Bioherbicidal ability and weed management of allelopathic methyl esters from *Lantana camara*

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Abstract

Allelochemicals are secondary metabolites which are not edible and can be used as growth regulators and bio-herbicides. The goal of current study was to assess allelopathic ability of *Lantana camara* (Sage-plant) flowers against weeds viz. *Avena fatua* (Wild oat), *Euphorbia helioscopia* (Sun-spurge), *Chenopodium album* (Goosefoot), *Phalaris minor* (Canary-grass), and *Rumex dentatus* (Knotweed). Bioassay analysis of three methanolic fractions of the Combi flash from *L. camara* was performed at 50%, 75% and 100% concentration using germination percentage parameters, inhibition of plumule and radicle size. The fraction II of Combi flash strongly suppressed all weeds with negligible effect on *T. aestivum*. Gas chromatography-mass spectroscopy was conducted for the fraction, and isolated compounds were used to perform bioassays. From fraction II GC–MS detected four methyl esters of allelopathic fatty acid viz. Methyl oleate, methyl palmitate, methyl stearate and methyl linoleate. The evaluation of physiological effects of the bioassay revealed substantial suppression of chlorophyll, antioxidant enzymes (superoxide, dismutase peroxidase) and protein material in all weeds by methyl palmitate. Bioassay activity and study of physiological parameters revealed that the effective bio-herbicidal compound in *Lantana camara* flowers is methyl palmitate. This is the first time that methyl palmitate (a fatty acid methyl ester) has been related to herbicidal activity in *L. camara* flowers. It is proposed that field studies based on hormesis research and the mechanism of action of this compound be carried out.

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1. Introduction

*Triticum aestivum* (Wheat) is one of the important cereal crops. Many factors including delayed sowing, water scarcity, less fertiliz-
losses of up to a remarkable economic threshold (Anwar et al., 2019b). Because of the limited resources and tracts available to farmers it seems very difficult to eradicate weeds completely in the region. Allelopathy is based on the fact that plants contain many chemicals that are harmful to the neighbouring species known as allelochemicals. Such chemical compounds are released into ecosystems by numerous processes including processes of exudation, leaching, and decomposition. Allelochemical substances may also be perfect agrochemicals.

*Lantana camara* (Verbenaceae) is an allelopathic plant. Allelochemicals are present in all parts of the shrub. When released in surrounding, these chemicals restrict germination of other species e.g. *Lemna paucicostata*, *Morrenia odorata*, *Eichhornia crassipes*, *Commelina benghalensis*, *Digitaria sanguinalis*, *Echinocloa colonaum*, *Panicum psilopodium*, *Microcystis aeruginosa*, *Abutilon theophrasti*, *Lepidium virginicum*, *Cyclosorus dentatus*, *Amaranthus hybridus*, *Parthenium hysterophorus* (Bais et al., 2006; Ambika et al., 2003; Kumar et al., 2011). The leaf, root and stem possess allelochemicals that suppress neighbouring plant germination and growth (Rusdy and Ako, 2017). Allelochemicals belong to a range of species, including phenolic, aromatic and alkaloid species, monoterpenes, triterpenes, sesquiterpenes, flavonoids, phenyl and iodinoid ethanoid glycosides (Ved et al., 2018). Such compounds display growth-inhibiting effects on germination and growth of adjacent plants through fluctuating microenvironments (Mishra, 2015; Murugesan et al., 2016; Saha et al., 2018).

*L. camara* is a dreadful weed that has a major negative impact on biodiversity (Choyal and Sharma, 2011). The toxins lantadene A and B found in its leaves and flowers make it unfit for ruminant herbivory. Because of the allelopathic effect of its root leachate, this weed stunts neighboring plants’ growth. When favorable conditions prevail, the seeds germinate. Pruning increases the density of the thicket. So far, almost every attempt to eradicate this plant has failed (Patel, 2011). So, management of this weed by utilization is required (Lakshmi and Sekhar, 2018). Recent studies have reported that *L. camara* improves soil quality by enriching it with nitrogen, exhibits termitcidic effect, acts as lignocellulosic substrate for cultivation of edible mushrooms, acts as potential insecticide and fumigant for grains storage against weevils, antifungal agent, and herbicide against water hyacinths. *L. camara* has bioactive ingredients exhibiting anticancer, anti- ulcerogenic, hypolipidemic, larvicidal and anti-inflammatory activity (Sharma et al., 2003).

