Environmental Engineering and Management Journal October 2021, Vol. 20, No. 10, 1611-1617
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

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AQUEOUS EXTRACTS AND RESIDUAL BIOMASS USE IN SUSTAINABLE AGRICULTURE: A CIRCULAR ECONOMY MODEL

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Abstract

The circular economy is based on sustainable production and consumption. In terms of circular economy, sustainable agriculture is achieved through the effective use of internal resources to self-produce and use, without waste production, green preparations for the fertilization and protection of crops. In this context, the project of a certified organic farm aims to cultivate medicinal plants to self-produce aqueous extracts of thyme and tansy, for use in crop protection, and reuse residues for composting and mulching. Regarding the environmental and economic impact, a virtuous process of cultivation and defense of agricultural crops was carried out (three-year rotation of zucchini-cabbage-chicory), to respond to market demand and to implement safety and health protection policies for farm workers and consumers. In a preliminary germination test, a stimulating effect was observed on *Vicia faba* seeds treated with thyme extract in which the greater elongation of the primary roots, relative to the control, was statistically significant. The effects of the extracts on the crops (weight and chlorophyll content) have always been significant. In particular, the chlorophyll content of chicory is highly significant for the remaining parameters measured for all horticultural crops in the field trial.

Key words: aqueous extracts, circular economy, composting, mulching, natural farming

Received: April, 2021; Revised final: July, 2021; Accepted: September, 2021; Published in final edited form: October, 2021

1. Introduction

In December 2015 the European Commission established an Action Plan for the circular economy, defining an economic development model in which resources must be exploited and waste used as raw material to create new production cycles and to protect the environment and reduce greenhouse gas emissions (GHG) (EC, 2015; Kirschenmann, 2010). The action plan also includes the EU Fertilizers Regulation (EC, 2018) which encourages production and trade of sustainable fertilizers (EC, 2009), including compost, produced by recycling organic waste, which can be used in agriculture (EC, 2016; Turcanu, 2018).

Agriculture, being a primary production sector, plays a fundamental role in terms of circular economy. Therefore, circular agriculture represents an innovative agricultural system in which everything is reused and regenerated, becoming a valuable resource. Savings is one of the main advantages of the circular

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economy in agriculture, since the reuse of waste and the self-production of fertilizers eliminates the problem of waste disposal at the end of the cycle, allowing a significant reduction in management costs and reducing the environmental impact caused by the use of synthetic products derived from non-renewable fossil sources.

In agriculture, too, the circular economy is becoming an increasingly relevant concept, as an important component of the transition to sustainable development. In fact, it is proposed to reduce the production of waste and residues deriving from productive activities (agroforestry, fishing, animal husbandry, processing of agricultural products, trade, agricultural services and consumption of products) (Albiach et al., 2000; Zaccardelli et al., 2007) as much as possible in order to develop an economic system based on recycling and sustainable agriculture.

Natural farming is an innovative method of cultivation that is suitable for the circular economy (Benyus, 2002), especially for horticultural crops, due to the self-production of the main technical means, such as compost and plant extracts, in order to improve productivity and the self-defense of the cultivation system.

Disposal of plant crop residues is an issue, especially in areas where there are many greenhouses with green biomass left outdoors to dry and decompose, instead of significantly reducing biomass residues and treating them through a recycling system (De Corato et al., 2018; Esparza et al., 2020). Since the chemical composition of biomass determines many of its properties, special emphasis should be placed on organic crop products and wastes that are free of synthetic substances present in plant protection products.

Within a certified organic farm, it is possible to apply the principles of the circular economy to self-produce and use effective plant preparations for crop fertilization and protection, using waste biomass for mulching and compost production. Chemical products are not used in natural agriculture, the treatments applied involve only self-produced technical means, such as compost, used as mulching material to create a layer of permanent homogenous soil, or aqueous plants extracts used as basic substances to support crop protection (Dayan et al., 2009; Gurjar et al., 2012). Plant extracts and substances that strengthen the plant's natural defenses (plant reinforcements), used in crop protection, could be included in the list of basic agricultural aid substances (Beni, 2020; Marchand, 2017).

Soil mulching with various types of bedding is done to prevent weed growth, protect soil from erosion, maintain soil moisture and prevent surface crusting, reduce compaction, safeguard structure, and increase soil temperature. Composting also makes it possible to transform the starting substrate into a stable product, similar to humus, used in soil fertilization (Sayara et al., 2020; Sleutel et al., 2003; Sleutel et al., 2006). The work being presented here is the result of a project for the construction of a sustainable farm based on a circular economy management principle. The experimentation started with preliminary tests and field tests carried out at the CREA experimental fields located in Tor Mancina (Beni et al., 2020), and then continued at an organic farm located in Poggio Mirteto, both near Rome.

