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An insecticidal composition comprising a first composition having an effective amount of at least one species of entomopathogen distributed in a matrix. A second composition selected from vegetable oil, crop oil and partially hydrogenated oil containing mono- and di-glycerides surrounds the first composition and significantly prolongs nematode viability of during storage. The matrix is a nematode-containing macrogel or paste of partially hydrogenated oils containing mono- and di-glycerides. The macrogel matrix is selected from a continuous polymer macrogel or microcapsule-containing macrogel. Partially hydrogenated oils containing mono- and di-glycerides, (e.g. Crisco^{R} shortening), can also be mixed with free nematodes to produce a paste formulation. Trapping nematodes in a Crisco^{R} matrix formulation along with the water retentive polymer results in increased protection against desiccation and significantly prolonged viability during storage.

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(57) Abstract

An insecticidal composition comprising a first composition having an effective amount of at least one species of entomopathogen distributed in a matrix. A second composition selected from vegetable oil, crop oil and partially hydrogenated oil containing mono- and di-glycerides surrounds the first composition and significantly prolongs nematode viability of during storage. The matrix is a nematode-containing macrogel or paste of partially hydrogenated oils containing mono- and di-glycerides. The macrogel matrix is selected from a continuous polymer macrogel or microcapsule-containing macrogel. Partially hydrogenated oils containing mono- and di-glycerides, (e.g. Crisco^{R} shortening), can also be mixed with free nematodes to produce a paste formulation. Trapping nematodes in a Crisco^{R} matrix formulation along with the water retentive polymer results in increased protection against desiccation and significantly prolonged viability during storage.

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STABILIZED INSECT NEMATODE COMPOSITIONS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates generally to insecticidal compositions containing nematodes which are useful for the biological control of noxious insects.

2. Description of Related Art

Insect nematodes (*Steinernematidae, Heterorhabditidae, Mermithidae*) have been shown to be highly effective bioinsecticides (Poinar, "Nematodes for Biological Control of Insects", CRC Press, Inc., Boca Raton, Fla., 1979). However, insect nematodes require moist conditions to survive and function. When nematodes are dried they lose activity rapidly; subsequent

rehydration fails to restore the lost activity. One method of preserving nematodes in a moist environment is via the microencapsulation processes of U.S. 4,615,883, 4,701,326, and 4,753,799. This art microencapsulates the nematodes in calcium alginate. The alginate microcapsules, however, have several drawbacks. They are fairly large and rigid and consequently are not appealing to insects as well as being out of the chewing range of smaller insects. Additionally, the ability of the alginate microcapsules to maintain moisture conditions conducive to survival of the nematodes has also been questioned (Dunkle and Shasha, Environ. Entomol., Vol. 17,120-126, 1988). To reduce the rate of desiccation, others have used water thickeners, mineral oil, or surfactants. See, for example, U.S. 4,178,366. With insect nematodes most of these approaches are ineffective in preserving the moisture content and consequently the biological activity. Some have adverse effects on biological activity or repel the target insects, thereby making them ineffective for their intended purpose.

Another factor greatly limiting the effective use of nematodes for biological control of insects is the inability of the nematodes to survive for an extended period of time at high temperatures. High temperatures are frequently encountered in the field during summer months and in unregulated storage warehouses.

U.S. Patent No. 3,271,243 (Cords et al.) discloses an oil-water suspension of *Bacillus thuringiensis*, including vegetable, animal or mineral oils, such as corn oil. The resulting insecticide is dependent on pH and salt concentration.

U.S. Patent No. 4,859,377 (Shasha et al.) discloses the encapsulation of corn oil and entomopathogens in starch. Encapsulation of *Bacillus thuringiensis* and of nuclear polyhedrosis virus is demonstrated in the presence of corn oil.

U.S. Patent No. 4,178,366 (Bedding) discloses an oil suspension of nematodes. The harvested, unprotected nematodes are stored in oil and/or wax. The patent also indicates that nematodes may be kept alive when suspended in water through which air is bubbled at a rate sufficient to insure that the nematodes are subject to some degree of agitation.

U.S. Patent No. 4,765,275 (Yukawa et al.) discloses an improved method for the storage and transport of nematodes. This patent recommends the storage of nematodes under substantially anaerobic conditions as a means for prolonging their viability.

It is an object of this invention to provide stable nematode formulations that can be stored at high temperatures without appreciable loss of activity. Preferably the nematode formulation should be stable for months at 32°C or for several weeks at 37°C.

Another object of the invention is to provide a means to maintain the nematodes in a moist environment for an extended period of time even at high temperatures.

