



Impact of Varied Microwave Exposures on Weed Species

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Abstract

Purpose This study explores the efficacy of microwaves as a method for the control of three weed species particularly widespread and problematic in the Italian farms cultivating IV range crops. These findings, based on laboratory experiments, are part of a project (MOPAS Project—Microwaves for Sustainable Agriculture) meant to realize a prototype for use in real field situations.

Methods Seeds from weed species (*Trifolium repens* L., *Portulaca oleracea* L., and *Solanum nigrum* L.) were subjected to microwave treatments at 480 and 900 W power levels for 10 and 30 s. The impact on seed germination and ion leakage was assessed. Histological changes induced by microwaves were also examined through microscopic analysis.

Results Significant germinability decreases were observed in *S. nigrum* and *T. repens* with increasing treatment duration and intensity. *P. oleracea* exhibited lower sensitivity, with detrimental effects only at the highest power levels suggesting that seed size and the associated differences in water absorption may play a crucial role in determining sensitivity to microwave exposure. *S. nigrum* membrane stability was affected only at the highest irradiation power, while all species showed increased ion leakage across treatments. Observations revealed wilting of stems and leaves, indicating cell turgidity loss in inner tissue layers.

Conclusion Microwave radiation may be a non-chemical weed control strategy, though efficacy is influenced by variability in seed traits. Further research is necessary to optimize treatment parameters and assess scalability under field conditions. This approach holds potential for reducing herbicide use and promoting sustainable agricultural practices.

Keywords Cell viability · Microwaves · Seed germinability · Weed control

Introduction

Some agronomic characteristics of horticultural species, such as low planting density, their small size, and slow initial growth, make it difficult for them to compete with weeds. In controlled growing environments, such as tunnels and greenhouses, weed propagation is particularly severe due to the elevated temperatures. Additionally, weeds frequently resist

containment measures such as fumigation, and in greenhouses, manual weeding is frequently conducted prior to harvest to ensure that the soil remains clean throughout subsequent cycles. At present, there are only a handful of herbicides that are specifically registered for use in greenhouses with mowed crops, and older compounds are employed as exceptions. Moreover, herbicides have the potential to pollute the environment, contaminating directly the soil (Gao & Su, 2004).

In leafy vegetables greenhouses, various weed species, both monocotyledonous and dicotyledonous, are commonly observed, particularly during the spring–summer season. In severe cases, weeds can hinder crop growth by competing for resources. Moreover, it has been shown that treating the soil with microwaves before sowing promotes crop growth by also acting on the soil biota (Brodie et al., 2019). Additionally, the presence of weeds in the harvested raw material may introduce foreign contaminants into the final product.

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The most common weed species include *Portulaca oleracea* L., *Sorghum halepense* L., various chenopods (i.e., *Chenopodium album* L.), *Xanthium orientale* L. and *Xanthium italicum* M., different *Amaranthus* species, *Galinsoga parviflora* Cav., *Solanum nigrum* L., *Capsella bursa-pastoris* L., *Echinochloa crus-galli* L., *Senecio inaequidens* DC, *Stellaria media* CYR, *Fumaria officinalis* L., *Ammi majus* L., wild lettuce (*Lactuca serriola* L.), *Sonchus oleraceus* L., and *Digitaria sanguinalis* L.

Some weed species that are not very aggressive in other crops such as *Portulaca oleracea* L., *Stellaria media* L., *Veronica* spp. L., *Capsella bursa-pastoris* L., and *Poa annua* L. are fearsome in horticulture. Equally significant in this context are the tolerance thresholds for plant species that are toxic to humans. For example, species like *Senecio vulgaris* L. and *Solanum nigrum* L. have a strict tolerance threshold of zero as contaminants in the final product.

The present study aims to assess the potential application of microwave radiation for the management of selected pest species, with particular consideration of their morphological and physiological traits as well as interspecific variation in sensitivity. Scientific interest in the interaction between high-frequency electromagnetic waves and biological systems can be traced to the late nineteenth century (Ark & Parry, 1940). Systematic investigations on plant material originated in the early decades of the twentieth century, initially concentrating on the effects of radiofrequency (RF) exposure. In numerous instances, brief treatments were reported to enhance germination and promote seedling vigor, whereas prolonged exposure typically resulted in significant reductions in viability or complete seed mortality (Nelson & Stetson, 1985).

One of the first experimental attempts to evaluate the lethal effects of microwave heating on seeds was conducted by Davis et al. (1973). In their study, seeds were exposed to microwave irradiation both in the presence and absence of soil, using a domestic microwave oven. The findings demonstrated that seed mortality was primarily dependent on the interaction between seed moisture content and the amount of energy absorbed per unit. Furthermore, the authors identified a strong correlation between seed susceptibility and specific physical properties, including mass and volume. Subsequent research (Wolf et al., 1993) suggested that this susceptibility may be associated with the seed's "radar cross-section," a parameter that regulates the extent to which seeds intercept and absorb microwave energy. Seeds characterized by larger radar cross-sections are therefore more likely to accumulate damaging energy loads, leading to increased mortality.

Barker and Craker investigated the use of microwave heating in soils with different moisture levels (from 10 to 280 g of water per kilogram of soil) to eliminate "Ogle" oats (*Avena sativa* L.) seeds along with an unspecified number of naturalized weed seeds present in their soil

samples (Barker & Craker, 1991). Their results indicated that a seed's susceptibility to microwave treatment is entirely temperature-dependent. Specifically, when the soil temperature reached 75 °C, there was a notable decrease in germination rates for both oat and naturalized weed seeds. At temperatures exceeding 80 °C, germination was completely suppressed for all species.

In a theoretical examination of the dielectric and density characteristics of seeds and soils, Nelson (Nelson, 1996) suggested that microwaves would not effectively target seeds for heating within the soil. He determined that any damage to seeds resulting from microwave exposure is purely thermal, stemming from the heating of the soil and the subsequent thermal conduction to the seeds (Brodie et al., 2007a, 2007b). The heating process can also affect the soil environment, with positive or negative effects on microorganisms, depending on the intensity, duration, and frequency of treatment (Jurburg et al., 2017; Khan et al., 2020, 2024; Nunes et al., 2018), hence influencing soil fertility.

