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PHYTOTOXICITY OF ABOVE - GROUND WEED RESIDUE AGAINST SOME CROPS AND WEEDS

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Abstract

Weed residues mixing with the soil after the death of weeds can inhibit growth and development of crop plants. A study was conducted to assess the allelopathic intrusion of *Nicotiana plumbaginifolia* above-ground residues on growth of selected crops (*Pisum sativum*, *Cicer arietinum*) and weeds (*Cassia tora*, *C. sophera*), nature of chemicals involved, role of nutrients and physico-chemical parameters. Growth responses of weeds and crops were analyzed for soil amended with different rates of *Nicotiana* residue (residue amended soil, RS) and residual extract (residue extract amended soil, RES). Likewise, the growth behaviour of test plants was also evaluated against extract of residue in hydroponics (residue extract, RE). Physiochemical screening of amended soils was conducted to look for concentration and bioavailability of essential nutrients. The soil amended with residues (RS and RES) showed inhibitory effect on test plants, however, residue extract (RE) had more inhibitory effects. The inhibition in growth (root length, shoot length and dry biomass) was concentration dependent and *C. tora* plants experienced the highest reduction among all test plants. A partial enrichment was observed in nutrient status and phenolic content as we increased the concentration of the soil amendment or the extract. This points out that growth inhibition occurred due to phenolic and not due to nutrient depletion. These findings indicate a role of putative phenolic allelochemicals forced allelopathic effects on *C. arietinum*, *P. sativum*, *C. tora* and *C. sophera*.

Key words: Allelochemicals, Growth behavior, Hydroponics, Phenolic content, Soil amendment.

Introduction

The allelochemicals are the bioactive chemicals discharged by the plants which pass into the soil and adversely influence the growth and development of the neighboring plants in the form of a biological interference (Secmen & Ozturk, 1978; Cheng & Cheng, 2015). Allelopathy can play a key role in weed management, crop protection, and crop restoration if employed properly. The allelochemicals with allelopathic potential exist in all parts of plants such as leaves, stem, roots, flowers and even pollen grains (Ozturk *et al*., 2007; Chon & Nelson, 2010) and are released as leachates from aerial parts, decomposition of residues, root exudation or even volatilization from plant surfaces (Arfan *et al*., 2009; Barkatullah *et al.*, 2010; Shinwari *et al*., 2013; Khan *et al.*, 2015 Mushtaq & Siddiqui, 2018).

Species from the genus *Nicotiana* are primarily native to the Neotropics and Australia (Chase *et al*., 2003). *N. plumbaginifolia* belongs to the section *Alatae*, which is considered a monophyletic group (Kaczorowski *et al*., 2005). The species epithet '*plumbaginifolia*' comes from the way in which the leaves resemble those of species in the genus *Plumbago* (Anon., 2017). *N. plumbaginifolia* is native to Mexico, South America and parts of the Caribbean (Anon.,_2017). Although it is reported as native to Cuba by Acevedo-Rodríguez & Strong (2012), Oviedo *et al*., (2012) list it as naturalized and invasive. *Nicotiana plumbaginifolia* Viv. belonging to the family Solanaceae is commonly known as Tex-Mex tobacco, curl-leaved tobacco or wild tobacco. Its other names are; *Nicotiana crispa* Cav., *N. minor* Sessé & Moc., *N. plantaginea* DC. ex Dunal, *N. tenella* Cav. This plant is an annual herb native to Mexico, South America and parts of the Caribbean. It is one of the most widespread species of the genus *Nicotiana*, reported as invasive in Cuba and naturalized in parts of Asia, Middle East and USA. *N. plumbaginifolia* reproduces via self-pollination and displays very widespread ecological dissemination throughout the globe. In India it is distributed in Assam, Delhi, Gujarat, Madhya Pradesh, Maharashtra, Manipur, Tamil Nadu, Uttar Pradesh and West Bengal in India (Anon., 2016). The species is considered a weed of humid wastelands and cultivated fields, occurs on wastelands near water also along river banks, railway tracks, roadsides and in cultivated fields (Sudhakar Reddy *et al*., 2008; Anon., 2017; Flora of Pakistan, 2017).

It is also included in a list of invasive species in India and described as an "aggressive colonizer" (Sudhakar Reddy *et al*., 2008). The species is reported from present in Asia, Africa, Europe, North America, Central America, the Caribbean and South America (Acevedo-Rodríguez & Strong, 2012; Anon., 2016; Anon., 2017; Anon., 2017f; Anon., 2017e; Anon., 2017g).

There is a lack of information on the distribution of this taxon and its environmental requirements, so it is not possible to properly assess the species' risk of introduction or its distribution limits. The fact that the species is used as a model species for molecular, genetic and plant physiology studies could lead to its spread, if it were to escape from sites where it is cultivated for

scientific purposes (Knapp & Clarkson, 2004). The chromosome number is n=10 (Kaczorowski *et al*., 2005). It is a wild relative of, and genetic resource for, tobacco (Anon., 2017g). Germplasm collections are stored at the USA's Agricultural Research Station facilities (Anon., 2017g). DNA barcode information for the species is available at the Barcode of Life Data Systems (Anon., 2017a). *N. plumbaginifolia* reproduces by seed (Anon., 2017), most seeds are produced through self-pollination (Kaczorowski *et al*., 2005; Figueroa-Castro & Holtsford, 2010). One plant can produce up to 100 capsules, with about 800-1000 seeds per capsule (Gairola *et al*., 2016). It flowers sporadically from March to November (Anon., 2017; Flora of Pakistan, 2017). Anthesis is nocturnal, with flowers opening at dusk and remaining open for two nights (Kaczorowski *et al.*, 2005). The seeds have a strong primary dormancy, requiring approximately a year of "afterripening" to be able to germinate (Grappin *et al*., 2000). Frey *et al*. (1999) reported that seeds stored for one year at 8°C yielded up to 100% germination. Seeds are likely to be dispersed as; can be transported through water or through movement on soil, clothes, footwear, vehicles or agricultural machinery.

