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# Encapsulation of bioactive components of thyme and clove essential oils is a potential biocontrol strategy for bacterial wilt disease in potato

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## Abstract
Potato (*Solanum tuberosum* L.) is an important food security crop in the world. Its productivity is however constrained by both biotic and abiotic stresses. Key among the biotic stresses is bacterial wilt disease caused by *Ralstonia solanacearum*. To date, there are no known conventional bactericides which provide effective control of this soil borne pathogen. Bioactive components of essential oils such as thymol, eugenol, carvacrol, cinnamaldehyde and citral have previously been reported to have a high antibacterial activity against pathogenic microbes and hence are potential antibacterials against *R. solanacearum*. However, their potential usage as antibacterials is limited due to their volatility, hydrophobicity, rapid degradation as well as poor solubility in water. In order to overcome these shortcomings, the use of nanotechnology is hereby proposed. This review examines the effect of nano-encapsulating these compounds in nanocarriers and recommends an appropriate bioactive compound of essential oil and its corresponding encapsulating agent that can be exploited as a bactericide for the management of *R. solanacearum*, the causative agent of bacterial wilt.

**Key words**: Bacterial wilt, *Ralstonia solanacearum*, nanotechnology, essential oils, nanocarriers

## Introduction

Potato is affected by a wide range of biotic factors including nematodes, bacteria, fungi and insects some of which are important pests and disease-causing agents. The most common diseases affecting potato are late blight, early blight and bacterial wilt. Of these diseases, bacterial wilt caused by *Ralstonia solanacearum* is the most destructive (Muthoni *et al*., 2014). It leads to severe losses in tropical, subtropical, and temperate regions estimated to close to 1 billion USD wordwide. It is one of the main causes of yield reduction in Africa (Harahagazwe *et al*., 2018). In Kenya, the disease has been reported to lead to up to 100% of yield losses (Muthoni *et al*., 2014).

The pathogen is mainly spread from infested to healthy fields by farm equipment, irrigation water and plant-to-plant through the rhizosphere. It is highly persistent and only antagonistic micro-organisms and environmental factors can affect its survival (Champoiseau *et al*., 2009). The pathogen is also spread through seed stocks (Patil *et al*., 2012).

*Ralstonia solanacearum* is a Gram-negative bacterium with a single polar flagellum, rod-shaped and 0.5-1.5 µm in length. The pathogen infects host plants mainly through the damaged roots arising from wounds caused by soil borne organisms like the nematodes or can also get into the plant through wounds formed by the emergence of lateral roots (Karim & Hossain, 2018). The seedlings from the pathogen free seeds can also get infected when planting is done in previously infested soil. The risk of infection is increased by the fact that *R. solanacearum* can remain in soil for a long period of time. Once it gets into the roots, it colonizes the plant by moving through the xylem in the vascular bundles and blocking water uptake resulting in wilting and eventual death. After colonization of the vascular system, the virulent strains also produce long polymer N-acetylated monosaccharides which are implicated in wilting (Meng, 2013). The bacteria also secrets cell wall degrading enzymes such as endoglucanase, pectin methylesterases, exopolygalacturonases, endopolygalacturonases, exoglucanases which are all associated with the virulent strains (Denny, 2006).

