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Insecticidal effects of essential oils against woolly beech aphid, *Phyllaphis fagi* (Hemiptera: Aphididae) and rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae)

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Abstract
Eleven essential oils (*Citrus aurantium*, *Citrus sinensis*, *Citrus limon*, *Eugenia uniflora, Ocimum gratissimum*, *Rosmarinus officinalis*, *Gaultheria procumbens, Thuja plicata*, *Pseudotsuga menziesii*, *Abies grandis, Bursera graveolens*) were tested against *Phyllaphis fagi,* as contact and residual toxins. *Abies grandis* (LC_{50}=0.006%) was significantly the most active contact toxin followed by the positive control, *R. officinalis* (LC_{50}=0.134%), *P. menziesii* (LC_{50} = 0.428%), *B. graveolens* (LC_{50}=0.907%), and *T. plicata* (LC_{50}=1.011%). Five of these essential oils were also tested against *Sitophilus oryzae* for fumigation effects. Based on the LC_{50} values of the oils, *G. procumbens* was the most active fumigant (LC_{50}=6.8 µl/l air). *Thuja plicata* and *B. graveolens* oils were equitoxic. *Rosmarinus officinalis* and *A. grandis* were the least toxic (LC_{50}=53.6 and 38.6 µl/l air respectively). In a grain-treated bioassay against *S. oryzae, G. procumbens* was the most active (LC_{50}=0.235 µl-g^{-1}) followed by *R. officinalis*. *Thuja plicata* and *B. graveolens* were equitoxic. *Abies grandis* was the least toxic. These oils exhibit different modes of action and have potential to be used as commercial insecticides against *P. fagi* and *S. oryzae*.

Keywords: *Phyllaphis fagi*, *S. oryzae*, contact toxicity, residual toxicity, essential oils, grain protectant

1. Introduction
The woolly beech aphid, *Phyllaphis fagi,* and rice weevil, *Sitophilus oryzae*, are serious pests worldwide. *P. fagi* is a specialist on the European beech tree, *Fagus sylvatica* [1] and is responsible for reducing the aesthetics of *F. sylvatica* in nurseries, causing economic losses to the nursery industry. It also attracts other insects to the site and may change the local community structure of the canopy shed [2]. This is especially critical in regenerating beech forests since younger beech trees are more prone to aphid infestation and damage [2]. Heavy infestations of *P. fagi* may also change the emission levels of biogenic volatile organic compounds (BVOCs) in *F. sylvatica* [1]. Infestation of *F. sylvatica* by *P. fagi* caused an increase in the release rate of terpenoids from 57 µg m^{-2} h^{-1} to 127 µg m^{-2} h^{-1}, possibly affecting global atmospheric levels [1]. Severe aphid infestation can reduce photosynthetic rates of host plants like *F. sylvatica* and thereby increases the overall carbon content in the atmosphere, having severe implications on climate change.
Sitophilus species cause considerable economic losses to stored wheat grain. Heavy infestation of these pests due to the lack of proper food hygiene and storage may cause weight losses of as much as 30–40% [3]. Direct feeding on the grain kernels may cause unfavorable effects on food quality, safety, and preservation [4]. Control of these insects relies heavily on the use of synthetic insecticides including organochlorines (lindane), organophosphates (malathion), carbamates (carbaryl), pyrethroids (deltamethrin) and fumigants including methyl bromide, phosphine, and sulfuryl fluoride. However, the indiscriminate application of synthetic products has led to various problems including toxic residual effects, environmental pollution, and development of resistance in insects [5]. Therefore, there is an urgent need to develop safe, convenient and low-cost alternatives. Considerable efforts have been focused on the use of plant-derived materials including essential oils as bioinsecticides.
Essential oils have demonstrated toxic effects against stored-product insects [6] as well as agricultural pests [7, 8]. They may act as fumigants [6, 9, 10], contact insecticides [9, 11], antifeedants [8, 12] or repellents [11, 13].

Essential oils also have a long history in medical and dietary uses [14] and in the USA are "Generally Recognized as Safe" [15]. Due to their low mammalian toxicity [16] they could be used as alternative sources for controlling a number of insect pests including stored-product insects [4]. Plant essential oils and their constituents have been shown to possess potential for development as new fumigants and they may have advantages over conventional fumigants in terms of low mammalian toxicity and low environmental impact.

