



## Formulation of botanicals for the control of plant-pathogens: A review

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### ABSTRACT

Essential oils and plant extracts contain a multitude of bioactive substances against fungi, bacteria and nematodes. In plant pathology research, botanicals are commonly used in their raw state. Without any type of formulation, bioactive compounds of plants can be degraded and volatilized rapidly under field conditions. Controlled-release liquid and solid formulations with plant compounds as active ingredients are common in some fields, such as medicine, pharmaceuticals, food technology and cosmetology. However, the use of controlled-release formulations is an under explored approach in plant pathology, although these technologies are interesting options for managing seed, soil-borne and post-harvest pathogens. In this review, we discuss the potential and options of formulations of botanicals against plant pathogens.

### 1. Introduction

Secondary metabolism of plants is responsible for the synthesis of numerous bioactive substances, which provide protection against insects, pathogens and limit the growth of other plants species. Essential oils and plant extracts contain a multitude of bioactive substances, including alkaloids, cyanogenic glycosides, glucosinolates, lipids, phenolics, terpenes, polyacetylenes and polythienyls. Scientists have been explored the diversity of these molecules and their use in integrated management of pests and pathogens (Isman, 2000; Zaker, 2016). Products based on plant extracts and essential oils are available for use in managing plant diseases in various countries. However, the number of botanical-based products remains restricted, despite the enormous potential for botanicals in the pesticide market, especially if we consider the increasing demand for ecofriendly options to manage agricultural pests.

The most common scenario in plant pathology research is to use extracts and oils in their raw state for managing fungi, bacteria and nematodes. In controlled conditions, extracts and essential oils from diverse plant species have shown efficiency in inhibiting plant pathogens (Isman, 2000; Zaker, 2016). However, the promising results obtained in laboratory or greenhouse are usually not observed in the field, with few exceptions (Jing et al., 2018). Degradation and volatilization of bioactive compounds are the major factors that reduce the efficiency of plant-based products under field conditions. Consequently, the potential suitability of certain plant material for use in agriculture ends up

being underestimated due to losses of bioactive substances. One option to avoid these drawbacks is to formulate bioactive plant products using polymers, plasticizers, stabilizers and biodegradable antioxidants.

Polymers, emulsifying agents, surfactants, solvents, stabilizers, defoamers and other components are used to ensure the stability, adherence and controlled release of the bioactive compounds, depending on the type of formulation (Knowles, 2008; Gasic and Tanovic, 2013). Examples of slow release liquid and solid formulations with plant compounds as active ingredients are common in some fields, such as medicine, pharmaceuticals, food technology and cosmetology (Arriola et al., 2016; Mikulcová et al., 2016). In the agricultural sector, the use of controlled release formulations is still in the initial stage, although these technologies are interesting options for managing seed, soil-borne and post-harvest pathogens (Knowles, 2008). In this type of formulation, active ingredients are released into the environment over time and this feature brings benefits such as reducing losses of the active ingredient, a longer period of activity and reduced toxicity to animals and plants (Knowles, 2008).

The formulation process may vary greatly according to the methods and materials used for encapsulation, but it is important that botanicals be formulated for use in experiments, rather than their use in raw state. In this review, we discuss the potential use of formulations of plant-based extracts, essential oils and isolated active compounds against plant pathogens. We present options for preparing formulations that may be used in plant pathology research and related fields.

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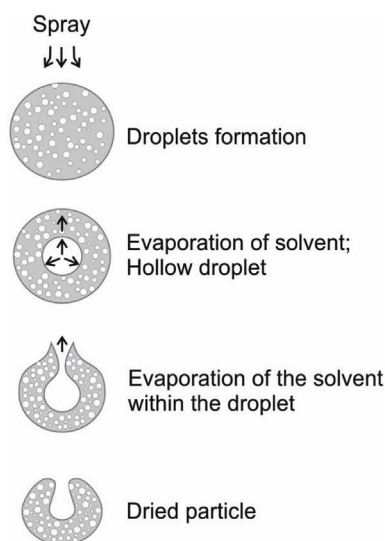


Fig. 1. Formation of particle by spray drying (adapted from Oliveira and Petrovick, 2010).

## 2. Principal methods of encapsulation

### 2.1. Atomization (spray drying)

This process consists of three steps: first, the product (e.g., extract and essential oil) is dispersed as droplets, which increases its surface area. Second, dispersed droplets came into contact with a heated air stream and in the third, the solvent is evaporated, resulting in the formation of the solid particle (Fig. 1) (Oliveira and Petrovick, 2010). This a low-cost process at industrial scale, especially for the micro-encapsulation of essential oils (Fernandes et al., 2014; Bakry et al., 2016).

Spray-drying formulations have not been extensively explored for the control of plant pathogens (Corrêa et al., 2016; Cortesi et al., 2017). In a few examples, formulations of coffee leaf extracts or gallic acid were used as plant resistance inducers (Corrêa et al., 2016) or in the management of *Pseudomonas syringae* pv. *tomato* (Cortesi et al., 2017).

