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(54) Title: SUNCIDE AGRI-PEST: A GREEN PESTICIDE FORMULA AGAINST AGRICULTURAL PESTS

(57) Abstract: This patent, introduces an innovative modality that combines both the effectivity and the efficiency for the control of agricultural pests with the highest levels of human safety and environmental friendliness through a new ecologically safe technology using natural plant extract porphyrin based photosensitizers. Field studies revealed that over an 85 % reduction of aphis and tunnel making insects (leaf miners) population was achieved by spraying 0.001 M/l of Suncide Agri-Pest concentration on the tomato field. Comparing the results of Suncide Agri-Pest and the other two commercially used pesticides against *Tuta absoluta* and *Aphis* sp reflect that the efficiency of Suncide Agri-Pest is greater than that of the other pesticides, making Suncide Agri-Pest a promising competitor in the field. This is conclusive proof of the high effectivity of Suncide Agri-Pest as a pesticide against agricultural pests. Suncide Agri-Pest is environmentally friendly and has the highest safety for human, animals and plants.

Suncide Agri-Pest: A green pesticide formula against agricultural pests

Technical Field:

This invention is related to the field of agriculture, specifically the control of the prevalence of noxious agricultural pests using novel models of photosensitizing drugs. It can be applied to different agriculture pests especially Aphis and tunnel making insects (Leaf miners).

Background Art:

Larvae of *Tuta absoluta* mine the leaves, flowers, shoots and fruits of tomatoes as well as the leaves and tubers of potatoes (Pastrana, 2004). After hatching, larvae penetrate apical buds, flowers, new fruit, leaves or stems. Conspicuous irregular mines and galleries as well as dark frass make infestations relatively easy to spot. Fruits can be attacked soon after they have formed, and the galleries made by the larvae can be colonized by pathogens that cause fruit to rot. The damage caused by this pest is severe, especially in young plants. When potato plants have completed the vegetative cycle, larvae of *T. absoluta* mine the tubers underneath the epidermis. Larval feeding can cause the tubers to rot (Pastrana, 1967).

Since its introduction into Europe in 2006, *Tuta absoluta* has continued to rapidly spread through the European and Mediterranean regions where it is a serious pest of field and greenhouse grown tomatoes. *Tuta absoluta* is "the major limiting factor for tomato production in South America" (Ferrara et al., 2001). It is a key pest of most greenhouse-grown tomatoes in Argentina (Botto, 2011b), and the key pest of tomato production in Chile (Estay, 2000). Without adequate controls, infestations of *T. absoluta* can result in 90 to 100 percent loss of field-produced tomatoes in Chile (Estay, 2000; Vargas, 1970). Tuta absoluta is considered one of the most important lepidopterous pests associated with processing tomatoes in Brazil (Torres et al., 2001)

Historically, *Tuta absoluta* has been controlled with chemicals. Organophosphates and pyrethroids were used during the 1970's and 1980's until new products

introduced in the 1990s (such as abamectin, spinosad, tebufonzide, and chlorfenpyr) became available (Lietti et al., 2005). At least 12 classes of insecticides control *Tuta absoluta* (IRAC, 2009a, 2009b). Control failures with organophosphates and pyrethroids in South America (Salazar and Araya, 2001) prompted research on the resistance status of *Tuta absoluta* (Lietti et al., 2005; Siqueira et al., 2000a, 2000b); however, newer classes of insecticides are providing better control of this pest (IRAC, 2009a).

Aphids constitute a large group of small, soft-bodied insects. They may measure up to six mm in total length. Aphids have piercing-sucking mouthparts that enable them to remove plant fluids from a host.

Aphids are common, persistent and sometimes key pests of ornamental plants. Most aphids cause damage to host plants by the removal of plant fluid, by the toxic action of their salivary secretions injected during feeding and by serving as vectors of plant diseases that are harmful to key plants. Feeding by aphids can stunt plant growth, deform leaves and fruit, or cause galls on leaves, stems and even roots. Many aphids also excrete a sticky, sugar-containing substance from their anus known as "honeydew." This material will drop onto the leaves, twigs, and fruit of a plant. A black, sooty mold soon begins to grow on this sugar-rich substrate.

