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Method for preparing linalool microcapsule for controlling plant soil-borne disease

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ABSTRACT OF THE DISCLOSURE

The present disclosure provides use of linalool in controlling plant soil-borne diseases, and relates to the technical field of pesticides. The present disclosure provides the use of linalool in controlling plant soil-borne diseases, and prepares a linalool microcapsule for controlling plant soil-borne diseases. The agent has excellent control effects on soil-borne diseases caused by *Phytophthora nicotianae* and *Ralstonia solanacearum Ralstonia solanacearum*and a better effect on fields where both types of diseases occur in a mixed manner. Compared with chemical agents, the agent has considerable efficacy, is environmentally friendly, and has no pesticide residue; the agent has superior efficacy to microbial agents and stable efficacy.

METHOD FOR PREPARING LINALOOL MICROCAPSULE FOR CONTROLLING PLANT SOIL-BORNE DISEASE

TECHNICAL FIELD

[01] The present disclosure belongs to the technical field of pesticides, and particularly relates to use of linalool in controlling plant soil-borne diseases.

BACKGROUND ART

[02] Soil-borne diseases seriously harm crop production; there are a variety of pathogens that have wide routes of transmission and are difficult to control. In particular, *Phytophthora* sp. and *Ralstonia solanacearum* can infect a plurality of crops and often lead to destructive diseases. Tobacco black shank and bacterial wilt are caused by these two pathogens, which are seriously harmful. Particularly, these two diseases occur in tobacco production areas of southern China in a mixed manner and are difficult to control. At present, control of these two diseases mainly depends on chemical agents. For example, control pesticides for tobacco black shank mainly include metalaxyl mancozeb, dimethomorph, propamocarb and the like; control pesticides for bacterial wilt mainly include copper agents such as thiediazole copper and copper hydroxide; these agents are very prone to pesticide residues, environmental pollution, and pesticide resistance of pathogens. Microbial pesticides deeply investigated so far have problems of poor control effect and unstable efficacy, which are difficult to replace chemical pesticides.

SUMMARY

[03] In view of this, an objective of the present disclosure is to provide use of linalool in controlling plant soil-borne diseases; linalool has excellent antibacterial and control effects on tobacco black shank and bacterial wilt and particularly a better effect on tobacco fields where both types of diseases occur in a mixed manner compared with the existing agents.

[04] To achieve the above objective, the present disclosure provides the following technical solutions:

[05] The present disclosure provides use of linalool in controlling plant soil-borne diseases.

[06] Preferably, the plant soil-borne diseases may include plant soil-borne diseases caused by *Ralstonia solanacearum* and/or *Phytophthora* sp.

[07] The present disclosure further provides a method for preparing a linalool microcapsule for controlling plant soil-borne diseases, including the following steps: mixing linalool, β-cyclodextrin and water in a mass ratio of 1:8:8, stirring for 40 min, vacuum drying and grinding a resulting mixture, and collecting a portion with a particle size of <200 μm to obtain the linalool microcapsule.

[08] Preferably, the vacuum drying may be conducted 70°C for 12 h.

[09] The present disclosure further provides a linalool microcapsule for controlling plant soil-borne diseases prepared by the foregoing preparation method.

[10] Preferably, the content of linalool in the linalool microcapsule may be 80 ± 5% by weight.

[11] The present disclosure provides use of the linalool microcapsule for controlling plant soil-borne diseases prepared by the foregoing preparation method or the foregoing linalool microcapsule in controlling plant soil-borne diseases.

[12] Preferably, the plant may include one or more of tobacco, peanut, and/or vegetables.

[13] Preferably, the soil-borne diseases may include tobacco black shank and/or bacterial wilt.

[14] The present disclosure provides a method for controlling plant soil-borne diseases, where a diluent of the linalool microcapsule for controlling plant soil-borne diseases prepared by the foregoing preparation method or the foregoing linalool microcapsule is applied in fields.

