Physiol Mol Biol Plants (January–March 2015) 21(1):83–92
DOI 10.1007/s12298-014-0272-0

RESEARCH ARTICLE

Phytochemical analysis of *Jatropha curcas* L. during different seasons and developmental stages and seedling growth of wheat (*Triticum aestivum* L) as affected by extracts/leachates of *Jatropha curcas* L

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Received: 2 September 2014 / Revised: 21 October 2014 / Accepted: 25 November 2014 / Published online: 24 December 2014

Abstract *Jatropha curcas* shows invasive characters and is a significant source of many phytochemicals with varying biological activities. Different plant parts of *Jatropha curcas* L exhibited variation in their phytochemical constituents. Leaves and ovary walls were found to contain higher contents of total phenols, tannins and phytic acid whereas free amino acids were greater in leaves. Young leaves of *Jatropha* show greater contents of all these metabolites. Further, plants exhibit seasonal differences as leaves collected during summer (May-June) have greater accumulation of total phenols, tannins and free amino acids however, phytic acid was more during rainy season. Leachates and extracts in their higher concentrations adversely affected the germination and growth of wheat seedlings however, lower concentrations were more or less stimulatory. These treatments not only decreased the length, fresh and dry weight of seedlings but also affected the chlorophyll contents and activity of enzymes such as nitrate reductase, aminotransferases in wheat seedlings however, the activity of superoxide dismutase and ascorbate peroxidases increased. Experiments indicate harmful allelopathic effects of *Jatropha* leachates /extracts on wheat seedlings, hence further experimentation and analysis is recommended before continued plantation of *Jatropha* particularly on fertile soils. However. Growth of *Jatropha* plants on saline soils and their potential for accumulating sodium, potassium and chloride are the attributes suggesting the possibility of use of *Jatropha* plants in improving saline soils.

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Keywords *Jatropha* · Seasonal changes · Nitrate reductase · Aminotransferases · Superoxide dismutase and Ascorbate peroxidase

Abbreviations

LE	Leaf Extract
SE	Stem Extract
RE	Root Extract
OWE	Ovary Wall Extract
Seed E	Seed Extract
LL	Leaf Leachate
SL	Stem Leachate
RL	Root Leachate
OWL	Ovary Wall Leachate
Seed L	Seed Leachate
SOD	Super Oxide Dismutase
APX	Ascorbate Peroxidase
ROS	Reactive Oxygen Species

Introduction

Allelopathy involves any direct or indirect, harmful or beneficial effect of one plant (including micro organisms) on others through release of some plant secondary metabolites into surrounding environment (Rizvi and Rizvi 1992). Allelopathy can play an important role on commercial plantation, on degradation of soil, reduction of productivity and biodiversity (Vesterdal et al. 2002).

Plants produce a wide spectrum of secondary metabolites. Many of these compounds are allelopathic in nature possessing a wide role in self defense of plant (Lovett 1982). Phenolic acids are potential allelochemicals having growth inhibitory properties because of their high water solubility

(Putnam and Tang 1986 and Inderjit 1996). Phenolic compounds have been identified to play key role in plant growth and development, nevertheless, their potential to increase crop productivity is yet to be revealed (Nanda and Kumar 1982).

Jatropha curcas L is a drought resistant perennial plant belonging to family euphorbiaceae. *Jatropha* plants can easily be grown on marginal soils to help reclaim land (Munch and Kiefer 1989). *Jatropha curcas* L is being cultivated as biodiesel crop in many tropical and sub-tropical areas (Heller 1996) and can also be seen growing as fence around crop plants in many regions of India. Non-edible oil produced from these seeds is used as feed stock for the production of bio-diesel. Press cake is used to improve soil and for the production of biogas.

Allelopathic potential of plant also changes with age of plant (Inderjit and Asakawa 2001). Various organs of the plants differ in contents of allelochemicals. Leaves of *Conyza albida* were found to be more allelopathic than its stem (Economou et al. 2002). Leaves of *Cassia tora* exhibit greater toxicity than stem and roots towards germination and growth of *Parthenium hysterophorus* (Thapar and Singh 2006).

Response of rye grass (*Lolium rigidum*) to water extracts of barley (*Hordeum vulgare* L) varies with variety, growth stage and plant parts (Burgos and Talbert 2000). Most crop plants release their allelopathic substances at an early stage of growth (Dekker and Meggitt 1983 and Economou et al. 2002). Fresh young leaves of pine show greater allelopathic activity than senescing leaves (Nektarios et al. 2005). Younger plants of *Eucalyptus grandis* (2–4 years of age) exhibit stronger allelopathic potential than older plants of 8–10 years of age (Zhang et al. 2010).

Present investigation focuses on phyto-analysis of *Jatropha curcas* along with evaluation of its allelopathic potential on seed germination and growth (including the activity of enzymes involved in nitrogen metabolism and antioxidant system) of wheat which happens to be a staple food in northern India and is grown over a large area. Alterations in phytochemical constituents of wheat resulting due to treatment of leachates of *Jatropha curcas* L have already been reported (Tomar and Agrawal 2013). Soil beneath *Jatropha curcas* L. reportedly (Uttam et al. 2014) affected the germination, seedling growth and vigour of some crops such as *Zea mays*, *Vigna radiata* and *Brassica compestris* and the most pronounced allelopathic effects were noticed on *Vigna radiata*. Dried leaves of *Jatropha curcas* L adversely affected the initial growth of lettuce particularly the hypocotyl and radical length (Matsuo et al. 2014).

