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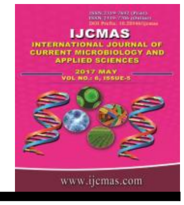

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Effect of Different Doses of Jatropha Leaf Extract on Growth and Development of French Bean (*Phaseolus vulgaris* L.) and Brinjal (*Solanum melongena*)

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A B S T R A C T

Experiments were conducted to determine the possible allelopathic effects of jatropha (*Jatropha curcas*) on french bean (*Phaseolus vulgaris* L.) and brinjal (*Solanum melongena*). In one experiment, aqueous extract of jatropha leaf at 5%, 10%, 15% and 20% (W/V) concentrations were bio-assayed against germination and seedling growth of French bean and brinjal. In both the crops, germination percentage, germination index, shoot and root length, fresh and dry weights of shoot and root were appreciably reduced by aqueous extract of jatropha leaf in a concentration dependent manner. However, germination of French bean seed was found to be more sensitive to jatropha leaf extract. In another experiments aqueous extract of jatropha leaf at 5%, 10%, 15% and 20% (W/V) concentrations were applied into soil to determine the allelopathic activity of jatropha on growth and development of French and brinjal. Plant growth of French bean in terms of plant height, leaf number, leaf area, root volume, shoot and root dry weights were reduced significantly by aqueous extract, particularly at higher concentrations. Relative leaf water content, total leaf chlorophyll content and leaf N P K content of French bean were also reduced by the aqueous extract. Moreover, pronounced negative allelopathic effects of jatropha on yield and different yield attributing parameters of French bean were recorded. However, no significant growth and yield reduction were recorded in brinjal with extract of jatropha leaf. From this investigation, it may be suggested that brinjal may be grown as an intercrop with jatropha.

Keywords

Allelopathic effect, *Jatropha curcas*, *Phaseolus vulgaris*, *Solanum melongena*, intercropping.

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Introduction

Several vegetable oils available commercially have been tested as fuel components for diesel engines. Some of these oils are soybean, cottonseed, sunflower, rapeseed, safflower, peanut, algal oil etc. (Spolaore *et al.*, 2006). Among various plants, Jatropha (*Jatropha curcas*) has been demonstrated as the most potential biofuel containing plant species which can be grown in diverse climatic conditions. As a bio-fuel crop, jatropha is grown in widely spaced rows at 3 m apart and after pruning; the newly emerged canopy does not cover the land adequately and hence needs frequent weeding (Singh *et al.*, 2007). This wide inter-row spacing can be effectively used to grow some inter-crop, which would not only reduce weed infestation but also the farmers would get good return from the land.

Therefore, it is suggested that growing of some intercrops with jatropha plantation could help in mitigating both food and energy crisis (Abugre and Sam, 2010).

The failure of most crops in an intercropping system has primarily been attributed to allelopathic interaction. Phytotoxicity is very old component of agriculture but it is described as allelopathy by (Molisch, 1937). The chemical compounds responsible for the phenomenon of allelopathy, collectively known as allelochemicals, is usually secondary plant metabolites (Ashrafi *et al.*, 2007). Jatropha extracts contain allelochemicals like tannins, glycosides, alkaloids and flavonoids (Igbinosa *et al.*, 2009) and such phytotoxic substances are reported to cause growth inhibition in various receiver plants (Javaid and Anjum, 2006). Thus, the research on allelopathic interactions of biofuel trees with intercropped food crops emerges as a major scientific and policy issue.

Materials and Methods

Aqueous extract of jatropha leaf was prepared following the method given by Maharjan *et al.*, (2007). Fresh jatropha leaves weighing 200 gm were ground homogeneously in a mortar and mixed with 1000 ml of distilled water and kept for 24 hours. Then the slurries were strained through two layers muslin cloth and were centrifuged at 4500 rpm for 10 minutes. The supernatant was considered as 20% aqueous extract. By subsequent dilution with distilled water, aqueous extracts of 15%, 10% and 5% were prepared and kept at 4°C till further use.

Aqueous extract bioassay

Bioassay of jatropha was carried out following the procedure of Rejila and Vijayakumar (2011). Surface of the French bean and brinjal seeds were sterilized by dipping in 0.10 percent (W/V) $HgCl_2$ for one minute and rinsed several times with distilled water. Ten seeds of French bean and brinjal were placed in separate glass Petri dishes (15 cm diameter) with 3 replications fitted with single layer of filter paper. The filter papers of different Petri dishes were moistened sufficiently by adding equal volume (15 ml) of aqueous extract of different concentrations. A control was set with distilled water. The Petri dishes were covered and kept in room temperature. The covered Petri dishes were opened periodically for aeration and to add stock solutions to keep the filter paper moistened.

Preparation of pot mixture

The collected soil was sun-dried, ground and screened to pass through a 2.5 mm sieve. The recommended doses of inorganic and organic fertilizer for French bean (30:40:20 kg of NPK ha-1 and 20 tonne of FYM ha^{-1}) were added to each pot containing 5 kg of soil.

