Screening of allelopathic activity of common weed species occurring in agricultural fields

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The investigation of allelopathic effects of different plant species is important to prevent serious crop losses that would arise. The usage of such materials as mulch, cover crops or residues will be useful to implement in integrated weed management strategies of agricultural fields. Therefore, this study was conducted to screen the allelopathic effect of selected plant species using seedlings growth of lettuce (Lactuca sativa L.) as an indicator. The sandwich method was used and 20 mg of dried plant material, resembling the leaf litter, from 27 species were placed in petri dishes. Twenty-eight treatments including the control treatment were arranged in a Completely Randomized Design (CRD). On the fifth day after the establishment of lettuce seeds, hypocotyl length (cm), radicle length (cm) and total height (cm) of seedlings were measured. The inhibitory percentage was calculated and a dendrogram with single linkage was developed. Results revealed that the lowest hypocotyl and radicle lengths with the highest inhibitory percentage were recorded for Ageratum conyzoides, Cassia occidentals and Clidemia hirta when compared to the control treatment (p<0.05). Weed species expressed varying degree of inhibitory effects on growth performances of lettuce seedlings. Further studies need to be carried out to explore the effects of allelopathy on crop plants.

Key words: Inhibitory percentage, lettuce, seedling growth, hypocotyl, radicle.

INTRODUCTION

Allelopathy is considered as an interference mechanism available to plants, which release chemicals that influence the growth and development of neighboring plants in both natural and agricultural ecosystems. This process happens through leaching, root exudation, volatilization, residue decomposition and other means by creating direct and indirect effects on the adjacent micro-environment due to (Khanh et al., 2007) chemical substances released by plants (Einhelling, 2008).

Allelochemicals results in harmful effects and is categorized into two, true allelopathy and functional allelopathy (Duke, 2015). The true allelopathy is the release of substances that are toxic in nature from its origin as they are produced in plants (Duke, 2015). Functional allelopathy is the release of toxic substances, which are resultants of transformation by microorganism (Inderjit et al. 2002; Jabran and Farooq, 2013). These chemicals accrue and persevere for a substantial time in the plant, thereby causing significant interference on growth and development of neighboring plants (Einhelling, 2008) which can be either a crop or a weed. With considerable work conducted in past decades, the

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presence of inhibitory compounds in a wide array of plant types and its parts at varying concentrations were investigated and with significant attention being paid to allelopathy (Inderjit et al. 2002) and its related effects.

As the understanding of the related mechanisms of these allelochemicals in plants grew, significant attention was given to the potential use of allelopathy as a weed management strategy in achieving sustainable agriculture, with weed control a challenging task, particularly in organic farming (Dayan et al., 2009). Integration of allelopathy into natural and agricultural management systems might help to reduce the use of synthetic agrochemicals. Ultimately, it will reduce the negative effects on the environment, especially pollution. Exploring crop allelopathy against weeds might be a useful strategy to eliminate problems created by synthetic herbicides (Anjum and Bajawa, 2005). Recently, several crops and weeds showed explicit weeds suppressive ability and with potential use in agricultural fields, by exuding allelochemical compounds either from living plants or from decomposing residues (Xuan et al., 2006). Further, this positive effect on weed control will definitely be helpful in introducing potential allelopathic crops into crop rotation programmes, either as a cover crop or mulch to smother crops, or as a green manure in sustainable agricultural practices. This is a very positive trend in agriculture, which could apply for the benefit of human beings by reducing chemical usage. Further, it helps to minimize severe health problems that could be caused due to unsafe handling of agrochemicals (Belz, 2007).

