

Antimicrobial efficacy of cinnamon, ginger, horseradish and nutmeg extracts against spoilage pathogens

Pouvoir antimicrobien d'extraits de cannelle, gingembre, raifort et muscade contre des agents pathogènes de conservation

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Résumé de l'article

Dans la recherche de moyens de remplacement à l'utilisation de fongicides de synthèse, des extraits aqueux d'épices ont été évalués pour leurs effets sur la croissance mycélienne de plusieurs agents pathogènes de conservation et pour leur efficacité *in vivo* de répression de la pourriture sèche de la pomme de terre et de la maladie de la tache de la carotte. Les travaux réalisés montrent que les extraits de cannelle, de gingembre et de muscade inhibent significativement la croissance mycélienne des hyphomycètes *Aspergillus niger* et *Fusarium sambucinum*, de l'oomycète *Pythium sulcatum*, ou du zygomycète *Rhizopus stolonifer*, alors que l'extrait de raifort n'a causé aucune inhibition à la concentration testée. Parmi les extraits les plus efficaces, 0,05 g mL⁻¹ d'extrait de cannelle a complètement inhibé *A. niger* et *P. sulcatum*, alors que 0,10 g mL⁻¹ d'extrait de cannelle a complètement inhibé *F. sambucinum*. Une concentration de 0,05 g mL⁻¹ de gingembre a également causé une inhibition de 100 % de *P. sulcatum*. Des essais *in vivo* ont montré que l'extrait de cannelle réduisait significativement les lésions de la pourriture sèche et de la maladie de la tache et que l'extrait de gingembre réduisait les lésions de la maladie de la tache. Cette étude suggère la possibilité d'utiliser des extraits aqueux de cannelle ou de gingembre comme produits de remplacement aux fongicides de synthèse pour la répression de certains agents pathogènes.

Antimicrobial efficacy of cinnamon, ginger, horseradish and nutmeg extracts against spoilage pathogens

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In the search for alternatives to the use of synthetic fungicides, aqueous spice extracts were evaluated for their effects on the mycelial growth of various spoilage pathogens and their ability to control potato dry rot and carrot cavity spot *in vivo*. Results showed that cinnamon, ginger and nutmeg significantly inhibited the mycelial growth of *Aspergillus niger* (Ascomycota), *Fusarium sambucinum* (Ascomycota), *Pythium sulcatum* (Oomycota) or *Rhizopus stolonifer* (Zygomycota), whereas horseradish extract did not lead to the inhibition of any microorganism at the tested concentration. Among the most effective extracts, 0.05 g mL⁻¹ of cinnamon extract completely inhibited *A. niger* and *P. sulcatum*, and 0.10 g mL⁻¹ of cinnamon extract completely inhibited *F. sambucinum*. A concentration of 0.05 g mL⁻¹ of ginger extract also caused 100% inhibition of *P. sulcatum*. *In vivo*, cinnamon extract significantly reduced lesions of potato dry rot and carrot cavity spot, and ginger extract reduced lesions of carrot cavity spot. These results indicate that aqueous cinnamon and ginger extracts could provide an alternative to the use of synthetic fungicides to control these pathogens.

Keywords: Cavity spot, cinnamon, dry rot, *Fusarium sambucinum*, ginger, *Pythium sulcatum*, spoilage pathogens.

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Mots clés: Agents pathogènes de conservation, cannelle, *Fusarium sambucinum*, gingembre, maladie de la tache, pourriture sèche, *Pythium sulcatum*.

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INTRODUCTION

Synthetic fungicides remain the most widely used control measure for food crop diseases. While relatively effective, these fungicides have major drawbacks, including their propensity to trigger resistance development in the targeted microorganism(s) as well as their adverse effects on health and the environment (see references in Avis 2007). In the particular case of food spoilage diseases in warehouses and cold storage units, many fungicides that are used in the field are not registered for use as postharvest treatments, and some prohibited chemicals have not been replaced (Janisiewicz and Korsten 2002). In this context, there remains an urgent need for disease control measures that present novel or alternative modes of action with reduced risks to health and the environment.