The objective of this study was to screen essential oils as effective contact or residual toxicants against the woolly beech aphid, *P. fagi*, and as fumigants against the rice weevil, *S. oryzae*. Species were chosen because of their world-wide pest status as well as their availability. Selection of essential oils for the experiment was based on prior knowledge of their insecticidal activities against related insect species. Families Lamiaceae (including *R. officinalis* and *O. gratissimum*), Rutaceae (including *C. aurantium* L., *C. sinensis* var. pera Osbeck, and *C. limon*), Myrtaceae (including *E. uniflora*), and Cupressaceae (including *T. plicata*) are specifically known for their insecticidal effects against hemipterans (including the aphids) and coleopterans (including the weevils) [16]. None of the oils used in our study have been tested against *P. fagi* and only some against *S. oryzae* for contact, residual, or fumigant effects, to the best of our knowledge.

2. Materials and Methods

2.1 Period of Study

This study was carried out during April-December, 2012.

2.2 Test substances

Essential oils used in the study (Table 1) were obtained from different sources. Rosemary, wintergreen and conifer oils were provided by Ecosafe Natural Products Inc. (Canada). *Citrus* and *Eugena* oils were provided by Dr. Claudio A. G. da Camara (Univ. Federal Rural de Pernambuco, Brazil).

2.3 Test Insects

Woolly Beech aphid

Aphids were collected from European beech trees, *Fagus sylvatica*, on the University of British Columbia campus during the months of May and June of 2012 under typical Vancouver spring weather. Early nymphal stages were distinguished from the mature stages by size and density of a wax-cover, particularly on their abdomens. Early staged nymphs and winged adults were not chosen in order to reduce variability. Approximately 100 third-fourth instar aphids were stored in a plastic container (diameter: 4cm; height: 3cm) and were immediately used in the experiments. Aphids used in the experiments were not redistributed into the general population to prevent potential resistance development to the essential oils.

Rice weevils

Rice weevils, *Sitophilus oryzae*, were obtained from Dr. Paul Fields (Agriculture and Agri-food Canada, Winnipeg) and were later stored in plastic boxes in the insect toxicology lab of the University of British Columbia at 23ºC and a 16:8 (light: dark) photoperiod. They were fed a mixture of whole wheat grain, white polished rice, and brown rice. Mature adults were separated based on size and were used in the fumigation bioassays.

Essential oils

2.4 Contact Toxicity Bioassay through direct spraying:

A filter paper (diameter: 4cm) was placed in a plastic container (diameter: 4cm; height: 3cm) and 30 aphids were transferred into the container. Aphids were sprayed with a methanolic solution of an essential oil at different concentrations. Control aphids were sprayed with methanol alone. Treated aphids were then transferred to a clean container with a beech leaf disc (2.5 cm diameter). Mortality was assessed after 24h. Three replicates were conducted for each oil; each replicate used 30 aphids. Experiments were conducted at room temperature (23ºC).

2.5 Residual Toxicity Bioassay

Clean European beech leaves were collected and cut into leaf discs. Each leaf disc (2.5cm diameter) was dipped into 50µL of a 1% methanolic solution of the essential oil and air dried for ~ 1.5 minutes. Leaf discs were then placed in a plastic container (diameter: 4cm; height: 3cm). Ten aphids were introduced on each leaf disc and the container was sealed with a plastic lid. Mortality was assessed after 24h. There were three replicates of 30 insects each.

2.6 Fumigation Bioassay

Thirty adult *S. oryzae* were collected from the colony and stored in a glass vial (length = 5.5cm, diameter = 2.5cm) for each treatment. The glass vial was tightly sealed by a mesh cloth immediately after weevil collection to prevent their escape. The mesh cloth was porous enough to allow fumes to penetrate into the vial and prevent weevils from coming into direct contact with an oil treated filter paper. Glass vials containing *S. oryzae* were hung from a pin stuck into a size 7 rubber stopper, used for sealing the fumigation chamber (500 ml Erlenmeyer flask).

