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# USE OF *EUCALYPTUS* ESSENTIAL OILS AS FUMIGANT FOR THE CONTROL OF THREE STORED FOOD LEGUME WEEVILS

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## ABSTRACT

The cowpea weevil *Callosobruchus maculatus* (Fabricius, 1775), the lentil weevil *Bruchus lentis* (Frolich, 1799) and the broad bean weevil *Bruchus rufimanus* (Boheman, 1833) are the most devastating and destructive primary insect pests of stored food legumes in Tunisia.

Control of these pests around the world primarily depends upon applications of organophosphorus, pyrethroid insecticides and the fumigants (methyl bromide and phosphine). These still the most effective treatments for stored food protection from insect infestation. Nevertheless, undesirable effects on non-target organisms, and fostered environmental and human health concerns were evoked. Thus, biological control using plant extracts mainly essential oils were investigated as an alternative to these chemicals. In this respect, the genus *Eucalyptus* is well known to possess various insecticidal activities including its fumigant action. The present work was carried out to investigate chemical composition and fumigant toxicity of two *Eucalyptus* essential oils namely *Eucalyptus camaldulensis* and *Eucalyptus leucoxylon* against adult of *C. maculatus, B. lentis* and *B. rufimanus*.

The GC-MS analyses showed that chemical composition varied with Eucalyptus species. The three essential oils contained α-pinene, α-terpineole and 1,8 cineole as major common compounds. Results demonstrated that fumigant toxicity varied with insect species, essential oil concentration and exposure time. At the lowest tested dose 26.31µl/l air, *B. lentis* is more sensitive than *C. maculatus* and *B. rufimanus*. *E. camaldulensis* and *E. leucoxylon* oils exhibited 100% mortality after 30 hours of exposure. At the highest dose 131.51µl/l air, *E. camaldulensis* oils achieved 100% mortality respectively after 6h for *B. lentis,* 30 h for *C. maculatus* and 24h for *B. rufimanus*. *E. camaldulensis* essential oil was more toxic against *B. lentis, C. maculatus* and *B. rufimanus*. $LC_{50}$ values were respectively 19.87, 24.83 and 36.13µl/l air. The results suggested that *Eucalyptus* essential oils may have potential as a control agent against these stored product weevils.

**Key words**: Food legume, Fumigation, weevil, Bruchidae, *Eucalyptus*

## INTRODUCTION

Recently, there has been a growing interest in research concerning the possible use of plant extracts as alternatives to synthetic insecticides. Essential oils are among the best-known substances tested against insects. These compounds may act as fumigants (Risha et al., 1990; Rice and Coats, 1994; Renault-Roger and Hamraoui, 1995; Shaaya et al., 1997) and may affect some biological parameters such as growth rate, life span and reproduction (Gunderson et al., 1985; Stamopoulos, 1991; Saxena et al., 1992; Renault-Roger and Hamraoui, 1995). Most of these substances were tested against insects attacking stored products in order to establish new control practices with lower toxicity and low persistence in the environment. In fact, management of stored product pests, using substances of natural origin, is nowadays a major research issue. Among the insects attacking stored products, Bruchidae and especially *Callosobruchus maculatus* (F.), *Bruchus lentis* Froelich and *Bruchus rufimanus* Boheman have attracted the attention of many scientists not only because they can easily be manipulated but also because of economic importance they have. The present work was carried out to investigate chemical composition and fumigant toxicity of two *Eucalyptus* essential oils namely *E. camaldulensis* and *E. leucoxylon* against adult of *C. maculatus, B. lentis* and *B. rufimanus*.

## MATERIALS AND METHODS

### Insect rearing

*C. maculatus* and *B. lentis* were reared in the laboratory respectively on chickpea and lentil grains. Insects were maintained at a temperature of 30.0 ± 2ºC and a relative humidity of 75.0± 2%. *Bruchus rufimanus* adults were obtained from bean fields.

### Essential oil extraction

The essential oils were extracted by water steam distillation using a Clevenger apparatus from leaves of *E. camaldulensis* and *E. leucoxylon* collected from natural populations at the flowering stage on November 2009 from the arboretum of Korbous (North Tunisia).

### Chemical analysis

The essential oils composition was analyzed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm; Agilent-Technologies, Little Falls, CA, USA).

### Fumigant toxicity

To assess fumigant toxicity, 2 cm diameter filter papers (Whatman No. 1) were impregnated with three oil doses: 1, 3 and 5 µl giving 26.31, 78.95 and 131.51 µl/l air in 38 ml Plexiglas bottles. The impregnated filter paper was then attached to the screw caps of the bottles. Ten adult insects one day old were used in each replicate. Each treatment and control was replicated five times. Mortality was recorded hourly until death. The mortality was calculated using Abbott's correction formula (Abbott, 1925) and probit analysis was used to calculate $LC_{50}$ values (Finney, 1971).

## RESULTS AND DISCUSSION

**Essential oils composition**

Oil yields based on dry matter weight were respectively 1.42% for *E. camaldulensis* and 0.61% for *E. leucoxylon*. GC-MS analysis of *E. camaldulensis* and *E. leucoxylon* essential oils is reported in Table 1.

A total of 90.96% from the constituents of *E. camaldulensis* essential oil were identified (Table 1). Among them 23.38% were monoterpene hydrocarbons, 27.93% oxygenated monoterpenes, 2.14% oxygenated sesquiterpenes and 16.54% sesquiterpene hydrocarbons. The major compounds were α-pinene (17.75%), 1,8-cineole (15.52%), Spathulenol (12.55%) terpinene-4-ol (6.84%) and γ-terpinene (5.63%). Regarding *E. leucoxylon* essential oil, results showed that 91.95% of the constituents were identified (Table 2). Among them 21.73% were monoterpene hydrocarbons, 31.23% oxygenated monoterpenes, 20.22% oxygenated sesquiterpenes and 4.04% sesquiterpene hydrocarbons. Major compounds were α-pinene (25.51%), camphene (5.22%), 1,8-cineole (15.64%), α-terpineol (6.08%) and globulol (14.38%). 1,8- cineole which is the major compound of the two essential oils, is recognized to be toxic to several insect species (Batish et al., 2008).