A possible alternative to the use of chemical fungicides involves the use of antimicrobial extracts from plant materials. In particular, spices from plant materials have been used as antimicrobial products both in traditional and herbal medicine (Cowan 1999). Among some of the more studied spices, chilies, cinnamon, clove, garlic, horseradish and wasabi have shown a high and generalized antimicrobial activity against a host of plant and human pathogens, including fungi, bacteria, protozoa and viruses (Cho *et al.* 2007; Cichewicz and Thorpe 1996; Cowan 1999; Dorman and Deans 2000; Naganawa *et al.* 1996; Yu *et al.* 2001). However, there is comparatively little information on the use of spice extracts as a means to control spoilage in stored produce.

Dry rot and cavity spot are among the most prevalent diseases in potato tubers and carrot roots, respectively. Dry rot is caused by various species of *Fusarium* (including *Fusarium sambucinum*) and is mainly controlled by postharvest applications of the fungicide thiabendazole. Many reports show increasing resistance of *F. sambucinum* to thiabendazole (Holley and Kawchuk 1996; Platt 1997) that makes the fungicide less effective in controlling this disease. Cavity spot is caused by different species of *Pythium* (including *Pythium sulcatum*). Symptoms of the disease generally appear in the field as the carrot root matures, and lesions can continue to develop during storage (Howard *et al.* 1994). Few efficient chemical options are available to control cavity spot (Martinez *et al.* 2005).

In view of the need for alternatives to synthetic chemical fungicides in a postharvest context, the objectives of this study were (i) to evaluate the effects of aqueous spice extracts on the mycelial growth of spoilage pathogens, and (ii) to evaluate the efficacy of these extracts in reducing potato dry rot and carrot cavity spot.

MATERIALS AND METHODS

Fungal material

Four postharvest pathogens were used to assess the antimicrobial activity of the prepared spice extracts. *Aspergillus niger* Tiegh. (Ascomycota), *Fusarium*

sambucinum Fuckel (Ascomycota), *Pythium sulcatum* R.G. Pratt & J.E. Mitch. (Oomycota), and *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. (Zygomycota) were maintained on potato dextrose agar (PDA; Becton Dickinson, Sparks, MD, USA) at 24°C.

Preparation of plant extracts

Extracts were prepared from cinnamon bark (*Cinnamomum verum* J. Presl), ginger rhizome (*Zingiber officinale* Roscoe), horseradish powder (*Armoracia rusticana* P.G. Gaertn., B. Mey. & Scherb), and nutmeg seed (*Myristica fragrans* Houtt.). Dried cinnamon bark sticks and nutmeg seed were reduced to a fine powder in a spice grinder. Ginger rhizomes were surface sterilized for 5 min in 70% ethanol, washed with water, and lyophilized prior to being reduced to powder using a mortar and pestle. Extracts were prepared by suspending 10 to 100 g of each dried powdered spice in 100 mL of sterile distilled water at 24°C for 36 h. The extracts were recovered by filtering the suspensions on Whatman paper (#1) followed by centrifugation at 3,100 x g for 15 min. The supernatant was recovered and cold sterilized through a 0.45 µm filter unit (Nalgene, Rochester, NY, USA).

Effect of spice extracts on mycelial growth

To determine their effect on *A. niger*, *F. sambucinum*, *P. sulcatum* and *R. stolonifer* mycelial growth, the produced extracts were incorporated into PDA. In an initial trial, spice extracts (cinnamon, ginger, horseradish and nutmeg) were incorporated at 0.05 g mL⁻¹ into warm (45°C) sterile PDA prior to pouring into Petri dishes. PDA without spice extracts served as controls. In a second trial, cinnamon and ginger extracts were incorporated into PDA (as described above) at the following concentrations: 0 (control), 0.05, 0.10 and 0.15 g mL⁻¹. Concentrations of extracts are expressed as the quantity of dried spice (in grams) per mL of PDA. Agar plugs (0.5 cm diam) covered with actively growing mycelia of the four pathogens were individually inoculated on the media and incubated for 2 (for *R. stolonifer*), 3 (for *A. niger*) or 4 d (for *F. sambucinum* and *P. sulcatum*) in the dark at 24°C. After the incubation period, mycelial growth of each pathogen was measured as the average of two perpendicular diam of the colony minus 0.5 cm (diam of the agar plug). All experiments were conducted according to a completely randomized design with five replications.