The removal of flowers and fruits delays plant senescence (Gupta & Chatterjee, 1971). In hot areas it grows in planters or cultivated fields that are well irrigated (Gairola *et al*., 2016). Considering its geographical distribution, its mean temperature limits are likely to be between 18°C and 35°C. It is reported as an agricultural weed (Anon, 2017c). The species is available for purchase for use in research (Knapp & Clarkson, 2004). It is invasive outside its native range, has a broad native range, pioneering in disturbed areas, tolerant of shade, has high reproductive potential, has propagules that can remain viable for more than one year.

N. plumbaginifolia genomic and cDNA libraries are sold for research purposes (Knapp & Clarkson, 2004). The species is also resistant to *Phytophthora nicotianae*, a fungus that causes the highly damaging disease 'black shank' in tobacco, making it a desirable species in the production of plant-made pharmaceuticals (Li *et al*., 2006). The plant is widely used as a model organism for research on plant physiology, genetics and molecular studies (Hérouart *et al*., 1994; Pinto *et al*., 1995; Farnsworth, 2000; Majira *et al.*, 2002; Knapp & Clarkson, 2004; Smigocki & Wilson, 2004; Lurquin & Kleinhofs, 2012; Anon., 2017g). Its widespread use is due to its short life cycle, fast growth in culture, regeneration ability, suitability for protoplast culture, low chromosome number, haploid genome and the fact that genetic map information is available for this species (Vasil *et al*., 1982; Lurquin & Kleinhofs, 2012).

In India, this species is used to remove leeches from the body (Teron & Borthakur, 2013). Other medicinal uses include the treatment of hemorrhoids, snake bites and wounds (Rothe, 2011; Singh *et al*., 2014). It is reported to have antibacterial properties (Singh *et al*., 2010) and is also used to treat animal wounds (Anon., 2017d).

Extracts from *N. plumbaginifolia* can inhibit the toxin production of the fungus *Aspergillus flavus*, which is known to infect cereal grains, legumes and tree nuts (Anon., 2017b). It is reported to be an insecticide (Rothe, 2011) and it also has the potential to be used in bioremediation of soils polluted with cadmium (Doroszewska & Berbec, 2004).

The plant in general largely establishes as a weed on moist soil along the roadsides, waste places, swamp areas and fallow lands, shades of buildings and amongst several crops (Mushtaq *et al*., 2018). Mushtaq *et al*., (2018, 2019) have demonstrated its strong allelopathic potential. However, the phytotoxicity of its above-ground residue and understanding of the role of macro and micronutrients on the behavior of allelochemicals is lacking information to a large extent. The effects of physico-chemical parameters on the phenomenon of allelopathy too have not been completely evaluated. This plant is rich in alkaloids in the form of pyridyl-pyrrolidines and pyridyl-piperidines; the chief alkaloids are nicotine, nornicotine, N-acetylnornicotine, anabasine and anatabine (Eckart, 2008). The methanolic extract of foliar part of *N. plumbaginifolia* contains secondary metabolites of several classes (Mushtaq *et al*., 2019).

N. plumbaginifolia is easily differentiated from other *Nicotiana* species by its whitish flowers and long corolla tubes. In contrast, the flowers of *N. tabacum* have rosy-white corollas. The vast majority of information available about this species relates to its use as a model plant for scientific research. More detailed information is required about its environmental requirements, risk of introduction and potential economic, social and environmental impacts. This study has been undertaken with this aim.

Studies on the allelopathic interference of this invasive weed, particularly through residue extract and its amendment with soil, are lacking and not much is known about its bioactive compounds. In the light of these facts, we have framed a hypothesis that different above-ground parts (leaf, stem and flower) of *N. plumbaginifolia* after the death of the plant fall to the ground (residue) and are eventually degraded by biotic/abiotic factors thereby releasing phytotoxic allelochemicals into the soil which prove harmful towards the expansion of associated plants covering the area in its neighborhood. Keeping this in view, an assessment was designed to investigate the allelopathic stress produced by extract and residues of *Nicotiana*, nature of chemicals involved and the possible role of nutrients if any, in modifying the allelopathic intrusion. For this purpose two important pulse crops i.e. *Pisum sativum* L., *Cicer arietinum* L. and two vigorous weeds, i.e. *Cassia tora* L., *Cassia sophera* L. were selected as test plants.

Materials and Methods

Collection of the material: The soil was gathered from an open area devoid of *N. plumbaginifolia*. It was air-dried, sieved through 2 mm mesh and divided into 1 kg lots. The seeds of crops (*P. sativum*, *C. arietinum*) and weeds (*C. tora*, *C. sophera*) were procured from Indian Agricultural Research Institute, New Delhi (India) and National Research Centre for Weed Science, Jabalpur (India). *N. plumbaginifolia* invaded site was chosen from the outskirts of campus of Aligarh Muslim University, Aligarh-India. Plant density and biomass were estimated by setting 20 quadrats (1 m^2/quadrat) of 1 m^2 after the

plants were completely dry at the completion of their life cycle. The naturally dried out plant residue (above-ground) was gathered, powdered and left in polyethylene sacks to be used later.

