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Phytochemistry and Allelopathic Effects of *Tanacetum vulgare* L. (Tansy) Extracts on *Lepidium sativum* L. (Garden Pepper Cress) and *Lactuca sativa* L. (Lettuce)

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Abstract: *Tanacetum vulgare* is a perennial plant growing wild along roadsides, pastures, and agricultural fields. Its prevalence is due to several factors: good climatic adaptability, high self-seeding potential, phenotypic plasticity, multiplying via underground rhizomes and its allelochemicals, which influence the seed germination, root development and the overall vegetation of the surrounding plants. The phytochemistry of tansy extracts and their allelopathic activity on the seed germination and growth of garden pepper cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.) were investigated. The major volatile compounds, 1,8-cineole, camphor and borneol were determined in tansy flower extracts. The leaf extracts contained appreciable amounts of 1,8-cineole and borneol. Feruloylquinic, (di)ferulic and dehydrocaffeoyl-5-caffeoylquinic acids, acacetin, ludovicin C and tanacetin were determined both in leaf and inflorescence extracts. Root extracts contained minor quantities of some terpenoids and polyphenols. Extracts of *T. vulgare*'s aerial parts showed strong allelopathic effects on model plants. The flower and leaf water extracts inhibited lettuce and pepper cress seed germination and growth the most. According to the fractions, the acidic solution had the strongest effect, followed by neutral and alkaline solutions. At the highest relative concentrations of 0.5 and 1.0 tansy leaf acidic fraction, lettuce seed germination and growth decreased by 89.93% (from 35.07 ± 4.79 to 3.53 ± 2.10 mm) and by 98.46% (from 35.07 ± 4.79 to 0.57 ± 0.98 mm) compared to the control, respectively. Tansy root extracts showed weak effects. Our results demonstrated that the allelopathic inhibitory potential of tansy extracts was higher on garden pepper cress than on lettuce. The presence of allelochemicals in *T. vulgare* may have a significant impact on plant communities and ecosystems.

Keywords: *Tanacetum vulgare* L.; Asteraceae; *Lepidium sativum* L.; *Lactuca sativa* L.; extracts; GC/MS; HPLC/DAD/TOF; allelopathy

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1. Introduction

Tanacetum vulgare L. (common tansy) is a well-known aromatic and medicinal plant, mainly distributed throughout the temperate regions of the Northern Hemisphere. The plant can be cultivated in gardens and used as a spicy herb. In the ethno-pharmacology of many European countries (including Lithuania), the plant has been used for many purposes: as an anthelmintic remedy, for the healing of neurological, digestive, skin, support-motion, and respiratory system diseases [1–6]. Recent investigations have shown that *T. vulgare* is a vast natural resource with a wide range of pharmacological properties, including antioxidant [2–5,7–11], antibiotic [3,10], anti-inflammatory [3,11], cytotoxic [1–

3,7,11], antimicrobial [1,2,10–12], anthelmintic [13], diuretic [14], vascular [15], antibacterial [8,10,16], enzyme inhibition [7,16] and neuroprotective activity [7,17].

Common tansy is a valuable source for veterinary, agricultural and food industry applications. Due to its antibacterial properties, the plant extract has been applied as a seed treatment to control bacterial leaf spot in tomatoes caused by xanthomonads (*Xanthomonas perforans*) [18]. The bioactive components present in tansy root extracts have demonstrated antibacterial activity against plant pathogens, such as *Xanthomonas euvesicatoria pv. euvesicatoria* and *Pseudomonas syringae pv. maculicola* strains [16]. The plant extracts and exudates showed in vitro effects on the spreading of some phytopathogenic fungi, such as *Alternaria alternata* (Fr.) Keissl., *Botrytis cinerea* Pers., *Phytophthora cambivora* (Petri) Buisman and *Fusarium oxysporum* Schltdl [19]. In laboratory and field tests, tansy leaf extracts in 50% ethanol showed significant repellent and insecticidal activity against wingless green peach aphids (*Myzus persicae* Sulzer) [20]. The aqueous extracts have demonstrated potent insecticidal properties against the pea leaf weevil (*Sitona lineatus* L.) and black bean aphid (*Aphis fabae* Scop.) [21]. The visible impact of tansy essential oils and extracts on eggs and larvae numbers, and on the behavior of grey knot-horn (*Acrobasis advenella* Zinck.) adults has been observed [22]. One study [23] evaluated the responses to the volatile organic compounds emitted by extracts of tansy leaves on the feeding of herbivores, in particular caterpillars and aphids (*Spodoptera littoralis* Boisduval and *Metopeurum fuscoviride* Stroyan). The effectiveness of *T. vulgare* essential oils was revealed against *Acrobasis advenella* in improving the yield and fruit quality of *Aronia melanocarpa* (Michx.) Elliott [24]. The in vitro anthelmintic activity of various tansy inflorescence and leaf extracts was estimated on Trichostrongylidae nematodes in sheep [25]. The cultivation of tansy (and other perennial wild plant species) is an important factor for sustainable bioenergy (biogas) production [26].

Furthermore, *T. vulgare* is an important and most widespread weed, growing wild along roadsides, hedges, wastelands and pastures, also in areas where major agricultural crops are cultivated. Weeds are common plants that are considered undesirable as they compete with the main crops for space, moisture, nutrients, and light in the same growing area. The widespread distribution of *T. vulgare* may be attributed to several factors, including its wide range of climatic adaptability and phenotypic plasticity, great self-seeding potential, ripening of numerous seeds, vegetative reproduction via rhizomes, etc. [27–30]. Additionally, successful tansy outspreading can be explained by its allelochemicals, which have an impact on the germination of seeds, the development of roots and the overall vegetation of surrounding plants, mainly caused by insufficient mineral and water intake, damage to cell membranes, imbalance of phytohormones, disruption of the photosynthesis process, etc. [31].

The term allelopathy was first used by Austrian scientist H. Molisch in 1937, when he published the results of research concerning the action of ethylene upon some plants [32]. Allelopathy is the phenomenon whereby organisms (mainly plants) liberate specific metabolites, known as allelochemicals into the environment that have an affect (positively or negatively) on the vegetation of other surrounding plants and all biological systems [30]. Allelochemicals are mainly primary and secondary metabolites, generally water-soluble chemicals belonging to a wide range of compound classes, such as amino and phenolic acids, terpenoids, alkaloids, flavonoids, anthocyanins, lignins, glucosinolates, tannins, etc. [33,34]. Numerous recent investigations focused on tansy volatile compounds and essential oils [1,3,6,8,12,13,22,27,35–40]. The phytochemical analyses of the plant extracts revealed that it contains flavonoids, tannins, phenolic compounds, carotenoids, and their derivatives [2,4,6,7,9–12,16,24].

