



(86) **Date de dépôt PCT/PCT Filing Date:** 2012/03/14
(87) **Date publication PCT/PCT Publication Date:** 2013/03/07
(85) **Entrée phase nationale/National Entry:** 2014/02/25
(86) **N° demande PCT/PCT Application No.:** IT 2012/000073
(87) **N° publication PCT/PCT Publication No.:** 2013/030854
(30) **Priorité/Priority:** 2011/08/26 (IT RM2011A000450)

(51) **Cl.Int./Int.Cl.** **A01K 51/00** (2006.01),
A01N 25/06 (2006.01), **A01N 63/00** (2006.01),
A01N 65/00 (2009.01), **A01N 65/22** (2009.01),
A01N 65/28 (2009.01), **A01N 65/42** (2009.01),
A01P 7/02 (2006.01), **A23K 1/18** (2006.01),
A61K 35/64 (2006.01)

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(54) **Titre : PROCÉDE, APPAREIL ET COMPOSITIONS POUR LA PREVENTION ET LE TRAITEMENT DU SYNDROME
D'EFFONDREMENT DES COLONIES D'ABEILLES**

(54) **Title: METHOD, APPARATUS AND COMPOSITIONS FOR THE PROPHYLAXIS AND TREATMENT OF COLONY COLLAPSE
DISORDER**

(57) **Abrégé/Abstract:**

This invention concerns a method for preventing and treating Colony Collapse Disorder, consisting in the use of an automated device that delivers a diet specifically calibrated for consumption by farm colonies of bees to be treated. The apparatus comprises a box-like container (10) accommodating in its interior at least one reservoir for liquid-tight, accessible from outside through a nozzle (4), an atomizing device of a liquid solution or suspension contained in the reservoir, means for the delivery (2, 1) of the liquid atomized solution or suspension into micrometric drops outside the apparatus, and a control unit programmed for timing the delivery of the solution or suspension to the outside, for the determination of the quantity of solution or suspension delivered and for emitting alarm signals in case of malfunction, the apparatus being powered DC with the energy supplied by a solar panel (5) located outside of the container. The liquid solution or suspension comprises tonic and nutrient ingredients, mainly consisting of milk powder, sugars and lower organic acids, antioxidant and antiseptic ingredients contained in plant extracts and healing ingredients to bees, such as essential oils of thyme and oxalic acid.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)
(19) World Intellectual Property Organization
International Bureau
(43) International Publication Date
7 March 2013 (07.03.2013)




(10) International Publication Number
WO 2013/030854 A1

- (51) International Patent Classification:
A01K 51/00 (2006.01) A61K 35/64 (2006.01)
A01N 25/06 (2006.01) A01P 7/02 (2006.01)
A01N 63/00 (2006.01) A01N 65/22 (2009.01)
A01N 65/00 (2009.01) A01N 65/28 (2009.01)
A23K 1/18 (2006.01) A01N 65/42 (2009.01)

(21) International Application Number:
PCT/IT2012/000073

(22) International Filing Date:
14 March 2012 (14.03.2012)

(25) Filing Language: Italian

(26) Publication Language: English

(30) Priority Data:
RM2011A000450 26 August 2011 (26.08.2011) IT

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report (Art. 21(3))

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**METHOD, APPARATUS AND COMPOSITIONS FOR THE PROPHYLAXIS
AND TREATMENT OF COLONY COLLAPSE DISORDER**

DESCRIPTION

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Technical Field

The present invention relates to a method for preventing and treating Colony Collapse Disorder, the apparatus for implementing such a method and the nutrient and therapeutic compositions to be distributed to the bees to be treated by this method. More specifically, the invention relates to a device that dispenses, in an automated way, a specifically calibrated diet for consumption by one or more colonies of domesticated honey bees, in substitution or in addition to natural nourishment that the insects derive from their normal activity of collecting and processing nectar, pollen and other natural materials. The consumption of nutrients and therapeutics according to the method proposed helps the treated bees to resist the disease known as "Colony Collapse Disorder"

Background of the invention

Bees are the kind of social insects most appreciated and studied since antiquity, whose usefulness is certainly known since prehistoric times. As other insects in the family *Apidae*, bees collect nectar and pollen to feed their offspring and to store them in their combs as food storage.

While it is not the only group of pollinators (insects which, with their activities, carry pollen from flower to flower allowing pollination and the subsequent formation of the fruit), honey bees are undoubtedly the most important for humans, also for the various products that their colonies develop from nectar and other material collected by foraging, including not only honey, but also beeswax, propolis, royal jelly. Unlike other social apidae like bumblebees, which perform similar functions as pollinators useful to agriculture but most of which do not survive the winter (with the exception of fertilized queens), the bees accumulate and process amounts of food stocks

to be sufficient to pass the winter, because their colony is able to winter along with their queen, which can live 4-5 years.

For these reasons, bees have been used since the dawn of civilization as real pets, and were reared according to ancestral and consolidated techniques over time, applying the knowledge which is a branch of animal husbandry, beekeeping.

Although the known species of the *Apidae* family are currently about five thousands, the genus of bees (*Apis*) is only one, and comprises only seven species recognized as distinct species, of which the best known are *Apis mellifera* (European honeybee), *Apis cerana* (Eastern honey bee or bee indica), *Apis florea* (dwarf honeybee, widespread in South Asia and southeast Asia) and *Apis dorsata* (giant bee of India). Only the first two species, *Apis mellifera* and *Apis cerana*, can be bred by humans and are actually made "domestic", the first at least, until the times of ancient Egyptians.

Apis mellifera is the most widespread species of the genus *Apis* in the world, native to Egypt, it spread millions of years ago in the Mediterranean and in tropical Africa, and then in the presence of man it has naturally populated Europe, Africa, middle East and part of Siberia. It was introduced in the seventeenth and the nineteenth century also in the American continent, where it was not originally present, and it was also brought in Australia and New Zealand by the colonizers. The most well-known European subspecies of *Apis mellifera* are identified by geographic areas, separated by mountains that swarms may not overcome, where they are native and have lived with a few external contacts. There are currently 28 recognized subspecies of *Apis mellifera*, which include the black bee (*Apis mellifera mellifera*), native of northern Europe, the Italian yellow honeybee (*Apis mellifera ligustica*), which occupies most of Italy, the carniolan bee (*Apis mellifera Carnica*) who is a native of Austria and Slovenia, the caucasian bee (*Apis mellifera caucasica*), which lives mainly in the Caucasus and Georgia, and so on. Mixed breeds and hybrids have been created by human action, either voluntary or not.

In sedentary beekeeping, hives are fixed and the area of collection of the bees does not exceed 2 or 3 km radius around the hive, which sets limits

to the collection. For this reason it is also practiced nomadic beekeeping, which involves moving the hives from site to site, depending on the presence of nectar plants (i.e. sugar bases to be provided to the bees). Such movements, in addition to increasing the productivity, allow the production of monoflower honeys, allowing a better offering of the final product. The transhumance is a very ancient farming technique, already practiced by nomads, carrying their hives in the back of an animal. In Italy on the Po, as in Egypt on the Nile, the hives were loaded onto special boats that sailed up the river toward regions with the more favorable honeydew. When a certain waterline was reached, the hives were full. Currently the movement of hives occurs on the road: they are loaded at nightfall (when all the bees have returned) and are downloaded at sunrise in the new site. The hives are often downloaded and re-housed in the new area chosen for the pasture, but sometimes, in order to reduce maintenance, the hives are left directly on vehicles equipped for this purpose.

