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Allelopathic effect of *Solanum nigrum* on *Pisum sativum, Eleusine coracana* and *Trigonella foenum graecum*

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ABSTRACT

Allelopathic effect of leaf ethanolic extract of *Solanum nigrum* was tested on test crops *Pisum sativum, Eleusine coracana* and *Trigonella foenumgraecum* and compared with control. The effect of the extract was tested by observing germination, radical length and total protein content. The Observations have been presented in Table and figure 1(Germination),2(Radicle length), and 3(Total protein content). The Seed germination showed both inhibitory and stimulatory influence on tested plants. Pisum sativum was the most sensitive crop to ethanolic extract of Solanum nigrum Phytotoxic effect and inhibitory influence was observed in Pisum sativum followed by Eleusine coracana. In Trigonella foenumgraecum, inhibitory influence was observed only in higher concentrations (80 & 100µg), whereas in lower concentrations no such influence was observed compared with that of control. The plant extract had higher inhibitory influence on the growth of Pisum sativum and was comparatively reduced in case of Eleusine coracana than Pisum sativum. Stimulatory influence on seeding growth was observed in lower concentrations (20 µg, 40 µg and 60 µg) with that of control in case of Trigonella foenungraecum. The change in concentration and phytochemical activity had modified the soil moisture, soil temperature and other soil factors, which had shown both positive and negative effect on the tested plant. There was a marked decrease in total protein content from lower to higher concentrations of the extract in Pisum sativum and in Eleusine coracana. The phytochemical tests were conducted for the leaf and the fruit extract. The secondary metabolites like tannins, terponoids, alkaloids, phytosterols and cardiac glycosides were answered positive for both the leaf and the fruit extract samples, whereas saponins were answered positive only in fruit extract. From the results observed secondary metabolites produced by plants are by-products of primary metabolic process. They have allelopathic effect on the same plant or neighbouring plants. Their effects are selective. The *Solanum nigrum* leaf extracted showed distinct allelopathic effect. Generally leaves are the most potent source of allelochemicals, however, the toxic metabolites are also distributed in all other plants in various concentration. In the crop fields, at ant given time there are at least more then one plant species growing together. In crop mixture or inter cropping systems, the major plants species are crops. besides, some weeds may also be presented. When the two plant species grow together they interact with each other either inhibiting or stimulating their growth or yield through direct or indirect allelopathic interactions.

Key words: Allelopathic effect, *Solanum nigrum, Pisum sativum, Eleusine coracana* and *Trigonella foenumgraecum*.

INTRODUCTION

Nearly all cultures, from ancient times have used plans as a source of medicine. In many developing countries traditional medicine is still the mainstay of healthcare, and most of the drugs and cures used come from plants. In developed countries too people are tuning to herbal remedies. Besides, modern scientific medicine still depends on plants and the knowledge gained from them, for some essential drugs according to WHO, more than one billion people rely on herbal medicine to some extent. The WHO has listed 21,000 plants worldwide, reported to have a medicinal uses .It also has a rich medicinal plant flora of some species, of which atleast 150 are used commercially for pharmaceutical purposes on a fairly large scale.

Solanum nigrum is a annual herb includes 85 genera and 2200 sps of alternative, sedative, diaphoretic, diuretic, hydragogue and expectorant. Leaves are employed as poultice over rheumatic and gouty joints, also as a remedy in skin diseases. Syrup of it is useful as a cooling drink in fever and to promote perspiration. Leaves are eaten as vegetables for curing stomach aches and back aches. It is also useful in dysentery, fevers and also promotes urination. The plant extract is laxative and improves appetite.

Allelochemicals refer mostly to the secondary metabolites produced by plants and are byproducts of primary metabolic processes (Levin, 1976). They have allelopathic effect on the growth and development of the same plant or neighboring plants, Allelochemicals most often impart plant resistance to insects, nematodes and pathogens. Their release into environment, some may regulate the distribution and vigour of plants. Usually plants come in contact with the allelochemicals in soil and their effect on crop plants may be modified by soil moisture, soil temperature and other soil factors (Patrick and Koch, 1958; Einhelling and Eckrich, 1984). The effects of secondary substances released by these mechanisms can be long lasting (Patric, 1971) or quite transitory (Kimber, 1973) and can ultimately influences practices like fertility, seeding and crop rotations.

