Section-II
OBJECTIVE

To study the phytotoxicity of naturally dried above-ground plant material (referred to as residue) of *P. hysterophorus* on the growth and establishment of some winter season crops *vis-à-vis* related changes in the physico-chemical properties of the soil.

OBSERVATION PARAMETERS

Following observations were made:

1. Estimation of the residue per unit area.
2. Phytotoxicity of residue-amended-soils (RS), residue-extract-amended-soils (RES) and the residue extracts (RE) towards five winter season crops namely *Cicer arietinum*, *Raphanus sativus*, *Brassica campestris*, *B. rapa* and *B. oleracea* var. *capitata* in terms of root length, shoot length and dry biomass. These crops were chosen as their sowing period coincided with the period of maximum residue formation i.e. during the months of October-November (post monsoons) when the life cycle of *P. hysterophorus* was over.
3. Dynamics of release of phenolics in residue-amended-soil, residue-extract-amended-soil and in residue extract.
4. Physico-chemical properties like pH, electrical conductivity, total water soluble phenolic acids, available macro- (N, P, K, Na, Ca, Mg, Cl and HCO₃⁻) and micro-nutrients (Zn, Fe, Mn and Cu) in residue-amended as well as residue-extract-amended-soils.
5. Elemental analysis (macro-nutrients- C, H, N, P, K, Na, Ca, Mg and micro-nutrients- Zn, Fe, Mn and Cu) of the residue.
6. Identification of allelochemicals from the residue.
MATERIAL AND METHODS

Collection of the Material

*P. hysterophorus* infested site was selected in and around Panjab University campus, Chandigarh. Plant density and biomass were measured by laying 20 quadrats of 1m² each in October-November when the plants were completely dried after the completion of its life cycle. The naturally dried plant residue (above ground) was collected, powdered and packed in polythene bags for further use.

Soil was collected from an open area free from *P. hysterophorus*. It was air-dried, sieved through 2 mm mesh and made the lots of 1 kg each.

Seeds of freshly harvested *C. arietinum*, *R. sativus*, *B. campestris*, *B. rapa* and *B. oleracea* were purchased from Plant Breeding Department and Seed Technology Unit of Punjab Agricultural University, Ludhiana.

Preparation of Residue-Amended and Residue-Extract-Amended-Soils

Under natural conditions, *P. hysterophorus*, upon death falls on the soil floor and gets mixed up there in it. In order to simulate these conditions 5, 10, 20, 30 and 40 g of the residues were added in 1 kg soil lot separately and thoroughly mixed so as to get 0.5, 1, 2, 3 and 4% residue-amended-soils. For the preparation of residue-extract-amended-soils, firstly, residue extracts were prepared. For this, 40 g powdered residue was immersed in pure water for 20 h at room temperature. It was filtered through muslin cloth followed by Whatman filter paper no. 1 to get 4% residue extract. Further dilutions with pure water were made so as to have 0.5, 1, 2 and 3% solutions. These were referred to as residue extracts. In 27 x 15 cm rectangular plastic trays, 500 ml of each of 0.5, 1, 2, 3 and 4% residue extract was added in one kg soil, separately and placed them for drying under shade for 30 h. After that, 250 g each of the respective residue-amended or residue-extract-amended-soil was taken in 6" diameter Petri dishes. The untreated soil was also taken in 6" diameter Petri dishes to serve as control.
The residue-amended-soil has been referred as ‘RS’ and residue-extract-amended-soil and unamended-soil as ‘RES’ and ‘US’, respectively.

Growth Studies in Amended-Soils

Seeds of *C. arietinum*, *R. sativus*, *B. campestris* and *B. oleracea* were used for growth studies. Thirty uniform seeds of the five crops were sown in ‘RS’ and ‘RES’ filled Petri dishes (6” diameter). Seeds sown in untreated soil served as control. For each treatment, four replicates were maintained in a completely randomized block design and placed in a chamber maintained at 25 ± 1°C, 75 ± 3% RH and 16/8 h light/dark photoperiod. Each Petri dish was sprayed daily with 25 ml water. After 10 days, seedlings were carefully uprooted ensuring minimal damage to the roots. Root and shoot lengths of five seedlings in each Petri dish were measured and their biomass determined after oven drying at 80°C for 24 h.

Preparation of Residue Extracts and Growth Studies under Laboratory Conditions

For preparation of the extracts, 4 g dried residue was dipped in 100 ml of pure water for 20 h at room temperature. It was filtered through a double-layer muslin cloth, followed by Whatman no. 1 filter paper. Further dilutions were made to get the concentrations of 0.5, 1 and 2%. Total phenolic content, pH and electrical conductivity of these extracts were measured. The effect of different concentrations of residue extracts on the growth and establishment of five above said crops were studied under laboratory conditions. For this, twenty seeds of test crops were treated with respective extracts for 20 ± 2 h. Seeds treated with pure water served as control. The treated seeds were spread out in 6” Petri dishes. Each Petri dish was lined with sterilized absorbent cotton wad and overlined with Whatman no. 1 filter paper. Each wad was moistened with 15 ml of the respective treatment solution ensuring no air trapping in the bed. Five replicates for each treatment were maintained in a completely randomized block design. The set-up was put in seed germinator maintained at 25 ± 3°C, and 75 ± 3% RH. After 10 days, when no more seeds germinated, lengths of roots and shoots of five uniform seedlings in each Petri dish were measured and dry biomass was determined after oven drying.
Estimation of Phenolics from Aqueous Extracts of *P. hysterophorus* Residue and in Amended Soils (RS and RES)