During the last decade, only a few papers have focused on the allelopathy of *T. vulgare* plants (Table S1 in Supplementary Materials) [41–47]. It should be mentioned that the allelopathic potential effects of tansy on neighbouring plants have not been sufficiently examined. Despite the fact that *T. vulgare* is a perennial, herbaceous, and profusely blooming plant that can reach a height of up to 1.5 m and is capable of

producing a considerable biomass. Common tansy may have a competitive advantage in a concurrence for space, light, moisture, nutrients, etc. over other species. These characteristics are of significant importance with regard to the growth conditions of the surrounding flora and the establishment of balanced ecosystems. Also, a very limited number of investigations included the full chemical composition of the tansy extracts applicable to allelopathic experiments. To the best of our knowledge, there are very few studies on allelopathy employing *T. vulgare* root extracts. The main objectives of the present research were the following: (i) to examine the detailed chemical composition of aqueous *T. vulgare* extracts prepared from separated morphological plant organs (inflorescences, leaves and roots), (ii) to estimate the allelopathic potential of the extracts with different pH values on the seed germination and growth of garden pepper cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.).

In agricultural systems, the allelopathic properties of *T. vulgare* can be employed successfully to control weeds, to inhibit plant diseases, to resist microbial pathogens, and to repel insect pests and animal herbivores.

2. Materials and Methods

2.1. Raw Herbal Material

T. vulgare plants (in quantity around 2.0–2.5 kg) were collected at full flowering stage (in August 2023) from an abandoned field (Eastern Lithuania, Trakai municipality, Padumblė village, 54°38′19.8″ N 24°59′50.7″ E). The growing locality (Figure S1 in Supplementary Materials) was marked on the Geographical Information System map. The area of common tansy population under investigation was approximately 100 m^2. The plants were gathered from random sampling sites where they were in full bloom. The raw material, which included both above- and below-ground plant parts, was air-dried for 2 weeks under shade conditions. The optimum storage temperature was maintained at 20–24 °C. Prior to the drying process, the herbal material was divided into three categories: leaves, inflorescences and roots.

The herbal material has been identified, a voucher specimen of *T. vulgare* was deposited at the Vilnius University Herbarium (WI, Lithuania), and the code number assigned to this item is P33884.

Garden pepper cress (*Lepidium sativum*) and lettuce (*Lactuca sativa*) were selected as the model plants for the allelopathic tests. Seeds for the plants were purchased from a local vegetable market.

2.2. Preparation of Extracts for Allelopathic Test

Fifteen grams of crushed herbal material (separately: roots, leaves and inflorescences) with 200 mL (150 mL in the case of roots) of distilled water were macerated in an ultrasonic bath at room temperature (21–24 °C) for a period of 45 min. The mixture was filtered through an 11 μm pore size filter paper and divided into three portions of 25 mL each.

Hydrochloric acid (HCl at 0.1 M concentration) and potassium hydroxide (KOH at 0.5 M) were applied to attain acidic (pH ca. 3.5) and alkaline (pH ca. 9.5) media of the solutions, respectively. A volume of 1.5 mL of the solution was considered as 1.0 of relative concentration used for the allelopathic experiments.

2.3. Preparation of Tansy Extracts for Analysis of Volatile Organic Compounds (VOCs)

Five mL of each aqueous solution (prepared according to the method described in Section 2.2) was extracted with 1 mL of diethyl ether by shaking in a separatory funnel for 5 min. Diethyl ether was purchased from C. Roth GmbH & Co. (Karlsruhe, Germany).

2.4. Preparation of Tansy Extracts for High-Performance Liquid Chromatography/Diode Array Detector/Time of Flight Mass Spectrometry (HPLC-DAD-TOF) Analysis

The different air-dried morphological parts (roots, leaves and flowers) of the tansy plant were ground to homogenous content. Herbal samples were protected from light and moisture in paper bags.

Approximately 2 g of ground herbal material was macerated with 20 mL of a solvent mixture (methanol/water, 1:1, *v*/*v*). The extraction procedure was carried out in an ultrasonic bath for 30 min without additional heating. The mixture was filtered through filter paper (pore size 11 μm) and the solutions were then purified through nylon syringe filters (0.22 mm) prior to chromatographic analysis.

Methanol was purchased from Honeywell (Seelze, Hanover, Germany).

2.5. Gas Chromatographic Analysis of VOCs in T. vulgare Extracts (Water/Diethyl Ether)

2.5.1. Gas Chromatography (GC/FID(Flame-Ionization Detector)) Analysis

A chromatograph (HP 5890II) interfaced with an FID (Hewlett Packard, Palo Alto, CA, USA), and fitted with HP-FFAP (polyethylene glycol 30 m × 0.25 mm i.d., film thickness 0.25 μm) and DB-5 ((5%-phenyl)-methylpolysiloxane; 50 m × 0.32 mm × 0.25 μm) capillary columns (Agilent, J&W Scientific, Santa Clara, CA, USA) was used for quantitative analyses of tansy extracts.

The GC oven temperature was programmed as follows: at 50 °C isothermal for 1 min, then increased at a rate of 5 °C/min up to 160 °C (held for 2 min), then heated to 280 °C at a rate of 10 °C/min, and finally, isothermal conditions maintained at 280 °C for 4 min. The temperature of the injector and detector was supported at 250 °C. The flow rate of carrier gas (hydrogen) was held 1 mL/min. At least 3 replicates ($n \geq 3$) per analysis were carried out.

2.5.2. Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

The Shimadzu GC-2010 PLUS (Shimadzu, Kyoto, Japan) chromatograph, equipped with a Shimadzu GC-MS-QP2010 ULTRA mass spectrometer (Shimadzu, Kyoto, Japan), and fitted with a capillary column Rxi-5 MS ((5%-phenyl)-methylpolysiloxane, 33 m × 0.25 mm i.d., film thickness 0.25 μm) (Restek, Bellefonte, PA, USA) was used for the qualitative analysis of VOCs in tansy extracts.

The chromatographic separation conditions were identical to those employed in the GC (FID) analysis. The temperature of the injector and detector was maintained at 250 °C throughout the experiment. The temperature of the ion source was held at 220 °C. The flow rate of carrier gas (helium) was maintained at 1 mL/min with a split ratio of 1:20. At least two replicates ($n \geq 2$) were conducted for each analysis. The mass spectrometer parameters were set to a mass range of 100–1700 m/z, with a rate of 0.97 scans per second. The spectra were generated at 70 eV.

2.5.3. Identification of Individual Components

Qualitative analysis of VOCs was established by comparing retention indexes on a polar and non-polar column, co-injection of n-alkane (C_8–C_{28}) mixture and some reference compounds, such as *α*-, *β*-pinene, *p*-*c*ymene, 1,8-cineole, camphor, *α*-, *β*-thujone, *α*-terpinene, verbenone, carveol and eugenol. Additionally, a comparison of the recorded mass spectra was performed with corresponding data presented in the computer mass spectra libraries (NIST, Flavors and Fragrance of Natural and Synthetic Compounds 2 (FFNSC 2) and Wiley) and in the literature [48]. The relative proportions of compounds are expressed as percentages, and the identification was accepted if the computer match with the mass spectrometry libraries gave a probability greater than 95%.