A Whatman (#1) filter paper (4.25 cm diameter) was treated with different doses of pure essential oil (6.25, 12.5, 25.0 and 50 µl). It was air-dried in the fumehood for ~ 2 minutes and placed in the centre of a 500ml Erlenmeyer flask. The glass vials containing the weevils were hung inside the flask. Vials were always positioned halfway in the chamber so that the distance between the fume source (filter paper) and the vial opening was consistentl for each trial. The rubber stopper tightly sealed the Erlenmeyer flask ensuring that no fumes would escape from the fumigation chamber. Mortality was assessed after 24h. There were three replicates of 30 *S. oryzae* each. Experiments were conducted at room temperature (23ºC).

2.7 Toxicity through treated grain assay

Ten grams of whole wheat seed were added into a glass vial (length = 5.5cm, diameter = 2.5cm) to occupy 50% of the vial volume. Essential oils were pipetted onto the surface of grains in the vial; the vial was immediately sealed, and thoroughly shaken to ensure that the oil mixed well with all the grains in the vial. Ten *S. oryzae* were transferred from the colony container into the prepared glass vial. The vials were gently shaken to ensure the *S. oryzae* were distributed throughout the vial. Each prepared vial was stored under laboratory conditions (23°C). Mortality was assessed at 24 and 48h. There were 3 replications of 30 insects each.

2.8 Statistical analysis

Percent mortality was calculated by using the ratio of dead insects to the total number of insects after 24 hours. Insects were considered to be dead if no movement was observed by touching with a probe under a magnifying glass for several seconds. Probit Analysis® was used to calculate LC_{50}

(concentration causing 50% mortality compared with the control) values and their confidence intervals.

3. Results

3.1 Residual and contact toxicity of essential oils against *P. fagi*

Most of the oils were not active as residual toxins against woolly beech aphid, *P. fagi* at the initial screening concentration. Bitter orange produced 40% mortality followed by western red cedar (28% mortality) and ≤15% for all others (Table 2) at the initial screening concentration of 1%.

Five oils were active as contact toxins exhibiting 97-100% mortality against *P. fagi* at the initial screening concentration of 1% (Table 2). *Abies grandis* (LC_{50} = 0.006%) was significantly the most active contact toxin followed by the positive control, *R. officinalis* (LC_{50} = 0.134%), *P. menziesii* (LC_{50} = 0.428%), *B. graveolens* (LC_{50} = 0.907%), and *T. plicata* (LC_{50} = 1.011%). *R. officinalis* (LC_{50} value=0.134%) and *P. menziesii* (LC_{50} value=0.428%) were equitoxic based on their overlapping confidence intervals. LC_{50} values for *B. graveolens* (0.907%) and *T. plicata* (1.011%) were not significantly different from *P. menziesii* based on their overlapping confidence intervals (Table 3).

3.2 Fumigant toxicity of essential oils against *S. oryzae*

Five oils were active as fumigants against *S. oryzae* exhibiting 70-100% mortality at the initial screening concentration of 100 µl/l air (Table 4). *Citrus aurantium* and *P. menziesii* produced 10% and 30% mortalities respectively at the initial screening concentration. Based on the LC_{50} values of the oils, *G. procumbens* was the most active fumigant (LC_{50} value = 6.8 µl/l air). *Thuja plicata* (LC_{50} value = 19.8 µl/l air) and *B. graveolens* (LC_{50} value = 21.4 µl/l air) oils were equitoxic (overlapping confidence intervals). *Abies grandis* and *R. officinalis* were the least toxic oils against *S. oryzae* (LC_{50} values = 38.6 and 53.6 µl/l air, respectively) (Table 4).

3.3 Toxicity of essential oils through treated grain (*S. oryzae*)

In the treated grain bioassay, *G. procumbens* was the most active (LC_{50} = 0.235 $\mu l\text{-}g^{-1}$) followed by *R. officinalis* (LC_{50} = 0.304 $\mu l\text{-}g^{-1}$) (Table 5). *Thuja plicata* (LC_{50} = 0.507 $\mu l\text{-}g^{-1}$) and *B. graveolens* (LC_{50} = 0.617 $\mu l\text{-}g^{-1}$) were equitoxic (overlapping confidence intervals). *Abies grandis* was the least toxic (LC_{50} = 0.753 $\mu l\text{-}g^{-1}$).