Sowing of seeds

French bean seeds (variety selection-9) were surface sterilized by dipping in HgCl2 (0.10 %) for 1 minute and ringed several times with distilled water. Then seeds were sown (10 seeds in each pot) at depth of 2 cm. However, after germination, three seedlings per pot were kept and transplant for recording different parameters. Throughout the entire experimental period, optimum level of moisture was maintained by adding water as and when required.

Details of treatment

Various concentrations of aqueous extracts of jatropha were applied in different pots (soil application, 500 ml pot-1) at 7 days after sowing (DAS), 14 DAS and 21 DAS following the procedure of Rejila and

Vijayakumar (2011). The experiment was carried out with three replications with the following treatments:

T1: 5.0% aqueous extract
T2: 10% aqueous extract
T3: 15% aqueous extract
T4: 20% aqueous extract

One set was kept as control without application of aqueous extract.

Results and Discussion

Experiment No. 1 and Experiment No. 2 (aqueous extract bioassay) were conducted under laboratory condition to ascertain the allelopathic effects of different concentrations of aqueous extract of jatropha leaf on germination behaviour of French bean and brinjal. It was observed that germination percentage of both French bean and brinjal were reduced by jatropha leaf extract. In both the crops, minimum and maximum reduction in germination percentage were observed with 5% and 20% concentrations of aqueous extract respectively, which revealed that inhibition of germination of French bean and brinjal by jatropha leaf extract was concentration dependent. This finding of the present investigation is in line with the results of other studies reported by several workers. For example, Abugre and Sam (2010) recorded similar reduction in seed germination of several crops by aqueous extract of jatropha leaf.

A perusal of the data in Table 1 and 2 gives the indications that this bioassay was conducted to ascertain the allelopathic effects of different concentrations of aqueous extract of jatropha leaf on seedling growth of French bean and brinjal in terms germination percentage, germination index, shoot and root length, shoot and root fresh and dry weights. It was observed that in both the crops, minimum and maximum reduction in germination percentage were observed with 5% and 20% concentrations of aqueous extract respectively, which revealed that inhibition of germination of French bean and brinjal by jatropha leaf extract was concentration dependent. This finding of the present investigation is in line with the results of other studies reported by several workers. For example, Abugre and Sam (2010) recorded similar reduction in seed germination of several crops by aqueous extract of jatropha leaf.

Germination index of French bean and brinjal, a criteria to evaluate the effect on rate of germination, was recorded in different concentrations of jatropha leaf aqueous extract. The speed of germination was retarded by aqueous extract of jatropha leaf as indicated by low germination index values. Inhibition in the growth of shoot and root of French bean and brinjal were recorded to be concentration dependent. Shoot and root length of both the test crops were reduced to a highest extent by 20% aqueous extract of jatropha leaf. Similar trend was recorded in case of fresh and dry weights of seedlings. It was observed that both shoot and root fresh and dry weights of French bean and brinjal were reduced by aqueous extract of jatropha leaf. In both the crops, minimum and maximum reduction in fresh and dry weights were observed with 5% and 20% concentrations of aqueous extracts respectively, which revealed that reduction in fresh and dry weights by jatropha leaf extract was concentration dependent. This finding is in line with the results reported by Abugre and Sam (2010). They recorded similar reduction in seedling weights of several crops by aqueous extract of jatropha leaf.

From the aqueous extract bioassay, it can be suggested that jatropha leaf contains water soluble phytotoxic substances which inhibit germination and early seedling growth under laboratory condition in a concentration dependent manner. Several researchers reported similar allelopathic effects of jatropha on other crops also. For example, Rejila and Vijayakumar (2011) reported that aqueous leaf extract of jatropha could strongly inhibit seed germination, shoot and root growth in Capsicum annum L. Abugre and Sam (2010) reported negative allelopathic effects of jatropha leaf extract on several receiver plants. They showed that aqueous extract of jatropha leaf had a strong inhibitory effect on germination and length of radicle and plumule of various test crops.

From the recorded data of the present investigation, it was observed that reduction in germination percentage in French bean with 20% concentration of aqueous extract was 34.48% over control, whereas in brinjal, it was only 24.00%. All the applied concentrations of aqueous extract of jatropha leaf exhibited pronounced effects on germination percentage, shoot and root length, fresh and dry weights of shoot and root of French bean compared to brinjal (Fig.1). Therefore, it is noteworthy to mention that germination and seedling growth in French bean, compared to brinjal, appeared to be more sensitive to aqueous extract of jatropha leaf.