2.6. HPLC-DAD-MS (TOF) Analysis of T. vulgare Extracts

Tansy root, leaf and flower extracts in 50% methanol were analyzed by the following HPLC/DAD (diode array detector)/TOF (time of flight detector) technique. The DAD of the Agilent 1260 Infinity model was produced by Agilent Technologies Corporation (Waldbronn, Germany) and the TOF is the Agilent 6224 TOF model (Agilent Technologies, Santa Clara, CA, USA). A reverse phase column ZORBAX Eclipse XDB (C18, size of particles was 5 µm, column parameters were 150 × 4.6 mm, Agilent Technologies, Santa Clara, CA, USA) was maintained at 35 °C during the chromatographic analysis. The solvents used for the procedure were A: deionized water containing 0.1% trifluoroacetic acid and B: acetonitrile containing 0.1% trifluoroacetic acid. Acetonitrile was purchased from Honeywell (Seelze, Hanover, Germany); trifluoroacetic acid—from Sigma Aldrich Co. (St. Louis, MO, USA). In the HPLC system, the following stepwise gradient elution method was applied: initial 45% (A)/55% (B); from 0 to 15 min from initial ratio to 25% (A)/75% (B); from 15 to 27 min to 0% (A)/100% (B), from 27 to 30 min to initial composition 45% (A)/55% (B) and isocratic mode for 1 min. The chromatographic separation was performed at a flow rate of 1.0 mL/min. Molecules were ionized in positive or negative mode using the Electrospray Ionization Interface (ESI). Extract volumes ranging from 5 to 15 µL were injected using an automated sampler. DAD was set at 254, 280, 325, 340, 364, 380, 420 and 450 nm.

The TOF acquisition parameters were set at mass range 100–1700 m/z, rate 1.42 spectra/s, time 704.2 ms/spectrum. Ionization source conditions were the following: nebulizer 30 psig, drying gas temperature 300 °C, drying gas flow rate 7 L/min and fragmentor voltage 125 V. An internal calibration solution with known reference masses was applied to achieve the mass accuracy of the recorded data.

2.7. Seed Germination Bioassay

Seeds of *Lactuca sativa* and *Lepidium sativum* were sterilized by soaking them in 70% ethanol for 2 min. The seeds were then washed three times with deionized water. Twenty seeds per dish were kept in the dark at 25 °C on the Whatman filter paper in glass Petri dishes (Ø 9 cm) with 1.5 mL of either treatment (extracts of different morphological tansy parts with adjusted pH values) or control solutions (deionized water with adjusted pH values) for five days. Three replicates were conducted for each concentration, and the number of germinated seeds was counted over five days after the incubation period. Germination was considered completed when the radical ruptured and emerged from the seed (Figure S2 in Supplementary Materials). Percentage of germination was determined by counting the number of germinated seeds after five days. To compare treatments, the radicle and plumule lengths of the model plants were measured using a caliper tool (Mitutoyo, Aurora, IL, USA). After 5 days of incubation, 20 seedlings per Petri dish were measured for their radicle length (RL) and plumule height (H), which were combined to determine plant length (PL). The relative germination (RG), germination rate (GR), and vigor index (VI) of *L. sativum* and *L. sativa* were calculated according to equations presented in Table 1 [49].

Table 1. Calculation model of germination rate (GR), relative germination (RG) and vigor index (VI) of garden pepper cress (*L. sativum*) and lettuce (*L. sativa*).

Index	Equation
Germination rate (GR), %	GR = final number of germinated seeds after 5 days of incubation/20 × 100%
Relative germination (RG), %	RG = GRtr (%)/GRcn (%) × 100 GRtr—mean seed germination for each treatment GRcn—mean seed germination for control
Vigor index (VI)	VI = PL (mm) × GR (%)

2.8. Statistical Analysis of Data

The obtained results were statistically processed by calculating the Pearson correlation coefficient (r); the results are expressed as average values, range intervals, and standard deviation (SD) values, using XLSTAT (Addinsoft 2014, Paris, France). The PAST version 4.03 was employed for the statistical analysis of the data on purpose to define the reliability and significant differences. The independent groups were compared using a one-way ANOVA test. The differences between the treatment groups and the control were compared using Tukey's test, with a significance level of $\alpha = 0.05$. The one-way ANOVA test was used to ascertain statistically significant differences in the estimation of germination rate, relative germination and vigor index.

3. Results and Discussion

3.1. Compositional Data of Volatile Organic Compounds (VOCs) in T. vulgare Water/Diethyl Ether Extracts

GC/FID and GC/MS techniques were used for the quantitative and qualitative analysis of the VOCs. The detailed chemical composition of the volatile compounds present in tansy flower and leaf extracts (at different pH values) is shown in Table 2. In total, around 50 different constituents were identified in tansy extracts from the aerial parts.

Root extracts contained minor quantities of some terpenoids, such as borneol, 1,8-cineole, spathulenol, humulene epoxide II, dihydroactinidiolide and α-bisabolol oxide A.

Table 2. Volatile organic compounds (VOCs) determined in *T. vulgare* inflorescence and leaf extracts (water/diethyl ether) of different acidities (n = 3, average mean ± SD).

Compound (RI $_{Exp}$) *	Flowers			Leaves		
	pH = 3.5	**pH = 4.9**	**pH = 9.5**	**pH = 3.5**	**pH = 6.0**	**pH = 9.5**
α-Pinene (935)	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.2	0.4 ± 0.1
β-Pinene (978)	0.4 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	0.4 ± 0.3	0.7 ± 0.3	0.5 ± 0.3
1,8-Dehydrocineole (990)	0.5 ± 0.2	0.5 ± 0.2	tr.	0.5 ± 0.2	0.2 ± 0.1	tr.
Yomogi alcohol (998)	0.7 ± 0.2	0.9 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	tr.	0.1 ± 0.0
α-Phellandrene (1006)	0.9 ± 0.6	tr.	tr.	0.2 ± 0.1	0.1 ± 0.0	tr.
α-Terpinene (1016)	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.3	0.8 ± 0.4	tr.	tr.
p-Cymene (1018)	0.8 ± 0.0	0.5 ± 0.3	0.3 ± 0.1	0.4 ± 0.2	0.6 ± 0.2	0.3 ± 0.1
1,8-Cineole (1033)	17.1 ± 2.6	15.5 ± 2.3	12.8 ± 2.1	28.1 ± 2.7	31.3 ± 3.3	30.0 ± 1.2
γ-Terpinene (1062)	tr.	0.4 ± 0.3	0.2 ± 0.1	0.9 ± 0.3	0.4 ± 0.2	tr.
Artemisia ketone (1062)	1.1 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	tr.	tr.	
Artemisia alcohol (1083)	0.5 ± 0.1	0.9 ± 0.2	0.2 ± 0.1			
Terpinolene (1088)	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
Linalool (1098)	0.9 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	tr.	0.2 ± 0.1	tr.
α-Thujone (1103)	0.4 ± 0.3	0.3 ± 0.2	tr.		tr.	
cis-Sabinene hydrate (1068)	tr.	0.6 ± 0.2	0.9 ± 0.2	0.5 ± 0.1	1.6 ± 0.4	2.4 ± 0.9
trans-Sabinene hydrate (1098)	0.2 ± 0.0	1.7 ± 0.2	1.5 ± 0.4	0.7 ± 0.2	2.4 ± 0.4	2.9 ± 1.1
β-Thujone (1115)	2.3 ± 1.5	1.9 ± 0.2	1.7 ± 0.2	0.3 ± 0.1	tr.	0.4 ± 0.2
Chrysanthenone (1124)	1.4 ± 0.2	1.0 ± 0.2	1.1 ± 0.3		tr.	
Nopinone (1136)	0.6 ± 0.2	0.7 ± 0.2	0.3 ± 0.2			
trans-Pinocarveol (1137)	1.4 ± 0.7	0.8 ± 0.1	0.6 ± 0.2	0.8 ± 0.4	0.5 ± 0.3	0.6 ± 0.3
Camphor (1145)	11.8 ± 2.3	10.9 ± 0.2	9.8 ± 1.5	0.6 ± 0.4	0.7 ± 0.1	0.8 ± 0.3
Isoborneol (1155)	tr.	0.3 ± 0.2	tr.	0.2 ± 0.1	0.6 ± 0.3	0.5 ± 0.1
Sabina ketone (1156)	0.3 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	tr.
cis-Chrysanthenol (1161)	2.2 ± 0.8	1.9 ± 0.2	1.7 ± 0.3	tr.	tr.	0.2 ± 0.1