4. Discussion

Some of the oils have demonstrated strong toxic effects against aphids and rice weevils. Essential oils were more active as contact than as residual toxins against *P. fagi*. *Abies grandis,* was the most active contact toxin. *P. menziesii* was equitoxic to the *R. officinalis* oil (positive control). Toxicity of the other two oils, *T. plicata* and *B. graveolens* did not differ from *P. menziesii*.

Comparing the residual and contact toxicity effects, most of the oils including *C. cinensis, C. limon, E. uniflora, O. gratissimum, G. procumbens* had very low or no residual effects against *P. fagi*. Some of these oils including *R. officinalis*, *P. menziesii, B. graveolens* and *A. grandis* were only active as contact toxins. *C. aurantium* was only active as a residual toxin and *T. plicata* demonstrated both contact and residual effects. Since *T. plicata* possesses both contact and residual effects it could be used as an effective crop protectant. *Gaultheria procumbens* oil was the most active in both fumigant and the treated-grain bioassays, probably due to the toxic vapours of *G. procumbens* oil. *Thuja plicata* and *B. graveolens* were equitoxic in both types of bioassays.

In general, efficacy of an essential oil depends upon its chemical composition and the ratio of the constituents present in the mixture. The chemical composition varies even within the phylogenetically close species of plants. Douglas fir and Grand fir belong to the same family (Pinaceae), yet share only a few common constituents such as α-pinene and camphene. *A. grandis* was significantly more active (LC_{50} = 0.006%) than *B. graveolens* through direct spraying against *P. fagi*.

Results from our study are consistent with previous work related to the insecticidal effects of *R. officinalis*. Rosemary oil is known for its insecticidal activities against a variety of insects and has been incorporated into many commercial insecticides [7]. Specifically, rosemary has been shown to act as a repellent, settling inhibitor, and contact toxin against green peach aphid, *Myzus persicae* (Sulzer)[17].

Although *R. officinalis* was an active contact toxin, it was not active as a residual toxin. This suggests that *R. officinalis* essential oil has a greater ability to penetrate through the cuticle of aphids than to be absorbed from the gut. Thus, the mode of action of *R. officinalis* essential oil could be different based on the method of application. The same reasoning may account for the observed lower residual toxicity for other essential oils like *C. limon*, *T. plicata, P. menziesii*, *A. grandis*, and *B. graveolens*. *R. officinalis* essential oil has also been shown to act as a contact neurotoxin against certain insects [18] and targets green peach aphids by damaging their olfactory senses [17].

Citrus oils used in our study were not active as residual or contact toxins. The source of *C. aurantium* essential is the bitter orange peel. Studies have shown that *C. aurantium* peel extracts and other related *Citrus* species' essential oils may be effective in pest management against a variety of insect pests [19]. Although, *C. aurantium* had no contact toxicity, it demonstrated a higher residual toxicity (40%) against *P. fagi*. However, the other citrus oils also showed relatively low toxicity against *P. fagi*. Essential oils of *C. limon* and *C. sinensis* showed low contact toxicity (6.7%). Limonene, which is a major constituent of the citrus oils used in this study, has demonstrated insecticidal activities against the palm aphid, *Cerataphis brasiliensis*, when used in combination with other oils and insecticidal soaps and sprays [20]. Interestingly *F. sylvatica* naturally emits limonene and when infested by *P. fagi*, limonene release is reduced [1]. Carvone, another constituent of citrus essential oil is a noted attractant to the related aphid *Carvarriella aegopodii* and is used in baits [21]. If used in combination with a toxic essential oil lacking repellent effects, carvone may act as an attractant for aphids to the source of toxin. Further research is needed to look at the insecticidal effects of other constituents of citrus oils against *P. fagi*. A variety of aphids are citrus pests and some have been observed on *C. aurantium* and *C. sinensis* [22]. The previous studies show citrus trees are able to attract aphid predators and/or parasites as kairomones. Testing our *Citrus* essential oil against natural predators/parasites of *P. fagi* may give us some insight on whether these essential oils could act as attractants for the natural enemies of *F. sylvatica*.