Material and methods

Different parts of *Jatropha curcas* L were collected from government nursery, Agriculture College, Gwalior (affiliated to Rajmata Vijayraje Scindhia Krishi Vishwa Vidhyalaya, Gwalior, M P). Leaves during different seasons of the year (May-June; Aug-Sep and Nov- Dec) and of different developmental stages were collected. Cultivar MP–4010 of wheat (*Triticum aestivum* L) released from Central Varietal Release Committee, New Delhi is widely grown in Gwalior-Chambal region of Madhya Pradesh has been selected as the test crop. Seeds of wheat were obtained from Krishi Vigyan Kendra, Rajmata Vijayraje Scindhia Krishi Vishwa Vidhyalaya, Gwalior (India).

Plant parts of both *Jatropha curcas* were washed and sun dried for 3 to 4 days and powdered using pestle and mortar and sieved through 2 mm sieve. This powder was kept in air tight polythene bags and used for the preparation of leachates. Total phenols were estimated following the method of Malik and Singh (1980) and tannins were worked out in accordance with Swain and Hills (1959). Free amino acids were determined following the method outlined in Sadasivam and Manickam (2004) and phytic acid was estimated following the method of Wilcox et al. (2000).

Preparation of aqueous extracts and leachates

Aqueous extracts

Freshly harvested parts were washed with distilled water to remove dust and were blot dried. 5.0 g of each part (leaf, stem and roots, ovary wall and seeds) was crushed separately in distilled water (50 ml), slurry is then filtered through two layers of cheese cloth. Filtrate is centrifuged at 3000 g for 15 min so as to obtain clear supernatant which was considered as 1/10 (w/v) extract and stored for 2–3 days at 4 °C for bioassay experiments.

Fresh leachates

Clean and blot dried fresh parts of *Jatropha* plant were cut into pieces. Plant parts (5.0 g) were soaked in 50 ml of distilled water and kept for 48 h at room temperature. After 48 h of soaking it was filtered through sieve and centrifuged at 3000 g for 15 min. Final volume was made up to 50 ml using distilled water and the supernatant so obtained is considered as 1/10 (*w/v*) fresh leachate and was stored for 2–3 days at 4 °C for bioassay experiments.

Dry leachates

Fresh parts were washed, sun dried and ground separately in a mechanical grinder. The powder was sieved through 2 mm sieve. Dry powder (5.0 g) was soaked in 50 ml distilled water for 48 h at room temperature. The

leachates were squeezed through cheese cloth and filtered through Whatman no. 1 filter paper. The filtrate is centrifuged at 3000 g for 15 min and final volume was made up to 50 ml using distilled water. This supernatant is considered as dry leachate 1/10 (w/v) and was stored for 2–3 days at 4 °C for use in bioassay experiments. Laboratory experiments were conducted using exracts, fresh and dry leachates from different parts of *Jatropha curcas* whereas only selected dry leachates were used for pot experiments.

Laboratory experiments

Uniform and healthy seeds of wheat (*Triticum aestivum* L cultivar MP-4010) were selected and surface sterilized using 0.01 % mercuric chloride solution for 4–5 min followed by washing with running water and finally rinsing with distilled water. Petriplates lined with two layers of Whatman no. 1 filter paper were used for laboratory experiments. Ten seeds were placed in each petriplate thereafter leachate or extract was added to petriplates and control was maintained with the same quantity of distilled water. Leachates/ extracts/ distilled water were added periodically and uniformly. Seed germination was observed after 2 days of soaking whereas, shoot/ root length and fresh weight of shoot / root were recorded on seventh day after seed wetting. Separated roots and shoots were kept in oven at 80 °C for 2 days and dry weight was recorded. Treatments which had greater impact were used for further experiments.

Chlorophyll contents were determined following Arnon's method (1949) and carotenoids were calculated following Kirk and Allen (1965). Activities of nitrate reductase (EC 1.6.6.1) (in vivo) was determined following Srivastava (1974) and that of aminotransferases [i.e. aspartate aminotransferase (EC 2.6.1.1) and alanine aminotransferase (EC 2.6.1.2)] were determined following Reitman and Frankel (1957). SOD (EC 1.15.1.1) activity was estimated following the method given by Dhindsa et al. (1981) and ascorbate peroxidase activity (EC 1.11.11.11) activity was estimated following Tor-Or et al. (1986). Protein estimation was done in accordance with Lowry et al. (1951). Dried roots and shoots of treated wheat seedlings were powdered and used for analysis of starch using anthrone method (Sadasivam and Manickam 2004) as followed by Pandey et al. (2004). Total free sugars were determined using anthrone method based on Fong et al. (1953) and Jain and Guruprasad (1989) and free proline estimation was done following Bates *et al*. (1973).