Pinocarvone (1163)	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.2	0.4 ± 0.1	0.3 ± 0.2	0.3 ± 0.1
Borneol (1165)	13.9 ± 2.7	11.1 ± 1.5	11.7 ± 1.8	20.9 ± 1.0	24.2 ± 2.5	24.2 ± 2.3
Terpinen-4-ol (1174)	3.4 ± 1.1	0.3 ± 0.1	1.8 ± 0.2	4.9 ± 0.5	1.4 ± 0.2	0.9 ± 0.3
p-Cymen-8-ol (1183)	0.3 ± 0.1	0.6 ± 0.2	0.2 ± 0.1	tr.	tr.	tr.
α-Terpineol (1190)	3.9 ± 1.6	3.6 ± 0.3	2.9 ± 0.7	3.6 ± 0.3	2.5 ± 0.3	3.7 ± 1.1
Z-Dihydrocarvone (1193)	2.9 ± 0.9	2.5 ± 0.9	3.9 ± 0.7	1.9 ± 0.7	1.4 ± 0.5	1.9 ± 0.3
E-Dihydrocarvone (1201)	8.5 ± 2.2	6.9 ± 0.9	5.1 ± 1.3	3.9 ± 1.9	3.4 ± 1.5	3.7 ± 1.3
trans-Piperitol (1205)	0.2 ± 0.1	0.4 ± 0.1	tr.	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.0
2-Hydroxycineole (1208)	0.2 ± 0.0	tr.	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.3
iso-Dihydrocarveol (1213)	0.7 ± 0.3	0.9 ± 0.4	0.9 ± 0.3	0.7 ± 0.3	1.5 ± 0.6	0.7 ± 0.4
trans-Carveol (1218)	1.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.2
neo-iso-Dihydrocarveol (1224)	0.5 ± 0.2	0.4 ± 0.1	1.1 ± 0.1	0.9 ± 0.4	0.3 ± 0.1	0.6 ± 0.2
cis-Ascaridole (1236)	0.6 ± 0.3	tr.	tr.			
Carvone (1242)	0.5 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Theaspirane (1255)				1.5 ± 0.3	tr.	0.3 ± 0.2
cis-Chrysanthenyl acetate (1264)	0.4 ± 0.1	2.0 ± 0.2	0.5 ± 0.3			
Bornyl acetate (1286)	0.7 ± 0.2	0.7 ± 0.3	0.3 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	0.1 ± 0.0
Eugenol (1359)	1.3 ± 0.4	1.1 ± 0.3	0.5 ± 0.2	0.9 ± 0.2	tr.	0.9 ± 0.3
Z-*β*-Damascone (1360)		tr.		0.5 ± 0.1	0.4 ± 0.2	0.3 ± 0.1
Dihydroactinidiolide (1498)	0.5 ± 0.3	0.6 ± 0.2	0.6 ± 0.3	1.9 ± 0.2	1.9 ± 0.3	1.9 ± 0.1
E-Nerolidol (1564)	0.6 ± 0.4	0.5 ± 0.3	0.7 ± 0.4	tr.	0.3 ± 0.1	tr.
Spathulenol (1577)	0.9 ± 0.3	0.2 ± 0.0	0.5 ± 0.3	0.4 ± 0.3	0.2 ± 0.0	0.3 ± 0.2
Humulene epoxide II (1607)	0.5 ± 0.1	1.5 ± 0.3	2.5 ± 1.3	1.3 ± 0.2	2.4 ± 0.7	1.9 ± 0.3
τ-Muurolol (1645)	1.1 ± 0.5	0.5 ± 0.2	0.5 ± 0.3		tr.	
Patchouli alcohol (1658)	2.0 ± 0.4	1.8 ± 0.3	tr.			
Cedroxyde (1705)	2.1 ± 1.0	1.9 ± 0.4	1.7 ± 0.4	1.9 ± 0.3	1.8 ± 0.2	2.1 ± 0.7

* RI $_{Exp}$: retention index on the Rxi-5MS column was determined experimentally. Components are listed in the order of their elution from a non-polar Rxi-5MS column. Compounds were identified by their mass spectra and retention indices on both columns (polar HP-FFAP and non-polar Rxi-5MS). tr.—trace amounts ≤ 0.05%.

Numerous studies have been conducted to examine the chemical composition and properties of tansy essential oils, and a remarkable intra-specific variation of them has been revealed [1,3,6,8,12,13,27,35–40]. However, data on the volatile composition of tansy extracts are rather limited [22,50]. Five chemotypes of VOCs were assigned to tansy leaf (cultivated in a multi cuvette system) extracts in hexane [22]. In the above study, the following terpenoids, (*Z*)-dihydrocarvone (>30%), myrtenol (~25%), myrtenyl acetate (>10%), camphor (>70%), sabinene (~20%), 1,8-cineole (~15%) and (*E*)-sabinene hydrate (>10%) dominated. In another study [50], the flowering tops of twenty Finnish tansy genotypes were extracted with petroleum ether. Fifteen were well-defined and five were classified as mixed chemotypes of the tansy genotypes under the investigation. The most commonly found monoterpene was camphor, with or without satellite compounds, such as the following: camphene, pinocamphone, 1,8-cineole, chrysanthenyl acetate, bornyl acetate and isobornyl acetate. Other chemotypes containing appreciable amounts of myrcene, tricyclene, *trans*-thujone, artemisia ketone, artemisyl acetate or davanone D were described [50]. In our research, tansy extracts could be attributed to the mixed chemotypes: 1,8-cineole (≤17.1%) + borneol (≤13.9%) + camphor (≤11.8%) for inflorescences; and 1,8-cineole (≤31.3%) + borneol (≤24.2%) for leaves (Table 2). The leaf extracts differed from the flower ones mostly in the amounts of camphor. At different pH values, the quantities of the main components also varied. The root extracts were low in VOCs. This is despite the fact that in many cases the root and aerial parts contain secondary metabolites of the same compound class, especially terpenoids, which are dominant in aromatic plants [51]. The data we obtained, showing that only minor

amounts of terpenoids were determined in the tansy root extracts, agreed well with the results obtained by Móricz, Á.M. et al., where none of the volatiles of the aerial part were identified in the tansy root extract [16].