Preparation of residue amended soil (RS), residue extract (RE) and residue extract amended soil (RES): Under natural environment, *N. plumbaginifolia* after death falls on the ground and gets mixed up with the soil. In order to mimic these conditions; 5, 10, 20 and 40 g of the residue was added to 1 kg soil lot. It was thoroughly blended in order to get 0.5%, 1%, 2% and 4% RS's respectively. For framing RES's, first stock of RE's was prepared. Out of the powdered residue 40 g were engrossed in 1000 ml of distilled water for 24 hours at room temperature followed by sifting through a twofold layer of muslin fabric and then Whatman no. 1 filter paper to acquire 4% extract. Further dilutions were made with distilled water to obtain 2%, 1% and 0.5% extracts. These solutions were referred to as RE's. A part of the RE's was left as a reserve for another experiment (growth studies with RE under laboratory conditions) and other part was used to make RES's. Five hundred ml of each of 0.5%, 1%, 2% and 4% residue extract were added to 1 kg soil in plastic trays (27×15 cm) and left for shade-drying for 30 hours. This was followed by adding 200 g each of RS or RES to petri dishes (15 cm diameter). The untreated (unamended) soil designated as "US" was also placed in the petri dishes to serve as the control.

Growth studies in amended soils: Ten uniform seeds of *P. sativum*, *C. arietinum*, *C. tora* and *C. sophera*, each were sown in 'RS' and 'RES' filled petri dishes (15 cm diameter) alongside US that served as the control. Five replicates were maintained for every treatment in a completely randomized block design (CRBD). These were left in a growth chamber at 25±2°C, 75±2% RH and 16/8 hour light/dark photoperiod. 15 ml of distilled water was sprinkled on each petri dish on daily basis up to 8 days. Following this the saplings were carefully uprooted ensuring negligible harm to the roots, root and shoot lengths of seedlings were measured using meter-scale and their biomass determined after oven drying at 80°C for 24 hours.

Growth studies with RE: The pH was recorded with EcoScan digital pH meter (Eutech Instruments, Singapore), the osmotic potential was determined as per the formula: osmotic potential = 0.36×conductivity (mS). And for determining conductivity, the electrode of an EcoScan Con 5 digital conductivity meter (Eutech Instruments, Singapore) was dipped into each aqueous extract. The total phenolic content of the REs determined with Folin-Ciocalteu reagent (Sisco Research Laboratories Pvt. Ltd., product code 39520) following a standard protocol (Sarwar *et al*., 2001). The seeds of test plants (*P. sativum, C. arietinum, C. tora, C. sophera*) were treated *in vitro* with different concentrations of extracts (0.5%, 1%, 2%, 4%) separately. Distilled water served as the control. Ten seeds of each of the test plant were placed evenly in petri dishes (15 cm diameter) lined with sanitized absorbent cotton wrapped in Whatman no. 1 filter paper and sprinkled with 15 ml of the respective treatment solution. Five replicates were maintained for each treatment in a completely randomized design. The whole set-up was kept in a seed germinator retained at 25±2°C and 75±2% RH. After 8 days, root and shoot length of the seedlings in each petri dish was measured and dry biomass was determined.

Estimation of phenolics from *N. plumbaginifolia* residue extracts and in amended soils (RS and RES): The total amount of phenolics was evaluated in four distinct parts. In the first part 500 ml of 4% residue extract was added to 1 kg soil (RS) and in the second part 40 g residue plus 500 ml distilled water were added to 1 kg soil to serve as RES. Five grams of the soil was taken from each part distinctly after 4, 8, 12, 20, 26, 32, 38, 42, 60, 72, 100, 120 and 140 hours, air-dried and used for extracting phenolic acids following the process of Keskin-Šašić *et al*. (2012) using Folin-ciocalteu reagent. In the third part 500 ml of distilled water was added to 1 kg soil and blended thoroughly (control) and afterwards tested for phenolic content. In the fourth part 40 g residue was added to 1000 ml distilled water and blended thoroughly (RE) of which 5 ml was removed after 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 40 and up to 72 hours and respective amount of phenolics was determined from each of these extracts. Five replicates were maintained from each treatment throughout the whole study.

Evaluation of physico-chemical characteristics of amended soils: Amended soils i.e. residue amended (RS), residue extract amended (RES) and unamended soils (US or Control) were analyzed for various parameters like pH (1:5 soil/water, w/v); osmotic potential (OP) (1:5 soil/water, w/v); organic matter (OM) as well as available nitrogen, phosphorus, potassium, calcium, magnesium, chloride, bicarbonate, iron, manganese, and zinc. pH was measured with pH meter (Eutech Instruments Pte Limited, Singapore), OP was determined as per the formula: Osmotic Potential=0.36×Conductivity (mS) and EC with an EcoScan digital conductivity meter. OM content was measured by rapid titration method (Pereira *et al*., 2006); amounts of available nitrogen by Kjeldahl method (Saez-Plaza *et al*., 2013); phosphorus by molybdenum blue method; potassium by ammonium acetate extract (pH 7); calcium, magnesium, chloride, bicarbonate (by titration methods) and atomic absorption spectrophotometry was used for determination of iron, manganese, and zinc (Allen, 1989).

Determination of elemental status of residue: Elements like carbon, nitrogen and hydrogen in the residue were resolved using a CHN analyzer and for phosphorus, potassium, sodium, calcium, magnesium and trace elements, wet diacid digestion of the residue was performed using nitric acid and perchloric acid. The status of phosphorus was determined from the residue duly digested (referred to as plant digest) using the colorimetric method with Vandamolybdate reagent. Sodium was analysed through flame photometry and calcium and magnesium in plant digest by the titration method. The concentrations of zinc, copper, iron and magnesium in plant digest were determined by Atomic Absorption Spectrophotometer (AAS).

Statistical analysis: The measurements of root length, shoot length and dry biomass were articulated with respect to control and analyzed by DMRT at $p<0.05$. The results attained from nutrient analysis were also subjected to DMRT as per Duncan (1955).

Results and Discussion

Growth studies

a. Growth studies in RS: Seed germination of all test plants (*P. sativum*, *C. arietinum*, *C. tora* and *C. sophera*) was recorded as 100% in control as well as in different treatments of amended soils so that the data has not been formulated and presented.

