume, interaction between species and application methods resulted not significative for t1 and t2, therefore the results were analyzed separately (fig. 17). For both t1 (3 DAT) and t2 (7 DAT), a high variability between species was observed in green cover area reduction data, but similar efficacy was observed especially for each species. For what concern green cover area reduction in t1, *Triticum aestivum* L. (63%) was the most controlled species, followed by *Cichorium intybus* L. (52.66%), *Lolium perenne* L. (41.33%), *Vicia sativa* L. (33.33%), *Setaria viridis* L. (28.33%), *Medicago sativa* L. (20.5%), and *Panicum miliaceum* L. (18.67%). Results in green cover area reduction 7 DAT (t2) underlined a specific effect of treatments on different species. Thus, all the species showed a green cover area reduction in the following 7 DAT (t2) mainly linked to acetic acid efficacy, except for *Triticum aestivum* L., for which a lower efficacy in green cover area reduction values was observed in t2 rather than t1. Consequently, the observed recovery (regrowth) in 7 DAT of *Triticum aestivum* L.

should be considered as a focal point for the adequate time selection, corroborating the importance of right application timing for acetic acid treatment, considering both crop and weeds developmental stage respectively, to minimize the damage on the principal crop. As previously reported, the response of wheat at the treatment may be linked to the effect of bioherbicides on sprouts and meristems (Evans et al. 2009). Product applications that contact only the larger leaf surfaces will burn foliage and reduce plant vigor, although regrowth from the shoot apex or axillary buds might be probable (Evans et al. 2009). Thus, in this case both wetted and sprayed methods did not allow the bioherbicide to enter in contact with crop meristems, but only with outer leaves, leading to regrowth and a decrease in herbicide activity. Positive results on weeds greenhouse trials with acetic acid were previously reported (Miller and Libbey, 2004), since greenhouse-grown weeds could be more susceptible to herbicide effect because they are not as hardened as in field conditions.

Focusing on the application method, no significative difference in green cover area reduction between wetted and sprayed method for both in t1 and t2 was observed (Fig 17, right side).

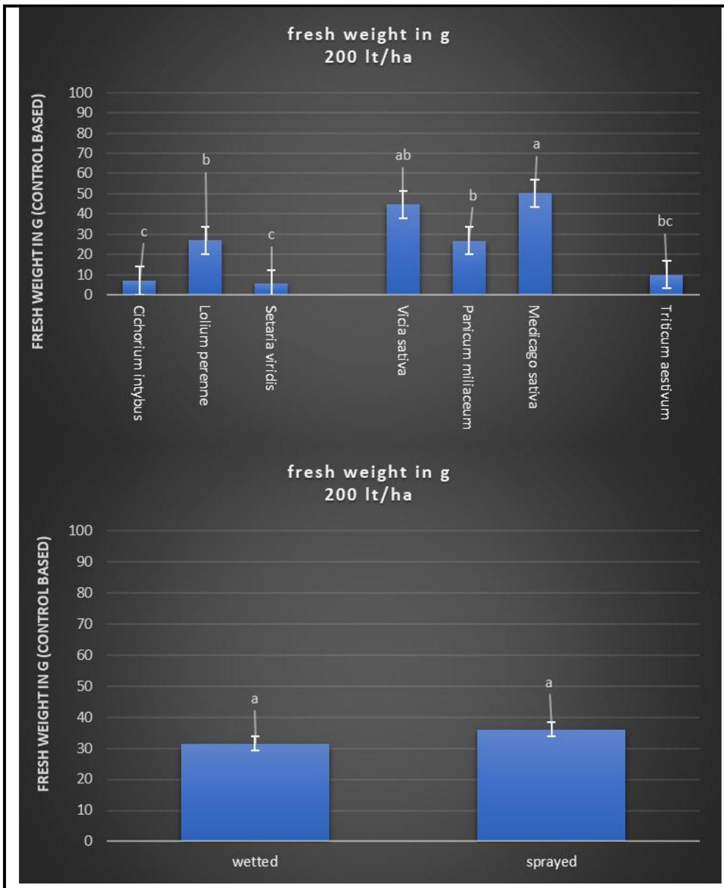

Fig. 18: fresh weight in % (control based) in t1 (3 DAT) and t2 (7 DAT) in different species and treatments

Interaction between species and application methods resulted not significative, so the results were reported separated (fig. 18). For what concern fresh weight reduction, higher impact of acetic acid treatment against *Cichorium inthybus* L., *Setaria viridis* L. and *Triticum aestivum* L. was observed. Thus, in this case a higher efficacy was obtained for wheat and weeds, not for cover crops, opening the possibility of a specific application.

As for green cover area, no significative difference in fresh weight between wetted and sprayed methods was obtained.

Considering 300 lt/ha irroration volume, interaction between species and application methods resulted not significative for t1 and t2, therefore the results were analyzed separately (fig. 19). For both t1 (3 DAT) and t2 (7 DAT), a high variability between species was observed in green cover area reduction data, but similar efficacy was observed especially for each species. For what concern green cover area reduction in t1, *Triticum aestivum* L. (95 %) and *Cichorium intybus* L. (90 %) were the most controlled species, followed by *Medicago sativa* L. (56 %), *Lolium perenne* L. (45 %), *Vicia sativa* L. (42 %), *Setaria viridis* L. (40 %), and *Panicum miliaceum* L. (35 %). Depending on thetreatment dose the response of the species was different in t1, except for *Triticum aestivum* L. that was the most impacted in both evaluated volumes for t1. As hypothesized, the effect on green cover area was higher with 300L/ha for t1 and t2.