Effect of spice extracts on postharvest diseases

To determine the inhibitory effect of cinnamon and ginger extracts on potato dry rot and carrot cavity spot, the extracts were sprayed on artificially inoculated potato tubers and mature carrot roots. In dry rot experiments, potato tubers (*Solanum tuberosum* L. var. Superior) were surface sterilized with a 0.5% solution of sodium hypochlorite for 15 min, rinsed with distilled water, and air dried. Wounds (0.5 cm diam, 4 mm deep) were performed on each tuber using a cork borer as previously described by Mecteau *et al.* (2008). Agar plugs (0.5 cm diam) covered with actively growing mycelia of *F. sambucinum* were placed in each wound. Agar plugs containing no fungi mycelia served as non-inoculated controls. In cavity spot experiments, mature carrot roots (*Daucus*

Table 1. Effect of aqueous spice extracts (0.05 g mL⁻¹) on mycelial growth of spoilage pathogens

Spice extract	Mycelial growth ^a (cm)			
	<i>Aspergillus niger</i> ^b	<i>Fusarium sambucinum</i> ^c	<i>Pythium sulcatum</i> ^c	<i>Rhizopus stolonifer</i> ^d
Control	2.2 a ^e	4.2 a	4.2 a	4.3 a
Cinnamon	0.0 b	1.3 b	0.0 c	1.6 c
Ginger	2.0 a	4.2 a	0.0 c	1.9 c
Horseradish	1.9 a	3.2 a	3.8 a	4.5 a
Nutmeg	2.1 a	3.9 a	3.0 b	2.4 b

^a Mean of two perpendicular diam of the colony.

^b Determined after a 3-d incubation period at 24°C.

^c Determined after a 4-d incubation period at 24°C.

^d Determined after a 2-d incubation period at 24°C.

^e Within a column, values followed by the same letter are not significantly different according to Fisher's protected LSD test ($P = 0.05$).

carota L. cv. Caropak) were surface sterilized with a 0.5% solution of sodium hypochlorite for 5 min, rinsed with distilled water, and air dried. Wounding was performed on each carrot root by gently scraping the epidermis with a sterile razor blade according to Benard and Punja (1995). Agar plugs (0.5 cm diam) covered with actively growing mycelia of *P. sulcatum* were placed directly on the wounded carrot epidermis. Agar plugs containing no *P. sulcatum* mycelia served as non-inoculated controls. The inoculated and non-inoculated potato tubers and carrot roots were individually transferred to plastic containers lined with moistened paper towels. Each potato tuber and carrot root was sprayed with 2 mL of cinnamon or ginger extracts at concentrations of 0.05, 0.10 and 0.15 g mL⁻¹. Sterile distilled water (2 mL) served as the control. Dry rot and cavity spot severity was measured as the average of two perpendicular diam of the lesions after a 7-d incubation period at 24°C or a 10-d incubation period at 15°C for dry rot and cavity spot, respectively. The experimental unit consisted of one potato tuber or one carrot root in an individual plastic container. The experiment was conducted according to a completely randomized design with four replications.

Statistical analysis

Analysis of variance (ANOVA) was carried out with the GLM procedure of SAS (SAS Institute Inc. 1999) and, when significant ($P < 0.05$), treatment means were compared using Fisher's protected least significant difference (LSD) test.

RESULTS

Effect of spice extracts on mycelial growth

In the initial *in vitro* trial, the four spice extracts (cinnamon, ginger, horseradish and nutmeg) were tested at a concentration of 0.05 g L⁻¹ against the four postharvest pathogens. At this concentration, cinnamon extract caused 100% inhibition of mycelial growth in *A. niger* whereas the other extracts did not inhibit the growth of this pathogen (Table 1). In *F. sambucinum*, only the cinnamon extract significantly inhibited mycelial growth when compared to the control (Table 1). Cinnamon and ginger extracts completely inhibited the growth of *P. sulcatum*. Nutmeg extract also significantly inhibited the growth of *P. sulcatum*, albeit to a lesser extent (28%) (Table 1).