Wintergreen oil, *G. procumbens*, is predominantly comprised of methyl salicylate (99%), which is a known repellent against aphids [36] and a contact toxin against many insects [1]. *G. procumbens* was not active either as a residual or contact toxin against *P. fagi* in the present study. Interestingly, Joó *et al.*'s study (2010) showed that *F. sylvatica* is capable of releasing methyl salicylate as a biogenic volatile organic compound. Since aphids in our study were obtained directly from an infested European beech tree, it is possible that *P. fagi* have already developed resistance to methyl salicylate prior to the testing in the lab. Insects are capable of developing resistance towards plant chemical defenses [23]. Therefore, resistance development by *P. fagi* against methyl salicylate could be a factor explaining the complete lack of toxicity against this species. This is a limitation of using field insects. Using laboratory raised, non-resistant aphids may give us more insight into the potential of methyl salicylate/wintergreen oil as a toxicant against *P. fagi*.

Ocimum gratissimum, commonly called "alfavaca", is believed to have originated in Central Africa or South East Asia and is known for its antimicrobial, medicinal and culinary uses [39]. The family Lamiaceae contains a number of species demonstrating toxicity against aphids [16] but *O. gratissimum* essential oil was not active as a contact or residual toxin against *P. fagi* in the present study. The main constituents in the *O. gratissimum* essential oil were eugenol (43.2%) and 1,8-cineole (12.8%) [39]. Toxic effects of eugenol and 1,8-cineole have been reported against many insects including *Sitophilus zeamais* and *Tribolium castaneum* [24].

Essential oil constituents are produced by a variety of coniferous trees for their own defense against insect pests [25]. When infested, various conifers upregulate the expression of terpenoid encoding genes, in turn releasing multifold levels of volatile terpenoids [23]. When attacked by insect pests *A. grandis* trees initially upregulate genes expressing toxic or repellent volatile monoterpenes and releases these products into the local environment [26]. The initial monoterpenes released may act as direct contact toxins and/or fumigants on insect pests, effectively reducing insect pest density on the tree.

Based on our study, *A. grandis* essential oil was the most active contact toxin against *P. fagi* producing 100% mortality at 0.01% down to 16.7% mortality at 0.001%. None of the other oils were active at such a low concentration. *Abies grandis* also demonstrated the lowest LC_{50} (0.006%) compared with other oils. Thus *A. grandis* essential oil has potential to be used as insecticide.

Abies grandis essential oil also exhibited fumigant toxicity to *S. oryzae* (LC_{50} 38.6 $\mu l.L^{-1}$). This finding demonstrates that *A. grandis* essential oil can be used as an effective fumigant against *S. oryzae*, consistent with the fact that *A. grandis* trees are capable of using volatile monoterpenes as a defense mechanism against insect pests in nature [26].

Thuja plicata, was the only essential oil demonstrating residual toxicity against *P fagi*. *Thuja plicata* essential oil caused 28% aphid mortality in the initial screening bioassays. Leaves of *T. plicata* have insecticidal effects on the white pine weevil, *Pissodes strobi*, [16] to a greater extent than other related conifers [25]. This may be due to different chemical constituents of each tree species' essential oil since (+)-3-thujone and (-)-3-isothujone is more predominant in *T. plicata* leaves (80-90%) than in other related conifers like *P. menziesii* [27]. Essential oils of *P. menziesii*, *T. Plicata* and *B. graveolens* were strong contact toxins against *P. fagi*. Related conifer essential oils like spruce exhibit toxicity against weevils and *T. plicata* essential oil is a deterrent to the white pine weevil, *P. strobi* [16].

Fumigant toxicity is evident for all oils tested and the positive control, *G. procumbens*, was shown to be highly effective against *S. oryzae*, consistent with previous findings for *G. procumbens* fumigant toxicity on other insects: the Coleopteran stored grain pest *Tribolium castaneum* [28] and the cecidomylid gall midge, *Camptomyia corticalis* [29].

Studies show *T. plicata* oil to be toxic to various insects and related weevil species like the cowpea weevil, *Callosobruchus maculata*, [30] and a known feeding deterrent to the white pine weevil, *P. strobi* [16]. Consistent with the previous reports, *T. plicata* demonstrated strong fumigant effects against *S. oryzae* in the present study. *T. plicata* and *B. graveolens* essential oils exhibited the lowest LC_{50} values (19.8 $\mu l.L^{-1}$ air for *T. Plicata* and 10.68 $\mu l.L^{-1}$ air for *B. graveolens*). *Thuja plicata* was a more active fumigant than *P. menziesii*. This may result from the difference in the chemical compositions of these oils. No (-)-3-isothujone and (+)-3-thujone was found in *P. menziesii* essential oil from France [27] while 80-100% of the *T. plicata* EO is found to be rich in the latter chemicals [16, 30].