The radicle length was measured largest in *C. arietinum* followed by *P. sativum*, *C. sophera* and *C. tora* when sown in unamended soil (control) while, a substantial decrease in its length was seen in RS (Fig. 1a). The root length declined along the concentrations, i.e. 0.5% to 4% with the largest reduction perceived in *C. tora* (31.61%) followed by *P. sativum* (25.61%), *C. arietinum* (21.53%) and least in *C. sophera* (19.72%) at 4% concentration when compared to the control (Fig. 1a).

The plumule length of target crops and weeds varied considerably both in control as well as in different treatments. It was maximum in *C. arietinum* followed by *P. sativum* in the case of crops while in weed plants the length was maximum in *C. sophera* and least in *C. tora* in control set as shown in Fig. 1b. Among all the test plants, plumule length got reduced with increase in concentration of residue amended in soil with 4%. It was the most inhibitory among all treatments. *C. arietinum* (33.21%) showed maximum reduction followed by *P. sativum* (29.31%), *C. tora* (27.85%) and *C. sophera* (16.27%) at 4% concentration as compared to control. The plumule length of all test plants decreased significantly with increase in concentration (Fig. 1b).

C. arietinum had maximum dry biomass followed by *P. sativum* and *C. sophera*, it was the least in *C. tora* (Fig. 1c). The largest inhibitory effect was recorded at 4% concentration and with *P. sativum* (50.36%) displaying the maximum reduction followed by *C. arietinum* (48.66%), *C. tora* (48.04%) and *C. sophera* (47.89%) (Fig. 1c). The reduction observed in dry biomass along different concentrations was statistically significant (Fig. 1c).

b. Growth studies in RES: The radicle length was reported to be largest in *C. arietinum* followed by *P. sativum*, *C. sophera* and *C. tora* when grown in control soil while a substantial decrease in its length was seen in RES (Fig. 2a). The root length declined significantly along the concentrations, i.e. 0.5% to 4% with the maximum reduction observed in *C. tora* (44.49%) followed by *C. sophera* (37.42%), *P. sativum* (33.20%) and least in *C. arietinum* (32.56%) at 4% concentration as compared to the control (Fig. 2a).

The shoot length of test plants (crops and weeds) varied substantially both in control as well as in different treatments. It was maximum in *C. arietinum* followed by *P. sativum* in the case of crop plants while in weeds the shoot length was recorded highest in *C. sophera* and least in *C. tora* in control set (Fig. 2b). Among all test plants, the shoot length decreased with increasing concentration of RES with 4% proving to be the most inhibitory among all the treatments. *C. arietinum* (38.59%) was observed to suffer maximum reduction followed by *C. tora* (38.21%), *P. sativum* (34%) and least in *C. sophera* (29.75%) at 4% concentration when compared to the control. In general, shoot length of all test plants decreased significantly when compared to control along different concentrations of RES (Fig. 2b).

C. arietinum had maximum dry biomass followed by *P. sativum* and *C. sophera* while the least dry biomass was recorded in *C. tora* (15.47±0.48 mg) in control setup (Fig. 2c). Among all test plants, largest inhibitory effect was reported at 4% concentration (Fig. 2c) with *C. sophera* (56.04%) showing the maximum reduction followed by *C. tora* (54.59%), *C. arietinum* (437%) and least in *P. sativum* (436%). The reduction observed in dry biomass along different concentrations was statistically significant (Fig. 2c).

c. Growth studies in RE: The seeds of test plants under study were measured to have the maximum radicle length in *C. arietinum* followed by *P. sativum*, *C. sophera* and *C. tora* in control. However, when the seeds were exposed to growth trial with different concentrations of RE in petri dishes, the decrease in root length was observed with increasing concentration of RE (Fig. 3a). Maximum reduction was observed at 4% in any of the cases ranging from 48.99% in the case of *C. tora* to 38.77% in *C. arietinum* as compared to the control.

The shoot length of seeds treated with RE was less in contrast to their respective controls. In all test plants sown in control, maximum length was recorded in *C. arietinum* followed by *P. sativum* and *C. sophera*. It was the least in *C. tora*. Moreover, similar to root length, the shoot lengths too were shorter in petri dishes exposed to different concentrations of RE when compared to their respective controls, with 4% RE proving to be the most inhibitory among all treatments (Fig. 3b). Maximum reduction was observed at 4% as recorded in *C. tora* (49.22%) and least in *C. sophera* (41.79%).

The dry biomass of test plants sown in control likewise varied considerably from the seeds exposed to RE as did the radicle length and plumule length. In control, the maximum dry biomass was measured in *C. arietinum* as compared to other plants followed by *P. sativum*, *C. sophera*, with the least recorded in *C. tora* (Fig. 3c). The dry biomasses of plants whose seeds were exposed to RE were found to be lower than their corresponding control values (Fig. 3c). The reduction observed in dry biomass was statistically significant and was concentration-dependent with the highest reduction observed at 4% RE. The 4% extract was more effective for *C. sophera* and *C. tora* showing reductions of 63.15% and 6.20% respectively.

