# EVALUATION OF GENOTOXIC EFFECT OF LAVENDER (LAVANDULA SPP.) ESSENTIAL OIL

**E. STURCHIO [1], P. BOCCIA [1], M. ZANELLATO [1], R. MANCINELLI [2], E. CAMPIGLIA [2] and A. CAVALIERI [2]**

[1] Department Industrial Installations and Interaction with the Environment (DIPIA), National Institute of Health and Safety at Work (ISPESL), Via Urbana 167, 00184 Rome, Italy,
[2] Department of Crop Production, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy
e-mail: elena.sturchio@ispesl.it

## EXTENDED ABSTRACT

Research has increased its effort to find out new and alternatives strategies in weeds control more friendly for the environment and human health. A possibility is to develop natural herbicides taking the advantage of allelopathic effect exerted by some plants. Evidence for allelopathic interactions in nature by plants containing volatile allelochemicals as been described frequently. This ecological phenomenon has been highlighted in many aromatic plants in their natural enviroments. Lavender essential oil were studied as an alternative strategy to the herbicides, usually overused.
In the last decade the most interesting aspect of genotoxicity assessment is its application to environmental toxicology.
The aim of the study was to develop biomarkers of genotoxicity to detect potential DNA damage due to the use of lavender essential oil. The genotoxic effects were evaluated on *Vicia faba* seedlings by two different short-term tests: Comet assay and Micronuclei test. The phytotoxicity was also investigated by the reduction in length of the primary root of *Vicia faba* seedlings.
In previous works, the Micronuclei test on this plant has been successfully used for detecting genotoxic damages in seedlings growth on a sandy soil added with different doses of chemicals from different starting materials.
*Vicia faba* was chosen as test plant because it has large chromosomes suitable for the study of chromosome aberrations and micronuclei in root tip cells following the mitotic division.
The results showed that there is not a dose-dependent relation between oil concentration and genotoxicity while at the higher concentrations was observed an increase of the phytotoxicity. This preliminary screening suggests that the essential oil could be useful as potential bioherbicides as an alternative strategy.

**Keywords**: *Vicia faba, Lavandula*, essential oil, Comet assay and Micronuclei test, Phytoxicity

## 1. INTRODUCTION

Modern and specialised agricultural systems relay productive function only by adding large auxiliary energy inputs such as synthetic fertilisers, pesticides, etc (1;2). To correct these negative tendencies it is necessary to return to environmentally friendly agriculture.
Due to the ban of synthetic herbicides in organic farming, research has increased its effort to find out new and alternatives strategies more friendly for the environment and human health (1). Among the new strategies, natural compounds have been screened for

developing natural herbicides. These natural compounds in fact relay their effect on allelopathy which is defined as “the direct or indirect effect of one plant upon another through the production of chemical compounds that escape into the environment” (3).
Essential oils are less hazardous to the environment and human health than synthetic pesticides (4). These are mixtures of different volatile aromatic compounds extracted by stream or hydrodistillation from plants.
Preparations of essential oils have been applied in pharmacology, medical microbiology, phytopathology, and food preservation (5), due to their antifungal, antimicrobial and insecticides properties.
The use of essential oils to control pests is gaining attention because of the increasing public concern over the level of pesticide residues in foods (6).
While the bioherbicide effect has been already demonstrated (7; 8; 9) has not been sufficiently investigated the possible consequence that these compounds could have on the main crop species.
In previous study lavender (*Lavandula* spp.) among the essential oils tested, have exerted a good inhibitory effect on several weed (7; 8; 9).
The oils are complex mixtures of several chemical compounds including terpenes, alcohols, aldehydes and phenols. Lavender oil, obtained from the flowers of *Lavandula angustifolia* (Family: Lamiaceae) by steam distillation, is chiefly composed of linalyl acetate (3,7-dimethyl-1,6-octadien-3yl acetate), linalool (3,7-dimethylocta-1,6-dien-3-ol), lavandulol, 1,8-cineole, lavandulyl acetate and camphor (10).
We have studied the possibility of using the short term tests of genotoxicity (Comet assay and micronuclei test) as methods for detecting potential DNA damage due to the use of lavander essential oil.
The evaluation of the genotoxic effects was performed in root meristems of *Vicia faba*.
*Vicia faba* was chosen as test plant because it has large chromosomes amenable to the study of chromosome aberrations in somatic cells during mitotic division and as micronuclei in root tip cells following the mitotic division.
The comet assay on plants has become a useful method for the assessment of the environmental and experimental genotoxic impact. The assay is ideal to detect DNA damage because of its high sensitivity and specificity and because it is a non-invasive technique. It may complement other test systems measuring different endpoints of genotoxicity (11).