Beneficial insects play an important role in aphid control. Lady beetles (both adults and larvae), lacewings, some flower flies (larvae), and tiny parasitic wasps will use aphids as a source of nourishment for the development of their offspring. Remember certain insecticide applications will destroy these beneficial insects as well as targeted pest species.

The use of insecticides is often the only effective means of managing an aphid infestation. A number of registered insecticide formulations are available for aphid control. However, to avoid damaging valuable plants, these insecticides are applied only to plants that are specified on their labels. (Gregory A. Hover, 2004)

Photosensitization:

Photosensitization process is a treatment involving the administration of a photoactive compound that selectively accumulates in the target cells and is followed by irradiation with visible light. The combination of the two absolutely nontoxic

elements, photosensitizer and light, in the presence of oxygen results in the selective destruction of the target cell **(Luksiene Z., 2006)**.

It is important to note that truly major advances have been made in photosensitized antimicrobial chemotherapy, in particular disinfection of the blood and blood products, and treating local infections **(Luksiene Z., 2006)**.

By any means, prevention of any disease by controlling its causative agents of prevalence, including the secondary – non human- hosts-, is of great importance. Thus, development of new antimolluscicidal methods is necessary. In this context, photosensitization has been shown to be really effective as different microorganisms such as drug-resistant bacteria, yeasts, viruses and parasites can be inactivated by this method. So far, a photosensitization process can open new and interesting avenues for the development of novel, effective and ecologically safe molluscicides' modality. As a rule, photosensitizers are usually aromatic molecules that can form long-lived triplet excited states. Despite the possible synthetic photosensitizers, there are many examples of natural photosensitizers that have evolved over the years in plants and fungi **(Fine et al., 2000)**.

Chlorophyll is the pigment that gives plants and algae their green color. More importantly, plants use chlorophyll to trap light needed for photosynthesis. The basic structure of chlorophyll is a porphyrin ring similar to that of heme in hemoglobin, however the central atom in chlorophyll is magnesium instead of iron. The long hydrocarbon (phytol) tail attached to the porphyrin ring renders chlorophyll fat-soluble and insoluble in water. Two different types of chlorophyll (chlorophyll a and chlorophyll b) are found in plants. The small difference in one of the side chains of the two molecules allows each type of chlorophyll to absorb light at slightly different wavelengths.

Chlorophyllin is a semi-synthetic mixture of water soluble sodium copper salts derived from chlorophyll. During the synthesis of chlorophyllin, the magnesium atom at the center of the ring is replaced with copper and the phytol tail is lost. Unlike natural chlorophyll, chlorophyllin is water-soluble. Chlorophyllin has been used orally as an internal deodorant and topically in the treatment of slow-healing wounds for more than fifty years without any serious side effects. Chlorophyll and chlorophyllin form molecular complexes with some chemicals known to or suspected to cause cancer and in doing so may block carcinogenic effects. Scientists are hopeful

that chlorophyllin supplementation will be helpful in decreasing the risk of liver cancer in high-risk populations with unavoidable, dietary aflatoxin exposure.

Little is known about the bioavailability and metabolism of chlorophyll or chlorophyllin. The lack of toxicity attributed to chlorophyllin led to the belief that it was poorly absorbed. However, significant amounts of copper chlorine were measured in the plasma of humans taking chlorophyllin tablets in a controlled clinical trial. Hence, more research is needed to understand the bioavailability and metabolism of natural chlorophyll and chlorine compounds in synthetic chlorophyllin.

1. Disclosure of invention:

This invention introduces a novel formula for the control of noxious insects. It was the pioneer that was concerned with the effect of chlorophyllin derivatives on the Aphis and leaf miners. The main aim of this work was to investigate the efficiency of Suncide Agri-Pest on two different insects' life style.

2. Methodology:

Field Evaluation of Suncide Agri-Pest:

The experimental area, which is about 1 acre, was divided into 20 equal plots. Each plot consisted of 30 rows, each 6 m long and 70 cm wide. One month old tomato seedlings were planted in rows separated by a 90 cm distance. All experimental plots received regular agricultural practices except for insecticide application. The treatments were performed two-month post planting of tomato seedlings and they were distributed as follows:

Five experimental plots were sprayed with Suncide Agri-Pest (10^{-3} M/L).

Fifteen experimental plots were sprayed with Suncide Agri-Pest (10^{-4} M/L).