The allelopathic effects are selective (Stowe, 1979; Melkania, 1983) and vary with different trees since these plants will vary in the amount of indigenous secondary metabolites and would release different amounts of the phytotoxins. So I was interested in analyzing allelopahic effect of this plant on plants like *Pisum sativum, Eleusine coracana and Trigonella foenumgraecum.*

Allelopathic effect of ageratum conyzoides L, *Cynodon dactylon* (L) *pers, Parthenium hysterophorus* L, *Solanum nigrum* L, were examined on seed germination, seedling growth, total protein content and protein profile on Ankur, Bhatt, Bragg, PK-416, PS-1042 and shilajeet varieties of soybean. Aqueous extracts of weeds showed both inhibitory and stimulatory influence on percent seed germination and seedling growth in different varieties of soybean (virma and Rao. 2006).

Chemical constituent of the non-saponins from *Solanum nigrum* L were studied through HPLC compounds isolated from 60% ethanol extract were identified as 6 methoxy – hydroxycoumarin (1)syringaresinol – 4-0-beta-D-glucopyranoside (2), Pinoresinol-4-0-beta-D-glucopyranoside (3), 3,4-dihydroxybenzoic acid (4), P-hydroxybenzoic acid (5), 3-methoxy-4-hydroxyenzoic acid (6)adenosine (7) [Zhong Yao Cai, 2007]

The major chemical constituent is steroidal alkaloid Solasodine about 2% and other include steroidal alkaloid Viz Solamargins, â-solamargine, solasonine; sterols viz cycloartenol, noscarpesterol, cholesterol and their derivatives (Verbist and monnet 1975; siddiqui *et al*., 1983)

MATERIAL AND METHODS

Collection of Plant Material

Solanum nigrum is an annual herb, cosmopolitan in distribution. It prefers acid, neutral and basic soils. Annual growing to 0.6 m by 0.3 m and it is in flower from July to September. Leaves were identified by its single, alternate, dentate, unicoscate and were collected from wastelands in Egmore. The leaves were washed with tap water and blotted dried and shade dried. The dried material was homogenized to fine powder and stored air tight containers.

Extraction of Plant Material

Weight of dried extract

The fine powdered plant material was weighed (20g) and soaked in ethanol. This was carried out for about 3 to 4 days and the solvent is extracted in saxhlet extraction apparatus. Ethanol solvent is used for extraction, At the end of extraction process the solvent was evaporated and the remaining residue was measured using the formula

$$\%\,\text{Yield} = \frac{\text{Weight of dried powder}}{\text{Weight of dried powder}} \times 100$$

Allelopathic Analysis

The selected fertile soil was autoclaved and allowed to cool and again autoclaved after 24 hours, So that the germinating fungal spores will be killed. Then the sterilized soil was allowed to cool and transferred to small pots. The seeds were

soaked in ethanolic extract of leaves of concentrations ranging from 20 µg, 40µg, 60µg, 80 µg,100µg and one control with ethanol. Seeds of different plants were then transferred to pots.

Estimation of Protein

Protein content is estimated by Bradford (1972) method. This method is based on the ability of proteins to bind to the dye coomassie brilliant blue G-250 and forms a complex whose extinction co-efficient is greater than free dye.

Preparation of Brilliant Blue reagent

10 mg of coomassie brilliant blue G-250 was mixed with 10 ml of 88% phosphoric acid 4.7 ml of absolute alcohol was added. The mixture was diluted to 100 ml with distilled water and stored in a dark bottle.

Preparation of BSA Standard

Bovine serum albumin (100 mg) was dissolved in 100 ml of 0.1N NaoH and concentration ranging from 10-100 µg/ml was made. To the different concentration, 2 ml of coomassie brilliant blue solution was added and blue coloured complex was read in a colorimeter at 595 nm. The values were plotted on to a graph taking concentration on X-axis and optical density on Y-axis and a straight line was obtained.

