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Phytotoxic Effects of Xanthotoxin on Germination and Seedling Growth of *Trifolium* and *Medicago sativa* (Alfalfa)

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Abstract

Allelopathy, the chemical interaction between plants that influences the growth and development of neighboring species, has emerged as a promising strategy for sustainable weed management. Xanthotoxin (8-methoxypsoralen), a furanocoumarin belonging to the phenylpropanoid group, is naturally present in Rutaceae and Apiaceae plants such as bergamot and wild carrot. While traditionally applied in medicine, its allelopathic potential as a bioherbicide remains underexplored. This study investigated the phytotoxic effects of xanthotoxin on two agriculturally important fodder plants, *Trifolium* (clover) and *Medicago sativa* (alfalfa). Seeds were sterilized and exposed to xanthotoxin solutions at concentrations of 0.001, 0.01, 0.1, and 1 mg/ml, with distilled water plus Tween 20 serving as the control. Germination rates were recorded daily for six days, and seedling growth was assessed by measuring root and shoot lengths. Statistical analysis was performed using SPSS v21 and Zanken's test. Results revealed a clear dose-dependent inhibition of germination and seedling development. Even at the lowest concentration, xanthotoxin reduced germination compared to the control, while complete suppression of both root and shoot growth occurred at 1 mg/ml. These findings confirm the strong phytotoxicity of xanthotoxin and highlight its potential as a natural herbicide. Compared to synthetic herbicides, allelochemicals such as xanthotoxin offer advantages including biodegradability, reduced environmental toxicity, and alignment with sustainable agricultural practices. Further research is needed to evaluate its selectivity, ecological safety, and field-level applicability, but this study provides foundational evidence supporting xanthotoxin's role in environmentally friendly weed management.

Keywords: Allelopathy, Xanthotoxin, *Trifolium*, *Medicago sativa*

1. Introduction

Allelopathy, first introduced by Molisch in the early 20th century, describes the chemical interactions between plants that result in the inhibition or stimulation of growth in neighboring species. The term derives from the Greek words *allelon* (meaning “of each other”) and *pathos* (meaning “suffering”), highlighting its role in plant competition. Allelopathic interactions are mediated by secondary metabolites known as allelochemicals, which can influence seed germination, root elongation, shoot development, and overall plant fitness. These compounds are increasingly recognized as important ecological drivers of plant succession, diversity, and productivity in both natural and agricultural ecosystems (1).

In modern agriculture, allelopathy has gained attention as a potential tool for sustainable weed management. Conventional herbicides, while effective, contribute to environmental pollution, soil degradation, and the evolution of herbicide-resistant weed populations. As a result, there is growing interest in natural bioherbicides derived from plant allelochemicals, which offer reduced ecological risks and biodegradability compared to synthetic chemicals (2). Recent reviews emphasize that allelopathy can be harnessed to maintain soil health, reduce pesticide dependency, and support ecological transitions in food systems, aligning with global sustainability goals.

Xanthotoxin, also known as 8-methoxypsoralen, is a furanocoumarin belonging to the phenylpropanoid group. It occurs naturally in plants of the Rutaceae and Apiaceae families, including bergamot (*Citrus bergamia*) and wild carrot (*Daucus carota*) (3). Traditionally, xanthotoxin has been used in medicine, particularly in psoralen therapy for skin conditions such as psoriasis and leprosy. However, its allelopathic potential as a phytotoxic agent has been less explored. Previous studies have demonstrated that coumarins, including xanthotoxin, can inhibit seed germination and seedling growth in species such as lettuce, suggesting their broader applicability as bioherbicides (1).

Trifolium (clover) and *Medicago sativa* (alfalfa) are among the most widely cultivated fodder plants, essential for livestock nutrition and soil fertility. However, their aggressive growth and persistence can also make them problematic in certain cropping systems, where they compete with primary crops for resources. Investigating the allelopathic effects of xanthotoxin on these species provides insight into its potential role in weed suppression and sustainable agriculture (4).

The present study aims to evaluate the phytotoxic effects of xanthotoxin on the germination and seedling growth of *Trifolium* and alfalfa. By assessing its inhibitory activity at different concentrations, this research contributes to the broader effort of identifying natural compounds that can serve as environmentally friendly herbicides. Such work is critical in advancing sustainable agricultural practices and reducing reliance on synthetic

inputs, thereby supporting ecological resilience and long-term productivity.