**Control of bacterial wilt of potato**

Currently, bacterial wilt management is mainly achieved through cultural control methods which includes the use of clean certified planting materials, growing tolerant varieties, use of drip irrigation, planting in uninfected production sites, reduced density of planting to reduce spread and crop rotation. Of these, selection of fairly resistant varieties is one of the most effective ways of controlling bacterial wilt (Patil *et al.*, 2012). In addition to cultural methods, breeding for resistance against bacterial wilt has also been attempted but has achieved limited success since the pathogen is constantly evolving, has a highly variable membrane protein, serological reactions and biochemical properties (Karim & Hossain, 2018). The use of chemicals in controlling bacterial wilt is difficult in potato because the bacteria is localized inside the xylem and also its ability to survive in the soil for prolonged periods of time. Hence until now, there is no effective chemicals to control potato bacterial wilt (Karim & Hossain, 2018). Many attempts have also been made to control the disease using antagonistic microorganisms and predators. For example, *Bacillus subtilis, Trichoderma viridae* and *Glomus mosseae* have yielded positive results against *R. solanacearum* (Dennis *et al*., 2016; Rostand *et al.*, 2018). However, their use in the field has been limited because biocontrol agents that are effective against the pathogen are yet to be identified. There is also lack of appropriate large scale inoculation methods and the challenge in determining the rate at which antagonistic predators can be applied (Karim & Hossain, 2018).

Despite concerted efforts to control the disease, these management strategies have limited efficacy and bacterial wilt continues to cause serious economic challenges in many countries in the world (Aguk *et al.*, 2018; Karim & Hossain, 2018). These limitations may be due to the complexity of *R. solanacearum* pathogenicity which enhances its resistance to the available control methods.

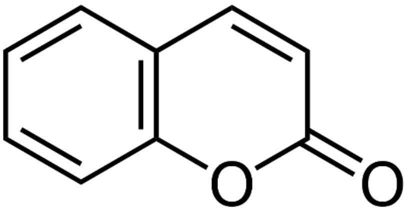
**Plant secondary metabolites**

These are different biologically significant chemical compounds that are naturally found in plants (Varma, 2016). Of great importance are the secondary metabolites produced by plants some of which are involved in protecting the plants against pests and diseases. Some of the secondary metabolites with antibacterial activity include phenolics, alkaloids, glycosides, terpenoids and essential oils (Cowan, 1999; Ohri & Pannu, 2010).

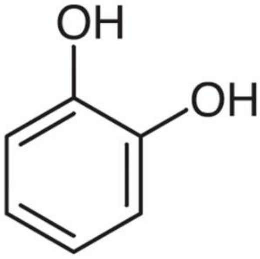
Plant phenolic compounds are a group of simple bioactive phytochemicals from plants which have a diversity of structures but their main characteristic is the presence of hydroxylated aromatic rings (Varma, 2016). Examples of phenolic compounds include coumarin, catechol, thymol, carvacrol, cinnamaldehyde and eugenol (Figure 1).

**Essential oils**

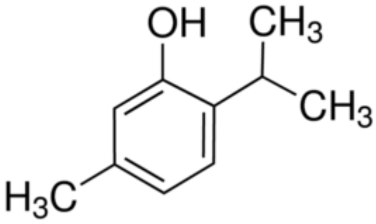
Phenolic compounds which possess $C_3$ side chain and do not have oxygen are classified as essential oils (EO) and have been shown to have antimicrobial properties. Examples in this category are eugenol, thymol, carvacrol and cinnamaldehyde (Cowan, 1999).