3.2. Chemical Composition of Non-Volatile Components in T. vulgare Extracts (Water/Methanol, 1:1, v/v)

In tansy leaf and inflorescence extracts prepared in accordance with the method described in Section 2.3, a total of about 25 compounds were identified. All of the components were detected by DAD and TOF with either the positive or negative ionization method (Table 3). Some compounds gave m/z ions with both (positive and negative) ionization modes.

Root extracts contained minor amounts of phenolic acids, such as succinic, quinic, isochlorogenic (A and B) and feruloylquinic. Also, luteolin, *O*-caffeoyl glucoside ($C_{15}H_{18}O_9$), pontica epoxide ($C_{13}H_{10}O$), 6-methoxykaempferol and 5,7,3′-trihydroxy-3,6,4′,5′-tetramethoxyflavone were identified in tansy root extracts in 50% methanol.

Table 3. List of constituents in *T. vulgare* leaf and inflorescence extracts examined by HPLC-DAD-TOF technique (n = 3, average mean ± SD).

Compound	Chemical Formula	Molecular Weight, g/mol	*m*/*z* ESI$^+$, Da	*m*/*z* ESI$^-$, Da
Acids:				
Succinic acid	$C_4H_6O_4$	118.09	119.08	
Quinic acid	$C_7H_{12}O_6$	192.17	194.15	191.017
3-Dehydrocaffeoyl-5-caffeoylquinic acid	$C_{25}H_{22}O_{12}$	514.10	514.30	
4-Dehydrocaffeoyl-5-caffeoylquinic acid	$C_{25}H_{22}O_{12}$	514.10	514.31	
Feruloylquinic acid	$C_{17}H_{20}O_9$	368.10	368.42	
Diferulic acid	$C_{20}H_{18}O_8$	386.4	387.11	384.94
Protocatechuic/gentisic acid	$C_7H_6O_4$	154.02	154.98	
Ferulic (hydroxycinnamic) acid	$C_{10}H_{10}O_4$	194.18	194.15	
Isochlorogenic (3,5-dicaffeoylquinic) acid A	$C_{25}H_{24}O_{12}$	516.45	517.13	515.12
Isochlorogenic (3,4-dicaffeoylquinic) acid B	$C_{25}H_{24}O_{12}$	516.45	517.13	515.12
Other compounds:				
p-Hydroxyphenylacetic acid 1-*O*-hexoside	$C_{14}H_{18}O_8$	314.09	314.08	
Luteolin	$C_{15}H_{10}O_6$	286.24	288.22	
Kaempferol	$C_{15}H_{10}O_6$	286.24	287.20	
Quercetin	$C_{15}H_{10}O_7$	302.24	304.21	
Acacetin	$C_{16}H_{12}O_5$	284.26	286.19	284.22
Ludovicin C	$C_{15}H_{18}O_4$	264.13	265.14	264.13
Hydroxyarbusculin	$C_{15}H_{22}O_4$	266.16	266.17	
6-Methoxykaempferol/Isorhamnetin	$C_{16}H_{12}O_7$	316.05	317.23	
Isorhamnetin 3-*O*-glucoside	$C_{22}H_{22}O_{12}$	478.10	478.92	
Tanacetin/armefolin	$C_{15}H_{20}O_4$	264.32	265.14	
5,7,3′-Trihydroxy-3,6,4′,5′-tetramethoxyflavone	$C_{19}H_{18}O_9$	390.3	391.28	

The majority of the compounds identified in tansy extracts in 50% methanol (Table 3) had previously been characterized [1,4–10]. It should be noted that some common flavonoids (apigenin, rutin, (iso)quercitrin, eupatorin, hispidulin, etc.), often present in *T. vulgare* herbal material, were not detected (or their levels were below detection limits) in the tansy extracts in this study.

3.3. Percentage of Relative Germination (RG) and Vigor Index (VI) of Lactuca sativa and Lepidium sativum Affected by Tansy (T. vulgare) Extracts

The extracts *of T. vulgare* had significant effects on the seed germination and growth of the tested lettuce (*Lactuca sativa*) (Figure 1) and garden pepper cress (*Lepidium sativum*) (Figure 2). The leaf and inflorescence extracts of *T. vulgare* had the strongest effect on the relative germination (RG) and vigor index (VI) of lettuce and pepper cress. Values for the RG, GR, and VI are presented in Tables S2–S4 in the Supplementary Materials.