Five experimental plots were sprayed with the insecticide "selecron" as a positive control. The rate of insecticide used (1 cm/liter) was recommended by the manufacturer.

Five experimental plots were sprayed with water as a normal control group.

Plant samples were taken before spraying and post spraying on the 1st, 3rd, 7th, 10th and 15th day. The sample consisted of 20 plants which were randomly selected from each plot and investigated for live insects. The third leaf of each seedling was carefully examined and the following observations were recorded:

1- The number of tunnels caused by tomato leafminer *Tuta absoluta.*

2- The number of Aphis adults and nymphs.

Data Analysis

Data was assayed by analysis of variance (ANOVA), with the means of separation using Duncan's Multiple Range criterion ($P<0.05$).

Brief Description of Drawings:

Figure 1 shows the results of the field study using Suncide Agri-Pest on Aphis spp. infested tomato plants. Two concentrations were used from Suncide Agri-Pest (0.001 and 0.0001 M/l) and one concentration of methomyl (1 cm/l) was used as positive control. The data of insects percent of reduction was taken on different days after treatment (1st, 7th, 10th and 15th day). Suncide Agri-Pest presented an effect better than that of the commonly distributed pesticide and the percentage of reduction caused by Suncide Agri-Pest increased from 90% to 100 % up to 15 days after treatment.

Figure 2 shows the results of field study using Suncide Agri-Pest on *Tuta absoluta* infested tomato plants. Two concentrations were used from Suncide Agri-Pest (0.001 and 0.0001 M/l) and one concentration of methomyl (1 cm/l) was used as a positive

control. The data of insect's percent of reduction were taken on different days after treatment (1^{st}, 7^{th}, 10^{th} and 15^{th} day). Suncide Agri-Pest showed an effect better than the commonly distributed pesticide and the percentage of reduction caused by Suncide Agri-Pest is more than 90 % up to 15 days after treatment.

Figure 3 shows some images of tunnel damage of *Tuta absoluta* after Suncide Agri-Pest treatment.

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Claims

1- Suncide Agri-Pest formula to control leaf miners and Aphids

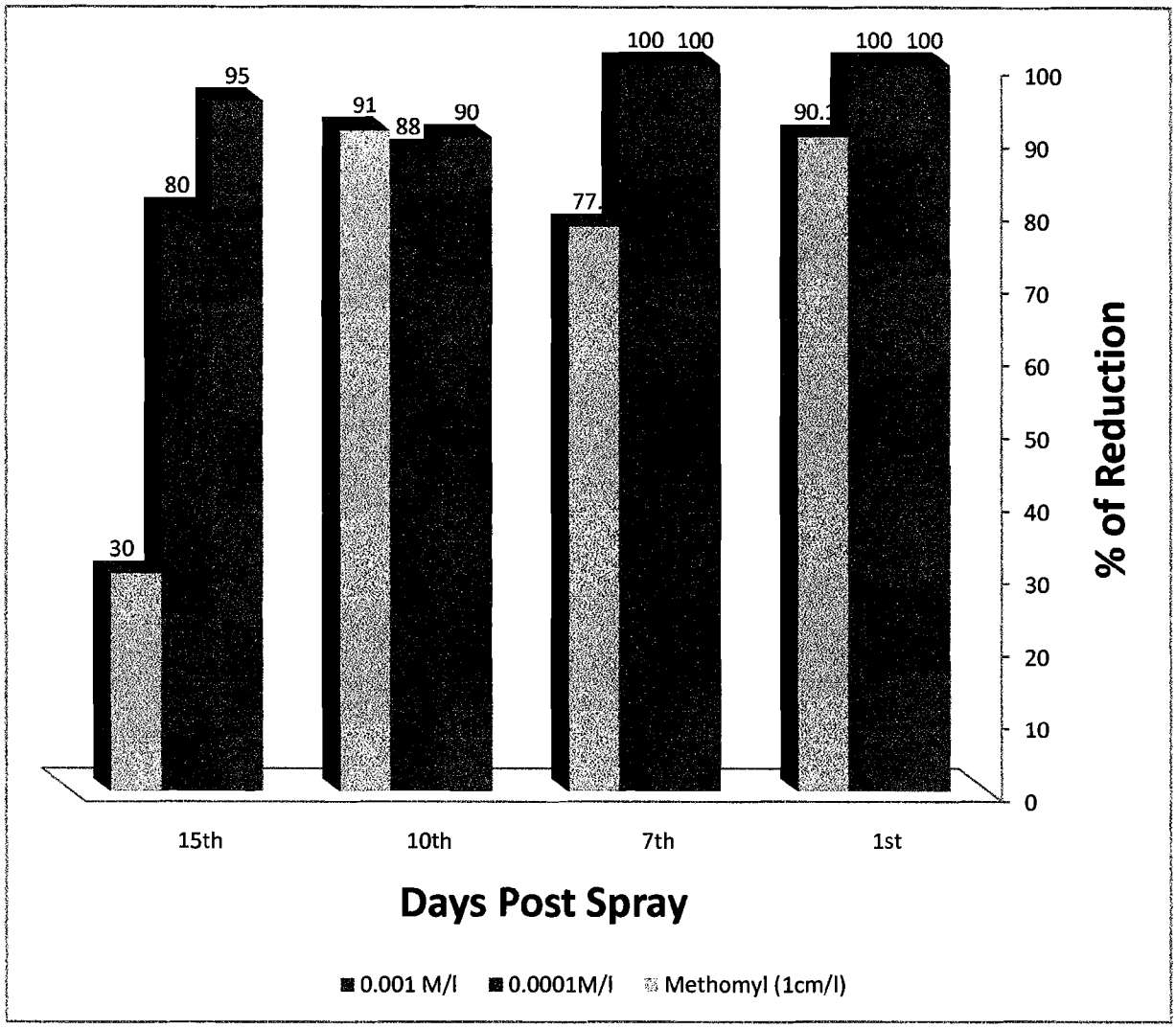
2- Composition of Suncide Agri-Pest formula according to Claim 1: tri-sodium Copper chlorophyllin and tri-sodium magnesium chlorophyllin as active ingredients and calcium carbonate as an inert material.