Results in green cover area reduction 7 DAT (t2) underlined a specific effect of treatments on different species, as well as previously observed for 200 lt/ha dose. Thus, all the species showed a green cover

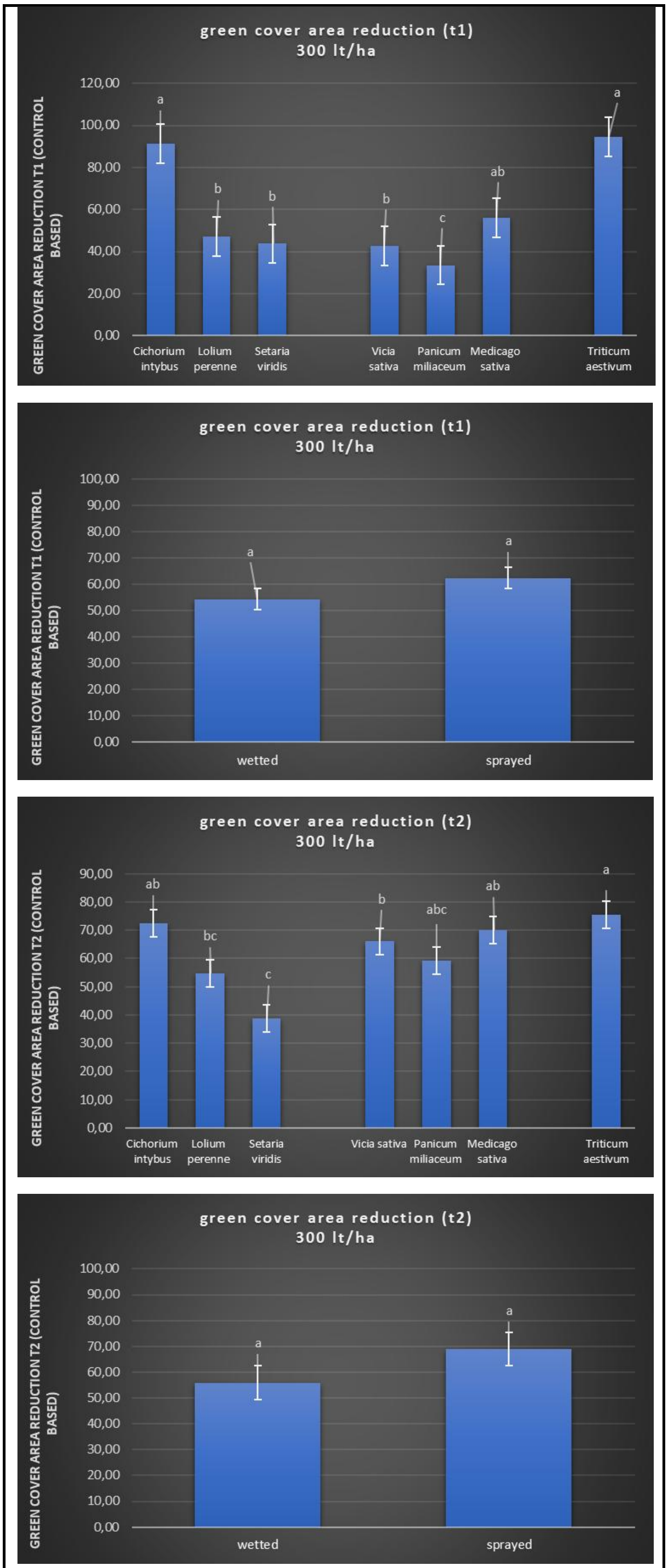

Fig. 19: green cover area reduction in t1 (3 DAT) and t2 (7 DAT) in species and application methods

area reduction in the following 7 DAT (t2) mainly linked to acetic acid efficacy, except for *Setaria viridis* L., for which no different efficacy in green cover area reduction values was observed from t1 to t2. The effect of the treatment on *Triticum aestivum* L. was different from the previous dose tested, since higher application volume of acetic acid may allow the herbicide reaching more fragile parts of the plant, not permitting wheat regrowth 7 DAT. The absence of recovery in 7 DAT of *Triticum aestivum* L. for 300 lt/ha pointed out the importance of the application volume to allow crop regrowth and maximize weeds damage. Research indicates that lower concentrations, such as 5% acetic acid, can effectively control very young weeds with only 1-2 leaves. However, as weed size increases, higher concentrations are necessary for effective control. For instance, 20% acetic acid applied at 935 L/ha resulted in weed control ranging from 44% to 63% (Webber et al. 2018). It's important to note that while acetic acid can provide immediate top kill of weeds, perennial weeds with extensive root systems may recover after treatment.

Acetic acid at varying concentrations has demonstrated potential as a natural herbicide, particularly effective against young, small weeds. However, its efficacy diminishes with larger weeds, and higher concentrations may adversely affect crop seed germination and growth (Abouziena et al. 2009).

Focusing on the application method, no significative difference in green cover area reduction between wetted and sprayed method for both in t1 and t2 was observed (Fig 19, right side).

