

Characterization of Thyme and Tansy Extracts Used as Basic Substances in Zucchini Crop Protection

Claudio Beni (Corresponding Author)

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di ricerca Ingegneria
e Trasformazioni Agroalimentari – CREA-IT

Via della Pascolare 16 - 00016 Monterotondo (RM) Italy.

E-mail: claudio.beni@crea.gov.it

Laura Casorri, Eva Masciarelli, Barbara Ficociello

Department of Technological Innovations and safety of plants, products and anthropic settlements
(DIT) - National Institute for Insurance against Accidents at Work (INAIL)

Via R. Ferruzzi, 38/40 - 00143 Rome, Italy.

E-mail: l.casorri@inail.it; e.masciarelli@inail.it; b.ficociello@inail.it

Olimpia Masetti, Ulderico Neri, Rita Aromolo

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di ricerca
Agricoltura e Ambiente – CREA-AA - Via della Navicella, 2 - 00184 Rome, Italy.

E-mail: olimpiade66m@gmail.com; ulderico.neri@crea.gov.it; rita.aromolo@crea.gov.it

Simona Rinaldi

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di Ricerca Zootecnia
e Acquacoltura – CREA-ZA - Via Salaria 31 - 00016 Monterotondo (RM) Italy.

E-mail: simona.rinaldi@crea.gov.it

Patrizia Papetti

Department of Economics and Law, Territorial and Products Analysis Laboratory (LAMEt),
University of Cassino and Southern Lazio, Via Sant'Angelo, Località Folcara, 03043 Cassino,
Italy

E-mail: papetti@unicas.it

Angelo Cichelli

Department of Medical, Oral and Biotechnological Sciences - DiSMOB. "G. D'Annunzio"
University of Chieti-Pescara - Via dei Vestini, Chieti, Italy.

E-mail: cichelli@unich.it

Received: July 6, 2020

Accepted: July 23, 2020

Published: July 28, 2020

doi:10.5296/jas.v8i4.17314

URL: <https://doi.org/10.5296/jas.v8i4.17314>

Abstract

Bio-sourced substances used for crop protection in natural and organic farming have a positive impact both on human and environment health: plant extracts are effective to stimulate plant defense against pests and diseases, with no toxic effect and they could be classified as basic substances. The chemical characterization of thyme and tansy extracts were carried out with ICP and NMR techniques for the analysis of elemental content and organic components. Antioxidant activity was evaluated by DPPH method. Phytostimulant effects of extracts were evaluated on zucchini crop in a preliminary field test. Vegetative plant conditions were assessed by measuring the chlorophyll content. The 1% extracts were chosen for field trial because of their maximum antioxidant activity (over 80%) and their cheapness compared to 2% concentration. NMR showed that the spectrum of tansy highlighted the phenolic fraction in 6-9 ppm region and, in particular, at 5.9 ppm the signals of thymol, a peculiar antioxidant compound; while the spectrum of thyme is less significant. The field trial results showed a higher fruit yield and a positive effect on plant growth in treated plants respect to control ones, due to the composition and antioxidant activity of both plant extracts. In fact, thyme extract treated plants showed a yield equal to 6,4 kg per plant, higher than no treated ones, 5,1 kg per plant; also tansy extract induced a higher yield respect to control plant, respectively 5,6 and 3,9 kg per plant. The results obtained indicate the role of the extracts investigated as basic substances.

Keywords: *Tanacetum vulgare*, *Thymus vulgaris*, aqueous extract, zucchini crop protection, NMR, ICP-OES

1. Introduction

Biological methods use to stimulate plant self-defense against pathogens and parasites is one of the main objectives to be promoted in compliance with Directive 2009/128/EC, regarding the sustainable use of pesticides (EU, 2009).

Synthetic pesticides impacts on the environment and on farmers' and consumers' health, especially in the production of fresh market vegetables, due to the high toxicity and the non-biodegradability of the major part of these products. Farm workers, who are at high risk of exposure to chemical pesticides, should always wear gloves and avoid contact with eyes

and skin, using these products. Therefore, it is necessary to develop sustainable agricultural practices (crop rotation, use of seasonal crop, etc.) to reduce chemical products use and to contrast the resistance induced on parasites by several classes of pesticides (Koul and Walia, 2009).

Bio-sourced and traditional plant extracts and plant defense enhancers used as crop protection are obvious candidates to be included in the list of basic substances, products which are already used for other purposes, e.g. as a foodstuff or a cosmetic, but which can also serve as a plant protection product (Marchand, 2017). For several substances traditionally used in amateur gardening and organic farming, a solution for a registration at EU level must be found. Article 23 of Regulation 1107/2009, that defines basic substances, could be a possible solution, in association with the implementation of the legislation at Member State level (IFOAM, 2016).

Plant extracts are known for their stimulating properties on plant growth and also for the protection of plants due to their bactericidal or fungicidal activity, depending on their composition (Koul et al., 2008). Plant extracts promote crop self-defense against attacks by fungi and bacteria. Aqueous extracts have been shown to be effective in agriculture (Beni C. et al., 2018).

Plant extracts are made up of a combination of several compounds (phyto-complexes), which act in synergy, as these mixtures have a higher activity than the single active substance, such as to determine a more effective response. This phenomenon, named synergism, has been observed for the constituents of essential oils (Hummelbruner and Isman, 2001; Singh *et al.*, 2009) which give better and longer lasting results in mixtures (Chockalingam *et al.*, 1990) compared to simple additive effect of the individual components.

Aqueous extracts of thymus and tansy could be particularly effective in the vegetable plant strengthening in an integrated defense approach, after rigorous and complete characterization and validation.