Preparation of Sample

Seeds of *Pisum sativm*, *Eleusine coracana* and *Trigonella foenumgraecum* from respective concentrations were uprooted, washed, bottled dried and weighed. The weighed sample from various concentrations was ground in mortar and pestle with 80% ethanol and was centrifuged at 10000 Xg for 10 minutes. The supernatant was collected and to 1 ml of the supernatant, 2 ml of coomassie brilliant blue G-250 solution was added and blue coloured complex was read in a colorimeter at 595 nm. The absorbance was plotted onto the BSA standard to estimate the protein content.

Thin layer chromatography

A crude ethanolic extract was spotted on a precoated silica gel TLC plate. The spots were allowed to dry. The plate was developed in a developing tank containing TolueneH e x a n e : Ethanol in 3:3:1 ratio, the chromatogram was developed for about 15 minutes. The Rf values of different coloured spots were calculated.

The Rf values are calculated using the formula

$$\text{Rf value} = \frac{\text{Solute Front}}{\text{Solvent Front}}$$

Phytochemical Analysis

The leaf and fruit extract was tested for secondary metabolites by the following phytochemical test.

Tannin

To the extract, few drops fo 1%Ferric chloride was added and observed for brownish green or blue black colour.

Saponin

2 ml of water was added to the extract and shaken vigorously till persistent froth was observed.

Phlobatannin

To the extract 1% HCL was added and observed for red precipitate.

Flavonoid

To the extract 5 ml of dilute Ammonium solution and concentrated Sulphuric acid was added and observed for yellow colour.

Steroid

2 ml of acetic anhydride and 2 ml of concentrated Sulphuric acid was added to the extract and observed for violet or blue or green colour.

Cardiac glycoside

To the extract 2 ml of Glacial acetic acid 1 drop of Ferric chloride, 1 ml of concentrated Sulphuric acid was added and observed for brown colour formed between 2 layers, Violet ring or Green ring may be present.

Terpenoid

2 ml of chloroform and concentrated Sulphuric acid was added to the extract and observed for reddish colour in interference.

Table 1: Effect of leaf extract on germination

S. No	Plants	Concentrations inµg	Germination rate in days 1	2	3	4	5
1.	*Pisum sativum*	C	-	-	+	+	+
		20	-	-	-	-	-
		40	-	-	-	-	-
		60	-	-	-	-	-
		80	-	-	-	-	-
		100	-	-	-	-	-
2.	*Eleusine coracana*	C	+	+	+	+	+
		20	-	+	+	+	+
		40	-	+	+	+	+
		60	-	-	+	+	+
		80	-	-	-	+	+
		100	-	-	-	-	+
3.	*Trigonella foenungraecum*	C	+	+	+	+	+
		20	+	+	+	+	+
		40	+	+	+	+	+
		60	+	+	+	+	+
		80	-	-	-	+	+
		100	-	-	-	-	+

Table 2: Effect of leaf extract on growth

S No.	Plants	Concentrations in µg	Length of radicle in cms No. of days 3rd	6th	9th
1.	*Pisum sativum*	C	-	2	4
		20	-	-	-
		40	-	-	-
		60	-	-	-
		80	-	-	-
		100	-	-	-
2.	*Eleusine coracana*	C	2	4	6
		20	1	3.5	5
		40	1	3.5	4.5
		60	-	2.5	3
		80	-	2	1.5
		100	-	1	1
3.	*Trigonella foenungraecum*	C	1	5.5	6.5
		20	3	7	9
		40	2	6.2	6.8
		60	2	6	6.5
		80	-	3.5	4
		100	-	2	2.5

Alkaloid

Formaldehyde: concentrated Sulphuric acid (1:10) was prepared and TLC plate was dipped into it and observed for brownspot.

Anthraquinone

10% Hydrochloric acid, few drops of cholorform and ammonium solution were added to the extract and heated in water bath and observed for appearance of pink colour.

Phytosterol

To the extract 2 ml of acetic anhydride and 2 drops of concentrated Sulphuric acid are added and observed for array of colour change.

Carbohydrates

To 0.5 ml of filtrate, Benedicts solution was added and heated in seam bath, and observed for red precipitate.

Phenol

To the extract 5 ml of distilled water and few drops of neutral 5% ferric chloride solution was added and observed for dark green colour.