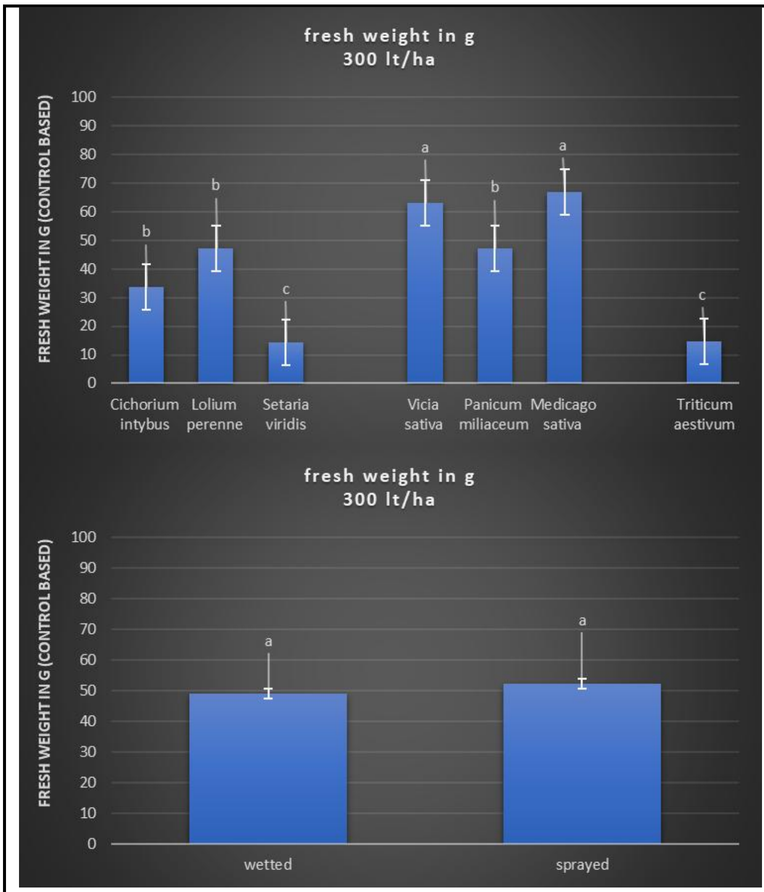

Fig. 20: fresh weight in % (control based) in t1 (3 DAT) and t2 (7 DAT) in different species and treatments

Interaction between species and application methods resulted not significative, so the results were reported separated (fig. 20). For what concern fresh weight reduction, lower impact of acetic acid treatment against *Vicia sativa* L. and *Medicago sativa* L. was observed with values of 63 and 67 %, respectively. Thus, in this case a higher efficacy was obtained for *Triticum aestivum* L. and *Setaria viridis* L. as reported for 200 lt/ha and lower effect for cover crops.

As for green cover area, no significative difference in fresh weight between wetted and sprayed methods was obtained. To conclude, for both doses (200 and 300 lt/ha) the effect of different methods did not impact both fresh weight and green cover area.

17 Conclusion

The challenges of modern agriculture, emphasizing the impact of climate change, population growth, soil degradation, and biotic/abiotic stresses on crop efficiency and quality. Declining soil quality and contamination from intensive farming and pesticide use contribute to environmental and food production issues. Weeds are a significant problem, reducing crop yield by competing for resources, with herbicides like glyphosate (GLY) widely used for management. However, glyphosate's environmental persistence, widespread presence in ecosystems, and potential health risks necessitate alternative solutions. Bioherbicides and eco-friendly products, such as acetic acid (vinegar), show promise as sustainable weed control methods. Acetic acid acts as a contact herbicide, effectively controlling small weeds but requiring precise timing and multiple applications for larger or perennial plants. Studies highlight vinegar's potential in reducing weed biomass and improving weed management when applied at optimal volumes and concentrations. Despite initial effectiveness, vinegar lacks the long-term control provided by synthetic herbicides.

The study aims to evaluate acetic acid's effectiveness in weed and cover crop termination, focusing on optimal application strategies for improving weed management. The effectiveness of acetic acid in weed and crop management was evaluated, examining two application volumes (200 and 300 L/ha) and two application methods (wetted and sprayed). High variability of control efficacy between the different species was observed. The efficacy observed for *Triticum aestivum* L. underlines the necessity to understand the effects at different development stage, in order to evaluate the best application time to minimize damage on crops and maximize effects on weeds. As reported, *Triticum aestivum* L. treated with acetic acid at the application volume of 200 lt/ha had lower efficacy at 7 DAT, in contrast with results obtained for all the other species. At 300 lt/ha, the highest application volume led to greater efficacy, with green cover area reduction of 95% for *Triticum aestivum* L. and 90% *Cichorium intybus* L. 3 DAT. Further, no regrowth was observed for all the species 7 DAT. Concerning application methods, no statistic difference was observed, despite sprayed method showed higher results. Wetted application method could be obtained different results in field application thanks to the possibility of lodging the plants. Sprayed method also could be implemented with precision agriculture system that allow to increase selectivity of the treatments, reducing application volume, costs and environmental pollution. In conclusion, acetic acid at 20% concentration with a 300 lt/ha application volume showed high herbicide efficacy, with low selectivity that may result in crop damage. For this reason, acetic acid might be a valuable alternative to conventional products in the weeds management as pre-emergence bioherbicide and in cover crop termination. Consequently, the best application method should be further investigated throught field application evaluating related costs and maximizing results.

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Chapter IV – Herbicide efficacy evaluation of natural compounds on weeds in semifield conditions

Mattia Alpi[1], Elettra Frassineti [1], Eros D'amen[1], Giovanni Dinelli[1], Ilaria Marotti[1*]

1.Department of Agricoltural and Food Science and Technologies, University of Bologna

* Corresponding author: Ilaria.marotti@unibo.it

ABSTRACT

Weeds present significant challenges in both agriculture and urban environments, leading to reduced crop yields, increased production costs, and functional disruptions such allergen production. While synthetic herbicides are widely used for weed control, their overuse raises serious environmental concerns, including contamination, health risks, and the development of resistance. This has prompted growing interest in sustainable alternatives, such as bioherbicides derived from natural compounds. Bioherbicides, which include organic acids, essential oils (EOs), and allelopathic substances, offer a promising solution due to their biodegradability and lower toxicity. This research investigates the herbicidal efficacy of acetic acid, both alone and in combination with essential oils and various adjuvants, for weed control under field conditions. Field trials were conducted at the University of Bologna's experimental station, where different formulations of 20% (v/v) acetic acid, combined with adjuvants such as lecithin, chabazite, chitosan, and EOs, were tested. The results indicated that acetic acid at 20% (v/v) concentration effectively reduced weed growth, with some adjuvants, particularly EOs and *Camelina sativa* L. oil, reaching the same efficacy. While acetic acid demonstrated rapid desiccation and low environmental persistence, its high evaporation rate and need for repeated applications present challenges for field-scale use. In conclusion, acetic acid shows strong potential as a bioherbicide, particularly for small-scale and organic farming systems, offering a promising alternative to synthetic herbicides. Further research is needed to optimize application

methods, reduce evaporation, and enhance consistency and efficacy under varying environmental conditions.

