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RESEARCH ARTICLE

INFLUENCE OF PETAL LEACHATE OF *DELONIX REGIA* (BOJ EX HOOK) RAF. ON GERMINATION AND SEEDLING GROWTH OF CHICKPEA

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ABSTRACT

Delonix regia (Boj Ex Hook) is a common tree growing along the roadside as well as farmside. There is frequent shedding of petals during flowering season. So an attempt was made to study the influence of petal leachate of *D. regia* on seed germination and seedling growth of important leguminous crop Chickpea (*Cicer arietinum* L.). In petridish bioassay seed germination and seedling growth with respect to root length, shoot length and fresh weight was inhibited due to petal leachate. In soil bioassay seed germination and seedling growth was inhibited due to petal leachate. In seedlings of Chickpea contents of total sugar, reducing sugar and soluble protein were also reduced due to petal leachate treatment. Petal leachates were analyzed for the presence of nitrate, nitrite, polyphenols and for the detection of phytochemicals. Petal leachate was found to contain appreciable amount of polyphenols and phytochemicals which may affect further growth performance of the crop.

INTRODUCTION

Under natural environmental conditions of modern age plants are frequently exposed to variety of environmental stresses. The phenomenon of allelopathy exerts a profound influence on seed germination, growth and yield of crop plants under natural conditions as well as intensive farming and agro forestry systems. Allelopathic interaction between crops and tree species plays vital role in establishment of the agro forestry systems (Rizvi *et al*., 1999). *Delonix regia* (Boj ex Hook) Raf. is mainly planted as an ornamental plant in avenues and gardens. It is grown on the lines of streets and also planted as a shade tree in farms. It is a nitrogen fixing tree useful for soil enrichment (HDRA No. TTS 18, 2002). This tree species is also recommended for the agro forestry programme throughout the country. This tree has a tendency of shedding of petals with frequent intervals during flowering period. Allelopathic potential of *D. regia* (Boj ex Hook) Raf. has been studied by many workers (Chou and Leu 1992; Eyini *et al*.,1996; Dhawan *et al*.,1998; Jadhav 2003; Mahadik and Jadhav 2003; Ramamoorthy *et al*., 2009; Oudhia, 2011). Chickpea (*Cicer arietinum* L.) is most important legume crop and having first rank with 10,671,503 ha under cultivation in the world. India is the largest chickpea producing country with 66% of global chickpea production (FAOSTAT, 2011). Chickpea widely used in human diet. It is rich source of protein and is considered as a healthy food in many developing and developed countries (Abbo *et al*., 2003). As having nitrogen fixing ability it plays important role in increasing soil fertitility (Maiti, 2001, Kantar *et al*., 2007). Hence an attempt has been made to study influence of petal leachate of *Delonix regia* on seed germination and seedling growth of Chickpea (*Cicer arietinum* L.).

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MATERIALS AND METHODS

Petals of *D. regia* were collected from lines of streets and farm side in Kolhapur District (Maharashtra State, India) in the month of April and May in 2013. Seeds of (*Cicer arietinum* L.) var. Kalyani (Prerana Seeds) were procured from local market.

For the preparation of petal leachate method given by Jadhav and Gaynar (1992) was followed. Ten grams of petals were washed with distilled water for 4-5 times to remove surface dust. The petals were soaked in 50 ml of distilled water for 24 hours and filtered through Whatman No.1 filter paper and used for further treatment. Healthy seeds of Chickpea were surface sterilized by treating with 0.1% Mercuric Chloride for 5 minutes. The seeds were rinsed with distilled water for 4-5 times. Ten seeds were placed in sterilized petriplates (having diameter of 9 cm) with moistened filter paper. Eight ml of petal leachate was added in each petriplate. The petriplates supplied with distilled water served as control. Petriplates arranged in triplicates for each treatment. Seeds were allowed to germinate at temperature range 24-28 ^{0}C under natural light and dark cycles and used for further analysis.