However, evidence indicates that microwaves can successfully eliminate various weed seeds present in the soil (Brodie et al., 2009), even if there is a scarcity of research focused on the efficacy of microwave energy in managing established weed plants (Brodie et al., 2012a, 2012b, 2021). The main scientific gaps of this research field may derive from the not complete knowledge about the microwave's penetration deep into the soil, depending on the effectiveness of the energy used for killing seeds and by the differences of soil types affecting how microwaves interact with seeds and soil particles.

Our research aims were as follows:

- (1) To determine the exact conditions (temperature, exposure time) needed to reliably control various types of weed seeds. The impact of microwaves on the germination ability was evaluated by cultivating seeds on an agar-based substrate, avoiding the temperature transfer effect from soil to seed and considering both germination rates and growth potential of the same weed species.
- (2) To evaluate the effect of exposure to different energy intensities and durations of microwaves on the germination of seeds and on plant pot trials by the evaluation of ion leakage measurement and light microscopy analysis.

These experiments were part of a project (MOPAS Project—Microwaves for Sustainable Agriculture), strongly desired by farms cultivating IV range crops that require a sustainable way to control weeds and soil diseases and parasites, aimed at realizing a prototype for use in real field situations.

Within this framework, the originality of the study resides in its contribution, through the availability of laboratory data, to the design and execution of experiments of the machinery to be used directly in the field for providing information on real-world applicability.

Materials and Methods

Weed Species Selection

A floristic survey was conducted on several farms producing type IV crops (leafy vegetables), including species such as rocket, red and green lettuce, spinach, and chard (such as red chard and Swiss chard). Weed species were identified through visual recognition of the main plant organs in both greenhouse and tunnel cultivation environments. For each weed species identified, the relative density was calculated by determining the exact number of individuals per unit area, thus defining the incidence of each species in relation to the total number of weeds sampled. In addition, weed species were identified in surrounding areas where they occurred naturally, such as semi-natural areas and field margins. Three weed species were selected based on their constant presence, density, and importance in cultivation areas. This selection highlighted the importance of understanding weed growth dynamics in IV range crop production in order to manage them effectively and ensure optimal growing conditions for the desired crops.

Plant Material

Seeds of the selected three weed species *Trifolium repens* L., *Portulaca oleracea* L., and *Solanum nigrum* L. obtained from the “seed bank” of the Apennine Center “Carlo Jucci” of the University of Perugia were used in this study. Surface sterilization of seeds was carried out by soaking them in 0.5–0.9% (v/v) sodium hypochlorite for 5 min. After discarding the bleach, seeds were rinsed three times in sterile distilled water and placed in the dark at 4 °C for 3 days. For plant cultivation, seeds were sown in sterile soil mix (Klasman-Deilmann GmbH, Geeste, Germany) into individual pots (5.5 cm diameter) and placed in a growth chamber at 28 ± 2 °C and 60–75% relative humidity, with 12-h light ($250 \mu\text{mol m}^{-2} \text{s}^{-1}$)/12-h dark cycles for 4 weeks.

Microwave Treatments

Treatments were carried out with a microwave oven (Model MW 545 De Longhi Perfecto Easy, 2.45 GHz), using two irradiation powers, an intermediate corresponding to 480 W and the maximum allowed 900 W. The microwave oven was utilized with a turntable in the cavity for rotating the

biological samples during heating to obtain a relatively uniform temperature, positioning also the samples in the same way inside the oven, not altering the treatment geometry and consequently the total energy dose delivered to the samples (Wu et al., 2020). Moreover, the plant samples, positioned in the same way inside the oven, were homogeneously subjected to the same emissions.

Each experiment consisted of five different treatments: control (CNT, no microwave treatment), treatment A (TA, 480 W for 10 s), treatment B (TB, 900 W for 10 s), treatment C (TC, 480 W for 30 s), and treatment D (TD, 900 W for 30 s).

Seed Germinability

Twenty sterilized seeds, as reported above, were placed in a 10-cm-diameter Petri dish containing 0.7% (w/v) agar in ultrapure water as substrate. After sealing the plates with Parafilm, the seeds were transferred into a growth chamber and subjected to controlled temperature (20 ± 2 °C for *T. repens* and 30 ± 2 °C for the other two species) and light conditions (light/night photoperiod 12/12, intensity $250 \mu\text{mol m}^{-2} \text{s}^{-1}$). Seed germination was assessed after 10 days; seeds were considered germinated when the radicle emerged through the tegument. The germination percentage (G%) was calculated according to the following equation: $G (\%) = \text{number of germinated seeds} / \text{total number of seeds} \times 100$. Three independent experiments were conducted, each with three replicates per treatment.

Cell Death Measurement in Plants

Cell death was assessed by measuring ion leakage from leaves. Immediately after microwave treatments, the leaves (area of approximately 13 cm^2) were excised, transferred into 15-mL Falcon tubes containing 10 mL of distilled water, and incubated for 1 h with shaking at 100 rpm at room temperature. The relative electrolyte leakage was measured using a Bibby MC1 conductivity meter and calculated as the percentage ratio between the value obtained after 1-h incubation and the total leakage evaluated after boiling the samples. Ion leakage was also assessed on untreated control leaves. The leaf damage after microwave treatments was also quantified by image analysis software ImageJ (Schneider et al., 2012). Three independent experiments were conducted, each with four replicates per treatment.

Light Microscopy Analysis in Plants

Transverse free-hand sections were meticulously prepared from both treated and untreated plant specimens. These sections were subsequently subjected to examination using a light microscope (DMLB, Leica, Wetzlar, Germany)

allowing for detailed observation of cellular structures. High-resolution photographs were captured during the analysis to document the morphological characteristics and any notable differences between the treated and untreated samples. This approach facilitates a comprehensive understanding of the histological changes induced by the treatment.