19 Introduction

Weeds pose a significant challenge due to their competitive nature, as they reduce crop yield and quality (Dew, 1972; Holt & Orcutt, 1991). Weeds interfere in agriculture production not only by directly compete with crops, but also directly such as higher cost for irrigation and fertilization, higher operational time during harvest blocking mechanical organs and higher use of pesticides due to the major number of spots for virus and pathogens on weeds. They are responsible for approximately 10% of global agricultural losses (Ekwealor, et al., 2009). Weeds can cause also functional damage in urban environments, by disrupting human activities, block stormwater drains, exacerbating drainage issues, contribute to allergies by releasing pollen, with species like grasses, Asteraceae, and Urticaceae being particularly problematic (Benvenuti S. 2004). In the European Union, the economic losses resulting from the absence of herbicides in agriculture are considerable amounting to billions of euros (Wynn and Webb, 2022). As a result, herbicides are the most commonly used pesticides; however, their overuse has led to environmental concerns, such as water contamination, pollution, and health risks (Rashid, et al., 2010). Despite their benefits, improper use of herbicides can cause soil, water, and air contamination, human health risks, and weed resistance (Myers et al., 2016; Morales et al., 2013; Velki et al., 2019; Huovinen et al., 2015). In response to these issues, the EU's Farm to Fork strategy aims to reduce chemical use, emphasizing alternative weed management methods. Organic farming practices combines mechanical operations, physical methods, antagonist organisms and natural compounds for weed management purpose (Liebman & Davis, 2009; Bàrberi, 2001). Natural compounds have been recently explored for their potential in weed control. Bioherbicides, typically non-selective, use a range of natural active ingredients in their formulations. These include natural chemicals like organic acids, essential oils, and allelopathic compounds (DeNux et al., 2024). These natural compounds, which are biodegradable and less toxic to both the environment and human health, offer a promising approach to sustainable weed management (Seiber et al., 2014; Dayan & Duke, 2014; Triolet et al., 2020), both in agricultural areas and urban areas. Bioherbicides might be advantageous thanks to their biodegradability and low environmental persistence, making them suitable for sensitive ecosystems, although they require multiple applications and act more slowly (Islam et al., 2024). Organic herbicides, typically non-selective, utilize natural ingredients such as organic acids and essential oils to damage plant tissues by breaking down cellular membranes, leading to rapid weed desiccation (Ciriminna et al., 2019). In particular, acetic acid resulted able to kill plants by damaging their cell membranes, leading to rapid desiccation of plant tissues (Owens, 2002), showing great potential as bioherbicide (Webber et al., 2012; Webber et al., 2024; Evans & Bellinder, 2009; Evans et al., 2009; Evans et al., 2011; Johnson et al., 2003; Radhakrishnan et al., 2002; Boyd et al., 2006; Bingaman et al., 2000; Moran and Greenberg, 2008; Patton and Weisenberger, 2012). Also, essential oils (EOs) have gained attention as a promising alternative to synthetic herbicides. The exact mechanisms through which EO components impact weeds are not yet fully understood. Most EO-based herbicides available on the market function as contact herbicides, disrupting weed cell membranes, inhibiting critical enzymes, or interfering with vital biochemical pathways. EO are non-selective, providing effective but short-term weed control (Lotha et al., 2024; Nikolova et al., 2021; Somala et al., 2023; Moura et al., 2025; Awojide et al., 2022; Ben Kaab et al., 2024; Souihi et al 2024; Benvenuti et al., 2017).

The aim of the research was the evaluation of the herbicide efficacy of acetic acid as well its combined application, including essential oils (EO) and adjuvants, for weed control. The was the evaluation of the on weeds of different formulation of acetic acid in field conditions. For this purpose, different adjuvants were added to acetic acid at 20% concentration, in order to understand the ability to control weeds.

20 Materials and Methods

20.1 Experimental design

Trials were conducted in an experimental station located at the Department of Agricultural and Food Sciences and Technologies (DISTAL) at the University of Bologna. This work reported some of the most significant trials aimed at testing the herbicidal effects of different formulations of acetic acid. The focus was the evaluation of the influence of acetic acid alone and combined with essential oils (EO) and several adjuvants as lecithin, chabazite and chitosan.

The treatments were obtained with a base vinegar solution containing 20% (v/v) of acetic acid. Each tested adjuvant was added to the acetic acid 20% (v/v) solution at a fixed concentration of 1,5% (v/v). Chitosan was added as powder and solubilized bringing the solution to 50°C at the final concentration of 1,5% (v/v). Lecithin and chabazite were added as powder and homogenously mixed in the final solution as well. Treatments were sprayed using a portable sprayer (drop diameter between 200-400 μm) at the final volume of 300 L/ha.

Acetic acid was provided formulated at 20% by the company Flortis (Italy), whereas eugenol geraniol and chitosan were purchased from Merck (Germany) and cabasite provided by Fitokem (Imperia, Italy). Camelina oil was kindly provided by Prof. Federica Zanetti, DISTAL).
Afterwards, two trials were performed with a randomized plot scheme with four replicates for each condition, and a control parcel sprayed with tap water was provided for each treatment. Each parcel was designed of 1 m2 surface and treated twice. The first treatment was performed on May 14, 2023, applying the different solutions to the surface area of the parcels, and the second treatment was then carried out on June 1, 2023, under the same conditions of the previous one. Further, pictures before and 7, 14 days after both treatments were taken, for each solution and replicate.