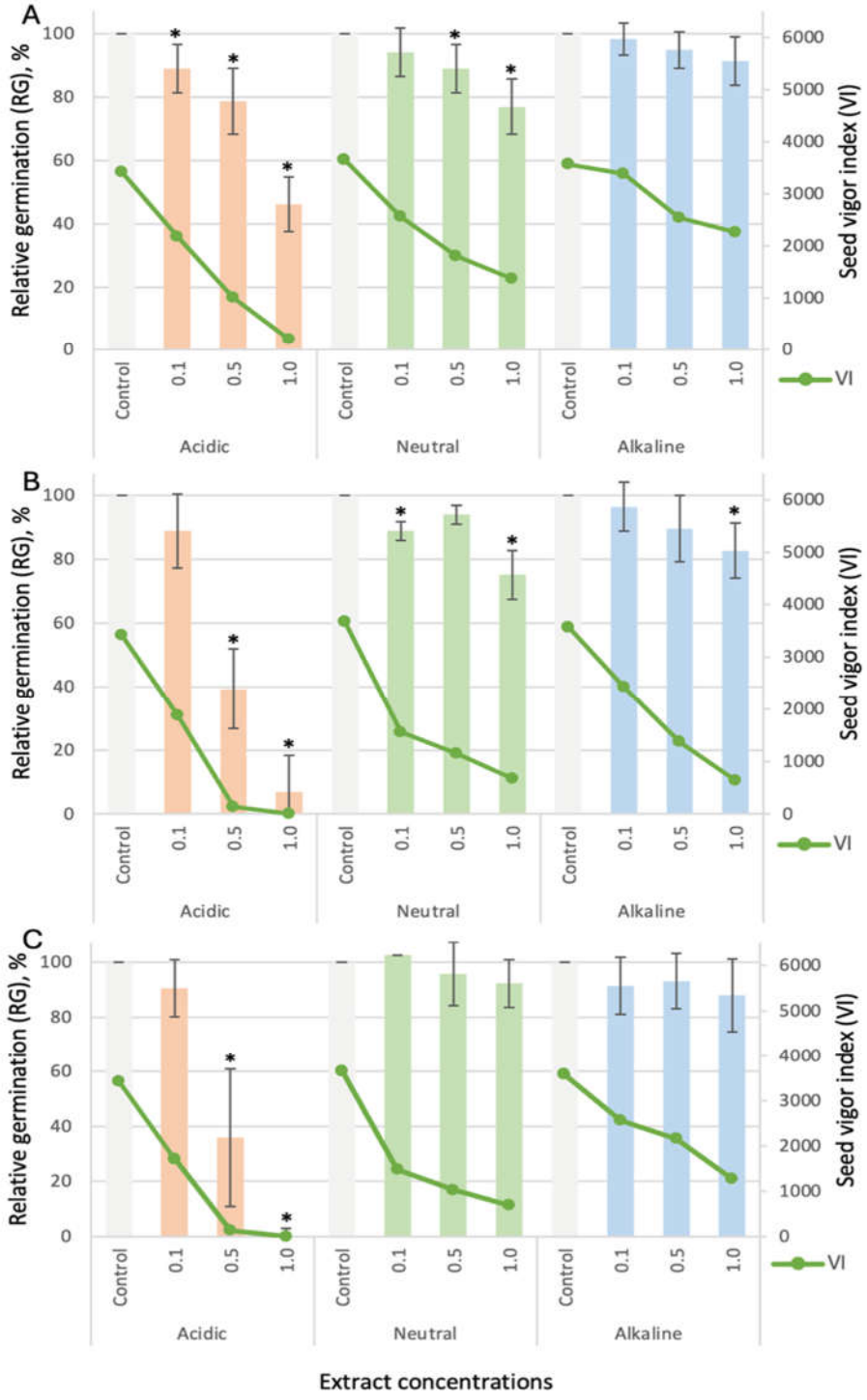

Figure 1. The inhibitory effect of *T. vulgare* aqueous extracts from roots (**A**), leaves (**B**) and flowers (**C**) on RG and VI of lettuce seeds was assessed using a one-way ANOVA test ($p < 0.05$). The data are presented as the means of repetitions (n = 60) ± SD (bars). Statistically significant differences

between the experimental and control groups (water with various pH values) are marked with asterisks (*).

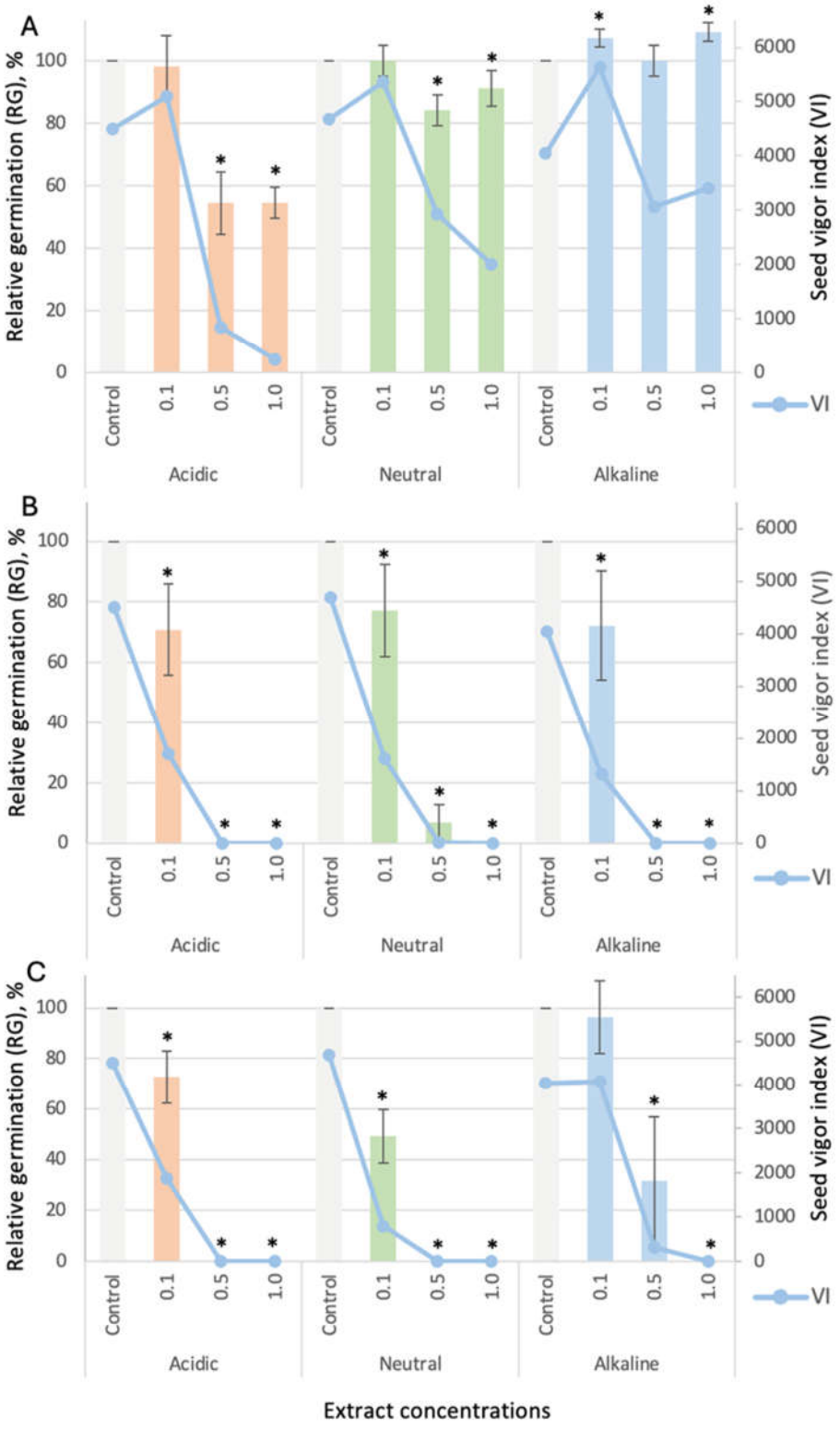

Figure 2. The inhibitory effect of *T. vulgare* aqueous extracts from roots (**A**), leaves (**B**) and inflorescences (**C**) on RG and VI of pepper cress seeds was examined using a one-way ANOVA test ($p < 0.05$). The data are presented as the means of treatments (n = 60) ± SD (bars). Statistically

significant differences between the experimental and control groups (water with various pH values) are indicated by asterisks (*).

The study assessed the effects of the aqueous extracts of tansy roots, leaves, and inflorescences on the germination of the model plant seeds. Allelopathic activity was investigated for different fractions: acidic, neutral, and alkaline, at relative concentrations of 0.1, 0.5, and 1.0. The tansy extracts demonstrated a notable impact on the seed germination and growth of the lettuce (Figure 1) and garden pepper cress (Figure 2). The data showed that the root extracts had the least impact on the RG and VI of lettuce and pepper cress.