Moreover, the concept of allelopathy can be utilized effectively to produce eco-friendly natural herbicides (Sodaeizadeh et al., 2009). Therefore, knowledge of allelopathic properties of crops or weeds will be beneficial to farmers who adopt different agronomic practices, which help to reduce the cost of production by eliminating the cost incurred for agrochemicals. Furthermore, allelochemicals as natural herbicides would help to mitigate the hitches created by synthetics. In addition, specific allelopathic chemicals enable the development of highly specific herbicides. In cultural and agronomic practices such as mulching, use of residues and cover crops, the specific allelochemicals in plants could also be exploited for weed management. The investigation into and development of new transgenic crop cultivars by incorporating allelopathic properties would reduce usage of synthetic herbicides causing benefits to the environment as well as to agricultural systems (Bhadoria, 2011). As reported by Duke et al. (2015), allelochemicals help to mitigate soil sickness and improve soil properties through controlling ion exchange capacity, organic matter, reactive mineral surfaces, inorganic ions, and also micro and macro fauna and flora of soil. More importantly, information on plant allelopathy could prevent serious crop damage that would arise due to buried biomass in soil in previous years as mulch, cover crop or residues (Jabran et al., 2015). The use of allelopathic traits in crops or weeds will provide a comprehensive understanding for further effective use in the agriculture sector. Particularly, allelopathy will create portentous opportunity to implement integrated weed management strategies and will be exploited as natural weapons to alleviate serious weeds problems in the agriculture sector.

During past decades, the suppressive ability of allelochemicals in weeds has drawn significant attention. However, the specific plant species and its unique responses were not clearly studied yet. Therefore, the focus of this study was to screen the allelopathic effect of naturally grown weed species that commonly occur in agricultural fields. The objective of the study was to study the allelopathic effect of selected common weed species on lettuce seedlings.

MATERIALS AND METHODS

Twenty-seven weed species were randomly collected from Glen Alpine Estate and University Farm (6.981° North, 81.077° East; 1120 m amsl altitude) and laboratory analysis were conducted at Uva Wellassa University. The study location belongs to Agro-ecological region of IM1a (Intermediate zone, mid country). Whole plants of twenty-seven weed species (Table 1) were collected fresh, placed in polythene bags separately, and then taken to the laboratory. The whole plants were cleaned and dried in an oven (VT 6025) at 40°C temperature for 48 h. After appropriate drying, plant materials of each species were kept in airtight plastic bags until further use. The dried plant samples were subjected to analysis for its allelopathic effects using the sandwich method, as described by Fujii et al. (2003). Rapido 344 variety of Lettuce (Lactuca sativa L.), 20 mg of dried plant material of each weed species were placed in autoclaved petri dish resembling the leaf litter. Five milliliters of 0.5% w/v agar was poured in two layers and three lettuce seeds were placed in each petri dish. Afterwards, the petri dishes were placed in the growth room under complete darkness at 25°C. In order to neutralize evaporation and changes to the composition of plant material, the caps of the petri dishes were sealed firmly.

Twenty-eight treatments (Table 1) including the control with seven replicates were arranged in a Completely Randomized Design (CRD). On the fifth day, hypocotyl length (cm), radicle length (cm) and total height (cm) of seedlings were measured. The percentage of inhibition in germination of lettuce seedlings was determined using the following formula as reported by Chung et al. (2001).

\[
\text{Inhibition percentage (\%) = } \frac{[(\text{Total length of sample-Total length of control})]}{\text{Total length of control}} \times 100
\]

Data were statistically analyzed in a one-way ANOVA procedure and significant differences were calculated using the Tukey test at 95% confidence intervals in Minitab 18 software. A dendrogram was created using scores of the mean values of hypocotyl length, radicle length and inhibitory percentage.