Herbicide efficacy was evaluated on green cover area reduction efficacy of different treatments. Growth and injury quantification of plants after treatments were observed with a commercial camera Nikon D5100. Photos of plants were taken before treatments at 14-22 BBCH growth stage (t0), 7 and 14 days after both treatments. The green cover area (leaf cover) of weeds was estimated by counting the number of green pixels. Each image was obtained at a height of 100 cm, covering an area of 1 m2. All pictures were processed with a public domain java-based image processing software “ImageJ”. The image was split into hue, saturation and brightness by using “Color Threshold” function. Color thresholds were adjusted: hue 40-110, saturation 0-255, brightness 0-255. Green leaf cover and background were segmented. Then hue values in color threshold, all background pixels were adjusted and eliminated, allowing noise reduction and improving the segmentation result of the image in binary format. On so doing, the binary format of the processed image allowed to examinate only the vegetation pixels of the weeds. Thus, pixels were counted to estimate the percentage of leaf cover capacity in each plot.

20.2 Species recognition and meteorological data

Before the treatment in each parcel weeds recognition was performed and occurring plants species were noted. Weeds species list is provided in the following table.

Tab. 4: weed species in the treated areas
Oxalis corniculata L.
Cersatium ramossimum Boiss
Gallium aparine L.
Erigeron canadiensis L.
Euphorbia prostrata Aiton
Trifolium arvense L.
Achillea millefolium L.
Digitaria sanguinalis (L.) Scop
Poa annua L.
Setaria viridis L.
Anisantha sterilis L.

The meteorological data (temperature and precipitation) for the entire duration of the experimental trial, comprising the multiple cycles in each location, was obtained from the Arpae weather station, located in Emilia Romagna (https://simc.arpae.it/dext3r/) (Figure 21).

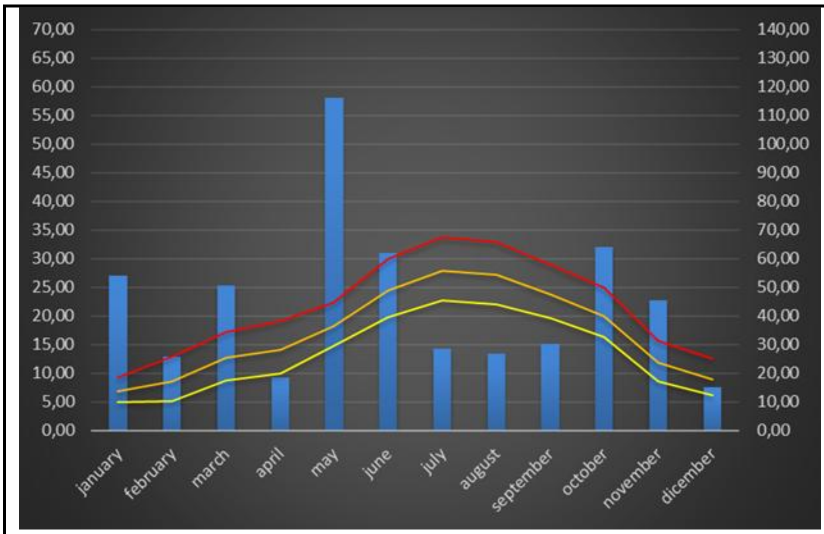

Figure 21. Monthly precipitation (mm) and average temperature (°C) at each location for all cultivation cycles between 2020 and 2022.

20.3 Statistical analysis

Statistical analyses were conducted using the Statistica 6.0 software (2001, StatSoft, Tulsa, OK, USA). Two way and one-way analysis of variance (ANOVA) in conjunction with Tukey's honest significant difference was performed. Significant differences between means were determined by least significant difference values for $p < 0.05$. Pearson's correlation coefficient (r) was calculated at significance level of $p < 0.01$.

21 Results and discussion

In the first trial, after the first treatment a significative reduction in green cover area ranged from 77% to 37% for acetic acid and acetic acid + chitosan, respectively, was observed 7 days after the first treatment. Greater effects on weeds were obtained by acetic acid without adjuvant and acetic acid with Cameline oil. Then, 14 days after the first treatment the effect on green cover reduction was less evident with values ranged between 60.75% and 23%, obtained for acetic acid + camelina oil and acetic acid + chabazite, respectively. The different efficacy between treatments after the second treatment was not as evident as the previous one, and 14 DAT the final green cover area reduction ranged between 84% and 34 % for acetic acid + camelina oil and acetic acid + lecithin, respectively. The maximize effect was obtained with a reduction of 85% and the effect was evident one month after the first treatment, almost eliminating weeds from the area. In general, dicotyledonous species were more susceptible to the treatments than monocotyledons, except for *Euphorbia prostrata*, for which a second treatment was required. All the remaining dicotyledonous species were controlled with only one treatment. Conversely, control of the two most abundant monocotyledonous species, *Digitaria sanguinalis* and *Poa* spp, required three and two treatments respectively. Smith-Fiola and Stanton (2024) reported that acetic acid sprayed at concentrations ranging from 5% to 20% showed higher efficacy for controlling small, young weeds, particularly broadleaf species. However, its efficacy decreased with mature weeds, and higher concentrations were required. Otherwise, in the present study significative effects were obtained with 20% acetic acid concentration for plants at 14-22 BBCH developmental phase. The effect might be influenced by environmental condition (sunny days and high temperatures), but certainly the application of a second treatment might be a valuable alternative to join 90% of reduction in GCA with the same concentration of acetic acid in solution. As previously reported (Webber et al. 2018) 20% acetic acid applied at 935 L/ha resulted in weed control ranging from 44% to 63%. In the present study, higher efficacy of acetic acid without adjuvant was demonstrated, multiple application may allow the molecule to act at different developmental phases not allowing regrowth. Since, acetic acid was demonstrated to provide immediate kill of weeds, but perennial weeds with extensive root systems may recover after treatment (Abouziena et al. 2009). Concerning adjuvants, the best results were obtained for essential oils and camelina oil compared to the others, even if their effect on green cover area reduction was comparable to the one obtained by acetic acid alone.