As already noted, the bees play a vital role in the reproduction of plants with entomophilous pollination. To understand the role of bees in agriculture around the world is enough to consider that the Food and Agriculture Organization of the United Nations (FAO) has estimated that on 100 species of plants that provide 90% of food worldwide, 71 are associated with the bee pollination. Over the last fifty years, the agricultural production independent of insect pollination has doubled and agricultural production that requires pollination of insects has increased fourfold, indicating that world agriculture has become more pollinator-dependent. Both the FAO and other independent research organizations in the industry have predicted that the economic value of pollination worldwide agriculture and related sectors is of the order of 180 billion U.S. dollars, of which 32 billion dollars just in the United States.

It is known that the population dynamic of a colony of bees is significantly influenced by its nutritional status, on which the development, production and survival of the colony depend. It is also well known that the necessary food for the bees are carbohydrates, proteins, lipids and vitamins:

the first provide the energy and are contained in nectar and honey, the other substances are present in pollen and are essential both for the production of larval food and for a life balanced run of the bee. In recent years, for various reasons (including illness, poisoning, reducing foraging areas, etc..) it has often become an indispensable requirement for the beekeeper to intervene with additional feed, which favors the survival of the hives or prepares them for a certain flowering (Frilli F. et al., *Confronto tra gli effetti di diversi tipi di alimento per le api*, Notiziario ERSa 3/2009). The supply of sugar is the most practiced by beekeepers; it consists in delivering syrups or candies (obtained from sugar by various sources) with the aim of integrating the energy needs of the bees. In relation to the need and to the administration time, sugar nutrition can be "stimulant", if carried out to increase the oviposition of the queen or to induce the colonies to recover after stress factors (poisonings, diseases, swarming, environmental adversity), or "compensatory", if the aim is to build up the winter stocks to avoid starvation of hives during periods of low availability.

The protein feeding, which compensates for a lack of pollen, is a nutrition technique less applied, but sometimes it can be of vital importance for a colony of bees, in fact, the lack of pollen may entail the reduction of the longevity of the bees and the reduction or total blockade of the brood, with consequent depopulation and collapse of the colonies. It should be kept in mind that in each case an artificial administration of proteins is not able to completely replace the pollen, and has effects only if it is carried out for a limited period of time.

The protein feeding can be done by providing to hives only pollen (pre-harvested), integrating the pollen (up to 25% by weight) with an artificial protein component (nutrition protein supplement) or by administering only artificial protein components (nutrition protein substitute). Very often the various protein components (soybean meal, sunflower meal, yeast, milk powder, etc.) are mixed together to achieve higher nutritional value, but it is important that the total quantity of protein food preparation is between 10 and 15 wt%, as higher values can lead to toxic effects on bees.

The protein nutrition can be done by placing the powdered food outside the hive in special containers, or placing the mixture inside the hive in deep frame feeders, or cakes over the combs, and covered by a cover-comb; in the latter case the food protein is almost always added with honey or sugar syrup until a pasty consistency and semi-solid candy-like ("protein cake").

About the choice of an appropriate food for a colony of honeybees, the International Patent Application Publication No. **WO 2006/073955** (The United States of America as Represented by the Secretary of Agriculture) entitled "Artificial diets for domestic honey bees" describes water dispersible preparations consisting of homogeneous mixtures of nutrients in effective amounts and proportions to support growth and development of farm bees. The proposed formulations are considered particularly advantageous for the feeding of bee colonies that are moved frequently in the area: in the absence of sufficient natural resources, such as during transfers, the preparations of artificial diet proposed should be able to provide all the necessary nutrients for the life of the colony. The components required in the described nutritional composition are proteins, lipids, carbohydrates, ash, cholesterol, ascorbic acid, an acidifier, an antimicrobial/antifungal agent for the conservation of the mixture and water, in the appropriate proportions, and as a source of proteins and lipids soy and/or egg are proposed.

One of the major problems of the apiary is to safeguard the health of the colonies. The honey bee diseases that may develop are numerous, as a result of several pathogenic organisms, including parasite insects, unicellular fungi, bacteria and viruses that can affect the bee in the different stages of its development.

The two best known pathogens to the beekeeping are the *Varroa destructor* acarus and *Nosema apis* microsporidio. The acarus *Varroa* is an external parasite, which attacks to the bee body and weakens it sucking the hemolymph, and during this process may also transmit viral RNA agents to the bee. The acarus was found also on other insect pollinators, such as bumblebees, beetles, and flies, but can only breed in a colony of honeybees. Once in the colony, the female acarus enters a brood cell of honey bees,

giving preference to a cell containing a male brood, i.e. larvae of kelp. Once the cell is capped, the acarus lays her eggs, after which the young mites leave the shell more or less the same moment the young bee develops, and this leaves the cell with his guests.

5 The population dynamic highlighted above shows that a large population of mites in autumn could lead to a crisis when kelp rearing ceases and the mites go to the larvae of worker bees, causing a rapid decimation of the population and often the death of the hive. For this reason the varroa mite is the parasite with the most pronounced economic impact in the apiary
10 industry.

To combat or prevent the infestation by *Varroa destructor* different physical or mechanical methods are known and used to control the number of mites in the colony, as well as acaricides products, both synthetic (pyrethroids, organophosphates) or of natural origin, as oxalic acid-based preparations, or
15 based on thyme essential oil (or by synthetic thymol).

The unicellular fungus *Nosema apis* (more recently found in a similar form also in *Apis cerana*, and called on this occasion *Nosema ceranae*) is characterized by a dormant state consisting of spores resistant to changes in temperature and humidity. The spores of *nosema*, in fact, cannot be destroyed
20 by freezing the contaminated combs. The spores are localized in intestinal epithelial cells and other cells of adult bees, where they begin the growth, heading for a series of cell divisions, invading the intestinal tract and thus causing the pathology known as noseemiasis. This is manifested with diarrhea evidenced by yellowish droppings outside the hive, a slow enhancement of the
25 colony, disjointed wings and distended abdomen in affected individuals. The mature spores come out with the feces, contributing to the propagation of the disease.

If untreated, the nosema infection may hit the queen, causing an early replacement by workers remained healthy. The disease hinders the digestion
30 of pollen, and therefore reduces the life of bees, and can be fought with greater difficulty in colder climates, where bees spend more time in the hive. In order to reduce the infection beekeepers use to increase the aeration in the

hive and remove, as much as possible, the honey gathered by bees for the winter, feeding them with sugar solutions in replacement. The pharmacological treatments available in case of needing are based on fumagillin, an antibiotic that was shown to be particularly effective for inhibiting the reproduction of spores in the host, but that is not able to kill them. Spores can be inactivated in the disinfestation of the beehive, treating them with acetic acid or formalin.