## 2. MATERIALS AND METHODS

### 2.1. Phytotoxicity

*Vicia faba* seeds were sowed in 500 gr of sandy control soil in aluminium basins, and were allowed to germinate in climatic chamber at 20º C ±1 for 5 days.
Each basin containing 50 seeds/basin was treated with a different concentrations (0%v/v; 0.02%v/v; 0.04%v/v; 0.06%v/v; 0.18%v/v; 0.54%v/v) of oil in water-emulsion of *Lavandula* spp. (120 ml of $H_2O$). Basins wetted only with water were used as negative controls. Three slides were evaluated per treatment and each treatment was repeated three times. In effort to permit the exchange of oxygen and $CO_2$ but not the volatilisation of the essential oil, each basin was sealed with laboratory film (Parafilm® M).
The seedlings were taken out and the phytotoxicity was calculated by measuring the primary roots length of *Vicia faba* seedlings exposed to the essential oil.

### 2.2. Genotoxicity

**Micronuclei Test**: The root tips of *Vicia faba* were fixed in ethyl alcohol and acetic acid 3:1 (v/v). The Feulgen method was used for staining. Micronuclei are Feulgen positive corpuscles, localised within the cell wall in the cytoplasmatic area surrounding the main nucleus. MC are formed by chromosome or chromosome fragments that are not incorporated into daughter nuclei at the time of cell division. The genotoxic effects were

evaluated by following the frequency of micronucleated cells in root meristems. The micronucleated cells frequency was scored from 15.000 cells (12 root tips, 400-500 cells for tip) (12). For each experimental group, means and standard deviations were obtained by Analysis Of Variance with Dunnett test significant at 0.05 level, that has been used for analysing the significant differences among the treated groups and negative control.
The **Comet test** was performed under alkaline unwinding/alkaline electrophoresis (A/A) protocol by Angelis K. et al (13). Briefly, root tips were chopped using a razor blade, the suspension with released nuclei was filtered through a 20 µm filter to remove most of the tissue debris. 50 µl of the filtrate were mixed with agarose and set on a microscopic slide. Nuclei embedded in agarose, were lysed for 1 hour and a half and then the slides were placed in the electrophoresis buffer at pH>13 for 40 min, before electrophoresis. The protocol was modified in the electrophoresis applying 300 mA, 25 V for 45 minutes instead of 30V for 20 min (14). Slides were stained with ethidium bromide (5µg/ml) and comets were viewed by an epifluorescence microscope and analyzed with the IAS 2000 analysis system (Delta Sistemi). The % DNA was used as a parameter of DNA damage (µm). For each experimental point, median and standard errors were calculated for at least 25 randomly chosen nuclei.
Data were analysed by ANOVA. If in a one-way analysis of variance test a significant F-value of $P< 0.05$ was obtained, a Dunnett's multiple comparison versus the control group analysis was conducted, using the statistical software SPSS (Chicago, IL).

## 3. RESULTS

The primary roots of germinated seeds exposed to essential oil were shorter than that of control seed. The results showed a phytotoxicity effect at all the concentrations tested.
Genotoxicity tests (Comet and Micronucleus assays) show that the higher concentrations of *Lavandula* spp. essential oil (0.18%v/v; 0.54%v/v) caused a significative DNA damage. At lower concentrations (0.02%v/v; 0.04%v/v; 0.06%v/v) the results showed an increment of DNA damage only at 0.02%v/v concentration, then there is not a dose-dependent relation between oil concentrations and genotoxic effect.

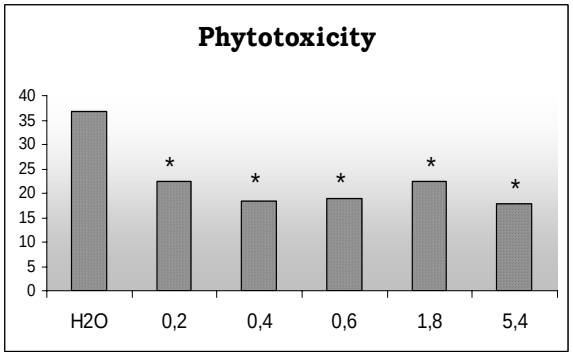


***Figure 1*** Phytotoxicity was calculated by measuring the roots length of *Vicia faba* seedlings exposed to the essential oil. An asterisk show significant differences with

