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TOXIC, REPELLENT AND ANTIFEEDANT ACTIVITIES OF *LAVANDULA ANGUSTIFOLIA* MILLER (LAMIACEAE) ESSENTIAL OIL AGAINST *SITOPHILUS GRANARIUS* (L.) (COLEOPTERA, CURCULIONIDAE) ADULTS

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RIASSUNTO

Dall'immediato dopoguerra, la difesa delle colture agrarie, facendo ricorso ad un uso intensivo di molecole di sintesi, ha creato seri problemi all'ambiente e all'uomo.

Recentemente con la crescente sensibilizzazione delle istituzioni comunitarie e nazionali agli aspetti ambientali e ai possibili effetti negativi sulla salute umana degli agrofarmaci sono state elaborate nuove normative in grado di guidare gli attori della filiera agroalimentare verso l'impiego più razionale e consapevole dei prodotti fitosanitari (PF) (direttiva 2009/128/CE e s.m.i., e D.M. 22 gennaio 2014, Adozione del Piano di azione nazionale per l'uso sostenibile dei prodotti fitosanitari, ai sensi dell'articolo 6 del decreto legislativo 14 agosto 2012, n. 150 recante: «Attuazione della direttiva 2009/128/CE che istituisce un quadro per l'azione comunitaria ai fini dell'utilizzo sostenibile dei pesticidi».).

La legge si propone di raggiungere obiettivi finalizzati alla riduzione dei rischi associati all'impiego dei PF, come diminuire gli impatti dei citati prodotti sulla salute umana, sull'ambiente e sulla biodiversità, promuovere l'applicazione della difesa integrata, dell'agricoltura biologica e di altri approcci alternativi, tutelare i consumatori e gli ecosistemi.

Particolari incentivazioni, in tal senso, vengono riservate agli operatori che utilizzano strategie di difesa basate su PF selettivi, a bassa tossicità e persistenza.

A seguito di tali normative molti principi attivi non rispondenti alle caratteristiche citate sono stati eliminati dal commercio o verranno gradualmente ritirati. Per ciò, diverse ricerche sono indirizzate a individuare nuove molecole con le caratteristiche sopracitate.

I principi attivi di origine vegetale costituiscono una interessante fonte di prodotti sicuri, degradabili e con diversi meccanismi di azione.

Ad esempio, gli oli essenziali (OE) sono una delle classi più antiche e conosciute di sostanze. Sono prodotti dalle piante aromatiche e rappresentano un sistema multicomponente di diversa natura. La complessità chimica giustifica la diversa attività biologica e le ampie e variegate modalità di applicazioni (cosmetica, farmaceutica, agricoltura, alimentazione). In particolare, in agricoltura, gli OE sono segnalati per possedere attività tossica, repellente, disappetente e deterrente su diversi organismi.

Lavandula angustifolia Miller, è un'importante specie della famiglia delle *Lamiaceae* ampiamente distribuita in tutto l'areale del Mediterraneo.

La pianta è tradizionalmente impiegata come espettorante, antispasmodico, carminativo, stimolante, antiostruente e ricostruente. L'olio essenziale è invece sfruttato in aromaterapia o come agente

antimicrobico, come analgesico e rimedio contro le coliche, espettorante e disinfettante delle ferite, oltre ad avere conclamate proprietà antibatteriche, antifungine, rilassanti, antidepressive ed essere utile in caso di ustioni e punture di insetti (Gattefossé 1937, Aazza et al., 2011).

Molte delle proprietà elencate sono state riscoperte, soprattutto per quanto concerne l'importante attività biologica del genere *Lavandula*.

Oltre queste interessanti caratteristiche, gli OE presentano una rapida volatilità e ossidabilità che devono necessariamente trovare risoluzione per una efficace applicazione pratica sugli insetti. Per tale motivo è necessario mettere a punto un opportuno formulato commerciale che preservi o esalti le loro caratteristiche biologiche.

Sitophilus granarius (L.), è un importante fitofago dei cereali in magazzino, in grado di provocare ingenti danni alle cariossidi di grano, le quali vengono divorate e svuotate dalle larve, causando una enorme riduzione quantitativa e qualitativa della granella.

Il controllo dell'insetto è difficile per le numerose generazioni che riesce a condurre negli ambienti confinati e per la vita endofitica degli stadi preimmaginali.

Scopo del presente lavoro è stato quello di effettuare uno studio completo sull'attività biologica dell'OE ottenuto, mediante distillazione in corrente di vapore, da infiorescenze di *L. angustifolia*.

Sono state, pertanto, eseguite prove di contatto, ingestione e inalazione valutando la mortalità, la repellenza, deterrenza e gli indici nutrizionali su adulti di S. *granarius*.

Inoltre, si è provveduto ad individuare e valutare un opportuno substrato minerale in grado di assorbire e rilasciare OE di lavanda in modo da potenziare e mantenere la tossicità e la repellenza osservate.

1. INTRODUCTION

During the past few decades, application of synthetic pesticides to control agricultural pests has been a standard practice. The repeated and intense use of synthetic insecticides has raised long-term human health and environmental concerns, mainly due to their slow degradation in the environment and toxic residues in the products, and the evolution of resistance to pesticides in pest populations (Isman, 2006). These effects have increased the need for effective and biodegradable pesticides and created a significant market opportunity for alternative products (Isman, 2000; Isman et al., 2011).

There is increasing interest in industry, academia and the health sciences in medicinal and aromatic plants. Many industries are involved such as forestry, agriculture, chemical, food, flavour, beverage, pharmaceutical, cosmetic and fragrance. The plant raw materials are roots, rhizomes, bulbs, leaves, stems, barks, wood, flowers, fruits and seeds. These yield gums, resins, essential (volatile) oils, fixed oils, waxes, juices, extracts and spices for medicinal and aromatic purposes. All these commodities are traded worldwide. Natural products do not mean safe products and account of this has to be taken by the above industries, which are subject to regulation. For example, a number of plants which are approved for use in medicine must not be used in cosmetic products. This may require absence of, or prescribed limits of, radioactive material, heavy metals, aflatoxin, pesticide residue, as well as the required level of active compounds. This analytical control is costly and tends to exclude small batches of plant material. Large-scale contracted mechanised cultivation with designated seed or plantlets is now preferable. Today, plant selection is not only for the yield of active substances, but for the plant's ability to overcome disease, climatic stress and the hazards caused by mankind.

Fascinating plant folklore and ethnopharmacology leads to medicinal potential. Examples are the muscle relaxants based on the arrow poison, from species of *Chondrodendron*, and the anti-malarials derived from species of *Cinchona* and *Artemisia*.

The medicinal traditions of ancient civilisations such as those of China and India have a large armamentaria of plants in their pharmacopoeias which are used throughout South-East Asia.

A similar situation exists in Africa and South America. Thus, a very high percentage of the world's population relies on medicinal and aromatic plants for their medicine. In Germany all medical practitioners have to pass an examination in phytotherapy before being allowed to practise. It is noticeable that throughout Europe and the USA, medical, pharmacy and health related schools are increasingly offering training in phytotherapy.

The practice of using botanical insecticides in agriculture dates back at least two millennia in ancient China, Egypt, Greece, and India.

They have the advantages of reducing risk to non target organisms due to their rapid degradation in the environment and providing novel and multiple mode of actions that reduce the probability of developing resistance in pest populations (Isman, 2006; Rajendran and Sriranjini, 2008; Ebadollahi, 2011).

1.1. ESSENTIAL OIL (EO)

The essential oils (EOs) are one of the most ancient and known classes of substances. They are natural plant products representing multi-component systems and consist of mixtures of several chemical compounds. Their constituents may be classified into two principal groups:

(a) hydrocarbons (mono-, di-, and sesquiterpenes),

(b) oxygenated compounds derived from these hydrocarbons including alcohols, aldehydes, esters, kethons, phenols, and so on.

They also contain many active phytochemicals such as flavonoids, terpenoids, carotenoids, coumarins, curcumines.

The complexity of such a chemical composition led to specific properties, responsible for the wide variety of applications.

The EOs possess pesticidal properties both herbicidal, insecticidal and antimicrobial as well as for the repellence and antioxidant activities. Combining the previous mentioned properties with the global concern for a safer and better life in a protected environment, it could be justify the great importance for the study of EOs applications.

EOs are volatile secondary metabolites that interfere with basic metabolic, biochemical, physiological, and behavioral functions in insects and have been demonstrated to possess contact, inhalation and ingestion toxicity, antifeedant activity, capacity to delay development, adult emergence and fertility, deterrent effects on oviposition and arrestant and repellent action (Tripathi et al., 2009 and references therein). EOs of aromatic plants were traditionally used against economically important pests and some of them have provided potential alternatives to currently used insect control agents (Isman 2006; Nerio et al., 2010; Isman et al., 2011).

1.2 PLANT

1.2.1 THE *LAMIACEAE* FAMILY

Lamiaceae or *Labiatae,* also called mint family, is a group of flowering plants.

The original family name is *Labiatae*, so given because the flowers typically have petals fused into an upper lip and a lower lip. Although, this is still considered an acceptable alternative name, most botanists now use the name *Lamiaceae* in referring to this family.

The leaves and stems of many species have a strong aroma when crushed. The leaves are opposite or may be arranged in a whorl. The flowers are clustered in whorls at the end of the flowering stalks or in the junctions of leaves and stems. They have both pollen-bearing and ovule-bearing parts and are zygomorphic (bilaterally symmetrical). There are usually 5 sepals and petals, with sepals that are fused together at the base. There are 2 or 4 stamens and an ovary with 2 carpels. The fruit is a schizocarp, and splits into 4 segments at maturity.

The family has a cosmopolitan distribution (Yuan et al., 2010). The enlarged *Lamiaceae* contains about 236 genera and 6.900 to 7.200 species. The largest genera are *Salvia* (900), *Scutellaria* (360), *Stachys* (300), *Plectranthus* (300), *Hyptis* (280), *Teucrium* (250), *Vitex* (250), *Thymus* (220) and *Nepeta* (200). *Clerodendrum* was once a genus of over 400 species but by 2010, it had been narrowed to about 150 (McKay and Blumberg, 2006).

Some are shrubs, trees, such as teak, or rarely vines.

Many members of the family are widely cultivated for ease of cultivation; these plants are among the easiest plants to propagate by stem cuttings. Besides those grown for their edible leaves, some are grown for decorative foliage, such as coleus. Others are grown for food purposes, but seeds are utilized instead of leaves.

1.2.2 *LAVANDULA* GENUS

Lavandula is a genus of 47 known species of flowering plants in the *Lamiaceae* family.

The English word lavender is generally thought to be derived from Old French *lavandre*, ultimately from the Latin *lavare* (to wash), referring to the use of infusions of the plants. The botanic name *Lavandula* as used by Linnaeus is considered to be derived from this and other European vernacular names for the plants or from Latin *livere*, "blueish".

It is native to the Old World and is found from Cape Verde and the Canary Islands, Europe across to northern and eastern Africa, the Mediterranean, southwest Asia to southeast India.

Flowers are borne in whorls, held on spikes rising above the foliage, the spikes being branched in some species. Some species produce coloured bracts at the apices. Flowers in addition to blue, violet or lilac in the wild species, can take blackish purple or yellowish colours (Fig. 1.1 and 1.2).

The calyx is tubular. The corolla is also tubular, usually with five lobes (the upper lip often cleft, and the lower lip has two clefts).

Many members of the genus are cultivated extensively in temperate climates as ornamental plants for garden and landscape use, for use as culinary herbs, and also commercially for the extraction of essential oils. The most widely cultivated species is *Lavandula angustifolia* Miller.

L. stoechas, L. pedunculata and *L. dentata* were known in Roman times.

From the Middle Ages onwards, the European species were considered two separate groups or genera, *Stoechas* (*L. stoechas*, *L. pedunculata*, *L. dentata*) and *Lavandula* (*L. spica* and *L. latifolia*), until Linnaeus combined them. He only recognised five species in *Species Plantarum* (1753), *L. multifida* and *L. dentata* (Spain) and *L. stoechas* and *L. spica* from Southern Europe; *L. pedunculata* was included within *L. stoechas.*

By 1790, *L. pinnata* and *L. carnosa* were recognised. The latter was subsequently transferred to *Anisochilus*. By 1826 Frédéric Charles Jean Gingins de la Sarraz listed 12 species in three sections, and by 1848 eighteen species were known. One of the first modern major classifications was that of Dorothy Chaytor in 1937 at Kew. The six sections she proposed for 28 species still left many intermediates that could not easily be assigned. Her sections included *Stoechas*, *Spica*, *Subnudae*, *Pterostoechas*, *Chaetostachys* and *Dentatae*.

However all the major cultivated and commercial forms resided in the *Stoechas* and *Spica* sections. There were four species within *Stoechas* (*Lavandula stoechas*, *L. dentata, L. viridis* and *L. pedunculata*) while *Spica* had three (*L. officinalis* (*now L. angustifolia*), *L. latifolia* and *L. lanata*). She believed that the garden varieties were hybrids between true lavender *L. angustifolia* and spike lavender (*L. latifolia*).

Fig. 1.1 - Dried lavender flowers

Based on more recent and current evidences (Upson et al, 2004) *Lavandula* is considered to have three subgenera. The most frequent form in cultivation is *L. angustifolia*; other commonly grown ornamental species are *L. stoechas*, *L. dentata*, and *L. multifida* (Egyptian lavender).

Commercially, the plant is grown mainly for the production of the EO.

L. angustifolia EO yields an essential oil with sweet overtones, and can be used in balms, salves, perfumes, cosmetics, and topical applications.

Lavandin, *Lavandula × intermedia* (also known as Dutch lavender), yields a similar essential oil, but with higher levels of terpenes including camphor, which add a sharper overtone to the fragrance.

In the *Lavandula* genus, the bioactivities towards insects, including Coleopteran stored-product insect pests, Lepidoptera, Rhyncota, and Diptera have been evaluated for EOs of *L. hybrida* Rev. (Papachristos and Stamopoulos, 2002a, 2002b; Papachristos et al., 2004; Cosimi et al., 2009), *L. angustifolia* (Shaaya et al., 1997; Pavela, 2005; Pugazhvendan et al., 2012; Laznik et al., 2012), *L. luisieri* (Rozeira) Riv. Mart. (Julio et al., 2014), *L. stoechas* L. (Ebadollahi, 2011), and *L. gibsoni* Grah. Ex. Dalz. & Gibs (Kulkarni et al., 2013).

The *Lamiaceae* EO yield and chemical composition can be changed by the environment, crop management and stress conditions (Delfine et al., 2005; Russo et al., 2013).

In particular, the composition of *Lavandula* species has been widely investigated and it varied according to the part of the plant analyzed (Skoula et al., 1996; González-Coloma et al., 2006), the method of extraction (Fakhari et al., 2005; Kim and Lee, 2002), the genetic type, the environmental factors (Munoz-Bertomeu et al., 2007), and according to the species (Touati et al., 2011).

The high chemodiversity of *Lavandula* EOs may result in different bioactivity and efficacy of applications in pest control.

1.2.3 *LAVANDULA ANGUSTIFOLIA* MILLER

L. angustifolia, common known as lavender, is an aromatic plant widely native and distributed in the Mediterranean area, and its EO was found to have medicinal, antibacterial, antifungal and pesticidal activities (Cavanagh and Wilkinson, 2002).

Fig. 1.2 - Flower spike before the petals emerge

The species name *angustifolia* is Latin for "narrow leaf".

Previously, it was known as *L. officinalis*, referring to its medicinal properties.

It is popular for its colourful flowers, its fragrance, and its ability to survive with low water consumption. It does not grow well in continuously damp soil and may benefit from increased drainage provided by inorganic matter. It does best in Mediterranean climates similar to its native habitat, characterized by wet winters and dry summers.