Other pathogens of *Apis mellifera* that were being investigated for their possible involvement in recent episodes of honeybees blight are viral agents, including Acute Bee Paralysis Virus (ABPV or APV), which is considered an agent infectious common for the bees, and the virus correlated thereof, described in 2004, known as Israeli Acute Paralysis Virus (IAPV) by the fact to have been identified in Israel for the first time. It was considered that the IAPV virus plays a critical role in cases of sudden collapse of honeybee colonies infested with the mite parasite *Varroa destructor*.

Another viral pathogen recently studied for similar reasons is the iridescent virus of invertebrates type 6 (IIV-6), which was identified in 2010 as an co-infectious agent in several colonies of honey bee collapsed as a result of infection by *Nosema ceranae*.

Over the last twenty years, parasite mites have certainly caused severe damage to beekeeping, including the fact that they transmit harmful viruses to bees, therefore causing significant losses of colonies each year. However, while most of the deaths during the winters of 2006/07 and 2007/2008 was mainly attributed to the parasitic mites, about 25-30% of colonies dead showed symptoms contrasting with mites or any other known cause.

Considering for instance the United States, in the thirty years from 1976 to 2006 it has been a drastic reduction in the number of wild bees (now almost extinct in the U.S.), and a significant, though gradual, decline in the number of families kept by beekeepers. This latter number, according to official data, declined from about 6 million in 1940 down to 2,3 million in 2008. This decline includes the cumulative losses from all factors such as

urbanization, pesticide use in agriculture, the acariosis and varroa, beekeepers retirement and the closure of businesses. However, between late 2006 and early 2007, the reduction rate has increased sharply, reaching proportions hitherto unknown, and the term "Colony Collapse Disorder" (CCD) was coined at that time to describe these sudden disappearances.

With Colony Collapse Syndrome beekeepers around the world are facing one of the toughest challenges in decades: to date a constant presence of colonies of healthy bees cannot be guaranteed, as a combination of causes, currently not fully understood, is causing mass deaths of bees constantly strong. It is a phenomenon not well known that, as noted, has been found for the first time in the colonies of bees in North America at the end of 2006, and that manifested itself in similar phenomena in Europe in subsequent years, for which families of bees (*Apis mellifera*) disappear abruptly.

Possible causes that have been suggested for the CCD comprise management practices of the beekeepers, especially the stress on the colonies due to environmental changes, malnutrition and nutritional deficiencies associated with the presence of extensive monocultures, heavy use of new pesticides neonicotinoid-based and related practices and procedures for application, various pathogens such as infestation by parasitic mites, nosema infections and viral infections (including IAPV virus), climate change, electromagnetic radiation from mobile phones or other devices created by man, genetically modified crops (GMOs) such as GM maize, new exotic pests and pathogens, decreased immunity to pathogens, and the subtle interactions between two or more of these factors. It is not yet known whether only one of these factors may be the real responsible, or it is a combination of factors which act independently in different areas affected by the CCD, or factors that act in association with each other, although more recent information suggest that a combination of several factors is the most likely hypothesis.

As a result of the foregoing, the Colony Collapse Disease was defined as a new syndrome of multifactorial kind which leads to the death of a

very large number of colonies of bees, and that caused, from 2007 to date, losses of colonies greater than the 35% a year. With regard to the diagnosis, a colony collapsed by the CCD tends to show all of the following signs:

- 5 a) presence of a brood of larvae abandoned (usually bees do not leave the larvae until they are fully opened);
- b) the presence of stockpiles of food, pollen and honey, that: -
 - are not immediately stolen by other bees,
 - when they are attacked by other insects, the attack is noticeably delayed;
- 10 c) presence of the queen in the bee hive (otherwise, the phenomenon is not attributable to the CCD).

At present there are not known treatments or specific solutions for the prevention and treatment of Colony Collapse Disorder, nor methods, techniques or procedures exist which the beekeeper can put in place with a reasonable expectation of success to protect his hives from a sudden event, and partly unexplained, as the collapse of a colony for CCD.

In the light of the foregoing, the present invention has therefore the aim of providing a method for the prevention and for the treatment of the Colony Collapse Disease in colonies of domestic honeybees that have not already undergone an episode of irreversible collapse, in view to safeguard the health of those families that have not yet been affected by the syndrome and to improve the immune resistance and overall nutritional status of bee breeding.

25 **Summary of the invention**

In the framework of the research that led to the present invention, it has been considered that, although not yet fully understood in its triggering mechanisms, the CCD is most likely a syndrome of multifactorial kind, and therefore an effective treatment must start from a general good health state in the colony and from the elimination of any possibility of nutritional deficiencies that may lead to lowering the immunity.

Furthermore, whereas the acariosis of *Varroa destructor* and the

endemic presence of Nosema, as well as that of viral pathogens such as IAPV and IIV-6, together with the effects of poisoning by the new generation pesticides (in particular, neonicotinoids) are among the most frequent or most presumable causes of colonies weakening, according to the present invention
5 it is proposed a method of prophylaxis and treatment of CCD colonies of honeybees consisting of continuously feeding the bees to be treated, an alimentary therapeutic composition especially formulated in aqueous solution, by an equipment designed for that purpose

The treatment method proposed according to the invention is carried
10 out through an automated equipment to be positioned in the proximity of the hive, which allows the atomization of a preparation (where such term means the transformation of a liquid into an aerosol having very fine drops), also a subject of the invention, made with ingredients with high nutritious, tonic, antioxidant and healing activities. The invention therefore concerns both the
15 proposed apparatus for atomizing the therapeutic and nutrient preparation and the formulation in terms of components of the preparation itself.

The solution or aqueous suspension to be distributed to the bees according to the invention is obtained through the optimal combination of: **a) nutrient and tonic** ingredients mostly powdered milk-based and/or yeast,
20 sugar and lower organic acids, **b) natural antioxidants** and antiseptics having high activity, contained in extracts of some plants known for their herbal properties, and **c) therapeutic** substances to bees, such as the extract of Thymus vulgaris, having the ability to prevent or counter the proliferation of external parasites mites and forms of fungal harmful to bees. The combination
25 of ingredients proposed is able to prevent nutritional deficiencies, to safeguarding the health state of the colonies treated and at least partially counteract the effect of contamination by pesticides such as neonicotinoids, drastically reducing the risk of contracting the CCD or mitigating the effects.

30 **Brief description of the drawings**

The specific features of the invention, as well as the advantages of the same and their operational modes, will be apparent with reference to the

detailed description presented below and to some specific embodiments of the relevant dispensing device illustrated by way of example in the accompanying drawings. In the accompanying drawings,

Figure 1 is a functional diagram of a first embodiment of the dispenser for the automated distribution of the therapeutic and nutrient preparation for the CCD according to the invention to the colonies of bees in the treatment, and

Figure 2 is a perspective view of a second embodiment of the dispenser apparatus of the therapeutic and nutrient preparation for the CCD according to the invention

Detailed description of the invention

Is therefore specific object of the present invention an automated equipment - to be positioned in the proximity of the hive - for the prevention and treatment of the syndrome of the Colony Collapse Disorder (CCD) through the administration of nutrients and therapeutic to bee colonies to be treated, comprising a box-like container made of atmospheric agents resistant material into which it is accommodated at least a first reservoir liquid-tight, equipped with stirrer and accessible from the outside through a first nozzle, a device atomizing a solution or a liquid suspension of nourishment contained in said reservoir, means for dispensing the atomized solution or liquid suspension into micrometric drops outside to the apparatus, and a control unit programmed for timing the delivery of the solution or suspension to the outside, for dosing the amount of solution or suspension delivered and for emitting alarm signals in case of malfunctions of the apparatus or in case of reduction of the solution or suspension of the liquid contained in reservoir below a predetermined level, said apparatus being powered DC with the energy supplied by a solar panel positioned on the outside of said container and electrically connected to said control unit.