A perusal of the data gives the indications that allelopathic effect of jatropha on growth, development and yield of French bean and brinjal. It was observed that at the early stages of crop growth (for example at 21 DAS), even the lowest concentration (5%) of jatropha leaf extract significantly reduced plant height of French bean. During the entire growth period of the crop, plant height of French bean was reduced in a concentration dependent manner (Fig. 2). However, in brinjal from 35 DAT to harvest, even the highest concentrations of aqueous extract (20%) failed to produce any inhibitory effect on plant height. It indicated that the inhibitory effect of aqueous extract on plant height of brinjal disappeared during this stage of growth. It may be because of the fact that allelochemicals released from aqueous extracts may not be sufficient to affect plant height of brinjal during the later stages of growth. Similar reduction in plant height by allelopathic interaction was observed by several workers. For example, Wang *et al.*, (2009), Kallil *et al.*, (2010), Rejila and Vijayakumar (2011) and Khan *et al.*, (2012) recorded similar type of reduction in plant height of various receiver plants grown under allelopathic influences of donor plants. It was observed that even at 70 DAS, except 5% concentration, all other applied concentrations showed significant reduction in leaf number of French bean. However, in case of brinjal, only the higher concentrations of aqueous extract exhibited such inhibition only at the early stages of growth. Similarly, aqueous extract of jatropha leaf showed pronounced inhibitory effect on leaf area development of French bean. Although with the progress in growth stages, leaf area of French bean was increased, but jatropha leaf aqueous extract reduced such increment in leaf area. In case of brinjal, aqueous extract failed to produce such inhibitory effect, especially at the later stages of growth. At 30 DAS, reduction in leaf area in French bean (with 20% concentration of aqueous extract) was 26.17% over control, whereas in brinjal (at 30 DAT), it was only 17.28% (Fig. 3). Ercisli *et al.*, (2005), documented similar reduction in leaf area because of allelopathic effect.

From the recorded data it is evident that at all the recorded phases of plant growth, shoot and root dry weights of French bean were significantly reduced by jatropha leaf extract in a concentration dependent manner (Fig. 4 & 5). In contrast, root and shoot dry weights of brinjal were reduced only at higher concentrations (Fig. 6 & 7). Moreover, this inhibitory effect was recorded only at early growth stage (30 DAT) of brinjal. Khan *et al.*, (2008), observed similar results; they recorded significant reductions in shoot and root fresh and dry weights of receiver plant by aqueous extract of donor species.

Leaf nitrogen, phosphorus and potassium contents of French bean and brinjal were affected by aqueous extract of jatropha leaf. At 30 DAS and 50 DAS, except 5%, all other applied concentrations of aqueous extract significantly reduced leaf nitrogen content of French bean. Similarly, leaf phosphorus content of French bean was significantly reduced by all the applied concentrations of aqueous extract both at 30 DAS and 50 DAS. However, such inhibitory effect on leaf nitrogen and phosphorus content of brinjal was not recorded in the latter stages of growth. At 50 DAS, all the applied concentrations of aqueous extract significantly reduced leaf potassium content of French bean. In case of brinjal, although at 30 DAT leaf potassium content was reduced by all the applied concentrations of aqueous extract, such inhibitory effect was not recorded in the latter stages of growth (Table 2 & 3).

Fig.1 Effect of 20% (W/V) concentration of aqueous extract of jatropha leaf on percent inhibition / reduction of germination (%), shoot and root length and fresh and dry weights of shoot and root of French bean and brinjal

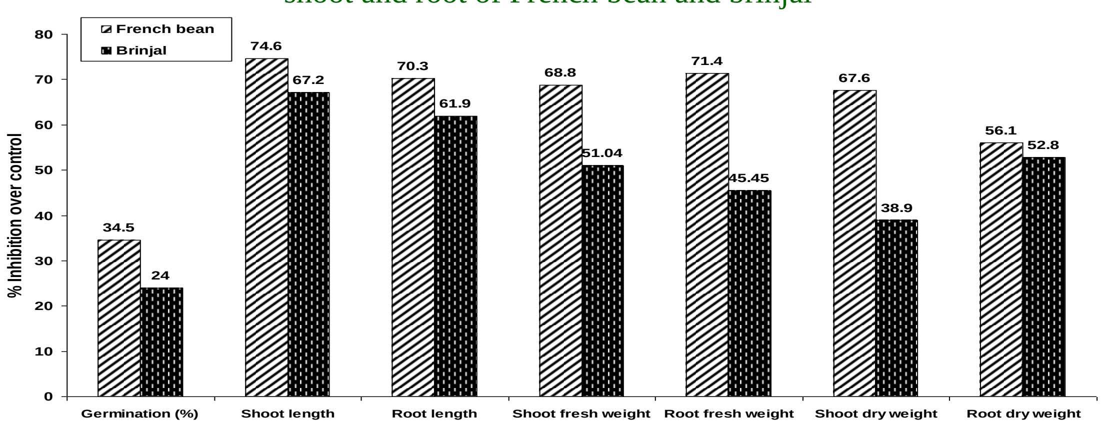


Fig.2 Effect of different concentrations of aqueous extract of *Jatropha curcas* on plant height (cm) of French bean. Data presented are means ± SEd (Vertical bars)