At the highest relative concentrations (0.5 and 1.0) of the tested root extracts, the RG of lettuce reached 78.64 ± 10.41% and 46.15 ± 8.66%, and the VIs were 1012.04 and 215.25, respectively (Figure 1A) (one-way ANOVA, $p < 0.05$). Aqueous root extracts on pepper cress at the same relative concentrations affected the RG and it reached 54.54 ± 10.01% and 54.54 ± 5.04%, VIs were 843.33 and 244.17, respectively (Figure 2A) (one-way ANOVA, $p < 0.05$).

In the acidic leaf fraction, the lettuce RG was suppressed, reaching up to 39.31 ± 12.58% and 6.84 ± 11.55% at 0.5 and 1.0 relative concentrations, respectively (Figure 1B). Similarly, in acidic inflorescence solutions, the lettuce RG was suppressed, leading to 35.90 ± 25.01% and 0.00% at 0.5 and 1.0 relative concentration, respectively (Figure 1C). Pepper cress RG was inhibited even more strongly by acidic leaf and inflorescence fractions (Figure 2B,C). At the same highest relative concentrations, the RG of pepper cress was inhibited up to 100%.

3.4. Allelopathic Effects of Tansy Extracts on Lactuca sativa and Lepidium sativum

The allelopathic effects of root, leaf, and inflorescence water extracts of *T. vulgare* on seed germination and growth of *L. sativa* and *L. sativum* are presented in Figure 3.

The root extracts demonstrated the lowest inhibitory activity on lettuce and garden pepper cress seed germination and growth (Figure 3A). The average length of the control lettuce in the acidic fraction was 35.07 ± 4.79 mm. Statistically significant differences were found between the effects of all tested concentrations (0.1; 0.5; 1.0) on lettuce, compared to the acidic water control (one-way ANOVA, $p < 0.05$). The plant length decreased by 28.14% (on average 25.20 ± 2.33 mm), 62.36% (on average 13.20 ± 2.77 mm), and 86.37% (on average 4.78 ± 0.78 mm) under the above mentioned conditions, respectively. The average length of the lettuce was 37.57 ± 4.91 mm in the neutral water control solutions. The suppressive impact was observed at all tested concentrations (one-way ANOVA, $p < 0.05$) compared to the neutral control. The length of the lettuce decreased by 25.34% (on average 28.05 ± 3.98 mm), 44.24% (on average 20.95 ± 3.30 mm), and 51.21% (on average 18.33 ± 1.53 mm), respectively. The average length of the control lettuce in the alkaline fraction was 36.93 ± 6.21 mm. The lowest concentration tested (0.1) did not differ significantly from the control (mean 35.62 ± 2.57 mm) (one-way ANOVA, $p > 0.05$). Garden pepper cress was inhibited only in the highest relative concentrations (0.5 and 1.0) (Figure 3A). Pepper cress length decreased by 65.63% and 90.06% in the acidic fraction (from 49.08 ± 3.27 mm in control to 16.84 ± 4.41 mm and 4.88 ± 2.55 mm in 0.5 and 1.0 relative concentrations, respectively). The neutral fraction also suppressed seed germination growth by 25.45% and 53.11% compared to control. The alkaline fraction showed the lowest inhibitory effect and seed growth was reduced by 23.95% and 22.72% (from 49.08 ± 3.27 mm in control to 34.10 ± 3.81 mm and 34.65 ± 3.57 mm in 0.5 and 1.0 relative concentrations, respectively).

Lettuce seed germination and growth in leaves in the acid fraction decreased by 89.93% (from 35.07 ± 4.79 to 3.53 ± 2.10 mm) at 0.5, and by 98.46% at 1.0 relative concentration (from 35.07 ± 4.79 to 0.57 ± 0.98 mm) compared to control (Figure 3B), while pepper cress was suppressed up to 100%. Inflorescence extracts also suppressed garden pepper cress seed germination and growth (Figure 3C). At a 0.5 relative concentration of

acidic and neutral fraction, the seeds were inhibited by 100%. At a 1.0 relative concentration, all three fractions (acidic, neutral, and alkaline) inhibited plant growth by 100%. Lettuce seeds at a 0.5 relative concentration of the acidic fraction were inhibited and plant length decreased by 89.36% (from 35.07 ± 4.79 to 3.73 ± 3.32 mm), while at a 1.0 relative concentration, plant growth was suppressed by up to 100% (Figure 3C).