Essential oils (EOs) can be used to successfully suppress weeds. According to recent research. These chemicals provide a viable commercial alternative for organic weed control. Fatty acid methyl esters (FAMES) are used as adjuvants in a number of consumer products based on the herbicidal action of EOs (Wang et al., 2015). Since FAMES have a strong affinity for fatty compounds, they can easily pass through the plant cuticle. When FAMES are added to a tank mixture, the herbicidal effect of commercial herbicides is greatly increased (Synowiec et al., 2017). Jones has proposed a composition and method for destroying unwanted plants using fatty acids and fatty acid esters as herbicides and carriers for herbicides (2008). Such compositions may be used in areas of both desirable and undesirable plants to destroy the undesirable plants without damaging the desirable ones. FAMES have also been shown to have antibacterial and antifungal properties (Sati et al., 2017).

Methyl palmitate is a fatty acid methyl ester with a nonpolar aliphatic carbon chain and a polar carboxyl group. It’s a natural botanical compound found in a variety of plants (Qin et al., 2000; Goswami and Fernandes, 2003; Lin et al., 2005). A number of insects have been shown to use methyl palmitate as a semiochemical. Insect repellents containing methyl palmitate have been proposed. Methyl palmitate, on the other hand, appears to be healthy for vertebrates, as shown by its widespread use in food, medicinal, cosmetic, and industrial products (Wang et al., 2009). The amount of methyl palmitate that is safe and effective for humans has been confirmed to be in the range of 0.1–10 mg/kg body weight (Usha and Nazarine, 2003). Various phytotoxic mites are poisoned by this selection. Human skin was only slightly affected by methyl palmitate, and fatty acids could be used as a complement to animal feeds. In conclusion, methyl palmitate is a promising botanical miticide that appears to be a good candidate for commercialization and agricultural use (Wang et al., 2009).

Keeping the facts in view, the current analysis was planned to test *L. camara* allelopathic ability against weeds viz. *Rumex dentatus*, *Euphorbia helioscopia*, *Phalaris minor*, *Chenopodium album* and *Avena fatua*.

### 2. Methods

The research was planned to assess the allelopathic ability of *L. camara* against *Rumex dentatus*, *Euphorbia helioscopia*, *Chenopodium album*, *Phalaris minor* and *Avena fatua* weeds. *L. camara* flowers were collected from the Rawalpindi district (latitude 33°36'N and longitude 73°02'E), Pakistan. The collected sample was dried at 30 °C in shade and pulverized with a heavy-duty blender (2 mm mesh size). Ground sample (300 g) was macerated in 2000 ml methanol. An aliquot of rotary evaporated methanol extract (15 g) on the Combiflash column (CombiFlash™ RF + by Teledyne Isco) gave us three fractions. The allelopathic ability of three CombiFlash fractions was tested against weeds (*Rumex dentatus*, *Euphorbia helioscopia*, *Chenopodium album*, *Phalaris minor* and *Avena fatua*) using germination rate, radicle, and plumule length parameters at 100%, 75%, 50% concentration. An aliquot (15 ml) was poured on 25 g of soil per petri plate using methanol as control for each concentration of the CombiFlash fractions. Ten seeds of each research weed species were added to each Petri dish. The petri plates were set at 25 °C for 15 days in growth chamber (NTS, MI-255). With each plant, the percentage of germination, length of the plumule and radicle was determined. Five replicates of the experiments were carried out. Completely randomized design was applied in STATISTIX v. 9 and ANOVA along Fisher’s LSD test was applied for means separation.