Statistical analysis

For each parameter three replicates were worked out and standard error determined.

Results and discussion

Phytochemical analysis of various plant parts of *Jatropha curcas* revealed variation in contents of total phenols, tannins, free amino acids and phytic acid. Leaves and ovary walls contain the maximum contents of phenols, tannins and phytic acid whereas, free amino acids were greater in leaves and seeds (Fig. 1). Compounds such as amino acids and carbohydrates may not act directly as allelochemicals but can modify the activity of allelochemicals like phenolic acids (Blum et al. 1993). A large number of reports suggest that phenolic acids exhibit allelopathic effects at proper concentrations and environmental conditions (Blum et al. 1999 and Dalton 1999).

Leaves of *Jatropha curcas* show change in their secondary metabolites with their developmental stage i.e. young leaves (3rd-4th) contain comparatively higher contents of phenols, tannins, phytic acid and free amino acids and a gradual decrease in these metabolites was found with advancing age of leaf i.e. from 3rd-4th to 9th-10th and subsequently 19th-20th leaf (Fig. 2).

Leaves of *Jatropha curcas* exhibited seasonal variation in terms of total phenols, tannins and free amino acids. Maximum level of these compounds were found in leaves collected during summer months (May-June) and least during rainy season (July-August) however, phytic acid contents were greater during rainy season (Fig. 3). Seasonal variation in plant secondary metabolites has also been reported earlier by Coder (1983) who found a change in juglone potential of black wall nut leaves from May to August.

Many biotic and abiotic factors along with age, part and variety of plant including method of extraction are reported to affect the concentration of phenolics in plant tissues (Guenzi and McCalla 1966 and Lodhi et al. 1987); Inderjit and Asakawa (2001) have reported a change in allelopathic potential of plants with age. Leaves of pine have been reported to contain higher contents of allelochemicals than their roots. *Pinus halepensis* synthesizes most of its phenolic compounds during early stage of colonization which may confer a competitive advantage (Fernandez et al. 2009); Abugre and Sam (2010) have reported higher contents of allelochemicals in the leaves of *Jatropha curcas* than its roots. In the present investigation leaves and ovary walls were found to contain higher contents of phenols and tannins than other parts such as stem, root and seeds which formed basis of selecting these parts for further experimentation. Older leaves (19th-20th) of *Jatropha curcas* contain less quantity of total phenols, tannins and phytic acid and free amino acids as compared to younger

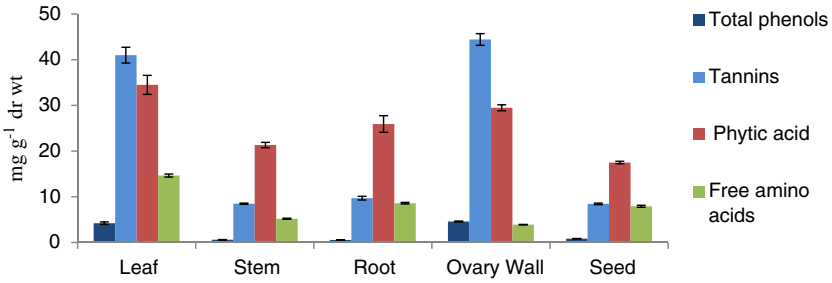

Fig. 1 Total phenols, tannins,phytic acid and free amino acids (mg g^{-1} dr wt) in different parts of *Jatropha curcas* expressed as catchecol eq

leaves which prompted us to select 9th-10th leaves as treatment i.e. for preparation of leachates to evaluate their allelopathic potential.

Treatments of higher concentrations (1/10, w/v) of fresh extracts from different parts (leaf, stem, root, seed and ovary wall) of *Jatropha curcas* reduced seed germination and seedling growth of wheat (*Triticum aestivum* L). Leaf extract (LE 1/10, w/v) reduced the growth of seedlings more followed by seed, root, ovary wall and stem extracts. Interestingly, lower concentrations of these extracts showed stimulation in growth of wheat seedlings (Table 1). Allelochemicals have been noticed to selectively affect the growth of plant species as they can inhibit the growth at certain concentration or stimulate the growth of same or different species at lower concentrations (Rice 1984 and Purvis et al. 1985); Abugre and Sam (2010) also noticed such results on germination and growth in *Capsicum annum* (green chilli) seedlings treated with *Jatropha curcas* leaf and root extracts. Reduction in germination and growth of seedlings could be attributed to higher concentrations of allelochemicals present in leachates and extracts. Allelochemicals probably interfere with enzymes involved in mobilization of nutrients necessary for germination, thereby affecting cell division consequently reducing the elongation of seedlings (Ashrafi et al. 2008).

Fresh leachates from various parts (leaves, stem, root, ovary wall and seed) of *Jatropha curcas* reduced the germination percentage and inhibited the growth of wheat seedlings in terms of root/shoot length, fresh and dry weight. Fresh leachates of all parts in higher concentration (1/10, w/v) reduced germination percentage and growth of seedlings, although treatment of lower concentrations of all these parts showed some stimulatory effects on seedling growth (Table 2).