Statistical Analyses

Data relative to seed germination, leaf ion leakage, and damage measure were submitted to two-way analysis of variance (ANOVA), using the extension for Excel® DSAASTA version 1.514 (Onofri & Pannacci, 2014), followed by Tukey's test. Experimental designs are described in the figure legends.

ANOVA statistical model was utilized to analyze the effects of the different combinations between microwave exposure time and power level on weed seed viability. This approach was suitable given the experimental design's factorial structure, allowing the evaluation of both main effects and potential interactions between treatment variables. Prior to analysis, assumptions of normality and homogeneity of variances were assessed to ensure the validity of the model.

Results

Effects of Microwaves on Seed Germinability

T. repens L. showed a germination rate of approximately 80% in the control sample (Fig. 1). The most significant decline in germination, around 75% from the control value, was noted after a 30-s exposure to microwaves at both intermediate and maximum power levels (TC and TD). In contrast, a shorter microwave exposure of 10 s resulted in a smaller reduction in germination rates (TA and TB), with a decrease of about 35% from the control value.

P. oleracea L., commonly known as purslane, exhibited a germination percentage of 63% in the control sample (Fig. 1). This percentage was not significantly changed when subjected to treatments at 480 W. Conversely, exposure to maximum power levels of 900 W had a significantly detrimental effect on germination, which was also influenced by the duration of the treatment. Specifically, after 10 s of treatment (treatment B), the germination rate drops to 13.3%, and after 30 s (treatment D), it was nearly completely inhibited, falling to just 1.7%.

The species *S. nigrum* exhibited a similar behavior to that of *T. repens* across various treatments (Fig. 1). In the control sample, it achieved a germination rate of approximately 70%. Notably, the reduction in germination rates in the other treatments was less pronounced than expected. Specifically, treatments A and B yielded germination rates of about 50%,

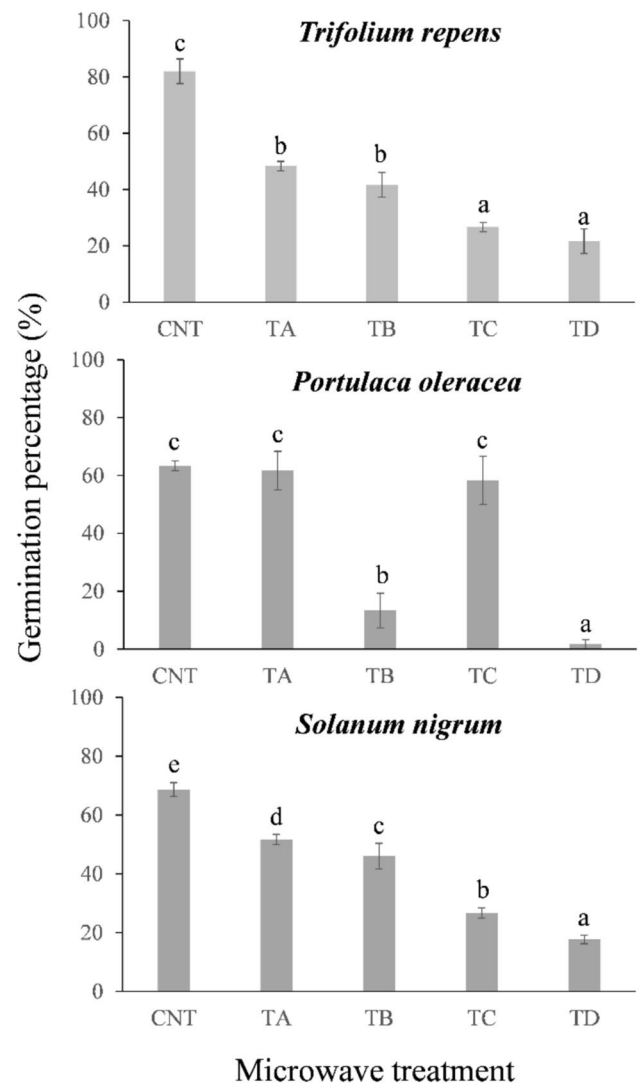


Fig. 1 Seed germinability results in the three selected weed species. Germination percentage of weed species was assessed after 10 days, and seeds were considered germinated when the radicle emerged through the tegument. In each one of the three independent experiments, three replicates of 20 seeds for treatment were performed. Data represent the mean $\pm$ SE of three independent experiments. Different letters indicate statistically different mean values ($p \leq 0.01$; ANOVA two-way, Tukey's HSD tests)

indicating a moderate decline. In contrast, treatments C and D resulted in significantly lower germination rates, dropping to below 30% and 20%, respectively.

Alteration of Cultivation Soil Temperatures After Exposure to Microwaves

Microwave irradiation induced a significant and progressive rise in soil temperature, which correlated directly with the intensity and duration of exposure (Table S1). This thermal effect was characterized by a measurable temperature

variation between pre-treatment and post-treatment conditions, reaching an increase of approximately 9 °C.

Impact of Microwaves on the Stability of Leaf Cell Membranes

The membrane permeability of *T. repens* was affected by all treatments. However, the greatest impact occurred after exposure to the highest power for the longest duration, when ion leakage increased sixfold compared to the untreated control (Fig. 2). In this case as well, the loss of membrane integrity resulted in cell death, as evidenced by the appearance of injuries, which progressively increased from approximately 22% to 55% (inset Fig. 2).

In *P. oleracea*, the trend of ion leakage mirrored that of *T. repens*. All microwave treatments produced an increased leaf ion leakage, rising from about a threefold increase after exposure to 480 W for 10 s to nearly a 6.5-fold increase following the highest intensity and duration of irradiation (900 W for 30 s) (Fig. 3). This result is mirrored in the observation of translucent or necrotic lesions, which increased with the power and duration of the treatment, reaching a damage rate of approximately 51% (inset Fig. 3).