RESULTS

Table 1 shows the result of the effect of leaf litter of different weed species on the growth of lettuce seedlings.
Table 1. Allelopathic effect of leaf litter of different weeds on growth and development of lettuce seedlings.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Botanical name</th>
<th>Plant family</th>
<th>Hypocotyl length (cm)</th>
<th>Radicle length(cm)</th>
<th>Inhibitory percentage(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>-</td>
<td>1.93±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.16±0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
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<tr>
<td>2</td>
<td>Ageratum conyzoides</td>
<td>Asteraceae</td>
<td>0.42±0.26&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.43±0.33&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>83.16&lt;sup&gt;g&lt;/sup&gt;</td>
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<td>3</td>
<td>Sphagneticola trilobata</td>
<td>Asteraceae</td>
<td>0.86±0.52&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.14±0.90&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>60.63&lt;sup&gt;bcdefg&lt;/sup&gt;</td>
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<tr>
<td>4</td>
<td>Chromolaena odorata</td>
<td>Asteraceae</td>
<td>0.82±0.43&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.57±0.67&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>52.97&lt;sup&gt;bcdefg&lt;/sup&gt;</td>
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<td>5</td>
<td>Crassocephalum crepidioides</td>
<td>Asteraceae</td>
<td>1.48±0.72&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.84±1.16&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>34.60&lt;sup&gt;abc&lt;/sup&gt;</td>
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<td>6</td>
<td>Erigeron sumatrensis</td>
<td>Asteraceae</td>
<td>1.28±0.45&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.71±0.60&lt;sup&gt;bcddef&lt;/sup&gt;</td>
<td>41.16&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
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<td>7</td>
<td>Bidens pilosa</td>
<td>Asteraceae</td>
<td>1.06±0.71&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.7±0.99&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>45.80&lt;sup&gt;bcddef&lt;/sup&gt;</td>
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<tr>
<td>8</td>
<td>Solanum americanum</td>
<td>Solanaceae</td>
<td>0.90±0.44&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.61±0.37&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>70.25&lt;sup&gt;cdefg&lt;/sup&gt;</td>
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<td>9</td>
<td>Cardiospermum halicacabum</td>
<td>Sapindaceae</td>
<td>0.77±0.64&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.10±0.62&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>63.25&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
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<td>10</td>
<td>Sida acuta</td>
<td>Malvaceae</td>
<td>1.41±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.36±0.17&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>45.54&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>11</td>
<td>Cleome aspera</td>
<td>Capparaceae</td>
<td>1.12±0.57&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.13±0.76&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>55.60&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
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<td>12</td>
<td>Cassia occidentalis</td>
<td>Fabacea</td>
<td>0.46±0.39&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.5±0.41&lt;sup&gt;bcde&lt;/sup&gt;</td>
<td>81.19&lt;sup&gt;cd&lt;/sup&gt;</td>
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<td>13</td>
<td>Cassia tora</td>
<td>Fabacea</td>
<td>1.32±0.54&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.47±0.37&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>45.17&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
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<td>14</td>
<td>Mimosa pudica</td>
<td>Fabacea</td>
<td>1.42±0.28&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.23±0.7&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>47.73&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
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<td>15</td>
<td>Cyperus rotundus</td>
<td>Cyperaceae</td>
<td>1.52±0.69&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.91±0.89&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>26.90&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>16</td>
<td>Achyranthus aspera</td>
<td>Amaranthaceae</td>
<td>1.30±0.37&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.62±0.84&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>42.48&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>17</td>
<td>Urena lobata</td>
<td>Amaranthaceae</td>
<td>1.17±0.54&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.36±0.67&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>50.35&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
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<td>18</td>
<td>Aerva lanata</td>
<td>Amaranthaceae</td>
<td>1.04±0.44&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.91±0.55&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>61.50&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
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<td>19</td>
<td>Amaranthus viridis</td>
<td>Amaranthaceae</td>
<td>1.00±0.27&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.78±0.2&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>65.00&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
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<td>20</td>
<td>Stachytarpheta urticaefolia</td>
<td>Verbenaceae</td>
<td>1.11±0.64&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.72±1.04&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>44.20&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
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<td>21</td>
<td>Lantana camara</td>
<td>Verbenaceae</td>
<td>0.96±0.58&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.13±0.49&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>58.88&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
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<td>22</td>
<td>Phyllanthus debilis</td>
<td>Euphorbiaceae</td>
<td>1.40±0.78&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1.83±0.92&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>36.49&lt;sup&gt;bcd&lt;/sup&gt;</td>
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<td>23</td>
<td>Connellina diffusa</td>
<td>Connellinaceae</td>
<td>1.03±0.42&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.5±0.58&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>50.13&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
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<tr>
<td>24</td>
<td>Cuscuta reflexa</td>
<td>Convalvulaceae</td>
<td>0.74±0.45&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.52±0.25&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>75.07&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
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<tr>
<td>25</td>
<td>Clidemia hirta</td>
<td>Melastomataceae</td>
<td>0.49±0.23&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.28±0.12&lt;sup&gt;g&lt;/sup&gt;</td>
<td>84.91&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>26</td>
<td>Osbeckia octandra</td>
<td>Melastomataceae</td>
<td>1.06±0.52&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.27±0.80&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>54.29&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
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<tr>
<td>27</td>
<td>Ocimum sanctum</td>
<td>Lamiaceae</td>
<td>0.89±0.52&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.6±0.91&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>50.13&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
</tr>
<tr>
<td>28</td>
<td>Hyptis suaveolens</td>
<td>Lamiaceae</td>
<td>1.24±0.46&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.29±0.7&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
<td>50.13&lt;sup&gt;bcddefg&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Means with same superscript in a column are not significantly different from each other while means with different superscript are significantly different at p<0.05.