**Purification of active CombiFlash fraction II by C-18.** An aliquot (0.2 g) of fraction II was chromatographed on a column of 2x60 cm silica gel (Silica gel mesh 70–230, Merck, Germany) eluted with (n)-hexane that had collective ethyl acetate amounts [20 percent per step, 300 ml per step]. On thin layer chromatography (Silica gel 60 G254; Merck) with a mixture of chloroform and acetic acid (90:10 v/v), the active fraction was eluted from the silica gel column (Ezhilan and Neelamegam, 2012). Residue dissolved in 20 per cent aqueous methanol (5 ml v/v) was loaded on C18 Sep-Pak cartridges in reverse phase (Waters Co., Milford, USA). High performance liquid chromatography was used for residue purification using 20, 40, 60 and 80 percent aqueous methanol.

**Gas Chromatography-Mass Spectrometry (GC–MS).** On Thermo GC-TRACE ultra ver. 2.2 (film thickness: 0.25 μm; DB 5-MS capillary standard non-polar column 30 Mts, ID: 0.25 mm) GC–MS was performed (Thermo Scientific Co.). The results were evaluated using National Institute of Standards and Technology (NIST) database mass-spectra.

2.1. Bio-Assay effect of isolated FAMES on seed germination and seedling growth

In mother solvent (methanol), the isolated compounds (methyl oleate, methyl stearate, methyl palmitate, and methyl linoleate) were dissolved. Ten seeds of each test species were placed on filter
paper (Whatman No. 1) in petri dishes (85 mm diameter). Assays for isolated compound were performed at 10 μM, 20 μM, 30 μM, 40 μM, and 50 μM. Wrapped with aluminium foil, the petri dishes were incubated in the dark at 28°C. After five days, germination, root and shoot-lengths were collected. All of the parameters were displayed as percentages. Non treated seeds were used as control. The student’s t-test analysed statistical differences between the treatments to determine the statistical significance of disparity between two sample means.

**Determination of chlorophyll contents.** One gram of the leaf material was ground in liquid nitrogen for test weed species followed by addition and centrifugation of 15 ml of 80 per cent acetone (extraction solution) (8,000 rpm). Readings were reported at 645 nm and 662 nm using a spectrophotometer (UV- 3802, UNIC, China) using 80 per cent blank acetone. The chlorophyll a, chlorophyll b and complete chlorophyll concentrations have been determined (Hanh et al., 2016):

\[
\text{Chl.a} = 11.24 \times \text{A661.6} - 2.04 \times \text{A644.8} (\text{mg/ml})
\]

\[
\text{Chl.b} = 20.13 \times \text{A644.8} - 4.19 \times \text{A661.6} (\text{mg/ml})
\]

Total Chl. Content = 4.0 × A661.6 + 18.09 × A644.8 (mg/ml)

**Quantification of peroxidase and superoxide dismutase contents.** For each test weed species, 0.2 g of leaf material was homogenized for 5 min at 4°C with 1 ml of 0.2 M perchloric acid, and then centrifuged (10,000 rpm) for 7 min. 0.2 M sodium hydroxide was used to change the supernatant’s pH to 7.5. An aliquot (100 μl) moved through a column of 0.4 ml at Dowex AG. The eluate was used for H₂O₂ assay. In 0.1 M ethylene diamine tetra acetic acid,
0.02 M sodium phosphate buffer (pH 7.8), 4% polyvinyl pyrrolidone and 0.2% Triton X-100, an aliquot (0.2 g) of test plant leaves was homogenised. Solutions have been filtered and centrifuged for 20 min (10,000 rpm). Superoxide dismutase was measured using standard method for separating isoenzymes from superoxide dismutases. On 7.5% acrylamide gel, non-denaturing polyacrylamide gel electrophoresis was performed. Superoxide dismutase activity reduction protocol for Nitroblue Tetrazolium was introduced (Zuo et al., 2012).