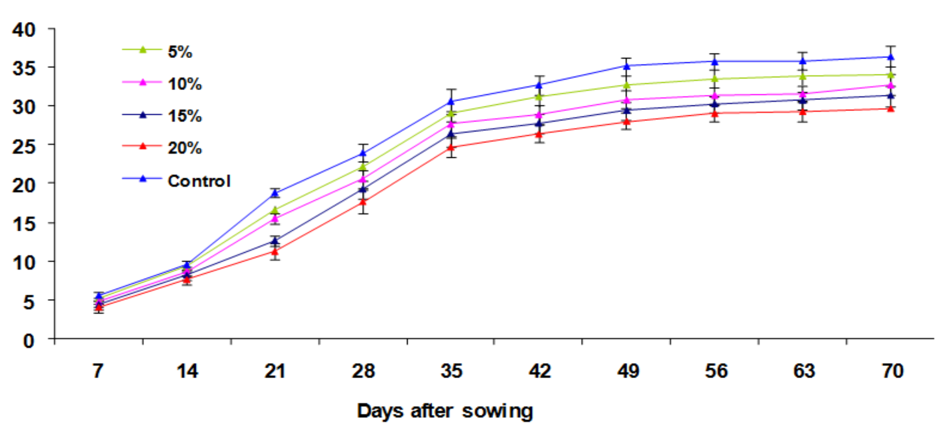


Table.1 Effect of different concentrations of aqueous extract of *Jatropha curcas* on germination percentage (%), germination index, shoot and root length (cm), shoot and root fresh weights (g seedling^{-1}) and shoot and root dry weights (g seedling^{-1}) of French bean

Concentration (W/V)	Germination * (%)	Germination Index	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g seedling^{-1})	Root fresh weight (g seedling^{-1})	Shoot dry weight (g seedling^{-1})	Root dry weight (g seedling^{-1})
5%	86.66 (68.85)	91.33	7.99	5.13	0.69	0.08	0.07	0.013
10%	76.66 (61.21)	80.00	5.11	3.58	0.45	0.06	0.05	0.010
15%	73.33 (59.21)	72.66	3.80	2.53	0.37	0.04	0.04	0.008
20%	63.33 (52.77)	71.33	2.33	2.05	0.27	0.03	0.03	0.007
Control	96.66 (83.85)	106.70	9.20	6.91	0.88	0.11	0.09	0.015
SEd ±	5.36	8.14	0.92	0.72	0.12	0.01	0.008	0.0009
CD (5%)	11.94	18.14	2.06	1.60	0.27	0.03	0.019	0.0020

***** Transformed values are in parentheses.

Table.2 Effect of different concentrations of aqueous extract of *Jatropha curcas* on germination percentage (%), germination index, shoot and root length (cm), shoot and root fresh weights (mg seedling^{-1}) and shoot and root dry weights (mg seedling^{-1}) of brinjal

Concentration (W/V)	Germination * (%)	Germination Index	Shoot length (cm)	Root length (cm)	Shoot fresh weight (mg seedling^{-1})	Root fresh weight (mg seedling^{-1})	Shoot dry weight (mg seedling^{-1})	Root dry weight (mg seedling^{-1})
5%	80.00 (63.43)	59.33	3.89	3.21	9.30	0.31	0.90	0.033
10%	76.66 (61.21)	55.33	3.56	2.86	8.60	0.29	0.81	0.031
15%	73.33 (59.00)	41.33	2.65	1.96	6.20	0.25	0.67	0.023
20%	63.33 (52.78)	32.67	1.28	1.25	4.70	0.18	0.58	0.017
Control	83.33 (66.14)	62.00	3.92	3.28	9.60	0.33	0.95	0.036
SEd ±	2.91	3.04	0.19	0.31	0.50	0.03	0.05	0.004
CD (5%)	6.48	6.77	0.44	0.69	1.00	0.08	0.11	0.008

***** Transformed values are in parentheses.

Table.3 Effect of different concentrations of aqueous extract of *Jatropha curcas* on leaf nitrogen (%, W/W), phosphorus (%, W/W) and potassium (%, W/W) of French bean

Concentration (W/V)	Leaf nitrogen content (%, W/W)		Leaf phosphorus content (%, W/W)		Leaf potassium content (%, W/W)	
	30 DAS	50 DAS	30 DAS	50 DAS	30 DAS	50 DAS
5%	4.09	3.44	0.15	0.11	1.95	1.76
10%	3.78	3.07	0.10	0.08	1.77	1.49
15%	3.51	2.68	0.08	0.07	1.62	1.29
20%	2.55	1.29	0.05	0.04	1.20	1.08
Control	4.39	3.45	0.19	0.18	2.06	1.85
SEd ±	0.17	0.08	0.006	0.003	0.07	0.03
CD (5%)	0.37	0.19	0.015	0.008	0.16	0.08

Table.4 Effect of different concentrations of aqueous extract of *Jatropha curcas* on leaf nitrogen (%, W/W), phosphorus (%, W/W) and potassium (%, W/W) of brinjal