Phytochemical Testing of bands eluted from TLC

The different bands in TLC were eluted and added with ethanol and are tested for Alkaloid, Flavonoid, Terpenoid, Tannin and Steroid. The tested Plant seeds were soaked in respective bands and then tested for their germination, growth and total protein content

Table 3: Effect of leaf extract on total protein content

Concentrations of extract in µg	MTotal Proein Content mg/g		
	***Pisum* sativum**	***Eleusine* coracana**	***Trigonella* foenungraecum**
C	1	0.97	1.18
20	-	0.91	1.26
40	-	0.83	1.24
60	-	0.80	1.20
80	-	0.74	0.81
100	-	0.62	0.68

Table 4: Phytochemical analysis of leaf and fruit extract

S.No	Secondary Metabolites	Leaf Extract	Fruit Extract
1	Tannin	+	+
2	Phlobatannin	-	-
3	Saponin	-	+
4	Flavanoids	-	-
5	Steroid	-	-
6	Terpenoids	+	+
7	Anthraquinone	-	-
8	Cardiac Glycosides	+	+
9	Phytosterols	+	+
10	Carbohydrates	-	-
11	Phenol	-	-
12	Alkaloid	+	+

Table 5: Rf values of leaf extract

S. No	Bands	Solute front	Solvent front	Rf Value
1	1	2.4	11.8	0.203
2	2	5.2	11.8	0.440
3	3	6.6	11.8	0.559
4	4	8.0	11.8	0.677
5	5	8.6	11.8	0.728
6	6	9.1	11.8	0.771
7	7	11.7	11.8	0.991

Table 6: TLC analysis

Secondary Metabolites	Bands						
	1	2	3	4	5	6	7
Terpenoids	+	-	-	-	-	-	-
Alkaloids	-	+	-	-	-	-	+
Tannin	-	-	+	-	-	-	-
Steroids	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-

RESULTS AND DISCUSSION

Allelopathic effect of leaf ethanolic extract of *Solanum nigrum* was tested on test crops *Pisum sativum, Eleusine coracana* and *Trigonella foenumgraecum* and compared with control. The effect of the extract was tested by observing germination, radical length and total protein content. The Observations have been presented in Table and figure 1(Germination), 2(Radicle length), and 3(Total protein content).

Germination

The test crops in various concentrations of ethanolic extract were observed for germination. In *Pisum sativu,* no germination was observed in all concentrations except control. In *Eleusine coracana, g*ermination was observed only in control for the 1st day followed by 20 µg, 40 µg on 2nd, 60 µg on 3rd day 80 µg on 4th day and 100 µg on 5th day. Germination was gradually delayed from lower to higher concentrations respectively. In *Trigonellla foenumgraecum,* germination was observed in control 20, 40 and 60 µg for the 1st day and 80 & 100 µg for the 4th and 5th day respectively. The observations have been presented in Table 1 and Fig. 1.

The Seed germination showed both inhibitory and stimulatory influence on tested plants. *Pisum sativum* was the most sensitive crop to ethanolic extract of *Solanum nigrum* Phytotoxic effect and inhibitory influence was observed in *Pisum sativum* followed by *Eleusine coracana*. In *Trigonella foenumgraecum,* inhibitory influence was observed only in higher concentrations (80 & 100 µg), whereas in lower concentrations no such influence was observed compared with that of control.

Radicle Length

Radicle length of each test crop were measured and compared with that of control for 6 days (Table 2). No growth was observed under all the concentrations of leaf extract except control in case of *Pisum sativum*.

In *Eleusine coracana* the radicle length was gradually decreased from lower to higher concentrations with that of the control. The results were recorded till 9th day, it was recorded that 4 cm (Control), 3 cm (20 µg), 2.5 cm (40 µg), 2 cm (60 µg), 1.5 cm (80µg) and 1 cm (100 µg). The observations have been presented in Table 2 and Fig. 2.