[15] The present disclosure provides use of linalool in controlling plant soil-borne diseases, and prepares a linalool microcapsule for controlling plant soil-borne diseases. The agent has excellent control effects on soil-borne diseases caused by *Phytophthora nicotianae* and *R. solanacearum* and a better effect on fields where both types of diseases occur in a mixed manner. Compared with chemical agents, the agent has considerable efficacy, is environmentally friendly, and has no pesticide residue; the agent has superior efficacy to microbial agents and stable efficacy.

BRIEF DESCRIPTION OF THE DRAWINGS

[16] FIG. 1 illustrates a fumigation-based inhibitory effect of linalool on *P. nicotianae*, where the left panel illustrates a fumigation-based inhibitory effect of 5 μl of linalool per Petri dish, and the right panel illustrates CK.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[17] The present disclosure provides use of linalool in controlling plant soil-borne diseases.

[18] The present disclosure has no particular limitation on the source of the linalool, and a commercially available reagent conventional in the art may be used. The soil-borne diseases of the present disclosure may preferably include plant soil-borne diseases caused by *R. solanacearum* and/or *Phytophthora* sp., more preferably tobacco black shank and bacterial wilt, and most preferably a mixed disease of tobacco black shank and bacterial wilt.

[19] The present disclosure further provides a method for preparing a linalool microcapsule for controlling plant soil-borne diseases, including the following steps: mixing linalool, β-cyclodextrin and water in a mass ratio of 1:8:8, stirring for 40 min, vacuum drying and grinding a resulting mixture, and collecting a portion with a particle size of <200 μm to obtain the linalool microcapsule.

[20] In the present disclosure, the vacuum drying may preferably be conducted at 70°C for 12 h. The linalool microcapsule of the present disclosure uses linalool as an active ingredient and β-cyclodextrin as an embedding agent.

[21] The present disclosure further provides a linalool microcapsule for controlling plant soil-borne diseases prepared by the foregoing preparation method.

[22] In the present disclosure, the content of linalool in the linalool microcapsule may preferably be 80 ± 5% by weight.

[23] The present disclosure provides use of the linalool microcapsule for controlling plant soil-borne diseases prepared by the foregoing preparation method or the foregoing linalool microcapsule in controlling plant soil-borne diseases. In the present disclosure, the plant may preferably include tobacco, peanut, and vegetables. In the present disclosure, the soil-borne diseases may preferably include tobacco black shank and/or bacterial wilt, and more preferably tobacco black shank caused by *P. nicotianae* and tobacco bacterial wilt caused by *R. solanacearum*.

[24] The present disclosure provides a method for controlling plant soil-borne diseases, where a diluent of the linalool microcapsule for controlling plant soil-borne diseases prepared by the foregoing preparation method or the foregoing linalool is applied in fields.

[25] In the present disclosure, a 400- to 800-fold diluent of the linalool microcapsule may preferably be applied in the fields, 50 ml of the diluent may be applied to each plant, roots may be watered every seven days, and the application may be conducted twice in total.

[26] The use of linalool in controlling plant soil-borne diseases provided by the present disclosure will be described below in detail in connection with examples, but they should not be construed as limiting the protection scope of the present disclosure.

[27] **Example 1**

[28] **Preparation of linalool microcapsule**

[29] (1) Linalool, β-cyclodextrin and water were mixed in a mass ratio of 1:8:8, and fully stirred for 40 min;

[30] (2) the above sample was dried in a 70°C vacuum oven for 12 h; and

[31] (3) dried product was gently ground and sieved to obtain a portion with a particle size of 200 μm, i.e., microcapsules, where linalool content was 80 ± 5%.

[32] **Example 2**

[33] **Inhibitory effect of linalool on *P. nicotianae***

[34] 1. Test methods

[35] 1.1 Plate antibacterial test for linalool

[36] Mycelium growth rate method. Linalool was dissolved in dimethylsulfoxide (DMSO) (0.5%, v/v) to prepare Oatmeal Agar Plates (OA, 30 g of oatmeal was boiled in 1,000 ml of water for 20 min,

filtered through four layers of gauzes, mixed with 17 g of agar, and sterilized at 121°C for 20 min) at different concentrations; final concentrations of linalool were 5, 10, 20, 40, 80, and 120 μl/L. *P. nicotianae* cakes (5 mm) were plated onto OA plates supplemented with different concentrations of *Chrysanthemum indicum* essential oil, cultured at 28°C for five days; using DMSO in the same dilution ratio as a control, colony diameters were measured by the cross method, and mycelial growth inhibition rate was calculated.