According to a preferred embodiment of the apparatus of the invention, the means for the delivery of the solution or liquid suspension of nutrient atomized outside comprise a container or supply chamber accessible

to the bees but sheltered from the outside environment, for example similar to those that are used as "feeders" to be positioned above the cover-comb in conventional hives, but with a way in for the bees provided from the outside. It is also possible to provide, above the container/nourisher itself a cover having the function of sunscreen, preferably to be colored in bright and floral colors, in order to better attract bees to the power source.

According to other preferred embodiments, the means for the delivery of the atomized solution or liquid suspension to the outside of the apparatus also include a telescopic tube extensible in length upwards, placed between the box-like container of the apparatus and said reservoir or chamber power, which allows to adjust the height of the point of delivery of the nutritious and therapeutic preparation of the invention.

Preferably, the proposed apparatus according to the invention comprises a second reservoir inside said box-like container, having smaller volume than the first tank, in communication with it and separately accessible from the outside through a second nozzle. The two separate accesses permit to reintegrate independently an aqueous concentrated solution or suspension of the nutrient and therapeutic preparation according to the invention through the second nozzle, while the first is used only for the connection to the water network.

The box-like container of the apparatus proposed is conveniently made of atmospheric agents resistant material, for example similar to the material of the hives near which it must be placed, and it is preferably externally colored by one or more colors that are attractive to insects pollinators, such as purple, blue, red or green. Preferably, said container is made of seasoned wood of fir, painted outside by the above mentioned colors.

To further facilitate the management of the automatic processing, the apparatus according to the invention may comprise further control devices in addition to those already mentioned, compatibly with the cost of such equipment. In particular, it may comprise one or more temperature and/or pH sensors, or multiparameter sensors, located inside the main tank, and the control unit may be programmed to monitor the measured values of the

relative magnitudes and for alarm signals outputting in the event that these values are beyond the set limits.

As it has to be positioned on the ground in the proximity of the bees colonies to be treated, the apparatus according to the invention preferably
5 comprises four wheels placed below the box-like container, and one or more handles for manual handling of said apparatus, so as to enable the beekeeper to move it in the most favorable place in relation to the hives, the water supply location, the position of the sun, and other requirements that may vary during the season or depending on other events.

10 The apparatus according to the invention is also described in the two exemplary embodiments illustrated in Figures 1 and 2 of the accompanying drawings (where corresponding elements are indicated with the same reference numeral). It consists of a special and automated atomizing system to be installed in the proximity of the colonies or hives in order to facilitate the
15 accessibility to the atomized liquid preparation, through the apparatus, by the families of bees to be subjected to the treatment.

The system is composed of a box or container (10), for example equipped with wheels (13) and handle (14) to allow its handling, and having a color suited to attract the bees and/or to be viewed by them, for example, in
20 purple, blue, red or yellow. The container (10) can be realized in different materials, possibly recyclable, such as fir seasoned wood or other timber suitable for the outdoor permanence and for the exposure to sunlight and rain.

Inside the container (10) are contained a first gas-tight reservoir (6), including from a minimum volume of 2 liters to a maximum of 20 liters, in
25 which it is poured, through the nozzle (3), the concentrate prepared according to the invention with chosen ingredients later detailed and that allows the correct dosage of the ingredients themselves.

In a second gas-tight reservoir (9), having a volume from a minimum of 20 liters to a maximum of 35 liters, drinking water is loaded through the
30 nozzle (4), possibly water with low hardness, by which the content of the first reservoir (6) will be diluted. Alternatively it may be used a single reservoir in which loading both the water and the therapeutic and nutritious preparation to

be diluted.

In the case where the tanks designed are two, they are connected together through a nozzle (11) and are supported on one another by means of media supports (8). On the bottom of the main reservoir (9) (in the case of two
5 reservoirs) or of the only reservoir (in the case of a single reservoir) there is a mixer (12) which is activated for short periods of time (within few minutes) and allow the preparations to be maintained mixed avoiding any form of sedimentation on the bottom.

The periodic distribution of the preparation according to the invention
10 to the bees to be treated is carried out by nebulization of the preparation through special nozzles, preferably placed within a nourisher-type container (1) similar to those normally used in the apiary in covercombs but accessible to the bees from the outside, for example, through the opening (17). The nebulization occurs through a nebuliser system(15) DC powered, by means of
15 rechargeable batteries in turn fed by a 5 W to 15 W solar panel (5), installed on the equipment, from 4,5 volts up to a maximum of 12 volts. The nebulization electrical system that pressurizes the contents of the reservoirs (8) and (9), or the one reservoir, is equipped with a telescopic tube (2) having, for example, a length up to 150 cm. The telescopic tube (2) allows to locate, at
20 the optimum height, the nozzles emitting a micrometer mist (aerosol) consisting of the preparation according to the invention in solution or in aqueous suspension.

As already noted, the energy required to operate the equipment is supplied by a solar panel (5), which allows to use the equipment also in open
25 country and where there is any network power supply. Therefore, the operator using the equipment, after the placement thereof in the optimal place in the proximity of the hives, has only to fill the reservoir with water and pour the mixture according to the invention. The preparation should be reinstated presumably every 2-3 months, while the water level is not to be reintegrated
30 more than once a month.

Through an electronic system control (7) present in the apparatus and by a programmed timer, the nutritious and therapeutic preparation according

to the invention is sprayed periodically and made available to the bees in micronized drops of solution or suspension. The control system (7) allows also to check the water and the nutritional and therapeutic preparation levels, and notifies the user by means of a light and/or sound signal and by sending
5 telephonic messaging and/or e-mail when a reservoir reaches the minimum level. The electronic control system (7) indicates by the same way, the nebulizer module efficiency (in particular, the possible clogging of the nozzles) signaling an abnormal electric absorption by the spray system or, alternatively, by means of a pressure device which shows an increase of pressure at the
10 nebulizer itself, a symptom of nozzles clogging.

The control system (7) also allows to check and report anomalies of the overall timing system (which may be digital or mechanical with mobile switches), the solar panel (5) functioning or the batteries charge levels. The control system (7), which can also be a simple PLC (programmable logic
15 controller), acquires the data of the multiparameter probe (16) with alarm for temperatures below 0°C and possible verification of the pH of the solution to be nebulized to control the exact dosage of the preparation components of the invention in relation to water.

The control system (7) can be further equipped with an interface for
20 remote control and verification that allows programming or management of alarm signals through smartphone applications, so as to have access to data 24 hours day, and especially to be able to check the proper functioning of the equipment.

The aqueous solution or suspension of the present invention to be
25 used for the supply through the automated apparatus described above is made by the combination of ingredients selected to provide nourishment in calibrated proportions, consisting of sugars, protein foods such as yeast and milk powder, essential oils containing active ingredients and antioxidants and having high tonic and healing effect.