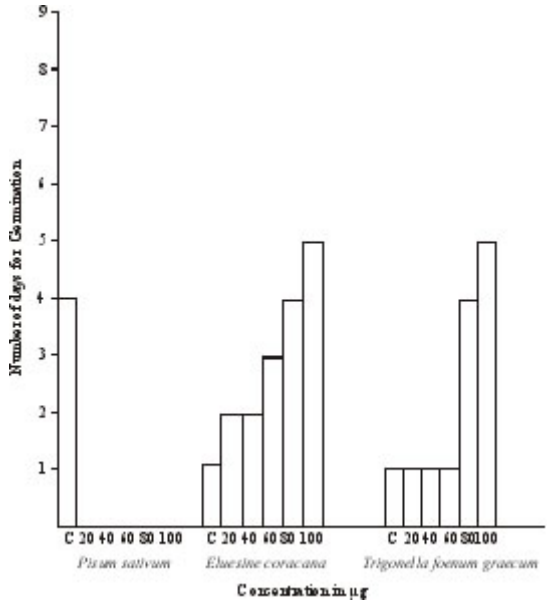

Fig. 1: Effect of leaf extract on germination

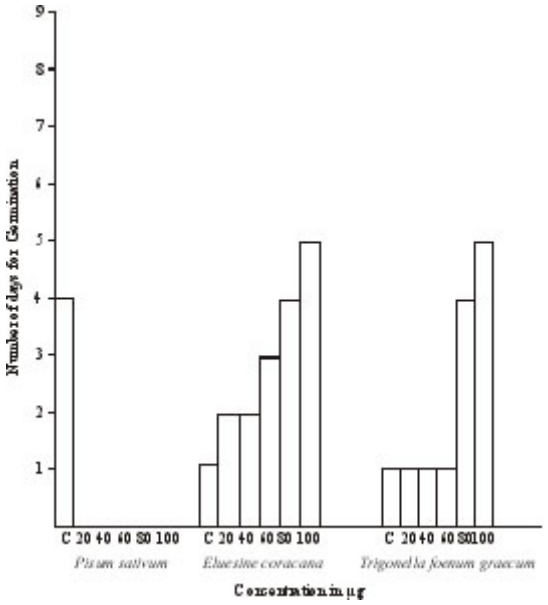

Fig. 2: Effect of leaf extract on radicle length

In *Trigonella foenumgraecum*, the radicle length was more in case of lower concentrations 9 cm (20 µg), 6.8 cm (40 µg) and 6.5 cm (60 µg) with that of control (6.5 cm) where as in higher concentration it was lesser than control (i.e) 4 cm (80 µg) and 2.5 cm (100 µg).