Treatment of dry leachates in higher concentrations (1/10, *w/v*) from all plant parts (leaves, stem, root, ovary wall and seed) of *Jatropha curcas* bring about significant reduction in germination percentage and seedling growth of wheat. However, leaves and ovary wall leachates were observed to be more inhibitory followed by roots, stem and seed leachates. All treatments at lower concentrations (1/100, *w/v*) somewhat stimulated the growth of seedlings excepting the treatment of seed leachates which still showed slight inhibition in root growth. Maximum inhibition was observed in treatments of leaf and ovary wall leachates. Lower concentrations from all plant parts were stimulatory (Table 3).

Tefera (2002) and Maharjan et al. (2007) have also noticed leaves to be the most potent parts for allelopathic interactions. Leaves of *Eclipta alba* have been reported to be more inhibitory to certain crops and weeds in comparison to its stem and roots (Gulzar and Siddiqui 2014).

Jatropha plants defoliate their leaves two times in a year pruning is also done to increase number of lateral branches to increase seed production. Further ovary walls are also discarded in larger quantities after separation of seeds. Bioassay experiments indicated strong inhibitory effect of leaves and ovary walls (1/10, *w/v*) on germination and seedling growth of wheat (Table 3). Biochemical analysis of various plant parts of *Jatropha* spp showed that leaves and ovary walls are rich in phenolic compounds and tannins (Fig. 1) which may have an allelopathic impact.

Phenolic acids are potential allelochemicals because of their high water solubility and plant growth inhibitory properties (Putnam and Tang 1986 and Inderjit 1996). These compounds often constitute the principle allelopathic agents in plants (Inderjit 1998 and Wang *et al.* 1998). Nevertheless, plants produce an array of chemicals with different bioactivities and a single compound is rarely responsible for a complicated biological process (Inderjit 2006).

Greater inhibition of germination and seedling growth due to treatment of dry leachates of leaves and ovary walls as

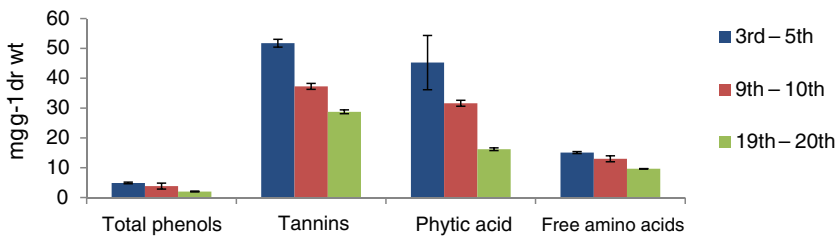

Fig. 2 in contents of total phenols, tannins, phytic acid and free amino acids (mg g^{-1} dr wt) in leaves of different developmental stage of *Jatropha curcas*

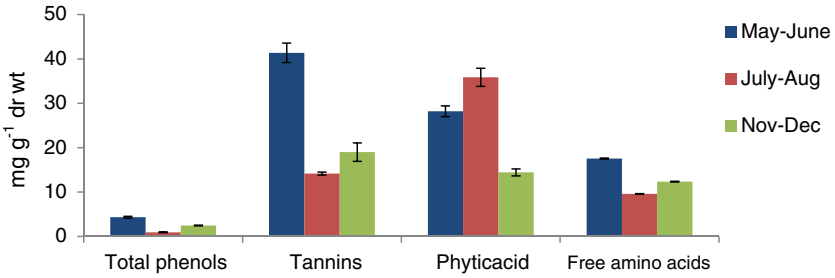

Fig. 3 Seasonal variation in contents of total phenols, tannins, phytic acid and free amino acids (mg g^{-1} dr wt) in leaves (9th) of *Jatropha curcas*

compared to fresh leachates and extracts was observed (Tables 1–3). Dry powder of *Brassica* spp has also been noticed to inhibit the growth of weed plants more effectively than their extracts (Jimenez-Osornio and Gliessman 1987). Concentration of allelochemicals, developmental stage of plant and environmental conditions affect the sensitivity of target species (Inderjit and Weiner 2001 and Inderjit 2001).

Tannins are phenolic substances associated with effects including reduced feed intake, growth retardation and impaired nutrient absorption (Butler et al. 1986). Phytic acid is also considered as antinutrient which adversely affects protein and starch digestibility and availability of essential minerals (Reddy and Pierson 1994). Greater concentrations of total phenols, tannic acid and phytic acid are found in leaves and ovary walls of both the *Jatropha* species (Fig. 1), these probable allelochemicals individually or synergistically may be reducing the growth of treated wheat seedlings and plants grown in field. Inhibitory effects of *Eucalyptus globulus* leaf leachates are attributed to higher contents of phenolic compounds whereas inhibition caused by *Acacia* species is considered to be due to the presence of tannins, flavonoids and phenolics (Ballester et al. 1982).