Germination percentage was recorded at 24 and 48 h stage. Seedling growth study with respect to root length, shoot length and fresh weight was performed at 120 h stage. Soil bioassays were carried by using plastic trays of dimension (22cm X 17cm X 4.2 cm). Trays were filled with 750 grams of soil. Soil was moistened with distilled water. Thirty seeds were sown in tray. Fifty ml of petal leachate was applied for 5 days (at alternate day). Then intact seedlings (10 days old) were uprooted carefully. Seedling growth with respect to root length, shoot length and fresh weight was recorded. For the estimation of total sugars and reducing sugars of seedling, method given by Nelson (1944) was followed. Five hundred mgs oven dried powder of seedlings of Chickpea was extracted with 80% neutral alcohol. The extract was filtered through Buchners funnel using Whatman No. 1 filter paper. The filtrate was condensed to 5 ml on waterbath of this 2 g lead acetate and potassium oxalate (1:1) were added for decolourization, 40 ml distilled water was added and aliquot was filtered. The volume of filtrate was measured and it served as an extract for determination of reducing sugars. Twenty ml of this extract was mixed with 4 ml concentrated HCl and autoclaved at 15 lb atmospheric pressure for half an hour. The content was cooled, neutralized with anhydrous sodium carbonate and filtered. The volume of the filtrate was measured and this filtrate was used for estimation of total sugars. For estimation of reducing and total sugars 0.4 ml filtrates were taken in test tubes. To this requisite amount of distilled water was added to make final volume 1 ml. To this 1 ml Somogyi's alkaline copper tartarate reagent was added and then the tubes were kept in boiling water bath for 10 minutes. After cooling to room temperature, 1 ml Arsenomolybdate reagent was added. The reaction mixtures were further diluted to 10 ml with distilled water and absorbance was read at 560nm. The amount of reducing sugars and total sugars were estimated with the help of standard curve and the values were expressed as mg g^{-1} dry tissue.

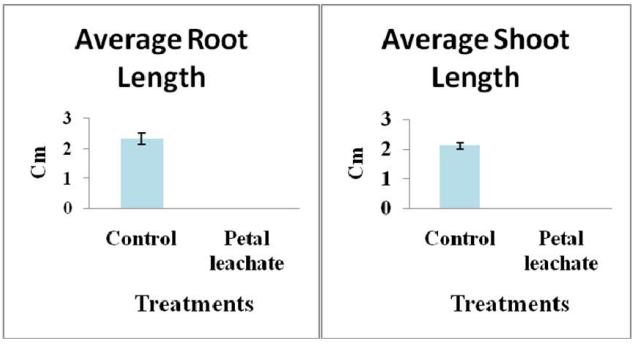
The soluble proteins were estimated by following the method of Lowry *et al*. (1951). Five hundred mg of dry powder was extracted in 10 ml phosphate buffer (pH-6.8). The homogenate was filtered through 4 layered muslin cloth and filtrate was centrifuged at 10000 rpm for 20 minutes. 0.1 ml extract was taken and was diluted to 1 ml with distilled water. To this 5 ml of reagent C solution was added, mixed well and allowed to stand for 15 minutes at room temperature. After 15 minutes 0.5 ml Folin and Ciocalteau phenol reagent was added with immediate mixing. This was allowed to stand for 30 minutes in dark and intensity of developed blue colour was measured at 660 nm. The values were expressed as mg g^{-1} fresh tissue. For estimation of nitrate content from petal leachate the method of Cataldo *et al*. (1975) was followed. 0.2 ml of petal leachate was mixed thoroughly with 0.8 ml salicylic acid- H_2SO_4 reagent. After 20 minutes 19 ml of 2 N NaOH was added slowly to raise the pH above 12. Then samples were cooled to room temperature and absorbance was read at 410 nm. Nitrate content was estimated with the help of standard curve and it was expressed as µg ml^{-1}. Nitrite content from petal leachate was estimated by the method of Nair *et al*. (1988). Three ml of leaf leachate was mixed with 3 ml of 1% sulfanilamide in 1 M HCl and 3 ml of 0.02% NEEDA. After 15 minutes the absorbance was read at 540 nm. Nitrite content was estimated with the help of standard curve and it was expressed as µg ml^{-1}. The total polyphenol content in petal leachate was estimated following the method of Folin and Denis (1915). Two ml of leaf leachate along with a series of standards (standard tannic acid 0.1 mg/ml) were taken in separate Nessler's tubes and to each tube 10 ml of 20% Na_2CO_3 and 2 ml of Folin Denis reagent were added. The final volume of reaction mixture was made 50 ml with distilled water. After 20 minutes absorbance was read at 660 nm with reagent blank. Total polyphenols were calculated with the help of std. curve of tannic acid and expressed as µg ml^{-1}. Aqueous leachate of petal (1:5 proportion) was condensed on water bath and remaining residue was mixed with methanol (1:5proportion) and was kept for 24 hours. Then filtered through Whatman No 1 filter paper and used for the detection of phytochemicals by using Gas Chromatography Mass Spectroscopy technique. The findings presented in text are mean of three independent determinations.