In this project, aqueous extracts from self-grown medicinal plants were investigated as bio-stimulants and adjuvants in agricultural crop protection. Plant extracts were chosen because, being a mixture of compounds, they provide greater activity than the single active ingredient, thus being more effective and not presenting particular toxicity to workers and the environment. This phenomenon, known a synergism (or synergy) (Hummelbrunner and Isman, 2001; Singh et al., 2009), induces better and longer lasting effects (Chockalingam et al., 1990) than a simple additive effect.

In a recent study, seaweed extracts improved abiotic stress tolerance of horticultural crops and acted as protective and growth-promoting factors, as well as antibacterial agents. The mode of action was related to shoot growth, root growth, fruit set and quality (Michalak et al., 2020). A two-year pot experiment was conducted to evaluate the effect of NPK fertilizer, garlic extract and their combinations on growth, flowering and chemical constituents of *Hedychium* plants. The fertilization of *Hedychium* plants with a NPK fertilizer (19:19:19) at 4 g pot^{-1} and spraying with a 50% garlic extract is considered the best treatment, also economical, used to enhance growth, flowering and improve the nutritional status of the plant (Attia et al., 2020).

Two experiments were conducted in Egypt to define the effect of neem, liquorice, turmeric, pomegranate and thyme extracts to control mechanical damage, increasing productivity and storability of potato plants. Foliar spraying with pomegranate peels or liquorice extracts (5%) significantly increased growth parameters, total tuber and marketable yields and decreased mechanical damage of potato. Application of pomegranate or thyme extracts significantly reduced the percentage of weight loss and decay, and increased starch and dry matter content compared to control and other treatments during storage at 4°C. This study recommends the use foliar applications with thyme extracts to increase storability and reduce weight loss, at 15-day intervals in spring (Ezzat et al., 2016).

In a study conducted on cucumber plants (*Cucumis sativus*, var. *Korinda*), the effects of *Artemisia absinthium* and *Tanacetum vulgare* extracts, against *Thrips tabaci*, were compared to a corresponding commercial product containing the same extracts, using a water treatment as a control (Helm, 2015). Histological characterization was carried out by checking the thickness of the leaf epidermis. Plants treated with the three treatments showed thickening of the cell walls of the epidermis compared to the control plants. The pectin colour of the leaves treated with the products was greater than those treated with water.

The manuscript presents the first results of innovative farms with a closed-cycle production, entirely inspired by the circular economy model. The farms self-produce most of the technical inputs used for growing vegetables and herbs. All fertilizers, substrates, crop-protection products, basic substances, phyto-stimulants and mulches used in the production cycle are of natural origin, obtained from primary production biodegradable waste. Even the seeds and cuttings for the reproduction and propagation of vegetables and aromatic plants, used as beneficial companion plants, are self-produced by the farms.

In previous work, 12 natural aqueous extracts (garlic, burdock, horsetail, hypericum, lavender, lantana, pomegranate, mint, oregano, sage, tansy and thyme) were initially tested (Casorri et al., 2014) and subsequently only the extracts of garlic, thyme, and tansy, were tested since they proved to be the most effective in controlling pests and diseases of agricultural plants (Beni et al., 2018a; Beni et al., 2018b; Beni et al., 2020; Casorri et al., 2014; Masciarelli et al., 2019; Rinaldi et al., 2019). The tests were carried out in the open field at CREA experimental farms, on zucchini (*Cucurbita pepo L.*) cv. Augusto (Romanesco type) to verify the effectiveness of 1% and 2% w / v aqueous extracts, as insecticides and as fungicides against *Podosphaera xanthii* (Casorri et al., 2014). The results obtained showed that all the zucchini plants treated with extracts of garlic, thyme, lavender, sage, mint, oregano and tansy showed greater resistance to parasitic attacks and bio stimulating effects compared to untreated plants (Casorri et al., 2014).

Indeed, treated plants, particularly with thyme, garlic and tansy extracts, were characterized by a greater vegetative vigor, a better floral induction, a higher chlorophyll content and a greater number of fertile flowers, compared to untreated plants. Furthermore, zucchini production per plant was statistically higher than controls (Beni et al., 2018b; Beni et al., 2020; Casorri et al., 2014; Masciarelli et al., 2019).