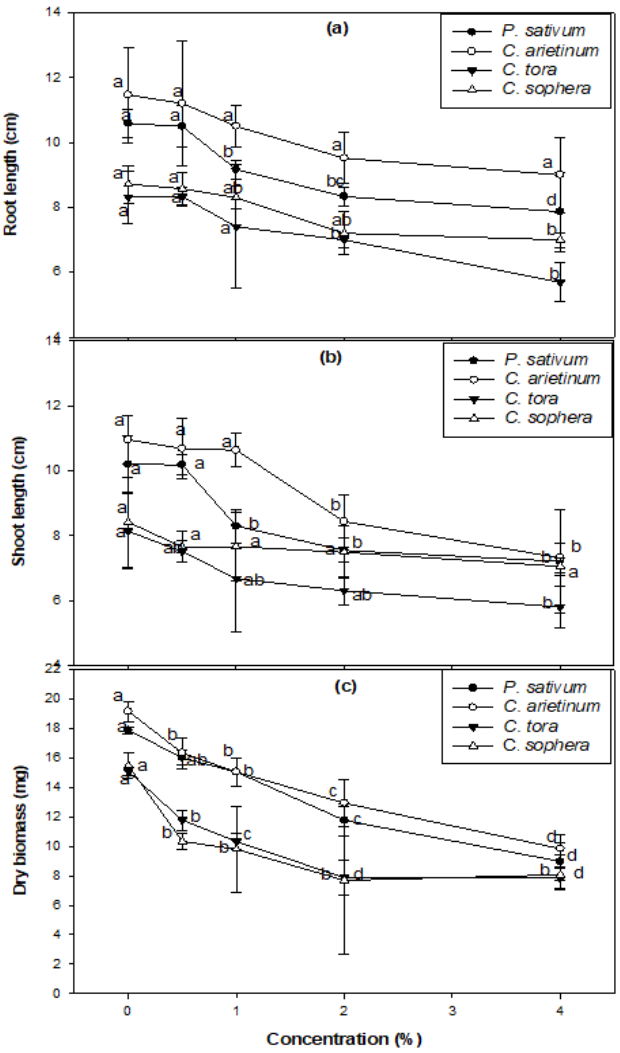

Fig. 1. Allelotoxic effect of residue amended soil, RS on (a) root length (b) shoot length and (c) dry biomass of target weeds and crops.
[Significant difference is represented by different superscript symbols among themselves along a curve at $p<0.05$ applying DMRT. Bars over lines represent standard deviation]

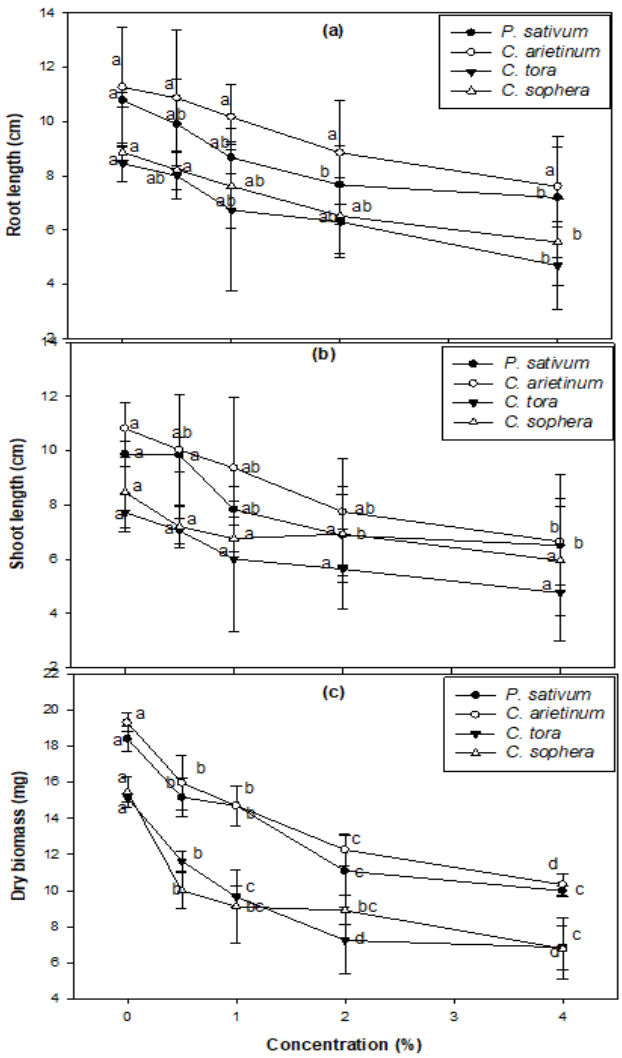

Fig. 2. Allelotoxic effect of residue extract amended soil, RES on (a) root length (b) shoot length and (c) dry biomass of target species (crops and weeds).
[Significant difference is represented by different superscript symbols among themselves along a curve at $p<0.05$ applying DMRT. Bars over lines represent standard deviation]

Dynamics of phenolics in amended soils and extract

a. Residue soil (RS): The control soil (US) was recorded to encompass least amount of phenolic content (95.98±0.57 µg/g) of all treatments. The total quantity of phenolic content minus phenolic content in unamended soil is presented in Fig. 4a. The highest amount of phenolic content (113.12±0.12 µg/g) was recorded under 38 hours of the amendment. Between zero to 38 hours, the substance demonstrated a steady increment. From that point onwards, a consistent decline was observed with the least value (88.65±0.48 µg/g) noted after 140 hours.

b. Residue extract soil (RES): The amount of phenolic content minus phenolic content in control soil is presented in Fig. 4b. The highest volume of phenolic content (184.25±0.55 µg/g) was estimated after 4 hours of the introduction of the extracts and afterwards phenolic content dropped gradually and continued up to 140 hours (78.42±0.45 µg/g) (Fig. 4b).

c. Residue extract (RE): The phenolic content was evaluated following 6 hours of adding distilled water to the above-ground residue (415.48±0.25 µg/ml). There was a very little difference in the amount of phenolics between 0-6 hours. However, at 12 hours the amount increased brusquely (486±0.26 µg/ml) with the maximum value noted after 20 hours (492.64±0.38 µg/ml) following which a gradual decrease was recorded up to 72 hours. The least phenolic content (325.68±0.15 µg/ml) was observed at 72 hours (Fig. 4c).