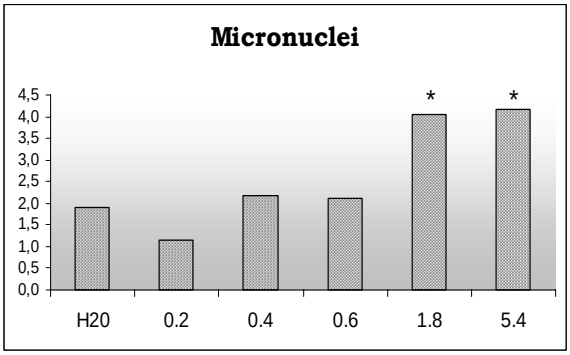


***Figure 2*** Frequency of micronucleated *Vicia faba* cell exposed to essential oil. An asterisk show significant differences with $P<0.05$.

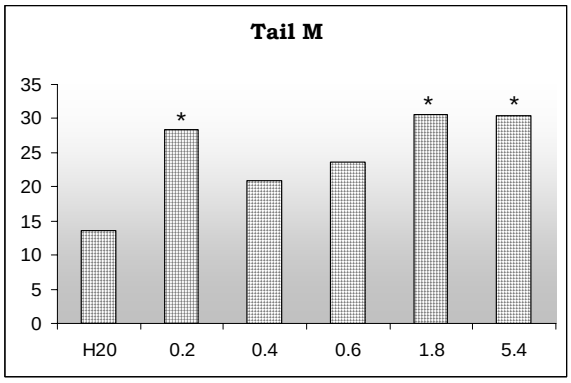


***Figure 3*** Comet test on *Vicia faba* cells An asterisk show significant differences with P<0.05.

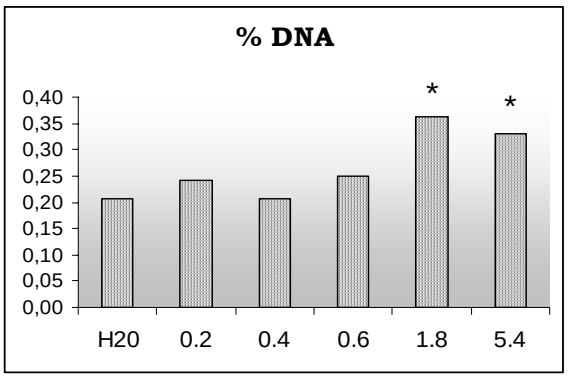


***Figure 4*** Comet test on *Vicia faba* cells (%DNA in the Tail) *a*n asterisk show significant differences with P<0.05.

## CONCLUSIONS

Lavender oil is considered to be one of the mildest of known plant essential oils. Concerns are building about the potential for irritant or allergenic skin reactions and cytotoxic effect with the use of lavender oil (10).

The acute toxicity of the lavender essential oil to Microtox luminescent bacterium (Vibrio fischeri) also was determined (data not show). The EC(50) values obtained were 1,4 %v/v corresponding to the significative genotoxicity results.

In previous studies, lavender (*Lavandula* spp.), among the essential oils tested, have exerted a good inhibitory effect on several weed (7). Essential oil inhibit both germination and cotyledon growth depending on concentration. The germination rate decrease with the increase of concentration; a lethal dose (0.54%v/v) able to inhibit totally the seeds germination was detected.

The results of this research showed a phytotoxicity effect at all the concentrations tested, while genotoxicity tests showed that the higher concentrations of *Lavandula* spp. essential oil (0.18%v/v; 0.54 %v/v) caused a significative DNA damage.

Furthermore, comet test results showed an increment of DNA damage at 0.02%v/v concentration. The specific molecular mechanisms of essential oils action are not well-understood because of the limited data reported in the literature. Probably, the initial genotoxic damage observed at 0.02% v/v concentration could lead to the activation of the cellular repair mechanisms at 0.04 and 0.06% v/v concentration.

The comet assay is a sensitive, rapid and economic technique for the detection of DNA damage, which is ideally suited as a biomarker of genotoxicity.

The comet assay seemed to be more sensitive than the micronuclei test to assess the DNA damage induced by chemicals.

In this study we have examined the first step towards a possible practical application of the essential oil, but further studies are still required to apply this technique to agriculture.

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