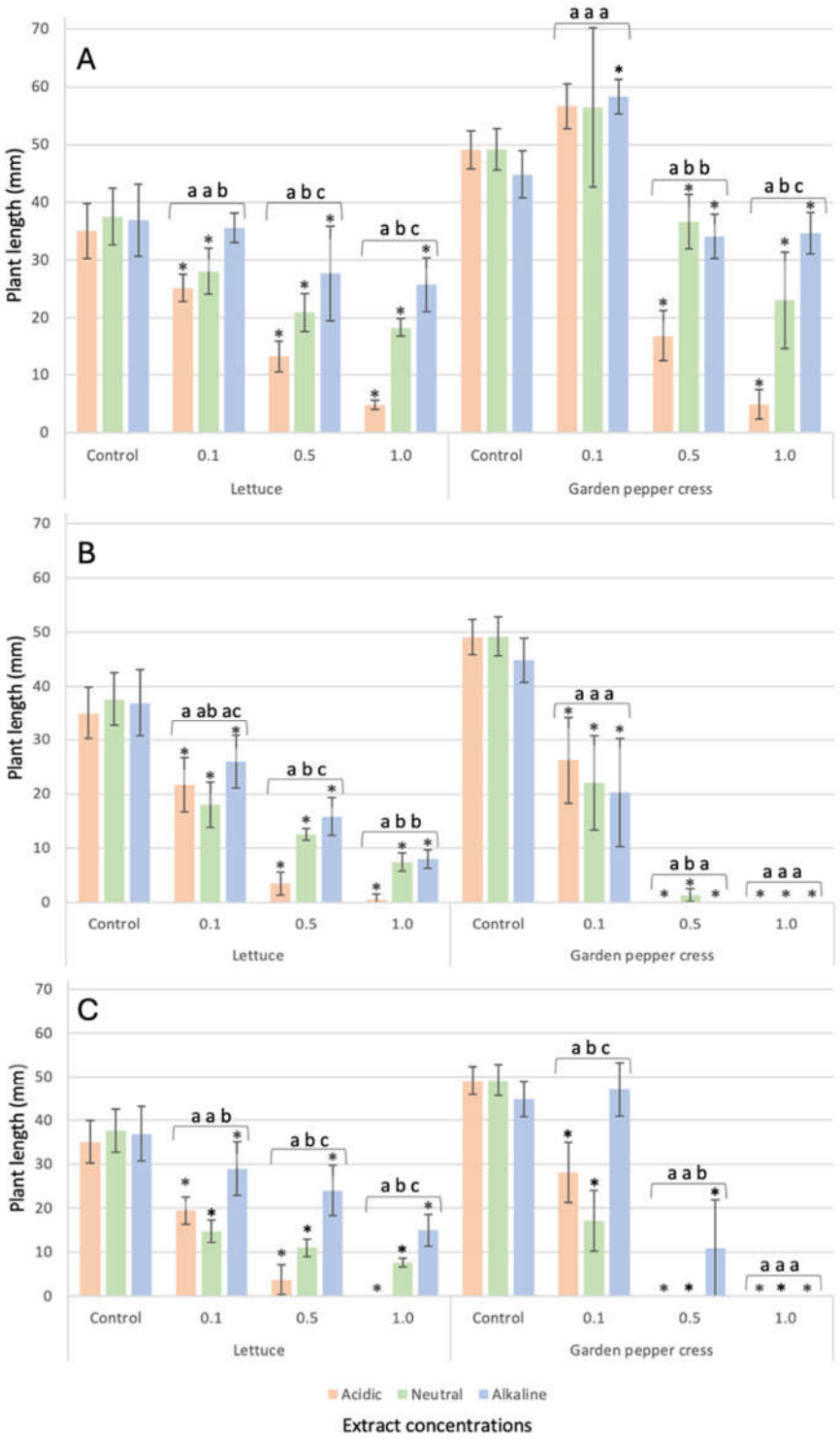

Figure 3. Inhibitory effect of *T. vulgare* aqueous extracts (acidic, neutral, and alkaline fractions at 0.1, 0.5 and 1.0 relative concentrations) from roots (**A**), leaves (**B**) and inflorescences (**C**) on germination and growth (mm) of lettuce and garden pepper cress (one-way ANOVA test, df = 9, $p < 0.05$). The data are presented as the means of repetitions (n = 60) ± SD (bars). Asterix (*) indicates

statistically significant difference compared to the control. Letters (a, b and c) denote statistically significant differences between fractions (Tukey's test, df = 3, $p < 0.05$).

Our results revealed that the seed germination and growth of garden pepper cress was more inhibited by tansy extracts than lettuce. The comparison of the different fractions (acidic, neutral, and alkaline) revealed that the alkaline fraction had the least inhibitory effect on lettuce seed RG and VI, followed by the neutral fraction (Figures 1 and 2). In contrast, the acidic leaf and inflorescence tansy extracts had the strongest inhibitory properties.

Traditionally, both the inflorescences and the leaves of *T. vulgare* are used for ethno-pharmacological purposes. Mostly flower and only in some cases leaf extracts can be used as bio-pesticides against agricultural pests, such as aphids [19,20,22] and lepidoptera [21,22]. The allelopathic potential effects of tansy extracts are usually realized through the use of the whole plant or just the leaves [39–45]. Furthermore, our research has demonstrated the significance of investigating the impact of plants' individual morphological parts and their extracts in varying pH solution media. The results obtained showed that not only leaf extracts have an inhibitory activity on seed germination and growth, but also tansy flowers possess the properties.

The analysis of VOCs established rather similar compositions for inflorescences and leaves. Major compounds 1,8-cineole, borneol and camphor were determined in the flower extracts, while 1,8-cineole and borneol predominated in the leaf extracts (Table 2). It was revealed by previous research that the monoterpenes 1,8-cineole [47,52], camphor and borneol [52] have germination and growth inhibitory potential. The results obtained by Islam, et al. demonstrated that the saturated aqueous extract of eight monoterpenoids (camphor, 1,8-cineole, α-terpineol, borneol, myrtenal, myrtenol, carvone and carveol) exhibited potent phytotoxic activity against certain plant species, such as *Lactuca sativa, Schizachyrium scoparium* (Michx.) Nash and *Disakisperma dubium* (Kunth) P.M. Peterson and N. Snow (syn. *Leptochloa dubia*). The investigation of allelopathy in plants' VOCs is still a relatively new field of research. Allelochemicals are an attractive prospect for the development of a new generation of herbicides or fungicides. In general, research into the allelopathic properties of *T. vulgare* is of particular importance to the future development of green agriculture.

4. Conclusions

The specific characteristics of *Tanacetum vulgare* plants, namely their perenniality, herbaceous nature, prolific flowering, high reproductive output, and capacity to produce a considerable biomass are of great importance to the growth conditions of surrounding plants and the formation of balanced ecosystems. The current study makes a contribution to the knowledge of the phytochemical profile and allelopathy of common tansy collected from spontaneous Lithuanian flora. The compositional characteristics of the volatile organic compounds, non-volatiles and polyphenols in aqueous tansy extracts (with various pH media) used for allelopathic tests were determined during the study. According to our results, tansy exhibited strong allelopathic effects on model plants. The seed germination and growth of garden pepper cress was more inhibited by tansy extracts than lettuce. *T. vulgare* flower and leaf extracts demonstrated the highest inhibitory activity, while root aqueous solutions showed minor effects. The different fractions (acidic, neutral, and alkaline) revealed that the alkaline tansy solutions had the least inhibitory effect on model plants, followed by the neutral fraction. The acidic leaf and inflorescence tansy extracts demonstrated the strongest inhibitory properties. The study provided new insights into the common tansy impact on the regeneration and diversity of neighboring plants, and more generally on plant communities and ecosystems. Increased knowledge of the evolution of aggressive weeds, such as *T. vulgare*, could help us to predict and better manage their spreading. On the other hand, the allelopathic properties of *T. vulgare* can be used for weed control, resistance to microbial

pathogens, plants diseases, pests and animal herbivores, and improving plants' defense mechanisms against stress. The application of allelopathy from plants or natural products derived from plants represents a more economical, environmentally friendly and effective approach to developing a green agricultural industry. Further research on the allelopathic potential properties of *T. vulgare* extracts on other model plants, such as perennial ryegrass (*Lolium perenne*), timothy grass (*Phleum pratense*) and/or white clover (*Trifolium repens*) would be promising and important for agronomy and agriculture.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10060538/s1. Table S1: Summary of previously reported data (from 2010) on allelopathic properties of various *T. vulgare* L. extracts; Table S2: The effect of acidic, neutral and alkaline fractions at different relative concentrations (0.1; 0.5; 1.0 M) of *T. vulgare* root aqueous extracts on germination rate (GR), relative germination (RG) and vigor index (VI) of garden pepper cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.) seeds; Table S3: The effect of acidic, neutral and alkaline fractions at different relative concentrations (0.1; 0.5; 1.0 M) of *T. vulgare* leaf aqueous extracts on germination rate (GR), relative germination (RG) and vigor index (VI) of garden pepper cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.) seeds; Table S4: The effect of acidic, neutral and alkaline fractions at different relative concentrations (0.1; 0.5; 1.0 M) of *T. vulgare* flower aqueous extracts on germination rate (GR), relative germination (RG) and vigor index (VI) of garden pepper cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.) seeds. Figure S1: The plant material collecting locality in Eastern Lithuania (Vilnius district, Trakai municipality, Padumblė), and Figure S2: Control samples of *Lactuca sativa* L. seeds in acidic (A), neutral (B), alkaline (C) water; and *Lepidium sativum* L. seeds in acidic (D), neutral (E) and alkaline (F) aqueous fractions.

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