The flowers and leaves are used as an herbal medicine, either in the form of lavender oil or as an herbal tea. Lavender essential oil, when diluted with a carrier oil, is commonly used as a relaxant; both the petals and the oil are the most popular ingredients in handmade soap.

L. angustifolia EO and some of its constituent compounds showed fumigant toxicity against *Sitophilus oryzae* (L.), *Rizopherta. dominica* (F.) and *Tribolium castaneum* Herbst (Abdelgaleil et al., 2009; Rozman et al., 2007; Pugazhvendan et al., 2012; Shaaya et al., 1997) and repellent activity against *Sitophilus zeamais* Motschulski, *T. castaneum, Cryptolestes ferrugineus* (Stephen) and *Tenebrio molitor* (L.) (Pugazhvendan et al., 2012; Cosimi et al., 2009).

1.3 INSECT

1.3.1 COLEOPTERA

The name Coleoptera derive from Greek *koleon*, that means "sheath" and *pteron* "wing"; Aristotle already called beetles "koleopteros" (κολεοπτερος) to refer to the hardened front wings protecting the membranous hind wings.

English beetle means "the little biter", derived from Old English britain, "to bite".

Coleoptera, order also commonly called beetles, weevils or fireflies, comprise 25% of all described animals and plants, and represent the primary contributor to Earth's biodiversity.

Beetles, like all insects, have an external skeleton called exoskeleton.

The body of insects is formed of 3 distinct regions the head, thorax, and abdomen. Each of these parts consist of a number of segments, which are most distinct on the abdomen. The head bears the eyes, the mouth parts, and one pair of *antennae*; the thorax bears three pairs of legs, and usually two pairs of wings.

Antennae. Beetle *antennae* function primarily as organs of smell or taste, but also serve as organs of touch. In some groups they may serve other functions; for example, in certain aquatic beetles they help circulate air under the body for use in breathing.

The large *antennae* of some long-horned beetles apparently help them maintain their balance.

The *antennae* are very useful in distinguishing the families of beetles. Their shape is often very distinctive for groups of families, and, sometimes, for a particular family.

Thorax. The thorax consists of 3 sections: *prothorax*, *mesothorax* and *metathorax.*

Each thoracic segment consists of 4 sclerites, or platelike areas set off by sutures.

The pronotum in beetles is so large and prominent that it may appear to be the entire thorax (the topmost sclerite is the notum, the 2 lateral sclerites are the pleura, and the bottom one is the sternum).

The nota of the mesothorax and metathorax are divided into 3 sclerites: the scutum, scutellum, and postnotum. The mesoscutellum (called simply the *scutellum*) in most beetles is conspicuous, and is shaped like a triangle or shield. It is visible at the base of the elytra, before the median suture.

Legs. Each leg consists of the *coxa*, *trochanter*, *femur*, *tibia*, and *tarsus*. The tarsal claws are sometimes useful in distinguishing different groups of beetles. They may be toothed, cleft, serrate, comblike, or bear ventral pads. Most claws, however, are simple, with no modifications.

Due to the constancy of the tarsal structure of a group of beetles, and the fact that this structure varies from one group of beetles to another, the tarsi are useful in classification, and are very important in

many family keys.

<u>Wings</u>. The front wings, or *elytra*, of beetles are greatly modified, and mainly provide protection for the membranous flying wings and the abdomen. The elytra are usually relatively hard or horny, but may be soft and pliable, and are nearly always opaque. In most beetle families the elytra loosely cover the tip of the abdomen; in some families (such as rove beetles, short-winged mold beetles, and others) the elytra are typically short and expose most of the abdomen. The sculpturing on the elytral surface is sometimes important in distinguishing groups of beetles; for example, in many beetles the elytra bear striae, that is, grooves or rows of punctures. The membranous flying wings of beetles are generally not used in distinguishing groups.

<u>Abdomen</u>. This region of the body consists of a series of segments composed of rings or partial rings. At the side of each segment is a spiracle, which is the opening of a trachea, another part of the breathing system. The pleura are normally very small, and are usually hidden.

Most beetles have 9 abdominal segments; these are most easily counted on the tergum.

Usually only 5 sternites are visible, for a number of them are small or fused together. The last dorsal segment of the abdomen is the *pygidium*. The reproductive organs are normally concealed within the tip of the abdomen. The genitalia (especially of the male) are often very useful in distinguishing closely related species, but are not used to distinguish genera, subfamilies, or families.

1.3.2 GROWTH AND DEVELOPMENT OF BEETLES

Beetles have the most advanced form of insect metamorphosis, called complete metamorphosis or holometabolous development. Great changes occur during this type of development, which includ four very distinct stages: egg, larva, pupa and adult.

<u>Egg</u>. The eggs develop in the ovaries of the female and are laid in a sheltered place where the young will have a food supply and favorable conditions for development. Eggs may be laid singly or in masses; hatching usually occurs after several days. The female nearly always goes on her way after egg-laying and leaves the young to care for themselves.

<u>Larva</u>. The larvae of different beetles vary greatly in appearance and habits. Generaly they have a distinct head that nearly always has opposable, chewing mouth parts. The skin stretches very little as the larva grows, so it has to be shed periodically to allow for a marked increase in size. Beetle larvae molt several times as they grow; the stages marked by this molting are called instars. The number of instars that a beetle larva passes through varies greatly (from 3 to many instars).

As a general rule, the last instar larva is the best developed and is easiest to recognize.

Most beetle larvae live on land in a wide variety of habitats, others are aquatic and feed on or within various parts of plants, both above and below ground. Some live on or within decaying vegetation or animal matter; others feed on stored food (in warehouses), while a few feed on fungi. Many larvae prey on other insects or small animals in aquatic or terrestrial habitats.

A small number of larvae are parasitic. During its development a parasitic larva feeds on or in a single individual, in contrast with a predaceous larva, which feeds on more than one individual.

In some cases the habits of the adults and larvae of a species are similar, but in most they are not. After the last instar, the larva changes into the next stage of development, the *pupa*.

Pupa. The pupa is called the "resting stage". The pupa of most species looks like a pale, mummified adult beetle. In a few species the pupa is covered by a cocoon made by the last instar larva. Beetle pupae are of the exarate type, that is, the appendage free and visible and do not cling to the body. Although the pupa is capable of doing little more than wriggling its abdomen, great changes are taking place internally, for larval tissues are breaking down to form adult structures.

Adult. The hardening of the body and darkening to adult colaration may take many hours. Once the adult exoskeleton hardens, the beetle neither grows nor molts. A beetle that has not yet assumed its full coloration is described as teneral.

Life history. One generation per year is typical for beetles, but some have 2 or 3 generations, or rarely more. The adult is generally present outdoors for a very limited time; it usually lives for just a few weeks, but some adults may live for several months. Beetles spend the winter in a dormant condition; the overwintering stage is most often the larva or the pupa, but in some species it may be the adult or even the egg. A period of dormancy at low temperatures may be essential for continued development; some species living in northern climates will not complete a generation without exposure to low temperatures.

Economic importance. Many beetles are regarded as major pests of agricultural plants and stored products. They attack all parts of living plants as well as processed fibers, grains, and wood products. Scavengers and wood boring beetles are useful as decomposers and recyclers of organic nutrients. Predatory species, such as lady beetles, are important biological control agents of aphids and scale insects.

Classification of Beetles. Suborders of beetles are recognized in: Archostemata, Adephaga, Myxophaga, and Polyphaga.

Most beetles belong to suborder Polyphaga; they vary greatly in form and habits.

Major families.

Curculionidae (weevils, snout beetles); herbivores; many species are pests of agricultural crops and

stored grains. Chewing mouthparts are at the tip of a proboscis.

Staphylinidae (rove beetles); scavengers and herbivores; elytra are characteristically shorter than the abdomen.

Carabidae (ground beetles), predators; many beneficial species including the fiery hunter, *Calosoma calidum*.

Chrysomelidae (leaf beetles), herbivores; includes many pests of agricultural crops. Most species have distinctive shapes or color patterns (e.g., Colorado potato beetle, *Leptinotarsa decemlineata*).

Scarabaeidae (lamellicorn beetles, June beetles, scarab beetles), herbivores; robust beetles with heavy spines on femur and tibia. Distinctive lamellate antennae. Usually live in the soil as larvae and feed on plant roots. Includes many pest species, including the Japanese beetle, *Popillia japonica*.

Tenebrionidae (darkling beetles), herbivores; found in flowers, rotting wood, and occasionally as pests of stored grain. Most abundant in arid climates.

Cerambycidae (longhorned beetles), herbivores; all larvae are wood borers. Adults have distinctively long antennae. A few species are pests of wood and wood products.

Elateridae (click beetles), herbivores; larvae are known as wireworms. Some species feed destructively on the roots of crop plants. When adults are turned on their back, they can snap (click) the head and abdomen against the substrate to right themselves.

Buprestidae (metallic wood borers), herbivores; larvae are known as flat-headed wood borers. Some species are forestry pests.

Coccinellidae (lady beetles), most adults and larvae are predators of aphids and scale insects, but a few species are pests of agricultural crops (e.g., Mexican bean beetle, *Epilachna varivestis*).

Cicindellidae (tiger beetles), predators.

Dytiscidae (predaceous diving beetles), large aquatic predators.

Gyrinidae (whirligig beetles), aquatic predators.

Hydrophilidae (water scavenger beetles), scavengers and predators.

Silphidae (carrion beetles), scavengers.

Lampryidae (fireflies), herbivores.

Dermestidae (carpet beetles), scavengers and herbivores.

Nitidulidae (sap beetles), scavengers and herbivores.

Meloidae (blister beetles), larval parasites, adult herbivores.

Scolytidae (bark beetles), herbivores.

Passalidae (wood-boring beetles), herbivores.

1.3.3 FAMILY CURCULIONIDAE, THE SNOUT BEETLES

Identification. Snout nearly always well-developed; broad and flat to (usually) very elongate and narrow. Antennal club of 3 segments, usually compact; antennae usually elbowed. Palps nearly always small and rigid, often concealed.

Similar families. Other beetles with a snout are most easily confused with these but they have straight antennae, palps flexible, pronotum often has a distinct margin at base. In primitive weevils, antennae beadlike, not distinctly clubbed; beak directed forward. Some narrow-waisted bark beetles have a beak. Seed beetles have a short beak; antennal club of 6/7 segments, or antennae sawtoothed or comblike.

Range and numbers. Snout beetles (*Curculionidae*) are the largest family worldwide, possibly the largest family in animal kingdom.

Habits. All our species are plant feeders, some are serious pests. Larvae feed on all parts of plants, most live inside tissues of host; a few feed externally on leaves. Larvae of broad-nosed weevils usually feed on roots; some cause galls on roots. Many larvae burrow into stems of plants; some feed in weakened trees, others in healthy trees. Many species feed under bark and in wood of dead trunks and limbs. Some are leaf-miners, others feed on dead leaves; a few feed in developing flower buds or seedpods. Fruits and nuts are hosts for some species. Adult weevils typically feed on leaves, pollen, flowers, developing fruit, or fungi; a few burrow into wood. Adults may feed on a plant different from the host on which the larva feeds. Some species feed on underwater plants throughout their life cycle. Parthenogenesis occurs in some groups, with no males ever having been discovered.

1.3.4 THE GRANARY WEEVIL, *SITOPHILUS GRANARIUS* (L.)

Sitophilus granarius or "grain weevil", is a typical member of the family Curculionidae.

Two other species in this genus, *S. oryzae* and *S. zeamais*, are also common grain storage pests.

The genus *Sitophilus* and its species may be identified using the keys of Gorham (1987) or Haines (1991).

Eggs, Larvae and Pupae. The developmental stages of *S. granarius* are all found within tunnels and chambers bored in the grain and are thus not normally seen. The larvae are white and apodous (Fig. 1.3). There are four larval instars. The general appearance of the larva and pupa is similar to that of *S. zeamais* and *S. oryzae*.

Fig. 1.3 - *S.granarius* larvae

<u>Adult</u>. Adults of *S. granarius* can vary considerably in size; between 2.5-5.0 mm in length, although 3 to 4 mm is usual. They have the characteristic rostrum and the *antennae* have eight segments and are often carried in an extended position when the insect is walking.

The body is sparsely covered with short, stout yellow hairs.

The head is prolonged into a slender snout. The dorsal surface of the rostrum is more closely and strongly punctured in males than in females. The prothorax has distinctly oval punctures.

Adults do not have wings, and cannot fly (Fig. 1.4).

Fig. 1.4 - *S. granarius* in coupling

<u>Distribution</u>. *S. granarius* is distributed throughout the temperate regions of the world. In tropical countries it is rare, being limited to cool upland areas. The FAO global survey of insecticide susceptibility recorded it from the UK, France, Italy, Spain, Denmark, Sweden, Poland, Algeria, Iraq, Canada, USA, Chile, Argentina, Swaziland, South Africa, Australia, Russia and Thailand (Champ

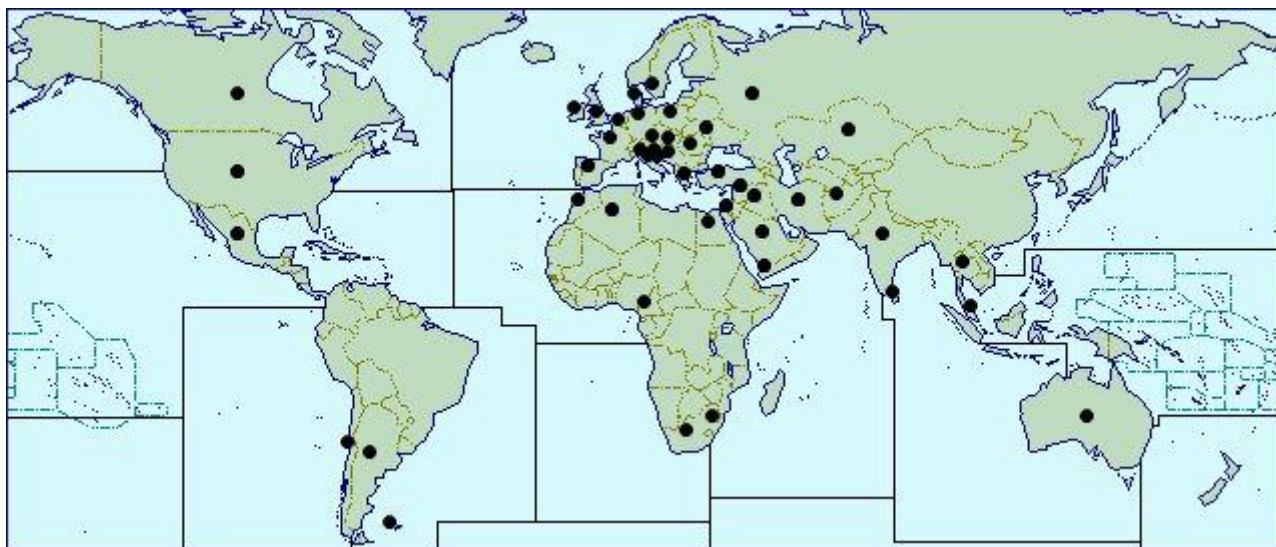and Dyte, 1976). It is also known from Yemen (Haines, 1981) (Fig. 1.5).

Fig. 1.5 - Distribution of *S.granarius*

Risk of introduction. Many countries, including those that have exclusively hot climates, have *S. granarius* as a named quarantine pest. This is presumably because it is not widely recognized that it will only establish itself in temperate climes. Nevertheless this species can cause serious damage to grain stocks under hot conditions even though it will eventually die out.