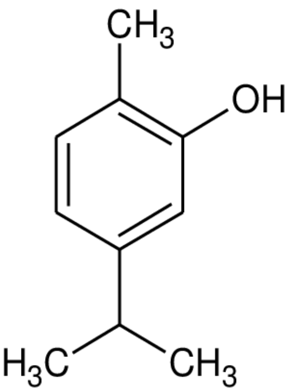
Coumarin



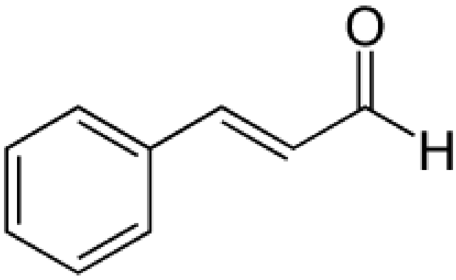
Catechol



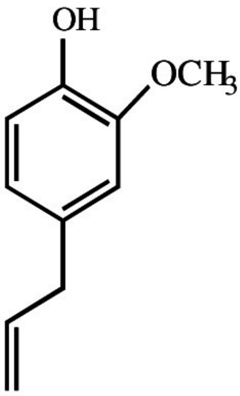
Thymol



Carvacrol



Cinnamaldehyde



Eugenol

**Figure 1:** Chemical structures of some phenolic compounds

An EO is a concentrated hydrophobic liquid from plants containing volatile aromatic compounds (Varma, 2016). They are the most common plant derived metabolites commonly synthesized in seeds, flowers and leaves and later stored in plant secretory cells and cavities (Dosoky & Setzer, 2018). EOs and aromatic plant extracts have a wide range of applications (Figure 2) (Pandey *et al.*, 2017). EOs are well recognized as natural antimicrobial preservatives and are classified by the US Food and Drug Administration as generally recognized as safe (GRAS) (Weiss *et al.*, 2009).

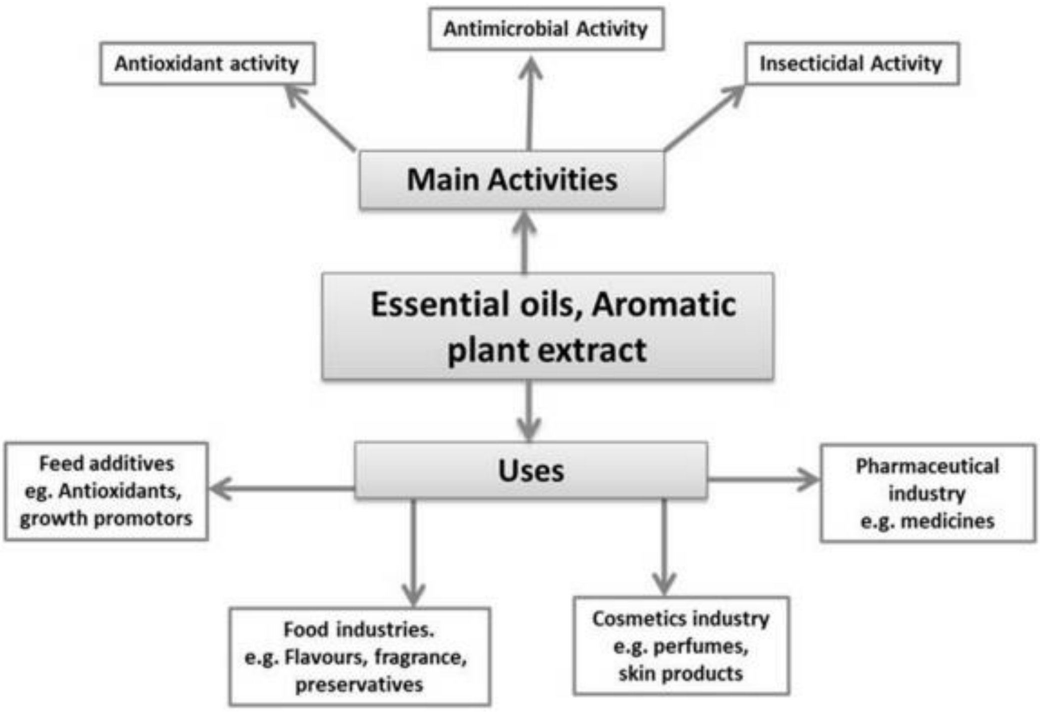



**Figure 2:** Activities and uses of EOs. Adopted from Pandey *et al.* (2017)

EOs have a pronounced antimicrobial activity because they are made up of a number of different active constituents which include terpenes, terpenoids, carotenoids, coumarins and curcumins among others (Pandey *et al*., 2017). In a given plant, EO is a mixture of between 20 to 60 different components which occur in different concentrations. However, they are characterized by the presence of between 2-3 major components which are present in higher concentrations (Chouhan *et al*., 2017).