30 Therefore, the invention further concerns a liquid solution or suspension of nourishment which may be delivered in a spray form through the automated apparatus proposed, for the prevention and treatment of the

Colony Collapse Disease (CCD), characterized in that it comprises in an unique aqueous solution or suspension:

- a) tonics and nutritious ingredients, mainly consisting of: milk powder and/or yeasts, sugars and lower organic acids;
- 5 b) natural antiseptic and antioxidant contained in the extracts of one or more aromatic and medicinal plants selected from: *Crocus sativus*, *Pelargonium graveolens*, *Monarda citriodora*, *Myristica fragrans*, *Origanum vulgare*, *Origanum majorana*, and
- c) curative substances to bees, selected from thymol, oxalic acid, extracts of
10 *Thymus vulgaris*, *Aloe arborescens* extracts, extracts of *Beta vulgaris* and mixtures of two or more thereof.

More specifically, the substances used in the preparation according to the invention are the following:

A. Nutrient and tonic substances

- 15 1) Yeast, for example, baker's yeast, and/or milk powder, which are very appetites by bees. The level of proteins of the same is about 50% with a fat content of which is around 7%. They are the most comprehensive in terms of amino acid and are rich in B vitamins
- 20 2) Sugar of glucose or fructose type to increase the palatability of the protein element to be administered. It is possible to add pollen and/or icing sugar (sucrose) in an amount ranging from 5% to 15% on the total dry weight of the formulation. As nutrients vitamin E and essential amino acids extracted from *Aloe arborescens* have also been used, which are indicated below between the substances having curative
25 activity.
- 30 3) Acetic acid and/or lower carboxylic or dicarboxylic acids, C₂-C₆. The acetic acid, tartaric acid and citric acid have the ability to separate the molecules of sucrose, promoting assimilation by the bees. Also, said acids are antifungal useful in combating the presence of the fungus *Nosema* and other fungal forms. Specifically, the formulation may contain acetic acid at a maximum concentration of 6% by weight. A source of ascorbic acid (vitamin C), such as lemon juice (which

contains it, in addition to the most abundant citric acid), may be included in the formulation as a vitamin compound, as well as an antioxidant.

- 4) Among the devices used to avoid the crystallization of the concentrated solution, it can be brought to a boil for fifteen minutes a part of water and two of sugar and add at the end a 5% of glycerol and/or propylene glycol (calculated on the total weight of the concentrated formulation). It also has the advantage of lowering the freezing point of the entire compound preventing the freezing of the preparation during the winter

B. Highly active **antioxidants and antiseptics**

Essential oils with a high content of antioxidants and/or antiseptic were extracted and added to the formulation, such as carotenoids of the type crocetin, crocin picrocrocin extracted from flowers and/or stigmas of saffron (extracts of *Crocus sativus*); oils essential as the limonene e.g. an extract from lemon; geraniol, citronellol, the terpineol and linalool, extracts from *Pelargonium graveolens* (geranium), or by *Monarda citriodora* var. *citriodora*; the myristicin, the elemicin, geraniol and/or safrole and other aromatic ethers extracted from *Myristica fragrans* (nutmeg); carvacrol, thymol and other minor phenols extracts from *Origanum vulgare* (ssp *hirtum*); and terpenes type terpineol, borneol, sabinene and linalool extracted from *Origanum majorana*.

These active ingredients can be extracted for example by the plant species above and usually have a purity exceeding 55%, or they can be made synthetically.

C. **Other therapeutic substances**

To counteract and/or prevent the mites, harmful fungal forms, viruses including the IIV6 and IAPV and side effects of neonicotinoids were used biologically active substances contained in *Thymus vulgaris* (common thyme) and those contained in *Aloe arborescens* (a species of Aloe less common of *Aloe vera*, but more rich in biological active substances). The first, whose

essential oil is already widely used in beekeeping, contains the two phenolic compounds to biocidal activity thymol and carvacrol, as well as cineol, borneol and other terpene compounds.

The second, *Aloe arborescens* contains many biologically active compounds, including acemannan (a mucopolysaccharide known for its antiviral activity having an immunomodulator action) and anthraquinones, including aloin and emodin, beta-sitosterol, in addition to acetylsalicylic acid. As already noted, the extracts of this plant also contain all the essential amino acids and vitamin E.

Moreover, a good effect was observed according to the invention in the use of extracts of common beet or *Beta vulgaris* cv *altissima* (beet sugar), containing flavonoids, trimethylglycine, compounds with antioxidant activity, oxalic acid and vitamins belonging the group B.

To these extracts can also be added oxalic acid in a quantity not exceeding 1% of the total of the concentrated formulation for increasing the disinfecting effect in synergy with the natural compounds cited.

Preparation of the concentrated solution or suspension - The concentrated solution or suspension, which must be subsequently diluted with water to reach the desired concentrations of the various ingredients, is prepared by the sequential addition of the various nutrients, after the antioxidants and curative compounds mixing with a stirrer bench in order to obtain an homogeneous solution (or suspension).

According to some specific embodiments of the nebulizable solution or suspension of the invention, such nutrients and tonic ingredients included in the formulation consist of milk powder, glucose and/or fructose, acetic acid, tartaric acid and citric acid. Preferably, as already noted, to the formulation is added a small amount of glycerine to avoid crystallization of the concentrated intermediate solution.

Preferably, the solution or suspension proposed contains the following aromatic and medicinal plants extracts in combination, with functions of primary antioxidants and/or antiseptics: *Crocus sativus* extract; *Pelargonium graveolens* extract; *Myristica fragrans* extract; *Origanum vulgare* extract and

Origanum majorana extracts. Optionally, to the quoted extracts it is also added the extract of *Monarda citriodora*

Additionally, the proposed solution or suspension contains, as curative substances to bees, *Thymus vulgaris* extracts, extracts of *Aloe arborescens* and oxalic acid and, according to a preferred embodiment, also extract of *Beta vulgaris* cv. *altissima*.

An exemplary formulation of the nutritional and therapeutic preparation of the invention is shown in the following table, with the fields of preferred concentrations for the various ingredients.

Table 1 – Preferred concentrated formulation

INGREDIENTS	PREFERRERED CONCENTRATIONS (% by weight)
Powder milk	5-10
Distilled water	28-33
Glucose and/or fructose	53-60
Vinegar	0.8-1.2
Glycerine	0.4-0.6
Liquid lemon sauce	0.4-0.6
<i>Crocus sativus</i> (crocus)	0.05-0.11
<i>Pelargonium graveolens</i> (geranium)	0.05-0.08
<i>Monarda citriodora</i> (monarda)	0.00-0.08
<i>Myristica fragrans</i> (nutmeg)	0.08-0.12
<i>Origanum vulgare</i> (oregano)	0.08-0.12
<i>Origanum majorana</i> (marjoram)	0.08-0.12
Thymol	0.00-0.04
<i>Thymus vulgaris</i> (thyme)	0.03-0.07
<i>Aloe arborescens</i>	0.10-0.20
<i>Beta vulgaris</i> (common beet)	0.00-0.30
Oxalic acid	0.00-0.10

According to another aspect complementary to the above, the present invention consists in a method of treatment of bees farmed colonies for the prevention and treatment of the Colony Collapse Disease through the administration of nutrients and therapeutic substances, which method consists

5 in the use the automatized apparatus of the invention described above for dispensing in a spray form a aqueous liquid solution or suspension containing:

a) tonics and nutritious ingredients, mainly consisting of: milk powder and/or yeasts, sugars and lower organic acids;

b) natural antioxidants and antiseptics contained in the extracts of one or more

10 aromatic or medicinal plants selected from: *Crocus sativus*, *Pelargonium graveolens*, *Monarda citriodora*, *Myristica fragrans*, *Origanum vulgare* and *Origanum majorana*; and

c) medicinal substances to bees, selected from thymol, oxalic acid, extracts of *Thymus vulgaris*, *Aloe arborescens* extracts, extracts of *Beta vulgaris* cv.