**Determination of protein contents.** 1 g of leaf material was combined with 10 ml of 2% anhydrous sodium carbonate dissolved in 0.1 M sodium hydroxide for each test weed. Protein suspension (0.5 ml) was combined with 0.5 ml reagent (1 ml 0.5% Copper sulphate, 1 ml 1% sodium potassium tartate, and 48 ml 2% anhydrous sodium carbonate dissolved in 0.1 M sodium hydroxide). The mixture had been permitted to stand 15 min at room temperature. Folin-Ciocalteau (0.5 ml) reagent was combined with solution and left to stand at room temperature for 30 min. Protein solution absorbance was measured on spectrophotometer at 700 nm (UV-3802, UNIC, China) (Javed, 2011).

### 3. Results

Three fractions were constructed from L. camara flowers methanol extract via CombiFlash® RF+ from Teledyne Isco.
(the chromatogram is shown in Fig. 1). Fraction I consisted of test tubes 6-8, fraction II consisting of test tubes 9–11 and fraction III consisted of test tubes 12-17. The fractions were collected using the rotary vacuum evaporator Buchi-Rotavapor R-300 to remove solvent and subsequent aqueous extract was lyophilized in vacuum (Stellar C210 Tray Type Freeze Dryer). Finally, an aliquot was obtained of 301 mg (fraction I), 245 mg (fraction II) and 154 mg (fraction III). These fractions were tested against selected weeds for allelopathic bioassays. The three fractions of Combi flash impacted germination and seedling growth parameters of weeds with no noticeable effect on T. aestivum as shown in Fig. 2.

For fraction I, P. minor, C. album and A. fatua exhibited 55%, 57% and 58% germination inhibition respectively while germination inhibition of R. dentatus and E. helioscopia remained unchanged. Maximum germination was noted in E. helioscopia and R. dentatus (98 percent), while A. fatua displayed modest germination, i.e. 42 percent. P. minor, C. album and A. fatua demonstrated a radicle reduction of 51 percent, 52 percent and 54 respectively. For E. helioscopia and R. dentatus the maximum radicle length (98 percent) was observed. Likewise, for A. fatua, radicle length of 46 percent was found. C. album, P. minor and A. fatua reported a reduction of 56%, 56.5% and 57%, while E. helioscopia and R. dentatus...
remained unchanged. It was observed that *E. helioscopia* and *R. dentatus* showed the maximum (98 per cent) plumule growth. For *A. fatua*, the least plumule length (49 per cent) was observed. Seed germination of *E. helioscopia*, *R. dentatus*, *A. fatua* and *P. minor* was affected as 66 percent, 67 percent, 68 percent, and 69 percent, respectively, while *C. album* remained unchanged. The highest germination was noted for *C. album* (96 percent), while *P. minor* was the lowest, i.e. 31 percent. *P. minor*, *A. fatua* and *C. album* showed substantial suppression of the radicle length by 68%, 67% and 55% while *E. helioscopia* and *R. dentatus* remained unaffected. *E. helioscopia* and *R. dentatus* showed the maximum radicle length (97 per cent). The experiments for *A. fatua* reported slightly lower radicle length (32 per cent). Fraction II significantly repressed the plumule length of *A. fatua*, *C. album*, *E. helioscopia* and *R. dentatus* by 66%, 66%, 67%, and 69% respectively, while *P. minor* remained unaffected. *P. minor* was found to have the maximum plumule length (95 percent). *R. dentatus* was most prone in terms of total plumule length (31 per cent). Fraction III substantially suppressed *E. helioscopia*, *R. dentatus* and *A. fatua* germination by 55 percent, 57 percent and 59 percent compared to control while *P. minor* and *C.
album remained unchanged. For C. album and P. minor the highest germination (96 percent) was noted while for A. fatua it was lowest i.e. 41 percent. The bioassays for A. fatua reported slightly lower radicle length (32 per cent). Fraction II significantly repressed the plumule length of A. fatua, C. album, E. helioscopia and R. dentatus by 66%, 66%, 67%, and 69% respectively, while P. minor remained unaffected. P. minor was found to have the maximum plumule length (95 percent). R. dentatus was most prone with plumule length of 31 per cent. Fraction III substantially repressed E. helioscopia, R. dentatus and A. fatua germination by 55 percent, 57 percent and 59 percent compared to control while P. minor and C. album remained unchanged. For C. album and P. minor the highest germination (96 percent) was noted while for A. fatua, it was lowest i.e. 41 percent. For fraction II, highest suppression effects were noted, therefore, its characterisation was done. The GC–MS chromatogram and NMR analysis of fraction II showed four peaks with different retention periods (Figs. 3 & 4). Four allelopathic fatty acid methyl esters (FAMEs) were found, i.e., methyl palmitate (R.T: 9.13), methyl stearate (R.T: 11.87), methyl oleate (R.T: 12.27), and methyl linoleate (R.T: 13.08).