Tab. 5: green cover area reduction for the different treatments

	efficacia 7 DAT (1 tratt)	efficacia 14 DAT (1 tratt) - fatto 2 tratt	efficacia 7 DAT (2 tratt)	efficacia 14 DAT (2 tratt)	efficacia a 28 DAT (1 tratt)
acetic acid (aa)	77.00 (a)	55.75 (a)	52.75 (a)	48.75 (ab)	85.75 (ab)
aa + eo	56.00 (a)	43.25 (a)	63.00(a)	64.00 (a)	80 (ab)
aa + Camelina sativa C. oil	58.50 (a)	60.75 (a)	63.75 (a)	84.50 (a)	93.50 (a)
aa + chitosan	37.50 (ab)	38.75 (a)	48.50 (ab)	41.50 (ab)	60.25 (ab)
aa + lecitin	53.25 (ab)	36.00 (a)	71.50 (a)	34.25 (ab)	59.25 (b)
aa + chabasite	56.00 (ab)	23.25 (ab)	68.25 (a)	46.50 (ab)	59.00 (b)
control	-4.50 (b)	-15.75 (b)	-0.25 (b)	-3.25 (b)	-19.50 (c)
LSD	39,170	29,050	32,936	34,976	21,255
p	*	***	**	***	**

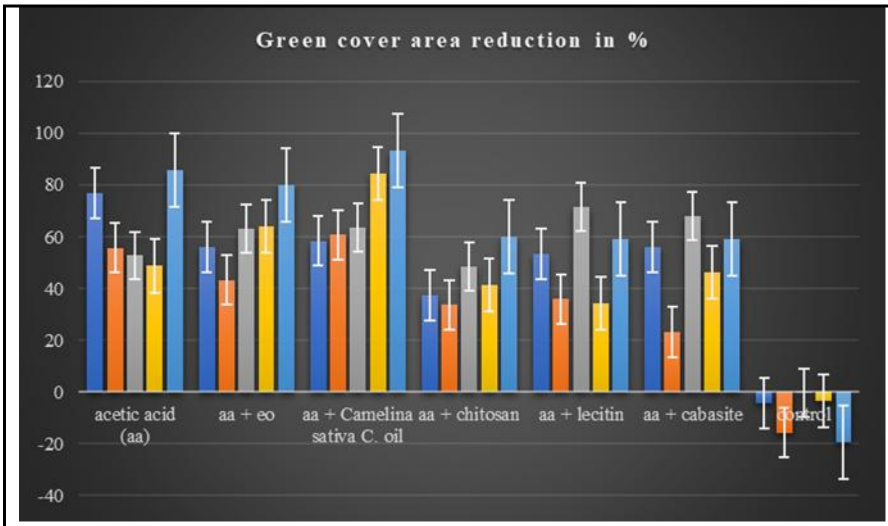

Fig. 22: evolution of green cover area for the different treatments in the first trials

Concerning the second trial, all the treatments showed great control efficacy 7 DAT, ranging between (28.50% and 78.75%) green cover area reduction both for the first and the treatment. As for the first trial, statistic difference was found between treatments efficacy at 7 DAT. Acetic acid showed the highest control in both treatments, respectively 78.75% (treatment 1) and 72.75% (treatment 2). The addition of adjuvants to acetic acid at 20% concentration didn't increased efficacy. It is to underline that essential oils (72.50% and 60.75%), *Camelina sativa* L. oil (62.00% and 64.75%) and chitosan (73.00 and 56.50%), showed in any case higher performance in front of chabazite (44.75% and 42.25%) and lecithin (29.25% and 28.50%) treatments. Different results were found for what concern the efficacy at 14 DAT. In general, as expected, at 14 DAT all the treatments showed a green cover area reduction lower, below 50%, than at 7 DAT, as weeds regrowth. In the first experiment no statistic difference was observed between treatment, contrarily in the second treatment statistic difference was observed, as acetic acid + *Camelina sativa* L. oil showed the highest green cover reduction (44.00% and 47.75%). No statistic difference was observed at 28 DAT. The efficacy of treatments on dicotyledonous species, compared to monocotyledonous species, was confirmed in the second trial and the same trend observed. Despite that several differences were obtained overall in green cover area reduction between treatments. In particular, acetic acid without adjuvants and acetic acid + *Camelina sativa* L. oil resulted as the most effective treatment against weeds for both the trials (first and second one). The high control efficacy of *Camelina sativa* L. oil it was probably due to the content of glucosinolates compounds. These molecules are found exclusively in dicotyledonous plants, with the highest concentrations in the families Resedaceae, Capparidaceae, and Brassicaceae, and showed to represent a viable source of allelochemical control for a variety of weeds, at different development stage (Adarsh P.V. et al., 2009; Brown P. and Morra M. 1995).

Between the first and the second trial a reduction of the effect of the treatments on green cover area was observed. This reduction in the activity of p.a. may be due to environmental factors, since in May 2023 precipitations were overall higher than in June but confined to a few days at the end of the month and temperatures in June were significantly higher (Fig.1). The amount of precipitations after the first trial and the rising of the temperatures allowed weeds growth, decreasing the treatment efficacy and needing higher doses (Smith-Fiola and Stanton, 2024). In addition, the higher temperatures observed for the second trial led to an easier evaporation of acetic acid. For instance, environmental factors such as temperature (higher than 20°), humidity, and wind speed significantly influence the rate of evaporation of acetic acid. High temperatures and windy conditions can accelerate evaporation, diminishing the contact time between the herbicide and the weed, thereby reducing efficacy (Roberts et al. 2022).