This work illustrates the experimentation, which is still in progress at the organic farm located in Poggio Mirteto, and concerns the protection of plants and their bio-stimulant effect by means of aqueous extracts of thyme and tansy, self-produced from officinal plants grown on the same farm and the reuse of residual biomass deriving from the cultivation and processing of all agricultural products of the farm, typical of the Mediterranean area for size and productive orientation. The phyto-stimulating properties of thyme and tansy extracts are able to activate a protective effect in the treated plants in response to adverse conditions (oxidative stress), which depends on the chemical composition of the phyto-complexes within the broad spectrum of molecules in solution in the extracts (Posmyk and Szafranska, 2016). Tansy (*Tanacetum vulgare*) has strong insect repellent properties. Through biological assays, the greater repellent efficacy was associated with the presence of 1,8-cineole, bornyl acetate, p-cymene, γ-terpinene and camphor, while the insecticidal properties to β-thujone. B-thujone (tanacetone) is present as a major component in the b-dextrorotatory form. It is a terpenoid containing a ketone group having two stereoisomeric forms known as (+) - 3-thujone or α-thujone and (-) - 3-thujone or β-thujone. It has a menthol smell and has an insecticidal effect (ants, aphids, lepidoptera, agrotids, moths and borer) and pesticide. Thanks to these properties it is used to disinfect gardens and vegetable gardens (Brewer and Ball, 1981; Dancewicz and Gabryś, 2008; Dancewicz et al., 2011; Kwiecień et al., 2020).

Thymol and carvacrol are the main constituents of thyme (*Thymus vulgaris*), responsible for effectiveness. (Saqvic et al., 2007). Thyme extract, due to its antiseptic, antibacterial, antifungal and antioxidant properties, can be used as a fungicide in agriculture, but it also has a good nematicide activity (Zaccardelli et al. 2007). Thymol is a monoterpenes' phenol characteristic of plants of the genus *Thymus*, contained throughout the plant and important for its antibacterial properties and characteristic odour. It is a particularly effective fungicide. Carvacrol (cymo-phenol) is also a monoterpenes' phenol present in *Thymus* and *Oregano*. Thymol and carvacrol act through a synergistic effect. The antioxidant properties of thyme are instead due to the content of polyunsaturated flavonoids (Kulisic et al., 2005; Szilvássy et al., 2013).

This work aims to show the possibility of creating an independent organic farm that can manage the production, protection, and control of crops in a sustainable regime and protect the environment and the health of agricultural workers and consumers and in the perspective of the principles of the circular economy.

2. Materials and methods

2.1 Preparation of the aqueous extracts and their chemical characterization

The extracts of tansy (*Tanacetum vulgare L.* subsp. *Vulgare*) and thyme (*Thymus vulgaris* var. *Verticillata* Willk) at 1% w/v were prepared in distilled water at a temperature of 25° C using thyme leaf powder and tansy flowering tops, dried in a thermostatic chamber at 40° C. Solute percentages were chosen based on guidelines provided by the producers' association in order to produce useful substances in organic and natural agriculture. The extracts, filtered after 48 h on filter paper, were either directly used or stored at + 4 ° C for 6-7 days. For crops treatments, the extracts were used without dilution, as indicated by the association of natural and organic producers (Nashwa and Abo-Elyousr, 2012).

The extracts were subjected to chemical analysis, phytotoxicity tests and field tests. (Beni et al., 2020).

2.2. Phytotoxicity test

In this experimentation, laboratory tests were performed to evaluate the potential toxicity of the aqueous extracts prepared with thyme and tansy produced within the organic farm. To perform phytotoxicity tests, fava bean (*Vicia faba* var. *minor* Beck) seeds were allowed to germinate in a quartz sandy soil (Sturchio et al., 2006) treated with thyme or tansy extracts. To perform the tests, 25 fava bean seeds were placed on 250 g of quartz sand in each of 9 aluminium boxes. Three boxes were treated with 100 mL of thyme extract, three boxes were treated with 100 mL of tansy extract, and three control boxes only with deionized water (100 mL) to wet seeds. Each box, sealed with Parafilm, was incubated for 5 days at 20±1 °C to allow seed germination.

The effects of both the aqueous extracts on seed germination were examined. In particular, the primary root length reduction (PRL) test was used to evaluate the phytotoxic effects through growth inhibition. After 5 days, 100% of the seeds had been germinated and each of the 75 primary roots was measured with a calliper, placing each root on a flat surface after washing.