Total phenolics were estimated in four different lots. In the first lot, 500 ml of 4% residue extract was added in one kg of dried soil. In the second lot, 40 g residue and 500 ml of pure water was added in one kg soil and in the third lot, 500 ml of pure water was added in one kg soil and thoroughly mixed. Five g of the soil was removed from each lot after 4, 8, 12, 16, 20, 24, 30, 36, 48, 60, 72, 96, 120 and 144 h and then air dried and subjected to extraction of phenolic acids following the method of Swain and Hills (1959) using Folin-Ciocalteu reagent. In the fourth lot, 40 g residue was added in one litre pure water. Five ml of residue extract was removed after 1, 2, 4, 8, 12, 16, 20, 24, 30, 36, 48, and upto 60 h and then phenolics were estimated from each of the residue extracts. Five replicates were maintained for each type of treatment.

Determination of Physico-Chemical Characteristics of Amended Soils

Amended soils namely residue-amended (RS), residue-extract-amended (RES) and unamended soils (US or control) were analysed for pH, electrical conductivity, organic matter and available macro- and micro-nutrients. The pH and electrical conductivity were measured with digital pH and conductivity meter from the soil paste in pure water in the ratio of 1:5 (w/v) by immersing the electrode in each of it. Total phenolic content was measured following the method of Swain and Hills (1959). Organic carbon and organic matter were measured using rapid titration method developed by Walkley and Black (1934). Available nitrogen was estimated by following AOAC, 1960 using alkaline KMnO₄. Available phosphorus was estimated by following the method of Olsen *et al.* (1954) using ammonium molybdenum solution, whereas the estimation of available potassium and sodium were done by following the method of Bower and Gschwend (1952), available calcium and magnesium by Versenate (EDTA) method and available chlorides and bicarbonates were determined by titration method. For the estimation of micro-nutrients, diethylene triamine penta acetic acid (DTPA) was used for the extraction and the content of these micro-nutrients in the extracted solution were
analyzed on an Atomic Absorption Spectrophotometer (AAS). For details, see section “Material and Methods”.

Elemental Analysis of Residue

Elements like carbon, nitrogen and hydrogen in the residue were determined using CHN analyser at Regional Sophisticated Instrumentation Centre (RSIC), Panjab University, Chandigarh. For the analysis of P, K, Na, Ca, Mg and trace elements, wet diacid digestion of the residue was done using nitric acid and perchloric acid. Phosphorus was estimated from the plant material Duly digested (referred to as plant digest) by colorimetric method using vandamolybdate reagent. K and Na were determined through flame photometry. Ca and Mg in plant digest were determined by titration method. Zn, Cu, Fe and Mn concentration in plant digest were determined by Atomic Absorption Spectrophotometer (AAS). For details, see section “Material and Methods”.

Identification of Allelochemicals in the Residue

Various allelochemicals i.e. phenolic acids and sesquiterpene lactone-parthenin from residue extract were separated in methanol and subjected to Thin Layer Chromatography (TLC), followed by High Pressure Liquid Chromatography (HPLC) by running standards of respective allelochemicals. Their RT values were compared with the standards for identification of phenolic acids. For details, see section “Material and Methods”.

Statistical Analysis

All the experiments were laid out in a completely randomized block design. The data of root length, shoot length and dry biomass were expressed with respect to control and were analyzed by DMRT at P < 0.05. The results obtained from nutrient analysis were also subjected to DMRT as per Duncan (1955) and two-sample-t-test. The values of correlation coefficient between concentration and respective parameters were also calculated.
RESULTS

The density of *P. hysterophorus* at the study site (with monoculture of *P. hysterophorus*) was measured to be 71.6 ± 38.8. Further, the above ground biomass per plant was estimated to be 9.51 ± 4.13 g and in terms of area, it was 68.09 ± 7.83 g/m² (= 6809 kg/ha).

Growth Studies

a) Growth Studies in RS

Hundred percent seed germination of all the test crops namely *C. arietinum, R. sativus, B. campestris, B. rapa* and *B. oleracea* was observed in control as well as in the treatments, except 4% concentration treated seeds of *C. arietinum* and *R. sativus*, where not even a single seed germinated. Since the speed of germination was not much affected, so data on these have not been tabulated and presented.

**Root Length**

The root length of plants sown in residue-free (control) soil was seen to be largest in case of *C. arietinum* (15.74 ± 0.96 cm) followed by *R. sativus* (11.67 ± 0.41 cm), *B. rapa* (10.34 ± 0.43 cm) and *B. campestris* (7.80 ± 0.43 cm). A significant reduction in the root length of seedlings of test crops was observed when grown in RS (Fig. 2.1 a). In case of *C. arietinum* and *R. sativus*, since none of the seeds germinated at 4% concentration so root length measurement was not possible. The rate of decline in the root length with respect to increasing concentration of amendment in soil was maximum in case of *C. arietinum* (apparent from the sharp fall of the curve) and least in case of *B. campestris* (Fig. 2.1 a). Strong negative correlation coefficient values ranging from - 0.980 to - 0.998 between root length and concentration were calculated (Fig. 2.1a).

**Shoot Length**

The shoot length of five crops varied appreciably. Shoot length of *C. arietinum* was measured to be 17.73 ± 1.08 cm whereas that of *R. sativus* was measured to be 12.72 ± 0.89 cm in control. In rest of the crops, the shoot length was less than 10 cm in control.
Fig. 2.1 Effect of different concentrations of residue of *P. hysterophorus* amended in soils on (a) root length (b) shoot length (c) dry biomass of test crops.