RESULTS AND DISCUSSION

Seed germination in Chickpea has been completely inhibited due to treatment of petal leachate of *Delonix regia* (Table 1).

Table. 1 Influence of petal leachate of *D. regia* on seed germination of Chickpea (*Cicer arietinum* L.)(Petriplate bioassay)

Treatment	Germination percentage	
	24 h	48h
Control	60± 10	97± 5.77
Petal leachate	-	-



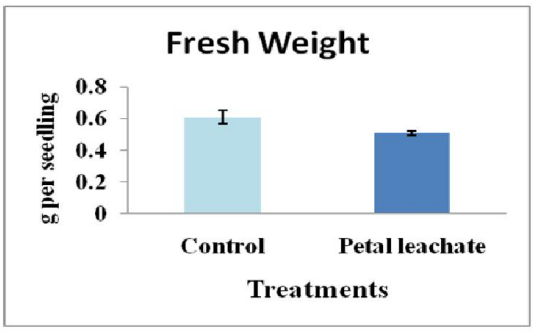


Fig. 1. Influence of petal leachate of *Delonix regia* on seedling growth of *Cicer arietinum* (Petriplate bioassay)

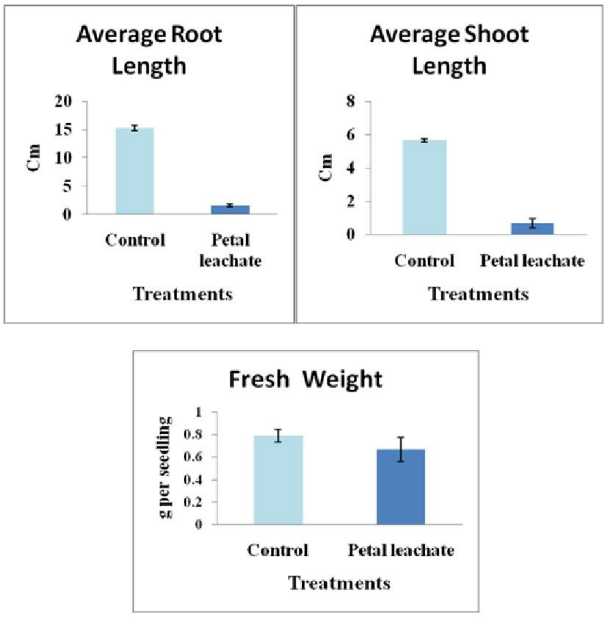


Fig. 2. Influence of petal leachate of *Delonix regia* on seedling growth of *Cicer arietinum* (Soil Bioassay)