Table 2. Effect of different concentrations of aqueous cinnamon extracts on mycelial growth of spoilage pathogens

Cinnamon extract (g mL ⁻¹)	Mycelial growth ^a (cm)			
	<i>Aspergillus niger</i> ^b	<i>Fusarium sambucinum</i> ^c	<i>Pythium sulcatum</i> ^c	<i>Rhizopus stolonifer</i> ^d
0	2.5 a ^e	4.2 a	4.0 a	4.7 a
0.05	0.0 b	1.1 b	0.0 b	1.4 b
0.10	0.0 b	0.0 b	0.0 b	0.8 b
0.15	0.0 b	0.0 c	0.0 b	0.0 c

^a Mean of two perpendicular diam of the colony.

^b Determined after a 3-d incubation period at 24°C.

^c Determined after a 4-d incubation period at 24°C.

^d Determined after a 2-d incubation period at 24°C.

^e Within a column, values followed by the same letter are not significantly different according to Fisher's protected LSD test ($P = 0.05$).

Table 3. Effect of different concentrations of aqueous ginger extracts on mycelial growth of postharvest pathogens

Ginger extract (g mL ⁻¹)	Mycelial growth ^a (cm)			
	<i>Aspergillus niger</i> ^b	<i>Fusarium sambucinum</i> ^c	<i>Pythium sulcatum</i> ^c	<i>Rhizopus stolonifer</i> ^d
0	2.2 a ^e	4.1 a	4.4 a	4.3 a
0.05	2.2 a	4.2 a	0.0 b	2.1 b
0.10	1.9 a	3.4 ab	0.0 b	0.8 c
0.15	1.7 a	2.9 b	0.0 b	0.0 c

^a Mean of two perpendicular diam of the colony.

^b Determined after a 3-d incubation period at 24°C.

^c Determined after a 4-d incubation period at 24°C.

^d Determined after a 2-d incubation period at 24°C.

^e Within a column, values followed by the same letter are not significantly different according to Fisher's protected LSD test ($P = 0.05$).

In *R. stolonifer*, cinnamon and ginger extracts caused the highest inhibition of mycelial growth relative to the control. Nutmeg extract also inhibited *R. stolonifer* growth, though significantly less so than cinnamon and ginger extracts (Table 1). At a concentration of 0.05 g mL⁻¹, horseradish extract did not cause a significant inhibition in any of the tested fungi or oomycota.

In a second *in vitro* trial, cinnamon and ginger extracts were assayed at different concentrations to assess their inhibitory effect on the tested pathogens. Significant statistical interactions between extracts and concentrations were found and the data are therefore presented for each extract. In general, cinnamon and ginger extracts inhibited the pathogens in a dose-dependent manner for *F. sambucinum* and *R. stolonifer*. Indeed, increasing the concentration in the medium from 0.05 to 0.15 g mL⁻¹ caused a significant decrease in mycelial growth in these two fungi (Tables 2 and 3). Results from this trial confirmed that 0.05 g mL⁻¹ of cinnamon extract was necessary to completely inhibit *A. niger* and *P. sulcatum*, whereas 0.10 and 0.15 g L⁻¹ were required to totally inhibit *F. sambucinum* and *R. stolonifer*, respectively (Table 2). Conversely, 0.05 g mL⁻¹ of ginger extract was necessary to completely inhibit *P. sulcatum* and 0.15 g mL⁻¹ was necessary to completely inhibit *R. stolonifer*. A concentration of 0.15 g mL⁻¹ of ginger extract significantly inhibited *F. sambucinum*, albeit by only 29%. None of the tested concentrations of ginger extract significantly inhibited the mycelial growth of *A. niger* (Table 3).

Effect of spice extracts on postharvest diseases

Various concentrations of cinnamon and ginger extracts were further tested to determine their suppression of potato dry rot (*F. sambucinum*) and carrot cavity spot (*P. sulcatum*). Significant statistical interactions between extracts and concentrations were found and the data are therefore presented for each extract. Cinnamon extract provided significant control of dry rot (approximately 20% relative to the water control) at 0.10 g mL⁻¹. A concentration of 0.15 g mL⁻¹ of cinnamon extract afforded the highest suppression of both dry rot and cavity spot, reducing lesions by 51 and 31%, respectively (Fig. 1). Ginger

extracts did not suppress dry rot at any of the tested concentrations (Fig. 2). A ginger concentration of 0.15 g mL⁻¹ significantly reduced cavity spot lesions by 35%.