Treatments of higher concentrations (1/10, *w/v*) of dry leachates of *Jatropha curcas* have resulted in reduced percentage moisture contents (Table 4), indicating osmotic effects of these treatments along with other possibilities. Extracts of *Ageratum conyzoides* and *Kochia scoparia* resulted in lowered RWC and leaf water potential in rice, soybean and sorghum respectively (Mary and Frank 1982 and Einhellig et al. 1982). Aqueous leaf extract of *Prosopis juliflora* adversely affects the germination and growth of wheat seedlings. Degree of inhibition was in proportion to the concentration of extract. Inhibitory effects were more conspicuous on the growth than on germination and such effects are attributed to the presence of allelochemicals in the extract. Many a time allelochemicals act in a synergistic way rather than as a single compound (Fag and Stewart 1994 and Siddiqui et al. 2009).

Plant phenolics constitute one of the major groups of compounds acting as antioxidants or free radical terminators. Leaves of *Jatropha curcas* contain tannins, alkaloids, phenols, flavonoids, glycosides and saponins (Miliauskas et al. 2004). Presence of phenolics in plant parts may be the reason for poor germination and growth of weeds (Yansen 2007). Decrease in germination is well correlated with increased membrane deterioration and enhanced lipid peroxidation (Kolahi and Kolahi 2008).

Total chlorophyll and carotenoid contents are reduced to the maximum in seedlings treated with dry leachates (1/10, *w/v*) of leaves and ovary wall of *Jatropha curcas* (Table 4). Reduction in growth, biomass and pigment content of wheat

Table 1 Effect of fresh extracts of different parts of *Jatropha curcas* L on germination and seedling growth (7DAS) of wheat (*Triticum aestivum* L)

Treatments	Germination %	Length (cm)		Fresh weight (g)		Dry weight (g)	
		Root	Shoot	Root	Shoot	Root	Shoot
Control	90.01±0.57	7.00±0.20	6.73±0.43	0.551±0.029	0.594±0.017	0.055±0.0020	0.0631±0.0030
LE (1/10)	66.64±0.66	1.94±0.15	4.76±0.53	0.112±0.003	0.396±0.016	0.023±0.0005	0.0450±0.0005
SE (1/10)	76.60±0.33	3.82±0.31	6.69±0.16	0.321±0.002	0.606±0.005	0.031±0.0004	0.0605±0.0004
RE (1/10)	70.00±0.57	3.11±0.25	5.77±0.33	0.276±0.017	0.588±0.012	0.030±0.0020	0.0576±0.0020
OW E (1/10)	78.33±0.33	5.61±0.24	6.49±0.08	0.450±0.020	0.509±0.021	0.041±0.0001	0.0514±0.0020
Seed E (1/10)	76.66±0.33	2.91±0.32	4.27±0.20	0.201±0.005	0.359±0.012	0.027±0.0004	0.0384±0.0004
LE (1/100)	83.33±0.33	6.70±0.16	7.07±0.47	0.535±0.018	0.687±0.018	0.046±0.0014	0.0659±0.0020
SE (1/100)	86.66±0.33	7.11±0.05	6.78±0.16	0.638±0.003	0.622±0.017	0.045±0.0010	0.0609±0.0019
RE (1/100)	83.33±0.33	6.68±0.18	7.25±0.18	0.565±0.039	0.604±0.016	0.047±0.0020	0.0682±0.0030
OW E (1/100)	83.33±0.33	6.31±0.27	6.53±0.04	0.580±0.003	0.540±0.044	0.052±0.0020	0.0612±0.0030
Seed E (1/100)	85.33±0.66	6.47±0.09	6.49±0.24	0.422±0.018	0.531±0.032	0.035±0.0009	0.0489±0.0010

Table 2 Effect of fresh leachates of different parts of *Jatropha curcas* L on germination and seedling growth (7DAS) of wheat (*Triticum aestivum* L)

Treatments	Germination %	Length (cm)		Fresh weight (g)		Dry weight (g)	
		Root	Shoot	Root	Shoot	Root	Shoot
Control	89.02±2.08	6.94±0.271	7.21±0.245	0.482±0.035	0.598±0.019	0.0445±0.0010	0.0600±0.0010
LL (1/10)	72.80±3.04	3.56±0.052	6.55±0.105	0.208±0.004	0.438±0.005	0.0223±0.0003	0.0452±0.0101
SL (1/10)	72.03±4.04	5.56±0.105	6.42±0.126	0.384±0.001	0.476±0.016	0.0341±0.0010	0.0462±0.0020
RL (1/10)	68.33±2.02	4.55±0.123	7.81±0.810	0.227±0.001	0.427±0.013	0.0368±0.0003	0.0431±0.0020
OW L (1/10)	75.66±2.33	4.85±0.202	7.94±0.093	0.237±0.028	0.498±0.002	0.0385±0.0010	0.0503±0.0004
Seed L (1/10)	78.02±3.05	5.73±0.021	7.15±0.226	0.498±0.001	0.433±0.008	0.0418±0.0010	0.0453±0.0030
LL (1/100)	88.52±1.15	6.93±0.156	7.51±0.200	0.489±0.012	0.609±0.023	0.0452±0.0006	0.0616±0.0020
SL (1/100)	89.02±2.02	7.51±0.157	7.85±0.282	0.467±0.011	0.619±0.017	0.0457±0.0010	0.0609±0.0004
RL (1/100)	88.33±0.66	7.54±0.031	7.89±0.106	0.611±0.016	0.621±0.006	0.0570±0.004	0.0614±0.0010
OW L (1/100)	82.00±1.15	7.19±0.116	8.00±0.179	0.577±0.018	0.678±0.032	0.0506±0.0030	0.0683±0.0030
Seed L (1/100)	84.06±3.37	8.04±0.141	7.69±0.172	0.587±0.007	0.564±0.016	0.0494±0.0007	0.0593±0.0006