on agar medium. There was a significant difference in the effect of allelopathy of weed species on growth and development of hypocotyl, radicle and percentage inhibition of lettuce seedlings. Many weed species tested showed significantly different responses on inhibitory percentage, hypocotyl and radicle lengths of lettuce plant compared to control in the experiment (Table 1). The highest hypocotyl and radicle lengths were recorded in control treatment (1.93±0.37 and 3.16±0.52 cm, respectively) family without any leaf litter. Except for *Cyperus*
rotundus, all other species showed a significant difference in the development of radicle length compared to control treatment (p<0.05).

Conspicuously two main clusters were formed in the dendrogram according to the varying degree of allelopathic effect of different plant species on seedling growth of lettuce (Figure 1). *C. rotundus* has shown very similar results as in control treatment where no leaf litter was used, with values of 1.52±0.69 cm hypocotyl length and 2.91±0.89 cm of radicle length (Table 1). In agreement with the above results, both control treatment and *C. rotundus* are in the same cluster representing its' close similarity on observed variables in the present study (Figure 1).

The lowest hypocotyl and radicle lengths with the highest inhibitory percentage were recorded in *Ageratum conyzoid* (0.42±0.26 cm, 0.43±0.33 and 83.16%), *Cassia occidentals* (0.46±0.39 cm, 0.5±41 and 81.19%) and *Clidemia hirta* (0.49±0.23 cm, 0.28±0.12 cm and 84.91%) which were significantly different from control treatment (p<0.05) (Table 1). Therefore, data suggests that there is a strong effect of allelopathy on growth and development of lettuce seedlings above three weed species (Figure 2), which commonly occur in cropping lands. Furthermore, these three species were grouped in one cluster (Figure 1) showing its similarity compared to the control as well as other weed species.

According to the results, it is apparent that leaf litter of *Solanum americanum* and *Cuscuta reflexa* exhibit significant effects on hypocotyl length (0.90±0.44 cm, 0.74±0.45 cm) and radicle lengths (0.61±0.37 cm, 0.52±0.25 cm), respectively with the second highest inhibitory percentages (70.25 and 75.07%) recorded in the study and appeared in the same sub cluster (Figure 1). Exhibiting close similarity to the hypocotyl and radicle lengths, *Aerva lanata* and *Amaranthus viridis*, which belongs to family of Amaranthaceae, have formed a sub-level in the same sub-cluster with *Solanum americanum* and *Cuscuta reflexa* as shown Figure 1.

Other weed species were grouped into another sub cluster, which consists of a number of ancillary sub clusters. Based on varying degree of similarity as shown in Figure 1. *Sphagnetocila trilobata*, *Cardiospermum halicacabum*, *Cleome aspera*, *Osbeckia octandra*, *Lantana camara*, *Chromolaena odorata*, *Ocimum sanctum* and *Commellina diffusa* are included in the same sub cluster, but connected with three separate limbs. Above mentioned weed species representing two limbs of the same sub cluster have shown a significant difference in radicle lengths and inhibitory percentage of lettuce seedlings compared to the control (p<0.05). However, there was no significant difference in hypocotyl length in *Osbeckia octandra* and *Cleome aspera* (1.06±0.52 cm and 1.12±0.57 cm, respectively) compared to the control treatment. *Chromolaena odorata*, *Commellina diffusa* and *Ocimum sanctum* in the same
level of the third limb of the above sub cluster have shown a negative effect on the growth of lettuce seedlings. According to the results generated by the Tukey test, there was a significant difference between above three weed species and the control treatment. It resulted statistically in different radicle lengths and inhibitory percentage except the hypocotyl length of Commellina diffusa, as it is statistically similar to the response of control treatment ($p<0.05$).