Concentration (W/V)	Leaf nitrogen content (%, W/W)		Leaf phosphorus content (%, W/W)		Leaf potassium content (%, W/W)	
	30 DAT	50 DAT	30 DAT	50 DAT	30 DAT	50 DAT
5%	1.84	1.68	0.09	0.112	2.26	2.26
10%	1.51	1.65	0.07	0.105	2.02	2.24
15%	1.13	1.62	0.06	0.104	1.88	2.22
20%	0.83	1.56	0.04	0.10	1.66	2.21
Control	2.06	1.71	0.12	0.113	2.58	2.28
SEd ±	0.039	0.043	0.002	0.004	0.015	0.38
CD (5%)	0.088	NS	0.005	NS	0.035	NS

Table.5 Effect of different concentrations of aqueous extract of *Jatropha curcas* on numbers of flower, numbers of pod (plant^{-1}), numbers of seed (pod^{-1}), total fresh and dry weights of pod (g plant^{-1}) and dry weight of seed (g pod^{-1}) of French bean

Concentration (W/V)	Numbers of flower (plant^{-1})	Numbers of pod (plant^{-1})	Numbers of seed (pod^{-1})	Total fresh weight of pod (g plant^{-1})	Total dry weight of pod (g plant^{-1})	Dry weight of seed (pod^{-1})
5%	34.66	21.00	5.33	47.55	4.29	0.58
10%	33.33	17.00	5.00	45.38	3.93	0.48
15%	31.33	14.33	5.00	43.73	3.36	0.36
20%	30.33	12.66	4.66	42.30	2.25	0.23
Control	36.33	25.66	6.66	48.75	4.68	0.72
SEd ±	1.61	2.33	0.36	1.68	0.38	0.016
CD (5%)	3.60	5.20	0.81	3.75	0.86	0.036

Table.6 Effect of different concentrations of aqueous extract of *Jatropha curcas* on numbers of flower ($plant^{-1}$), numbers of fruit ($plant^{-1}$) and fresh and dry weights of fruit ($g\ plant^{-1}$) of brinjal

Concentration (W/V)	Numbers of flower ($plant^{-1}$)	Numbers of fruit ($plant^{-1}$)	Fresh weight of fruit ($g\ plant^{-1}$)	Dry weight of fruit ($g\ plant^{-1}$)
5%	15.67	8.00	416.58	28.54
10%	14.67	7.67	412.30	28.22
15%	14.00	7.33	410.32	28.01
20%	13.67	6.67	407.54	27.76
Control	16.33	8.33	418.45	28.66
SEd ±	0.91	0.56	3.51	0.68
CD (5%)	NS	NS	NS	NS

Fig.3 Effect of 20% (W/V) concentration of aqueous extract of jatropha leaf on percent inhibition / reduction of leaf area, shoot and dry weight and total chlorophyll content of French bean and brinjal (Data used in this figure were recorded at 30 DAS and 30 DAT for French bean and brinjal, respectively)

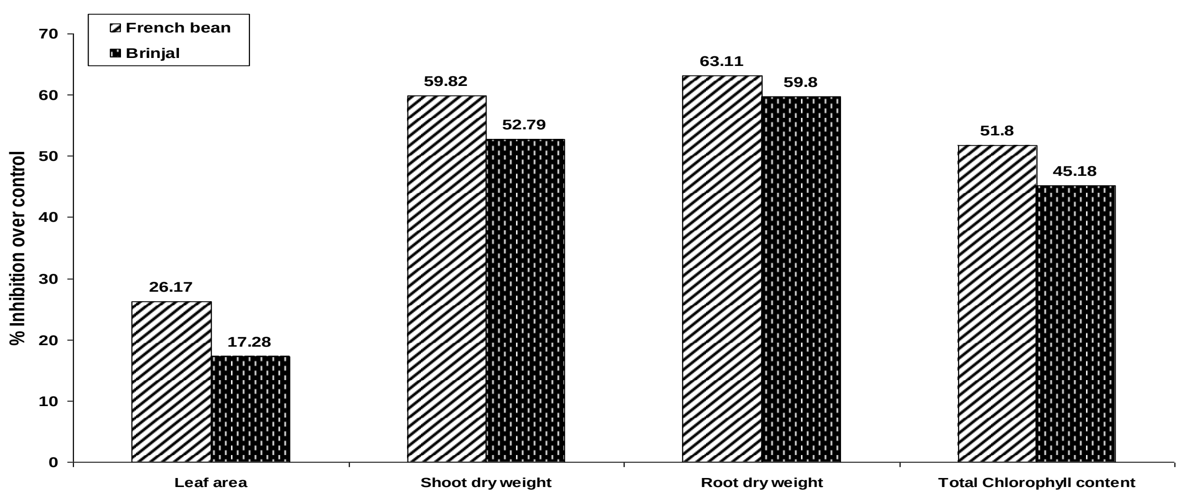


Fig.4 Effect of different concentrations of aqueous extract of *Jatropha curcas* on shoot dry weight (g plant^{-1}) of French bean. Data presented are means ± SEd (Vertical bars)