Still a further object is to provide means to attract insects to the formulations and, once attracted, to induce the insects to consume the moist and viable nematodes.

SUMMARY OF THE INVENTION

This invention is drawn to an insecticidal composition comprising a first composition having an effective amount of at least one species of entomopathogen distributed in a matrix, and further including H_2O_2. A second composition selected from vegetable oil, crop oil and partially hydrogenated oil containing mono- and di-glycerides surrounds the first composition.

The matrix is a nematode-containing macrogel or paste of partially hydrogenated oil containing mono- and di- glycerides. The macrogel matrix is selected from a continuous polymer macrogel or microcapsule-containing macrogel.

The addition of nematode macrogels or capsules to vegetable or crop oil such as corn oil, soybean oil, palm oil and cottonseed oil; or partially hydrogenated oil containing mono- and di-glycerides significantly prolong nematode viability during storage.

When partially hydrogenated oils containing mono- and di- glycerides (e.g., Crisco^R shortening) are mixed with free nematodes a paste formulation results. Trapping nematodes in a Crisco^R paste formulation along with the water retentive polymer results in increased protection against desiccation and significantly prolonged viability during storage.

Additionally, the inclusion of from 0.01 to 1.0% hydrogen peroxide in nematode matrix or paste formulations generates oxygen which is trapped and made

available to prolong the life of the nematodes. Alternatively, nematodes may be encapsulated in the presence of hydrogen peroxide. The nematodes are entrapped inside a polymer made of either gellan gum, carrageenan or calcium alginate, together with the trapped oxygen.

When stored in vegetable oil, crop oil or hydrogenated oil containing mono- and di-glycerides, such nematode continuous matrices or microcapsules are stable at high temperatures for many months.

The invention further provides a formulation comprising a nematode-containing matrix surrounded by at least one member selected from the group consisting of vegetable oil, crop oil and partially hydrogenated oil containing mono- and di- glycerides.

The invention further provides a formulation comprising a paste of partially hydrogenated oil containing mono- and di- glycerides and nematodes.

The invention further provides a formulation comprising a nematode-containing matrix and H_2O_2.

The invention further provides a method for controlling insects comprising applying to an area an effective amount of the above-mentioned formulation.

The invention further provides a use of the above-mentioned formulation for controlling insects.

The invention further provides a commercial package comprising the above-mentioned formulation together with instructions for controlling insects.

DETAILED DESCRIPTION OF THE INVENTION

The problem with desiccation and inactivation of entomopathogens such as insect nematodes, bacteria, baculoviruses or fungal pathogens can be substantially ameliorated by this invention. While Bedding uses high concentrations of paraffin wax and paraffinic oil to retard evaporation of insect nematodes, such a formulation, however, is not appealing to insects. We have unexpectedly found that partially hydrogenated vegetable oil or crop oil containing mono-and di-glycerides, such as Crisco^{R} shortening, not only are conducive to nematodes as insecticides, but also significantly prolong the viability of nematodes during storage. The presence of additional vegetable oil or crop oil in the formulations renders them highly attractive to certain insects thus the insects are attracted to the baits containing the nematodes. In addition, vegetable oil and crop oil, including partially hydrogenated oils, also have a beneficial effect in

preserving the viability of nematode and hydrogen peroxide containing formulations.

The active ingredient in one embodiment of this invention comprises the entomopathogens distributed within a matrix. Such matrices include a macrogel matrix, wherein nematodes are distributed continuously throughout a polymer matrix or encapsulated in a polymer matrix; and a Crisco^{R} paste matrix which entraps nematodes. Thus, the macrogel matrix may be a continuous polymer macrogel or a microcapsule-containing macrogel. The problem of desiccation of the entomopathogens is substantially ameliorated by the addition of vegetable oil, crop oil or partially hydrogenated oil containing mono- and di- glycerides during storage.

The entomopathogens which are distributed and immobilized in a continuous insect-consumable matrix, one of the matrices of the present invention, along with a source of water for the entomopathogens, have significantly enhanced viability. The final product is a continuous matrix in which the nematodes, or the like, are embedded, together with a source of moisture, and, optionally, other additives, such as our newly discovered insect feeding stimulant (raffinose) and other insect attractants and such stabilizers as may be required by the contemplated use of the insect bait device.

The continuous macrogel matrix, an insect-consumable macrogel, is produced by methods whereby, the entomopathogens are suspended in an aqueous solution of a gel-forming matrix in the presence of an inert water retaining compound. Gelation is then induced by whatever means are appropriate for the selected matrix. The

resultant insecticidal continuous matrix then contains a distribution of entomopathogens and water reservoirs. The matrix may be stored for an indefinite period without adverse effects on the viability of the entomopathogens and may be cut into smaller pieces as desired.