Weed species of Crassocephalum crepidioides, Phyllanthus debilis, Erigeron sumatrensis, Achyranthus aspera, Bidens pilosa and Stachytarpheta urticaefolia formed another sub cluster indicating close similarity as shown in Figure 1. Crassocephalum crepidioides of Asteraceae family and Phyllanthus debilis of Euphorbiaceae family have similar responses on inhibitory percentage, hypocotyl and radicle length of lettuce seedlings than that of other weed species of that sub cluster and thus grouped into a same sub-level. Erigeron sumatrensis, Achyranthus aspera, Bidens pilosa and Stachytarpheta urticaefolia exhibit statistically similar results (Table 1) and in the same limb of the sub cluster. The group of Sida acuta, Mimosa pudica, Cassia tora, Urena lobata and Hyptis suaveolens rested in a separate sub cluster laid far from the control compared to other weed species.

Diverse responses have resulted in weed species, which belong to different plant families in the present study. Six-tested weed species representing Asteraceae family (Ageratum conyzoides, Sphagneticola trilobata, Chromolaena odorata, Crassocephalum crepidioides, Erigeron sumatrensis and Bidens pilosa) exhibit diverse effects on the overall performance of lettuce seedlings. It showed a wide range of inhibitory action compared to the control treatment, which varied from 34.60 to 83.16% (Table 1) and subjected to fall in different sub clusters in the dendogram analysis (Figure 1).

The present study shows that leaf litter of four species of Amaranthaceae family (Achyranthus aspera, Urena lobata, Aerva lanata and Amaranthus viridis) resulted in non-significant responses for length of hypocotyl, radicle and inhibition percentage. However there was a significant difference between four species mentioned above and the control treatment only for radicle length and inhibitory percentage of seedlings (at $p<0.05$) (Table 1). However, no significant difference in hypocotyl length compared to control treatment was observed, except for Amaranthus viridis.

According to the results, there was a significant difference between Cassia occidentalis and other tested weed species of Fabaceae family (Cassia tora and Mimosa pudica) on all three observations and the inhibitory percentage ranged from 45.10 to 81.19% as observed in the emergence of lettuce seedlings. There was no significant difference between Clidemia hirta and Osbeckia octandra weed species of Melastomataceae family on the observed parameters of lettuce seedling growth ($p>0.05$). A similar response was exhibited in members of Verbanaceae and Lamiaceae families as there was no significant difference between two weed species of each family (Table 1) and formed part of separate sub clusters in the dendogram (Figure 1).

Comparable allelopathy effects were accrued by weed species belonging to Solanaceae and Convolvulaceae families to the observed parameters on seedling growth of the study and formed the same level of similarity in the dendogram (Figure 1).

**DISCUSSION**

Effect of leaf litter of weed species belong to different plant families on growth of lettuce seedlings in sandwich method was studied. The present study found that leaf litter of the Ageratum conyzoides of the family Asteraceae, C. occidentalis from Fabaceae and Clidemia hirta of the family Melastomataceae resulted in over 80% of inhibition of the growth of lettuce seedlings and

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**Figure 1** Three common weed species with high allelopathic effect (a) A. conyzoides (b) C. occidentalis (c) C. hirta.
ranked first among the weed species tested in this study. *Ageratum conyzoides* is a tropical herb that is very common in Asia and called as Billy Goat weed, which has allelopathy potential in nature. In Sri Lanka, *A. conyzoides* is traditionally used as green manure and raw material in compost production. Some researchers reported that the ability of green manure of *A. conyzoides* to upsurge crop yields and generally it is intercropped as understory with perennial crops to subdue weeds and control of pests (Kong et al. 1999). *A. conyzoides* performs to be a valued agricultural resource, as it comprises a wide range of secondary metabolites including flavonoids, chromenes, benzo furans and terpenoids (Kong et al., 1999). Among these secondary metabolites, some are allelochemicals, which impede the growth of other organisms (Okunade, 2002). *A. conyzoides* can biosynthesize and release non-volatile allelochemicals into the soil. Thus, it inhibits the growth of other plants and microorganisms in soil. It was found that polymethoxy flavones, ageratocromene and its analogues are rare in natural products but they have been discovered in *A. conyzoides* (Okunade, 2002). In fact, *A. conyzoides* possesses the ability of applying in different aspects of agriculture, potentially of allelopathy in agro-ecosystems. The findings of the present study it suggests the negative affects seedling growth. Hence, the use of these plant materials in compost production might affect the early growth of seedlings and the farmers can expect positive effects. However, with all these studies, it has shown the possibility of using this plant species to control other competitive weeds in croplands as an alternative approach for improved weed management especially in organic farming.