The pH of the REs was more or less near to neutral however displayed a gradual increase from 5.45 to 6.50 for 4% and 0.5% respectively. The extracts presented different osmotic potentials with a range of -0.38 to -0.89 bars. The phenolic content also varied along the concentration from 357.12 at 0.5% to 677.05 µl/mg at 4% RE (Table 1). The increase in values along the concentration was observed to be linear in all these parameters.

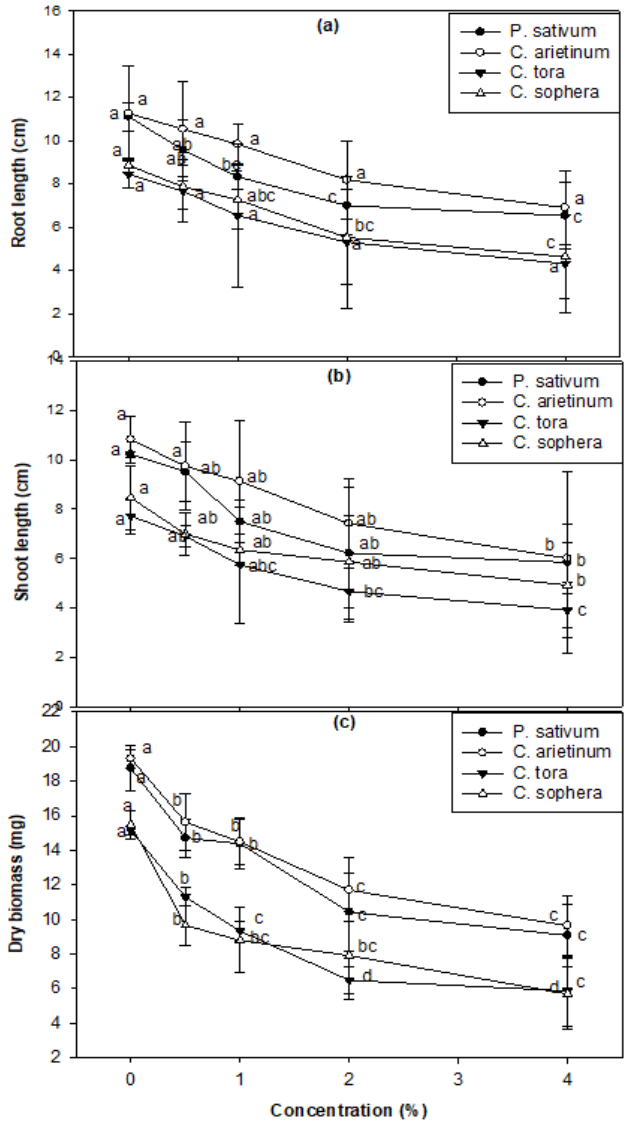

Fig. 3. Allelotoxic effect of residue extract, RE on (a) root length (b) shoot length and (c) dry biomass of target weeds and crops. [Significant difference is represented by different superscript symbols among themselves along a curve at $p<0.05$ applying DMRT. Bars over lines represent standard deviation]

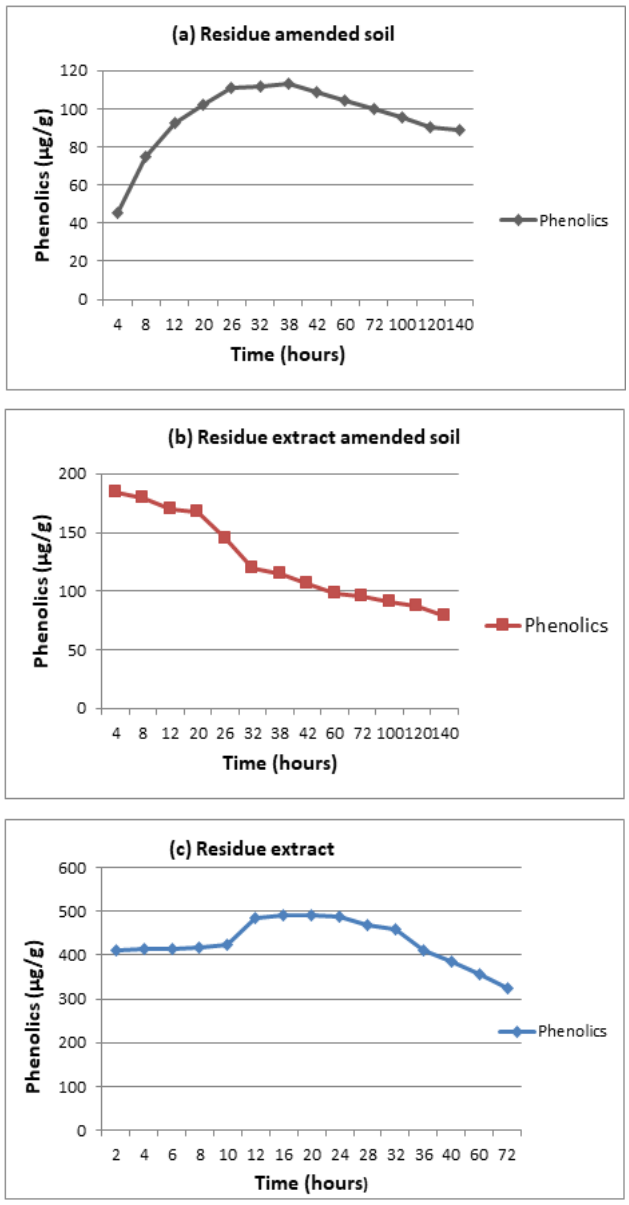

Fig. 4. Dynamics of phenolics in (a) Residue amended soil (b) Residue extract amended soil and (c) Residue extract.

Table 1. Values of pH, OP and phenolic content in REs of *N. plumbaginifolia*.