Tansy (*Tanacetum vulgare* L.) is a perennial herbaceous flowering plant of the Asteraceae family. It is also known as common tansy, bitter buttons, cow bitter, mugwort, or golden buttons and belongs to the subfamily Tubiflore, characterized by flower heads with peripheric ligulate flowers and central tubular flowers and fruits with pappus.

Tansy is native to Eurasia; present in almost all parts of mainland Europe while in Asia it is common especially in western areas. This plant was introduced to North America either for use in natural remedies or as an ornamental plant. It is an invader of disturbed sites and is commonly found on roadsides and is listed as a “noxious weed” in several countries.

Phytochemical studies have shown that *T. vulgare* contain several biologically active metabolites, mainly sesquiterpene lactones, essential oils (borneol, thujone, camphol), sesquiterpene lactones, pyrethrins, flavonoids (quercetin, jaceidin), vitamin C, citric acid, butyric acid, oxalic acid, malic acid, resin, tannins (Schrearer, 1984; Hough-Goldstein, 1990).

Thyme (*Thymus spp* L.) belongs to the family Lamiaceae, originated from Mediterranean region. This genus includes various species among which *T. serpyllum*, *T. citriodorus*, *T. zygis*, *T. herba barona* e *T. vulgaris*. Thyme species are well known strongly aromatic and medicinal plants.

T. vulgaris grows preferably in places hills and mountains, while the *T. serpyllum* is more common in the plains. These species are both used for their therapeutic and aromatic properties. The main constituents of thyme, responsible of its properties, are the phenols thymol and carvacrol. Other constituents are resin, linalool, chimol, cymene, timene, apinene, luteolin, flavonoid glycosides, flavonoids, tannins, triterpenes, and saponins with antibiotic properties. If the plant is harvested during the winter the phenolic content is low with predominance of thymol, if harvested in summer it has a high concentration of phenols and especially carvacrol. For its biological and pharmacological properties thyme is widely used as an antioxidant, antiseptic and antifungal (thymol) effects (Saqvic et al., 2007).

In amateur and natural farming, thyme and tansy are proposed for use to strengthen self-defense in vegetable crop systems and their use could be extend in organic farming as basic substances.

The aim of this work is to evaluate the antioxidant properties of thyme and tansy aqueous extracts and to define chemical composition of the extracted phyto-complex, using ICP-OES and NMR analytical techniques. Moreover, the phytostimulant effects of both thyme and tansy phytocomplexes in the extracts were evaluated in a preliminary open field trial on zucchini (*Cucurbita pepo* L.) crop.

2. Materials and Methods

2.1 Preparation of the Aqueous Extracts

Extracts were prepared using thyme or tansy (*Thymus vulgaris* var. *verticillata* Willk. and *Tanacetum vulgare* L. subsp. *vulgare*) dried powder at two concentrations of 1% and 2% w/v, using distilled water at room temperature (298°K). The dry powder was obtained by drying the plant material in a thermostatic chamber at 313°K for five days. The material was subsequently pulverized in a mill. The percentages of the solute were chosen based on the empirical indications provided by the producer associations on the use in agriculture of dried officinal plants to produce basic substances for natural and organic agriculture, ranging from 1% to 2%. Each mixture was allowed to stand for 48h to complete the extraction process, then the extracts were filtered with filter paper.

Plants were cultivated in a synergistic garden at CREA farm located in Monterotondo (Rome), Italy. Regarding thyme, only leaves were used to produce the extract, while for the tansy, the whole flowering top was used.

For the determination of antioxidant activity both the above defined 1% and 2% w/v extracts were used while for the other chemical analysis and for field trial only the 1% w/v concentration extracts were used.

The solution used for field application was prepared by placing the dried thyme or tansy

powders in water at the concentration of 10 g L⁻¹ (1% w/v) and the mixture was allowed to stand for 48 hours for a more complete extraction. After filtration, the aqueous extracts could be used immediately or stored for 6-7 days at +4°C. Dried plants were used at the concentration of 10 g L⁻¹ because its levels of chemical compounds seem optimal for spraying on plants and this concentration is generally used in open field experiments as reported in previous works (Beni C. et al., 2018; Nashwa and Abo-Elyousr, 2012). The crude extracts were used as they are, without further dilution, as indicated by the natural and organic producer groups.

2.2 Measurement of Antioxidant Activity

The antioxidant activity of aqueous extracts was evaluated by DPPH (1,1-diphenyl-2-picryl-hydrazyl radical) method (Katalinic et al., 2006; Nikolova et al., 2011).

The spectrophotometric method of DPPH is based on the reduction of the stable free-radical 2,2-Difenil-1-picrilidrazil (DPPH·) by antioxidant molecules. Color change from dark purple to yellow measured at the spectrophotometer is proportional to the antioxidant capacity of the sample.

1 ml of a 0.15 mM DPPH methanolic solution was mixed with increasing doses of extract (ranging from 20 to 100 µL) and adding methanol to the final volume of 1.5 mL. The mixtures were shaken vigorously and left standing at room temperature for 30 min in the dark.

The absorbance was measured at 517 nm by UV–VIS double-beam spectrophotometer (Uvikon XL Secomam S.a.s. ®, Alés Cedex, France), using methanol as blank. Analyses were performed in triplicate. All the reagents used were characterized by a quality standard grade.

The antioxidant activity was expressed as a percentage of inhibition of DPPH radical calculated according to the following equation:

$$\% \text{ antioxidant activity} = (A_0 - A_s) / A_0 \times 100$$

where A₀ is the absorbance of the control (containing all reagents except the extract), and A_s is the absorbance of the tested sample.