*C. occidentalis* L. is a nitrogen-fixing plant belonging to the subfamily Caesalpinioideae of the family Fabaceae. Many studies have established to demonstrate the allelopathic inhibition by different plant parts, residue and aqueous extracts (Gurib-Fakim, 2006). Inhibition of growth of lettuce seedlings in the present study might be due to the above stated allelopathic influence of chemicals released by leaf litter of *C. occidentalis* L. Allelopathic. Action of *C. occidentalis* can be substantiated due to soluble carbohydrates and amino acids present in the plant, which indirectly causes damage to the seed membranes of lettuce seed (Nayek, 2014). The membrane is the most significant site of a seed, which appears to be affected first by leachates having strong allelopathic action (Nayek, 2014). Anjum and Bajwa (2005), who studied the similar allelopathic effect of Cassia occidentalis, support the observations of the present study. Farooq et al. (2008) have also reported comparable outcomes during several allelopathic studies.

*C. hirta* is a noxious alien invasive weed species native to Central and South America, which has great potential to adversely alter natural mesic and hydric habitats and cause adverse impacts on cultivated lands on a large scale (Breaden, et al., 2012). The tested lettuce seedlings were affected by leaf litter of *C. hirta* that negatively affected the emergence of immature root and shoot upon its supplement. According to the studies done by Faravani et al. (2008), the inhibition of root and shoot growth were observed in the Barnyard grass seed which used the extracts isolated from shoot and root of *C. hirta*. The results of the present study also are in agreement with previous research findings as strong suppression of growth of lettuce seedlings were reported by leaf litter of *C. hirta* (Chon et al., 2002).

The tested crop species of the present study is lettuce (*Lactuca sativa* L.), an annual herbaceous plant of Asteraceae and interestingly, the weed species belong to the same family, family *Ageratum conyzoides*, *Sphagneticola trilobata*, Chromolaena odorata, *Crascocephalum crepidioides*, *Erigeron sumatrensis* and *Bidens pionsa* suppressed the growth of lettuce seedlings. That might be attributed by the interference of allelochemicals present in these species. Further findings of the present study are in agreement with the findings that ensured the allelopathic effects on plants that belong to the Asteraceae family (Inam et al., 1989). Moreover, it indicates that the strong growth inhibition on crop seeds might be the result of the plant species, which belongs to same plant family.

Results revealed that *Solanum americanum* of Solanaceae family and *Cuscuta reflexa* of the Convalvulaceae family exhibits statistically similar percentage of inhibitory behavior on growth of lettuce seedlings. *Cuscuta reflexa* is a common agricultural weed throughout the world, causing reduction of yields of many crops and if the infestation is heavy, causes the death of host. Kumari et al. (2017) also found the allelopathic potential of *C. reflexa* that caused an inhibitory effect on host plants of *Ziziphus mauritiana*, *Cajanus cajan* and *Ficus glomerata*. Further it was reported that 60-65% yield reductions in chilies, 31-34% in Greengram/ Blackgram, 60-65% in Niger, 87% in lentil, 86% in chickpea, 72% in tomato, and 60-70% in alfalfa due to the infestation *Cuscuta* (Mishra, 2009). *Solanum americanum* is one of the most widespread and morphologically variable species belongs to Solanum (Edmonds and Chweya, 1997).

In Fabaceae plants, allelopathy is regarded as a natural strategy that is shielding the plants against environmental enemies as well as competing with other plants (Xuan et al., 2006). According to the results of the present study, weeds have shown varying degrees of inhibitory behavior that ranges 45-81% within the species of Fabaceae family. This may be due to the different concentration of coumarins present in plant species of Fabaceae family which responsible for allelopathic behavior (Harborne, 1998).

