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Phytotoxicity of encapsulated essential oil of rosemary on germination and morphophysiological features of amaranth and radish seedlings

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ABSTRACT

Recently, there has been a growing interest in plant-derived substances and their environmentally-friendly application to crops, as alternatives to the use of chemical herbicides. Here, the essential oil (EO) of rosemary (*Rosmarinus officinalis* L.) was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS). The EO was then evaluated to examine its phytotoxic effects under greenhouse conditions. For the first time in the scientific literature, starch was used in order to encapsulate the EO and to make it function as a bio-herbicide which, accordingly, would be released slowly in the soil as a result of its encapsulation. The EO was successfully encapsulated in the starch matrix for the first time and the released profile of the EO was evaluated as a core material. This study revealed that the encapsulation efficiency (EE) and the loading capacity (LC) of the starch matrix loaded with EO were about 99.99 and 5.26%, respectively, when encapsulation occurred by using 1 g of EO per 1 g of starch. The starch-coated capsules containing the EO were mixed with pot soil at various concentrations (0, 0.5, 2, 3.5 and 5 g of capsules per kg of soil). Furthermore, the inhibitory effects of the encapsulated EO (ENCEO) were tested on the germination rate and percentage of seeds, fresh and dry weights of the roots and stems, root length, leaf area, chlorophyll content, proline content, hypocotyl length and diameter in radish, as well as the relative membrane permeability (RMP) of the leaf in amaranth (*Amaranthus retroflexus*) and radish (*Rhaphanus sativus*) under greenhouse conditions. The results showed that the increase in the concentration of ENCEO caused the significant decrease in germination rate and percentage, leaf area, fresh and dry weights of roots and stems, root length and chlorophyll content in both species ($P \le 0.05$). However, there was a significant increase in the amounts of proline and RMP ($P \le 0.05$) in the tested species.

1. Introduction

One of the most prominent problems in the production of horticultural crops is the accumulation of toxins and chemical residues in fresh products such as vegetables and fruits. Common herbicides are dangerous pollutants concerning horticultural crops (Hazratia et al., 2017). On the other hand, weeds can interfere with the productivity of horticultural fields, and they reduce the quality and quantity of fresh and processed products. Therefore, finding safe and effective methods for weed control is of prime interest in the production of horticultural crops (Hazratia et al., 2017; Mahdavikia et al., 2017). The control of weeds usually could be performed through various chemical, biological and cultural methods (Singh et al., 2005). Natural products released from the residues of medicinal plants may help to reduce the use of synthetic herbicides for weed management and, as a result, cause less pollution in addition to providing grounds for safer agricultural products (Sodaeizadeh et al., 2010).

The term allelopathy refers to the detrimental effect of a plant on another plant through the production of chemical retardants (Bostan et al., 2013; Murrell et al., 2011). By common definition, allelopathy is any direct or indirect effect of plants on other surrounding plants through their influence on germination and growth, as plants create chemical compounds (Hassannejad and Ghafarbi, 2013). Chemicals released from plants and microorganisms are termed allelochemicals (Kruse et al., 2000).

Secondary products or waste products often comprise allelochemicals which act through the metabolic pathways of higher plants. Allelochemicals are a diverse group of complex compounds which include but are not restricted to alkaloids, phenolics, jasmonates, momilactones, flavonoids, hydroxamic acids, brassinosteroids,

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glucosinolates, salicylates, carbohydrates, EOs and amino acids. Many physiological and biochemical processes are made possible or altered by the presence of allelochemicals. The degree to which allelochemicals become toxic depends on factors such as their concentration, age, the climate, the metabolic state of the plant and various environmental conditions. In fact, the growth and development of plants can be adversely affected by the stress caused by these biochemical compounds. Weeds continue to acquire resistance against herbicides, and allelochemicals can alleviate the dilemma by serving as useful agents to generate secure countermeasures against weeds (Hassannejad and Ghafarbi, 2013). *Rosmarinus officinalis* L. (Lamiaceae), more popularly known as “rosemary”, is an aromatic evergreen herb with the potential to provide EO. It is widely distributed in the Mediterranean region (Alipour and Saharkhiz, 2016). Rosemary EO includes 1,8-cineole, α-pinene, linalool, camphor, bornyl acetate, and camphene as the major components (Zargari, 1990; Moghtader and Afzali, 2009; Jafarzadeh et al., 2010). The allelopathic competence of many plants that can yield essential oils is an issue that has been well documented in reports concerning laboratory and greenhouse experiments. For instance, Saharkhiz et al. (2009) showed the allelopathic effects of four species including *R. officinalis* against three weed species. Hassannejad and Ghafarbi (2013) reported the allelopathic effects of EO from plants in the Lamiaceae family; the EO had been used as growth inhibitors affecting the post-emergence seedling phase of *Cuscuta campestris.* Similarly, Hazratia et al. (2017) confirmed the herbicidal activity of *Satureja hortensis*, as the nanoemulsion of its EO showed high phytotoxic effects and interfered with the germination, growth and physiological processes of two weeds, i.e. *Amaranthus retroflexus and Chenopodium album* under greenhouse conditions. Additionally, a similar report exists on the strong phytotoxic effects of EOs from *S. hortensis* and other species of *Satureja* against the seed germination of weed species (Taban et al., 2013).