In contrast, *S. nigrum* showed significant membrane damage only after prolonged microwave exposure, with passive ion efflux increasing from roughly 2.5 times at the lowest treatment level to nearly 9 times at the highest (Fig. 4). Indeed, the development of damaged areas on the leaves was

observed only in the TC and TD samples, with percentages of 3% and 28%, respectively (inset Fig. 4).

Macro and Micro Observations on Individuals of *Trifolium repens* L.

For the species *T. repens*, the greatest damage is evident, as with the previous species, at the maximum power and duration of microwave emission (Fig. 5). The stem appears translucent and prostrate just after treatment, with browned and withered leaves (Fig. 5B). After 24 h, more darkening and water loss are evident, with severely dehydrated leaves (Fig. 5C). Figure 5A shows the control thesis for comparison with the treated theses.

Sections of the stems of “control” plants showed the presence of several cribro-vascular bundles of different sizes where lymphatic conduction elements (cells with a large cell lumen) were evident. Clusters of sclerenchyma cells are borne in the outer part of each bundle to defend the conduction elements, with the presence of considerable percentages of cellulose that raise the fiber content of the stems considered (Fig. 5D).

Although macroscopic analysis also showed clear wilting and withering of the stems in this species, microscopic investigation revealed instead the absence of cellular damage with the presence of regularly shaped stomatal cells. The internal vascular tissues showed tracheal elements without

Fig. 2 Effect of microwaves on the stability of leaf cell membranes in *Trifolium repens*. The measurement of ion leakage was carried out immediately after the microwave treatments. An inset with leaf damage percentage quantified by ImageJ software is shown inside the figure. Values are the mean $\pm$ SD of three independent experiments with four replicates for treatment in each experiment. Different letters indicate statistically different mean values ($p \leq 0.01$; ANOVA two-way, Tukey's HSD tests). A representative image of the leaves is shown below the histogram

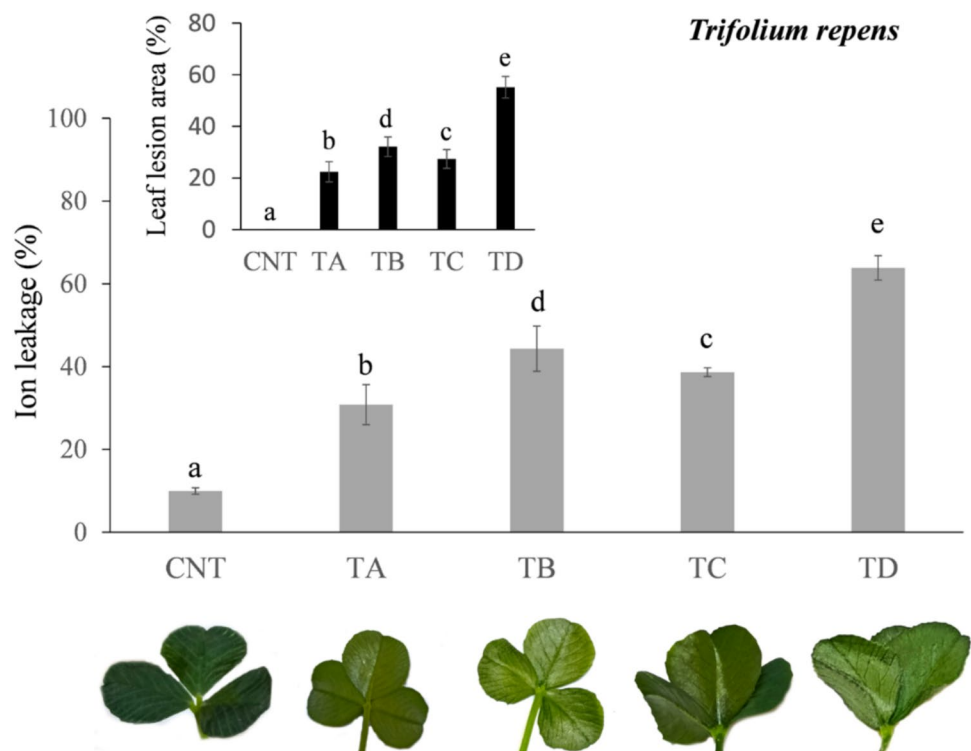


Fig. 3 Effect of microwaves on the stability of leaf cell membranes in *Portulaca oleracea*. The measurement of ion leakage was carried out immediately after the microwave treatments. An inset with leaf damage percentage quantified by ImageJ software is shown inside the figure. Values are the mean $\pm$ SD of three independent experiments with four replicates for treatment in each experiment. Different letters indicate statistically different mean values ($p \leq 0.01$; ANOVA two-way, Tukey's HSD tests). A representative image of the leaves is shown below the histogram

