Herbicidal activity of *Citrus limon* essential oil extracted from Sicilian lemon industry

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Weeds could reduce yield of annual crops by up to 70%. The main features of these species include high seed production, easily seed dispersal, variable degrees of seed dormancy and high competitivity by rapid seedling growth and higher growth rate than crops. Weed management is a significant challenge faced for crop yield and quality since they compete for water, light, soil nutrients and space. Environmental constraints of crop production systems have stimulated interest in alternative weed management strategies. In fact, the continued use of synthetic herbicides may threaten sustainable agricultural production and has resulted in serious ecological and environmental problems, such as the increased incidence of resistance in weeds to important herbicides and increased environmental pollution and health hazards. Public awareness and demand for environmentally safe herbicides with less persistence and less contaminating potential lead to the search of new weed control strategies.

The European legislation also supports weed control by means of Integrated Pest Management. Essential oils (EOs) are natural plant products, biodegradable, that contain natural flavours and fragrances. Some of them are classified as Generally Recognized As Safe (GRAS) for ingestion by the U.S. Food and Drug Administration's (FDA).

In this work, the herbicidal potential of *Citrus limon* (L.) Osbeck essential oil from a Sicilian industry was tested against the weed species *Amaranthus retroflexus, Portulaca oleracea, Echinochloa crus-galli and Avena fatua*, which are among the ten worst annual weeds of the temperate agricultural regions of the world. The essential oil was obtained by cold pressing method; consist of crushing and pressing the peels thus leading to the formation of a water emulsion. Then, the emulsion is centrifuged to separate out the EOs. Since no external substances are needed, this process ensures that the resulting oils retain all their properties.

The experiments were performed in vivo, in greenhouse conditions during May, June and July 2018. The oil was applied to the weeds grown in pots (8x8x7 cm) in postemergence, irrigated and sprayed. The soil used for the experiment was collected from the topsoil (first 5 cm) of a citrus orchard non treated with herbicides. The soil was air-dried and sieved at 1 cm. Weed seeds were purchased from Herbiseed (England), and germinated in a germination-growth chamber, at 30 ± 0.1 °C, 16 h in light and 20 ± 0.1 °C, 8 h in dark for *A. retroflexus*, *P. oleracea* and *E. crus-galli*, while for *A. fatua* seeds the germination conditions were - 23.0 ± 0.1 °C, 8 h in light and 18.0 ± 0.1 °C, 16 h in dark. After a week of incubation, the weed seedlings emerged were placed on the pots, previously filled with 2 cm of perlite and 5 cm of soil. Ten replicates per treatment were prepared. Pots were placed in a greenhouse of the Universitat Politècnica of Valencia (UPV). In order to find the most effective dose of oil and its adequate mode of application, different concentrations were used: 12, 18, 24 and 30 µL/mL. Fitoil was used as emulsifier at a concentration of 0.05% (v/v).

Plants of *A. fatua* and *E. crus-galli* were treated at two-leaf stage (growth stage 12) and *A. retroflexus* and *P. oleracea* were treated at five-leaf stage (growth stage 15). To monitor the experiments, photos were taken after 24, 48 and 72 hours; and then once a week. The photos were processed with Digimizer software to take different data: efficacy of the treatment, level of damage, height of plants, root length.