DISCUSSION

Spice extracts have been known to provide antimicrobial activity in traditional and herbal medicine (Cowan 1999). However, little is known of their ability to control plant pathogens and, in particular, those that contribute to food spoilage during storage. Synthetic chemical fungicides are still the most widely used control methods against produce spoilage, but they generally have the potential inconvenience of triggering resistance development in the targeted pathogen and causing risks to health and the environment.

The present study showed that spoilage pathogens are strongly affected by various aqueous spice extracts. In particular, cinnamon was the most effective extract as it significantly inhibited the mycelial growth of *A. niger*, *F. sambucinum*, *P. sulcatum* and *R. stolonifer* by 63 to 100%. Aqueous cinnamon bark extracts had previously shown *in vitro* effects against a host of microorganisms, including *Aspergillus candidus* Link, *A. niger* and *Fusarium culmorum* (W.G. Sm.) Sacc. (Magro *et al.* 2006). Various aqueous and alcohol extracts or essential oil distillates from cinnamon leaves have also shown antimicrobial activity against *R. stolonifer* (Rodríguez *et al.* 2008) and *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hansen (Fawzi *et al.* 2009). To our knowledge, this is the first report of antimicrobial activity of aqueous cinnamon bark extracts against *F. sambucinum* and *P. sulcatum*.

Among the tested extracts, aqueous ginger extracts also highly inhibited the mycelial growth of *P. sulcatum* (100%) and *R. stolonifer* (56%), and it significantly inhibited the mycelial growth of *F. sambucinum* (29%). Essential oils obtained from hydrodistillation of ginger or aqueous extracts from ginger seed had previously shown antimicrobial activity against a wide range of spoilage pathogens, including *A. niger*, *Aspergillus flavus* Link, *F. oxysporum*, *Fusarium roseum* Link:Fr. and *R. stolonifer* (Okigbo and Nmeka 2005; Tripathi *et al.* 2008). However, the aqueous

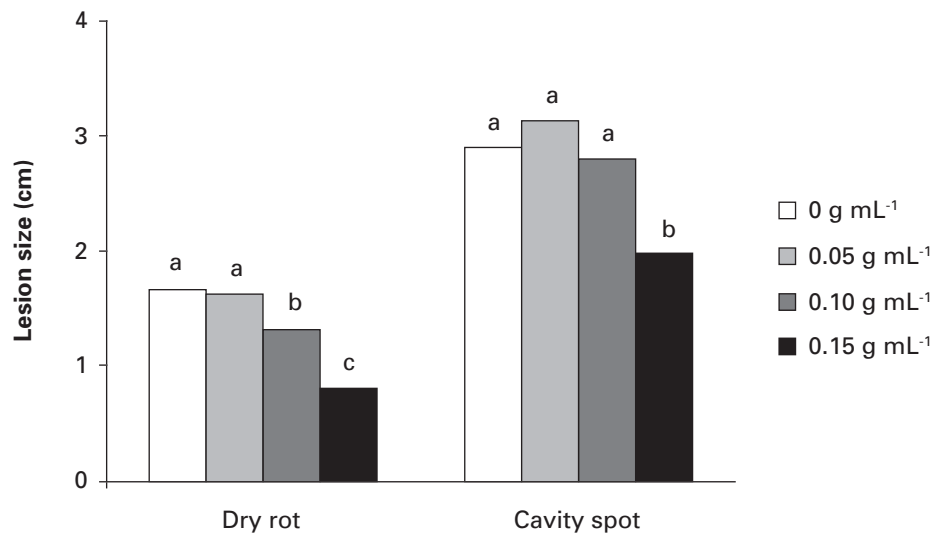


Figure 1. Effect of aqueous cinnamon extracts on the development of potato dry rot and carrot cavity spot. For each disease, lesion sizes with the same letter are not significantly different according to Fisher's protected LSD test ($P = 0.05$).

ginger rhizome extracts in this trial did not inhibit *A. niger* mycelial growth. To our knowledge, this is the first report of antimicrobial activity of aqueous ginger extracts against *F. sambucinum* and *P. sulcatum*.