15 *altissima* and mixtures of two or more thereof.

In the method of treatment according to the invention, preferred solutions are those in which such ingredients nutrients and tonics consist of milk powder, glucose and/or fructose, acetic acid, tartaric acid and citric acid. Moreover, as already noted, such nutrients and tonics ingredients preferably

20 comprise also glycerin.

According to some preferred embodiments of the method of treatment and prophylaxis of the invention, the liquid solution or suspension contains the extracts of aromatic or medicinal herbs already mentioned, in combination: *Crocus sativus* extract; *Pelargonium graveolens* extract; *Myristica fragrans*

25 extract; *Origanum vulgare* extract and *Origanum majorana* extract. Moreover, as already noted, the liquid solution or suspension may comprise in addition extract of *Monarda citriodora*.

Still according to some specific embodiments, a method of prophylaxis and therapy of the invention, these substances curative for the bees are

30 preferably extracts of *Thymus vulgaris*, extracts of *Aloe arborescens* and oxalic acid. In addition to these, in a preferred variant, even the extract of *Beta vulgaris* cv. *altissima*.

By way of examples, which are not to be considered limitative but useful to clarify the therapeutic and prophylactic measures for the CCD proposed according to the invention two formulations of exemplary solutions are reported in the following, which are adapted to be provided through the device proposed according to the method of the invention, together with the results of experiments conducted on the same formulations in field tests.

EXAMPLE 1

Concentrated nourishing, antioxidant and curative formulation No. 1

A first formulation to produce **a liter of concentrated preparation** to be diluted in 30 liters of water for the final spraying on a number of hives from 10 up to a maximum of 15 contained the following ingredients in the following proportions.

A) Nutritious and tonics- for a total of 425 ml:

15	▪ aqueous solution at 15% by weight of milk powder	80 ml
	▪ aqueous solution at 90% by weight of sugar	260 ml
	▪ aqueous acetic acid at 6% by weight	20 ml
	▪ glycerin	50 ml
	▪ tartaric acid	5 ml
20	▪ citric acid	10 ml

B) Antioxidants – for a total of 255 ml:

Essential oils in aqueous solution:

	▪ <i>Crocus sativus</i>	55 ml,
	▪ <i>Pelargonium</i>	45 ml,
25	▪ <i>Monarda citriodora</i>	35 ml,
	▪ <i>Myristica fragrans</i>	45 ml,
	▪ <i>Origanum vulgare ssp hirtum</i>	40 ml,
	▪ <i>Origanum majorana</i>	35 ml;

C) Therapeutic compounds - for a total of 320 ml:

Essential oils in aqueous solution:

30	▪ <i>Thymus vulgaris</i>	100 ml;
	▪ <i>Aloe arborescens</i>	175 ml;

- *Beta vulgaris* cv *altissima* 25 ml;
- aqueous oxalic acid at 3% by weight 20 ml.

Nutrients contained in the milk	FORMULA I
lipids	2.61 g
proteins	2.39 g
sugars	13.52 g

5 The process to obtain the solution involves the sequential addition of the various nutrients in the order as indicated above, followed by the antioxidants and to terminate by the addition of the curing compounds. Once added within a container, they are stirred at room temperature for 5-10 minutes with a stirrer bench in order to obtain an homogeneous solution of 10 1000 ml. At this point the preparation is ready to be further diluted with water, preferably of low hardness, or with demineralized water.

EXAMPLE 2

Concentrated nourishing, antioxidant and curative formulation No. 2

15 A second formulation to make **a liter of concentrated preparation** to be diluted in 29 liters of water for the final spraying on a number of hives from 10 up to a maximum of 15 contained the following ingredients in the following proportions.

- A) Nutritious and tonics- for a total of 425 ml:
 - aqueous solution at 15% by weight of milk powder 70 ml
 - 20 ▪ aqueous solution at 90% by weight of sugar 250 ml
 - aqueous acetic acid at 6% by weight 20 ml
 - glycerin 50 ml
 - citric acid 10 ml

B) Antioxidants – for a totale of 245 ml:

25 Essential oils in aqueous solution:

- *Crocus sativus* 55 ml,
- *Pelargonium* 35 ml,

- *Myristica fragrans* 55 ml,
- *Origanum vulgare ssp hirtum* 75 ml,
- *Origanum majorana* 25 ml;

C) Therapeutic compounds - for a total of 355 ml:

5 Essential oils in aqueous solution:

- *Thymus vulgaris* 90 ml;
- *Aloe arborescens* 245 ml;
- oxalic acid at 3% by weight in aqueous solution 20 ml.

Nutrients contained in the milk	FORMULA II
lipids	2.28 g
proteins	2.03 g
sugars	11.83 g

10

The process to obtain the final solution involves the sequential addition of the various nutrients in the order as indicated above, followed by the antioxidants and to terminate by the addition of the curing compounds. Once added within a container, they are stirred at room temperature for 5-10 minutes with a stirrer bench in order to obtain an homogeneous solution of 1000 ml. At this point the preparation is ready to be further diluted with water, preferably of low hardness, or with demineralized water.

Experimentation in the field

20 Tests have been made since 2009 in Italy in Tuscany region at a location about 200 meters above sea level. The apparatus according to the invention has been positioned in the proximity of active spruce 10 hives, about 3 meters from the same and was fed continuously with batches of nutritious and curative preparation formulated as in Example 1, and thereafter with
25 batches of preparation formulated as in Example 2.

This equipment has been active until the early months of 2011 and in the colonies there were no problems related to the CCD, while in other bees

farms in the vicinity there was an average loss of more than 20% over the same two years. At the end of 2010 the number of colonies in the apiary subjected to treatment according to the invention was increased up to a total of 14, with a net increase of 40%.

5 At the beginning of 2011 the apparatus according to the invention has been removed, and as of late March 2011, the same hives were gradually affected by the depopulation of the CCD, and their total number was reduced to only four units in the month of July 2011. Thus this finding confirms the initial hypothesis that the treatment method of the invention, as implemented
10 through the proposed automatic spray equipment properly positioned in the vicinity of colonies of honey bees to be treated, and fed with the described nutrient solutions, antioxidants, and curative is effective to prevent and combat the Colony Collapse Disease.

 It follows that the prophylactic and therapeutic solution proposed
15 according to the invention, further to counter the aforementioned problems of the mites, fungi and viruses IIV-6 and IAPV, also provides a remedy to the shortage of food and tonics, and counters the negative effects of poisoning by pesticides of new generation, allowing them to prevent and combat the onset of Colony Collapse Disorder.