Different superscript symbols along a curve represent significant difference among themselves at $P < 0.05$ applying DMRT.

$\pm$ represents standard deviation, $r$ represents value of correlation coefficient.

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The shoot length of each of the five crops was seen to decline with increasing concentration of amendment of the residue in the soil. The decline was very sharp and consistent, especially in *C. arietinum* and *R. sativus* (Fig. 2.1b). It was apparent even from the strong values of correlation coefficient between concentration and shoot length. The sharpest decline, right from the lowest till the highest concentration of amendment, was in case of *C. arietinum* and *R. sativus* (Fig. 2.1b).

**Dry Biomass**

The variations in the dry biomass of the seedlings among the crops were more compared to root and shoot lengths of 10 days old plants. In control, *C. arietinum* had a biomass of $48.66 \pm 4.04$ mg. In contrast, *R. sativus* was just $14.02 \pm 0.53$ mg and *B. campestris* was $8.02 \pm 0.25$ mg and in case of *B. oleracea* and *B. rapa*, the biomass were measured to be $3.89 \pm 0.20$ mg and $3.22 \pm 0.18$ mg, respectively (Fig. 2.1c). The trend of changes in dry weight with respect to increasing concentration of amendment of residue in the soil was almost the same as that of root and shoot lengths. Maximum reduction in dry biomass was seen in case of *C. arietinum* and least in case of *B. oleracea*. In all the crops, the values of correlation coefficient between concentration and dry biomass was reciprocal and relatively strong showing, thereby, some element of consistency (Fig. 2.1c).

**b) Growth Studies in RES**

The growth studies in RES indicate a significant effect on the root and shoot lengths and dry biomass of test crops compared to their respective controls. In general, a decline in root length was observed in all the treatments and in all crops (Fig. 2.2a). Maximum effect was observed on *C. arietinum* where none of the seed germinated at 4% concentration of residue extract amended in soil. It was followed by *R. sativus* and *B. campestris*. A significant decline was also observed in case of *B. rapa*. In all the test crops, a strong correlation coefficient values were calculated, indicating almost consistent decrease in root length with increasing concentration of amendment (Fig. 2.2a).
Fig. 2.2 Effect of different concentrations of residue extracts of *P. hysterophorus* amended in soils on (a) root length (b) shoot length (c) dry biomass of test crops.

(a) Root Length

Different subscripts symbols along a curve represent significant difference among themselves at P < 0.05 applying DMRT.

± represents standard deviation.

r represents value of correlation coefficient.
The trend of changes in shoot length was similar to that of root length. The strong reciprocal correlation values also point to the similarity of response between root and shoot lengths of the crops under study (Fig. 2.2b). As regards the dry biomass of the plants, the similar trend of inhibition was observed as in case of root and shoot lengths (Fig. 2.2c).

c) Growth Studies in Residue Extracts (RE)

Radicle Length

The radical length in control was measured to be 11.93 ± 0.47 cm in R. sativus and 13.09 ± 0.80 cm in B. campestris. In rest of the three crops, the values ranging between 8.13 to 10.50 cm were observed when grown in pure water treated control set up. When the set-up was subjected to the treatment of aqueous extracts of residue, the lengths of roots were measured to be relatively shorter than their respective control (Fig. 2.3a). The decline was very sharp during the initial concentration upto 1% beyond that the decline continued, however, the sharpness of the decline was not as acute as till 1% of residue extract treatment. In case of C. arietinum, the roots did not come up when the seeds were treated with 4% of the residue extract. In all the five crops, the values of correlation coefficient between root length and concentration of the treatments were very strong and reciprocal (Fig. 2.3a).

Plumule Length

The length of plumule among the crops was the least in case of C. arietinum. After 10 days of germination, it was around two cm in control. However, in others, it ranged between 3.5 cm to 5.3 cm in water treated control. The seeds of C. arietinum that showed cent per cent germination in control and when treated with residue extract solution of 2 to 4% did not show any plumule initiation or growth. Even in 0.5 or 1% extract solution, the plumule length showed reduction by about 62.3 and 24.4% to that of control, respectively (Fig. 2.3b). In other crop plants also, the plumule length was relatively shorter in case of those treated with residue extract solution compared to water treated control. However, the rate of decline in these crops was not as sharp as in C. arietinum (Fig 2.3b). Nevertheless, in all the cases, with increasing concentration of
Fig. 2.3 Effect of different concentrations of aqueous extracts of residue of *P. hysterophorus* on (a) radicle length (b) plumule length (c) dry biomass of test crops.

Different superscript symbols along a curve represent significant difference among themselves at $P < 0.05$ applying DMRT.

$\pm$ represents standard deviation.

$r$ represents value of correlation coefficient.
extract *arietinum* (Fig 2.3b). Nevertheless, in all the cases, with increasing concentration of extract solution, there was a significant decline in plumule length. This was also apparent even from reciprocal correlation coefficient values (Fig. 2.3b). However, in *C. arietinum*, the value was as strong as those of four other crops, perhaps for the reason that beyond 1% of the extract treatment the plumule did not emerged.

**Dry Biomass**

The trend of changes in dry biomass of seedlings of all the five crops under study with respect to the concentration was almost the same as those of radicle and plumule lengths (Fig. 2.3c). In other words, the values decreased with increasing the concentration of residue extracts.