2. Materials and Methods

2.1. Seed Preparation and Sterilization

Seeds of *Trifolium* (clover) and *Medicago sativa* (alfalfa) were obtained from the Pakan Seed Company in Isfahan, Iran. To ensure sterility and minimize microbial contamination, seeds were first disinfected in a sodium hypochlorite solution for three minutes. They were then thoroughly rinsed with distilled water to remove residual disinfectant. This step was critical to prevent external factors from influencing germination and growth responses to xanthotoxin.

2.2. Preparation of Xanthotoxin Solutions

Xanthotoxin, a furanocoumarin compound, was weighed precisely (20 mg) using an analytical balance and transferred into a microtube. Because of its limited solubility in water, two drops of Tween 20 were added as a surfactant to facilitate dissolution. The mixture was then transferred into a volumetric flask, diluted to 20 ml with distilled water, and shaken for approximately 11 minutes to achieve homogeneity. This stock solution had a concentration of 1 mg/ml. From this stock, serial dilutions were prepared to obtain working concentrations of 0.001, 0.01, and 0.1 mg/ml. For consistency, the control solution also contained Tween 20 in distilled water, ensuring that any observed effects were attributable to xanthotoxin rather than the solvent.

2.3. Experimental Design and Cultivation

Sterile Petri dishes were prepared by autoclaving and lined with Whatman filter paper. Eleven seeds were placed in each dish, with three replicate dishes per treatment group, including the control. In total, twelve Petri dishes were used for each species. After seed placement, the dishes were moistened with the respective xanthotoxin solutions and transferred into an incubator maintained at 21°C. Germination was monitored daily for six consecutive days. At the end of the incubation period, the lengths of the shoots (plumules) and roots (radicles) were measured using a millimeter ruler to assess growth inhibition.

2.4. Statistical Analysis

All measurements were compiled and analyzed using SPSS v21 software. Differences between treatment groups and the control were evaluated using Zanken's test, with statistical significance set at $p < 0.05$. This analysis allowed for the determination of whether observed reductions in germination and seedling growth were statistically meaningful.

3. Results

3.1. Germination Response

Exposure to xanthotoxin resulted in a clear, concentration-dependent inhibition of seed germination in both *Trifolium* and *Medicago sativa*. Even at the lowest concentration tested (0.001 mg/ml), germination rates were reduced compared to the control. As concentrations increased, the inhibitory effect became more pronounced. At 0.1 mg/ml, germination was

severely restricted, and at 1 mg/ml, germination was completely suppressed in both species, with no seeds showing signs of sprouting.

3.2. Shoot Growth (Plumule Length)

The elongation of shoots was significantly affected by xanthotoxin treatments. In control conditions, *Trifolium* plumules reached an average length of approximately 3.5 cm, while *Medicago sativa* plumules averaged around 3 cm. With increasing xanthotoxin concentrations, shoot length declined progressively. At 0.01 mg/ml, both species exhibited marked reductions, and at 0.1 mg/ml, shoot elongation was minimal. At 1 mg/ml, no shoot growth was observed, confirming complete inhibition (Fig. 1).

3.3. Root Growth (Radicle Length)

Root elongation was similarly inhibited by xanthotoxin. In the control group, *Trifolium* radicles averaged 2.5 cm, while *Medicago sativa* radicles averaged 2 cm. At 0.001 mg/ml, root growth was already reduced compared to the control, and at 0.01 mg/ml, the inhibition became more pronounced. By 0.1 mg/ml, root elongation was strongly suppressed, and at 1 mg/ml, radicle growth was entirely absent (Fig. 2).