Although work conducted using other extraction methods had previously demonstrated their antimicrobial activity (Cho *et al.* 2007; Tripathi and Dubey 2004), nutmeg and horseradish extracts revealed low or no activity against the studied pathogens under the tested conditions.

Cinnamon extracts significantly decreased dry rot (*F. sambucinum*) and cavity spot (*P. sulcatum*) lesions in infected potato tubers and mature carrot roots, respectively. Although cinnamon strongly inhibited the growth of *F. sambucinum* and *P. sulcatum* *in vitro* at the lowest tested concentration (0.05 g mL⁻¹), a

concentration 2- to 3-fold higher was necessary to significantly reduce both diseases *in vivo*. Ginger extracts were able to reduce the lesions of carrot cavity spot only at a concentration 3-fold higher than the concentration that was necessary to completely inhibit *P. sulcatum* growth. Although ginger extracts caused significant inhibition of *F. sambucinum* *in vitro*, no tested concentration allowed such an inhibition in potato dry rot *in vivo*. These results suggest that some extracts would lose their efficacy when applied to the infected produce or that higher concentrations would be required to afford the same level of inhibition as the one obtained *in vitro*. Of particular interest, cinnamon extracts reduced potato dry rot more efficiently than carrot cavity spot, although this extract was less inhibitory toward *F. sambucinum* when compared to *P. sulcatum*. While this may

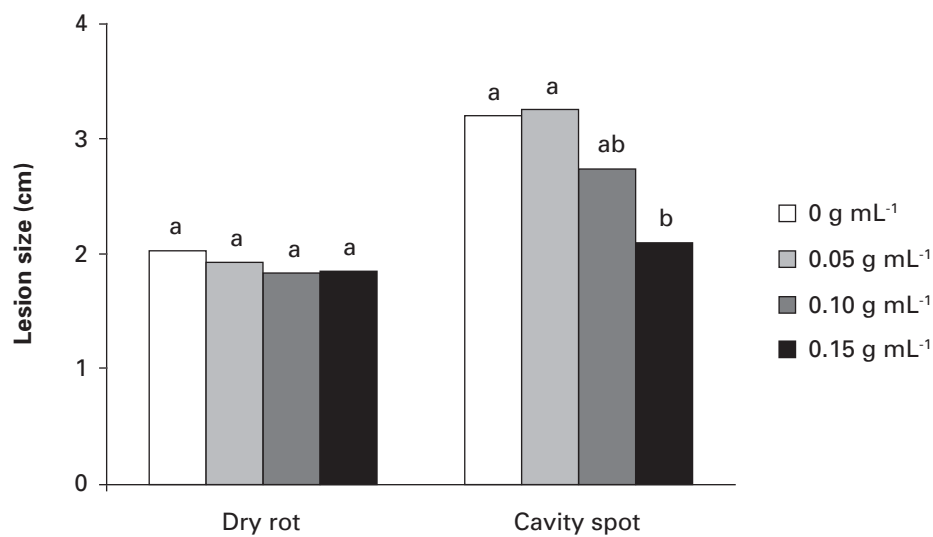


Figure 2. Effect of aqueous ginger extracts on the development of potato dry rot and carrot cavity spot. For each disease, lesion sizes with the same letter are not significantly different according to Fisher's protected LSD test ($P = 0.05$).

simply be a product of the tested pathosystem, it is possible that cinnamon extracts may also heighten molecular and biochemical reactions that may contribute to disease control through activation of the potato tuber defense reactions, as observed with other antimicrobial treatments (Mecteau *et al.* 2009).

Overall, this study revealed that aqueous spice extracts (cinnamon, ginger and nutmeg) have direct antimicrobial activity against *A. niger*, *F. sambucinum*, *P. sulcatum* and/or *R. stolonifer*. Among the tested spices, cinnamon extract was effective in reducing potato dry rot and carrot cavity spot, whereas ginger extract significantly reduced carrot cavity spot. This indicates the possibility of applying cinnamon and ginger extracts to control or limit the development of these spoilage diseases in warehouses and cold storage units. However, further research is required to determine the inhibitory compounds that are responsible for the mode of action of these extracts, to evaluate their efficacy on a larger scale, and to determine the organoleptic properties of the produce receiving the spice extracts.

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