Seedling growth with respect to root length and shoot length was also completely reduced due to treatment of leachate of petals (Fig.1) in petriplate bioassay. In soil bioassay seedling growth of Chickpea was also inhibited due to petal leachate treatment (Fig. 2). Inhibition of seed germination and seedling growth in chickpea due to treatment of different parts of tree species was observed by many workers. Lisanework and Michelsen (1993) have reported that seed germination and radicle growth of chickpea was reduced due to aqueous extract of *Cupressus lusitanica, Eucalyptus globulus, E. camaldulensis* and *E. saligna*. Dry weight shoot and root was also reduced due to 10 weeks treatment of leaf extract of tree species. Reduction in seed germination, vigor and growth rate of seedling of Chickpea due to aqueous extract of *E. camaldulensis* Dehn. was observed by Ahmed *et al*. (2004). Oudhia (2001) and Veenapani (2004) have given account of inhibition of seed germination and seedling growth of chickpea due to *Euphorbia helioscopia*. Shajie and Saffari (2007) described allelopathic potential of aqueous extract of leaves and stem of Cocklebur (*Xanthium strumarium* L.). Aqueous extracts reduced the germination, root and shoot lengths of Chickpea. Haoula *et al*. (2008) have studied allelopathic effects of leaves + shoots, roots, seeds and ground seeds of Fenugreek on seed germination and early seedling growth of Chickpea under lab conditions and reported that seed germination and early seedling growth were reduced due to aqueous extracts of various parts. Babar *et al*. (2009) pointed out the allelopathic potential of *Asphodelus tenuifolius* Cav. and stated that when Chickpea seeds were soaked in root extract took more time for germination than the seeds soaked in stem and fruit extract. Siddiqui *et al*. (2009) have showed inhibitory effects of different concentrations of leaf extracts of *Ficus infectoria, Emblica afficinalis* and *Acacia leucophloa*. Tanveer *et al*. (2012) have studied effect of organic solvent fraction and aqueous fraction of *Euphorbia dracunculoides* Lam. on germination attributes and seedling growth of Chickpea and explained that aqueous fractions are more phytotoxic than organic fractions and inhibited seed germination, root length, shoot length and dry weight of Chickpea. Jalali *et al*. (2013) have noted that higher concentration of aqueous extract of root, stem, leaf and flowers of Beebalim (*Puleguim vulgare* L.) and Fennel (*Foeniculum vulgare* L.) inhibited percentage and rate of germination, length of radicle and hypocotyls and time to end of germination of Chickpea (cv. Seffid). Mangal *et al*. (2013) revealed that leaf extract of *Moringa oleifera* at lower concentration (2.5%) caused negligible effect and higher concentration (5.0, 7.5, 10.0%) showed inhibitory effect on seed germination, seed biomass and radicle length of Chickpea. Inhibition of seed germination and seedling growth of *Cicer arietinum* L. due to aqueous extracts of different parts of weeds has been studied by Oudhia *et al*., 2000; Ahmed *et al*., 2007 and Devi *et al*., 2013. Due to petal leachate of *D. regia* seed germination and seedling growth of Chickpea was also reduced which may further affect overall performance of the crop. Total sugar and reducing sugar contents in seedlings of Chickpea were reduced due to treatment of petal leachate of *D.regia* (Fig 3and 4). Das *et al*. (2012) have reported that in Chickpea seedlings soluble sugar content was reduced due to treatment of 100% (v/v) of leaf leachate of *A. auriculiformis, A. occidentale, A. lebbek, E. citridora, E. officinalis, S. robusta* and *T. grandis*. In seedlings of Chickpea soluble protein content was also reduced due to treatment of petal leachate in both petriplate and soil bioassays (Fig. 5). In Chickpea protein content was also reduced due to treatment of different concentrations of *Moringa oleifera* leaf extract (Mangal *et al*., 2013).

Petriplate Bioassay **Soil Bioassay**

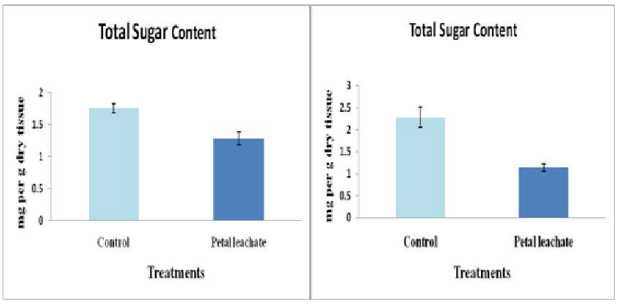


Fig.3. Influence of petal leachate of *Delonix regia* on Total sugar content in seedlings of *Cicer arietinum*