It has been demonstrated [30] that some constituents of *T. plicata*, predominantly thujone and its isomers, have long lasting effects after *T. plicata* leaves have fallen onto the substratum and gone into the soil [30]. Thus, in the practical situation of *S. oryzae* infestation such as in storage areas of grain or rice, *T. plicata* essential oil fumes would be active for a longer period of time than oils with shorter-lasting terpenes. From our results, at least for higher concentrations of *T. plicata* applied, fumigation methodology can be considered as a viable pest management approach against *S. oryzae*, consistent with previous claims of this methodology being effective, economical and convenient against stored insect pests [31]. Fumigation has been considered to be effective due of the ability of volatiles to penetrate into the commodity with minimal residues [32]. Moreover the fumes in a stored system would also not easily dissipate out into the exterior environment.

In our treated grain bioassay, *G. procumbens* was the most active oil followed by *R. officinalis*. *T. plicata* and *B. graveolens* were equitoxic. *Abies grandis* was the least toxic as a grain protectant. Future studies should focus on testing the constituents of these oils against *P. fagi* and/or *S. oryzae* as effective environmentally friendly crop protectants.

5. Conclusion

Abies grandis, P. menziesii, *R. officinalis* (positive control), *B. graveolens,* and *T. plicata* were found to be strong contact toxins against *P. fagi*. *G. procumbens, T. plicata*, *P. menziesii*, *A. grandis, B. graveolens* were active fumigants against *S. oryzae*. In a grain-treated bioassay against *S. oryzae, G. procumbens* was the most active oil followed by *R. officinalis*. *Abies grandis* was the least toxic in grain-treated bioassays against *S. oryzae*. These oils exhibit more than one mode of action and may constitute a "multichemical defense" against a variety of potential herbivores. Since the oils are composed of mixtures of compounds, they will be more effective than individual compounds in terms of forestalling and diluting resistance and habituation [33, 34, 35] for long-term use. These oils have been tested for the first time against woolly beech aphids to the best of our knowledge. Some of these oils have never been tested against rice weevils. This study has explored the potential for development of essential oils especially from conifers to be effective, economically and environmentally friendly commercial insecticides.

6. Acknowledgement

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Table 1: List of essential oils used in the study: their origin and major constituents

Essential Oil			Origin	Major Constituents
Scientific Name	Common Name	Family Name		
Citrus aurantium [36]	Bitter Orange	Rutaceae	Tunisia	limonene (89.8%)
Citrus sinensis [36, 37]	Sweet Orange	Rutaceae	Southeastern Asia (China)	limonene (93.5%)
Citrus limon [37]	Lemon	Rutaceae	Asia (Southern India, Burma, China)	limonene, β-pinene
Eugenia uniflora [38]	Brazillian Cherry	Myrtaceae	Tropical South American East Coast	germacrene B (21.2%), seline-1,3,7-trien-8-one oxide (19.3%), β-caryophyllene (12.6%)
Ocimum gratissimum [39]	Clove Basil	Lamiaceae	Eastern Africa	eugenol (75.1%)
Rosmarinus officinalis [40]	Rosemary	Lamiaceae	Morocco	1,8-cineole (26.54%), α-pinene (20.14%), camphor (12.88%), camphene (11.38%), β-pinene (6.95%)
Gaultheria procumbens [36]	Wintergreen	Ericaceae	Northeastern North America	methyl salicylate (96-99%)
Thuja plicata [41]	Western Red Cedar	Cupressaceae	Western North America	α-thujone (62%), β-thujone (10%)
Pseudotsuga menziesii [42]	Douglas Fir	Pinaceae	Western North America	bornyl acetate (34.6)%, camphene (29.8%), α-pinene (11.6%), limonene (4.5%), β-pinene (2.7%)
Abies grandis [43]	Grand Fir	Pinaceae	Western North America	β-pinene (20.3-31%), bornyl acetate ((12.7-26.2%), β-phellandrene (13.7 - 25.2%), camphene (8.3-11.5%), α-pinene (4.4-7.4%)
Bursera graveolens [44]	Palo Santo	Burseraceae	South America	limonene (59%), α-terpineol (11%)