2.3 Chemical characterization of Aqueous Extracts

The metabolic profiling of both the prepared thymus and tansy extracts was performed using ICP-OES and NMR for their chemical characterization. These techniques have been chosen to characterize the entire metabolic profile of the extracts with fast and simultaneous methods, given that the basic substances are used in agriculture precisely for the synergistic effect of the chemical components, rather than for the effect of a single component.

2.3.1 Elemental Analysis with ICP-OES and Kjeldahl Method

All chemicals as analytical quality reagents and commercial stock element solutions used were provided by Sigma-Aldrich Co. (St. Louis, MO, USA). All solutions were made with high purity water (Millipore, Molsheim, France).

Macronutrients (P, Mg, Ca, Na and K), micronutrients (Fe, Mn, Cu, Zn and Ni) and heavy metals (Al, Cd, Cr, and Pb) were determined in the filtered extracts using the inductively coupled plasma optical emission spectrometry (ICP-OES) equipped with a ICAP 6100 (Thermo Scientific, Waltham, MA, USA) spectrometer. The analytical parameters of ICP-OES were an applied power of 1.3 kW, a nebulizer flow rate of 0.8 L min⁻¹, a plasma gas flow of 15 L min⁻¹ and an auxiliary gas flow of 2.0 L min⁻¹. Prior to each sample evaluation, the ICP-OES instrument was calibrated with a blank and four multi element standard solutions. Total N was determined using Kjeldahl digestion (Kjeldahl, 1883).

2.3.2 NMR Analysis

0,5 mL of extract (1% w/v) was placed in a standard 5mm NMR glass tube and added of D₂O (0.5 mL) which was used for locking and shimming the B₀ magnetic field. NMR spectra of thyme and tansy extracts were recorded at the ¹H frequency of 400.13 MHz using a Bruker AVANCE spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) equipped with a 5 mm broadband probehead. The-3-(trimethylsilyl) propionic-2,2,3,3-d₄ acid sodium salt (TSPA) was dissolved in D₂O and used as reference for peaks assignment (methyl groups signal appeared at δ=0.00 ppm).

The spectra were recorded at room temperature (298 °K) with a spectral width of 10.847 ppm (4340.278 HZ), accumulating 512 transients with a recycle delay of 2 s and they were acquired by using a water suppression pulse sequence in order to increase the signal-to-noise-ratio (Mannina, 2017).

2.4 Field Trial

The effects of the extracts were evaluated for the first time on zucchini crops (*Cucurbita pepo* L.) in an open field trial.

Extracts concentration of 10g L⁻¹ has levels of chemical compounds which seem optimal for spraying on plants and this concentration is generally used in open field treatments as reported in previous works (Beni C. et al., 2018; Nashwa and Abo-Elyousr, 2012). Furthermore, extracts at a concentration of 1% are less expensive than those at a concentration of 2% and still have a good maximum antioxidant activity.

Zucchini plants (*Cucurbita pepo* L.) var. Augusto (Romanesco type) were planted in open field at CREA farm of Tor Mancina near Rome (lat. 42°062 N, long. 12°402 E, alt. 43 m a.s.l.), in April 2014, cultivated under integrated farming. The chemical characteristics of the soil were analyzed according to the official methods of soil chemical analysis (Mi.P.A.A.F., 2000). Soil has a clay-loamy texture, with sub alkaline pH (7.6), and a high cation exchange capacity (31.41 meq 100 g⁻¹). At the transplant, a single operation of local fertilization was

carried out, distributing 30 g per plant of bi-ammonium phosphate (DAP 21-54), due to high soil concentration of nitrogen and potassium.

The plants were divided into 6 plots (3 plots for each extract) of 4 plants with three replicates in a random design. In each plot two plants were treated weekly for two months with thyme or tansy aqueous extracts and two plant were untreated as control.

The plants were periodically inspected to verify the presence of diseases due to fungal infections or attacks by insects. The fruits were collected during the months of June and July (12 harvests), weighed and measured (length and diameter).

2.5 SPAD Measurements

To assess the vegetative conditions of each plant, the *in vivo* chlorophyll content was measured using the SPAD 5200 portable fluorimeter (Konica Minolta® Business Solution Italia S.p.a., Milan, Italy), after its calibration in the laboratory by spectrophotometric measurements of chlorophyll content in leaf extracts (Figure 1).

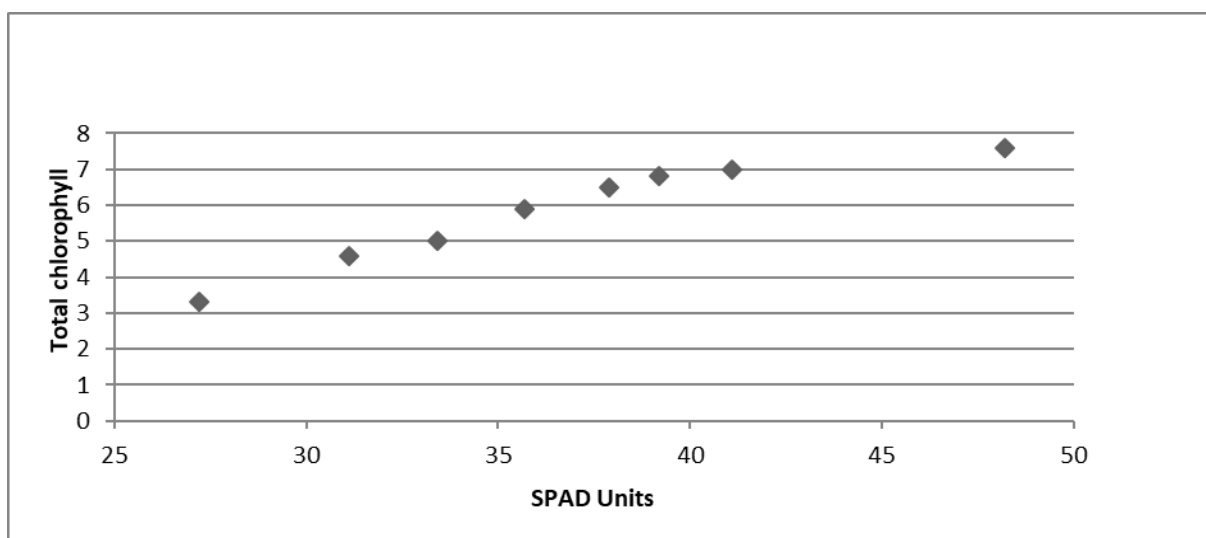


Figure 1. Calibration SPAD units towards real total chlorophyll content (mg L⁻¹) in leaf samples