[37] 1.2 Measurement of fumigation effect

[38] The fumigation activity of linalool against *P. nicotianae* was measured by the sealed plate method. *P. nicotianae* cakes with a diameter of 5 mm were plated onto OA plates supplemented with 0.625, 1.25, 2.5, 5, 10, 20, and 40 μl (equivalent to 4.92, 9.84, 19.69, 39.37, 78.74, 157.48, and 314.96 μl/L) of linalool, sealed with sealing film in triplicate, and cultured at 28°C for five days. Colony diameters were measured by the cross method, and IC_{50} was calculated; a plate without linalool was used as a control.

[39] Mycelial growth inhibition rate was calculated according to the following formula:

$$\text{Inhibition rate (\%)} = \frac{(\text{Control diameter} - 5\text{ mm}) - (\text{Treatment diameter} - 5\text{ mm})}{\text{Control diameter} - 5\text{ mm}} \times 100\%, \text{Formula I.}$$

[40] 2. Results and analysis

[41] Measured by the mycelium growth rate method, a curve of virulence of linalool against *P. nicotianae* is $y = 0.6431x + 3.5278$ ($R^2 = 0.9935$), where x is a logarithmic concentration, and y is a probability value; the IC_{50} is 18.03μl/L, indicating high antibacterial activity against *P. nicotianae*. In addition, it is found that linalool has a better fumigation effect on *P. nicotianae*, where the virulence curve is $y = 2.7340 + 2.2765x$ ($R^2 = 0.9959$), and IC_{50} is 9.8941 (8.8932-11.0076). FIG. 1 illustrates that treatment with 5 μl of linalool per Petri dish can completely inhibit the growth of *P. nicotianae*; therefore, linalool has excellent inhibitory and fumigation effects on *P. nicotianae*.

[42] **Example 3**

[43] **Inhibitory effect of linalool on *R. solanacearum***

[44] 1. Materials and methods

[45] The virulence of linalool against *R. solanacearum* was measured by the contact method. Preserved *R. solanacearum* was activated on a Luria-Bertani (LB) agar (at 28°C); a single colony was picked into an LB broth, and cultured under shaking at 160 rpm and 28°C for 48 h; the concentration of the cell suspension was adjusted to 1×10^9 CFU/ml for use. Using the pesticide solution-cell suspension mixing method, linalool was dissolved in sterile water with 0.2% DMSO to formulate into a 400 μl/L mother liquor, which was diluted to 200, 100, 50, 25, and 12.5 μl/L, respectively. 5 ml each of diluents were charged into test tubes with 5 ml of LB broth, and 10 μl of *R. solanacearum* suspension was charged thereinto; each concentration was repeated in triplicate. A culture medium

without pesticide solution was used as a negative control; a culture medium without pesticide solution but with *R. solanacearum* was used as a positive control. All test tubes were subjected to shaking culture on a shaker (at 160 rpm and 28°C) and removed until the absorbance (OD_{600}) of the positive control was around 1.2; absorbance after treatment with each agent was measured spectrophotometrically; antibacterial effect and EC_{50} were calculated. Antibacterial effect (%) = (Absorbance of positive control - Absorbance of treatment group)/Absorbance of positive control × 100, Formula II.

[46] 2. Results and analysis

[47] After measurement, a curve of virulence of linalool against *R. solanacearum* is y = 1.9377x + 2.5111, where x is a logarithmic concentration, and y is a probability value; the correlation coefficient is 0.9972, the EC_{50} is 19.2518 μl/L, and the 95% confidence interval ranges from 17.6625 to 20.9842. It is indicated that linalool has strong virulence against *R. solanacearum* and potential application value.