Petriplate Bioassay **Soil Bioassay**

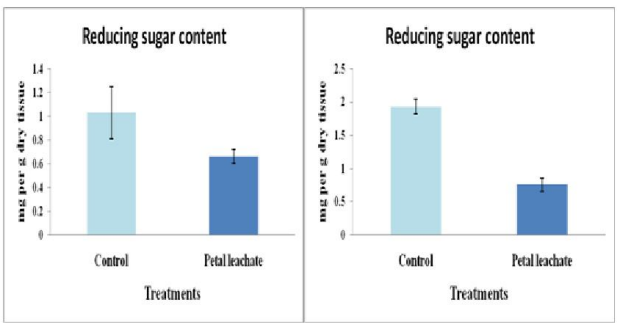


Fig.4. Influence of petal leachate of *Delonix regia* on Reducing sugar content in seedlings of *Cicer arietinum*

Petriplate Bioassay **Soil Bioassay**

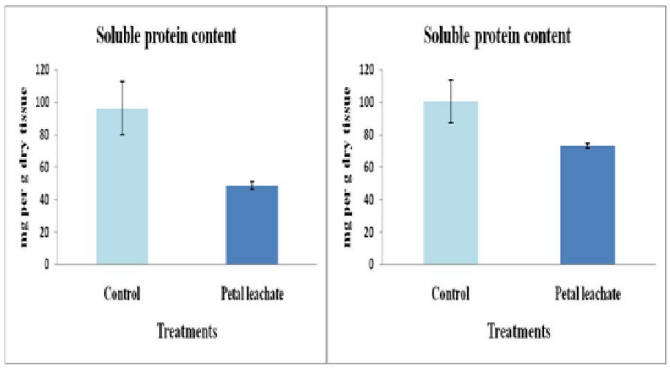


Fig. 5. Influence of petal leachate of *Delonix regia* on Soluble protein content in seedlings of *Cicer arietinum*

Sugars are forming building blocks for amino acids, fatty acids and other compounds in plants. As sugars and proteins are conceiving as substrates for all metabolisms in germinating seeds, the overall germination performance may be ruined due to disturbances in metabolism caused by decline in sugar and protein content by the treatment of petal leachate treatment. Germination of seeds is fundamentally important in agriculture as cultivation of most of the crop species relies on the germination of seeds. Reduction in seed germination and seedling growth of Chickpea due to treatment of petal leachate may further affect growth and yield of Chickpea. Petal leachate contained nitrate, nitrite and polyphenols. Appreciable amount of polyphenol was found in petal leachate (Table 2).

Table.2 Chemical properties of petal leachate

Nitrate Content µg per ml	Nitrite Content µg per ml	Total polyphenol content µg per ml	Phytochemicals detected
66.66±6.506	17.23±0.183	97.92±28.7	Tetrahydrocyclopenta [1,3]dioxin-4-one, Itaconic acid anhydride, Citrconic acid anhydride, 4-Heptenal, 1-Adamantal, 4-(1,2-Dimethyl-cyclopent-2-enyl) butan-2-one, Pilocarpine, p-Hydroxybenzoate, m-Ethoxybenzoic acid, Benzoic acid, 3-hydroxy, 1,4-Naphthoquinine,2-butyl-3-hydroxy, 1-Benzosuberone, 5-Methyl-2-Phenyl-2-hexenal, 4-Methyl-4-phenyl-2,3:5,6-diepoxycyclohexanone