Encapsulation may be defined as a process by which one substance (core) is entrapped within another substance (wall material). The encapsulating substance is often called the coating, membrane, shell, capsule, carrier material, external phase or matrix (Nedovica et al., 2011; Fang and Bhandari, 2010). Microencapsulation is also defined as a process in which tiny particles or droplets are surrounded by a coating. Alternatively, they can be a type of embedded substance in a homogeneous or heterogeneous matrix which is used in order to form small capsules with many useful properties. Four reasons are proposed for applying microencapsulation: to reduce the core’s reactivity with environmental factors, to reduce the transfer rate of the core material to the outside environment, to promote easier handling and to control the release of the core material (Gharsallaoui et al., 2007). There are different microencapsulation processes such as spray-drying, spray-cooling, spray-chilling, air suspension coating, extrusion, centrifugal extrusion, freeze-drying, coacervation, rotational suspension separation, co-crystallization, liposome entrapment, interfacial polymerization and molecular inclusion. (Desai and Park, 2005). Carbohydrates such as starches, corn syrup solids and maltodextrins have been usually used as encapsulating agents. These materials are considered as good encapsulating agents as they exhibit low viscosities at high solid contents and good solubility (Yoshii et al., 2001). Starch is a polymeric carbohydrate widely used as a solid matrix for encapsulated materials (Almeida et al., 2013). Dong et al. (2011) reported the encapsulation of peppermint EO in coacervation of various matrices consisting of gum Arabic and gelatin. Beirao da Costa et al. (2012) also reported that Oregano EO can be encapsulated in rice starch, inulin and gelatin/sucrose capsules by spray drying. Fernandes et al. (2014) created microencapsulates that encased rosemary EO; the microencapsulates were created by spray-drying using maltodextrin and modified starch as carriers. The encapsulated oil and its composition proved to be quite similar to pure oil. In another study, the influence of wall material concentrations (10–30%), inlet temperature (135–195 °C) and feed rate (0.5–1.0 $L.h^{-1}$) were evaluated to examine the properties of rosemary oil microencapsulated by spray-drying, while gum Arabic was used as the carrier. The optimized conditions were a wall material concentration of 19.3%, an inlet air temperature of 171 °C and a feed flow rate of 0.92 $L.h^{-1}$. Under this condition, the particles exhibited no fissures and the compositions of pure and microencapsulated oil were similar (Fernandes et al., 2013).

Almost all of the previous studies focused only on the general phytotoxic effects of secondary plant metabolites by *in vitro* experiments. However, in the present work, we encapsulated the essential oil of rosemary with starch as a suitable and economic polymer for the first time and then practically utilized the formulation in the pot soil to control the growth of an index plant (radish) and an important weed (amaranth) under greenhouse conditions.

Briefly, the main objectives of the present study were to determine (i) rosemary EO encapsulated with starch by the cabinet dryer, encapsulation efficiency (EE), released profiles of EO from the starch matrix, and (ii) to examine the phytotoxic effects of the ENCEO on several physiological and growth parameters of amaranth and radish under greenhouse conditions. The achievements of this work proved the possibility of applying the encapsulated essential oil of rosemary as a pre-emergence agent for weed control in the cultivation systems of horticultural crops.

2. Materials and methods

2.1. Plant material

The aerial parts of *R. officinalis* were collected at fruit-set stage (green fruits) on the 6th of May 2012, from Sadra Medicinal and Aromatic Plants Botanical Garden (Shiraz, Iran) at an altitude of 1846 m above sea level, and was geographically coordinated at 29.8° north and 52.4° east. The mean value for annual temperature was 14.5 °C, and the mean value for annual rainfall was 437 mm. All of the harvested materials were air-dried at room temperature (at less than 25 °C) in the shade for 14 days.

The seeds of amaranth weed (*A. retroflexus*) and radish (*R. sativus*) were prepared from the Research Field of the Agriculture College, Shiraz University, Shiraz, Iran. The samples were used for phytotoxic experiments.

Amaranth was selected for phytotoxic experiments as it is one of the most important weeds in the world. It is an aggressive and competitive weed among a variety of crops. It causes substantial yield loss in soybean, maize, cotton, sugar beet, sorghum and many other horticultural crops. It is an alternative host for a number of pests that inhabit crops and cause diseases. Such pests and diseases include the green peach aphid, *Myzus persicae*, and the cucumber mosaic cucumovirus in peppers (Weaver and McWilliams, 1980). Most previous cases of research on plant toxicity have used radish seeds as an index plant. The inhibition of germination and seedling growth of radish is taken as an indicator of toxicity in plants because it is very susceptible to phytochemicals at low concentrations. Radish is known for its easy growth and high rate of germination. Therefore, it is commonly used in the bioassays of phytochemicals since its preparation for experiments is convenient (Mahdavikia et al., 2017).

The rosemary plant was considered to have the potential to provide the required EO in phytotoxic experiments. It was identified and authenticated by A. Khosravi, a plant taxonomist at Shiraz University, Shiraz, Iran. The voucher specimen (No: 24,987) was deposited in the herbarium.

2.2. Extraction of EO

The shade-dried aerial parts of rosemary were subjected to water distillation (hydro distillation) for 3 h using an all glass Clevenger-type apparatus to extract EO according to the method outlined by the European Pharmacopoeia (Anonymous, 1997). The extracted oil was