20 The present invention has been described with particular reference to some embodiments thereof but it should be understood that changes and modifications may be made by those skilled in the art without departing from the scope of the invention as described in the appended claims.

CLAIMS

1. An automated apparatus for the prevention and treatment of the Colony Collapse Disease (CCD) through the administration of nutrients and therapeutic to bee colonies to be treated, comprising a box-like container (10) of a material resistant to atmospheric agents which accommodates in its interior at least a first reservoir (9) for liquid-tight fitted with a stirrer (12) and accessible from the outside through a first nozzle (4), an atomizing device (15) of a liquid solution or suspension of nourishment contained in said tank, means for the delivery (2, 1) of the liquid solution or suspension atomized into micrometric drops outside the apparatus, and a control unit (7) programmed for timing the delivery of the solution or suspension to the outside, for the determination of the amount of solution or suspension delivered and for emitting alarm signals in case of malfunctions of the apparatus or reduction of the solution or suspension in a liquid contained in the reservoir (9) below a predetermined level, said apparatus being powered DC with the energy supplied by a solar panel (5) located outside of said container and electrically connected to said control unit (7).

2. An apparatus according to claim 1, wherein said means for dispensing (2, 1) the liquid solution or suspension atomized outside the apparatus include a container or supply chamber (1) accessible to the bees but sheltered from the external environment.

3. An apparatus according to claim 2, wherein said means for dispensing (2, 1) the liquid solution or suspension atomized outside the apparatus include a telescopic tube (2) extendable in length upwards, placed between said container box (10) and said container or supply chamber (1).

4. An apparatus according to claims 1 or 2, comprising within said box-like container (10) a second reservoir (6) of smaller volume of the first reservoir (9), in communication with said first reservoir and separately accessible from the outside through a second nozzle (3).

5. An apparatus according to claim 4, wherein said box-like container (10) made of a material resistant to atmospheric agents is colored externally in

one or more of the colors violet, blue, red or green.

6. An apparatus according to claim 5, wherein said box-like container (10) made of a material resistant to atmospheric agents is made of wood of seasoned spruce.

5 7. An apparatus according to claims 1 or 2, further comprising one or more sensors (16) for temperature and/or pH placed within said first reservoir (9), and wherein said control unit (7) is programmed to monitor the detected values of the relative magnitudes and for emitting alarm signals in the case where these values go beyond the preset limits.

10 8. An apparatus according to claims 1 or 2, comprising four wheels (13) placed at the bottom of said box-like container (10) and one or more handles (14) for the manual handling of said apparatus.

9. A liquid solution or suspension of nutrients that can be delivered in a spray form through the automated apparatus of claim 1 for the prevention
15 and treatment of the Colony Collapse Disease (CCD), characterized in that it comprises in a single aqueous solution or suspension:

a) tonics and nutritious ingredients, mainly consisting of: milk powder and/or yeasts, sugars and lower organic acids;

b) natural antioxidants and antiseptics contained in the extracts of one
20 or more aromatic or medicinal plants selected from: *Crocus sativus*, *Pelargonium graveolens*, *Monarda citriodora*, *Myristica fragrans*, *Origanum vulgare* and *Origanum majorana*; and

c) substances medicinal to bees, selected from thymol, oxalic acid, extracts of *Thymus vulgaris*, extracts of *Aloe arborescens*, extracts of *Beta*
25 *vulgaris* cv. *altissima* and mixtures of two or more of the same.

10. A liquid Solution or suspension that can be delivered in a spray form according to claim 9, wherein said nutrient ingredients and tonics consist of milk powder, glucose and/or fructose, acetic acid, tartaric acid and citric acid.

30 11. A liquid solution that can be delivered in a spray form according to claim 10, wherein said ingredients nutrients and tonics further comprise glycerin.

12. A liquid solution or suspension that can be delivered in a spray form according to claim 9, containing the following aromatic or medicinal plant extracts in combination: of *Crocus sativus* extract; *Pelargonium graveolens* extract; *Myristica fragrans* extract; *Origanum vulgare* extract and *Origanum majorana* extract.

13. A liquid solution or suspension that can be delivered in a spray form according to claim 12, further containing extract of *Monarda citriodora*.

14. A liquid solution or suspension that can be delivered in a spray form according to claim 9, wherein said curative substances for the bees are extracts of *Thymus vulgaris*, extracts of *Aloe arborescens* and oxalic acid.

15. A liquid solution or suspension that can be delivered in a spray form according to claim 14, wherein said curative substances for the bees com-furthermore take extract of *Beta vulgaris* cv. *altissima*.

16. A method for the prevention and treatment of the Colony Collapse Disease (CCD) through the administration of nutrients and therapeutic to bee colonies to be treated, consisting in using the automated apparatus of claim 1 or automated apparatus of claim 2 to deliver in a spray form a solution or know-aqueous liquid suspension containing:

a) tonics and nutritious ingredients, mainly consisting of: milk powder and/or yeasts, sugars and lower organic acids;

b) natural antioxidants and antiseptics contained in the extracts of one or more aromatic or medicinal plants selected from: *Crocus sativus*, *Pelargonium graveolens*, *Monarda citriodora*, *Myristica fragrans*, *Origanum vulgare* and *Origanum majorana*; and

c) substances medicinal to bees, selected from thymol, oxalic acid, extracts of *Thymus vulgaris*, extracts of *Aloe arborescens*, extracts of *Beta vulgaris* cv. *altissima* and mixtures of two or more thereof.

17. A method for prevention and treatment according to claim 16, wherein said nutrient and tonic ingredients consist of milk powder, glucose and/or fructose, acetic acid, tartaric acid and citric acid.

18. A method for prevention and treatment according to claim 17, wherein said nutrient and tonic ingredients further comprise glycerin.