The tubers of *C. rotundus* releasing harmful substances and allelopathic interactions play a key role in the defining the dissemination of plants in nature and yield of
different crops (Xu et al., 2008). The observations made in the present study are not in close agreement with previous findings as leaf litter has been used in the present study and it might not be strong enough to express significant negative effect on seedling growth of lettuce and results were on par with the control treatment. Furthermore, C. rotundus contains a strong inhibitor of AChE in their tubers, which possibly acts as an agent to suppress other plants and become the worst invasive weed in the world (Sharma and Gupta, 2007). As leaf litter was only taken into an account in the present study, this might cause less allelopathy towards the germination of lettuce seedlings.

The lower level of allelopathic effects were reported in C. crepidioides and P. debilis that showed 34.6 and 36.4% of inhibitory percentages on growth of lettuce seedlings. Chen et al. (2009) stated that although C. crepidioides has been listed as an invasive plant, it also cultivated as a vegetable in China, which indirectly indicated the less toxicity compared to other weed species. However, scientific evidence available on the allelopathic effect of P. debilis is very limited in the literature. The Amaranthaceae family is well recognized for a smothering effect in vegetable fields and some Amaranthus spp. are promising ground covers that suppress weeds in tropical countries (Fischer and Quijano, 1985). The higher inhibition percentage (65%) showed by A. viridis compared to other tested species of the Amaranthaceae family. Different species have shown varying degree of allelopathy ranging from 42.48% to 65%. Furthermore, C. halicacabum showed similar results as A. viridis indicating 63% of inhibitory reaction.

Lantana camara and Stachytarpheta urticaefolia are notorious and invasive weeds and belong to the Verbenaceae family which retard germination, growth, development or metabolism of crops due to the discharge of allelochemicals (Qasem, 2001). Ocimum sanctum and H. suaveolens are aromatic weeds, which are noxious, and world’s exotic persistent species invading the natural ecosystems (Rodrigues, et al., 2012). Results of the present study revealed a reduction in seedling height of lettuce under the allelochemical stress of both weed species, which is similar to the earlier reported allelopathic studies (Singh et al., 2013; Rodrigues, et al., 2012).

As per the results obtained in the present study, leaf litter of all twenty-seven weed species possesses allelopathic potential. The reduction in hypocotyl and radicle length might be attributed with allelochemicals by interfering major physiological processes of plant metabolism, viz. respiration and photosynthesis. Various inhibitors present in plants having allelopathic property reduce the overall metabolism of plants or plant parts, and predominantly anabolic activities are reported to be strongly decreased (Nayek, 2014). Therefore, use of green manure, compost or mulch using materials of weed species commonly found in croplands would be taken into serious consideration, as there might be growth retardation at any stage of plant growth particularly at initial stages such as seedling emergence. Furthermore, as allelochemicals are the secondary metabolites produced by plants, the understanding of plant interactions is important to enable the potential use of such allelochemicals by reducing dependency on herbicides in future cropping systems.

Conclusions

Leaf litter of most weed species considered in the present study showed adverse influence on lengths of hypocotyl and radicle and inhibitory percentage of lettuce seedlings under laboratory conditions. The growth of lettuce seedlings was strongly inhibited by plant species of A. conyzoides, C. hirta and C. occidentalis. Weed species belonging to the same family of lettuce also expressed a varying degree of inhibitory effects on growth performances of lettuce seedlings.

SUGGESTIONS

Further studies should be carried out to explore the mechanism of allelopathic activities of crop species as there is little or no information on laboratory and field studies. Purification of allelochemicals, identification of bioactive molecules and their effects should be investigated. Advance research is also needed to explore the allelopathic potential of the plant species in order to discover new molecular structures to be used in the control of invasive weeds and pests in agriculture systems, thus minimizing the damage caused by the hazardous synthetic agrochemicals. Moreover, there is a need for genetic and molecular studies of allelopathic plants, in order to investigate the bio-systemic pathways towards the defense against competitors, as well as to identify allelopathic genes for the transgenic improvement of economically important agricultural crops.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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