For the instrument calibration, 0.5 g of fresh leaves were homogenized in an aqueous solution of 80% acetone. The extracts were analyzed at wavelengths of 660.0 nm and 642.5 nm by UV-VIS spectrophotometer Uvikon XL (Secomam S.a.s.®, Alés Cedex, France), against a blank consisting of 80% acetone.

The value of total chlorophyll was expressed in mg L⁻¹ by the formula:

$$\text{Total chlorophyll} = 7.12 A(660.0) + 16.8 A(642.5)$$

where $A(660.0)$ and $A(642.5)$ are, respectively, the absorbance values at 660 nm and 642.5 nm.

Field measurements with SPAD were carried out in the intermediate phase of the vegetative cycle of zucchini crops at three points in the central leaf portion of two plants.

2.6 Statistical Analysis

Since two plants, on the half plot, were treated with thyme and tansy extract and the other two plants were not treated, an experimental spatial dependence as a result of closeness of plants has been introduced in the design. The plots were repeated for three times at different points of the area of the field test. The differences in yield and biometric data between treated and not treated plants were tested statistically using the t test for paired samples (p value < 0.05) as a result of spatial dependence between the treatments. SPAD measurements were submitted to Levene's test to check homogeneity of variances and subsequently to two ways ANOVA (Treatment and leaf position – α level = 0.05) to compare the effect of each extract on the chlorophyll content of the treated plants compared to the control.

3. Results

3.1 Antioxidant Activity

Free radical scavenging activity of plant extracts has been extensively evaluated by DPPH method. The antioxidant activity of thyme and tansy extracts maintains a similar trend by increasing the dose of extract in all the extracts considered while the 2% (w/v) extracts showed higher antioxidant activity compared to those at 1% (w/v) especially in the lower doses (Figure 2).

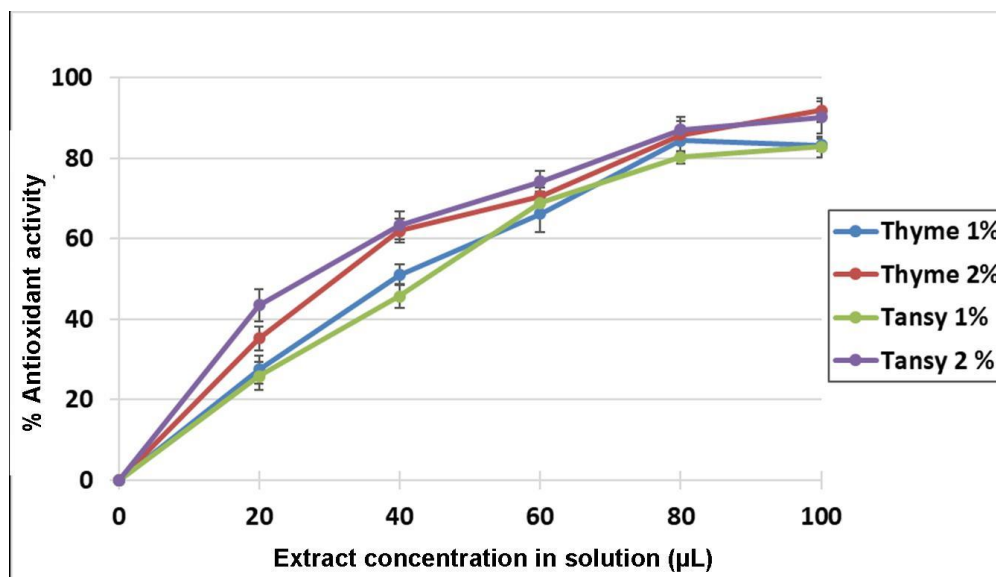


Figure 2. Antioxidant activity of thyme and tansy extracts at the concentrations of 1% and 2% w/v. Values are expressed as mean \pm standard deviation (sd) ($n = 3$)

The use of 1% extracts in field trial were chosen because of their good maximum antioxidant activity (over 80%) and their cheapness as only half of the amount of dried thyme and tansy is used compared to 2% concentration.

3.2 Elemental Content

Table 1 shows that both extracts have a slight concentration of macro-elements, such as N, P, K, Ca and Mg. In particular, tansy presents, for the first three cited elements, a double

concentration with respect to thymus. Moreover, in the two aqueous extracts, many beneficial elements for the plant are present, such as Fe, Cu, Zn, Mn, B and Al. These elements are coenzymes capable of activating several enzymatic systems within the plant cells.