Species Affected. *S. granarius* is a frequent pest of wheat and barley. It can attack other cereals such as maize, sorghum and rice, but it does not compete well with the other two *Sitophilus* species on these grains.

Symptoms. The developmental stages of *S. granarius* are not normally seen as they occur inside intact grains. Adult emergence holes with irregular edges are apparent some weeks after initial attack. Adults can be found wandering over the surface of grain especially if the grain has been disturbed.

Biology and ecology. The biology and behaviour of *S. granarius* is similar to the tropical species *S. oryzae* and *S. zeamais*, except that it cannot fly.

Adults live for 7 to 8 months on average. Females usually lay around 150 eggs, and up to 300 eggs, throughout their lives. Eggs are laid individually in cavities that the female bores in the grain kernels. Cavities are sealed by a waxy egg plug, which the female secretes. Eggs incubate for about 4-14 days before hatching, depending on temperature and humidity. One larva develops in each infested kernel. Feeding larvae excavate a tunnel and may keep feeding until only the hull remains. Pupation occurs inside the grain. The newly emerged adult chews its way out of the grain, leaving a characteristic exit hole. In warm summer conditions the life cycle can be completed within 4 to 6 weeks, but can take

as long as 17 to 21 weeks in the winter.

Adults can survive for a month or more without food in cooler conditions.

Optimum conditions for development are similar to other tropical species of *Sitophilus*, about 30°C and 70% R.H. (Relative Humidity) (Richards, 1947), but in tropical areas it is apparently not able to compete with *S. oryzae* and *S. zeamais*. It seems that its distribution is limited more by its commodity associations with cool climate crops than by its direct response to temperature. However, it can develop at temperatures down to 11°C, and is therefore successful in temperate regions that are too cool for other *Sitophilus* species (Howe and Hole, 1968). Being flightless, *S. granarius* is not usually able to infest crops in the field before harvest. Adult *S. granarius* cannot disperse by flight, although they are very active walkers. They are transported within grain as eggs, larvae or pupae. They can readily spread in grain residues.

Impact. It sometimes infests sunflower seeds, dried beans, chickpeas, groundnuts, acorns, chestnuts, pasta products, ornamental dried corn and birdseed. Feeding damage by *S. granarius* can make grains vulnerable to attack by other pests, such as the weevil *Caulophilus oryzae* (Gyllenhal), which are unable to penetrate intact grains.

Detection and inspection. Granary weevil infestations in stored cereals are generally difficult to detect, particularly in the initial stages, since the life cycle mainly takes place (from egg to pupa) inside the kernel. Pitfall traps placed on the grain surface and probe traps inserted into grain bulks have been used successfully to detect adult *S. granarius* (e.g. Buchelos and Athanassiou, 1999; Wakefield and Cogan, 1999). Larval stages in the grain may be detected using hidden infestation detection techniques. These can involve squashing the grain against indicator papers, testing for changes in specific gravity, or using X-ray machines (Haff and Slaughter, 1999).

Rotundo et al. (2000) described a serological method for detecting the immature stages of *S. granarius* in kernels, while Ridgway and Chambers (1996) described a detection method involving near-infrared reflectance spectroscopy.

Similarities to other species. *S. granarius* can be separated from *S. zeamais* and *S. oryzae* by the absence of wings and by the presence of oval, rather than circular, punctures on the prothorax; *S. oryzae* and *S. zeamais* almost always 4 reddish spots on the elytra (Fig. 1.6).

The larvae cannot be easily separated by superficial features; only characters accessible to a microscope are useful (Gorham, 1991).

All insects are cosmopolitan species, while *S. granarius* prefers temperate climates, *S. oryzae* and *S. zeamais* warmer ones. Adults of *S. oryzae* and *S. zeamais,* thanks to functional wings can attack the grain even, also in open fields, in the hot zones.

Fig. 1.6 - *S. granarius*   *S. zeamais*   *S. oryzae*

1.3.5 PREVENTION AND CONTROL *OF S.GRANARIUS*

Chemical control. Grain may be protected by the admixture of insecticides.

S. granarius has susceptibility to organophosphorus compounds, such as pirimiphos-methyl and to synthetic pyrethroids. Grain stocks may be fumigated with magnesium phosphide and aluminium phosphide to eliminate existing infestation. However, fumigation treatments provide no protection against reinfestation. Carbon dioxide fumigation, in controlled-atmosphere storage, (Kishino et al., 1996) or cold storage can also be used to control *S. granarius* in stored grain, although the weevil is more resistant to this treatment than other storage pest species.

Inadequate fumigation or controlled-atmosphere treatments are likely to result in some survival.

Cultural control and sanitary methods. It's important to take preventive and fight techniques against the granary weevil. The rooms used as storage must be perfectly impenetrable by insects and a good storage hygiene, play an important role in limiting infestation by *S. granarius*. The removal of infested residues from the previous season's harvest is essential as well as ensuring grain is well dried at intake. In stores or in workplace it is essential to use food, bright and sexual traps for a massive and monitoring capture.

In addition, there are known a number of natural enemies capable of predating or parasiting *S. granarius* (Tab. 1.1).

Natural enemies	Type	Life stages
Acaropsellina docta Zaher	Predator	
Anisopteromalus calandrae Howard	Parasite	Larvae
Bacillus thuringiensis thuringiensis Kurstaki	Pathogen	
Beauveria bassiana (Bals.-Criv.) Vuill	Pathogen	Adults
Cephalonomia tarsalis Ashmead	Parasite	Larvae
Choetospila elegens Westwood	Parasite	Larvae
Lariophagus distinguendus Förster	Parasite	
Lonchaea corticis Taylor	Parasite	
Pteromalus cerealellae Ashmead	Parasite	
Theocolax elegans Westwood	Parasite	Larvae

Tab. 1.1 - Known natural enemies of *S.granarius*

1.4 INERT DUSTS

1.4.1 GROUPS

There are groups of inert dusts which can be differentiated by their chemical composition or by their level of activity.

<u>Non-silica dusts</u> include katelsous (rock phosphate and ground sulphur), lime (calcium hydroxide), limestone (calcium carbonate) and common salt (sodium chloride). For example, lime is layered with maize cobs in the Philippines and is also used to protect grain in Honduras (Golob and Webley, 1980); katelsous has been tested as a carrier dust for conventional insecticides in Egypt (El Halfaway and El Attal, 1973) though it possesses insecticidal properties as well (Mostafa and Al'Moajel, 1988; Fam et al., 1974).

<u>Sand, kaolin, zeolite, paddy husk ash, wood ash and clays</u> constitute a group of materials which are used commonly by small-scale farmers in the developing world as grain protectants. Characteristically, large quantities, in excess of 5% by weight, are required for application in order to exert an effect (Golob and Webley, 1980).

In particular, Kaolin is a white, nonabrasive, nonporous, nonswelling, fine-grained aluminosilicate mineral ($Al_4Si_4O_{10}(OH)_8$) that easily disperses in water and is chemically inert over a wide pH range (Glenn and Puterka, 2005). Coating grade kaolin is >90% pure and has a high brightness quality of >85%. Mined kaolin has traces of 2 metals, Fe_2O_3 and TiO_2; the former must be removed to obtain white brightness qualities >85% that is required for various industrial applications (Harben, 1995). Technical advances within the past decade have made it possible to produce kaolin particles with specific sizes, shapes and light reflective properties. It has the environmental advantage of being an inert product and non-toxic to vertebrates (Gonzàles-Nùñez et al., 2008).

Kaolin particles are engineered with specific properties for use in the paper, paint, cosmetic, and plastic industries, but have been largely ignored by the agricultural industry. These developments have opened new possibilities for the use of mineral particles for pest control in agriculture.

The particle film coating, formed by kaolin, would serve as a physical barrier that would conceivably repel arthropods, or suppress infestations, by making the plant visually or tactually unrecognizable as a host.

Further, insect movement, feeding, and other physical activities can be severely impaired by the attachment of particles to the arthropods body; so, applied against insects, it acts primarily as physical barrier and reduces the oviposition rate of several pests. For example, in olive orchards, it is used to control *Bactrocera oleae* (Gonzàles-Nùñez et al., 2008).

Zeolite are crystalline, hydrated aluminosilicates of alkali and earth metals that possess infinite, three-dimensional crystal structures. It is further characterized by an ability to lose and gain water reversibly and to exchange some of their constituent elements without major change of structure. Natural zeolites have found applications as fillers in the paper industry, as lightweight aggregate in construction, in pozzolanic cements and concrete, as ion-exchangers in the purification of water and municipal sewage effluent, as traps for radioactive species in low-level wastewaters from nuclear facilities, in the production of high purity oxygen from air, as reforming petroleum catalysts, as acid-resistant absorbents in the drying and purification of natural gas, and in the removal of nitrogen compounds from the blood of kidney patients. Based on their high ion-exchange capacity and water retentivity, natural zeolites have been used extensively in Japan as amendments for sandy soils.

The pronounced selectivity for large cations, such as ammonium and potassium, has also been exploited in the preparation of chemical fertilizers that improve the nutrient-retention ability of the soils by promoting a slower release of these elements for uptake by plants.

Diatomaceous earths, diatomite refers to the fossilized remains of diatoms, composed mainly of amorphous hydrated silica, but also other minerals including aluminium, iron oxide, magnesium, sodium and lime. Sources of these dusts are either of marine or fresh-water origin; the former are said to be more effective. Diatomaceous earths of marine origin are effective against storage insects at about 0.1% (w/w), but commercially available products are often enhanced by other compounds, e.g. ammonium fluorosilicate. Other diatomaceous earths usually contain about 90% SiO_2 but very high quality synthetic silicates and precipitated silicas are now manufactured, which have a SiO_2, content of 98% or more. These purified compounds are both dense and expensive and have many industrial applications, including as anti-caking and free-flow agents, but not as grain protectants.

Silica aerogels are produced by drying aqueous solutions of sodium silicate. They are very light, hydrophobic powders which are effective at lower rates than diatomaceous earths. The very low dust density has prevented the widespread application of these materials in the past because of the potential hazards which would occur as a result of inhalation.

1.4.2 APPLICATIONS IN AGRICULTURE

In recent years, awareness of the consequences of environmental pollution, the increasing cost of storage insecticides and the growing problem of insect resistance has led to pest management specialists reappraising inert dusts. Unlike conventional contact insecticides, inert dusts function through their physical properties and are generally slower acting (Maceljski and Korunic, 1972). Insect mortality is induced primarily as desiccation: water loss is a consequence of the destruction of the cuticle. Silica aerogels adsorb the waxy particles from the cuticle surface (Maceljski and Korunic, 1972; Le Patourel et al., 1989) and although diatomaceous earths, having small dense particles of silicon dioxide, were said to abrade the cuticle (Alexander et al., 1944), they also function by adsorption of wax (Ebeling, 1971).

The action of these materials is not dependent on metabolic pathways, it has been postulated that insects will not be selected genetically by the action of these dusts, so that physiological resistance will not occur. Nevertheless, it may be possible for insects to develop a behavioural response to the dust and avoid contact (Ebeling, 1971).

Another advantage over conventional insecticides is the low mammalian toxicity of these materials (Subramanyam et al., 1994); in the USA, diatomaceous earths are 'Generally Recognised as Safe' by the US Food and Drug Administration and are registered for use as food additives (Banks and Fields, 1995).

In antiquity, elemental sulfur or sulfur compounds mixed with bitumen were heated to produce fumes that repelled insects from vines and trees (Smith and Secoy, 1975; 1976). Diatomaceous earth (diatomite), was applied to plants and structures for pest control in China as early as 2000 B.C.E. (Allen, 1972). Toxic preparations of arsenic and arsenic salts were used around 900 C.E.in China and incorporated in Europe in 1699 (Cassida and Quistad, 1998). Powdered limestone (calcium carbonate) was added to grain to deter insects in the 1st century.

One of the primary insecticides and fungicides of early agriculture, dating to the Hellenistic Era, was the mixture of hydrated lime [$Ca(OH)_2$] with sulfur (S) (Secoy and Smith, 1983).

Chemically reactive hydrated lime and sulfur were applied independently or together in mixtures with a range of other materials such as tobacco, wood ash, linseed oil, soap, and cow dung. These concoctions were applied as paints or washes to fruit trees and grape vines to protect them from insect and disease damage.

From the late 1500s to the 1800s, slaked lime (calcium hydroxide) and burned lime (calcium oxide) were used against household, stored grain, and crop insect pests.

The discovery of the insecticidal properties of the pigment Paris green in 1897 marked the beginning of the modern use of insecticides (Little, 1972). The bright green powder, prepared by combining copper acetate and arsenic trioxide to form copper acetoarsenite, was extremely poisonous and had to be made and used with caution. The minerals schultenite (lead arsenate) was first prepared as an insecticide and used against the gypsy moth in 1892 and was a widely used general insecticide for crops up to 1940, when it was replaced with the synthetic insecticide, diclorodiphenyltrichloroethane (DDT) (Peryea, 1998). Inorganic chemists were unknowingly synthesizing chemical compounds such as hexachlorocyclohexane during the early 1800s that were later found to be insecticidal in 1942 (Cassida and Quistad, 1998). The discovery of this and other insecticidal compounds such as tetrahethylthiuram disulfide (Guy, 1936) and DDT in 1939 (Cassida and Quistad, 1998) spurred a major exploration into inert mineral carriers. Lead arsenate, sulfur, nicotine, and hydrated lime, alone or in mixtures, were still the predominant insecticidal materials used in agriculture in the early 1900s. During the first quarter of the 20th century few other insecticidal materials were used and pesticide delivery was also in its infancy.

Dust applications gained favor over liquid sprays in the 1920s because of the speed of dusting operations, economy in labor, good plant coverage, and comparable insect control with liquid sprays (Giddings, 1921; Headly, 1921; Parrott, 1921). Other research that increased interest in using dusts to deliver insecticides proposed that chemically active particles of sodium fluoride and borax (Shafer, 1915) and toxin impregnated minerals (Marcovitch, 1925; Mote et al., 1926) reacted with the insect cuticle and caused a "self-cleaning" response due to the irritation, and, in the process, insects ingested particles and died. Particle ingestion led to a more rapid killing action by insecticide-laced dusts than by the insecticide (lead arsenate) alone (Mote et al., 1926).

Research in the 1930s established that certain inert dusts alone had toxic activity against insects when ingested during the process of selfcleaning (Boyce, 1932; Richardson and Glover, 1932).

Suffocation by inhalation was not an important factor, and it was found that the inert dust itself had a desiccating action (Hockenyos, 1933). This highly significant observation would later become regarded as one of the major mechanisms of how dusts kill insects.

A number of "so-called inert materials" caused high mortalities of stored grain weevils by desiccation (Chiu, 1939) that summarized the modes-of-action of inert materials as:

(1) ingestion of the dust into the digestive system (Boyce, 1932; Richardson and Glover, 1932),

(2) desiccation (Zacker and Kunike, 1931; Hockenyos, 1933),

(3) chemical reaction with the body wall of the insect (Shafer, 1915),

(4) direct mechanical action (Germar, 1936).

Another important discovery related to mechanisms was that as particle size decreased from 37.0 to 2.9 µm in diameter, insect mortality increased (Chiu 1939, a-b). Research in the 1930s brought about the realization that fine mineral dusts were misclassified by insect physiologists and that inert dusts had many unexpected properties in relation to insects (Briscoe, 1943) that established that mortalities by dust ingestion and suffocation were negligible in grain weevils and that dusts increased water transmission through the insect's cuticle causing desiccation.

Insect mortalities increased as particle size decreased and as intrinsic hardness of the materials increased. The mechanisms of how particles caused desiccation of insects was finally attributed to either their adsorption of the epicuticular waxes of the cuticle or abrasion of the cuticle (Kalmus, 1944; Wigglesworth, 1944).