Tab. 6: green cover area reduction for the different treatments

	efficacia 7 DAT (1 tratt)	efficacia 14 DAT (1 tratt) - fatto 2 tratt	efficacia 7 DAT (2 tratt)	efficacia 14 DAT (2 tratt)	efficacia a 28 DAT (1 tratt)
acetic acid (aa)	78.75 (a)	40.50 (a)	72.75 (a)	39.25 (ab)	65.50 (a)
aa + eo	72.50 (ab)	49.00 (a)	60.75 (a)	24.25 (ab)	61.25 (a)
aa + Camelina sativa C. oil	62.00 (ab)	44.00 (a)	64.75 (a)	47.75 (a)	72.50 (a)
aa + chitosan	73.00 (ab)	35.75 (a)	56.50 (a)	30.25 (ab)	59.00 (a)
aa + lecitin	29.25 (bc)	17.00 (a)	28.50 (ab)	23.25 (ab)	36.50 (a)
aa + chabasite	44.75 (ab)	3.00 (a)	42.25 (ab)	31.25 (ab)	39.75 (a)
control	-12.25 (c)	-5.00 (b)	6.25 (b)	-10.50 (b)	-16.25 (b)
LSD	39,170	29,050	32,936	34,976	21,255
p	***	*	**	ns	***

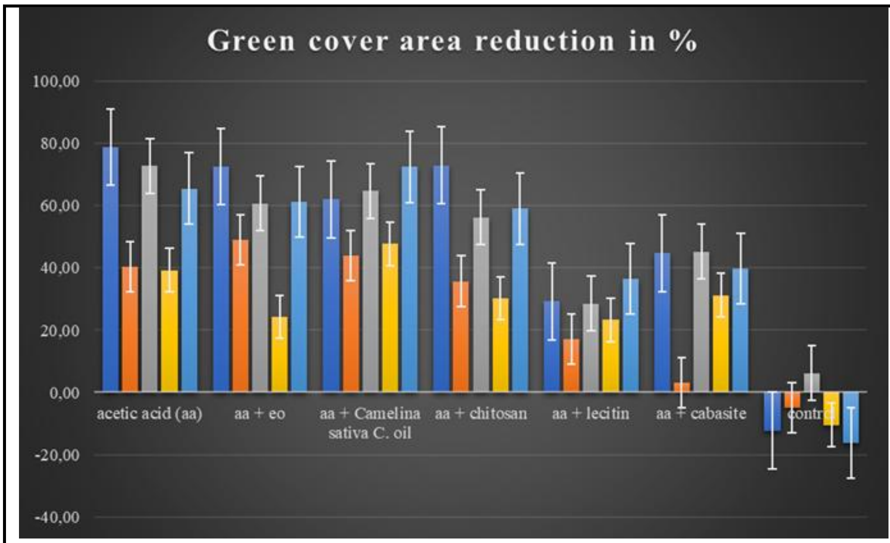

Fig. 23: evolution of green cover area for the different treatments in the second trial

22 Conclusion

Weeds significantly challenge agriculture and urban environments, causing reduced crop yield, increased production costs, and functional urban damage such as drainage blockage and allergens. They are responsible for 10% of global agricultural losses and lead to billions of euros in economic impact within the EU. While synthetic herbicides are effective, their overuse raises environmental concerns, including contamination, health risks, and weed resistance. In response, sustainable alternatives like organic farming and bioherbicides have gained attention. Bioherbicides, which include natural compounds like organic acids and essential oils (EOs), are biodegradable and less harmful to ecosystems but require multiple applications and act more slowly. Acetic acid, in particular, has demonstrated strong potential as a bioherbicide, damaging plant cell membranes and causing rapid desiccation. EOs also show great potential due to their herbicidal properties, although their mechanisms require further exploration. This research focused on evaluating acetic acid formulations, both alone and with adjuvants like EOs, for weed control under field conditions. The study tested different combinations to assess their efficacy and the role of adjuvants in enhancing weed management.

As demonstrated acetic acid alone at 20% concentration proved to be highly effective in reducing weeds GCA with substantial effects observed after a single application. The role of the adjuvant such as essential oils, *Camelina sativa* L. oil, and chitosan provided some enhancement in weed control, and their impact was comparable to acetic acid alone. Essential oils and Camelina oil showed higher performance compared to other adjuvants like lecithin and chabazite. However, the efficacy of acetic acid (alone and with adjuvants) varied based on the developmental stage of weeds and environmental conditions. Thus, environmental factors such as temperature and precipitations significantly affected the herbicidal performance. Indeed, higher temperatures in the second trial led to increased evaporation of acetic acid, reducing its contact time with weeds and necessitating repeated applications. Additionally, dicotyledonous weeds demonstrated to be more susceptible to the treatments compared to monocotyledons, for which multiple treatments were required to enhance treatments efficacy. Sequential treatments improved overall weed control and minimized regrowth. Acetic acid demonstrated immediate weed desiccation and low environmental persistence, making it a promising alternative for sensitive ecosystems. However, its rapid evaporation and the requirement for repeated applications pose challenges for field-scale use. While adjuvants showed some potential to enhance efficacy, they did not consistently outperform acetic acid applied alone. Further research should explore optimizing adjuvant formulations to improve stability and reduce evaporation rates. The observed decrease in treatment efficacy during the second trial highlights the need for strategic application timing. In conclusion, acetic acid presents a viable bioherbicide option, particularly for small-scale and organic farming systems, due to the low persistence in the environment. While effective against a broad spectrum of weeds, further studies are necessary to refine its application methods, optimize formulations, and mitigate environmental influences to enhance its consistency and efficacy under field conditions.