EOs containing major compounds such as eugenol and thymol have highest antibacterial activity (Bassolé & Juliani, 2012). There is evidence however, that minor components also contribute to the entire antimicrobial effect of EOs (Pradhanang *et al*., 2003). The differences in the concentration of active constituents of EOs are mainly related to factors such as agronomic practice, variety, seasons and processing conditions (Verma *et al*., 2011). However, the high variability both in antimicrobial activity and in composition may limit the use of EOs in controlling bacterial wilt.

**Antibacterial effect of essential oils against *R. solanacearum***

Both thyme and clove EOs were reported to show antibacterial activity against both gram negative and gram positive bacteria *in vitro* using disk diffusion method (Oulkheir *et al*., 2017). Using agar diffusion, a strong antibacterial activity of thyme oil was reported against

120 clinical strains of bacteria in the genus *Escherichia*, *Enterococcus, Pseudomonas* and *Staphylococcus* (Sienkiewicz *et al*., 2012). Lee *et al.* (2012) showed that clove EO applied at a rate of 0.005%−0.01% exhibited potent *in-vitro* antibacterial activities against *R. solanacearum* thereby significantly decreasing tomato bacterial wilt. It can therefore be concluded that clove and thyme EOs have notable antibacterial activity against *R. solanacearum* which can be harnessed to protect potato against bacterial wilt.

**Active components of thyme and clove essential oils**

The antimicrobial effect of thyme and clove EOs correlate to the occurrence of major compounds thymol and eugenol in them respectively (Bai *et al.*, 2016; Pradhanang *et al*., 2003)

***Thymol***

Thymol is a phenolic compound and a major volatile component of EO extracted from thyme (*T. vulgaris* L.), basil and oregano which are all medicinal plants. It is a colorless crystalline compound whose IUPAC name is 2-isopropyl-5-methylphenol with a structure shown in Figure 2.3. Thymol has been used as medicine in many countries for many years and research has proved that it possesses antibacterial, antifungal, antiseptic activities (Nagoor *et al*., 2017). Its excellent antimicrobial activity has been linked to its hydroxyl groups which can interact with the cell membrane of bacteria leading to the disruption of its integrity and hence cause leakage of cellular components (Di Pasqua *et al*., 2007).

***Eugenol***

Eugenol is a phenolic compound which was extracted for the first time from the buds and leaves of *Eugenia caryophyllata* commonly known as clove (Khalil *et al*., 2017). It is the major bioactive component of clove EO which accounts for 45-90% of its constituents. A part from clove EO it can also be obtained from a number of other plant sources which include extracts from thyme, ginger, turmeric, cinnamon extract among other plants (Charan *et al*., 2015; Khalil *et al*., 2017).

Chemically, eugenol (4-Allyl-2-methoxyphenol) ($C_{10}H_{12}O_2$) is a compound belonging to a class of phenylpropanoids (Figure 2.3) with a molecular mass of 164.2g $mol^{-1}$. The compound is also known as caryophyllic acid, allylguaiacol, 4-allylcatechol-2-methyl ether. Its partially soluble in water and its colour ranges from clear to pale yellow (Charan *et al*., 2015). Eugenol exhibits strong antibacterial activity against a number of both gram-positive and gram-negative bacteria (Charan *et al*., 2015).

**Limits and challenges of eugenol and thymol oils**

The use of EOs in inhibiting the pathogenicity of bacteria is limited because they are highly volatile, not very soluble in water and are also poorly stable when exposed to factors such as heat, moisture, and oxygen (Amro, 2017). Some of the drawbacks of eugenol are low solubility and susceptibility to sublimation (Charan *et al*., 2015). These drawbacks can be overcome by use of nanotechnology. EOs can be protected against oxidation, reduction and easy loss under natural conditions by covering it in a polymer wall (Figure 3) (Vishwakarma *et al*., 2016).