Table 1. Content in macro, micro elements and heavy metals in thyme and tansy extracts, mean and standard deviation (sd)

Element	Thyme mg L ⁻¹		Tansy mg L ⁻¹	
	mean	sd	mean	sd
N	68.4	0.80	130.6	0.72
P	5.6	0.04	9.6	0.01
K	94.0	0.72	186.1	0.70
Ca	47.0	0.28	38.3	0.18
Mg	16.4	0.16	10.1	0.03
Na	16.6	0.17	12.5	0.06
Fe	0.149	0.0017	0.054	0.0001
Cu	0.028	0.0004	0.026	0.0007
Zn	0.098	0.0006	0.106	0.0002
Mn	0.224	0.0025	0.117	0.0004
B	0.044	0.0006	0.083	0.0008
Al	0.194	0.0020	0.033	0.0003

3.3 ¹H-NMR Spectra

NMR in liquids is a powerful technique for determination of natural compounds in plant extracts and complex mixtures, due to rapidity of data acquisition and easiness of purifications or extractions.

Water-soluble extracts of the two analyzed plants mainly contains amino acids and aliphatic components (0.5 to 3.5 ppm); vitamins as ascorbic acid (vitamin C); organic acids as citric and formic acids; carbohydrates as sucrose and glucose (3.5 to 5.5 ppm), aromatic and phenolic compounds (6.0-8.5 ppm). The phenolic fraction is more abundant in tansy extracts respect to thyme ones because flowers were present in tansy sample used, while thyme sample contained only leaves. A very recent study on antioxidant activity of tansy shows that flowers extract has a higher total phenolic content with a consequently higher antioxidant activity (Anca Mot et al., 2018).

Excluding the intense peaks of α - β glucose and sucrose, the ¹H-NMR spectrum of thyme and tansy extracts shows the signals of these metabolites: ethanol (1.2 and 3.6 ppm), isoleucine and leucine (at 1.0 ppm), valine (at 1.0 and 2.3 ppm), serine (at 3.8-4.0 ppm), alanine (at 1.5, 1.7, 1.9, 3.2 and 3.7 ppm); glutamate (at 2.0, 2.1, 2.4 and 3.7 pm); threonine (at 1.3 ppm), arginine (at 1.7 and 1.9 ppm), glutamine (at 2.1 and 2.5 ppm), glutamic acid (at 2.0-2.1 and 2.4 ppm), aspartic acid (at 2.6 and 3.8 ppm), lactic acid (at 1.3 ppm), acetic acid (at 1.9 ppm), malic acid (at 2.4 and 2.7 ppm), citric acid (at 2.4 and 2.7 ppm), succinic acid (at 2.5 ppm), quinic acid (at 1.8-1.9 ppm), ascorbic acid (at 3.6 and 3.8 ppm), GABA (at 1.9, 2.3 and 3.0

ppm), choline (at 3.2 ppm), histidine (7.0 ppm), and tryptophan (at 7.2-7.3 ppm, 7.5, 7.7 ppm). Especially in tansy spectrum, the signals of some phenolic compounds are visible: rutin (at 6.4 and 7.6 ppm), quercetin (at 7.0, 7.6 and 7.8 ppm), kaempferol (at 6.30, 6.50, 7.0 and 8.0 ppm), apigenin (at 6.6-6.8, 7.1, 7.8 ppm), riboflavin (3.7-4.0, 7.4 ppm), pyrocatechol (6.8-6.9 ppm), caffeoyl quinic acid (6.4, 7.1 and 7.7 ppm), naringenin (6.8 and 7.3 ppm), chlorogenic acid (6.4 and 6.9 ppm). The ethanol in the extraction mixture allowed to solubilize from tansy also the thujone which shows signals at 2.1-2.3 and 2.6-2.7 ppm (Monakhova et al., 2011).

In Figure 3, the spectrum of tansy is shown in an expanded view in order to highlight the phenolic fraction. The spectrum of thyme is not reported because it is less significant and interesting in the 6—9 ppm region. In particular, at 5.9 ppm the signals of thymol, a peculiar antioxidant compound, are very weak probably due to the applied water suppression pulse and not to its concentration in thyme extracts. In fact, this sequence efficiently suppresses water signal, but it has also effects on neighboring resonances. Conversely, ¹H-NMR spectrum of tansy is more intense in phenolic zone because this spectrum shows also the signals of flavonoids in flowers, while thyme extracts comes only from leaves without flowers.

