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# Early physiological response of broccoli leaf to foliar application of clove oil and its main constituents

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ABSTRACT

Clove oil and its principal constituent, eugenol, display herbicidal contact action on annual broadleaf weeds. In the present study, we examined the early effects of clove oil (CL) and its main constituents: eugenol (E), eugenol + humulene (E + H) and eugenol + caryophyllene (E + C) on the physiological state of broccoli leaves during the first 60 min after application. We also addressed the hypothesis that synergism exists between the main components of clove oil and is responsible for its phytotoxic effect. The response of plant tissues was recorded using isothermal calorimetry (TAM III) during the first 60 min and chlorophyll *a* fluorescence (FluorCam) imaging at three time points: 20, 40 and 60 min after application. The changes in three fluorescence parameters were visually analyzed: $QY_{max}$-maximum photochemical efficiency of photosystem II, NPQ–non-photochemical quenching, and Rfd-fluorescence decrease ratio. All of the treatments caused a decrease of both heat emission and chlorophyll *a* fluorescence. The visible physiological changes in broccoli leaves were observed as early as 20 min after treatments. Based on a multiple regression analysis, we demonstrated a strong correlation ($R^2$ = 0.81–0.96) between heat emission and $QY_{max}$, as well as heat emission and Rfd. The strongest phytotoxic effect was observed for CL. We did not observe the existence of synergism between eugenol and any of the other main constituents, a phenomenon believed to increase phytotoxic effects.



## 1. Introduction

Clove oil and its principle constituent eugenol are the components of several natural herbicides used in weed control, which are registered in the US market (Copping and Duke, 2007; OMRI, 2013). Clove oil shows a contact action on weeds, particularly annual weeds. Its effect is strongly dependent on the species of plant (Boyd and Brennan, 2006), its development phase, thickness of the cuticle on the leaf surface (Bainard et al., 2006), and atmospheric conditions at the time of application (Brainard et al., 2013). All of the aforementioned factors modify the effectiveness of clove oil applied in field conditions (Brainard et al., 2013; Johnson et al., 2013).

The physiological effects of clove oil have been extensively studied. Only Park et al. (2011) compared the effects of its application on cucumber seedlings with that of paraquat herbicide. They found that in as little as one hour after the herbicide was applied, seedlings wilted and there was a significant loss of water in their tissues. These effects were independent of the presence/absence of light. Clove oil stimulated superoxide dismutase (SOD) activity but decreased catalase activity (Park et al., 2011).

Eugenol, a principal component of clove oil, is most responsible for the herbicidal effects of clove oil (Stokłosa et al., 2012). In a series of bioassay treatments Ahuja et al. (2014) showed that eugenol inhibits root growth in wild oat (*Avena fatua*) seedlings through the generation of reactive oxygen species-induced oxidative damage.

The herbicidal effect of clove oil is, however, stronger than the effect of eugenol applied in its pure form in concentrations equivalent to its proportion in the oil. This finding suggests the existence of synergism between particular constituents of clove oil. Apart from eugenol, two other main constituents of clove oil are β-caryophyllene and α-humulene (Jirovetz et al., 2006). Stokłosa et al. (2012) found that, when applied on leaf surfaces, and in proportions corresponding to those in oil, these two compounds did not exert a phytotoxic effect. It was only when they were applied in concentrations markedly exceeding their percentage proportions

*Abbreviations:* CL, clove oil; E, eugenol; H, α-humulene; C, β-carryophyllene; HE, heat emission; PSII, photosystem II; QY, $_{max}$maximum quantum yield of PSII photochemistry; Qp, photochemical quenching; NPQ, non-photochemical quenching; Rfd, fluorescence decrease ratio.