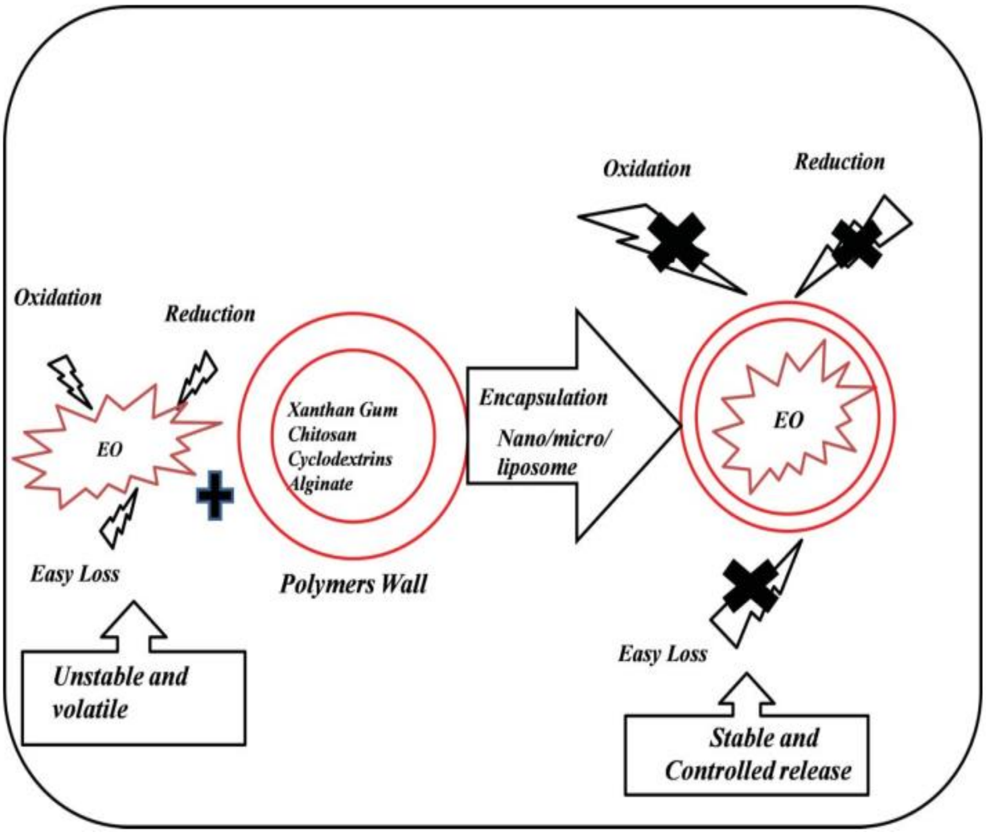



**Figure 3:** Protection of EOs from release under natural conditions by covering it in a polymer wall. Adopted from Vishwakarma *et al*. (2016).

**Nanotechnology in Agriculture**

Blecher *et al*. (2011) provisionally defined nanotechnology as the design, production and application of materials that are in the nano-scale range (<1–100 nm). However, in the agricultural point of view, nanoparticles are defined as particulate dispersions or solid particles with a size in the range of between 10 and 1,000 nm (Noori *et al*., 2014).

Nanotechnology has for many years been utilized in medicine but its application in agriculture has received relatively less attention. Currently, in agriculture, research studies are exploring the use of nanotechnology in controlled release of agrochemicals, plant hormone delivery, seed germination, transfer of target genes and nanosensors (Worrall *et al*., 2018). The nanostructured systems have the potential of enhancing the efficacy of EO and their components by promoting their sustained release, reduced dosage, improved activity and minimized side effects (Bonifácio *et al*., 2014; Worrall *et al*., 2018).

There are two mechanisms through which the use of nanoparticles can protect plants: the use of nanoparticles in providing crop protection and use of nanoparticles as carriers for existing active plant compounds such as pesticides, fertilizers, antibacterial and antifungal compounds (Worrall *et al*., 2018). When used as carriers, they enhance shelf-life, improve water solubility, reduce toxicity, and boost site-specific uptake into the target microbe (Worrall *et al.*, 2018). The nanoparticles or the nanocomposites can be applied to the plants through soaking the seeds, irrigating the roots or foliar spray (Chen *et al*., 2019).