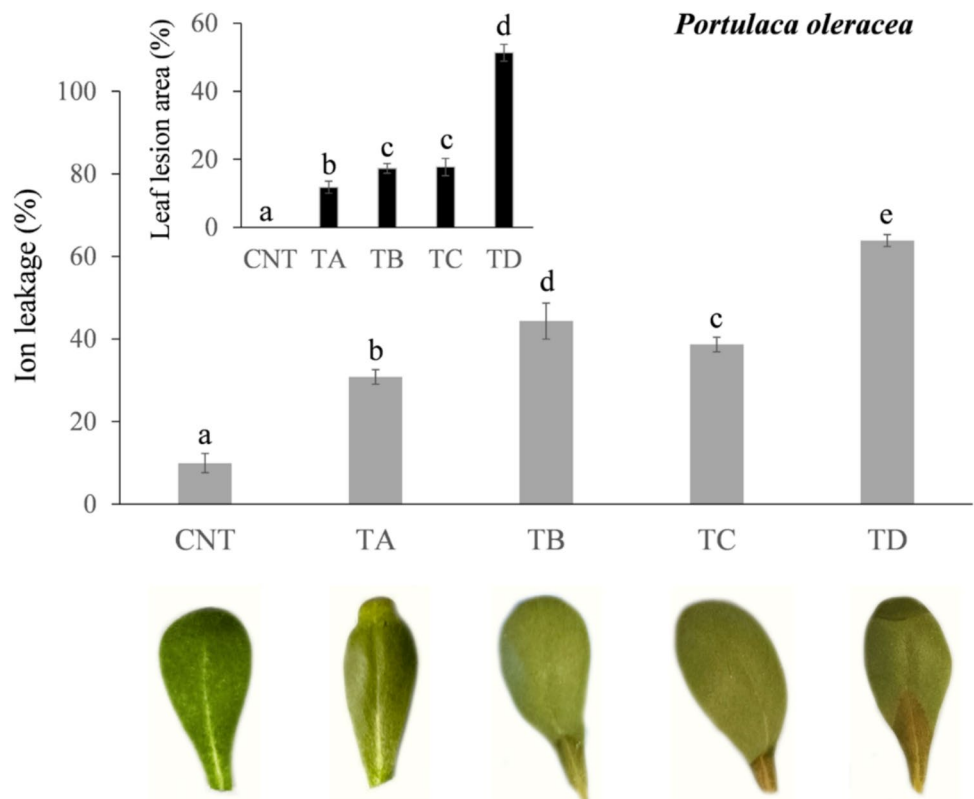
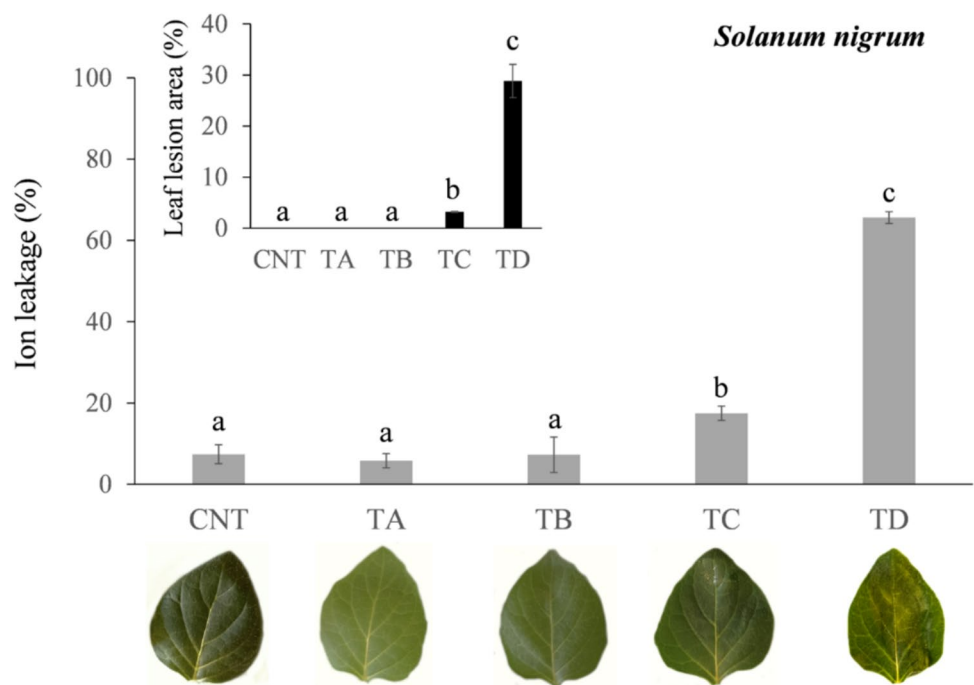


Fig. 4 Effect of microwaves on the stability of leaf cell membranes in *Solanum nigrum*. The measurement of ion leakage was carried out immediately after the microwave treatments. An inset with leaf damage percentage quantified by ImageJ software is shown inside the figure. Values are the mean $\pm$ SD of three independent experiments with four replicates for treatment in each experiment. Different letters indicate statistically different mean values ($p \leq 0.05$; ANOVA two-way, Tukey's HSD tests). A representative image of the leaves is shown below the histogram

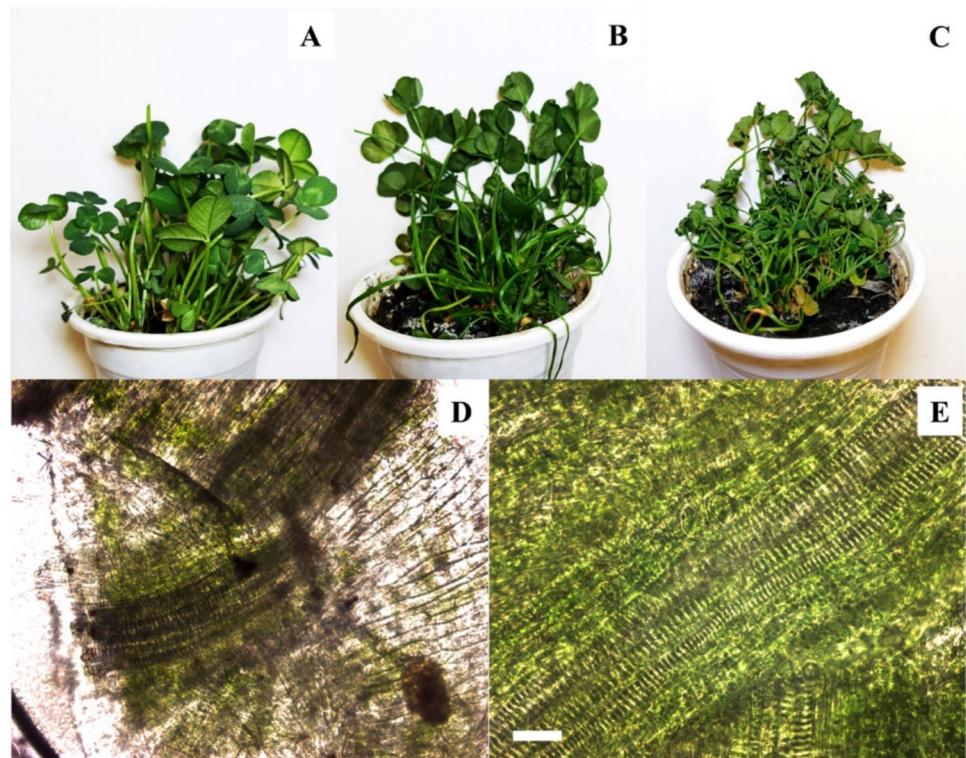


the presence of lacerations (bursts due to excessive internal pressure) but with continuity in the same elements (Fig. 5E).