**Dynamics of Release of Phenolic Content**

**RS**

The amendment-free (control) soil was found to contain 19.2 ± 0.23 µg/g dry weight of phenolic content. The total amount of phenolic content minus phenolic content in control soil is presented in Fig. 2.4a. Maximum amount of phenolic content (77.6 ± 0.06 µg/g) was estimated to be present in amendment after 48 h. Between zero to 48 h, the content showed a gradual increase. After 48 h a sharp decline was, however, noticed. Thus, after 144 h. the value was calculated to be just 56.8 ± 0.08 µg/g dry weight.

**RE**

It was observed that after 1h of adding pure water to residue, 313.3 ± 3.8 µg/ml phenolic content could be estimated. However, after 2 h of the amendment, the value increased to 391.3 ± 2.5 µg/ml and thereafter it kept on increasing till 20 h, when it was calculated to be 443.8 ± 2.8 µg/ml (Fig. 2.4b). After that, the amount of phenolics declined and rate of decline was sharp upto 36 h. After this, the amount remained unchanged.
Fig 2.4 Dynamics of release of phenolic content w.r.t. time in (a) residue amended soil (b) aqueous extract and (c) residue extract amended soil of *P. hysterophorus*.

(a) Residue amended Soil

(b) Aqueous Extract

(c) Residue Extract amended Soil

\[ \text{Amount of Phenolics (pg/g)} \]

\[ \text{Amount of Phenolics (pg/ml)} \]

\[ \text{Amount of Phenolics (pg/g)} \]

\[ X \text{ represents standard deviation} \]
Based on the above that the maximum amount of phenolic content leach out of the residue after 20 h in aqueous medium. The suspension of residue in water, was filtered after 20 h. It (filtrate) was added to the soil and mixed thoroughly. Maximum amount of phenolic content (73.1 ± 0.59 µg/g of dry weight) were measured after 30 h of addition of the extracts. After 30 h, the values of phenolic content declined. The decline was, however, slow. After 144 h, the content of phenolics was measured to be 60.1 ± 0.42 µg/g (Fig. 2.4c).

**Characteristics of Residue Extracts**

**pH**

The pH of the extracts was found to be near neutral ranging from 6.95 ± 0.04 to 6.69 ± 0.05 (Table 2.1). It is clear from the table 2.1 that not much change in pH could be observed with increasing concentration of extracts from 0.5 to 4%.

**Table 2.1 Values of pH, electrical conductivity and phenolic content in residue extracts of *P. hysterophorus*.**

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>pH</th>
<th>Electrical Conductivity (mS)</th>
<th>Phenolic Content (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>6.95 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.30 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>70.11 ± 0.95&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>6.80 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.24 ± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>126.26 ± 5.91&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>6.77 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.16 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>231.72 ± 10.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>6.69 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.30 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>474.99 ± 13.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different superscript symbols along a column represent significant difference applying DMRT at P < 0.05 level.

± represents standard deviation.

**Electrical Conductivity**

The electrical conductivity increased with increasing concentration of extracts from 1.30 ± 0.07 mS to 7.30 ± 0.13 mS (Table 2.1). This increase was seen to be linear.
Phenolic Content

The amount of phenolics in the extract concentration of 0.5% was calculated to be 70.11 ± 0.95 µg/ml. With increasing concentration of extracts, the value increased and at 4% concentration, it was nearly 7 times more than in 0.5% (474.99 ± 13.47 µg/ml) (Table 2.1). This increase was also linear, like electrical conductivity.

Elemental Analysis of the Residue

The residues prepared from the above ground *P. hysterophorus* plants were estimated to contain 37.66% total carbon, 4.8% total hydrogen and 1.25% of total nitrogen.

Table 2.2 Content of elements in *P. hysterophorus* residue per dry weight.

<table>
<thead>
<tr>
<th>Element (Units)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total C (%)</td>
<td>37.66</td>
</tr>
<tr>
<td>Total H (%)</td>
<td>4.80</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>1.25</td>
</tr>
<tr>
<td>Available P (%)</td>
<td>0.168 ± 0.06</td>
</tr>
<tr>
<td>Available K (%)</td>
<td>1.163 ± 0.12</td>
</tr>
<tr>
<td>Available Na (%)</td>
<td>0.02 ± 0.001</td>
</tr>
<tr>
<td>Available Ca (g/100g)</td>
<td>36.6 ± 0.34</td>
</tr>
<tr>
<td>Available Mg (g/100g)</td>
<td>24.9 ± 0.24</td>
</tr>
<tr>
<td>Available Zn (ppm)</td>
<td>0.70 ± 0.004</td>
</tr>
<tr>
<td>Available Cu (ppm)</td>
<td>0.25 ± 0.002</td>
</tr>
<tr>
<td>Available Fe (ppm)</td>
<td>14.5 ± 0.27</td>
</tr>
<tr>
<td>Available Mn (ppm)</td>
<td>3.5 ± 0.08</td>
</tr>
</tbody>
</table>

± represents standard deviation.

The residue of *P. hysterophorus* when analyzed for the available elements, showed 0.168 ± 0.06% phosphorus, 1.163 ± 0.12% potassium, 0.02 ± 0.001% sodium per dry weight (Table 2.2). Macro-nutrients, like calcium and magnesium constituted 36.6 ± 0.34% and 24.9 ± 0.24%, respectively. Among the micro-nutrients, Fe was maximum.
with a value of $14.5 \pm 0.27$ ppm. It was followed by Mn ($3.5 \pm 0.08$ ppm). Zn and Cu were estimated to be 0.7 and 0.25 ppm, respectively, on the dry weight basis (Table 2.2).