[48] **Example 4**

[49] **Fumigation experiment for linalool in the control of tobacco black shank**

[50] 1. Materials and methods

[51] Soil fumigation experiment: 1% and 2% diluents (diluted with 5% Tween 80 solution) of linalool microcapsule (prepared in Example 1) were prepared; sterile soil with *P. nicotianae*-infected grains was potted (3 g/pot), and each pot contained 500 g of soil; 50 ml each of diluents of linalool microcapsule was poured into the pots; the pots were sealed with plastic film and fumigated for 5 days; at 5 days, the film was uncovered for sampling; after sampling, 50 ml each of diluents were poured into the pots again, sealed, fumigated for two days, and sampled again.

[52] Pot experiment: Tobacco seedlings of uniform size were transplanted in the fumigated soil and sampled at 5 days; the disease index was recorded at 15 days.

[53] 2. Results

[54] From Tables 1 and 2, the linalool microcapsule has a better control effect on tobacco black shank and a concentration effect, i.e., the inhibitory effect of the linalool microcapsule on *P. nicotianae* is enhanced as the concentration increases. The control effect of the linalool microcapsule is 82.61% 15 days after transplanting. Meanwhile, 2% addition leads to pesticide injury, and 1% addition shows a better control effect.

[55] Table 1 The copy number of DNA fragments of *P. nicotianae* in the fumigated soil

Treatment	Fumigation for 5 days	Fumigation for 7 days	Potting for 5 days
CK	11,827,274.97 ± 247,720.52a	12,527,258.76 ± 980,953.52a	11,796,836.01 ± 392,644.87a

1%	5,517,261.79 ± 76,709.39b	602,992.56 ± 10,953.22b	614,390.14 ± 29,411.72b
2%	603,049.13 ± 9,405.51c	72,389.78 ± 4,869.53b	128,774.24 ± 10,354.35b

[56] Table 2 The control effect of linalool microcapsule on tobacco black shank (at 15 days)

Treatment	Disease index	Control effect (%)
1%	12.18	82.61
CK	70.05	-

[57] **Example 5**

[58] **Pot experiment for linalool in the control of tobacco bacterial wilt**

[59] The linalool microcapsule prepared in Example 1 was diluted 400-, 600- and 800-fold, and poured into roots of healthy potted tobacco seedlings at the 5-to-6-leaf stage; 20 ml of each diluent was poured per seedling; the procedure was repeated in triplicate every 15 seedlings were treated. After the seedlings were treated with the agent for 24 h, several holes were punched around the caudex using a sterile needle, and 10^8 cfu/ml *R. solanacearum* suspension was poured along the caudex, 20 ml per seedling. The seedlings were cultivated in a phytotron at 28°C; once the control incidence was more than 90%, the number of diseased seedlings was recorded and the corrected efficacy was calculated.

[60] Corrected efficacy (%) = (Treated incidence – control incidence)/Control incidence × 100, Formula III.

[61] The control effect is shown in Table 3. The 400-, 600- and 800-fold diluents of linalool microcapsule have some control effects on tobacco bacterial wilt, and particularly, the 400-fold diluent has the optimal control effect, which reaches up to 69.05%.

[62] Table 3 Control effect of linalool on tobacco bacterial wilt (12 days)

Treatment	Incidence (%)	Control effect (%)
400-fold diluent of linalool	28.89 ± 3.85	69.05
600-fold diluent of linalool	42.22 ± 3.85	54.76
800-fold diluent of linalool	53.33 ± 6.67	42.86
CK	93.33 ± 6.67	-

[63] **Example 6**

[64] **Control effect of linalool on fields suffering from both tobacco bacterial wilt and tobacco black shank**

[65] The test was conducted in the Zheng'an tobacco field, Zunyi in 2017. Tobacco black shank

and bacterial wilt were occurred in a mixed manner in experimental fields. The tobacco cultivar planted was K326. Test pesticides were a 400-fold diluent of linalool microcapsule prepared in the example, a 400-fold diluent of 58% metalaxyl mancozeb (a major pesticide for controlling tobacco black shank), and a 400-fold diluent of 20% thiediazole copper (a major pesticide for controlling bacterial wilt), respectively. Plots were arranged randomly, area of each plot was 30 m^2, and tests were done in triplicate. Pesticide application began once tobacco plants had symptoms in the fields; each pesticide was applied twice every seven days; roots was watered and 50 ml of the pesticide was applied to each plant.