However, if absorption was a factor, many researchers believed it must be augmented by cuticular abrasion for cause desiccation in most insects (Beament, 1945; Wigglesworth, 1944; Hurst, 1948). While many researchers had focused efforts on determining the mechanisms of how inert dusts killed pest insects (Beament, 1945; Kalmus, 1944; Wigglesworth, 1944; Hunt, 1947; Hurst, 1948), others had noticed that inert dusts affected insects in different ways and could actually cause increases in pest infestations (Callenbach, 1940; Flanders, 1941; Halloway et al., 1942; Halloway and Young, 1943). Crops coated with dusts from dirt roads or intentional dust applications exhibited increased levels of codling moth, *Cydia pomonella* (L.) (Callenbach, 1940), Citrus red mite, *Panonychus citri* (McGreggor) (Halloway et al. 1942), and purple scale, *Lepidosaphes beckii* (Newman) (Halloway and Young 1943).

Flanders (1941) proposed that the pest increases were a result of dusts interfering with the efficacy of natural enemies. The efficacy of natural enemies was influenced by dusts via at least four mechanisms:

(1) dusts impeded movement of legs and mouthparts (Germar, 1936),

(2) dusts invoked the self-cleaning response (Marcovitch, 1925; Mote et al., 1926),

(3) the mineral film presented a physical barrier to natural enemy attack (Driggers, 1928),

(4) dusts caused direct mortality of natural enemies (Zacker and Kunike, 1931).

Insecticidal dusts were the primary means of delivering insecticides in the 1940s and interest in the toxicity of mineral dust diluents established the need to better classify these diluents. Watkins and Norton (1947) found diluents and carriers fell into two basic categories, botanical flours (e.g., walnut shell flour) and minerals (e.g., attapulgite). A cornerstone study by David and Gardiner (1950) on the physical properties of dust carriers for insecticides summarized that particle size, shape, specific gravity, bulk density, surface area, hardness, and moisture relations were all factors that affected the

toxicity of dusts alone or in combination with DDT. These results were confirmed by Alexander et al. (1944a), who established that abrasive dusts with sharp angular structure caused insects to die from desiccation most rapidly and that low mortalities were associated with high humidities.

Abrasive dusts like alumina-aluminum oxide (Al_2O_3) or silica oxide (SiO_2) were the best carriers for DDT (Watkins and Norton, 1947).

After World War II, the development of synthetic pesticides superceded the use of minerals in the control of plant pests. Despite the common usage of synthetic pesticides, diatomaceous earth (suh as Celite®), wettable sulfur, and hydrated lime are still used as insecticides in some crops.

Was found that nonabrasive sorptive dusts like montmorillonite and attapulgite removed the thin lipid layer covering the epicutical of dry wood termites, *Incistermes minor* (Hagan) (Ebling and Wagner, 1959). Sorptive-dust treated termites died from desiccation more rapidly than through contact with insecticides like parathion. Certain silica aerogels (synthetic oxides of silicon), especially those impregnated with fluoride, were more lethal than mineral dusts at high humidities (Ebling and Wagner, 1959).

Stored grain pests such as the rice weevil, *S. oryzae*, household pests such as the western drywood termite, or american cockroach, *Periplanta americana* (L.), and ectoparasites affecting livestock such as the northern fowl mite, *Ornithonyssus sylvarium* (Can. and Fan.), were ideally suited for control by sorptive dusts (Ebeling, 1971).

Interest in the control of insects with inert dusts transitioned from minerals to synthetic compounds like silica aerogels and fumed silicas by 1970. Although dusts for insect control may have had the greatest potential for the pest control needs of the grain industry, inexpensive fumigants became widely used instead. Much of the research on mineral particles after 1970 was limited to pesticide formulations where mineral particles were used as carriers for synthetic insecticides (Kirkpatrick and Gillenwater, 1981; Margulies et al. 1992) or microbial agents (Studdert et al., 1990; Tapp and Stotzky, 1995) and in the use of minerals as whitewash sprays for preventing plant virus diseases that were vectored by aphids (Moore et al., 1965; Johnson et al., 1967; Adlerz and Everett, 1968; Bar-Joseph and Frenkel, 1983) and thrips (Smith et al. 1972).

Various studies on the efficacy of inert dusts have been reported in literature. Soil dusts have long been used as insect repellents by primitive people, mammals, and birds that took "dust baths" regularly to ward off biting insects (Ebeling, 1971).

Actualy the clay powders used in agricolture are: kaolin, bentonite, zeolite and attapulgite.

Kaolin has found application for crop protection and pest control also in the open field (Glenn et al., 1999). It is usually mixed with water and sprayed on the leaves of the fruit trees to combat and protect

plants from damage by water stress and by insects. The kaolin form a non-abrasive barrier which is marked repellent action and carry out irritating against a large number of insects (Glenn et al., 1999; Knight et al., 2000; Puterka et al., 2000; Unruh et al., 2000).

Bentonite powder has been shown to as a adhesive properties, a carrier of other products (copper sulfate) and for its drying capacity creates an environment unsuitable for the development of cryptogamic and insects, also thanks to its alkaline reaction. The application is very useful in the presence of damp and cold environments, where it performs the dehydrating and drying activities, thus reducing the possibility of the development of fungal hyphae. The bentonite can also be used in the compost, in the cultivation of molds, in the production of pulp by brushing to the trunks, as well as in biodynamic preparations.

Based on their high ion-exchange capacity and water retentivity, natural zeolites have been used extensively in Japan as amendments for sandy soils. The selectivity of clinoptilolite, the most common element of the natural zeolite for large cations, such as ammonium and potassium, has also been exploited in the preparation of chemical fertilizers that improve the nutrient-retention ability of the soils by promoting a slower release of these elements for uptake by plants. The addition of zeolite, therefore, resulted in a marked improvement in the soil's ammonium retentivity. Although additions of both montmorillonite and mordenite increase the cation-exchange capacity of upland soils, the greater stability of the zeolite to weathering allowed this increase to be retained for a much longer period of time than in the clay-enriched soils. The high adsorption capacities in the dehydrated state and the high ion-exchange capacities of many natural zeolites make them effective carriers of herbicides, fungicides, and pesticides.

Attapulgite is used in agriculture, generally in a formulated granular. This vector is extremely porous and develops an exceptional surface, highly absorbent, able to incorporate a substantial amount of toxic substances. The active substance is retained safe from a rapid environmental degradation and is released slowly. This helps to protect longer the crops from the attack of the harmful organisms with the same amount of active ingredient.

Attapulgite-based clay dust was shown to control *Corcyra cephalonica* (Stainton), *T. castaneum* and *Caryedon serratus* (Olivier) when applied to groundnuts at 0.5% (w/w) (Mittal and Wightman, 1989) and *Callosobruchus chinensis* (L.), *S. oryzae*, *Oryzaephilus surinamensis* (L.), *R. dominica* and *Lasioderma serricorne* (F.).

2. AIM OF THE WORK

The goal of this study was to carry out a complete study on the biological activity of EO obtained by vapour steam distillation, from inflorescences of *L. angustifolia* grown in the eastern side of the Italian Apennines.

EOs present wide variability according to areal of distribution, growing methods and soil properties, for this reason we considered appropriate to chemically characterize its by gaschromatography coupled with mass spectrometry (GC-MS).

Biological activities of EO were evaluated by several bioassays to determine contact and fumigant toxicities, repellent, antifeedant and nutritional effects against insect.

As insect test we used the granary weevil, *S. granarius*, one of the most damaging pest of stored cereals worldwide that causes major quantitative and qualitative losses by its feeding activity and excretory products.

Furthermore, to preserve its promising properties it was identified and assessed a suitable mineral substrate to absorb and release EO, in order to improve and maintain the activity observed against *S. granarius* useful for pratical application.

3. MATERIALS AND METHODS

3.1. PLANT MATERIAL

Flower spikes of *L. angustifolia* were collected from plants grown in the garden of the University of Molise (Campobasso, south-central Italy) located at 650 m a.s.l. in the eastern side of the Apennines watershed (Fig. 3.1). Overall, weather conditions reflected the specific orographic position (distance from the sea, Eat-West appearance, elevation above the sea level) of the experimental site. The area has an average annual rainfall of 700 mm, and mean annual temperature of 14.9 °C. The soil is characterized by a clay texture and the organic matter content was 1.2%. The soil profile was overall uniform, containing medium amount of total N (nitrogen, 0.11%), low amount of available P (phosphorous, 11.5 µg/g) and medium quantity of exchangeable K (potassium, 133 µg/g).

Soil had very low active $CaCO_3$, and pH was average neutral; salinity was low.

After ploughing (30 cm depth), 70 kg P/ha, 70 kg K_2O/ha and 60 kg N/ha were applied. Planting of rainfed lavender was done at 2 plants/m^2 (Delfine, 2009). The field was surrounded by a buffer strip to allow for uniform growing conditions. Weeds were manually controlled. Yield values were based on a hand-made harvesting. The dry flower spikes were obtained after oven drying at 35 °C for 72 h.

Fig. 3.1 - Lavender plant grown in the garden of the University of Molise

3.2. EXTRACTION

The flower spikes (500 g) of *L. angustifolia* samples (n = 3) were hydrodistilled for 3 h using a Clevenger-type apparatus (Fig. 3.2) according to the method recommended in the current *European Pharmacopoeia* (2010). The oils were combined and stored under N_2 at +4°C in the dark until tested and analysed. The EO density was 0.8981 g/L.

EO
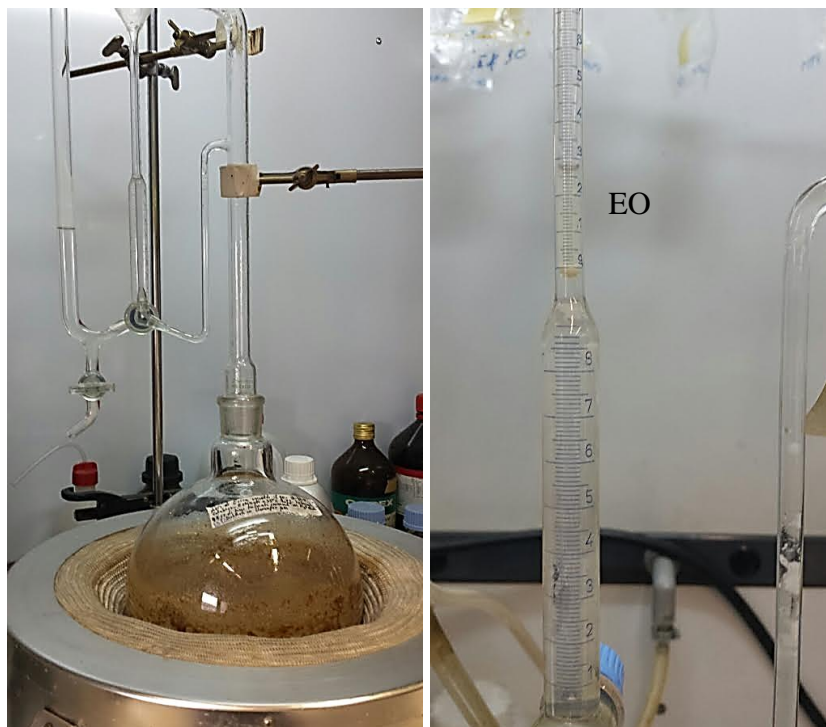
Fig. 3.2 - Clevenger-type apparatus used for collected EO (left). Particular of EO in condenser (right).

3.3 CHEMICAL ANALYSIS

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) - The oil was diluted 1:100 with dichloromethane-hexane (2:3) and a 2 µL sample was injected in the GC system. A 6890N series gas chromatograph (Agilent Technologies) with an Agilent 5973 mass selective detector (MSD) and equipped with a HP-INNOWAX capillary column (60m x 0.25mm I.D, 0.25µm film thickness, J&W Scientific Inc., Folsom, USA) was used. The carrier gas was helium at a flow rate of 1.0 mL/min. The injection was made in the splitless mode, the injector temperature was 250°C. The column oven temperature was initially held at 40°C, then it was programmed to 230°C at 2.5°C/min, with a final holding time of 20 min. Spectra were recorded in the electron impact mode (ionization energy, 70eV) in a range of 30–500 amu at 3.2 scans/s. A solvent delay time of 10 min was used to avoid overloading

the mass spectrometer with solvent. The identification of the volatile compounds was achieved by comparing mass spectra with those of the data system library (NIST 98, P>90%) and retention indexes with published data. Component relative percentages were calculated based on GC peak areas.

3.4. INSECT

Sitophilus granarius were reared on wheat grains for several generations (Fig. 3.3), in glass cylindrical containers (Ø 15x15 cm) closed by metallic net (1 mm) and maintained in the dark a climatic chamber set at at 25±2 °C and 60±5% r.h. Adult beetles, 2-4 weeks old, were used for the experiments.

Fig. 3.3 - Grain infested by *S. granarius*

3.5. BIOASSAY TO EVALUATE ACTIVITY OF EO

3.5.1 CONTACT TOXICITY

The contact toxicity of lavender EO to granary weevil adults was determined by topical application. The EO was dissolved in acetone to obtain two-fold serial dilutions from 898.1 to 56.13 µg/µL.

A 0.5 µL droplet of an EO solution was applied onto the pronotum of an adult weevil in thanatosis using a Hamilton's syringe (700 series, MicroliterTM Hamilton Company, USA) (Fig. 3.4). For each EO solution, 60 insects divided in 12 replicates were used. Concentrations were expressed as µg of EO per adult (average adult weight 1.98±0.02 mg). Insects treated with acetone alone were used as control. After topical application, the insects were confined in a Petri dish within a metal ring (Ø 4.0 x 2.5 cm) covered with metallic net (mesh 1 mm) to prevent insects escape, provided with 5 wheat kernels and maintained in the dark at 26±2°C and 60±5% r.h. The number of dead insects was recorded after 24 and 48 h. The percentage mortalities were transformed to arcsine square-root values for repeated measures analysis of variance (ANOVA). Treatment means were compared and separated by Tukey HSD test. The Lethal dose 50 (LD_{50}) and 90 (LD_{90}) values, the confidence upper and lower limits, regression equations and chi-square (χ^2) values were calculated using probit analysis (Finney, 1971).

Fig. 3.4 - Bioassay of contact toxicity. Topical application (left). Petri dishes containing kernels of grain and treated *S. granarius* adults (right)

3.5.2 FUMIGANT TOXICITY

The fumigant toxicity of lavender EO to granary weevil adults in the absence and in the presence of wheat (*Triticum durum* var. Simeto) grains was assessed using the method described in previous studies (Germinara et al., 2007; Germinara et al., 2012a). A glass container (600 mL) was used as a fumigation chamber. A filter paper (Whatman No. 1) disc (Ø 2.0 cm) was suspended in the centre of the chamber by an iron wire attached to the under surface of its aluminium screw cap (Fig. 3.5). Twenty adult insects were placed in the chamber, the paper disc treated with an appropriate volume of lavender EO and the glass container tightly closed. In tests with wheat grains, intact kernels (100

g) were placed on the base of the fumigation chamber together with the insects. Test doses were volumes of EO yielding concentrations of 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 mg/L volume, respectively. An untreated paper disc was used as a control. Five replicates of each dose and the control were set up. Bioassays were carried out in the dark at 26 ± 2 °C and $60\pm5\%$ r.h. for 24 h. Dead insects were counted after exposure to fresh air in Petri dishes for 12 h. This allowed for recovery of insects immobilized and apparently dead immediately after exposure to the EO.

The percentage mortalities were submitted to two-way ANOVA with substrate presence or absence and dose as the two subjects factors. For each set of experiments, treatment means were separated by Tukey's HSD test. The LC_{50} and LC_{90} values, expressed as mg EO/L volume, the confidence limit of upper and lower confidence levels, regression equations and chi-square (χ^2) values were calculated by probit analysis (Finney, 1971).