All this recorded evidence does not therefore allow the transport elements to be identified as the main culprits in

the wilting of *T. repens* stems, while the loss of water in the innermost parenchyma tissue could be identified as the cause of the reduction in cell turgor of the entire medullary cylinder with 'wilting' of the entire plant.

Fig. 5 Macroscopic observations and microscopic analysis in *Trifolium repens*. **A** Control thesis, **B** plants treated at 900 W for 30 s, **C** plants treated at 900 W for 30 s after 24 h. The unit of measurement equivalent to 5 microns is given



Macro and Micro Observations on Individuals of *Portulaca oleracea* L.

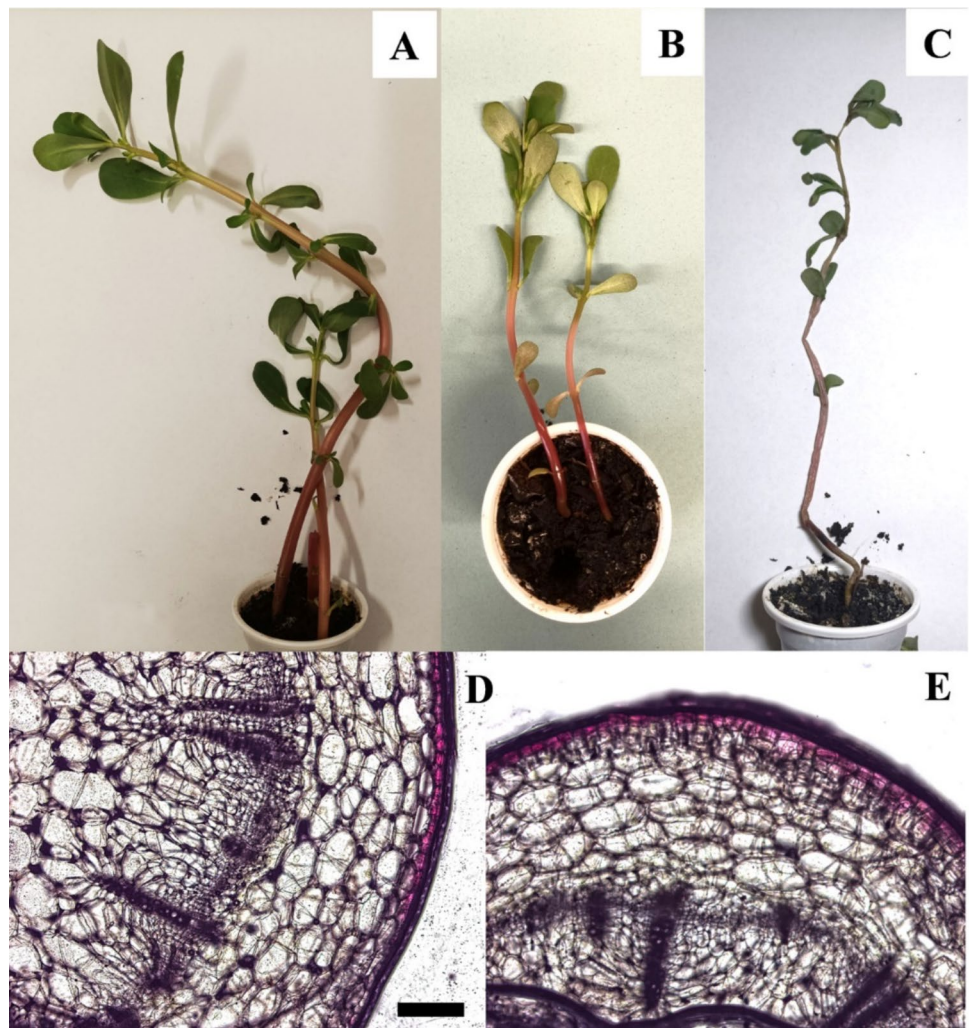
The macroscopic observation (Fig. 6) indicates that pot plants subjected to maximum power and time (TD) exhibited darkening at the base of the leaves and a translucent appearance in the stems. This effect is likely due to the significant energy absorption by water molecules during treatment (Fig. 6B). After 24 h of exposure at maximum settings, both the stems and leaves showed major damage, appearing severely dehydrated and adopting a brownish color (Fig. 6C). Figure 6A provides a control reference for comparison with the treated samples.

The stem micro-analysis of *P. oleracea* involved nearly circular cross-sections observed under various magnifications (5×, 10×, and 20×), allowing for the identification of different stem regions in both untreated and microwave-treated specimens, highlighting potential cellular damage. The sections revealed polygonal epidermal cells surrounded externally in a thick, red-pigmented cuticle. Beneath the epidermis, several layers of collenchyma cells were present, with a well-developed cortical cylinder layer. The central cylinder contained prominent vascular bundles, while a large parenchymatous medulla, composed of larger polygonal cells than those in the cortical cylinder, was also noted (Fig. 6D).

Despite the visible wilting and alterations in plants exposed to high radiation intensities during macroscopic analysis, microscopic examinations displayed that stem cells do not exhibit significant structural damage (Fig. 6E). Specifically, the chlorophyll parenchyma retained its cell size and chloroplast content. The cells within the central cylinder also appeared unchanged, with vascular bundles arranged similarly to those in untreated plants, including a cambial circle between the cortical and central cylinders (Fig. 6E).