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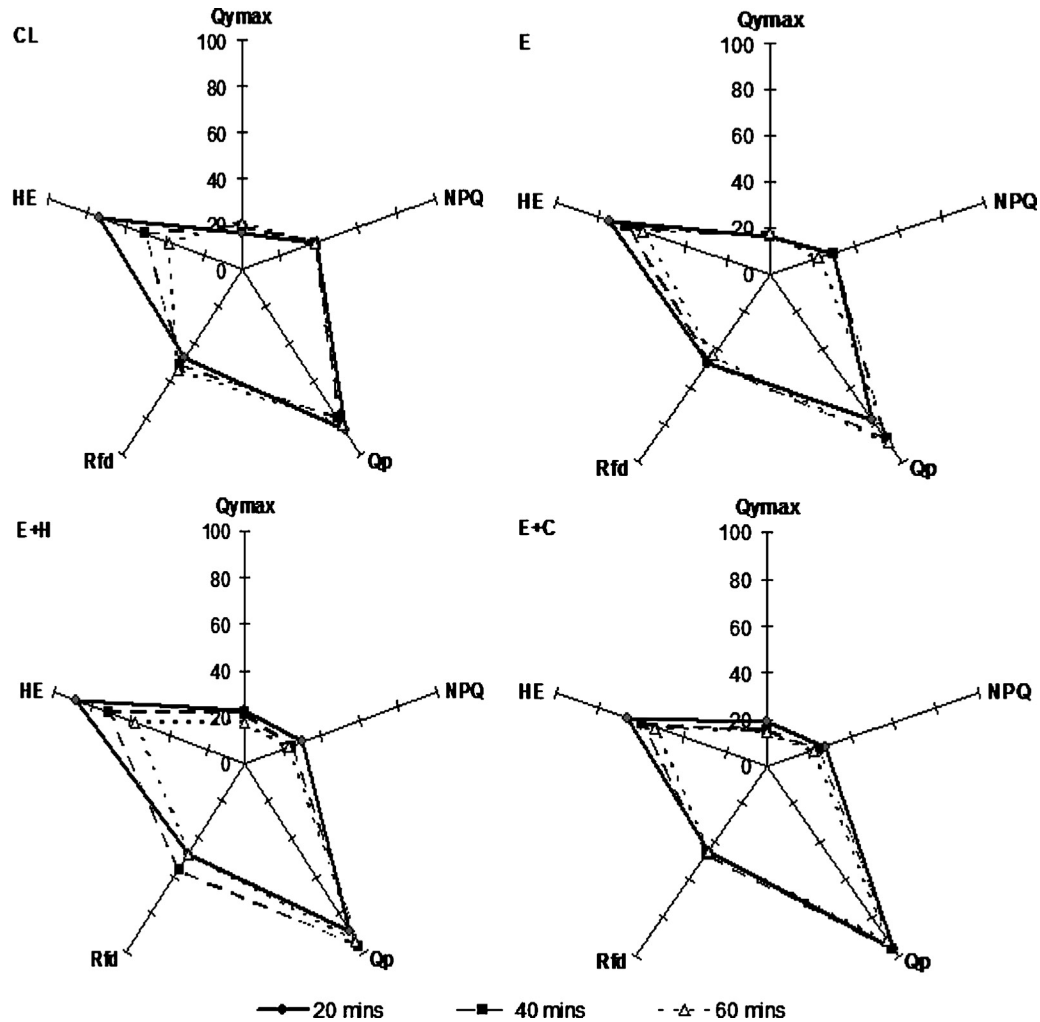


**Fig. 1.** The percentage values, as per control treatment (100%), of all the physiological parameters tested in the experiment.
Abbreviations: CL-clove oil; E-eugenol, E + H-eugenol + humulene; E + C-eugenol + caryophyllene; HE-heat emission; $QY_{max}$- maximum quantum yield of PSII photochemistry, Qp-photochemical quenching, NPQ-non-photochemical quenching, Rfd-fluorescence decrease ratio.

in the clove oil that they produced visible effects (Stokłosa et al., 2012).

For the above reasons, it is interesting to analyze and compare the early physiological effects of eugenol both alone and in combination with each of the remaining constituents of clove oil, compared with the effect produced by eugenol alone. Moreover, it is of interest to determine the dynamics of the herbicidal action induced by clove oil, i.e., the timing of the appearance of the first visible changes on leaves, as well as the rate of deterioration. This timing may be of importance during the foliar application of clove oil, which can be modified by weather conditions, i.e., lower relative humidity causes a decrease in the herbicidal potential of clove oil (Brainard et al., 2013).

Modern analytical equipment makes it possible to monitor the course of early changes in plants, both at the level of total metabolism, as well as at a level of specific physiological processes. In recent years, the isothermal calorimeter has become a useful tool to show the outflow of heat from plant tissues, or even whole organisms, that are subjected to various types of stress, both abiotic (Baltruschat et al., 2008; Schabes and Sigstad, 2005; Stokłosa et al., 2006;) and biotic (Janeczko et al., 2007; Troć et al., 2011; Skoczowski et al., 2011). Another useful parameter is the level of chlorophyll *a* fluorescence. Various types of fluorimeters used to measure continuous and modulated fluorescence, i.e., switched on and off with a certain frequency, together with a multitude of fluorescence indicators (Kalaji et al., 2014; Schreiber et al., 1995), can precisely characterize the changes that occur in photosynthetic apparatus, being the most sensitive indicator of stress in plants (Brestic et al., 2015; Lichtenthaler, 1988). The method of image analysis of chlorophyll fluorescence overcomes the limitations of point-measurements of chlorophyll fluorescence, and comprehensively characterizes the plant material (Lenk et al., 2007; Nedbal et al., 2000; Scholes and Rolfe, 2009).

In the present study, we investigated the temporality of the herbicidal action of clove oil and its main constituents, based on the physiological tests listed above (isothermal calorimetry and the imaging of chlorophyll *a* fluorescence). Additionally, we evaluated the hypothesis that synergism between the main constituents of clove essential oil exists and is responsible for the herbicidal effect. The above problems were addressed using the physiological state of leaves of broccoli (*Brassica oleracea* L.), a model plant.

## 2. Materials and methods

Laboratory studies were carried out on the second fully developed leaf of broccoli *(Brassica oleracea* L.*)* cv. 'Ironman F1', at the 3–4 leaf stage. Broccoli was chosen as a model plant because of its large leaf blade and the presence of a thick cuticle on the leaf