Fig. 3.5 - Glass containers used as a fumigation chambers

3.5.3 REPELLENCY ON FILTER PAPER DISC

Repellence activity of lavender EO was evaluated using the area preference method (McDonald et al., 1970). A filter paper disc (Whatman No. 1, Ø 8.0 cm, area = 54.4 cm^2) was divided in half. One half was treated as uniformly as possible with 500 µL of an EO acetone solution using a micropipette and the other half was treated with an equal volume of acetone used as control. Both treated and control halves where air-dried for about 10 min to allow complete solvent evaporation, joined with transparent adhesive tape and the full disc fixed on the bottom of a Petri dish (Ø 9.0 cm). Ten granary weevil adults were confined to each filter paper disc within a metal O-ring (Ø 8.0 x 4.0 cm) covered with metallic net (mesh 1 mm) to prevent insect escape (fig. 3.6). The experiment was run in the dark

at $26\pm2°C$ and $60\pm5\%$ r.h. Seven EO acetone solutions were tested corresponding to the doses of 0.055, 0.110, 0.221, 0.441, 0.883, 1.765 and 3.531mg/cm^2, respectively. Each bioassay was replicated 4 times. The number of weevils on the treated (N_t) and control (N_c) portion of paper disc was recorded at 30-min intervals during the first 2 h.

Percentage repellency (PR) values were calculated as follows:

$PR = (N_c-N_t)/(N_c+N_t) * 100$

Positive PR values indicate repellency whereas negative values indicate attraction. For each test dose, the mean PR value was calculated and assigned to repellency classes from 0 to V (Talukder and Howse, 1993): class 0 (PR < 0.1%), class I (PR = 0.1 - 20%), class II (PR = 20.1 - 40%), class III (PR = 40.1 - 60%), class IV (PR = 60.1 - 80%), class V (PR = 80.1 - 100%). PR values were submitted to repeated measures analysis of variance (ANOVA). For each exposure time, mean PR values were separated by Tukey's HSD test.
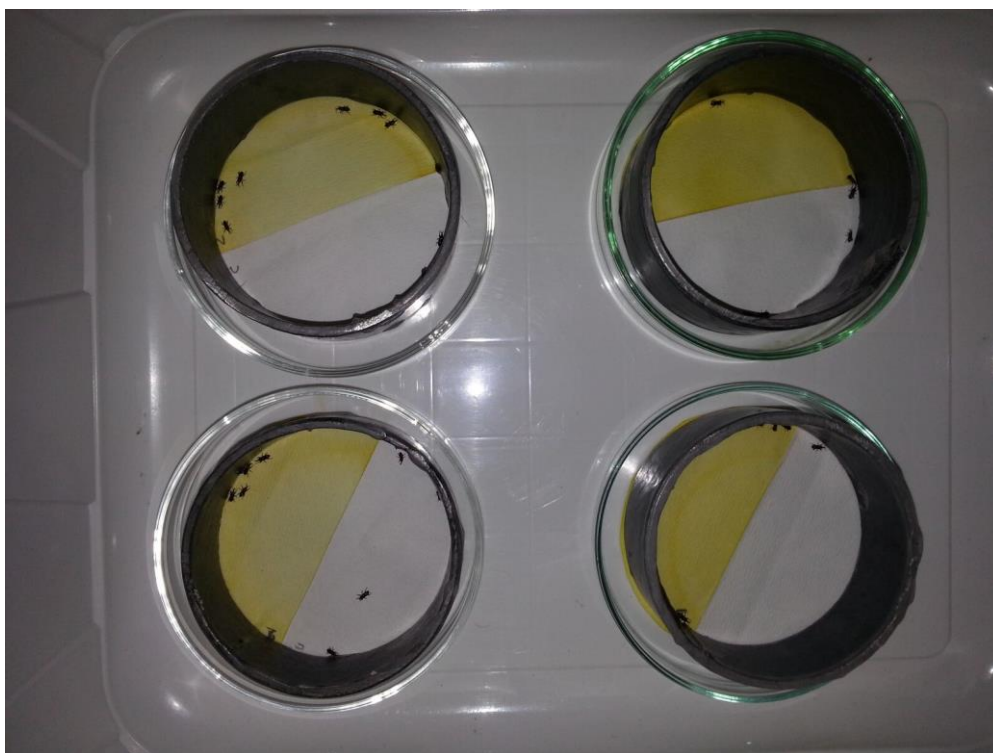

Fig. 3.6 - Repellence activity on filter paper disc

3.5.4 REPELLENCY IN ARENA

The repellent activity of different lavender EO solutions to granary weevil adults and their ability to disrupt insect orientation to odors of wheat grains were evaluated in a two-choice pit-fall bioassay similar to that described in previous study (Germinara et al., 2008). The test arena was a steel container (Ø 32 cm × 7 cm height) with two diametrically opposed holes (Ø 3 cm) located 3 cm from the side wall. A filter paper disc (Ø 0.7 cm) was suspended at the center of each hole by a cotton wire taped to the lower surface of the arena. Glass flasks (500 mL), assigned to collect the responding insects, were positioned under each hole. The inside necks of the collection flasks were coated with mineral oil to prevent insects from returning to the arena. Thirty insects of mixed sex, left for at least 4 h without food, were placed under an inverted Petri dish (Ø 3 cm x 1.2 cm high) at the center of the arena and allowed 30 min to acclimate prior to release. During the assay, the arena was covered with a steel lid to prevent insects from escaping (Fig. 3.7).

In a first set of experiments, insects were presented with a given dose of EO (10 µL of an acetone solution) adsorbed onto a filter paper disc and acetone (10 µL) adsorbed onto the opposed paper disc as control. In a second set of experiments, insects were given a choice between the odors emitted by wheat grains (200 g; 14.5% moisture content) left in a collection flask alone or plus a set dose of EO (10 µL of acetone solution), adsorbed onto the overlying filter paper disc, and acetone (10 µL) adsorbed onto the opposed paper disc as control. In both set of experiments five doses (0.561, 1.122, 2.245, 4.490, 8.981 mg) of lavender EO were assessed. Tests lasted 3 h and were carried out in the dark at 26±2 °C and 60±5% r.h.

There were five replicates of each assay, and insects were only used once.

In each experiment, a response index (RI) was calculated by using $RI = [(T - C)/Tot] \times 100$, where T is the number responding to the treatment, C is the number responding to the control and Tot is the total number of insects released (Phillips et al., 1993). For each bioassay, the mean numbers of insects in the treatment and control were compared by Student's *t*-test for paired comparisons. The mean numbers of insects found in the treatment and in the control and the mean RIs at different doses of EO alone and in the presence of wheat grain odors were subjected to ANOVA and ranked according to Tukey's HSD test.
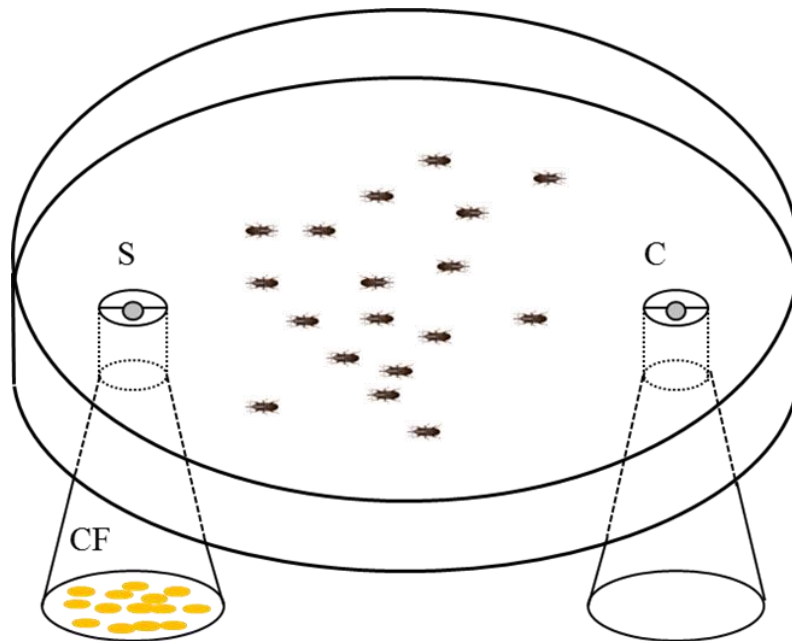
Fig. 3.7 - Repellence bioassay in arena, where S = stimulus, C = control, CF = wheat kernels

3.5.5 ANTIFEEDANT AND NUTRITIONAL EFFECTS

Effects of lavender EO on the feeding activity and nutrition of granary weevil adults were evaluated by the flour disk bioassay (Xie et al., 1996). Wheat flour (10 g) was uniformly suspended in distilled water (50 mL) by stirring. To obtain flour disks, aliquots (200 µL) of suspension were dropped onto a plastic Petri dish and left overnight at 26±2°C and 60±5% r.h. to dry.

Disks were treated with EO acetone solutions (5 µL) corresponding to different concentrations (4.490, 2.245, 1.125, 0.563, 0.281 mg/disk) or acetone alone as control. Disks were held at room temperature for 2 h for solvent evaporation. In a pre-weighed glass vial (Ø 2.5 x 4.0 cm) two flour disks and 10 group-weighed weevil adults were introduced. Each vial was then re-weighed and maintained at 26±2°C, 60±5% r.h. for 3 days. The glass vials with flour disks and live insects were weighed again and the number of dead insects recorded. Glass vials containing treated flour disks but without insects were prepared to determine any decrease in weights due to evaporation of acetone and essential oil. For each EO concentration and control 5 replicates were set up (Fig. 3.8).

The following nutritional indices were calculated: relative growth rate (RGR) = (A - B)/(B x day), where A = mean weight (mg) of live insects on third day, B = original mean weight (mg) of insects; relative consumption rate (RCR) = D/(B x day), where D = biomass ingested (mg)/ no. of living insects on the third day; efficiency conversion of ingested food (ECI) = (RGR/RCR) x 100; feeding

deterrence index (FDI) (%) = [(C-T)/C] x 100, where C = consumption of control disks and T = consumption of treated disks (Farrar et al., 1989; Huang and Ho, 1998).

Data were submitted to ANOVA followed by Tukey's HDS test for mean comparisons. Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences) v.10.0.7 for Windows (SPSS Inc., Chicago, IL).
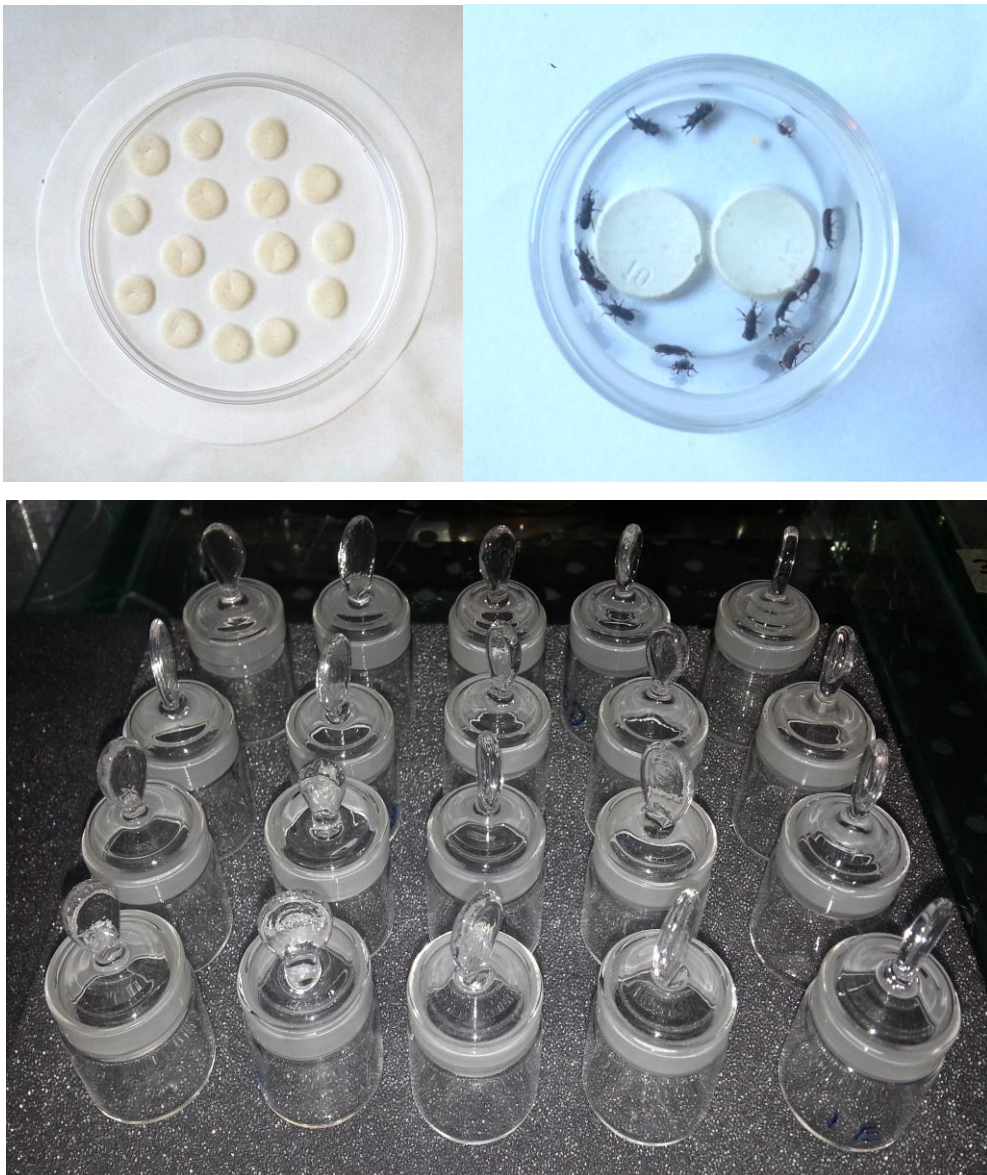
Fig.3.8 - Ingestion bioassay. Dried flour discs (top left); weighs glass filter containing two dried flour discs and adults of *S. granarius* (top right); weighs glass filter (bottom)

3.6. FORMULATION

3.6.1 AROMATIZAZION OF POWDERS

Metod 1 - Commercial Kaolin (2-4 µm, purity >98%, containing 950 g/kg of kaolin) was obtained by Progetto Geovita S.a.s. (Scanzano Ionico, Italy) and Zeolite (5-10 µm) from Lazio (Italy).