seedlings as a result of treatments of leachates of *Jatropha curcas* is an indicator of its phytotoxicity to wheat seedlings. Roots being in direct contact of leachates are affected to a greater degree as compared with shoots. Reduction in chlorophyll contents in response to allelochemicals has been reported by others as well (Tyagi and Agarwal 2011).

Interestingly, in majority of leachate treatments promotory effects were noticed at lower concentrations, may be due to the presence of some nutrients in these leachates. Greater amount of potassium in leaf extract of *Ruellia tuberose* L have been reported to have synergistic effect with indole acetic acid (Cocucci and Dalla-Rosa 1980). Nutrient status of plants has a regulatory role in plant resistance against environmental stresses (Marschner 1995).

Treatment with leachates of higher concentrations (1/10, w/v) led to decreased nitrate reductase activity in wheat seedlings (Table 5). Reduction in nitrate reductase activity in response to weed extracts treatment has been reported by Gogoi et al. (2002) also.

Higher concentrations of leaf and ovary wall leachates of *Jatropha curcas* (1/10, *w/v*) inhibited the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The inhibition was more pronounced in roots than shoots (Table 5). Aminotransferases serve as a link between carbohydrate and amino acid metabolism and play an important role in nitrogen metabolism in plants because of their involvement in synthesis of different amino acids from glutamate which may also contribute in synthesis of proline that accumulates under stress conditions (Gajewska et al. 2009).

As evident from the above results exposure of wheat seedlings to higher concentrations of leachates (1/10, *w/v*) of *Jatropha curcas* brings down the activity of nitrate reductase

Table 3 Effect of dry leachates of different parts of *Jatropha curcas* L on germination and seedling growth (7DAS) of wheat (*Triticum aestivum* L)

Treatments	Germination %	Length (cm)		Fresh weight (g)		Dry weight (g)	
		Root	Shoot	Root	Shoot	Root	Shoot
Control	90.00±0.82	10.20±0.256	8.60±0.063	0.700±0.068	0.709±0.041	0.054±0.0001	0.077±0.0020
LL (1/10)	52.00±0.14	0.98±0.019	4.54±0.170	0.038±0.001	0.239±0.014	0.010±0.0004	0.035±0.0001
SL (1/10)	56.00±3.33	2.53±0.156	7.33±0.177	0.230±0.002	0.372±0.041	0.013±0.0001	0.058±0.0006
RL (1/10)	63.33±1.30	1.89±0.092	7.26±0.257	0.093±0.005	0.362±0.018	0.018±0.0004	0.056±0.0001
OW L (1/10)	50.50±0.57	0.63±0.025	4.13±0.206	0.023±0.050	0.251±0.010	0.007±0.0004	0.028±0.0009
Seed L (1/10)	63.33±2.33	3.83±0.025	9.08±0.261	0.262±0.025	0.604±0.018	0.020±0.0007	0.060±0.0060
LL (1/100)	93.33±1.55	10.61±0.125	10.11±0.082	0.821±0.014	0.810±0.038	0.056±0.0010	0.091±0.0001
SL (1/100)	83.30±3.77	10.25±0.215	9.82±0.436	0.550±0.020	0.782±0.018	0.045±0.0013	0.086±0.0020
RL (1/100)	90.10±3.70	10.32±0.666	10.43±0.291	0.716±0.022	0.771±0.032	0.049±0.0040	0.095±0.0130
OW L (1/100)	90.00±4.15	10.04±0.413	10.13±0.192	0.815±0.042	0.799±0.032	0.055±0.0030	0.094±0.0020
Seed L (1/100)	86.60±3.60	9.80±0.458	9.69±0.165	0.705±0.056	0.696±0.025	0.046±0.0015	0.088±0.0003

Table 4 Moisture (%), Total chlorophyll and carotenoid contents (mg g^{-1} fr wt) in wheat (*Triticum aestivum* L) seedlings (7DAS) treated with dry leachates of different parts of *Jatropha curcas* L