No substantial differences were observed in the tracheal elements between treated and untreated plants, indicating no ruptures in conduction tissues or bursting of transport cells due to excessive internal pressure from heated lymph. Thus, these observations suggest that transport elements are not primarily responsible for wilting in *P. oleracea* stems. Instead, water loss from the medullary region may be a contributing factor to this phenomenon, particularly considering the thermal effects of microwaves. The collapse of the medullary zone—composed of highly vacuolated cells with significant water content and relatively thin walls—could be a key factor leading to alterations in the parenchymatous tissues forming the central column of *P. oleracea* plants. The escape of water from these parenchyma cells can quickly diminish turgor pressure throughout the medullary cylinder, resulting in wilting of the stem itself (Fig. 6E).

Fig. 6 Macroscopic observations and microscopic analysis in *Portulaca oleracea*. **A** Control thesis, **B** plants treated at 900 W for 30 s, **C** plants treated at 900 W for 30 s after 24 h. The unit of measurement equivalent to 1 micron is given



Macro and Micro Observations on Individuals of *Solanum nigrum* L.

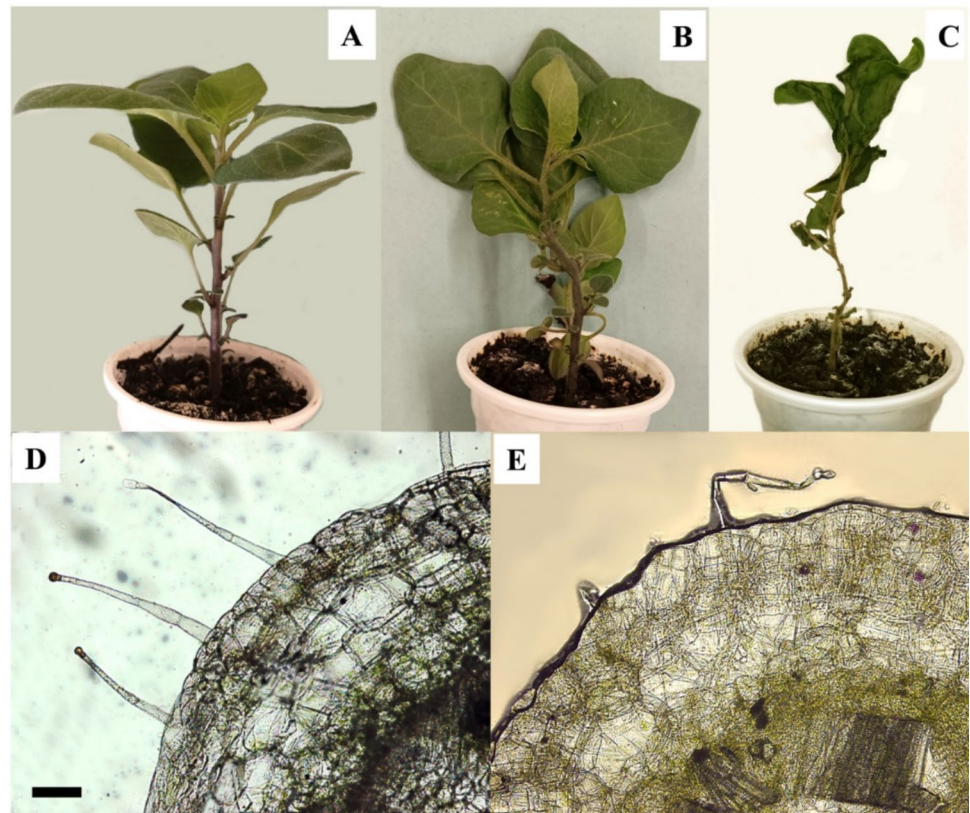
The results of the pot trials for the species *S. nigrum* showed at macroscopic level a wilting state of the plants subjected to higher radiation intensities (Fig. 7). In particular, plants treated with the highest power and at longer times (TD) showed a folding of the stems and a slight wilting of the leaves (Fig. 7B). Twenty-four hours after treatment at maximum power and duration, greater damage appeared on both the stem and the leaves where, due to loss of water and cell turgor, the leaves appeared strongly dehydrated and took on a brownish color (Fig. 7C). Figure 7A shows the control thesis for comparison with the treated theses.

The shape of the stem in the species *S. nigrum* was quadrangular, with a cross-section consisting of a uniseriate epidermis covered with cuticle and many pluricellular trichomes of considerable size (Fig. 7D). The cortical cylinder consisted of two layers: the first, below the

epidermis, composed of collenchyma tissue; the second, more internal, composed of parenchymatous tissue. The medulla, located in the center of the stem, was composed of parenchymatous cells with large lacunae (Fig. 7D). Anatomical sections made in “control” plants highlighted the outer layers of the cortical cylinder with the presence of multicellular trichomes. The cells of the parenchymatous layer were in good condition up to the cambial rim (Fig. 7D).

Sections taken from plants subjected to maximum intensity treatment (30 s, 900 W) showed trichomes with symptoms of evident thermal stress (folded trichomes with interrupted functional continuity) (Fig. 7E). The cells of the parenchymatous layer of the cortical cylinder were also damaged by the treatment, with evident cell damage and rupture of the cell membranes. These effects at the cellular level certainly induced the overall wilting of the plants treated with microwaves at maximum power (Fig. 7E).