Table 1. Chemical fractions, others constituents and total identified compounds of the essential oil obtained from leaves of *E. camaldulensis* and *E. leucoxylon* (%)

| N° | Compound | *E. cam. %* | *E. leuc. %* | KI |
|---|---|---|---|---|
| Monoterpene hydrocarbons | | 23.38 | 25.73 | |
| 1 | α-pinene | 17.75 | 20.51 | 939 |
| 2 | γ-terpinene | 5.63 | 2.3 | 1075 |
| Oxygenated monoterpenes | | 27.93 | 31.23 | |
| 3 | 1,8-cineole | 15.52 | 15.64 | 1084 |
| 4 | Camphene | ---- | 5.22 | 1206 |
| 5 | Terpinene-4-ol | 6.84 | 3.57 | 1217 |
| Sesquiterpene hydrocarbons | | 2.14 | 4.04 | |
| 6 | Aromadendrene | 2.14 | 4.01 | 1468 |
| Oxygenated sesquiterpenes | | 16.54 | 20.22 | |
| 7 | Spathulenol | 12.55 | ---- | 1618 |
| 8 | Viridiflorol | 3.28 | 4.9 | 1624 |
| Other compounds | | 11.33 | 10.73 | |
| Total | | 90.96 | 91.95 | |

Table 2. $LC_{50}$ values calculated for mortality within 24 h of exposure of *C. maculatus, B. rufimanus* and *B. lentis* adults to *E. camaldulensis* and *E. leucoxylon* essential oils

| | *E. camaldulensis* | *E. leucoxylon* |
|---|---|---|
| *C. maculatus* | 24.87 ( 11.80 - 34.85) | 28.85 |
| *B. rufimanus* | 36.13 (28.06 - 43.03) | 95.32 (76.55 - 145.08) |
| *B. lentis* | 19.87 (8.90 - 27.38) | 20.71 (9.10 - 28.82) |

**Fumigant toxicity**

Results presented as percentage of mortality of adults after 24 h showed that essential *E. camaldulensis* and *E. leucoxylon* oils were toxic to *C. maculatus, B. rufimanus, B. lentis* adults (Fig. 1). These results corroborate the findings of Mahfuz and Khalequzzaman (2007) who reported the toxic effect of *Eucalyptus* essential oil on *C. maculatus*. Moreover, *E. camaldulensis* was more toxic than *E. leucoxylon* for all the concentrations and *B. lentis* was the most sensitive species. At the lowest concentration (26.31 µl/l air), adults of *B. lentis* all died after 24h of exposure for *E. camaldulensis* compared with respectively 66.66% and 80% mortality of *C. maculatus* and *B. rufimanus*.

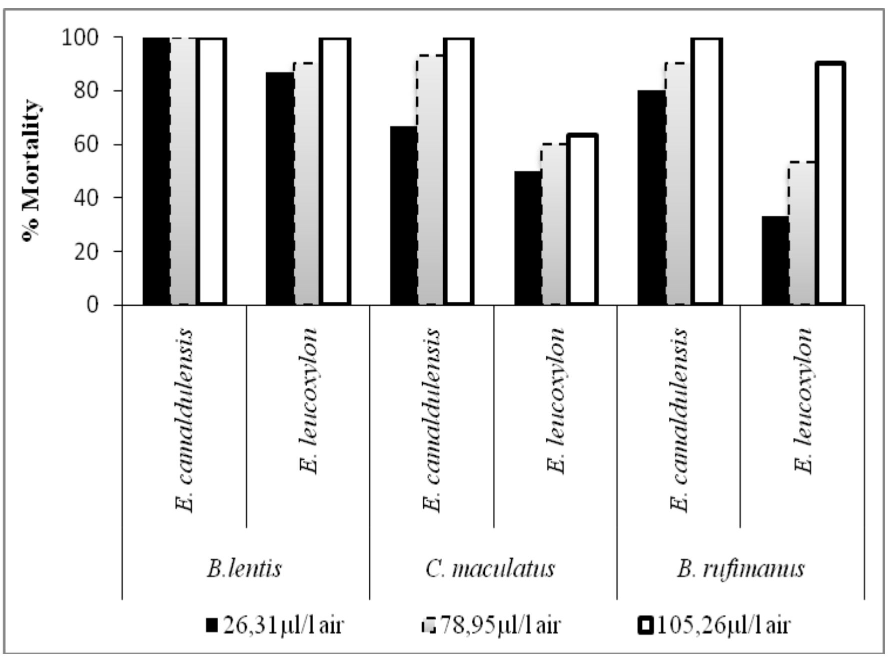


Fig. 1- Percentage of adult mortality after 24 hours exposure

For the concentration 78.95 µl/l air, *B. lentis* all died after 18h of exposure with *E. camaldulensis* and *E. leucoxylon* oils compared with 93.33% and 90% mortality of respectively *C. maculatus* and *B. rufimanus* after 24 h of exposure with *E. camaldulensis* (Fig. 1).

Probit analysis showed that *B. lentis* was more sensitive to *E. camaldulensis* essential oil than *C. maculatus* and *B. rufimanus*. The corresponding $LC_{50}$ values were 19.87, 24.83 and 36.13 µl/l air, respectively (Table 2).

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