Treatments	*Jatropha curcas*			
	Moisture (%)		Total Chlorophylls mg g^{-1} fr wt	Carotenoids mg g^{-1} fr wt
	Shoots	Roots		
Control	89.49±0.060	93.24±0.149	0.5240±0.0176	0.1976±0.0090
LL (1/10)	87.22±0.097	85.98±0.588	0.2899±0.0222	0.1249±0.0026
SL (1/10)	86.81±0.105	89.88±0.154	0.4665±0.0224	0.1338±0.0026
RL (1/10)	86.98±0.101	90.03±0.120	0.4186±0.0193	0.1162±0.0031
OW L (1/10)	84.97±0.088	84.49±0.089	0.2001±0.0011	0.0912±0.0075
Seed L (1/10)	88.50±0.069	92.40±0.067	0.4196±0.0083	0.1648±0.0075
LL (1/100)	89.60±0.119	93.32±0.102	0.5402±0.0059	0.1645±0.0227
SL (1/100)	88.94±0.109	92.13±0.105	0.5399±0.0111	0.1667±0.0048
RL (1/100)	89.36±0.110	92.51±0.115	0.4742±0.0045	0.1194±0.0234
OW L (1/100)	89.38±0.098	93.05±0.180	0.5493±0.0111	0.1679±0.0338
Seed L (1/100)	89.39±0.062	93.26±0.173	0.4562±0.0107	0.1146±0.0147

(NR), aspartate and alanine aminotransferases. However, slight increase in activity of these enzymes was noticed in treatments of lower concentrations of leachates. Change in activity and function of enzymes has been noticed in response to phenolic allelochemicals (Politycka 1998). Effects of allelochemicals on enzyme activity have been reported earlier also (Peng et al. 2004 and Niakan et al. 2008).

Increased activity of SOD in shoots of wheat seedlings in response to treatment of leachates of leaves and ovary walls of *Jatropha curcas* indicated some kind of stress imposition due to the presence of allelochemicals in these leachates (Table 6). Probably allelochemical stress triggers the production of ROS in wheat seedlings. Superoxide dismutase (SOD) is the enzyme involved in the detoxifying process catalysing the dismutation of O_2^- to H_2O_2 and O_2. Increase in activities of SOD possibly constitutes an adaptive advantage against oxidative stress and is considered to be an essential component of antioxidant defense system in plants. Greater SOD activity has been associated with stress tolerance in plants because it neutralizes the activity of O_2^- produced under stress (Bowler et al. 1992).

Production and consumption of ROS is regulated by antioxidants nevertheless, under stress conditions production of ROS increases causing oxidative stress. Non-enzymatic antioxidant defence encompass ascorbate, glutathione, carotenoids (lycopene and carotene), α-tocopherol and many other phenolic compounds where as enzymatic antioxidant system includes super oxide dismutase (SOD), catalase (CAT), and enzymes of ascorbate-glutathione cycle (Noctor and Foyer 1998).

Many a time allelochemicals simulate environmental stress and can increase the production of O_2^- in cells. O_2^- plays an important role in the formation of other reactive oxygen species (ROS) which can induce changes in cellular metabolism through oxidative damage to membranes, proteins and nucleic acid and may result in lipid peroxidation and protein denaturation leading to senescence (Fridovic 1986). Increase in level of free oxygen radicals and activity of antioxidant

Table 5 Activity of nitrate reductase and aminotransferases in wheat (*Triticum aestivum* L) seedlings (7DAS) treated with dry leachates of *Jatropha curcas* L

Treatments	NR Activity (μ mol NO_2 produced h^{-1} g^{-1} fr wt)		Alanine aminotransferase activity (μ mol pyruvate released min^{-1} mg^{-1} protein)		Aspartate aminotransferase activity (μ mol pyruvate released min^{-1} mg^{-1} protein)	
	Shoots	Roots	Shoots	Roots	Shoots	Roots
Control	1.147±0.060	0.622±0.029	135.08±3.00	171.24±2.16	37.88±0.46	34.26±1.16
LL (1/10)	0.790±0.025	0.492±0.026	114.52±2.70	93.38±0.78	27.00±0.24	19.98±0.26
OW L (1/10)	0.775±0.015	0.457±0.017	103.82±2.20	90.93±0.30	28.10±0.72	20.50±0.40
LL (1/100)	1.248±0.045	0.797±0.006	138.22±1.02	178.08±0.08	37.92±1.60	41.76±0.90
OW L(1/100)	1.268±0.063	0.821±0.059	136.22±2.22	173.54±3.12	37.74±0.96	37.96±0.38

Table 6 Activity of superoxide dismutase and ascorbate peroxidase in wheat (*Triticum aestivum* L) seedlings (7DAS) treated with dry leachates of *Jatropha curcas* L

Treatments	Superoxide dismutase activity (EU mg^{-1} protein h^{-1}) Shoot	Ascorbate peroxidase activity (EU mg^{-1} protein h^{-1}) Shoot
Control	0.357±0.024	0.253±0.016
LL (1/10)	0.394±0.003	0.325±0.007
OW L (1/10)	0.420±0.008	0.314±0.007
LL (1/100)	0.388±0.017	0.298±0.026
OW L (1/100)	0.358±0.003	0.301±0.011

Table 8 Total free sugars (mg g^{-1}dr wt) and free proline (μ mol g^{-1} dr wt) in wheat (*Triticum aestivum* L) seedlings (7DAS) treated with dry leachates of *Jatropha curcas* L

Treatments	Total free sugars (mg g^{-1}dr wt)		Free proline (μ mol g^{-1} dr wt)	
	Shoots	Roots	Shoots	Roots
Control	90.6±2.9	42.0±2.0	135.52±8.10	105.03±8.64
LL (1/10)	218.7±4.6	77.6±1.4	181.44±7.02	188.20±7.02
OW L (1/10)	220.6±7.3	80.0±2.8	194.14±2.70	195.76±5.94
LL (1/100)	136.6±1.7	46.0±2.8	145.24±9.00	139.98±1.19
OW L (1/100)	138.3±9.2	45.0±1.4	149.83±6.21	141.06±3.66

enzymes in response to stress imposed by allelochemicals has been reported in *Lycopersicum esculantum* (Lara-Nunez et al. 2006).