A number of encapsulating agents have so far been reported (Hasani & Hasani, 2018). Among them, chitosan is the most cost effective carrier and has been used in both pharmaceutical and agricultural fields (Dennis *et al.*, 2016). Chitosan has been used as an encapsulating carrier agent in managing different plant pathogens (Hasani & Hasani, 2018; Jamil *et al*., 2016; Kalagatur *et al*., 2018). Chitosan is preferred as an ideal carrier system due to its properties including biodegradability, biocompatibility, availability, cationic charge safety, large surface area for adsorption and innate antimicrobial potential (Jamil *et al*., 2016). It also induces plants leaves to release hydrolytic enzymes β-glucanase and chitinase which actively contribute to the defense of plants against a variety of plant pathogens (Mandal *et al.*, 2013). Previously, chitosan which is a deacetylated form of chitin has been extensively used to encapsulate bioactive compounds including EOs and their components such as *Ocimum basilicum*, *Origanum vulgare* and carvacrol through ionic gelation (Hosseini *et al.*, 2013; Keawchaoon & Yoksan, 2011; Rasaee *et al*., 2016).

Cinnamon EO microencapsulated in chitosan were found to be highly stable and had a significantly high antibacterial activity against *R. solanacearum* (Tu *et al.*, 2020). Encapsulation of thymol has also been reported to protect it from volatilization and sustenance of its antioxidant and antimicrobial properties (Bhalerao & Wagh, 2018). These limitations are the reason why majority of the previous studies on the antibacterial activities of active compounds of EOs have been limited to *in vitro* evaluation.

## 2.1 Nanotechnology

The use of nanotechnology in plant biotechnology and agriculture has shown great promise in the recent past (Das & Bimal, 2016). Currently, efforts are being made to explore its use in agriculture. Some applications being explored include its use as means of delivering plant hormones, controlled release of agrochemicals, nanobarcoding and nanosensors (Worrall *et al*., 2018).

Clay, silica, organic polymers such as cellulose and chitosan are some of the materials used for encapsulation to enhance the efficacy of the EOs (Gatahi *et al*., 2016). The nano-materials are preferred due to their ability to penetrate cellular walls of plants and affinity for microbes thus precision in delivering the biochemicals to the pathogen infected areas within a plant. In addition, some encapsulating materials such as chitin and silica possess antimicrobial activity on pathogens and may act synergistically with the EO components in the control of the pathogens. The encapsulated materials also play nutritional roles in plants as they provide plant nutrients such as nitrogen, silicates, carbon and other micro nutrients enhancing plant productivity (Gatahi *et al*., 2016).

### 2.4.1 Essentials oils loaded in nanosystems

Different materials can be used as nanocarriers. However, organic nanocarriers are preferred because of their biocompatibility and high biodegradability. The organic nanocarriers used to encapsulate EO components are classified into lipid and polymer based nanoparticles (Figure 4) (Bilia *et al*., 2014).