The continuous matrix-forming polymer is selected from natural, naturally derived, and synthetic polymers, with the provisos that the matrix per se and the gelation conditions are neither harmful to the entomopathogens nor interfere with the effectiveness of the pathogens. Suitable matrix-forming polymers include, but are not limited to, agarose, carbopols, carrageenan, dextran, guar gum, and other heteropolysaccharides, such as gellan gum. One advantage associated with the use of the natural polysaccharides is that these are often attractive as food for the insects whose demise is desired.

A preferred source of the matrix-forming polymer is the cationically gellable hetero-polysaccharides, such as those disclosed in U.S. 4,326,052 and U.S. 4,326,053.

A suitable variety of this material is available commercially as Gel-Gro^{R} gellan gum from ICN Biochemicals, Cleveland, Ohio.

The gelation time of the Gel-Gro^{R} gellan gum is easily controlled by varying the polymer concentration, the concentration and type of gelling agent, and the temperature. Preferably, the Gel-Gro^{R} liquid polymer concentration is between 0.2% and 5.0% by weight, the gelling agent is a cation, and the concentration of gelling agent is from 0.1 mM to 500 mM. Most preferably,

the polymer concentration is from about 0.6% to 1.2% by weight, the gelling agent is a divalent cation, and the cation concentration is from about 0.5 mM to 25.0 mM. The most preferable conditions result in gelation times of about 1 to 15 minutes. When spraying formulations are desired, a cation concentration in excess of 25 mM is preferred to obtain rapid gelation.

Suitable divalent cations include barium, calcium, copper(II), iron(II), magnesium, manganese, and zinc(II). Monovalent cations such as ammonium, cesium, lithium, potassium, and sodium, may also be used to induce gelation, albeit at higher concentrations. Trivalent ions such as aluminum and iron(III) are also useful.

In another embodiment of the invention, Crisco^{R} is used in the process of producing a nematode-containing paste matrix such that Crisco^{R} takes the place of the matrix-forming polymer. Accordingly, ions are unnecessary for the formation of the Crisco^{R} matrix.

The hydrated, water retentive polymer which is incorporated into the matrices, polymer and Crisco^{R}, as the water reservoir for the entomopathogen is typically a water-absorbing compound, such as a hydrophilic acrylic, acrylamide, polyurethane or starch-based polymer. Such polymers, commonly known as hydrogels, will absorb and retain several hundred times their weight in water and will slowly release the absorbed water. Representative examples of these materials are California Crystals^{R}, a water-absorbing acrylic polymer available from J & G Agrow-tek, Rancho Cordova, Cal. and Water Grabber^{R}, a water-absorbing acrylamide from FP Products, Inc.,

Atlanta, Ga. Other materials which exhibit similar affinities for water may be substituted. The amount of hydrated, water retentive polymer present in the matrix is generally about 25% to about 75%, although the choice and concentration of pathogen and the envisioned environment may lead to significant departures from these norms.

This invention may also be used in the process of microencapsulating nematodes as well as in the storage thereof. H_2O_2 may be incorporated into micro-encapsulation techniques as well. Furthermore, microencapsulated nematodes may be stored in vegetable oil or crop oil, such as corn oil, soybean oil, palm oil, cottonseed oil and the like; or partially hydrogenated oil containing mono- and di- glycerides. Accordingly, any vegetable oil or crop oil that is compatible with nematode capsules, such as capsules prepared with gellan gum carrageenan and calcium alginate may be used.

As previously noted, the entomopathogen is selected from among those pathogens which control noxious insect infestations. Baculoviruses, such as nuclear polyhedrosis virus; bacteria, such as *Bacillus thuringiensis;* fungal pathogens, such as *Beauveria bassiana, Metarhizium anisopliae,* and *Nomuraea rileyi;* and nematodes, such as *Steinernema carpocapsae,* (also known as *Steinernema feltiae* and *Neoaplectana carpocapsae*) and *Heterorhabditis bacteriophora* (also known as *Heterorhabditis heliothidis)* are among the more useful pathogens. Selection of the entomopathogens is not limited to those described herein, but is well within the purview of one skilled in the art of natural predation. Nematodes are particularly well-suited for

the practice of this invention. However, the only limitations on the pathogens are that they not be inactivated by the conditions of gelation or the composition of the matrix or microcapsule. Since the nematodes will reproduce in the insect host, only a few need be incorporated in a discrete sample to provide control. Of course, millions of pathogens may be easily incorporated. In the practice of this invention, we have found that nematode concentrations from a few up to about 500,000 per milliliter are most useful. For other pathogens, such as *Bacillus thuringiensis*, the macrogel may contain as much as 20% by weight.