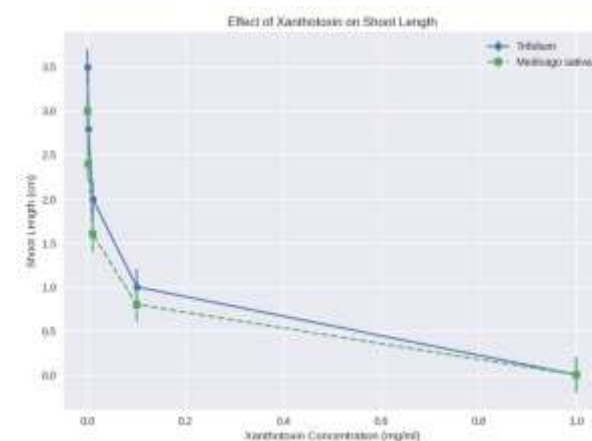


Figure 1. Effect of xanthotoxin concentration on shoot (plumule) length of *Trifolium* and *Medicago sativa*. Seeds were treated with increasing concentrations of xanthotoxin (0, 0.001, 0.01, 0.1, and 1 mg/ml). Shoot length was measured after 6 days of incubation at 21°C. Values represent mean $\pm$ standard deviation ($n = 3$). Both species showed a dose-dependent reduction in plumule length, with complete inhibition at 1 mg/ml.

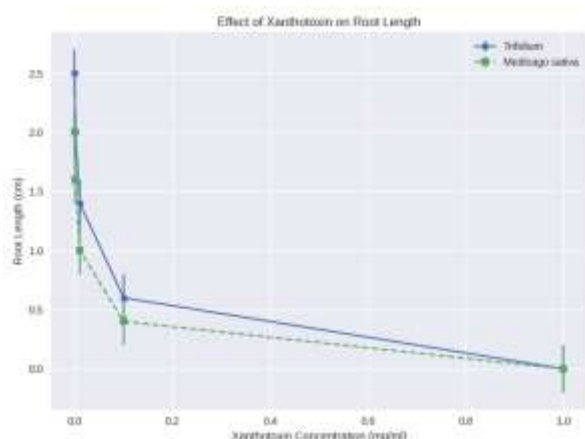


Figure 2. Effect of xanthotoxin concentration on root (radicle) length of *Trifolium* and *Medicago sativa*. Seeds were treated with the same concentrations as in Figure 1. Radicle length was measured after 6 days. Values represent mean $\pm$ standard deviation ($n = 3$). Root elongation was strongly inhibited at concentrations ≥ 0.1 mg/ml, with complete suppression at 1 mg/ml.

The results consistently demonstrated that xanthotoxin exerts strong phytotoxic effects on both germination and seedling development. The inhibitory effect was dose-dependent, with complete suppression of both root and shoot growth at the highest concentration tested. These findings highlight the potential of xanthotoxin as a natural herbicide candidate.

4. Discussion

The present study demonstrates that xanthotoxin exerts strong phytotoxic effects on both *Trifolium* and *Medicago sativa*, with complete inhibition of germination observed at 1 mg/ml. These findings are consistent with earlier reports on coumarins' allelopathic activity, such as Razavi (2011) (5), who showed that plant-derived coumarins significantly reduced lettuce germination. Our results extend this evidence to fodder legumes, highlighting the potential of xanthotoxin as a natural herbicide.

The dose-dependent suppression of root and shoot growth observed here reflects the broader mechanisms of allelopathy, which include interference with cell division, disruption of membrane permeability, and inhibition of protein synthesis. Such physiological disruptions have been documented in wheat and rice systems, where allelochemicals reduced seedling vigor and altered nutrient uptake (2, 3, 6).

From an applied perspective, the use of allelopathic compounds such as xanthotoxin aligns with the growing demand for bioherbicides in sustainable agriculture. Synthetic herbicides, while effective, contribute to soil contamination, biodiversity loss, and herbicide resistance. In contrast, natural compounds are biodegradable and often exhibit species-specific activity, reducing collateral damage to non-target organisms. Recent reviews emphasize that allelopathy can be strategically harnessed to reduce pesticide dependency and improve soil health, thereby supporting ecological resilience (7).

However, several limitations must be acknowledged. First, this study was conducted under controlled laboratory conditions; field-level validation is necessary

to assess the persistence, selectivity, and ecological safety of xanthotoxin. Second, the solvent (Tween 20) may interact with seed physiology, and future work should include solvent-only controls to isolate its effects. Third, while clover and alfalfa are agriculturally relevant, testing across a wider range of weed and crop species would provide a more comprehensive understanding of xanthotoxin's selectivity.

Despite these limitations, the findings contribute to the broader effort of identifying natural allelochemicals for weed management. As allelopathy research advances, compounds like xanthotoxin could be integrated into crop rotation systems, cover cropping strategies, or formulated bioherbicides. Such applications would support sustainable agriculture by reducing reliance on synthetic inputs, mitigating environmental pollution, and enhancing long-term productivity.

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