2.3. Field trial

An open field trial was conducted on a certified organic farm located in Poggio Mirteto, about 50 km north of Rome. The farm began converting a portion of the vegetable garden to natural farming in 2016. Natural farming aims at the self-reinforcement of undisturbed soil fertility. Tillage is not carried out, except for the preparation of the cultivation beds at the beginning of the conversion, prepared by taking the topsoil from permanent walkways. None of the chemicals allowed in organic farming were used. The only application made concerns self-produced technical means, such as green compost, integrated into the mulch in order to accelerate the creation of a permanent homogenous topsoil layer, or plant aqueous extracts used as basic substances to support crop defense (Beni et al., 2018b; Cappello, 2019; Fiebrig et al., 2020; Fini et al., 2016; Hazelip, 2014; Mancin, 2012; Marchand, 2017; Pinamonti, 1998; Rezendes et al., 2020; Rinaldi et al., 2019; Shyam et al, 2019).

By means of analyses conducted according to official methods (MiPAAF, 2000), the soil was characterized as a clayey-silty texture with a high content of total organic carbon (4.7%), a sub-alkaline pH (7.4) and a significant cation exchange activity (35.86 meq 100 g^{-1}).

The soil was only tilled prior to conversion, to shape raised beds approximately 0.4 m high, 1 m wide and 5 m long. On the beds, mulching is carried out with self-produced green compost and renewed every 6 months, before each productive cycle. This operation has the dual purpose of containing the germination and growth of wild plants and improving soil fertility by creating a homogenous top-soil layer. The compost is self-produced by the farm, using the biodegradable waste from the pruning of olive and fruit trees and vegetable residues. The compost is turned over and moistened weekly for the first 21 days during the stabilization phase, then it is left to mature for six months to be used as mulch. In this last phase, passive ventilation is carried out by placing perforated pipes inside the pile of biomass being oxidized. The characteristics of the compost produced in the period 2018-2020 are described in Table 1.

Table 1. Chemical composition of the self-produced compost

Parameter	*Measurement unit*	*Value*
Moisture	%	35-42
pH	-	6.5-8.2
TOC	% d.m.	24-31
Total N	% d.m.	1.7-2.3
C/N	-	13-16
P	% d.m.	1.1-1.8
K	% d.m.	2.2-3.5
Cu	mg kg^{-1}	91-112
Zn	mg kg^{-1}	254-311
Germination rate (dilution 30%)	%	73-88
Salmonella	MPN	Absent
Escherichia coli	UFC g^{-1}	< 1.000

Evaluation of the effects of the extracts was carried out in the open field on zucchini-cabbage-chicory plants in a three-year rotation. On each bed, the following plants were respectively transplanted: 6 courgette plants (*Cucurbita pepo L.*) cv. Augustus (Romanesco type) on 9 April, 2018; 40 cabbage plants (*Brassica oleracea L.*) cv. Savoy King on April 16, 2019; and 50 chicory plants (*Cichorium intybus L.*) cv. Catalonia on May 7, 2020. Nine beds were divided into three blocks, and the plants on one bed per block were sprayed weekly with thyme or tansy extracts at a concentration of 10g L^{-1}. As a control, a bed per block was used whose plants were sprayed with water and compared with those treated with thyme and tansy extracts. Three zucchini plants, nine cabbage and chicory plants were randomly selected and marked to detect yield. Zucchini were harvested at 14 different times, from the end of May to the end of July, and the harvest data were summarized.

2.4. SPAD measurements

To evaluate the vegetative state of each plant, in vivo measurements of chlorophyll content was carried out with the SPAD 5200 portable fluorimeter (Konica Minolta Business Solution Italia Spa, Milan, Italy) on the leaves of the three species. Measurements were made for courgette in the middle of the harvest period and before harvest for cabbage and chicory, with three replicates per plant on each cultivation bed.

2.5. Statistical analysis

The experimental data of plant weight (g $plant^{-1}$), SPAD units, and phytotoxicity tests (PRL test), were previously analyzed by graphical analysis (box plot) and descriptive statistics to estimate the variability and determine possible outliers. Homoscedasticity of the data was checked using Levene's test. Two ways ANOVA was performed to check the effect of two extracts on the vegetable plants on the field and on bean seeds, compared to the untreated control. Comparisons between treatments means were done when test F of ANOVA was significant (α level=0,05) using Tukey's HSD test.

3. Results and discussion

The results of the phytotoxicity test and the three-year rotation field test are presented in Table 2 and Table 3.

3.1. Phytotoxicity test

The Primary root length (PRL) test was significantly affected by the effect of the extracts, as shown by the ANOVA F test (Table 2). No repressive induction on primary root growth was detected compared to the control, thus excluding any phytotoxic activity. On the contrary, a stimulation effect was observed on seeds treated with thyme extract in which the greater elongation of the primary roots, compared to the control, was statistically significant.