**Physico-Chemical Properties of Amended Soils**

**pH**

The value of pH declined in the amended soils compared to unamended (control) field soil where it was measured to be $7.28 \pm 0.06$. In case of RS, the change in pH was insignificant up to 2% compared to US. At the highest concentration, however, pH changed significantly. On the other hand, in case of RES, the change in pH was statistically insignificant to the extract concentration of 1%. Beyond that, however, a significant decline in pH compared to US was observed (Fig. 2.5a). The soils in which 4% residue and its extract were amended, the values of pH were measured to be $6.80 \pm 0.03$ and $6.91 \pm 0.04$, respectively. The difference in pH values of RS and RES were significant only at 3 and 4% concentrations. Less than these concentrations, no significant change in value of pH was found. The values of correlation coefficient between concentration of extract/residue amended in soil and the value of pH were strong and reciprocal.

**Electrical Conductivity**

The electrical conductivity of the US was measured to be $360.0 \pm 0.12$ μS. On addition of the residue or its extracts in the soils, the EC values were seen to be increased with increasing concentration. The increase in EC in RS at the highest concentration of residue i.e. 4% was nearly $1761.6 \pm 0.39$ μS compared to US. However, in case of RES, at the same concentration, this increase was only 4 times i.e. $1505.1 \pm 0.24$ μS. While comparing the EC values of RS and RES, differences were statistically significant except at 0.5% (Fig. 2.5a). The correlation coefficient values were + 0.987 and + 0.974 for RS and RES, respectively, reflecting a strong correlation.
Fig. 2.5 (a) pH and electrical conductivity (b) phenolic content and organic matter of the soils amended with different concentrations of residue and extract of *P. hysterophorus.*

(a) pH and Electrical Conductivity

Different superscript symbols along a curve represent significant difference among themselves at $P < 0.05$ applying DMRT.

* represents significant difference between residue amended and residue extract amended soils at respective concentration applying two sample t-test.

$r$ represents value of correlation coefficient.

(b) Phenolic Content and Organic Matter

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Phenolic Content

The content of phenolics in the control soil was 2.97 ± 0.16 mg/100g of dry weight. In contrast, the amount of phenolic content was significantly more in the amended soil irrespective of the amendement made with extracts or residues (Fig. 2.5b). It increased with the increasing concentration of amendments. At the highest concentration of amendment i.e. 4% extract and residue, the amount of phenolics increased 3.5 and 4 times, respectively (Fig. 2.5b). The difference in the amount of phenolics at respective concentration of RS and RES was significant, except, at 2% concentration. Further, the values of correlation coefficient in this case were very high and negative indicating a strong reciprocal correlation.

Organic Matter

The amount of organic matter in the control soil was measured to be 1.45 ± 0.13% on dry weight basis. With the amendment of 4% extract or residue of *P. hysterophorus*, the organic matter increased but not as sharply as in total phenolics. In the RS and RES, the amount of organic matter at 4% concentrations were estimated to contain 3.46 ± 0.19 and 2.56 ± 0.28%, respectively (Fig. 2.5b). The values of correlation coefficient in this case also were strong and 0.930 and 0.961, respectively, for RS and RES. Further, the differences in organic matter in RS and RES at respective concentrations were statistically significant (Fig. 2.5b).

Available Nitrogen and Phosphorus

The available nitrogen in *P. hysterophorus*-free (US) soil was measured to be 168 ± 7.09 kg/ha. The value of total available nitrogen in RS and RES were statistically insignificant at lower concentrations of 0.5 and 1%, respectively (Fig. 2.6a). Beyond these, a statistically significant increase in available nitrogen was observed. At 4% amendment in both the cases of RS and RES, the amount of nitrogen was 237.3 ± 10.87 and 220.5 ± 7.73 kg/ha, respectively. Between RS and RES, the variations were significant only at 3 and 4% concentrations (Fig. 2.6a).
Fig. 2.6 (a) Available nitrogen and phosphorus (b) available potassium and sodium of the soils amended with different concentrations of residue and extract *P. hysterophorus*.

(a) Available Nitrogen and Phosphorus

(b) Available Potassium and Sodium

Different superscript symbols along a curve represent significant difference among themselves at $P < 0.05$ applying DMRT.

* represents significant difference between residue amended and residue extract amended soils at respective concentration applying two sample t-test.

$r$ represents value of correlation coefficient.
In case of available phosphorus, the content in the control soil (US) was measured to be 208.3 ± 6.55 kg/ha. No significant change could be observed up to 2% extract amendment soil. At 3% and 4% concentration, however, significant increase in available phosphorus could be seen compared to US (Fig. 2.6a). In contrast, the samples amended with residues of *P. hysterophorus* till 2% showed appreciable increase in available phosphorus content. The residue amendment beyond 2% showed little variation, which was statistically insignificant. Significant differences were observed at respective concentration of RS and RES (Fig. 2.6a).

**Available Potassium and Sodium**

The content of potassium in the US was 270.1 ± 5.84 ppm where as that of sodium was 60.2 ± 0.41 ppm. In the amended soils, however, the amount of potassium increased significantly. With every increasing concentration of RS and RES, the values showed statistically significant increase. However, the increase in the value of potassium ions was relatively less in RES compared to RS. The difference between the two was however, significant at respective concentration. The strong values of correlation coefficient were also calculated (Fig. 2.6b).

In case of sodium, the trend of changes was similar as that of potassium ions. However, the dimensions of the change were relatively less i.e. with every increasing concentration of RS and RES, the sodium ions showed gradual increase, especially in case of RS, compared to RES (Fig. 2.6b). The difference between the two i.e. RS and RES at their respective concentrations was statistically significant.