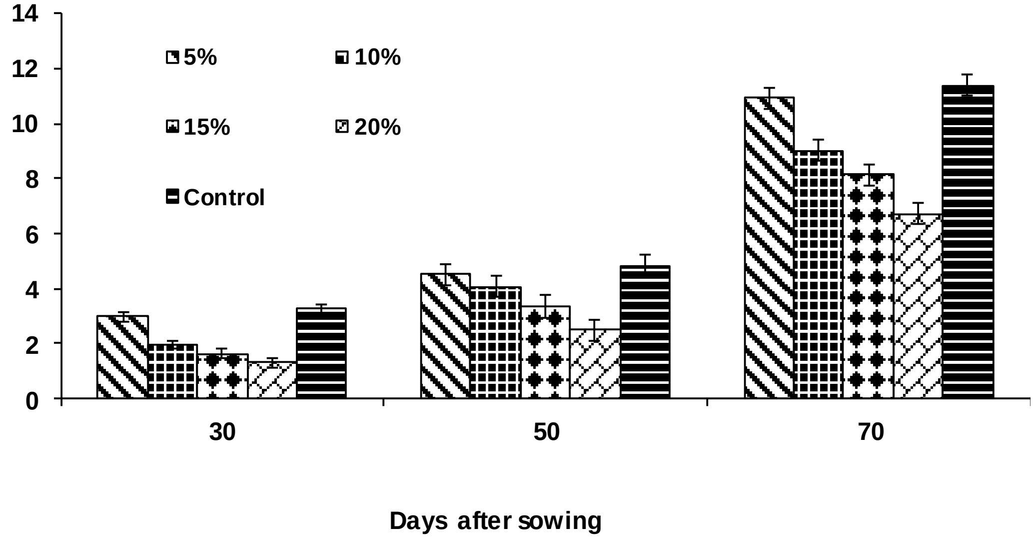


Fig.5 Effect of different concentrations of aqueous extract of *Jatropha curcas* On root dry weight (g plant^{-1}) of French bean

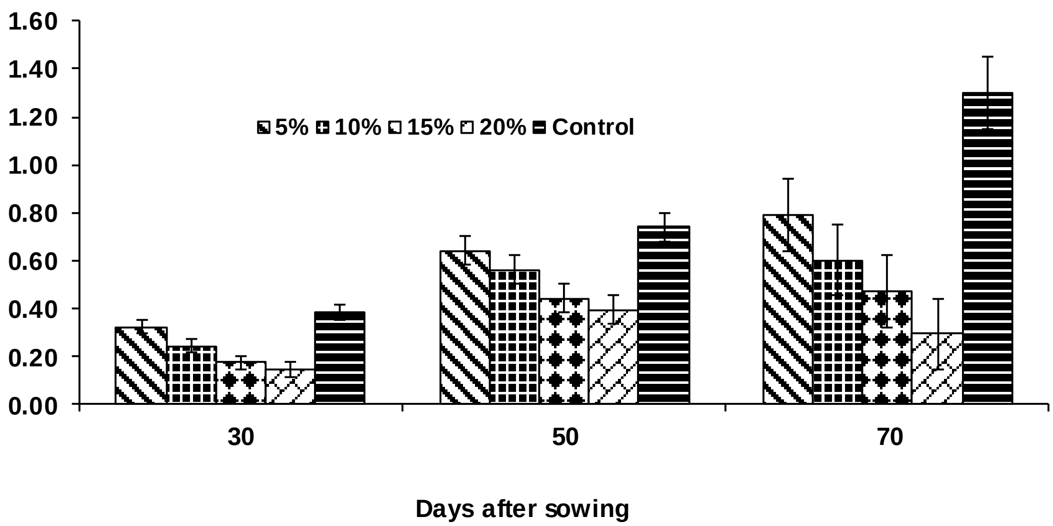


Fig.6 Effect of different concentrations of aqueous extract of *Jatropha curcas* on shoot dry weight (g plant^{-1}) of brinjal. Data presented are means ± SEd (Vertical bars)