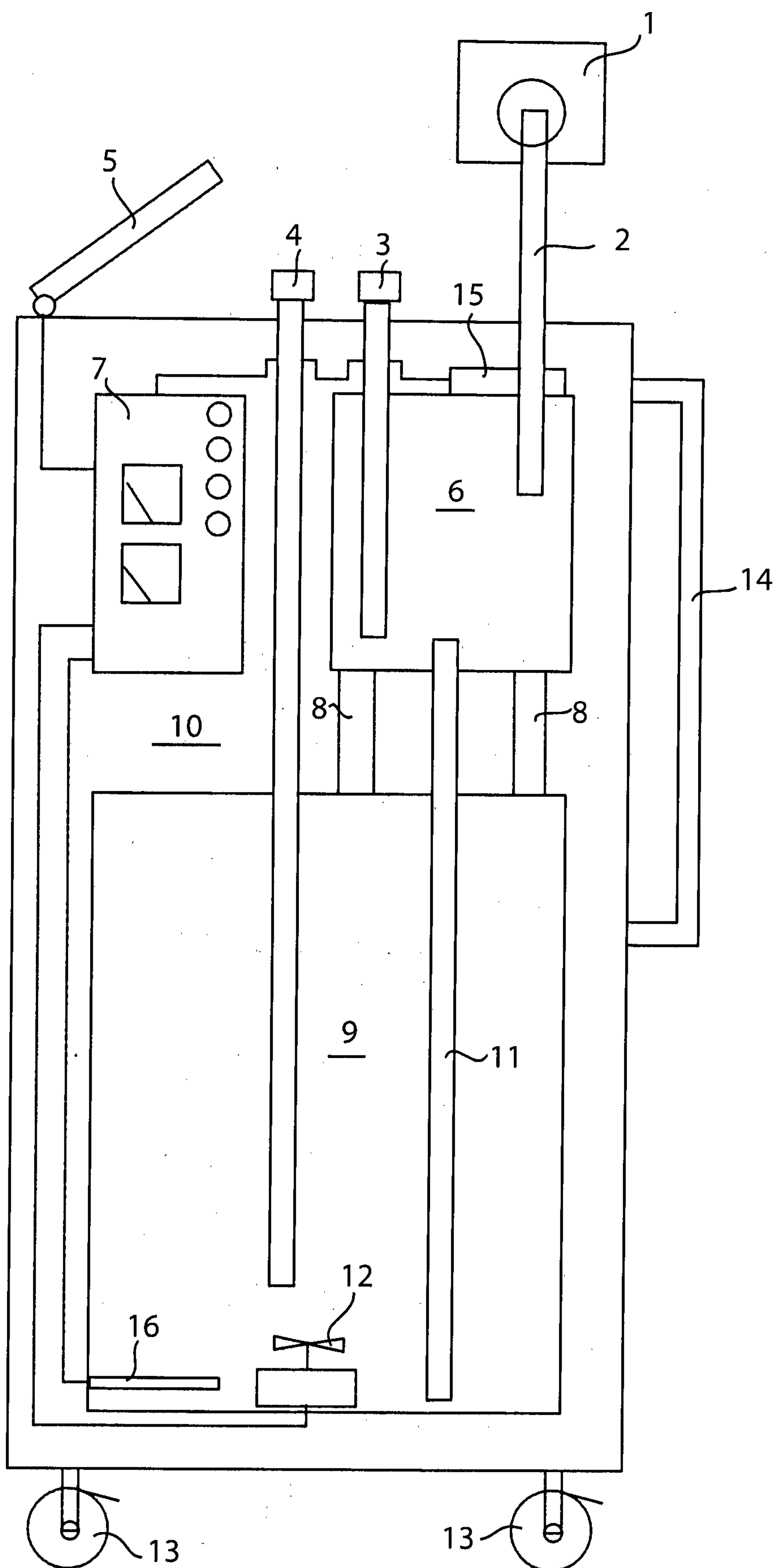
19. A method for prevention and treatment according to claim 16, wherein said liquid solution or suspension contains the following extracts of aromatic and medicinal plants in combination: *Crocus sativus* extract; *Pelargonium graveolens* extract; *Myristica fragrans* extract; *Origanum vulgare* extract and *Origanum majorana* extract.

20. A method for prevention and treatment according to claim 19, wherein said liquid solution or suspension also contains extract of *Monarda citriodora*.

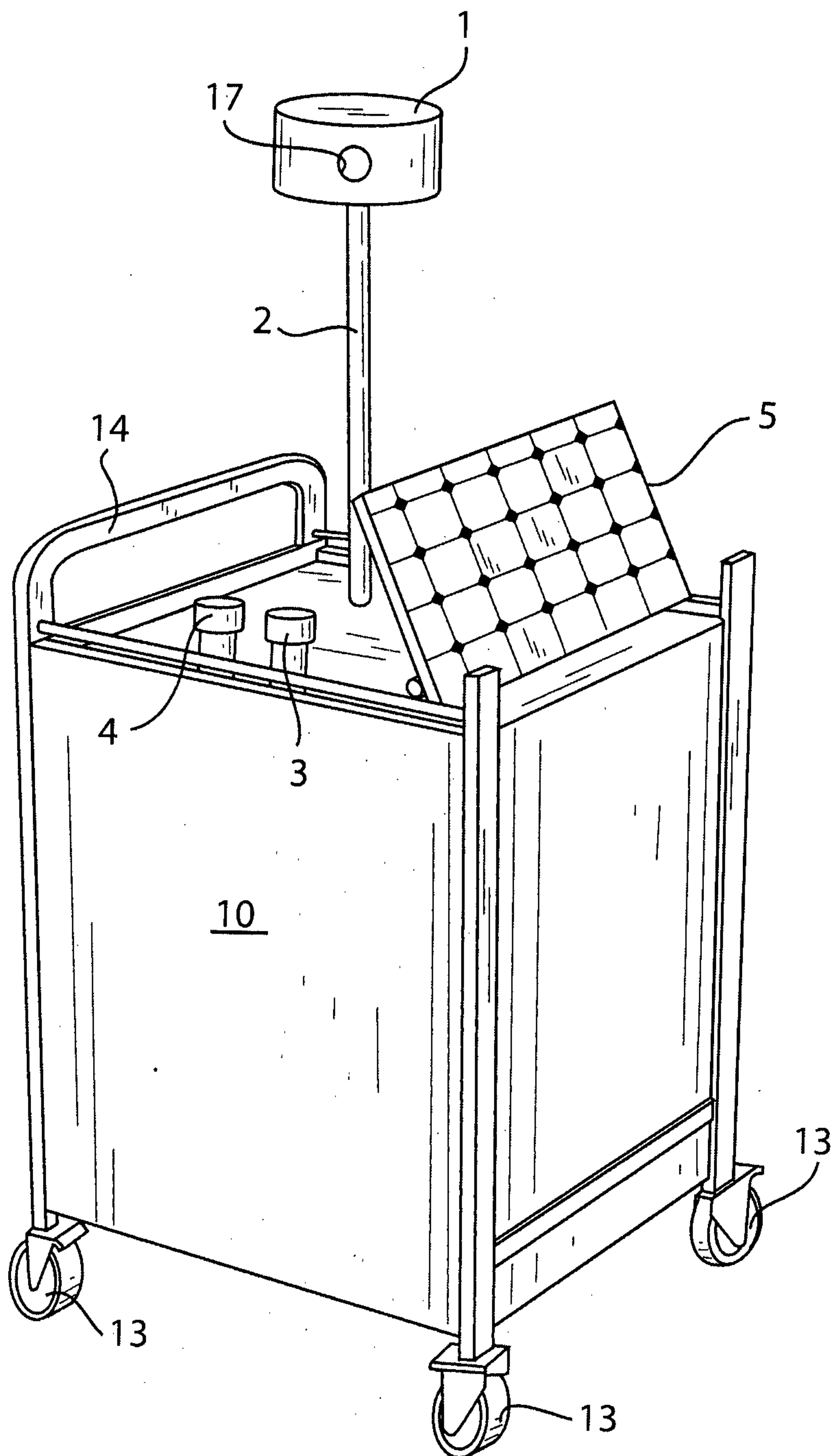
21. A method for prevention and treatment according to claim 16, wherein said curative substances for the bees are extracts of *Thymus vulgaris*, extracts of *Aloe arborescens* and oxalic acid.

22. Method for prevention and treatment according to claim 21, wherein said curative substances to bees also include extract of *Beta vulgaris* cv. *altissima*.

1/2

**Fig.1**

2/2

Fig.2