**Available Calcium and Magnesium**

The control soil was measured to have 12.5 ± 1.21 g/100g of dry weight of calcium and 4.85 ± 0.56 g/100g dry weight of magnesium. In both the cases i.e. the amendments with residue of *P. hysterophorus* or its extracts showed higher values than that of US. With increasing concentration of amendments of residue and its extracts, the values of Ca and Mg showed a trend towards increase (Fig. 2.7a). The linearity of
Different superscript symbols along a curve represent significant difference among themselves at P < 0.05 applying DMRT.

* represents significant difference between residue amended and residue extract amended soils at respective concentration applying two sample t-test.

r represents value of correlation coefficient.

Fig. 2.7 (a) Available calcium and magnesium (b) available chlorides bicarbonates of the soils amended with different concentrations of residue and extract of *P. hysterophorus.*
Increase was seen in case of RS rather than RES. It was also apparent from the values of correlation coefficient, which were stronger in case of RS compared to RES.

**Available Chlorides and Bicarbonates**

The control soil (US) was estimated to possess 3.28 ± 0.39 and 12.9 ± 0.67 g/100g of chlorides and bicarbonates, respectively. However, when residues of *P. hysterophorus* were added, the content of ions of chlorides and bicarbonates showed an increase (Fig. 2.7b). This increase was seen to be gradual but consistent at least up to 3% of residue amendment. In the sample where the amendment by the residue was at 4%, the content of bicarbonates was measured to be maximum i.e. 27.01 ± 1.36 g/100g. In case of RES, the amount of bicarbonates increased up to 1% and thereafter, it declined, whereas in RS, amount of bicarbonates increased with every increase in concentration of residue (Fig. 2.7b).

**Available Micro-nutrients (Zn, Cu, Fe and Mn)**

The amount of Zn, Cu, Fe and Mn was measured to be 3.5 ± 0.13 ppm, 0.6 ± 0.002 ppm, 12.75 ± 0.35 ppm and 15.7 ± 0.007 ppm, respectively in unamended (control) soil. In amended soil, however, the amount of microelements increased, by and large, except in a few cases such as 4% concentration of RES for Mn and Zn. The values of correlation coefficient between concentration of RS and RES and content of microelements were strong, (more than 0.8) except, between RES and amount of Cu, where it was only 0.509 (Fig. 2.8).

In case of Fe ions, with increasing concentration of amendments by the residue extracts, the content did not show much change. However, when residue was added, the trend towards increase in the content could be noticed.

In case of Zn, the content was more in RES of *P. hysterophorus* compared to control. It increased with every increasing concentration of up to 3% of RES. Thereafter, it declined. In case of RS, however, similar trend of increase was observed till 3% of amendment (Fig. 2.8). At 4% amendment, little change in the amount of Zn was observed.
Fig. 2.8 Micronutrients (Cu, Fe, Zn and Mn) of the soils amended with different concentrations of residue and extracts of *P. hysterophorus*.

Different superscript symbols along a curve represent significant difference among themselves at $P < 0.05$ applying DMRT.

* represents significant difference between residue amended and residue extract amended soils at respective concentration applying two sample t-test.

$r$ represents value of correlation coefficient.
In case of Cu, no appreciable change in the amount could be observed between the amended (RS and RES) and unamended (control) soils (Fig. 2.8).

**Identification of Allelochemicals**

In addition to parthenin—a major sesquiterpene lactone and one unidentified, eight phenolic acids namely ferulic acid, chlorogenic acid, p-coumaric acid, gallic acid, syringic acid, caffeic acid, vanillic acid, p-hydroxybenzoic acid were identified from the dried residue of *P. hysterophorus* (Table 2.3). These have been arranged in order to their retention (RT) values given in Table 2.3. Among these, chlorogenic acid had lowest RT value of 2.831. The highest RT value (4.272) was that of p-hydroxybenzoic acid (Table 2.3).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Allelochemical</th>
<th>RT Value</th>
<th>Mol. Weight</th>
<th>Mol. Formula</th>
<th>Derivative Type/Chemical Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chlorogenic acid</td>
<td>2.831</td>
<td>354.30</td>
<td>C_{16}H_{18}O_{9}</td>
<td>Cinnamic acid</td>
</tr>
<tr>
<td>2</td>
<td>Ferulic acid</td>
<td>2.935</td>
<td>194.18</td>
<td>C_{10}H_{10}O_{4}</td>
<td>Cinnamic acid</td>
</tr>
<tr>
<td>3</td>
<td>p-Coumaric acid</td>
<td>2.975</td>
<td>164.15</td>
<td>C_{9}H_{8}O_{3}</td>
<td>Cinnamic acid</td>
</tr>
<tr>
<td>4</td>
<td>Gallic acid</td>
<td>3.705</td>
<td>170.12</td>
<td>C_{7}H_{6}O_{3}</td>
<td>Cinnamic acid</td>
</tr>
<tr>
<td>5</td>
<td>Syringic acid</td>
<td>4.097</td>
<td>271.1</td>
<td>C_{9}H_{11}O_{5}</td>
<td>Cinnamic acid</td>
</tr>
<tr>
<td>6</td>
<td>Caffeic acid</td>
<td>4.157</td>
<td>180.15</td>
<td>C_{9}H_{8}O_{4}</td>
<td>Cinnamic acid</td>
</tr>
<tr>
<td>7</td>
<td>Vanillic acid</td>
<td>4.235</td>
<td>168.14</td>
<td>C_{9}H_{8}O_{4}</td>
<td>Benzoic acid</td>
</tr>
<tr>
<td>8</td>
<td>p-Hydroxybenzoic acid</td>
<td>4.272</td>
<td>138.31</td>
<td>C_{7}H_{6}O_{3}</td>
<td>Benzoic acid</td>
</tr>
<tr>
<td>9</td>
<td>Parthenin</td>
<td>8.199</td>
<td>262.31</td>
<td>C_{15}H_{18}O_{4}</td>
<td>Sesquiterpene lactone</td>
</tr>
<tr>
<td>10</td>
<td>Unidentified</td>
<td>12.618</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**DISCUSSION**