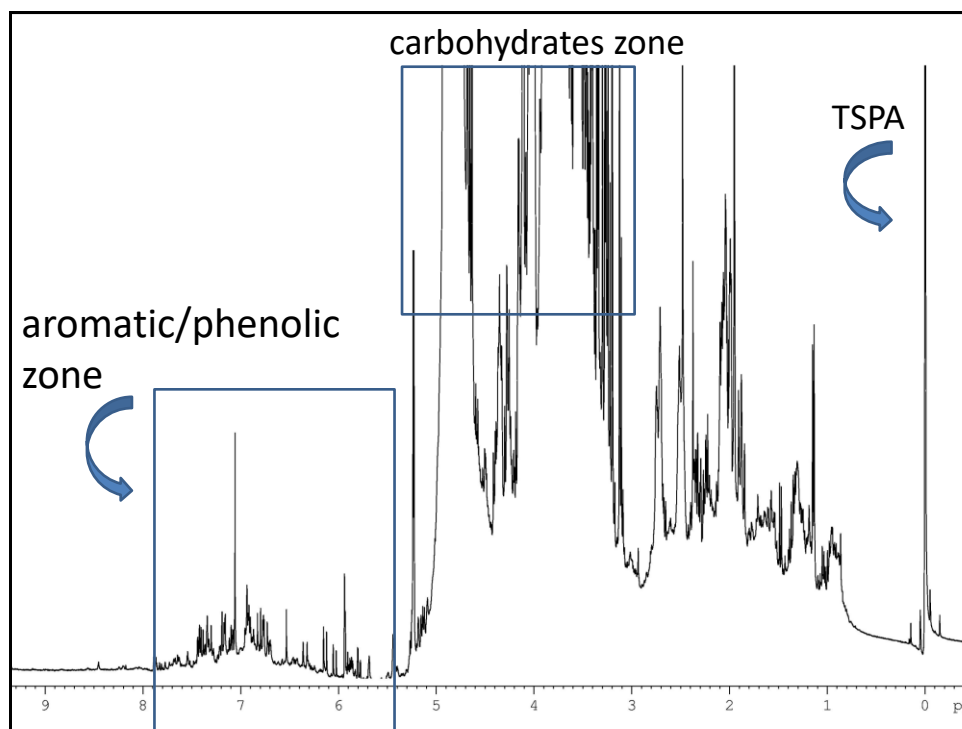


Figure 3. ¹H-NMR spectrum of aqueous tansy extracts

3.4 Field Production

The field test results showed a significantly higher fruit yield in thyme and tansy (Table 2) treated plants compared to control plants due to the increase in both the number of fruits per plant and the fruit mean weight. The fruits had higher diameter and lengths in treated plants compared to control plants (Table 2). However, only the difference of the average production per plant was statistically significant (*p* value < 0.05).

Table 2. Production of zucchini, fruit parameters and SPAD (mean data per plant) in plants treated with thyme and tansy extract - mean and standard deviation (sd)

	Thyme				Tansy			
	Treated plants		Untreated plants		Treated plants		Untreated plants	
	mean	sd	mean	sd	mean	sd	mean	sd
Yield (g) §	6354 *	1352	5089	1044	5591 *	2021	3877	970
Number of fruits (n) §	31.0	2.6	30.0	4.4	27.7	8.7	23.0	5.6
Fruit weight (g)	165.4	84.9	137.1	75.0	160.7	87.3	143.9	65.4
Fruit length (cm)	15.1	7.1	14.2	7.2	15.1	6.3	14.6	7.4
Fruit diameter (cm)	3.3	1.5	3.1	1.6	3.2	1.6	3.2	1.3
SPAD units	37.9 **	3.7	33.2	3.0	36.6 **	2.9	33.3	3.1

* Significant t test for paired samples (p value < 0.05)

** Significant F test ANOVA (p value < 0.01)

§ Sum of all harvests

Moreover, treated plants had greater vegetative vigour and upper chlorophyll content in leaves as evidenced by significantly higher SPAD values (p value < 0.01) both in thyme and tansy treated plants respect to those not treated (Table 2).

Examining the data of production for the single harvests, the t-test has shown statistically significant differences (p value < 0.05) in three harvests (number 5-6-8) both in the plants treated with tansy and thyme compared to the untreated ones (Figure 4). In a particular harvest, the number 7 (June 24) zucchini production was much lower than the others for both treatments, due to a strong reduction of the minimum air temperature (287 °K) in the days prior to the harvest.

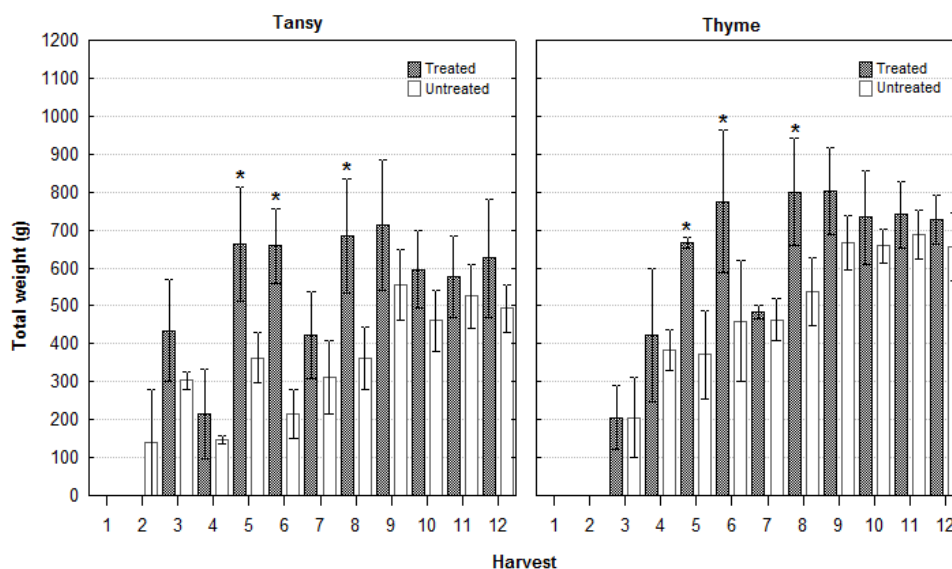


Figure 4. Means (Bars) and standard errors (Whiskers) of Production of zucchini fruits at each harvest

* Significance to the t test for paired samples (p value < 0,05)

4. Discussion

Both thyme and tansy extracts showed good antioxidant properties and seem to have a biostimulant effect on plant growth and fruit production, confirming the possibility of their use as basic substances for the integrated protection of vegetable crops.

The high antioxidant activity of plant extracts as thyme and tansy have been reported in several studies (Sokmen et al. 2004, Kaur and Mondal, 2014) and the DPPH radical scavenging method is commonly used for comparing the antioxidant properties of different plant extracts (Katalinic et al., 2006; Nikolova et al., 2011). Although this method is unable to detect specific antioxidant components, it is interesting to study the total antioxidant capacity of the extracts due to the contributions of the different compounds present in the mixture.

The chemical characterization analysis showed that the aqueous extracts analyzed contains amino acids and aliphatic components, vitamins as ascorbic acid (vitamin C), organic acids and carbohydrates, aromatic and phenolic compounds. The phenolic fraction is more abundant in tansy extracts respect to thyme ones because flowers and leaves were used for the preparation of the solution, while thyme extract contains only leaf compounds.