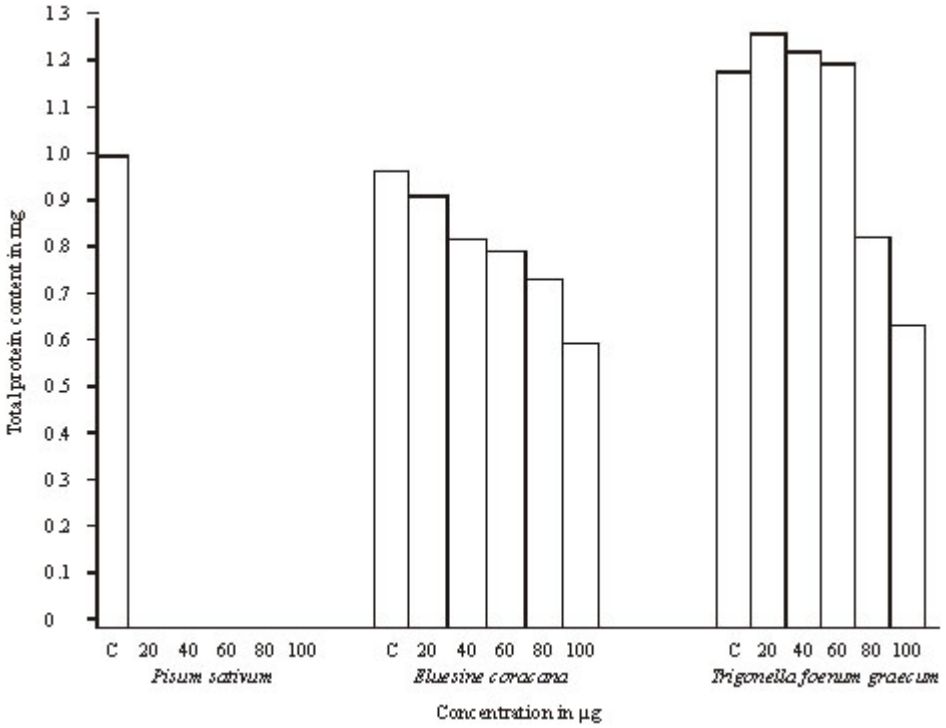

Fig. 3: Effect of leaf extract on total protein content

The plant extract had higher inhibitory influence on the growth of Pisum sativum and was comparatively reduced in case of Eleusine coracana than Pisum sativum. Stimulatory influence on seeding growth was observed in lower concentrations (20 µg, 40 µg and 60 µg) with that of control in case of Trigonella foenungraecum.

The change in concentration and phytochemical activity had modified the soil moisture, soil temperature and other soil factors, which had shown both positive and negative effect on the tested plant.

Total protein content per gram of sample was estimated in all concentrations of the extract by using Brad ford Method (1972). The observations have been recorded in Table and Fig. 3.The blue coloured complex hat produced after adding coomassie brilliant blue G-250 to 1 ml of supernatant was read in calorimeter at 595 nm.

Total protein content in control was 1 mg/g of plant sample in case of Pisum sativum and the results were recorded in Table 3.

In Eleusine coracana the total protein content per gram of sample was estimated as follows; 0.97 mg (Control), 0.91mg (20 µg), 0.83 mg (40 µg), 0.80 mg (60 µg), 0.74 mg (80 µg) and 0.62 mg (100 µg). There was a marked decrease in total protein content from lower to higher concentrations of the extract.

In Trigonella foenungraecum, the total protein content per gram of sample was estimated as follows: 1.18mg (control), 1.26 mg (20 µg), 1.24 mg (40 µg), 1.20 mg (60 µg), 0.81 mg (80 µg) and 0.68 mg (100 µg).It was observed that the total protein content was more in lower concentration (20 µg, 40µg and 60 µg) when compared to that of control, whereas in higher concentrations (80 µg and 100 µg) it was lesser than control.

Phylochemical Analysis

The phytochemical tests were conducted for the leaf and the fruit extract. The secondary metabolites like tannins, terponoids, alkaloids, phytosterols and cardiac glycosides were answered positive for both the leaf and the fruit extract samples, whereas saponins were answered positive only in fruit extract. The observations have been presented in Table 4.

Chromatographic analysis

Using Thin layer silica gel plates that various metabolites were separated as distinct bands. Approximately seven different bands were separated. Their Rf values from Band 1 to 7 were found to be 0.203, 0.440, 0.559, 0.677, 0.728, 0.771 and 0.991 respectively. The Observations have been presented in Table 5

TLC analysis

The different bands separated were individually tested for their phytochemicals. The band 1 answered for terpenoids, bands 2 and 7 answered for alkaloids and band 3 answered for tannin. *Terpenoids* and *Alkaloids* showed negative influence or inhibitory effect on the test crops. The observations have been presented in Table 6.

From the results observed secondary metabolites produced by plants are by-products of primary metabolic process. (Levin, 1976). They have allelopathic effect on the same plant or neighbouring plants. Their effects are selective. The *Solanum nigrum* leaf extracted showed distinct allelopathic effect. Generally leaves are the most poten source of allelochemicals, however, the toxic metabolites are also distributed in all other plants in various concentration.

The secondary compound released from litter are formed by microbes decomposing the litter will be influenced by the type of crop being leached or decomposed type (Putnum and Duke, 1978). Infact litter leaching and decay are the major pathways of the release of allelochemicals from plants. Harbone (1977) proved that higher plants (tree crops) release some phytotoxins into soil, which adversely affect the germination and yield of crops. Such type of crop interactions called phytochemical ecology/ecological biochemistry. Agricultural losses are being experienced by marginal and submarginal farmers, who are concerned about the adverse effect of weeds on cultivated land and standing crops (Bhatt *et al*, 1993).