Concentration (%)	pH	Osmotic potential (bars)	Phenolic content (µg/ml)
0.5	6.50a	0.38a	357.12a
1	6.25b	0.65b	401.14b
2	5.98c	0.79c	411.25c
4	5.45d	0.93d	677.05d

Different alphabets within in a column represent significant difference at p<0.05

Elemental analysis of the residue: *N. plumbaginifolia* above-ground residue was assessed to comprise 26.80±0.34% of total carbon. Total hydrogen and total nitrogen were estimated to be 5.95±0.45% and 3.06±0.66% respectively. The available elements in the residue had phosphorus (0.16±0.68%), potassium (2.92±0.64%), sodium (0.10±0.10%) (Table 2). Macronutrients, like; calcium and magnesium were 14.86±0.39 % and 14.97±0.42 % respectively. Iron (19.02±0.26 ppm) was measured to stand at highest concentration among the micro-elements, followed by manganese (2.98±0.68 ppm), zinc (0.85±0.15 ppm) and copper (0.36±0.10 ppm) (Table 2).

Effect of *N. plumbaginifolia* amended soils on the soil nutrients and amount of phenolics: The influence of residue amendment on the availability of soil nutrients has revealed that there is no decline in the nutrient status (Table 3). The amended soils (RS and RES) were significantly richer in OM and every nutrient was higher in quantitaty (Table 3). The levels of available nitrogen, phosphorus, potassium, calcium, magnesium, chloride,

iron, manganese and zinc increased in the amended soils as compared to the control, the highest increment was recorded at 4% concentration of both types of amended soils (Table 3). pH increased in the amended soils (RS and RES) as compared to control and was approaching neutral with increasing concentration (Table 3).

Table 2. Elemental status of *N. plumbaginifolia*.

Element(Units)	Value (%)
Total C (%)	26.80 ± 0.34
Total H (%)	5.95 ± 0.45
Total N (%)	3.06 ± 0.66
Available P (%)	0.16 ± 0.68
Available K (%)	2.92 ± 0.64
Available Na (%)	0.40 ± 0.09
Available Ca (g/100g)	14.86 ± 0.39
Available Mg (g/100g)	14.97 ± 0.42
Available Zn (ppm)	0.85 ± 0.15
Available Cu (ppm)	0.36 ± 0.10
Available Fe (ppm)	19.02 ± 0.26
Available Mn (ppm)	2.98 ± 0.68

± Represents standard deviation

N. plumbaginifolia residue amended soils (RS and RES) encompassed a substantial quantity of water-soluble phenolics (Table 3). Amount of phenolics nearly doubled at 4% concentration of RS (98.12±0.78 mg g^{-1} soil) as compared to the control (39.24±0.67 mg). In the case of 4 % RES, the phenolic amount increased many folds (128±0.56 mg) compared to the control (unamended soil) (Table 3).

Seedling development and dry biomass decreased due to the phytotoxic effect of residue extract in different concentrations when compared with the control. Our results clearly revealed that seedling growth of the test plants was expressively diminished when comaped with the unamended soil. The target plants displayed differing degrees of inhibitions, but the highest retardation was recorded in *C. tora*. The maximum decrease in the growth parameters in the test plants was observed at 4% concentration of the extract, which could be attributed to the allelochemicals aggregating in the soil at bioactive concentration and realising the inhibitory impact on the plants (Batish *et al*., 2009). These findings show that *N. plumbaginifolia* residue contains some water-soluble phytotoxic chemicals which upon discharge accrue in the soil upto bioactive concentration and inhibit crop and weeds growing in the proximity. Similar findings have been reported by Batish *et al*., (2009). Adding residue or blending it with soil leads to putrefaction and their contents gradually mix and build up in the soil, which may be responsible for the inhibitory impact on the plants. So as to assess this, the growth of the test plants was evaluated against exposure to the soil amended with *N. plumbaginifolia* residue. The growth of all target plants in this experiment has revealed that there was an affect on growth of plants, particularly *C. tora* and *C. sophera* got affected considerably and significantly. These findings are supported by earlier reports published by Batish *et al*., (2009) and Amb & Ahluwalia (2016). They have reported a negative impact upon target plants by the integration of residues from the donor plants. Similarly, the residues (both above and below-ground) of an invasive weed (*Ageratum conyzoides*) have been reported to significantly reduce the growth, nodules (number and weight) and leg hemoglobin concentration of target species (Batish *et al*., 2009). This demonstrates that some plants show variability in their reaction with reliance on a few variables like size of seeds and hereditary variations. The differential reaction of crop plants towards aqueous leachates or some other phytotoxic material has previously been reported for different crops (Mushtaq *et al*., 2018).

Table 3. Changes in the soil physiochemical properties in the *N. plumbaginifolia* residue-amended soils in comparison to control soil.