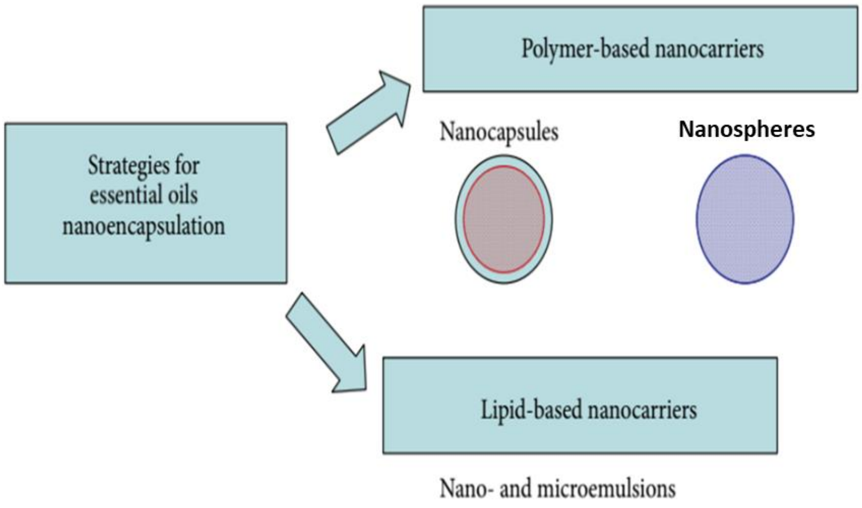



**Figure 4:** Nanosystem platforms for EOs. Adopted from Bilia *et al.* (2014)

Two major categories of polymer based nanocarriers exist (Bilia *et al*., 2014), these include nanocapsules and nanospheres (nanoparticles) (Figure 4). Nanospheres are composed of matrix system while nanocapsules have an outer polymeric membrane which is an encapsulating material (Amro, 2017). This encapsulating material can allow the incorporation of EO or their components within the polymeric membrane. Different EOs or their components have successfully been loaded into different polymeric nanocarriers. For example, in a study by Woranuch & Yoksan (2013), the thermal stability of eugenol was improved when loaded into chitosan nanoparticles. Rassu *et al*. (2014) evaluated encapsulation of thymol in a shell made of cyclodextrin and a copolymer based on dimethyl aminoethyl methacrylate. The purpose of encapsulation was to control powderisation, solubilisation, and taste-masking properties. Different biodegradable polymers such as xanthum gum, poly vinyl alcohol, gelatine, starch, sodium alginate, ethyl cellulose and chitosan have previously been used to encapsulate thymol (Bhalerao & Wagh, 2018; Hasani & Hasani, 2018).

Nanoparticles made from polysaccharide is one major class of biocompatible polymers which is preferred carrier material due to their unique properties such as biodegradability, safety, non-toxicity, stability, abundance in nature and low processing cost. It comprises of compounds from plant, animal or microbial origin. Those from plant origin include cellulose and starch compounds together with their derivatives while polysaccharide compounds from animals include chitosan and xanthan gum (Bilia *et al*., 2014). Chitosan is a polymer which is processed through deacetylation of chitin using an alkali. The polymer has been used in medicine in delivery of enzymes, genes and drugs. CNPs have a larger surface which increases their utilization (Li *et al*., 2010). CNPs are preferred because they are non-toxic, have a potential of enhancing host plant resistance, are easily assimilated by the plant root hairs thus delivery of the adsorbed materials and are also naturally abundant (Gatahi *et al*., 2016). Eugenol has previously been encapsulated into chitosan nanoparticles with an average size of less than 100 nm and was found to be thermally stable (Woranuch & Yoksan, 2013).

Encapsulation has been reported to enhance the biocidal activities of EOs or their components. For example, when compared to free EO, the antibacterial and antioxidant activities of thyme EO against *Staphylococcus aureus* and *E. coli* increased after being nanoencapsulated in chitosan (Hasani & Hasani, 2018). In a study conducted by Woranuch & Yoksan (2013), the thermal stability and radical scavenging activity of eugenol encapsulated in chitosan was significantly enhanced when compared to naked eugenol.

**Conclusion**

This review study has shown that the effects of bacterial wilt disease in potato has continued to be felt by farmers despite the current management practices which include the use of cultural control methods, breeding for resistant varieties amongst others. It has also been shown that the use of active compounds from essential oils such as thymol and eugenol could be used as an agrochemical solution against *R. solanacearum*. However, they are limited by their volatility, hydrophobicity and instability when exposed to the environment. Therefore, their encapsulation in biopolymer compounds such as chitosan could protect them from the surrounding medium, enhance their antibacterial activity as well as facilitate their controlled release from the nanocapsules. Nano encapsulation of active essential oil compounds therefore is a promising strategy to control bacterial wilt in potato.

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