Some species improve the soil but at the same time some species may cause adverse effect on long-term basis (Gill, 1992; Mughal, 2000). The component plants species in agricultural field depends on the same reserve of growth resources such as light, water and nutrients and hence there will be influence of one component of a system on the performance of the other components as well as system as a whole. These interactions may be positive or negative (Basavaraju and Gururaju, 2000). The balance between these positive or effects determines the overall effects of the interactions. Production of agricultural crops is the main sources of subsistence in India, because 80% population was dependant upon agriculture. Combining of weeds, crops and livestock is in practice since long and getting benefits on sustained basis.

In the crop fields, at ant given time there are at least more then one plant species growing together. In crop mixture or inter cropping systems, the major plants species are crops. Besides, some weeds may also be presented. When the two plant species grow together they interact with each other either inhibiting or stimulating their growth or yield through direct or indirect allelopathic interaction. Among the studies conducted for several species, Baker (1966) reported the *Eucalyptus globulus* produces volatile emanations that inhibit root growth of *Cucumis* species seedlings and also the growth of hypocotyls, but not the roots of *Eucalyptus* seedlings. Singh and Bawa (1982) found leaf leachates of *Eucalyptus globulus* to be inhibitory to seed germination of *Glaucium flavus.* Many other species also reported for allelopathic to plant growth are *Celtis laevigata***,** *Rhododendron albiflorum***,** *Grevillea robusta Quercus falcate***,** *Quercus alba* (Rice, 1974, 1979). *Pinus roxburghii, Cedrus deodara, Quercus leucotrichophora, Myrica esculenta* (Melkania, 1983). Various workers have been reported allelopathic influences on certain tree crops (Saxena and Singh, 1987: Melkania, 1984; Suresh and Vinaya Rai, 1987; Bhatt and Todaria, 1990) for the different parts of the country. Kaletha *et al* (1996) also done the similar study for aqueous extract of leaves and bark of *Grewia oppositifolia, Ficus roxburghii, Bauhinia variegata* and *Kydia calycina* on test crops *Echinochloa frumentacea, Eleusine coracana, Zea mays, Vigna unguiculata* and *Glycine max* and found that the bark and leaf aqueous extracts of tree species were most toxic to food crops. Similarly Bhatt and Chauhan (2000) found allelopathic influenced of *Quercus* speciies on *Triticum aestivum, Brassica campestris* and *Lens culinaria* and found leaf and bark extract suppressed the germination, plumule and radicle length of all food crops.

As literature revealed that numerous plant species released organic compounds in the soil. Although these toxic substance may be useful to control weed, insect nematodes and disease pathogens. Crop-rotation in monoculture soil sickness often occurs due to imbalance of soil micro-organism leads accumulation of soil toxins, mineral deficiency or abnormal soil pH which reduced soil productivity. Some time application of nutrient also found suitable to reduced phytotoxic effect. Besides that the weeds provides harmful effects, should be lopped, at the time of growing crops which will reduced the toxic effects from the place.

REFERENCES

1. Ahmad, A; Pillai K.K. Najmik, A.K; Ahamad, s; Pal, S.N; Balani, D.K., Evaluation of hepatoprotective potential of Jigrine post treatment against thioacetamide included hepatic damage. *Journal of Ethnopharmacology*, V. **79**(1): 35-41 (2002).
2. Basavaraju, TB & R Gururaja. Tree-crop interactions in agroforestry systems: A Brief Review, *Indian Forester* **126** (11): 1155-116 (2000).
3. Bhatt BP & NP Todaria. Studies on the allelopathic effects of some agroforestry tree crops of Garhwal *Himalaya. Agroforestry system* **12**: 251-255 (1990).
4. Bhatt, BP, DS Chauhan & NP Todaria. Allelopathic effects of *Quercus* species on

crops of Garhwal Himalaya. *Allelopathy Journal* **7**(2) 265-272 (2000).