Pre-emergence bioassay with allelopathic fatty acid methyl esters (FAMES): The seed germination of Avena fatua, Chenopodium album, Euphorbia helioscopia, Rumex dentatus and Phalaris minor was inhibited by methyl oleate, methyl stearate, methyl palmitate and methyl linoleate in a dose-dependent manner.

Methyl palmitate has demonstrated the highest inhibition effects; physiological parameters (chlorophyll a & b, peroxidase content, and protein content) have also been tested for this compound. The effect of methyl palmitate on the content of chlorophyll a & b and total chlorophyll content is shown in Fig. 5. The chlorophyll content of P. minor, A. fatua, E. helioscopia, C. album and R. dentatus was substantially suppressed by 66%, 64%, 63%, 62% and 60%. For C. album, R. dentatus, A. fatua, E. helioscopia, and P. minor chlorophyll b content was suppressed by 63%, 64%, 66%, 67%, and 68% respectively. For P. minor, E. helioscopia, A. fatua, C. album, and R. dentatus chlorophyll content was suppressed by 67%, 68%, 65%, 63%, and 62% respectively.

The role of antioxidant enzymes and the suppression of protein content by methyl palmitate is shown in Fig. 6. A. fatua, R. dentatus, E. helioscopia and P. minor peroxidase content were suppressed by 60 percent, 61 percent, 64 percent, 65 percent and 67 percent respectively at T1, while for C. album, R. dentatus, E. helioscopia, A. fatua and P. minor peroxidase content was suppressed by 46 percent, 52 percent, 62 percent, 66 percent and 72 percent at T2. At T1, 62 percent, 63 percent, 64 percent, 66 percent, and 68 percent suppression of superoxide dismutase content was noted for R. dentatus, A. fatua, P. minor, E. helioscopia, and C. album respectively. Superoxide dismutase was 42 percent, 47 percent, 52 percent, 60 percent and 66 percent respectively at T2 for A. fatua, E. helioscopia, P. minor, R. dentatus and C. album. Methyl palmitate substantially suppressed E. helioscopia, A. fatua, R. dentatus, C. album and P. minor protein content by 61%, 63%, 64%, 67% and 68% respectively (Fig. 7).