It is clear from the study that large amount of residue of *P. hysterophorus* accumulate on the substratum, especially after the completion of its life cycle. Since the
plant is known to be allelopathic, it is expected that its residues would affect the succeeding vegetation or crops (Mersie and Singh, 1987b; Pandey et al., 1993a,b; Kohli and Batish, 1994; Kohli and Rani, 1994; Batish et al., 2002a). In order to test this, a number of growth studies were undertaken under laboratory and greenhouse conditions such as effect of extracts and amended soils (amended with residue and its extracts). The crops were also carefully chosen since the large amount of residue formation occurs in October-November after completion of the life cycle of *P. hysterophorus*. Thus, the winter season crops like *C. arietinum*, *R. sativus*, *B. campestris*, *B. rapa* and *B. oleracea* were selected for assessing the phytotoxicity.

The extracts prepared from residue in different concentrations were found to be phytotoxic to all the test crops, as the early seedling growth and dry biomass were found to be significantly reduced compared to control. Among the crops, greater inhibitory effect was observed on *C. arietinum* and *B. rapa*. In case of *C. arietinum*, complete inhibition was observed in 4% residue extracts. Whereas in other crops no complete inhibition was observed in any of the concentrations tested. This indicates that the response of different crops is variable and dependent upon several factors, like size of seeds and genetic differences or variability. The differential response of crops towards extracts, leachates or any other phytotoxic material has already been reported in a number of other crops (Qureshi et al., 1987; Qasem, 2001; Kiemnec and McInnis, 2002; Ambika et al., 2003; Xuan et al., 2004).

As regards the phytotoxicity of residue extracts, again a number of reports are available indicating that decomposing residue of crops or weeds or even trees release some inhibitors in the environment that may be toxic to the other plants (Rice, 1984; Varshney and Saxena, 1994; Singh, 1996; Singh et al., 1999a,b; An et al., 2000a; Qasem and Foy, 2001; Batish et al., 2002b; Singh et al., 2003a,b; Tawaha and Turk, 2003; Tsuzuki and Dong, 2003). In the present study, the residues comprised dried stem, leaves, parts of inflorescence that remained after the completion of its life cycle. When the weed is green, the leaves, stem, inflorescence are all reported to be allelopathic in nature, releasing allelochemicals through various mechanisms, such as leachation, death and decay and even exudation (Einhellig, 1988; Singh et al., 2001; Kobayashi, 2004). From
the results of the present study, it becomes evident that allelochemicals or inhibitors are
released through leachation and these are biologically very active. Nature of
allelochemicals could be either phenolics or sesquiterpene lactones that leach out of the
plant as already reported (Kanchan, 1975; Mersie and Singh, 1987b; Rani, 1990). Since
upon leachation, they enter the soil medium, therefore, bioefficacy studies were also
undertaken in soil amended with extracts to ascertain whether the allelochemicals or
inhibitors upon release accumulate in soil in bioactive concentration and cause inhibitory
effect on the test crops. In this case also, it was found that growth of the test crops was
significantly reduced compared to unamended soil. Here also, maximum retardatory
effect was observed on C. arietinum followed by varying degree of inhibition in the other
crops. Maximum retardatory effect on all the crops was observed at the highest
concentration of extract-amended soil. All the parameters under study i.e. root length,
shoot length and dry biomass, were significantly inhibited indicating the phytotoxicity of
soils amended with extracts. Thus, this study confirms that the allelochemicals
accumulate in soil at bioactive concentration and bring about the inhibitory effect on
other crops. Since the residues are in the stage of decomposition and their biomass
gradually mixes with the soil, these incorporation or mixture in the soil may also be
responsible for inhibitory effect on other crops. In order to test this, the powdered residue
was also amended in the soil and growth of test crops was checked. As expected in this
set-up of experiments, the growth of all the test crops especially, C. arietinum and R.
sativus was significantly and adversely affected. In the soil amended with residue powder
at the rate 4%, none of the seeds of C. arietinum and R. sativus germinated, indicating a
complete inhibition. While in the amendments involving lower concentrations the growth
of these crops was significantly reduced compared with that of unamended soil. In case
of other test crops, a clear retardatory effect was observed at all the concentrations, but
complete inhibition could never be observed.