5. Bhatt, BP & DS Chauhan & NP Todaria., Phytotoxic effects of tree crops on germination and radicle extension of some food crops. *Tropical Science* **33**: 69-73 (1993).
6. Einhellig, FA & DC Eckrich., Interactions of temperature and ferulic acid stress on grain sorghum and soybean. *Journal of Chemical Ecology* **10**: 161-170 (1984).
7. Gill, AS., *Allelophathy in agrofoestry "Allelopathy in Agroecosystem"* Tauro P & SS Narwal (Eds), Indaian Society of Allelopoathy, Haryana Agriculture University, Hissar, India, 9 (1992).
8. Harbore, JB., *Introduction to Ecological Biochemistry.* Academic Press, New York (1977).
9. Jainu, M: Shyamala Devi, C.S, Antiulcerogenic and ulcer healing effects of *Solanum nigrum* (L) on experimental ulcer models: Possible mechanism for the inhibition of acid formation. *Journal of Ethanopharmacology*, **104**(1-2): 156-163 (Eng : 44 ref.) (2006).
10. Ji YB, Gao SY, Ji CF, Zoux., Induction of apoptosis in HepG(2) cells by solanine and Bcl-2 protein. *Journal of Ethnopharmacol,* Epub ahead of print (2007).
11. Joshera : A.J; Vinodh, D.R; Pravina K; Suja, R.S : Goudar, K.S; Pande, C.B: Amit, A., Lipotropic activity of natchol, a polyherbal formulation in albino wistar rats. *Journal of Natural Remedies*, **6**(2): 221-226 (2007).
12. Kapoor, B.B.S; Khatri, J.S; Bhumika; Ranga. P, New source of ascorbic acid. *Journal of phytological research.* **17**(1): 111-112 (2004).
13. Kulkarnai, D.S. Manisha., To study the effect of Hridyarnava – Ras in ischaemic heart disease. *Deerghayu International.* **22**(88): 109-113 (2006).
14. LiJ, LiQ, Feng T, Zhang T, Lik, Zhao R, Hanz, Gao D, Antitumor activity of crude polysacchardies isolated from *solanum nigum* L on U 14 cervical carcinoma bearing mice. *Journal of phytotherapia*. **21**(9): 832- 40 (2007).
15. Lim KT., Glycoprotein isolated from *Solanum nigrum* L, Kills HT-29 cells through apoptosis **8**(2): 215-26 (2005).
16. Melkania, NP., Allelopathy and its significance on production of agroforestry plants association. *Proceeding workshop of Agroforestry for Rural Needs.* Feb. 22-26, 1986, New Delhi. ISTS, Solan, pp. 21-224 (1986).
17. Mughal, AH., Allelopathic effect of leaf extract of *Morus alba* L. on germination and seeding growth of some pulses. *Range Management and Agroforestry* **21**(2): 164-169 (2000).
18. Patrick, ZA & LW Koch., Inhibition of respiration, germination and growth by substances arising during the decomposition of certain plant residues in soil. *Canadian Journal of Botany* **36**: 621-647 (1958).
19. Putnum, AR & WB Duke., Allelopathy in agroecosystems *Ann. Rev. Phytopathol.* 16: 431-451 Rice, EL. 1974. Some roles of allelopathic compounds in plant communities. *Biochemical Systematic and Ecology.* **5**: 201-206 (1978).
20. Ravi, C.S; Sreeramu. B.S; Gowda. A.P.M., Genetic variability parameters for alkaloid yield contributing characters in makoi (*Solanum nigrum* Linn.) genotypes. *Biomed*, **1**(3): 223-227 (2006)
21. Rice, EL., Alleopathy-An update. *Botanical Review,* **45**: 15-109 (1979).
22. Stowe, LG., Allelopathy and its influence on te distribution of plants in an Illinois old field. *Journal of Ecology* **67**: 1065-1085 (1979).
23. Suresh, KK & RS Vinaya Rai., Studies on the allelopathic effects of some agroforestry tree crops. *The International Tree Crops Journal* **4**: 109-115 (1987).
24. Verbist; J.F. and Monnet R., plants medicinales et phytotherapic plant Med. *Phytotherapy.* **9**: 59 (1975).
25. Verma M,Rao PB., Allelopathic effect of four weed species extracts on germination,growth and protein in different varieties of *Glycine max* (L). **27**(3): 571-577 (2006).
26. Wang LY, Wang NL, Yao XS., non saponins from *Solanum nigrum L. Journal of Zhong Yao cai.* **30**(7): 792-794 (2007).