Table 2: Residual effects of essential oils at 1% against *P. fagi*

Essential Oils	Mortality (%)
Control	3.4
Citrus aurantium	40
Citrus sinensis	15
Citrus limon	0
Eugenia uniflora	15
Ocimum gratissimum	10
Rosmarinus officinalis	0
Gaultheria procumbens	0
Thuja plicata	28
Pseudotsuga menziesii	0
Abies grandis	0
Bursera graveolens	2.8

n= 3 replicates of 30 insect

Table 3: LC_{50} values and the corresponding confidence intervals (CI) for oils exhibiting >50% mortality through direct spraying against *P. fagi* at the initial screening concentration (1%)

Essential oils	Mortality at Initial Screening Concentration (%)	LC_{50} (%)*	Confidence Interval
MeOH Control	1.1	-	-
Blank	0	-	-
Citrus aurantium	0	-	-
Citrus sinensis	6.7	-	-
Citrus limon	6.7	-	-
Eugenia uniflora	9.1	-	-
Ocimum Gratissimum	0	-	-
Rosmarinus officinalis	100	0.134	0.101–0.168
Gaultheria procumbens	0	-	-
Thuja plicata	97	1.011	0.643–3.201
Pseudotsuga menziesii	100	0.428	0.122–2.506
Abies grandis	100	0.006	0.001–0.010
Bursera graveolens	97	0.907	0.660–1.641

*LC_{50} values (concentration causing 50% mortality); 4-5 concentrations (0.001-1%) were used to calculate the LC_{50} values; n= 3 replicates of 30 insect

Table 4: LC_{50} values and the corresponding CI (confidence intervals) for oils exhibiting >50% mortality through fumigant toxicity against *S. oryzae* at initial screening concentration (100µl/L air)

Essential Oils	Mortality at Initial Screening Concentration (%)	LC_{50} µL.L^{-1} air (Confidence interval)	X^2 (calculated)	Slope ± Stand. Error
Blank	2.5	-	-	-
Citrus aurantium	10	-	-	-
Citrus sinensis	0	-	-	-
Citrus limon	0	-	-	-
Eugenia uniflora	0	-	-	-
Ocumum gratissimum njoroke	0	-	-	-
Rosmarinus officinalis	100	53.6 (42.2 – 73.2)	8.4	4.8 ± 0.86
Gaultheria procumbens	100	6.784 (5.4 – 8.6)	7.22	4.936 ± 0.67
Thuja plicata	100	19.84 (13.6 – 29.2)	11.8	5.2 ± 1.04
Pseudotsuga menziesii	30	-	-	-
Abies grandis	70	38.6 (30.4 - 49.6)	9	5.06 ± 0.84
Bursera graveolens	100	21.358 (13.58 – 29.8)	0.186	7.38 ± 2.096

*LC_{50} values (concentration causing 50% mortality); 4-5 concentrations (15-100 µL.L^{-1} air) were used to calculate the LC_{50} values; n= 3 replicates of 30 insects

Table 5: LC_{50} values and the corresponding CI (confidence intervals) for oils through introduction of *S. oryzae* on treated grain

Essential oils	LC_{50} (CI)* (mg/kg wheat)	LC_{50} (CI)* µL.g^{-1}	X^2 (calculated)	Slope ± Stand. Error
Rosmarinus officinalis	3.04 (2.64 - 3.43)	0.304 (0.264 -0.343)	2.26	5.66 ± 0.85
Gaultheria procumbens	2.35 (2.02 - 2.67)	0.235 (0.202 – 0.260)	0.35	5.75 ± 0.85
Bursera graveolens	6.17(5.30 – 6.50)	0.617 (0.530 – 0.650)	7.86	6.29 ± 2.05
Thuja plicata	5.07 (4.42 - 5.66)	0.507 (0.442 – 0.566)	1.98	7.22 ± 1.11
Abies grandis	7.53 (6.60 - 8.55)	0.753 (0.660 – 0.855)	0.33	4.97 ± 0.77

*LC_{50} values (concentration causing 50% mortality); 4-5 concentrations were used to calculate the LC_{50} values; CI= confidence interval

n= 3 replicates of 30 insects

Concentration = amount of EO/ amount of wheat = mg EO/kg wheat

7. References

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