23 Bibliography

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FINAL CONCLUSIONS

The challenges posed by weed management in modern agriculture are multifaceted, extending beyond the immediate needs of crop protection to broader concerns about environmental sustainability, economic viability, and public health. The dominance of synthetic chemical herbicides over recent decades has revolutionized agriculture, offering efficient and cost-effective solutions to weed control. However, their widespread use has come with significant drawbacks, including environmental contamination, health risks, and the emergence of herbicide-resistant weed species. These issues are exacerbated by increasing regulatory restrictions, such as those outlined in the European Green Deal, which aim to reduce chemical pesticide usage and expand organic farming practices. Against this backdrop, the search for sustainable, effective alternatives to synthetic herbicides has become an urgent priority, with natural compounds such as organic acids and essential oils (EOs) gaining attention as potential bioherbicides. The research presented in these studies highlights both the promise and the challenges of utilizing natural compounds in weed management. Acetic acid, a naturally derived organic acid, emerged as a particularly effective bioherbicide. Its mechanism of action involves damaging plant cell membranes, leading to rapid desiccation and wilting of the weeds. In experimental trials, acetic acid demonstrated remarkable efficacy, particularly when applied at a concentration of 20%. This concentration was sufficient to reduce the green cover area of various weed species significantly, with results visible shortly after application. Its rapid action and biodegradability make it an attractive candidate for integration into sustainable agricultural practices. However, the performance of acetic acid showed some limitations. One of the primary challenges identified in the studies is its variability in efficacy, which is influenced by factors such as environmental conditions, weed species, and application timing. For example, higher temperatures were shown to accelerate the evaporation of acetic acid, reducing its contact time with the plants and necessitating repeated applications to achieve the desired level of weed control. Similarly, different weed species exhibited varying levels of susceptibility to acetic acid, with dicotyledonous weeds generally more affected than monocotyledons. These findings underline the importance of understanding the interaction between the herbicide, the target species, and the environmental context to optimize its use. The addition of adjuvants such as essential oils, Camelina oil, and other natural compounds was explored as a means to enhance the performance of acetic acid. Essential oils, known for their herbicidal properties, were particularly promising when combined with acetic acid at lower concentrations (5% and 10%).

These combinations showed increased efficacy in controlled environments, suggesting a potential synergy between the two compounds. However, at higher concentrations of acetic acid (20%), the addition of adjuvants did not consistently improve performance, indicating that their utility may be limited to specific scenarios. Moreover, the high production costs of essential oils, driven by the labor-intensive process of distillation and low yields from aromatic plants, remain a significant barrier to their widespread adoption in agriculture. The comparative analysis of other organic acids, such as pelargonic acid, citric acid, and lactic acid, provided further insights into the potential of natural herbicides. Pelargonic acid demonstrated high efficacy similar to acetic acid, but its production costs are currently prohibitive for large-scale application. Citric and lactic acids, while less effective overall, showed selective potential against certain species, making them candidates for targeted weed management strategies. These differences in efficacy can be attributed to the chemical properties of the acids, such as their pH levels and ability to penetrate plant tissues. Acetic acid's higher acidity likely contributes to its superior performance, particularly in damaging cellular structures. While the results of these studies are promising, they also underscore the complexities of transitioning to natural herbicides as a primary tool for weed management. One of the most significant challenges is the need for repeated applications. Unlike synthetic herbicides, which often provide long-lasting control, natural compounds tend to act quickly but lack residual activity. This characteristic, combined with their susceptibility to environmental factors like temperature and precipitation, necessitates careful planning and strategic application to achieve effective results. Precision agriculture technologies, which allow for targeted and efficient herbicide application, could play a crucial role in overcoming these challenges and reducing costs. The broader implications of these findings are closely tied to the principles of sustainable agriculture. The adoption of bioherbicides such as acetic acid aligns with the goals of reducing chemical dependency and minimizing environmental impact. Acetic acid, in particular, has the advantage of being readily available as a byproduct of other industries, which could lower its production costs and make it economically viable for large-scale use. This characteristic contrasts with the high costs associated with essential oils and other bioherbicides, making acetic acid a more accessible option for farmers, especially those transitioning to organic practices. However, the integration of bioherbicides into mainstream agricultural practices will require more than just technical efficacy. Economic feasibility remains a key concern, particularly for smallholder farmers who may struggle to absorb the higher costs associated with natural herbicides compared to synthetic alternatives. Policy support and incentives, such as subsidies for bioherbicide use or research grants for improving production processes, will be essential to facilitate this transition. Additionally, educational initiatives to raise awareness among farmers about the benefits and limitations of bioherbicides can help build confidence in their use. The need for further research is evident. While these studies have provided valuable insights into the efficacy and potential applications of natural herbicides, they also highlight gaps in knowledge that must be addressed to fully realize their potential. For example, large-scale field trials under diverse environmental conditions are necessary to validate the findings from controlled environments and small-scale experiments. Investigating the interactions between natural compounds and various crops, as well as their impact on soil health and non-target organisms, will provide a more comprehensive understanding of their long-term effects. Moreover, innovation in formulation and application technology could significantly enhance the utility of bioherbicides. For instance, developing formulations that improve the stability of acetic acid and reduce its evaporation rate could mitigate some of the challenges identified in these studies. Similarly, exploring the use of novel adjuvants or delivery systems could further enhance the efficacy of natural herbicides while minimizing costs and environmental impact. In conclusion, the transition from synthetic herbicides to sustainable alternatives represents a critical step toward achieving environmentally responsible agriculture. The studies presented here demonstrate the considerable potential of natural compounds such as acetic acid and essential oils in weed management, offering a pathway to reduce chemical dependency and mitigate the environmental and health risks associated with synthetic herbicides. However, the journey toward widespread adoption of bioherbicides is not without its challenges. Addressing issues of cost, consistency, and scalability will require a concerted effort from researchers, policymakers, and agricultural practitioners alike. By fostering innovation, supporting policy initiatives, and promoting education, the agricultural sector can take meaningful strides toward a more sustainable and resilient future.

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