### 2.2. Lyophilization (freeze-drying)

In the freeze-drying process, the product (extract or oil) is rapidly frozen, thus preserving its chemical characteristics. In the following step, the frozen material is subjected to a partial vacuum. Then, the ice or other frozen solvents are removed from the material through sublimation and the product is dried to approximately 2% wet basis. The dehydrated solid material is milled until reaching the desired particle size.

Freeze-dried extracts of some plants have fungicidal activity. Freeze-dried extracts of *Ruta graveolens* reduces the mycelial growth of the phytopathogenic fungi *Fusarium solani*, *Pyrenochaeta lycopersici*, *Thielaviopsis basicola*, *Verticillium dahliae* and *Penicilium* sp. (Oliva et al., 1999), while those from *Pelargonium* sp., *Salvia officinalis*, *Lavandula officinalis*, *Mentha pulegium* and *Mentha arvensis* reduces up to 85% the germination of *Phakopsora pachyrhizi* spores (Borges et al., 2013).

### 2.3. Liposome inclusion (emulsions)

#### 2.3.1. Emulsions

An emulsion is defined as a thermodynamically unstable system containing at least two non-miscible liquid phases, where one phase contains colloidal particles dispersed in the other phase. Nanoemulsions are the most studied form of emulsions. They are colorless emulsions with droplet sizes ranging from 50 to 200 nm, while conventional

emulsions appear as blue droplets with size between 1 and 100  $\mu\text{m}$ . In comparison to conventional emulsions, nanoemulsions have higher kinetic and thermodynamic stability, greater ease of diffusion and nanoparticle transport, enhanced incorporation and protection of both hydrophilic or lipophilic molecules in their dispersed phases. The transport of phytochemicals across cellular membranes, for example, is facilitated when the products are encapsulated in nanoemulsions (Huang et al., 2010).

Emulsions of essential oils and plant extracts are valuable options for controlling plant diseases (Lu et al., 2013; Elshafie et al., 2015; El Ouali et al., 2017; Jing et al., 2018). For example, nanoencapsulated essential oils of cinnamon, lemon and bergamot have antifungal activity toward *Aspergillus niger* (Ribes et al., 2016). Other notable examples are the suppression of *Xanthomonas fragariae* by palmarosa oil nanoemulsion (Luiz et al., 2017) and the inhibition of *Rhizoctonia solani* and *Sclerotium rolfsii* by nanoemulsions of oils of *Azadirachta indica* A. Juss and *Cymbopogon nardus* (L.) Rendle (Ali et al., 2017).

### 2.4. Extrusion – casting

In this method, an emulsion/extract core and coating material (alginate, acetate, starch, etc.) is applied through pipette or nozzle at high pressure into an ionic solution under agitation, such as calcium chloride. Gel beads are collected after 20 min and dried. The resistance of the bead wall depends on the components of the formulation and the contact time between particles and the ionic solution. Care must be taken to minimize or avoid losing active compounds during the encapsulation processes and storage (Arriola et al., 2016; Pasukamonset et al., 2016).

Plant extracts and essential oils are encapsulated by extrusion and used in food preservation (Arriola et al., 2016; Pasukamonset et al., 2016), e.g. minimally processed apples and mushrooms (Raybaudi-Massilia et al., 2008). However, this technique still has not been explored in the formulation of botanicals for plant disease management.

### 2.5. Fluidized bed

The fluidized bed coating consists of spraying an encapsulated agent on a fluidized powder bed (Hemati et al., 2003). The material to be encapsulated is suspended in solid state by a current of gas at a given temperature and sprayed with fine droplets of the encapsulating material liquid, forming a fine liquid film on the particle. Finally, the materials undergo wetting and drying, thus forming a solid homogeneous layer (Benelli et al., 2015). The rate of solid circulation, nozzle atomization pressure, humidity and coating temperature may interfere in the efficiency of the coating (Guignon et al., 2002). Fluidized beds are widely used in the pharmaceutical and food industries, as well for synthesizing agrochemicals, dyes and other industrial chemicals.

Granules obtained through this technique have higher bioactive compound retention, better flow properties and higher coating efficiency compared to those obtained by spray drying (Benelli et al., 2015). It is possible to prepare controlled-release formulations using this process (Hemati et al., 2003), which could be used in products for managing soil-borne pathogens. Another advantage of this method is the possibility of large scale applications, by presenting a short circulation time of particles and high heat transport, in addition to being highly controllable and automated (Capece and Dave, 2011). Śmigielski et al. (2011) observed that more than 40% of the essential oils of lavender (*L. angustifolia*) are lost during the drying process. However, if the fresh biomass of a plant is dried by fluidized bed in a system of closed circuit containing a drying agent and a heat exchanger, the generated product will contain more volatile and biologically active substances than other processing methods (Śmigielski et al., 2011).

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