4. Discussion

Plant allelopathy can have positive effects, e.g., in agricultural management, such as weed control, crop restore or crop protection. Allelochemicals may possibly be used as regulators for production, insecticides, herbicides and crop protection products. Here we appraised L. camara methanol extract from flowers as a source of natural herbicide against selected weed species viz. A. fatua, Chenopodium album, Phalaris minor, Euphorbia helioscopia and Rumex dentatus. The degree of phytotoxicity was dependent upon concentration. Past studies have reported that extract of methanol from L. camara suppress Raphanus sativus, Phaseolus mungo, Cicer arrietinum, Cucumis sativa, Brassica juncea, Eichhornia crassipes, Phaseolus mungo and Microcystis aeruginosa (Mishra, 2015). In our case, Combiflash fraction II from L. camara flowers had the greater inhibitory ability than fractions 1 and 3 (see section MM for the fractionation procedure). Methanol extracts of L. camara flowers depressed growth parameters, protein content, chlorophyll content and antioxidant enzymes in test species. Reduction of chlorophyll...
by phytochemicals is possibly due to degradation of chlorophyll, retardation in chlorophyll production along with photosystem II malfunction. Allelochemicals reduce chlorophyll content by 52%–62% in young leaves and 72%–92% in mature leaves (Biljana and Kragujevac, 2015; Anwar et al., 2018). In addition, phytochemicals produce reactive oxygen species (ROS) which cause Ca²⁺ signalling cascade leading to gene expression manipulation and shoot death (Nekonam et al., 2014). The effects of cinnamic acid on the ability of the enzyme to scavenge ROS, the rate of generation of ROS and subsequent growth in cucumber were recorded. Allelochemical stress is linked to ROS production and oxidative stress. Phytotoxicity enhances the potential for SOD which causes H₂O₂ deposition and increased membrane peroxidation (Zhang et al., 2018). Lantana leaf extract’s toxic potential is probably due to oxidative stress. L. camara leaf extract substantially suppressed POD activity. The POD is linked to biochemical and physiological activities such as cell formation, fruit production, ethylene biosynthesis and growth, and the response to environmental toxins and stresses (Zaytseva and Neumann, 2016). In Lycopersicon esculentum, Phaseolus vulgaris and Zea mays the substantial reduction in protein content is stated earlier by the leaf methanol extract of L. camara (Zuo et al., 2012). Allelochemicals inhibit cell division and cellular processes including enzyme activity, membrane permeability, respiratory and photosynthetic ETC retardation, ion uptake, protein damage, and cell death due to DNA (Li et al., 2018; Maiti et al., 2010). Allelochemicals change enzyme functionality. Reduction of the permeability of the cell membrane, protein formation, gibberellins and indole acetic acid caused by allelochemicals leads to reduced mitotic activity and growth rate (Alcântara et al., 2017). In recent years, analytics technology has enabled the identification of minute amounts of allelochemicals. GC–MS study of L. camara flowers methanol extract (CombiFlash fraction II) classified four methyl esters of fatty acids (FAMEs) as possible allelochemicals viz. Methyl linoleate, methyl stearate, methyl palmitate and methyl oleate. There is no earlier evidence of allelopathic ability of these compounds. From other plant species, including T. aestivum, Cucumis sativus and Echinochloa crusgalli, esters and long fatty acids with allelochemical properties are reported earlier (Cheng et al., 2016). At a concentration of 1000 μg/mL, methyl oleate and methyl linoleate are reported to inhibit radicle by 80% and plumule by 60%. Alpha-linolenic acid and linoleic acid (essential fatty acids) have been reported to be allelochemical compounds...
from *Typha domingensis* leachates (Nea et al., 2017). However, no phytotoxic activity of methyl palmitate from *Lantana* flowers has ever been identified. These compounds may have inhibitory effects on ATP generation and electron transport in chloroplasts and mitochondria, similar to other phytotoxins (Qureshi et al., 2021). Allelochemicals usually work through pathways that synthetic compounds don’t, making natural compounds a potential source of new herbicide leads. It is proposed that the action mechanism of an isolated compound be investigated as only a few of the hundreds of allelochemicals known have had their mode of action determined.

### 5. Conclusion

Allelochemicals have wide potential for enhancing crop production, defense and biocontrol. In the current study, allelochemical potential of methanol extract from flowers of *Lantana camara* was evaluated against selected weeds viz. *Phalallis minor*, *Chenopodium album*, *Avena fatua* and *Rumex dentatus*. Results provide evidence that methyl palmitate isolated from *L. camara* flowers has herbicidal potential. Studies are suggested on the degree and extent of the phytotoxicity of isolated compounds in agronomic conditions at different stages of development.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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