Parameter	Control	Residue amended soil				Residue extract amended soil			
		0.5%	1%	2%	4%	0.5%	1%	2%	4%
pH	6.15±0.04a	6.62±0.12a	6.75±0.23a	6.89±0.01a	6.90±0.15a	6.55±0.14a	6.68±0.12a	6.85±0.23a	6.95±0.01a
OM (%)	1.37±0.01a	1.61±0.07a	1.94±0.02b	2.30±0.10b	4.28±0.56c	1.40±0.11a	1.87±0.17b	1.97±0.18b	2.68±0.40c
Availabe N (g Kg^{-1}soil)	55.23±2.30a	57.16±4.10b	62.15±6.25c	64.50±5.24d	64.88±2.54d	57.43±2.30b	66.16±4.10c	69.00±6.25d	72±5.24e
Available P (mg $100g^{-1}$soil)	10.53±0.42a	13.87±0.51a	14.72±0.56 a	15.22±0.66a	18.42±0.76b	11.53±0.82a	18.87±0.58b	20.72±0.76c	25.62±0.66d
Available K (mg $100g^{-1}$ soil)	38.36±3.45a	39.52±5.64a	40.36±10.74ab	43.45±7.41b	46.45±7.41c	38.98±3.45a	42.52±5.64b	48.36±2.74c	52.45±3.41d
Ca (meq $100g^{-1}$ soil)	3.41±0.23a	3.52±0.33a	3.61±0.60a	3.73±0.44a	12.98±0.54b	3.71±0.23a	4.52±0.33ab	4.61±0.65ab	18.67±1.84c
Mg (meq $100g^{-1}$ soil)	4.10±0.10a	4.26±0.65a	4.50±0.66a	5.95±0.39b	8.89±0.42c	4.80±0.10a	5.26±0.65ab	6.48±0.66b	10.95±0.55c
Cl (meq $100g^{-1}$ soil)	1.43±0.26a	1.51±0.17a	2.31±0.35b	2.65±0.49b	3.95±0.58c	1.48±1.26a	1.68±0.17ab	2.12±0.65b	4.68±1.24c
Fe (ppm)	4.47±0.77a	4.63±0.55a	4.80±0.39a	5.80±0.66b	6.98±0.72c	4.67±0.77a	4.83±0.55a	4.90±0.32a	8.40±0.2 b
Mn (ppm)	2.42±0.32a	5.57±0.10b	9.31±0.71c	11.50±0.68d	14.20±0.58e	2.52±0.32a	6.57±0.10b	10.31±0.61c	15.90±0.72d
Zn (ppm)	1.13±0.87a	1.22±0.32a	1.27±0.37a	1.30±0.55a	1.33±0.57a	1.17±0.52a	1.21±0.30a	1.27±0.45a	1.35±0.24a
Phenolics (mg g^{-1} soil)	39.24±0.67a	42.84±.16b	58.68±0.54c	62.98±0.12d	98.12±0.78e	44.87±0.66b	62.89±0.14c	85.56±0.88d	128±0.56e

Different alphabets within in a row represent significant difference at $p<0.05$ applying DMRT. ± represents standard deviation

Many other reports are available regarding the phytotoxicity of residue extracts 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 (Singh *et al.,* 2003a, b; Belz, 2007; Uddin *et al*., 2014; Ramachandran, 2019). In our study we found that after the completion of life cycle, the plant residue gets accumulated and comprises of dried stem, leaves, as well as parts of inflorescence. Due to the allelopathic nature of weed, the allelochemicals are released through various mechanisms such as leachation, death, decay and even exudation (Einhellig, 2002; Uddin *et al*., 2014; Safdar *et al*., 2019) leading towards the inhibitory effects. Allelochemicals released as leachates are biologically very active as is evident from the results of present study. These are either phenolics or sesquiterpene lactones in nature that leach out of the plants (Li *et al*., 2010). Therefore, bio-efficacy studies were undertaken by us in the extract amended soil to ascertain whether the inhibitory effect exerted on the test plants is due to the allelochemicals or inhibitors that accumulate in the soil in bioactive concentration after their release through leachate.

Analyses of the physiochemical properties of the amended soils in our studies clearly indicated that the status of nutrients is not a growth limiting factor for the observed reduction in test plants (Table 3). The amended soils (RS and RES) were found comparatively richer in nutrients and enhanced status of OM was recorded in contrast to unamended control soil (Table 3). This finding runs parallel to the earlier reports related to the soil incorporated with residues/decomposing material from plants with allelopathic potential, which have shown an increase in nutrient status (Batish *et al*., 2007, 2009). The present study shows a significant increase in the available nitrogen in the amended soils. However, we did not analyze the possible effect of increased nutrient content on water potential of the amended soils. Nevertheless, the estimation of water-soluble phenolics from soils amended with above-ground residues and their observed concentration-dependent phytotoxicity against test-plants indicates their possible involvement in the allelopathic effect (Figs. 1 and 2). These results run parallel with earlier studies discussing the water-soluble phenolics as ubiquitous organic based biomolecules released by roots, even decaying plant residues, and broadly associated with allelopathic interactions (Batish *et al*., 2007, 2009; Djurdjevic *et al*., 2008).

Phenolic content has shown more or less gradual upsurge over time attaining peak (Khaliq *et al*., 2013, 2014) at their corresponding time after which a reduction in phenolic content was witnessed in RS, RES and RE. This distinction might be because of their relative discharge from the respective treatments. The structure and amount of allelochemicals may differ/fluctuate considerably over time or with dynamic environmental factors which is in line with previous studies (Khaliq *et al*., 2013, 2014; Trezzi *et al*., 2016). Khaliq *et al*. (2013, 2014) suggested that residue-mediated suppression could happen only if the susceptibility phase of the target plant corresponds with the inhibitory peak period of an allelopathic plant. Since phenolics are the key group of water-soluble allelochemicals accountable for the most allelopathic action, their occurrence has been measured over time after integration (Khaliq *et al*., 2013, 2014). In this way, the occurrence of allelochemicals in *N. plumbaginifolia* residue is the principal cause for the growth inhibition of target plants.

Conclusions

The results highlighted in this study lead to the conclusion that, an appreciable quantity of *N. plumbaginifolia* residue is amassed under field conditions, i.e. after the completion of its life cycle. These residues are similar to its fresh parts and show allelopathic potential. Allelochemicals from this plant are discharged through leaching and putrefaction and have the potential to negatively affect the early growth of target plants by liberating water-soluble phenolic allelochemicals into the soil without any decrease in the available soil nutrients. These findings indicate role of putative phenolic allelochemicals forced phytotoxic effects on different crops including *C. arietinum*, *P. sativum*, *C. tora* and *C. sophera*.

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