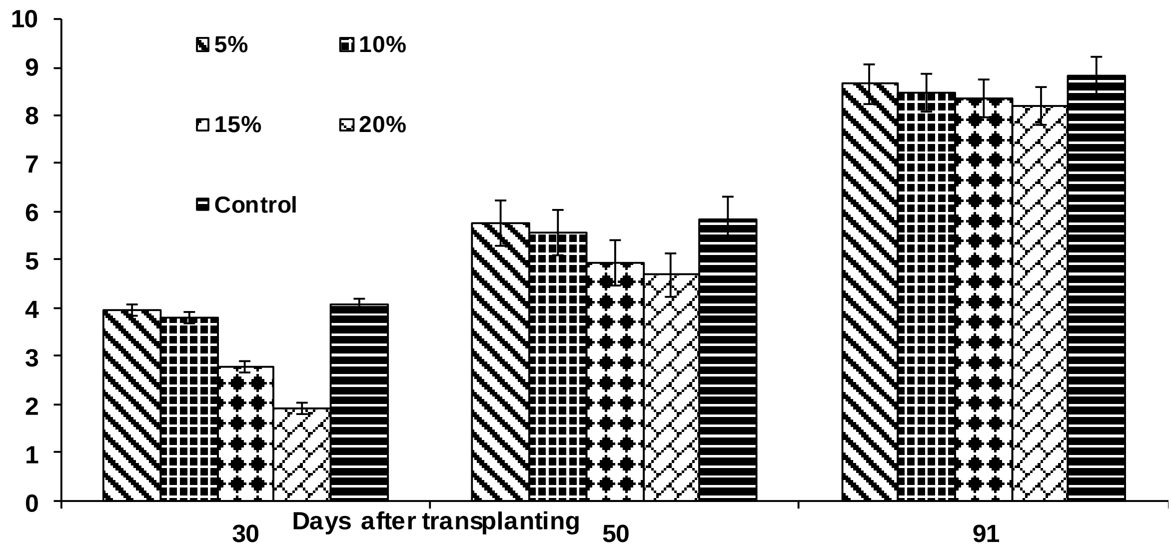


Fig.7 Effect of different concentrations of aqueous extract of *Jatropha curcas* on root dry weight (g plant^{-1}) of brinjal. Data presented are means ± SEd (Vertical bars)

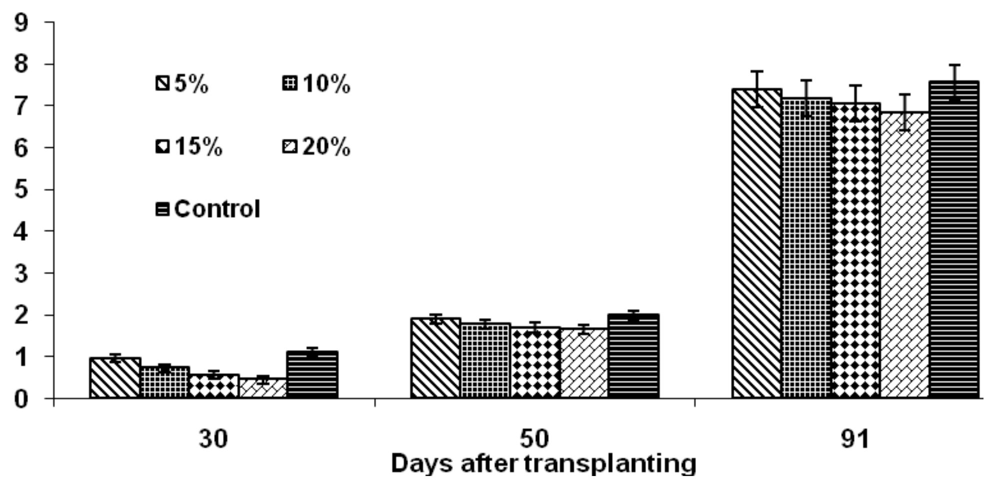
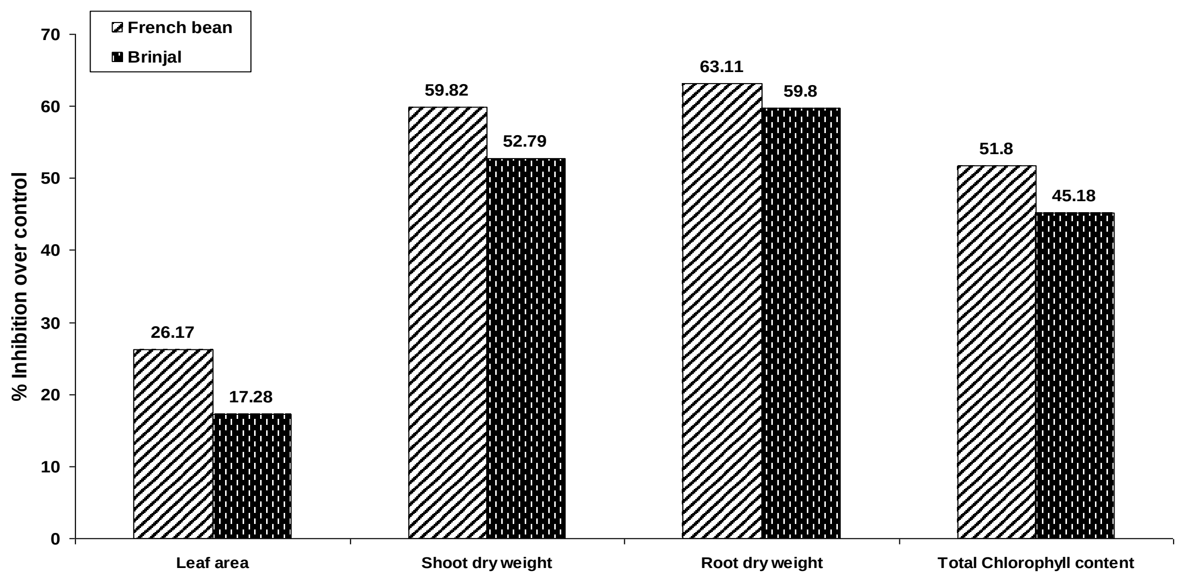