Powders were dried in dessicator with silica gel for one night. An aliquot of each powder (2g) was placed in flask (50 mL) and 200 µL EO, dissolved in 5 mL of acetone. The mixture was homogenized with a vortex for 2 h and the pH was recorded (5.50). The solvent was aspirated by rotavapor (30°C). The flavoured powders placed in the flask, (Fig. 3.9)wrapped with alluminium foil, were kept cool until use (Gueye et al., 2012).
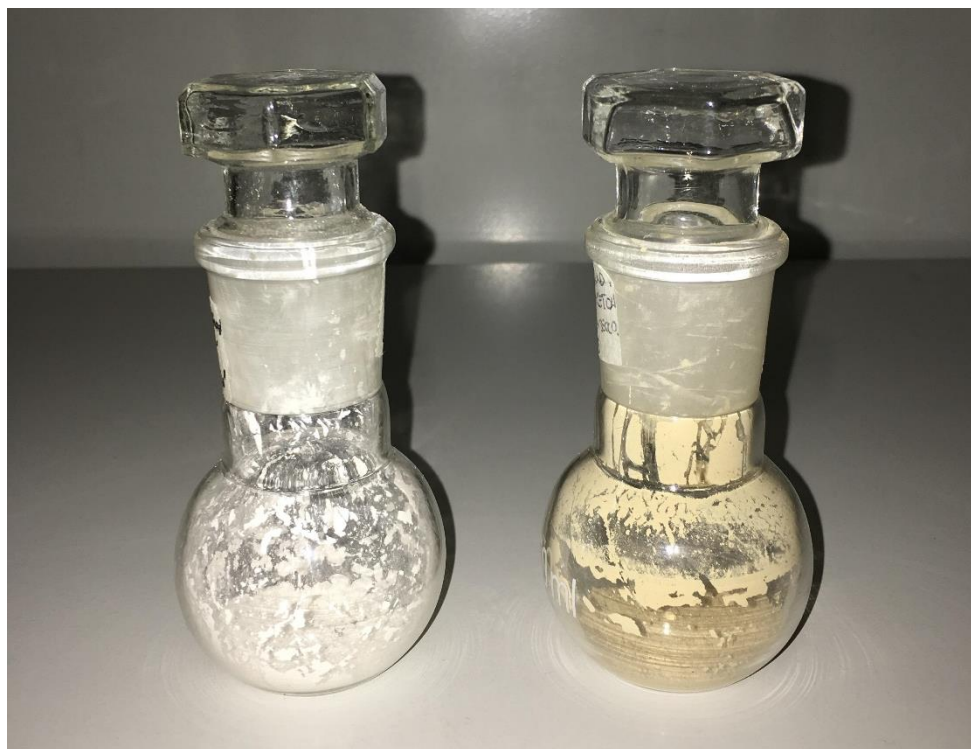
Fig. 3.9 - Flask with aromatized Kaolin (left) and Zeolite (right)

Metod 2 - Kaolin was dried in dessicator with silica gel for one night.

An aliquot of the dried powder (2 g) was placed in flask (50 mL) and mixed with different doses of lavender OE (50-100-200 µL dissolved in 5 mL of acetone).

The mixture was homogenized with a vortex for 2 h and the pH was recorded (5.50) and was centrifuged (2000 rpm for 15 min), the supernatant was removed (Nguemtchouin et al., 2009) and

aromatized powder has been subjected to a stream of N_2 (30"). The formulations obtained were stored until use in the dark and refrigerated at 4° C.

3.7 BIOASSAY TO EVALUATE ACTIVITY OF FORMULATION

3.7.1 PETRI DISH

The test was performed in two ways; in plastic Petri dish (Ø 5 cm) covered by lid with hole (Ø 2 cm) closed by a plastic net or without hole sealed with parafilm. *S. granarius* adults (n. 10) were placed in dish with a layer of flavoured or unflavoured powders (0.02 g) (Fig. 3.10) and untreated dishes was used as controls. Mortality was recorded every 24 h for 4 days to treatment.

The percentage mortalities, for the different powders formulation, were subjected to analysis of variance (ANOVA). Treatment means were compared and separated by the LSD test (P= 0.05).
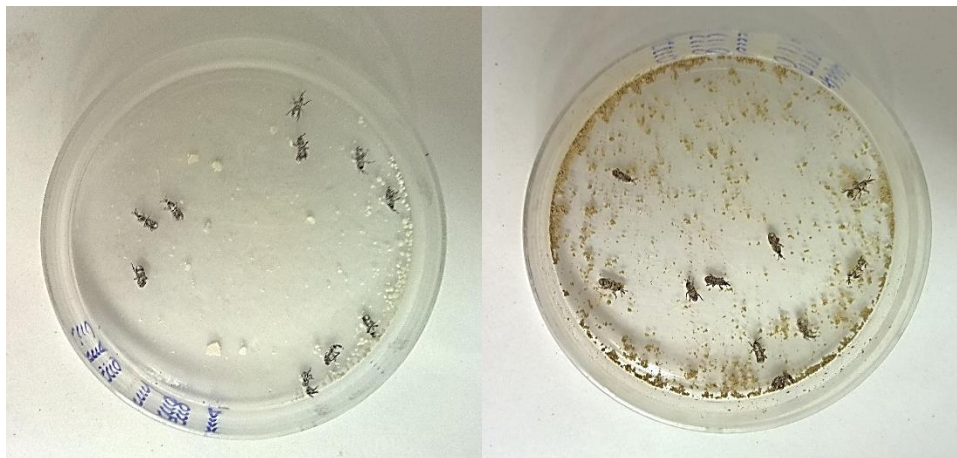
Fig. 3.10 - *S. granarius* mortality in Petri dish bioassay with aromatized Kaolin (left) and Zeolite (right)

3.7.2 FLASK

The toxicity test with different formulations was conducted in 500 ml flasks. The grain (200 g) was placed in a flask and treated with different amounts of kaolin powder (100, 250, 500 mg) and / or flavored with different concentrations of OE (2.5-10-15%). The flask was covered with a Petri dish (Ø 5 cm) with a hole (Ø 2 cm) closed by a plastic net (0.2 mm) (Fig. 3.11).

Adults of *S. granarius* (n. 30 x 4), placed into the flask, were monitored every day for 5 days and was recorded dead individuals. The bioassay was performed in a room with temperature and humidity controlled (26±2 °C; 60±5 UR).

The percentage mortalities, for the different doses of EO formulation, were subjected to analysis of variance (ANOVA). Treatment means were compared and separated by Tukey HSD test (P= 0.05).

Fig. 3.11 - Flask with treated (left) and untreated grain (right)

3.7.3 ARENA

The repellency test was developed in a two-choice olfactometer as already described (Fig. 3.7).

Glass flasks (500 mL), assigned to collect the responding insects, were positioned under each hole; an empty flask represented the control, while the other contained grain (200 g) treated with different percentage of kaolin (100, 250 and 500 mg) and / or flavored with OE (10, 15%).

Thirty insects of mixed sex, left for at least 4 h without food, were placed under an inverted Petri dish (Ø 3 cm x 1.2 cm high) at the center of the arena and allowed 30 min to acclimate prior to release.

During the assay, the arena was covered with a steel lid to prevent insects from escaping.

There were five replicates of each assay; insects were used once.

Every day the bioassay was rearranged and were used new adults.

In each experiment, a response index (RI) was calculated by using RI = [(T - C)/Tot] × 100,

where T is the number responding to the treatment, C is the number responding to the control and Tot is the total number of insects released (Phillips et al., 1993). The IR values can vary from -100, for a total repellency, +100 if there is total attraction.

The mean numbers of insects found in the treatment and in the control, for the different doses of EO formulation, were subjected to ANOVA and ranked according to Tukey's HSD test (P=0.05).

4. RESULTS

4.1. EO COMPOSITION

The flower spike EO of L. angustifolia accession studied contains noticeable percentages of linalool (23.8%), 1,8-cineole (12.0%), borneol (10.7%), terpinen-4-ol (10.0%), linalyl acetate (6.9%), (E)-β - ocimene (6.2%), (E)-β-farnesene (3.5%), and camphor (2.8%) (Tab. 4.1). Overall, 53 constituents were identified accounting for 98.3% of the whole EO.

Compound	R.T.	%
Tricyclene	10.80	0.04
α-Pinene	11.35	1.33
α-Thuiene	11.45	0.33
Camphene	13.00	0.72
β-Pinene	14.77	1.29
β -Phellandrene	15.28	0.46
3-Carene	16.57	0.50
β-Myrcene	17.19	0.89
α -Phellandrene	17.32	0.06
3-Hexenol	18.80	0.01
D-Limonene	19.11	1.92
1,8-Cineole	19.97	11.97
(E)-β-Ocimene	20.93	6.16
γ-Terpinene	21.44	0.28
(Z)- β-Ocimene	21.62	0,75
3-Octanone	21.81	0.05
o-Cymene	22.63	1.30
Terpinolene	23.00	0.03
(+)-4-Carene	23.31	0.37
Hexyl-iso-butyrate	26.28	0.53
Allo-Ocimene	27.89	0.42
(Z)-3-Hexen-1-ol	28.48	0.01
p-Cymen-7-ol	29.28	0.04
Hexyl butyrate	30.07	1.50
Hexyl-2-methyl butyrate	30.97	0.72
1-Octen-3-ol	31.86	1.36
(Z)-β-Terpineol	32.76	0.35
(Z)-Linalool oxide	33.06	0.14
Camphor	35.51	2.84
β-Bourbonene	36.13	0.05
Linalool	36.93	23.76
Linalyl acetate	37.74	6.90
(Z)-α-Bergamotene	38.18	0.03
(-)-α-Santalene	38.39	0.25
Bornyl acetate	38.67	0.33
Terpinen-4-ol	39.73	10.00

(Z)-β-Farnesene	42.24	0.74
(E)-β-Farnesene	42.57	3.46
Lavandulol	42.88	2.14
Borneol	44.44	10.72
Germacrene D	44.79	1.15
Geranyl acetate	44.97	0.11
Geraniol butyrate	45.76	0.11
Lavandulyl acetate	46.34	1.04
Nerol	48.32	0.12
Carveol	49.83	0.04
p-Cymen-8-ol	50.11	0.06
Geranyl acetate	50.28	0.17
3,7-Octadiene-2,6-diol, 2,6-dimethyl-	54.30	0.10
Caryophyllene oxide	56.27	0.35
p-Cymene-7-ol	60.51	0.06
Carvacrol	63.43	0.02
α-Bisabolol	64.77	0.34
Others		1.73
Total		**100.00**

Tab. 4.1 - Chemical composition of EO obtained from *L. angustifolia* flower spikes (R.T. = retention time in minutes).

4.2. EO BIOLOGICAL ACTIVITY

4.2.1 CONTACT TOXICITY

The contact toxicity of lavender EO by topical application significantly increased with dose and exposure time increase (Tab. 4.2). The interaction dose x exposure time was not significant at $P = 0.05$ level. At the highest dose, adult mortality reached 91.7 and 100% after 24 and 48 h exposure, respectively (Tab. 4.3). LD_{50} and LD_{90} values were 83.8 and 379.7 µg/adult after 24 h and respectively decreased to 58.3 and 208.3 µg/adult after 48 h (Tab. 4.3).

Source	df	Type III SS	Mean square	F-value	p-value
Dose	5	148266.667	29653.33	83.781	< 0.001
Error	55	19466.667	353.939		
Exposure time	1	2844.444	2844.444	17.17	0.002
Error	11	1822.222	165.657		
Dose x exposure time	5	155.556	31.111	0.538	0.746
Error	55	3177.778	57.778		

Tab. 4.2 - Repeated measures analysis of variance between subjects effects for the contact toxicity of *L. angustifolia* EO against *S. granarius* adults at the doses of 449.05, 224.52, 112.26, 56.13, 28.06 µg/adult after 24 and 48 h exposure, respectively.

Exposure time (h)	Dose (µg/adult)	% mortality (mean±S.E.)	Regression equation	χ^2	LD_{50} (95% F.L., µg/adult)	LD_{90} (95% F.L., µg/adult)
24	449.05	91.7±3.0 a	y=1.95x-3.76	4.54	83.8	379.7
	224.52	81.7±5.2 a			(68.2-101.3)	(283.0-580.1)
	112.26	53.3±6.7 b				
	56.13	46.7±6.7 b				
	28.06	13.3±3.7 c				
	Control	6.7±2.8 c				
48	449.05	100.0±0.0 a	y=2.32x-4.10	7.59	58.3	208.3
	224.53	91.7±3.0 a			(28.6-91.7)	(124.3-854.4)
	112.26	63.3±5.4 b				
	56.13	58.3±5.2 b				
	28.06	21.7±4.6 c				
	Control	11.7±3.9 c				

Tab. 4.3 - Contact toxicity of different concentrations of *L. angustifolia* EO against *S. granarius* adults 24 and 48 h after topical application. For each exposure time, mean mortality values followed by the same letter are not significantly different at P = 0.05 (Tukey HSD test).

4.2.2. FUMIGANT TOXICITY

The fumigant toxicity of lavender EO significantly increased with dose and significantly decreased in the presence of wheat grains (Tab. 4.4) (Fig. 4.1). The interaction dose x substrate was significant at P = 0.001 level. A 100% mortality was reached at the doses of 11.9 and 47.5 mg/L volume in the absence and the presence of wheat grains, respectively (Tab. 4.5) (Fig. 4.1). The LC_{50} and LC_{90} values were respectively 1.6 and 4.1 mg/L volume in the absence of wheat grains and 10.9 and 47.6 mg/L volume in the presence of grains.

Source	df	Type III SS	Mean square	F-value	p-value
Dose	7	53622.917	7660.417	342.047	< 0.001
Substrate	1	13668.750	13668.750	610.326	< 0.001
Dose x substrate	7	12289.583	1755.655	78.392	< 000.1
Error	32	716.667	22.396		

Tab. 4.4 - Two-way analysis of variance between subjects effects for the fumigant toxicity of *L. angustifolia* EO against *S. granarius* adults at the doses of 47.52, 23.76, 11.88, 5.94, 2.97, 1.49, 0.74, 0.00 mg/L volume in the absence and presence of food substrate (100 g wheat grains), respectively.

Substrate	Dose (mg/L volume)	% mortality (mean±S.E.)	Regression equation	χ^2	LD $_{50}$ (95% C.L., mg/L)	LD $_{90}$ (95% F.L., mg/L)
Absence	47.52	100.0±0.0 a	y=3.07x-0.61	20.08	1.57 (1.05-2.37)	4.12 (2.66-10.89)
	23.76	100.0±0.0 a				
	11.88	100.0±0.0 a				
	5.94	93.3±3.3 a				
	2.97	33.3±6.0 b				
	1.49	28.3±6.0 b				
	0.74	6.7±1.7 c				
	Control	1.7±1.7 c				
Presence	47.52	100.0±0.0 a	y=2.00x-2.07	38.52	10.89 (5.45-40.60)	47.62 (18.89-1897.4)
	23.76	46.7±1.7 b				
	11.88	20.0±2.9 c				
	5.94	10.0±0.0 d				
	2.97	10.0±2.9 d				
	1.49	5.0±2.9 dc				
	0.74	1.7±1.7 dc				
	Control	0.0±0.0 c				

Tab. 4.5 - Fumigant toxicity of different concentrations of *L. angustifolia* EO against *S. granarius* adults in the absence and the presence of food substrate (100 g wheat grains). For each set of experiments, mean mortality values followed by different letters are significantly different at P = 0.05 (Tukey HSD test) (ANOVA F = 177.91 - 304.21; df = 7; P < 0.001).
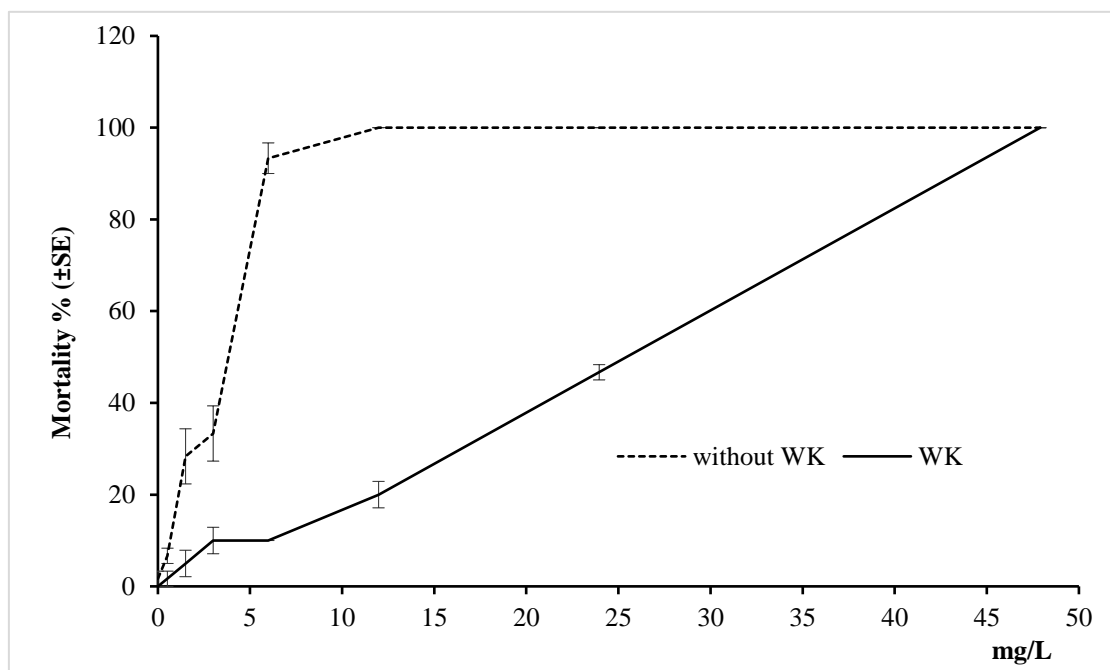
Fig. 4.1 - Fumigant activity (mean ± SE) of Lavender EO, at different concentrations, against *S. granarius* in 600 mL fumigation chambers, with and without wheat kernels (WK).