EXAMPLE 1

Approximately 200,000 nematodes (*Steinernema carpocapsae* strain All) in 5 ml water containing 1% raffinose (an insect feeding stimulant) together with 5 ml of crushed, fully swelled and expanded water retentive polymer, (WRP-nematodes), were dispersed thoroughly by mixing into 10 ml of partially hydrogenated oil containing mono- and di- glycerides (e.g. Crisco^{R} shortening). To attract corn rootworms, a different composition containing 2 ml of corn oil (Mazola^{R}) and 8 ml of Crisco^{R} shortening were mixed with 10 ml of above WRP-nematodes solution containing raffinose. The mixtures were then put into plastic tubes and each placed into soil containing 10 corn rootworms (*Diabrotica* spp.) and a ten day old corn plant in a 500 ml plastic beaker.

Experiment	Day 0	Day 7
1. Crisco-WRP only (control)	All 10 rootworms are alive	All 10 root-worms are alive
2. Crisco-WRP and nematodes (no raffinose)	"	9 rootworms dead 1 rootworm alive
3. Crisco-WRP-nematodes and raffinose	"	All 10 root-worms dead
4. Crisco-WRP-nematodes with raffinose and corn oil	"	All 10 root-worms dead

While subsequent dissection of dead rootworms showed that they contained over 200 nematodes in each dead body, significant differences in both the root structure and plant height were noticed. In both Experiments 3 and 4, the root structure and plant height ten days after treatment were the same as those in control plants without corn rootworms. In contrast, considerable damage in root structure and greatly reduced plant height was seen in Experiment 1. Nematode paste formulation without raffinose (Cricso-WRP and nematodes) showed more than 50% damage to the root structure and the plants were 30% lower when compared to control plants.

EXAMPLE 2

Stability of nematodes at high temperatures.

Until now, nematodes could only be stored at low temperatures (e.g. 16°C or lower) with a short shelf life. When stored at 32°C, nematodes are inactivated quickly, usually within one day. We unexpectedly discovered that the addition of hydrogen peroxide allows nematodes to be stored at 32°C for many months. Stable nematode preparations can be prepared as follows: approximately 20,000 nematodes (*Steinernema carpocapsae* strain All) in 0.5 ml deionized water were mixed with cool 2.5 ml of a 1% gellan gum (e.g. Gel-Gro^{R}) solution containing 0.1% hydrogen peroxide (final concentration). The Gel-Gro^{R} solution was prepared by dissolving 0.1 g in 10 ml deionized water followed by either heating in a waterbath or autoclaving for 3 minutes.

A solution of 0.2 M calcium chloride containing 0.1% hydrogen peroxide (final concentration) was then prepared. The above nematode solution was dropped into the calcium chloride solution. To insure that nematodes are concentrated at the center of the finished capsule, the initial stirring speed should be very slow (50 to 80 rpm). After about 3 to 5 minutes at this low speed, the stirring speed was then increased to about 300 to 500 rpm and maintained at this speed for an additional 20 minutes. The resultant nematode Gel-Gro^{R} capsules were then stored in corn oil. The nematode capsules were stable for many months when stored at 32°C. The capsules may also be stored in soybean oil or in partially hydrogenated oil with mono- and di-glycerides (such as Crisco^{R}). In contrast, when nematode capsules without

H_2O_2 were stored in oil at 32°C, they only lasted for several days.

Other nematode capsules were also prepared. They include carrageenan and calcium alginate capsules all containing 0.1% hydrogen peroxide. *Heterorhabditis* instead of *Steinernema* nematodes were also used and similar results were obtained.

When the nematode capsules originally stored at 32°C were transferred to 38°C, the nematodes in these capsules survived for more than one week at this high temperature.

EXAMPLE 3

The benefit of H_2O_2 on the viability of nematodes in Crisco^R paste formulation during storage at 32°C is demonstrated in the following experiment. Two paste formulations were made by mixing 0.5 ml of nematodes (*Steinernema carpocapsae* strain A11, 40,000 nematodes/ml) with 4.5 ml of Crisco^R shortening. One of the paste formulations contained 0.1% H_2O_2 (final concentration) by adding 0.17 ml of 3% H_2O_2 whereas the other contained 0.17 ml of water. When the two paste formulations were stored at 32°C, the one without H_2O_2 was inactivated in one week, whereas the one containing 0.1% H_2O_2 was still active after three weeks.