Fig.8 Effect of 20% (W/V) concentration of aqueous extract of jatropha leaf on percent inhibition / reduction of leaf area, shoot and dry weight and total chlorophyll content of French bean and brinjal (Data used in this figure were recorded at 30 DAS and 30 DAT for French bean and brinjal, respectively)



Norby and kozlowski (1980) observed that phosphorus concentration in red pine was reduced when red pine trees were watered with aqueous extract of *Lonicera taturica* or *Salidago gugntia* foliage. Chlorophyll molecules embedded in the thylakoid membrane absorb light energy. These molecules are the most important pigments for absorbing the light energy used in photosynthesis. Any changes in chlorophyll content are expected to bring about change in photosynthesis (Reigosa *et al.,* 2006). In the present investigation, total chlorophyll content of French bean leaves was found to be reduced by all applied concentrations of aqueous extract of jatropha leaf. This inhibitory effect was observed both at early and later stages of growth. However, in case of brinjal, aqueous extract failed to produce such inhibitory effect, especially at the later stages of growth. Various allelochemicals such as caffeic, t-cinamic, p-coumaric, ferulic, gallic and vanillic acid were also reported to reduce chlorophyll content of soybean (Patterson, 1981).

It has been reported that allelochemicals can reduce chlorophyll accumulation in plants by three ways: 1. inhibition of synthesis, 2. stimulation of degradation and 3. both inhibition of synthesis and stimulation of degradation (Yang *et al.,* 2002). Einhellig and Rasmussen (1979) suggested that reduction in chlorophyll content occurred only after some other physiological processes were altered by allelochemicals, but they could not conclude whether the reduction was because of degradation or reduction in synthesis of chlorophyll. The importance of plant water status has widely been recognized for the maintenance of cellular turgidity, which is required for normal growth and survival of plant. From this present investigation, it is evident that relative leaf water contents of two tested crops were altered by aqueous extract of jatropha leaf. At 50 DAS, all the applied concentrations of aqueous extract showed significant reduction in RLWC of French bean. Although, RLWC of brinjal was reduced by aqueous extract at 30 DAT, such inhibitory effect was not recorded in the latter stages of growth of the crop. From the present investigation, it is observed that yield and all yield-attributing parameters of French bean were negatively affected by jatropha leaf extract. Flower number of French bean was significantly reduced by aqueous extract of jatropha leaf. Considerable inhibition in the numbers of pod and numbers of seed per pod of French bean were also recorded under allelopathic effect of jatropha. Aqueous extract of jatropha leaf significantly reduced total fresh weights of pod (final yield) of French bean (Table 3). However, in case of brinjal aqueous extract of jatropha leaf failed to show such inhibitory effect on yield and yield attributing parameters (Table 4). Some workers hypothesized that reactive oxygen species (ROS) status is an important mechanism involved in the interspecific difference in response to allelochemicals. Some plants have found ways to reduce the effects of allelochemicals produced by neighboring plants. Detoxification mechanisms that are used by plants include the conjugation, sequestration or secretion of carbohydrates, and the oxidation of the phytotoxic compounds (Inderjit and Duke, 2003). Detoxification products are then released into the environment, where they are presumably metabolized by soil microorganisms, in root exudates (Sicker *et al.,* 2001). It is well documented that plants generate more ROS when exposed to stressful conditions such as sub-optimal temperature, high light, salt, and pathogen infection (Rhoads *et al.,* 2006). These ROS are either toxic by-products of aerobic metabolism or key regulators of growth, development, or the defence pathway (Mittler *et al.,* 2004) which can affect membrane permeability, cause damage to DNA and protein, induce lipid peroxidation, and ultimately lead to programmed cell death (PCD).

One of the probable reasons for such variations is the differential allelopathic responses exhibited by different crop species. Results obtained from the present investigation revealed that jatropha aqueous leaf extract significantly reduced growth and yield of French bean, whereas in brinjal it could not produce such inhibitory effects. Based on this result, it can be concluded that brinjal is more suitable for intercropping in jatropha plantation than that of French bean.

In the previous section, it has been mentioned that germination and seedling growth of French bean, compared to brinjal, were appeared to be more sensitive to aqueous extract of jatropha leaf. Similar trend was recorded from the pot culture experiment also. Aqueous extract of jatropha leaf noticeably reduced several growth and yield parameters of French bean compared to brinjal (Fig. 8). It is interesting to note that in case of brinjal, such inhibitory effects of jatropha leaf extract were not recorded (Table 6). This result of our study clearly demonstrated the differential responses of two tested crop species towards jatropha leaf extracts.

In conclusion, results of aqueous extract bioassay and pot culture experiment clearly indicate that brinjal was less sensitive to allelopathic effects of jatropha compared to French bean. Therefore, it can be suggested that brinjal may be grown as intercrop with jatropha plantation. However, further research in field condition will be required to confirm the results obtained from our laboratory and pot culture experiments.

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