An increase in ascorbate peroxidase in shoots of wheat seedlings was registered as a result of treatments of dry leachates of leaves and ovary walls. Increase was more conspicuous in seedlings receiving higher concentrations of leachates (Table 6). Ascorbate peroxidase is involved in scavenging of H_2O_2 thus forming an important part of the antioxidant system (Dabrowska et al. 2007).

Decrease in starch contents was noticed in all wheat seedlings treated with higher concentrations (1/10, w/v) of leachates. Starch contents were greater in shoots as compared to roots. Seedlings treated with leachates (1/100, w/v) showed greater amount of starch indicating that stimulation is provided by leachate (1/100, w/v) treatments (Table 7).

Treatments of higher concentration of leachates of leaf and ovary wall of *Jatropha curcas* resulted in accumulation of free sugars in wheat seedlings (Table 8). Higher levels of free sugars probably contribute towards maintenance of turgor and osmotic adjustment in seedlings and accumulation of free sugars has been reported under stress conditions (Pandey et al. 2004 and Jatav et al. 2012).

Plants often accumulate compatible solutes under unfavorable conditions which protect membrane functionality and induce osmotic adjustment. Our results show increased free proline and free sugars in seedlings treated with leachates of *Jatropha curcas*. Wheat seedlings exposed to varying concentrations of leaf and ovary wall leachates of *Jatropha curcas* accumulated proline which was greater in shoots than roots (Table 8). Increase in proline contents of marigold seedlings has been reported in response to treatment of leaf leachates of *Jatropha curcas* (Wang et al. 2009)

Wheat seedlings treated with higher concentrations of leachates (LL and OWL 1/10, w/v) and potassium had greater proline contents in comparison to seedlings receiving treatments of lower concentrations of leachates (LL and OWL 1/100, w/v) reflecting some kind of stress caused by leachate treatment of higher concentration (Table 8). Accumulation of proline has been considered as one of the adaptation mechanisms to withstand stress (Aspinall and Paleg 1981) protecting enzymes against denaturation (Paleg et al. 1984) and contributing to osmotic adjustment and maintenance of leaf water potential under water stress (Pandey and Agarwal 1998).

Phytochemical analysis of *Jatropha curcas* exhibited organ (maximum in leaves and ovary walls/seeds) and developmental stage (greater in young leaves) dependent variation in total phenols, tannins, free amino acids and phytic acid contents. Seasonal changes were also noticed in these constituents i.e. maximum phenols, tannins and free amino acids in leaves were found during summer (May-June) and least during rainy season (July –August), on the other hand, phytic acid contents were greater during rainy season.

Greater degree of inhibition of germination and seedling growth of wheat was evident due to treatment of dry leachates of leaves and ovary walls of *Jatropha curcas* in comparision to extracts and leachates of fresh parts. Such a trend was also observed in total chlorophyll, carotenoid contents and in the activity of nitrate reductase and aminotransferases. Concomitant to this an increase in antioxidant enzymes i.e. superoxide dismutase and ascorbate peroxidase was noticed as reported in many stressful situations.

Hence present experiments are suggestive of adverse influence of *Jatropha* plants from allelopathic view point and care must be taken to select the location for *Jatropha curcas*

Table 7 Starch (mg g^{-1}dr wt) in wheat (*Triticum aestivum* L) seedlings (7DAS) treated with dry leachates of *Jatropha curcas* L

Treatments	Starch (mg g^{-1}dr wt)	
	Shoots	Roots
Control	136.4±0.5	89.1±0.9
LL (1/10)	91.4±1.2	65.6±1.6
OW L (1/10)	89.4±0.9	63.8±1.8
LL (1/100)	145.5±3.0	94.5±2.7
OW L (1/100)	150.9±2.3	93.8±0.4

plantation, as continued plantation may lead to accumulation of such constituents and such a situation warrants need for more analysis and experimentation particularly in the context of fertile soils. *Jaropha* plants grow for 30–35 years, therefore long term effect of *Jaropha* plantation regarding fertility of soil needs further work. Nevertheless, *Jaropha* plants accumulate sodium, potassium and chlorides and grow well on saline soils, and therefore possibility of these plants to improve saline soil cannot be ruled out.

Acknowledgments Thanks are due to Ex-Head School of Studies in Botany Prof Rekha Bhadauria, for providing necessary facilities, Prof. GBKS Prasad Dean Life Sciences, Jiwaji University, Gwalior for useful interactions and MPCST, Bhopal for funding a project during a part of this work.

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