EXAMPLE 4

Stable nematode preparations were also prepared in the presence of active water reservoir by mixing nematodes with partially hydrogenated vegetable oil

together with mono- and di- glycerides (e.g. Crisco^{R} shortening) and hydrogen peroxide as follows:

5 ml of nematodes (*Steinernema carpocapsae* Strain All, 40,000 nematodes/ml) were mixed for 5 minutes at room temperature with 5 ml of swollen pieces of water retentive polymer (WRP) in the presence of 0.2% hydrogen peroxide and 1% raffinose. The nematode-WRP mixture was then added into 10 ml of Crisco^{R} shortening. After gentle mixing for 5 minutes, a nematode paste was formed with nematodes evenly distributed in the paste. Such nematode pastes are stable for six to twelve months when stored at 16°C or lower. When stored at room temperature, they are stable for at least two months.

EXAMPLE 5

Stability of nematode paste formulation in the field.

Nematode paste formulation was prepared as described in EXAMPLE 4. Six paraffin section tubes (1.5 ml capacity) were used in this field study. Each tube contained a large piece of previously swollen water retentive polymer gel at the bottom (to provide additional water source) and the remaining tube space was covered with nematode paste (approximately 1.0 ml). The tubes were place three inches below the soil surface on May 14, 1990 in a suburb of Philadelphia. The experiment was stopped on June 19, 1990. The daytime temperature during this period ranged from 75° to 95°F.

Days	% Nematode Survival
0	100%
8	100%
16	100%
23	95%
29	85%
35	80%

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and equivalent variations of this invention may be devised by those skilled in the art without departing from the true spirit and scope of this invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

1. An insecticidal formulation comprising a first composition having an effective amount of at least one species of entomopathogen distributed in a matrix, and further including H_2O_2, said first composition surrounded by a second composition comprising at least one member selected from the group consisting of vegetable oil, crop oil, and partially hydrogenated oil containing mono- and di- glycerides.

2. The formulation of claim 1 wherein said vegetable oil is selected from the group consisting of corn oil, soybean oil, palm oil and cottonseed oil.

3. The formulation of claim 1 or 2 wherein said matrix comprises a paste of partially hydrogenated oil containing mono- and di- glycerides.

4. The formulation of claim 1 or 2 wherein said matrix is a macrogel.

5. The formulation of claim 4 wherein said macrogel is selected from the group consisting of a continuous polymer macrogel and a microcapsule-containing macrogel.

6. The formulation according to any one of claims 1 to 5 wherein said entomopathogen is selected from the group consisting of nematodes, bacteria, baculoviruses, and fungal pathogens.

7. The formulation according to any one of claims 1 to 5 wherein said entomopathogen is selected from the group consisting of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*.

8. The formulation according to any one of claims 1 to 5 wherein said entomopathogen is nuclear polyhedrosis virus.

9. The formulation according to any one of claims 1 to 8 wherein said H_2O_2 is from 0.01% to 1.0% by weight.

10. The formulation according to any one of claims 1 to 9 wherein said partially hydrogenated oil containing mono- and di- glycerides and said vegetable oil or crop oil independently prevent desiccation.

11. The formulation according to any one of claims 1 to 10 further including an insect feeding stimulant.

12. A formulation comprising a nematode-containing matrix surrounded by at least one member selected from the group consisting of vegetable oil, crop oil and partially hydrogenated oil containing mono- and di- glycerides.

13. The formulation of claim 12 wherein said matrix further includes H_2O_2.

14. The formulation of claim 12 or 13 wherein said nematode-containing matrix is a paste of partially hydrogenated oil containing mono-and di- glycerides.

15. The formulation of claim 12 or 13 wherein said nematode-containing matrix is a continuous polymer macrogel.

16. The formulation of claim 12 or 13 wherein said nematode-containing matrix is a microcapsule-containing macrogel.

17. A formulation comprising a paste of partially hydrogenated oil containing mono- and di- glycerides and nematodes.

18. The formulation of claim 17 wherein said matrix further includes H_2O_2.

19. A formulation comprising a nematode-containing matrix and H_2O_2.

20. A method for controlling insects comprising applying to an area an effective amount of the formulation according to any one of claims 1 to 19.

21. The method of claim 20 wherein the insects to be controlled are proximate to the area to which the formulation is applied.

22. The method according to claim 20 or 21 wherein the formulation is applied to an insect bait device.

23. Use of the formulation according to any one of claims 1 to 19 for controlling insects.

24. A commercial package comprising the formulation according to any one of claims 1 to 19 together with instructions for its use in controlling insects.