The presence inside the solutions of many beneficial elements for the plant, such as Fe, Cu, Zn, Mn, B and Al, gives to the extracts the capability of activating numerous enzymatic systems within the plant cells (Welch and Shuman, 1995; Broadley et al., 2012). In fact, treated plants showed better physiologic conditions.

An interesting aspect to evaluate was the absence of insect attacks on the plants, such as

fungal and bacterial diseases, despite the high rainfall and the high moisture content of the air in the period in which the trial was held. The zucchini plants seemed to have taken advantage from the repellent and preventive effect, of extracts used, against insect vectors. Components with phenolic structures, like carvacrol, eugenol, and thymol, are reported to have antimicrobial effect and are highly active against the pathogens, probably acting as plant defense mechanisms against pathogenic microorganisms (Das et al., 2010; Gurjar et al., 2012).

The biostimulant effect of considered extracts may depend on a protective effect against the oxidative stress, due to the synergistic action of the mixture of its chemical components. Natural extracts displayed biostimulant properties, thus improving and stimulating plant life processes through mechanisms that are different from those of fertilizers or phytohormones, depending on the wide range of molecules contained in the solution (Posmyk, M.M. and Szafranska, K., 2016). Many reports indicate that unfavorable environmental conditions may cause oxidative stress due to excessive formation of ROS (Reactive Oxygen Species).

The extracts investigated in this work contains a consistent concentration of polyphenols and vitamins, in particular ascorbic acid, with high antioxidant activity and different aminoacids that are known to have biostimulant effects (Brown, P. and Saa, S., 2015; Posmyk, M.M. and Szafranska, K. 2016). The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (Sokmen et al. 2004, Kaur and Mondal, 2014). Furthermore, radical-scavenging activity is an effective defense mechanism of the plant from environmental stress, due to the action of various antioxidant components, thereby creating a synergistic effect (Sokmen et al. 2004).

Both thyme and tansy extracts treatments have improved plant growth and vigor and zucchini productivity, probably through biostimulant and nutritional effects of their components that could be effective in strengthening of plants self-defense.

Biostimulators often increase chlorophyll content, which is crucial for proper course of photosynthesis. This effect was observed in cowpea seeds, after pre-soaking in carrot extract (Abbas, 2013), and in rocket (*Eruca sativa*) treated with *Moringa oleifera* extract (Abdalla, 2013). Moreover, algae extracts from *Ascophyllum nodosum* or *Laminaria digitata* stimulated growth and nutrition of the treated plants and could induce natural defense reactions (Joubert and Lefranc, 2008). The studies on their way of acting showed that these products acted as enzymatic phyto-activators, stimulating N and P uptake and an increased chlorophyll content.

In the context of integrated agricultural management of crop protection, field crops production could be enhanced by stimulation of plant growth and development, in order to improve the plant self-defense by harmful organisms and abiotic stresses.

5. Conclusions

The application of thyme and tansy extracts seems to have phytostimulant effects on plant growth, with an increase in zucchini yield. Composition and good antioxidant capacity of extracts suggest a positive effect against plant oxidative stress, related to the polyphenols content.

The gradual replacement of conventional chemicals with bio-sourced substances, such as plant extracts, will bring significant benefits with regard to natural resources, environment and human health. The introduction of plant extracts, experimentally tested and technically and economically produced on a large scale, will also lead to the opening of new and attractive markets. To achieve this, it is necessary to improve quality control and adopt appropriate strategies for standardization.

In Mediterranean countries, that are particularly rich in endemic plants and biodiversity, these natural compounds can have their greatest impact in a system of integrated agricultural management, given their safety for humans, non-target organisms and the environment.

References

Abbas S. M., & Akladios, S. A. (2013). Application of carrot root extract induced salinity tolerance in cowpea (*Vigna sinensis* L.) seedlings. *Pakistanian Journal of Botany*, 45(3), 795-806.

Abdalla M. M. (2013). The potential of *Moringa oleifera* extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in rocket (*Eruca vesicaria* subsp. *sativa*) plants. *International Journal of Plant Physiology and Biochemistry*, 5(2), 42-49. <https://doi.org/10.5897/IJPPB2012.026>

Anca Mot, C., Lupitu, A. I., Bungau, S., Iovan, C., Copolovici, D. M., Purza, L., ... Copolovici, L. (2018). Composition and Antioxidant Activity of Aqueous Extracts Obtained from Herb of Tansy (*Tanacetum Vulgare* L.). *Revista Chimica (Bucharest)*, 69(5), 1041-1044. <https://doi.org/10.37358/RC.18.5.6257>

Beni, C., Casorri, L., Masciarelli, E., Ficociello, B., Masetti, O., Rinaldi, S., ... Cichelli, A. (2018). Characterization of garlic (*Allium sativum* L.) aqueous extract and its hypothetical role as biostimulant in crop protection. *Journal of Food Agriculture and Environment*, 16(3-4), 38-44.

Broadley, M., Brown, P. H., Cakmak, I., Maj, F., Rengel, Z., & Zhao, F. J. (2012). *Beneficial elements*. In: Marschner's Mineral Nutrition of Higher Plants. ED. Marschner P., London, Academic Press, ISBN 9780123849052, 191-248. <https://doi.org/10.1016/B978-0-12-384905-2.00008-X>

Brown, P., & Saa, S. (2015). Biostimulants in agriculture – *Frontiers on Plant Sciences*, 6, 671. <https://doi.org/10.3389/fpls.2015.00671>

Chockalingam, S., Thenmozi, S., & Sundari, M. S. N. (1990). Larvicidal activity of different products against mosquito larvae. *Journal of Environmental Biology*, 11, 101-104.