4.2.3 REPELLENT ACTIVITY

In filter paper bioassays, the repellent activity of lavender EO significantly increased with dose increase whereas it was not significantly affected by increase of time exposure (Tab. 4.6). The interaction dose x time was significant at P = 0.05 level. Mean PR values were higher than 80% (V repellent class) starting from the 0.441 mg/cm^2 dose and significantly higher (F = 18.81 - 41.68; df = 6; P < 0.001) than those recorded at the lowest doses 60 min after the experiment start (Tab. 4.7).

Source	df	Type III SS	Mean square	F-value	p-value
Dose	6	114442.857	19073.810	33.126	< 0.001
Error	18	10364.286	575.794		
Exposure time	3	1296.429	432.143	3.25	0.074
Error	9	1196.429	132.937		
Dose x exposure time	18	6928.571	384.921	3.477	< 0.001
Error	54	5978.571	110.714		

Tab. 4.6 - Repeated measures analysis of variance between subjects effects for the repellent activity of *L. angustifolia* EO against *S. granarius* adults on filter paper disc bioassays at the doses of 3.51, 1.77, 0.88, 0.44, 0.22, 0.11, 0.06, mg/cm^2 30, 60, 90 120 min exposure, respectively.

Dose (mg/cm^2)	Exposure time (min)			
	30	60	90	120
3.531	95.0 ± 5.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	95.0 ± 5.0 a
1.765	95.0 ± 5.0 a	100.0 ± 0.0 a	95.0 ± 5.0 a	100.0 ± 0.0 a
0.883	100.0 ± 0.0 a	95.0 ± 5.0 a	100.0 ± 0.0 a	95.0 ± 5.0 a
0.441	85.0 ± 9.6 ab	100.0 ± 0.0 a	90.0 ± 5.8 a	90.0 ± 5.8 a
0.221	55.0 ± 12.6 b	60.0 ± 0.0 b	55.0 ± 12.6 b	45.0 ± 17.1 c
0.110	60.0 ± 8.2 b	25.0 ± 9.6 c	15.0 ± 12.6 c	5.0 ± 9.6 c
0.055	20.0 ± 8.2 c	25.0 ± 9.6 c	25.0 ± 9.6 bc	20.0 ± 11.6 c
F	15.65	41.68	25.73	18.81
Df	6	6	6	6
P	<0.001	< 0.001	< 0.001	< 0.001

Tab. 4.7 - Percent repellency (PR) (±S.E.) of different concentrations of *L. angustifolia* EO against *S. granarius* adults on filter paper disc bioassays after different exposure times. Values in the same column followed by different letters are significantly different at P = 0.05 (Tukey HSD test).

In arena behavioral bioassays, increasing EO concentrations elicited significant reductions in the number of insects in the treatment and significant increases in the number of insects in the control both in the absence (Tab. 4.8, Fig. 4.2, Fig. 4.3) and the presence of odors of wheat grains (Tab. 4.8, Fig. 4.3). In both sets of experiments, mean RIs were negative at all doses tested and significant (*t*-test; P = 0.05) starting from the 1.12 µg dose, indicating actual repellence.

Stimulus	Treatment	Control	Student's *t*-test t-value	Student's *t*-test P-value	Response Index
Acetone	8.8 ± 1.1 a	8.5 ± 0.5 a	0.18	0.867	2.5 ± 4.4 a
0.56 mg EO	2.0 ± 0.4 b	4.8 ± 1.7 a	1.84	0.163	-9.2 ± 5.0 ab
1.12 mg EO	0.8 ± 0.5 b	7.3 ± 1.2 ab	7.51	0.005	-21.7 ± 2.9 b
2.24 mg EO	0.0 ± 0.0 b	9.0 ± 0.7 ab	12.73	0.001	-30.0 ± 2.4 bc
4.49 mg EO	0.3 ± 0.3 b	14.3 ± 2.0 b	6.60	0.007	-46.7 ± 7.1 c
8.98 mg EO	0.0 ± 0.0 b	14.8 ± 2.2 b	6.78	0.007	-49.2 ± 7.2 c
	F = 41.37	F = 6.90			F = 15.68
	df = 5	df = 5			df = 5
	P < 0.001	P = 0.001			P < 0.001
WG	25.3±1.5 a	2.0±0.7 a	10.33	0.002	77.5±7.5 a
WG + 0.56 mg EO	8.8±1.6 b	9.0±1.0 b	0.15	0.890	-1.2±5.8 b
WG + 1.12 mg EO	6.3±0.9 bc	11.5±0.3 b	8.35	0.004	-17.5±2.1 bc
WG + 2.24 mg EO	4.3±0.5 bc	11.8±0.9 b	8.66	0.003	-25.0±2.9 c
WG + 4.49 mg EO	3.5±0.3 c	12.0±1.3 b	5.47	0.012	-28.3±5.2 c
WG + 8.98 mg EO	2.8±0.3 c	13.0±0.9 b	8.82	0.003	-35.0±3.4 c
	F = 71.61	F = 66.6			F = 76.1
	df = 5	df = 5			df = 5
	P < 0.001	P <0.001			P < 0.001

Tab. 4.8 - Behavioural responses of *S. granarius* adults to ascending doses of *L. angustifolia* EO alone and in the presence of odors emitted by 200 g of wheat grains (WG) in two-choice bioassays. In a row, significant differences between treatment and control responses are indicated by Student's *t*-test. For each set of experiments, means in the same column followed by different letters are significantly different at P = 0.05 (Tukey's HSD test).
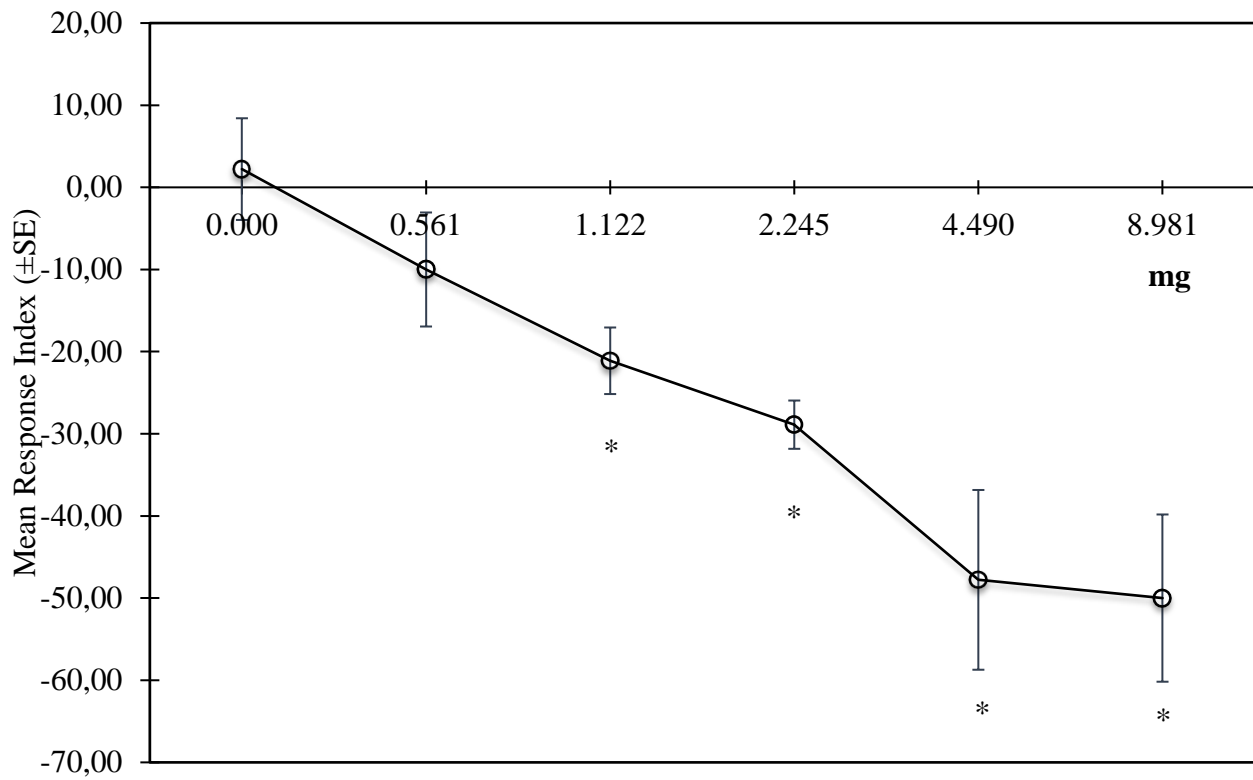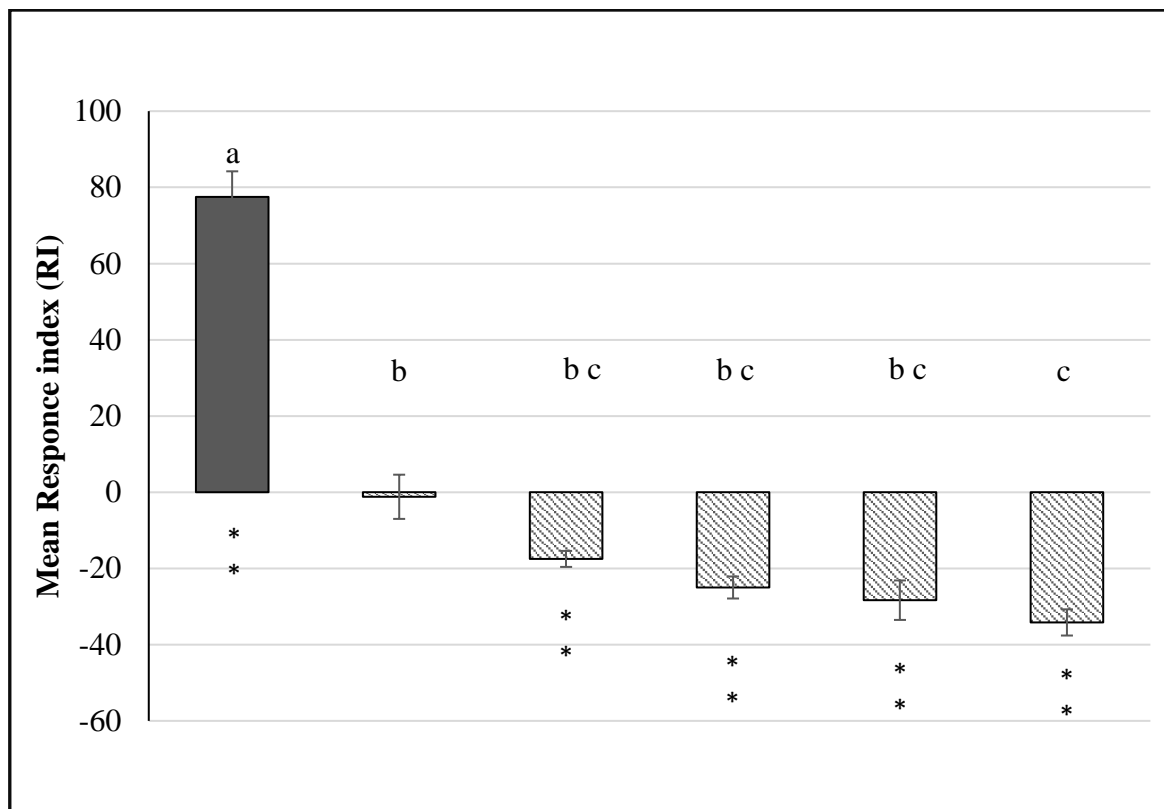
Fig. 4.2 - Responses of *S. granarius* to ascending doses of *L. angustifolia* EO in arena bioassay. Significant differences between treatment and control response to a set test dose are indicated by *P=0.05; (Student's *t*-test)

Fig. 4.3 - Responses of *S. granarius* adults to odours emitted by 200 g of wheat kernels (WK) alone and in the presence of ascending doses of *L. angustifolia* essential oil (EO) in two-choice bioassays. Mean with no letters in common are significantly different (P=0.05; Tukey's test). Significantly RI:** P=0.01.

4.2.4 INGESTION TOXICITY, ANTIFEEDANT AND NUTRITIONAL INDICES

In flour disk bioassays, the EO induced a significant increase of mortality with dose increase that reached 74.8 and 100 % levels at the 2.245 and 4.490 mg/disk doses, respectively (Tab. 4.9). At the 1.125 mg/disk dose the EO antifeedant activity was 8.9 % and not significantly different than those recorded at the lower doses. In the range of sublethal doses between 1.125 and 0.281 mg/disk, RGR, RCR, and ECI values did not vary significantly and were similar to those of control.

Concentration (mg/disk)	Mortality (%)	FDI (%) ± S.E.	RGR (mg/mg/day) ± S.E.	RCR (mg/mg/day) ± S.E.	ECI (%) ± S.E.
4.490	100±0.0 a	-	-	-	-
2.245	74±8.1 ab		-	-	-
1.125	54±14.7 b	8.9±3.7 a	0.013±0.018 a	0.397±0.039 a	3.872±5.678 a
0.563	2±2.0 c	9.7±6.1 a	0.017±0.111 a	0.360±0.011 a	4.638±3.119 a
0.281	4±2.4 c	- 6.9±6.1 a	0.003±0.006 a	0.432±0.023 a	0.862±1.437 a
Control	0±0.0 c	-	0.024±0.005 a	0.426±0.021 a	5.625±0.980 a
F	38.16	3.021	0.642	2.692	0.375
df	5	2	3	3	3
P	< 0.001	0.087	0.599	0.081	0.772

Tab. 4.9 - Mortality, feeding, growth and dietary utilization of *S. granarius* adults fed for 3 days on flour disks treated with increasing concentrations of *L. angustifolia* EO. Values in the same column followed by the same letters are not significantly different at P = 0.05 (Tukey HSD test).

4.3. BIOLOGICAL ACTIVITY OF FORMULATIONS

4.3.1 MORTALITY IN PETRI DISH

Aromatized Kaolin and Zeolite powder, in Petri dishes bioassay (closed and aerated), increased the percentage of mortality than powders during the first two days after treatment, in particular for Kaolin formulation (Tab. 4.10 and 4.11).