3.2. Yield and chlorophyll content of crops in three-year rotation

The two-way ANOVA has never detected any significance for the effects regarding the blocks of beds, while the effects of the extracts on vegetable weight and chlorophyll content (SPAD units) in all horticultural crops were always significant. In particular, the ANOVA test F, shown in table 3, confirms that the effects of the extracts were significant for the SPAD units of chicory (*p value* <0.05) and remarkably significant (*p value* <0.01) for the remaining parameters measured for all horticultural crops in the field trial.

The data in Table 3 shows the effects of the extracts on the yield expressed as weight of the edible portion per plant and on chlorophyll content of crops. In particular, for zucchini, the thyme extract significantly increased the weight of the vegetable by 22.44% compared to the control; also, the tansy extract increased the vegetable weight by 8.04% compared to the control but without statistical significance. Regarding the SPAD units, treatments with tansy and thyme extracts both significantly increased the values of this parameter by 21.9% and 25.17%, respectively. In cabbage, both extracts, highly significantly (test F, *p value* <0.01) and similarly, increased the weight of the vegetables and the SPAD units, compared to the control.

Table 2. F ANOVA and PRL test (mm) in fava beans treated with tansy and thyme Extracts (mean and standard deviation sd)

F ANOVA[(a)]	***7.652 (p value < 0.01)***		
Extracts	***N. of seeds***	***PRL (mm)***	
		Mean	***sd***
Control	75	89.89 a	10.27
Tansy	75	88.09 a	11.30
Thyme	75	94.55 b	9.79

Means marked with different letters are significant on Tukey's HSD test (p-value <0.05);
[a)]Degree or freedom 2;216 (extracts: error);

Table 3. F test ANOVA, Vegetable weight and SPAD units in plants treated with thyme and tansy extracts (mean and standard deviation sd)

Vegetable Crops (year)		***Vegetable weight (g plant-1)***		***SPAD Units***	
Zucchini (2018)	***F ANOVA (a)***	***7.357 *****		***18.33 *****	
		mean	***Sd***	***mean***	***Sd***
	Control	5215.89 a	768.08	33.34 a	2,73
	Tansy	5635.22 ab	529,43	40.64 b	2.53
	Thyme	6386.33 b	661.89	41.73 b	4.18
Savoy cabbage (2019)	***F ANOVA (a)***	8.177 **		13.417 **	
		mean	*Sd*	*mean*	*Sd*
	Control	1329.22 a	87.87	25.67 a	4.22
	Tansy	1520.44 b	166.19	36.29 b	5.61
	Thyme	1593.89 b	130.85	36.59 b	5.14
Chicory (2020)	***F ANOVA (a)***	8.129 **		5.297 *	
		mean	*Sd*	*mean*	*Sd*
	Control	486.22 a	88.27	41.43 a	5.29
	Tansy	651.78 b	74.56	50.29 b	5.07
	Thyme	566.44 ab	86.56	44.21 ab	5.86

***(p value < 0.01); * (p value < 0.05), The means marked with separate letters, in the columns concerning the same crop, were significant in Tukey's HSD test (p value <0.05), (a) Degree or freedom 2;18 (extracts: error)*

As a mean of two treatments, weight gain was 17.15% (+227.95 g) and in SPAD units 41.96% (+10.77 units), compared to control. In chicory, tansy extract significantly increased the yield of the edible portion by 34.05%, compared to the control. Thyme extract also allowed yields that were 16.50% higher than the control, although not statistically significant. Similarly, the SPAD units in chicory were significantly increased by 21.39% from the tansy extract compared to the control, while the 6.71% increase induced by the thyme extract, compared to the control, was not statistically significant.

4. Conclusions

The concept of circular economy takes inspiration from the natural cycle and is perfectly applicable to the agricultural sector. Agricultural biomass can be used for the autonomous production of plant extracts useful for crop defence, and their waste exploited in mulching and fertilization operations. Plant extracts have proved to be effective auxiliary technical means in the defence of crops. Furthermore, the extracts showed no toxic effects; on the contrary, they were antioxidants and bio stimulants, effective in increasing the growth and production of vegetables. The treated plants had, compared to the untreated, a greater vegetative vigour and a greater production of flowers and fruits.

The gradual replacement of conventional plant protection products and fertilizers with natural preparations, such as plant extracts, will provide significant benefits in protecting human health and reducing occupational and environmental risks, via pest management with low pesticide intake. This virtuous process of agricultural defence is more advantageous in terms of circular economy and provides for a more efficient use of resources that responds to the needs of the market, environmental safety, and health protection policies for consumers and agricultural workers.

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