Das, K., Tiwari, R. K. S., & Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, 4, 104-111.

European Union (EU) (2009). *Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the*

sustainable use of pesticides. Official Journal of European Union.
<https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32009L0128>

Gurjar, M. S., Ali, S., Akhtar, M., & Singh, K. S. (2012). Efficacy of plant extracts in plant disease management. *Agricultural Sciences*, 3(3), 425-433.
<https://doi.org/10.4236/as.2012.33050>

Hough-Goldstein, J. A. (1990). Antifeedant Effects of Common Herbs on the Colorado Potato Beetle (Coleoptera: Chrysomelidae). *Environmental Entomology*, 19(2), 234-238.
<https://doi.org/10.1093/ee/19.2.234>

Hummelbruner, L. A., & Isman, M. B. (2001). Acute, sublethal, antifeedant and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Journal of Agriculture and Food Chemistry*, 49, 715-720.
<https://doi.org/10.1021/jf000749t>

IFOAM EU Group (2016). *Plant health care in organic farming. Position paper.*
https://www.ifoam-eu.org/sites/default/files/ifoameu_policy_position_paper_plant_health_201604.pdf

Joubert, J. M., & Lefranc, G. (2008). *Seaweed biostimulants in agriculture: recent studies on mode of action of two types of products from algae: growth and nutrition stimulants and stimulants of plant defense reactions* - Proc. Book of Abstracts of Conference: Biostimulators in Modern agriculture, Arysta Life Science Poland, Warsaw.

Katalinic, V., Milos, M., Kulisic, T., & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry*, 94, 550-557.
<https://doi.org/10.1016/j.foodchem.2004.12.004>

Kaur, S., & Mondal, P. (2014). Study of Total Phenolic and Flavonoid Content, Antioxidant Activity and Antimicrobial Properties of Medicinal Plants. *Journal of Microbiology and Experimentation*, 1(1), 00005. <https://doi.org/10.15406/jmen.2014.01.00005>

Kjeldhal, J. (1883). "Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern" (New method for the determination of nitrogen in organic substances). *Zeitschrift für analytische Chemie*, 22(1), 366-383. <https://doi.org/10.1007/BF01338151>

Koul, O., & Walia, S. (2009). Comparing impacts of plant extracts and pure allelochemicals and implications for pest control, CAB Reviews: Perspectives in Agriculture. *Veterinary Science, Nutrition and Natural Resources*, 4, No. 049.
<https://doi.org/10.1079/PAVSNNR20094049>

Koul, O., Walia, S., & Dhaliwal, G. S. (2008). *Essential Oils as Green Pesticides: Potential and Constraints.* *Biopesticides Int.*, 4(1), 63-84.

Mannina, L., et al. (2017). *NMR technologies in food analysis.* In: Analytical Chemistry: Developments, Applications and Challenges in Food Analysis, Chapter 5, 104-148. Nova Science Publ. Editors: Marcello Locatelli and Christian Celia. ISBN: 978-1-53612-267-1.

Marchand, P. A. (2017). Basic Substances under EU Pesticide Regulation: An Opportunity for Organic Production? *Organic Farming*, 3, 16-19. <https://doi.org/10.12924/of2017.03010016>

Mi, P. A. A. F. Italian Ministry for Agriculture and Forest Policies (2000). National Observatory on Soil Science and Soil Quality. *Methods of Soil Chemical Analysis*. Franco Angeli Editor, Rome, Italy.

Monakhova, Y. B., Kuballa, T., & Lachenmeier, D. W. (2011). Rapid Determination of Total Thujone in Absinthe Using ¹H NMR Spectroscopy. *International Journal of Spectroscopy*. Article ID 171684, 5 pages. <https://doi.org/10.1155/2011/171684>

Nashwa, S. M. A., & Abo-Elyousr, K. A. M. (2012). Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. *Plant Protection Science*, 48(2), 74-79. <https://doi.org/10.17221/14/2011-PPS>

Nikolova, M., Evstatieva, L., & Nguyen, T. D. (2011). Screening of plant extracts for antioxidant properties. *Botanica Serbica*, 35(1), 43-48.

Posmyk, M. M., & Szafranska, K. (2016). Biostimulators: A New Trend towards Solving an Old Problem. *Frontiers on Plant Sciences*, 7, 748. <https://doi.org/10.3389/fpls.2016.00748>

Saqvic, K. M., Kosalec, L., Mastelic, J., Pieckova, E., & Ppelinac, S. (2007). Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. *Letters in Applied Microbiology*, 44(1), 36-42. <https://doi.org/10.1111/j.1472-765X.2006.02032.x>

Scheerer, W. R. (1984). Components of oil of tansy (*Tanacetum vulgare*) that repel Colorado potato beetles (*Leptinotarsa decemlineata*). *Journal of Natural Products*, 47, 964-969. <https://doi.org/10.1021/np50036a009>

Singh, R., Koul, O., Rup, P. J., & Jindal, J. (2009). Toxicity of some essential oil constituents and their binary mixtures against *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae). *International Journal of Tropical Insect Science*, 29, 93-101. <https://doi.org/10.1017/S1742758409990087>

Sokmen, A., Gulluce, M., Akpulat, H. A., Daferera, D., Tepe, B., Polissiou, M., Sokmen, M., & Sahin, F. (2004). The in vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. *Food Control*, 15(2004), 627-634. <https://doi.org/10.1016/j.foodcont.2003.10.005>

Welch, R. M. & Shuman, L. (1995). Micronutrient Nutrition of Plants. *Critical Reviews in Plant Sciences*, 14(1), 49-82. <https://doi.org/10.1080/07352689509701922>

Copyright Disclaimer

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).