3- Application according to Claim 1, using Suncide Agri-Pest formula as a photopesticide against Aphis and Leaf miners and some other agricultural insects.

4- The field study according to Claim 1 using Suncide Agri-Pest on tomato fields to control aphis and leaf miners.

5- Enhancing the efficiency of active ingredients of Suncide Agri-Pest according to Claim 2, using calcium carbonate as an inert material.

6- The mode of action of Suncide Agri-Pest according to Claim 1 on the agricultural pests using sunlight which activates the active ingredient to produce cytotoxic reactive oxygen species causing oxidation tress on the agricultural pest.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40
30
20
10
0
% of Reduction
15th
10th
7th
1st
Days Post Spray
0.001 M/l
0.0001M/l
Methomyl (1cm/l)

Fig. 1

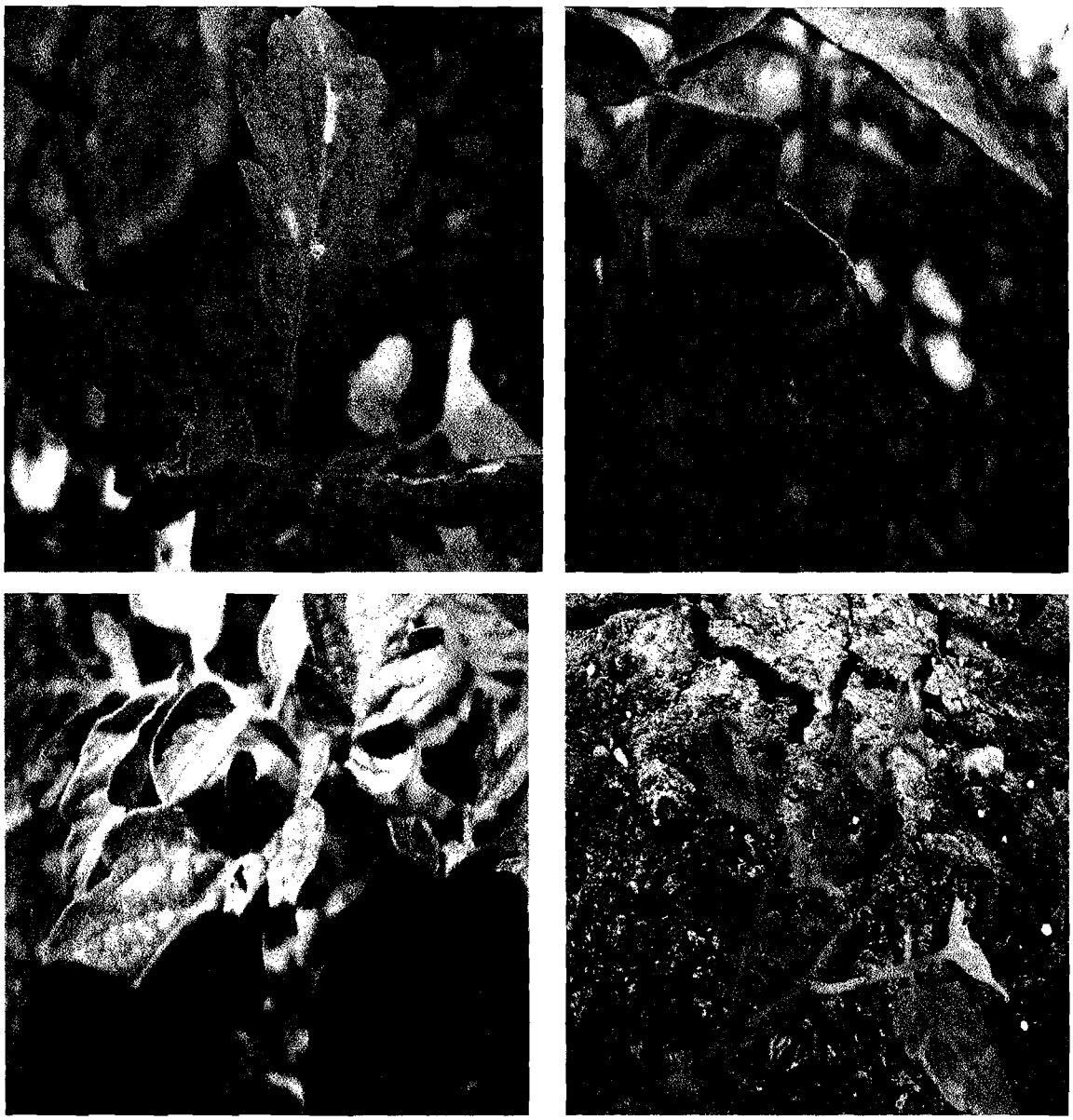

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EG2014/000004

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01N43/90 A01N55/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/075805 A1 (FUNDACAO EDUCACIONAL DA REGIAO DE JOINVILLE FURJ [BR]; DONAT PETER HAE) 30 June 2011 (2011-06-30) page 10 - page 13; claims; examples -----	1-6