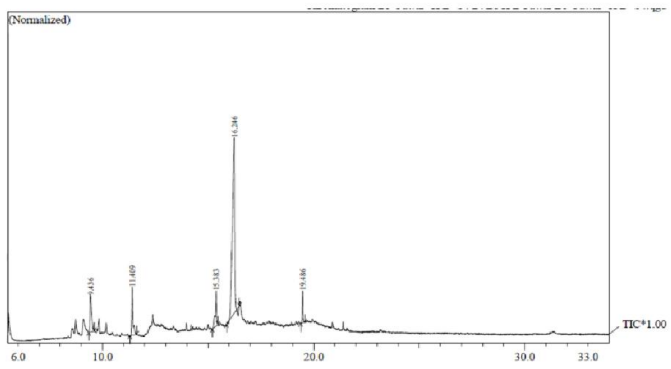
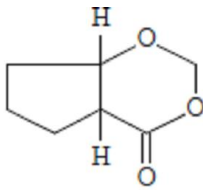
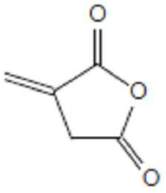


Fig. 6. Phytochemicals detected in petal leachate of *D. regia*

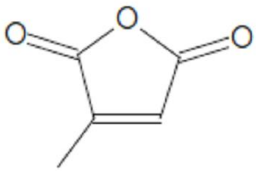
Tetrahydrocyclopenta [1,3]dioxin-4-one



Itaconic acid anhydride

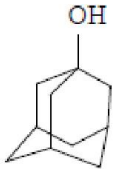


Citrconic acid anhydride

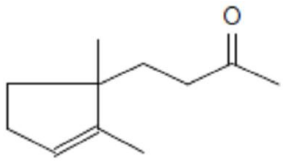


4- Heptenal

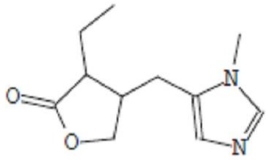
1-Adamantanol



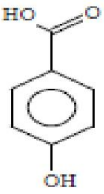
4-(1,2-Dimethyl-cyclopent-2-enyl) butan-2-one



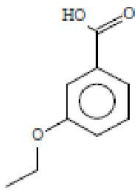
Pilocarpine or Almocarpine



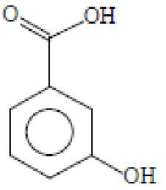
p-Hydroxybenzoate



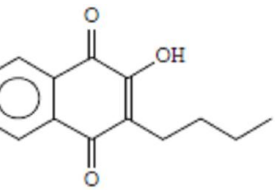
m-Ethoxybenzoic acid



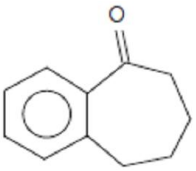
Benzoic acid, 3-hydroxy



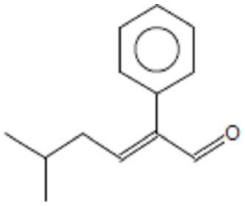
1,4-Naphthoquinine,2-butyl-3-hydroxy



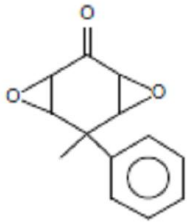
1-Benzosuberone



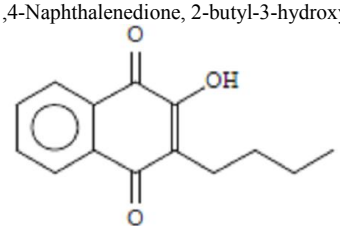
5-Methyl-2-Phenyl-2-hexenal



4-Methyl-4-phenyl-2,3:5,6-diepoxycyclohexanone



1,4-Naphthalenedione, 2-butyl-3-hydroxy



The phytochemicals (Table 2 and Fig 6) in petal leachates wereTetrahydrocyclopenta [1,3]dioxin-4-one, Itaconic acid anhydride, Citrconic acid anhydride, 4- Heptenal, 1-Adamantal, 4-(1,2-Dimethyl-cyclopent-2-enyl) butan-2-one, Pilocarpine, p-Hydroxybenzoate, m-Ethoxybenzoic acid, Benzoic acid, 3-hydroxy, 1,4-Naphthoquinine,2-butyl-3-hydroxy, 1-Benzosuberone, 5-Methyl-2-Phenyl-2-hexenal, 4-Methyl-4-phenyl-2,3:5,6-diepoxycyclohexanone. Chemical constituents and phytochemicals present in petal leachate may synergistically affect the seed germination and seedling growth of Chickpea.

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