Powder	Mortality (%±S.E.)			
	24 h	48 h	72 h	96 h
Kaolin+200µL EO	12.5±7.5 a	85.0±5.0 a	92.5±2.5 a	100.0±0.0 a
Zeolite+200µL EO	15.0±6.4 a	85.0±2.9 a	90.0±4.1 a	100.0±0.0 a
Kaolin	0.0±0.0 b	57.5±7.5 b	82.5±7.5 a	95.0±5.0 a
Zeolite	2.5± 2.2 b	52.5±2.5 b	90.0±4.1 a	100.0±0.0 a
Control	0.0±0.0 b	2.5±0.0 c	2.5±2.1 b	18.0±4.8 b

Tab. 4.10 – Toxicity of Kaolin and Zeolite powder aromatized with 200 µL of *L. angustifolia* EO against *S. granarius* adults in areated Petri disches bioassay. Observations were carried after 1, 2, 3

and 4 days of treatment. Means in the same column with the same letter are not significantly different at the 0.05 level determined by the LSD test.

Powder	Mortality (%±S.E.)			
	24 h	48 h	72 h	96 h
Kaolin+200µL EO	20.0±4.1a	90.0±4.1 a	97.5±2.5 a	100.0±0.0 a
Zeolite+200µL EO	5.0±5.0 b	55.0±2.9 b	85.0±2.9 bd	87.5±2.5 b
Kaolin	2.5±2.5 b	20.0±10.9 c	72.5±2.5 cd	90.0±0.0 b
Zeolite	2.5±2.5 b	25.5±5.0 c	82.5±6.3 bcd	90.0±0.0 b
Control	2.5±2.5 b	10.0±0.0 c	17.5±2.5 e	20.0±0.0 c

Tab. 4.11 – Toxicity of Kaolin and Zeolite powder aromatized with 200 µL of *L. angustifolia* EO against *S. granarius* adults in closed Petri disches bioassay. Observations were carried after 1, 2, 3 and 4 days of treatment. Means in the same column with the same letter are not significantly different at the 0.05 level determined by the LSD test.

4.3.2 MORTALITY IN FLASK

The unflavoured kaolin powder showed, in the bioassay of toxicity in flask with grain, a growing percentage mortality according to the doses and hours of exposure (Tab. 4.12).

The percentage of mortality was than 75% after the 4 [th] day for the dose of 500 mg.

The lower dose (100 mg) presented no-significant mortality (<10%) in time and significantly less those of other quantities (Tab. 4.12).

The kaolin treated with the solvent (acetone) showed a mortality in same trend to the unflavoured kaolin powder (Tab. 4.12).

Flavored kaolin in the first 24 h of exposure exhibited a lower mortality rate at 3.3%; after 48 h, the percentage of mortality increased significantly (from 45.00 to 66.67%) for the doses of 250 and 500 mg 15% of flavoring and with significant differences between them; after 72 h, for the same dose and to 500 mg at 10%, the percentage of mortality increased further (63.33 to 93.34%) with significant values compared to the other treatments but not between them; after 96 h, the previous dose, 250 mg of 10% and 500 mg of unflavoured kaolin, showed a mortality (75.96 and 98.33%) significantly superior to the other treatments but not between them; after 120 h, for the same doses, the percentage

of mortality increases further (90.84 to 100%) and reaches 100% for 500 mg of flavored to 15 and 10% kaolin (Tab. 4.12).

Grain 200g + Kaolin mg (% aromatization)	Mortality (%)±SE				
	Hours				
	24	48	72	96	120
100 (0.0%)	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	0.84±0.84 a	9.17±3.69 a
250 (0.0%)	0.00±0.00 a	5.00±2.15 a	16.67±3.60 ab	27.50±4.38 abd	61.67±14.56 cd
500 (0.0%)	0.00±0.00 a	12.50±3.94 a	40.84±5.99 bcd	81.79±8.77 e	94.17±5.83 ef
100 (acetone)	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	7.50±2.85 ab	20.00±5.61 ab
250 (acetone)	0.00±0.00 a	3.34±1.92 a	26.67±11.30 abc	49.17±11.49 cd	65.00±10.67 cde
500 (acetone)	0.00±0.00 a	5.00±3.97 a	40.84±2.50 bcd	75.96±3.53 de	90.84±4.58 def
100 (10%)	0.83±0.83 a	6.67±4.71 a	24.17±6.85 abc	37.50±5.67 c	49.17±4.78 bc
250 (10%)	0.83±0.83 a	15.00±4.19 a	42.50±11.34 bcd	78.33±5.18 e	90.00±4.30 def
500 (10%)	3.34±1.36 a	15.67±1.67 a	63.33±13.74 cde	98.33±1.66 e	100.00±0.00 f
100 (15%)	0.83±0.83 a	6.67±1.36 a	20.00±1.36 ab	30.00±1.92 bc	45.00±0.96 bc
250 (15%)	1.67±0.96 a	45.00±4.81 b	69.17±14.99 de	82.50±10.12 e	94.17±3.43 ef
500 (15%)	3.34±1.36 a	66.67±2.72 c	93.34±3.04 e	98.33±0.96 e	100.00±0.00 f

Tab. 4.12 - Bioassay of mortality, of formulations based on kaolin and kaolin aromatized with lavender OE, in 500 ml flask with 200 grams of grain, covered by lid with hole (Ø 2 cm) closed by a plastic net. The mean in the same column with the same letters are not significant for the level of 0.05 determined by Tukey test.

4.3.3 RI IN ARENA

In arena bioassay, the mean of RI of adult of *S. granarius* to 200 grams of wheat kernels is between 63.83 and 90.00 with significantly higher values in the different hours of exposure, compared to grain treated with kaolin (Tab. 4.13).

After 24 h, the RI decreased from 30.83 to 14.16, when the grain is treated with various amounts (100-250-500 mg) of kaolin, kaolin-acetone, or with kaolin aromatized (100 mg and 250 mg) to 10%; RI is further reduced between 8.33 and 7.00 for 500 mg and 100-250 mg 10% to 15% and reaches negative values (-10.00), but not significant compared to the previous, with 500 mg 15%.

After 48 h, RI values of the treated wheat ranged between 21.67 and -9.16 but not significant between them.

After 72 h, 96 h and 120 h of treatment the RI values of wheat aromatized with kaolin reported values between 59.17 and -5.00 and wheat with kaolin or kaolin-acetone between 40 and -0.83. The statistical analysis showed a significance value of IR, but do not allow to draw the answers dose correlations.

Grain 200 g + Kaolin mg (% aromatization)	RI (%)±SE				
	hours				
	24	48	72	96	120
Grain	64.66±3.74 a	63.83±2.82 a	90.00±4.08 a	86.33±3.96 a	76.00±2.21 a
Grain+100 (0.0%)	24.17±2.85 bcd	21.67±7.01 b	29.17±5.50 bc	19.17±2.85 bcd	24.17±4.38 bcd
Grain+250 (0.0%)	30.00±1.36 bc	21.67±3.19 b	40.00±7.07 b	24.17±4.38 bcd	25.00±2.15 bcd
Grain+500 (0.0%)	30.83±5.51 b	15.00±4.41 b	30.00±3.60 bc	25.83±5.99 bcd	15.00±2.89 cd
Grain+100 (acetone)	14.75±1.84 bcd	4.17±7.98 b	7.50±4.59 ce	0.83±5.67 d	7.50±4.78 d
Grain+250 (acetone)	16.67±5.61 bcd	1.67±4.99 b	17.50±4.98 bcde	22.50±7.74 bcd	5.83±3.15 d
Grain+500 (acetone)	14.16±6.58 bcd	16.67±5.61 b	0.01±7.82 de	5.00±3.19 d	0.83±10.31 d
Grain+100 (10%)	23.33±5.27 bcd	10.00±7.07 b	26.67±7.07 bc	47.50±11.58 b	59.17±5.51 ab
Grain+250 (10%)	21.67±5.69 bcd	5.00±8.87 b	10.83±6.85 cde	15.00±9.67 cd	50.00±11.22 abc
Grain+500 (10%)	8.33±5.85 cde	16.67±2.72 b	12.50±3.69 cde	41.67±0.96 bc	22.50±11.57 cd
Grain+100 (15%)	7.91±1.67 de	9.16±9.46 b	5.00±4.19 e	20.83±5.34 bcd	51.67±7.76 abc
Grain+250 (15%)	7.00±1.96 de	0.00±6.80 b	10.83±0.83 cde	16.67±1.36 cd	13.33±7.82 cd
Grain+500 (15%)	-10.00±4.08 e	0.83±4.38 b	21.67±4.19 bcd	26.67±4.91 bcd	33.33±14.33 abcd

Tab. 4.13 - Response Idex (RI), in arena of adult *S. granarius* to grain (200 g) treated with kaolin and / or aromatized with lavender OE for several hours of exposure (24, 48, 72, 96, 120 h). The mean in the same column with the same letters are not significant for the level of 0.05 determined by the Tukey test.

5. DISCUSSION

The major constituents of EO extracted from *L. angustifolia* flower spikes collected in the Italian Apennines were linalool, 1,8-cineole, terpinen-4-ol, linalyl acetate, (E) - β-ocimene, (E)-β-farnesene, and camphor.

Linalool, linalyl acetate, 1,8-cineole and camphor have been already recorded as major components of flower EOs of different lavender cultivars (Charles et al., 2002; Dušková et al., 2016) even if in different proportions. The high variability of lavender EOs is known (Lis-Balchin, 2002) and some compounds (e.g. linalyl acetate) have been recognized as highly variable components depending on cultivation area and plant genotypes (Tucker et al., 1984; Dušková et al., 2016).

Topical application of lavender EO to adult granary weevils induced dose-dependent contact mortality that significantly increased with the exposure time. This toxicity was lower than those reported by Ziaee (2014) for *Carum copticum* L. and *Cuminum cyminum* L. EOs against the same pest. In that study, however, EOs were topically applied onto the ventral surface of the thoracic segments instead onto the pronotum of adult weevils. The contact toxicity of lavender EO to *S. granarius* was comparable with those observed against the congener *S. zeamais* for the EOs of other aromatic plants including *L. hybrida* (Rossi et al., 2012) various *Artemisia* species (Liu et al., 2010; 2014; Chu et al., 2012, 2013) and *Pelargonium hortorum* Bailey (Liu et al., 2013) but about 20 times less than that obtained using a pyrethrum extract (Liu et al., 2010).

The EO exhibited a strong fumigant toxicity against granary weevils with a 24h LC_{50} value of 1.6 mg/L volume in the absence of wheat grains. This value was lower than those recorded for other lavender EOs against stored-product insect beetles including *O. surinamensis* (LC_{50} 11.3 md/L air), *R. dominica* (LC_{50} 11.4 mg/L air), *S. oryzae* (LC_{50} >15 mg/L air), *T. castaneum* (LC_{50} >15 mg/L air) (Shaaya et al., 1997; Rozman et al., 2007; Abdelgaleil et al., 2009; Pugazhvendan et al., 2012) and *S. granarius* itself (Laznik et al., 2012).

In this latter study, the LC_{50} value was 16.1 mg/L air even after 72 h exposure at 30 °C and 55% R.H. suggesting that variation in chemical composition can be responsible for marked differences in EO toxicity. The fumigant toxicity of lavender EO was about 10-fold reduced by the presence of wheat grains (LC_{50} 10.9 mg/L volume). A similar effect of wheat grain presence on the toxicity of some aliphatic ketones was observed by Germinara et al. (2012a) and it is probably due to the sorption of EO vapours to starch (Maier and Bauer, 1972) and cellulose (Demovaya and Eltekov, 1988) of wheat grains or to a their reduced diffusion through the interstitial spaces of grains (Lee et al., 2003).

The repellent activity of the EO to granary weevil adults was studied using both filter paper and arena bioassays. The filter paper bioassay permits a visual control of the repellent effect of the test stimulus over regular time intervals whereas the large volume of the arena bioassay permits to evaluate the repellence even in the presence of an attractive source (Germinara et al., 2007; Benelli et al., 2012; Bedini et al., 2016). A strong repellent effect was found in both bioassays. In the arena, the EO exhibited repellency even in the presence of wheat grains indicating the capability to effectively disrupt granary weevil orientation to the attractive host substrate. In the nutritional experiments, sublethal concentrations of EO did not significantly affect feeding and growth of adult granary weevils. This suggests that the toxicity observed at the highest doses tested in flour disks bioassays was not due to ingestion, but to inhalation of EO vapours and contact with treated flour disks.

The toxic and repellent effects of the lavender EO to granary weevils could be attributed to its major constituents since for some of them different bioactivities towards several stored-product insect pests have been recognized. For example, a remarkable fumigant toxicity was reported for 1,8-cineole, linalool, borneol, champhor, and linalyl acetate against *S. oryzae* and *R. dominica* (Rozman et al., 2007). Fumigant toxicity of linalool and 1,8-cineole have also been found for *Blattella germanica* (L.) and *O. surinamensis* (Lee et al., 2003). Moreover, 1,8-cineole and linalool have been shown to inhibit acetylcholinesterase (AChE) from *S. oryzae* adults and *T. castaneum* larvae (Abdelgaleil et al., 2009). Contact toxicity and repellent activity of 1,8-cineole have been reported in studies with *S. granarius*, *S. zeamais*, *Tribolium confusum* Jacquelin du Val and *Prostephanus truncatus* (Horn) (Obeng-Ofori et al., 1997). Good repellent effects were shown for linalool against *T. castaneum* and *R. dominica* (Ukeh and Umoetoka, 2011) and borneol towards *Bradysia* sp. nr. *coprophila* (Lintner) (Diptera Sciaridae) (Cloyd et al., 2011). Aromatized kaolin and zeolite powder, in Petri dishes bioassay (closed and aerated), increased the percentage of mortality than powders during the first two days after treatment, in particular for kaolin formulation. This increased mortality is due to a release of toxic volatile substance, present in EO, from aromatic powders. In closed Petri dishes mortality is little more low than aerated dish, problably for the stagnation of umidity which lowers the activity of the powders. The interesting insecticidal activity of aromatized kaolin, in the first days, was also observed in flask bioassay in the presence of food substrate, compared to the simple kaolin powder. Kaolin is also able to decrease the attractiveness of the grain, very probably due to the kaolin powder ability to absorb or hinder the release of volatile substances from the substrate. Aromatized kaolin (500 mg to 15%/200gr grain) shows also a good repellent activity that, in the first days of treatment, remove *S. granarius* adults from food substrate.

6. CONCLUSIONS

The flower spike EO of *L. angustifolia* exhibited good fumigant and contact toxicity against granary weevil adults confirming potential as a natural alternative to synthetic insecticides for the control of stored-product insect pests. In addition, a strong repellent activity able to disrupt granary weevil orientation to an attractive host substrate was shown, indicating possible applications to flush out insect infestation from empty stores before fresh grain is introduced.

This could create a chemical barriers able to mask grain odours to insects, and to incorporate it into packaging materials to prevent insect infestation of packaged foods (Cox, 2004; Hou et al., 2004; Germinara et al., 2012b; 2015). Moreover, it is worth noting that the use of lavender EO to control stored-product insect pests should be safe since it is already employed by food industries in flavouring beverages, ice-cream, candy, baked goods, and chewing gums (Kim and Lee, 2002; Da Porto et al., 2009) and has many medicinal, pharmaceutical and aromatherapy uses (Hassiotis et al., 2010).

Aromatized kaolin, with lavender EO, it has allowed to obtain an interesting formulation that consents take the advantage of the rapid toxic and repellent activity of OE (during first days), combine by slow insecticidal activity of kaolin, that shows after 4 days of treatment.

These activities help break down the insects present and remove its from food substrate.

In addition, the determined doses of aromatized kaolin in lab to control *S. granarius* are more less ($\leq$ 2,5%) than simple kaolin (5-10%) recommended as an insecticide on foodstuffs.

However, the practical application such a formulation, should be further evaluated on larger quantities of food substrates.

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