X	LOPEZ-CARBALLO G ET AL: "Photoactivated chlorophyllin-based gelatin films and coatings to prevent microbial contamination of food products", 15 August 2008 (2008-08-15), INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, ELSEVIER BV, NL, PAGE(S) 65 - 70, XP023315597, ISSN: 0168-1605 [retrieved on 2008-05-11] Results and Discussion -----	1-6

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EG2014/000004

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ONG T ET AL: "Comparative antimutagenicity of 5 compounds against 5 mutagenic complex mixtures in Salmonella typhimurium strain TA98", 1 January 1989 (1989-01-01), MUTATION RESEARCH/GENETIC TOXICOLOGY, ELSEVIER, PAGE(S) 19 - 25, XP025563633, ISSN: 0165-1218 [retrieved on 1989-01-01] the whole document	1-6

A	SARKAR D ET AL: "Differential protection of chlorophyllin against clastogenic effects of chromium and chlordane in mouse bone marrow in vivo", 1 January 1993 (1993-01-01), MUTATION RESEARCH LETTERS, ELSEVIER, NL, PAGE(S) 33 - 38, XP025208130, ISSN: 0165-7992 [retrieved on 1993-01-01] the whole document	1-6

A	US 5 200 427 A (REBEIZ CONSTANTIN A [US] ET AL) 6 April 1993 (1993-04-06) claims	1-6

A	US 5 300 526 A (REBEIZ CONSTANTIN A [US] ET AL) 5 April 1994 (1994-04-05) claims	1-6

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EG2014/000004

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011075805 A1	30-06-2011	NONE	
US 5200427 A	06-04-1993	NONE	
US 5300526 A	05-04-1994	NONE	