Fig. 7 Macroscopic observations and microscopic analysis in *Solanum nigrum*. **A** Control thesis, **B** plants treated at 900 W for 30 s, **C** plants treated at 900 W for 30 s after 24 h. The unit of measurement equivalent to 3 microns is given



Discussion

This study aimed to assess the potential impact of microwaves on the control of selected weed species. Increasing attention has been focused on the effects of microwaves on living systems. Despite various findings, the exact mechanism of action of this radiation, especially on plant systems, is still unclear. The most widely accepted hypothesis is that microwaves, depending on their frequency, act through two different mechanisms: ionic conduction and dipolar rotation (Fan et al., 2024). In the case of ionic conduction, the generation of an electric field that continually alternates its orientation produces oscillations of ions and electrons with collisions between the various molecules and consequent dispersion of energy in the form of heat (Zhang et al., 2011; Horikoshi et al., 2025). In dipolar rotation, polar molecules, particularly water molecules, tend to realign along the direction of the oscillating electric field, causing internal friction and resulting in heating (Horikoshi et al., 2025; Kubo et al., 2020). Furthermore, although data are conflicting, the literature reports non-thermal effects of microwaves, unrelated to temperature increases, that can alter biochemistry and physiology of biological systems. In fact, it has been shown that under the action of high-power microwaves, but at low temperatures, membrane stability is altered, resulting in changes in cell morphology (Guo et al., 2020; Rougier et al., 2014; Shaw et al., 2021). In contrast, other studies do

not show significant non-thermal effects of microwaves on microbial metabolism (Kubo et al., 2020).

In this study, microwave irradiation induced a significant and progressive rise in soil temperature, which correlated directly with the intensity and duration of exposure. The energy generated by microwaves interacts with soil constituents, mainly water molecules, which undergo dipole rotation and friction due to alternating magnetic fields. In the soil, electromagnetic energy is therefore converted into thermal energy, causing what is known as dielectric heating, which is linked to factors such as soil texture and composition (Khan et al., 2024). Several studies suggest that the impact of microwaves on plants is mainly caused by an increase in the temperature of the soil, water, and plants (Suzuki et al., 2025). In our study, only the change in soil temperature was monitored and not that within the plant structures, so we can only assume that the observed effects on the seeds and plant are related to the heating of the potting soil or the water used to imbibe the seeds, without, however, excluding possible non-thermal effects.

Our data revealed that microwave treatments with different exposure times and doses showed significant changes in various parameters such as seed germinability and cell membrane stability in three weed species. The impact of microwaves on seed germinability has already been investigated. At low power and short exposure times, microwaves increase seed germination and seedling vigor,

whereas with increasing power and treatment time, seed germination and biomass growth decrease (Abu-Elsaoud & Qari, 2017; Ibrahim et al., 2020; Kretova et al., 2018; Lu et al., 2024; Ragha et al., 2011; Sahin, 2014). It has recently been shown that exposure to controlled microwaves increased β -glucanase activity and protein content and inhibited the expression of ethylene biosynthetic genes, promoting the germination of okra seeds (Iwuala et al., 2021). Transcriptomic and metabolomic analyses in *Arabidopsis* have shown that low microwave irradiation can influence auxin and gibberellin production and circadian clock function, increasing both plant growth and stress tolerance (Suzuki et al., 2025). Prolonged microwave treatments for minutes produce higher soil temperatures, resulting in inhibition of weed plant germination (Bebawi et al., 2007; Brodie et al., 2007a, 2007b). Also, in the present study, microwave irradiation caused reduced seed germinability of the three weed species even though they showed different susceptibility. *T. repens* and *S. nigrum* were found to be most susceptible to microwaves in terms of germination, while *P. oleracea* only reduced its germination rate at the highest power level. The different responses of these weed species suggest that both seed size and differences in water absorption are crucial in determining sensitivity to microwave exposure. Since *P. oleracea* seeds are smaller than those of the other two species, they absorb less water and consequently experience less heating during treatment. As a result, their germination is inhibited only under particularly intense exposure. Therefore, in order to achieve effective germination inhibition through microwave treatment, it is necessary to take into account the dielectric characteristics of the target weed seeds.

This study also showed that microwaves produced altered membrane stability, leading to leaf cellular death. In fact, higher treatment power and/or longer duration led to increased ion leakage, clearly indicating the severity and progression of leaf damage. Cell membrane stability has long been taken as an indicator of the effect of different stresses (Farooq & Azam, 2006), and ion leakage measurement was used here to assess the action of microwaves on leaf cell permeability. Microwave energy is known to cause increased cellular pressure due to water vaporization, resulting in altered structure of biomolecules, such as proteins and lipids, as well as changes in the permeability of cell membranes (Pakhomov et al., 1998; Ungureanu et al., 2009). In fact, microwaves have recently been widely used in plant metabolite extraction procedures (Carpentieri et al., 2021). Damage to the membrane in plant tissues often occurs due to the production of reactive oxygen species and the consequent activation of ion channels with a significant loss of calcium and potassium ions,

as well as the activation of hydrolytic enzymes involved in cell death (Demidchik, 2018; Demidchik et al., 2014).

The present study showed that the entire plant exhibited evident wilting, likely due to heat-induced damage to the stems. Microscopic analysis revealed a significant loss of cell turgor, particularly in the inner medullary tissue rather than in the outer parenchymatic layer. Medullary tissue, characterized by thin cell walls and large vacuoles, is essential for providing structural support to herbaceous plants. As its cells are particularly vulnerable to heat stress, their deterioration plays an important role in the overall wilting of the plant.



Fig. 8 Prototype of the machine that was developed from the results obtained within MOPAS project. Its features cannot be disclosed at the moment

Conclusions

The results of this study contribute to understanding the use of microwaves as an alternative method for weed control in sustainable agriculture, considering the increasing resistance to herbicides and the environmental impact of chemical treatments. The effect of microwave radiation varies depending on the specific parameters of each plant species, highlighting the importance of preliminary laboratory evaluations to determine the optimal power and time combination for effective weed control. The results of this work provide the basis for technology transfer aimed at developing a prototype (Fig. 8). Specific details on the operation of the machinery are not yet available, as the prototype is still in the early stages of development and refinement to identify practical techniques and commercial machinery capable of efficiently and economically applying microwaves over large areas of land. Given the potential implications for soil health, microbial communities, and ecosystem balance, a thorough assessment of any long-term effects remains essential.

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Data Availability The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

Ethics Approval Not applicable

Consent to Participate Not applicable

Competing interests The authors declare the following financial interests/personal relationships, which may be considered potential competing interests:

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