[66] The incidence was investigated 10 days after the second pesticide application, and disease index and control effect were calculated. Results are shown in Table 4. Because both tobacco black shank and bacterial wilt occurred in the tobacco field and the control effect of each major pesticide for a single disease was undesired in production, the linalool microcapsule had a significantly better effect than both pesticides and exhibited an advantage of controlling both diseases. However, as the diseases were aggravated at a later stage, none of pesticides could effectively control disease development, but linalool microcapsule-treated tobacco plants exhibited milder disease severity than those treated with others.

[67] Table 4 Control effect of linalool on tobacco bacterial wilt (12 days)

Treatment	Incidence (%)	Control effect (%)
400-fold diluent of linalool	8.37 ± 1.17d	61.50a
400-fold diluent of 58% metalaxyl mancozeb	12.32 ± 1.39c	48.10b
400-fold diluent of 20% thiediazole copper	15.32 ± 1.82b	37.95c
CK	29.53 ± 3.39a	-

[68] The foregoing descriptions are only preferred implementations of the present disclosure. It should be noted that several improvements and modifications may further be made by a person of ordinary skill in the art without departing from the principle of the present disclosure, and such improvements and modifications should also be deemed as falling within the protection scope of the present disclosure.

WHAT IS CLAIMED IS:

1. A method for preparing a linalool microcapsule for controlling plant soil-borne diseases, comprising the following steps: mixing linalool, β-cyclodextrin and water in a mass ratio of 1:8:8, stirring for 40 min, vacuum drying and grinding a resulting mixture, and collecting a portion with a particle size of <200 μm to obtain the linalool microcapsule.

2. The preparation method according to claim 1, wherein the vacuum drying is conducted at 70°C for 12 h.

3. A linalool microcapsule for controlling plant soil-borne diseases prepared by the preparation method according to claim 1 or 2.

4. The linalool microcapsule according to claim 3, wherein the content of linalool in the linalool microcapsule is 80 ± 5% by weight.

5. Use of the linalool microcapsule for controlling plant soil-borne diseases prepared by the preparation method according to claim 1 or 2 in controlling plant soil-borne diseases.

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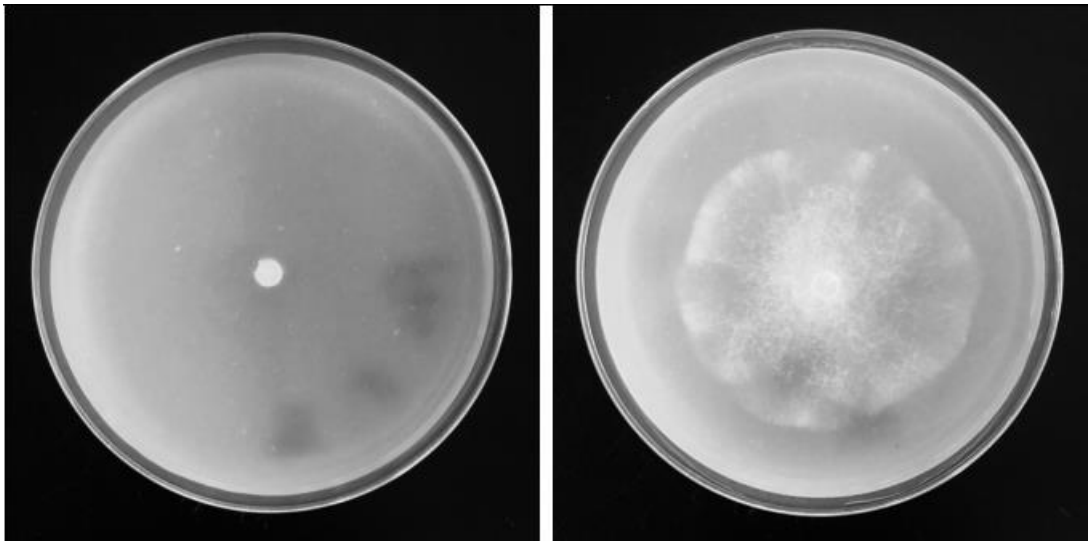

FIG. 1