From all the bioefficacy studies, it becomes clear that residue of P. hysterophorus
exert an inhibitory effect on test crops by releasing probably the allelochemicals that
accumulate in the soil in concentration enough for bringing retardatory effects on the
crops. In order to find out the allelochemics or phytochemicals in the extracts and
amended soils, some specific tests, such as presence of phenolics, were conducted. A number of studies indicate that phenolics are ubiquitous in plants and are well known group of allelochemicals that are easily leachable and may also release through root exudation and decomposition (Sequiera et al., 1991; Appel, 1993; Reigosa et al., 1999; Singh et al., 2001; Ambika et al., 2003). In our study a significantly high amount of phenolics were found to be present in aqueous extracts of residue. The studies on the dynamics of release of phenolics indicate that their release is maximum after 20 h of their solution made in water. Likewise, phenolics were also found to be present in amended soils (both residue-amended and residue-extract-amended soils) whereas their amount was negligible in the unamended soil. The dynamics of release of phenolics in soil was, however, different compared to simple extracts. In case of RS, the dynamics of release of phenolics was calculated to be maximum in 48 h whereas 30 h in case of RES. This difference may be due to their relative release from the respective treatment. For example, in case of extract amended in soil, release of phenolics would be relatively easier than from soil residue. The composition and quantity of allelochemicals may vary substantially over time or with changed environmental conditions (Wojcik-Wojtkowiak et al., 1990; Blum, 1998; Dalton, 1999; Okumura et al., 1999).

In case of Vulpia, however, the presence of phenolics in any type of the residues indicates their role in causing observed inhibition of growth on test plants (An et al., 1996a,b; 1997; 2000a,b).

In P. hysterophorus, the presence of phenolics have been indicated by several studies conducted with fresh parts (Kohli et al., 1985; Kumari et al., 1985; Kumari and Kohli, 1987; Rani, 1990). However, their presence in the residue of the plant is of utmost ecological significance, as even after the completion of the life cycle of plant, the phytotoxicity prevails. Besides, phenolics, reports indicate the presence of sesquiterpene lactones, especially, parthenin that is in fact the major constituent of the weed (Kanchan, 1975; Picman and Picman, 1984; Mersie and Singh, 1988; Kohli and Batish, 1994). Since there is no rapid and separate test of sesquiterpene lactones, hence no such test was conducted. However, their presence in the extracts and amended soils cannot be ruled out.
Several questions arise from these results:

- Does allelochemicals bring growth inhibitory effects directly?
- Do changes in the nutrient status affect the allelochemical action or does allelochemicals interact with or through soil nutrients?
- What is the nature of allelochemicals involved?

To find the answers to these questions, it could be stated that since drastic changes in the growth of test crops have been observed with extracts of residues and amended soils. It can be clearly stated that allelochemicals are responsible for bringing inhibitory effect. However, it is difficult to say whether these interact with soil nutrients and cause changes in the physico-chemical properties of the soil, until specific experiments are conducted.

In order to test this, specific studies were conducted to find out the nutrient status in amended soils as well as the residue itself and determination of pH and EC of extracts. The pH of aqueous extracts of concentration 0.5 to 4% was near neutral varying from 6.95 to 6.69, which is optimum for the plant growth and thus plays no role in causing any inhibition to test plants. Likewise, the EC of extracts varied from 1.3 to 7.3 mS which is again optimum. These results rule out the possibility of any ionic effect or extreme pH in retarding the growth of test plants. Therefore, most likely, the presence of phenolics in the extracts (as already indicated) is the sole reason for causing growth retardatory effect. The studies conducted on available elements of *P. hysterophorus* residues reveal that various macro- and micro-nutrients were present in sufficient amounts in the residues. In case of amended soils, the differences in physico-chemical properties were apparent. Though, the differences in pH were not very sharp, the electrical conductivity was enhanced several times, both in RS and RES, indicating that the amendments improved ionic status of soil several times. Likewise, a significant increase in organic matter was also seen, though, it was not as sharp as other nutrients and phenolics in the soil. A sharp increase in available, N, P and K was observed and in each case, the increase was more in RS compared to RES showing, thereby, that the amounts of N, P and K were sufficient enough not to cause any growth retardatory effect. Besides macro-nutrients, the amounts
of various micro-nutrients like Zn, Mn, Fe and ions like Cl and HCO₃ also increased many folds in amended soils compared to unamended soil. The amount of Cu, however, did not show much change in amended and unamended soils. Besides, the presence of phenolics in amended soils, the amount was also detected as already indicated. All these observations point to the fact that phenolics are responsible for growth retardatory effects since all the amended soils had nutrients in sufficient amounts that is unlikely to cause any growth inhibition. Thus, the presence of allelochemicals in P. hysterophorus residues is the primary reason for the growth retardation of test plants. As regards their identification, based on the tests conducted through TLC and HPLC, in addition to one unidentified, eight phenolic acids namely ferulic acid, p-coumaric acid, chlorogenic acid, gallic acid, syringic acid, caffeic acid, vanillic acid, p-hydroxybenzoic acid were detected. Earlier their presence was also reported in fresh parts (Rani, 1990; Kohli and Batish, 1994). Besides these, the presence of parthenin was also detected through HPLC. Thus, the results highlighted in the present section, indicate the followings:

1. Appreciable amount of residue of P. hysterophorus is accumulated under field conditions during the months of October-November i.e. after the completion of major life cycle in post-rainy season. However, little amount of residue could be seen throughout the year.

2. The residue of P. hysterophorus, like its fresh parts was allelopathic in nature causing significant retardatory effect on the winter season crops.

3. Allelochemicals are released through leachation/decomposition, as established through this experiment.

4. Chemically, the allelochemicals were phenolic acids namely ferulic acid, p-coumaric acid, syringic acid, gallic acid, p-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, besides, major sesquiterpene lactone parthenin.

5. There is unlikelyhood of any resource depletion as all the soils amended with extracts or residues were sufficiently rich in macro-and micronutrients. Thus, the residue of P. hysterophorus exerts its allelopathic effects like its fresh parts.