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Allelopathic Interaction of Spinach (*Spinacia oleracea* L.) with *Trigonella* and *Coriandrum sativum*

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Abstract

The negative allelopathic interaction of spinach with other crop plants (fenugreek and coriander) was studied under laboratory conditions. The seeds of *Spinacia oleracea* were grown in combinations with trigonella and coriander seeds in the seed extracts of all three plants in dilutions of 1:10, 1:20 and 1:50 and in distilled water as control. The seedling growth were studied in terms of radicle length, dry weight, total nitrogen and carbohydrate contents at 3rd and 7th day immediately after radicle emergence. It was found that the growth of spinach was inhibited by *Trigonella* and coriander seedlings. In their own seed extract the growth inhibition was not more as compared to distilled water grown control and with *Trigonella* or coriander seedlings. The total nitrogen and carbohydrate contents were lower in spinach seedlings grown in the seed extracts of *Trigonella* and *Coriandrum* both, as compared to control.

Key Words: Allelopathy, Coriander, Spinach, *Trigonella* and seed extract

Introduction

As we proceed into the 21st century and year beyond 2080, the world demand for food will increase because growth in population will exceed growth of food crops. The inequitable distribution of the world's population, wealth and agricultural production are major disappointments for the hungry world. There is a loss of yield due to some insects, weeds diseases and rodents, but some of these are phytotoxic resulting in a yield loss of crops. Negative alleopathic interactions are common in crops growing in the same season. The residual parts of these crops were negatively interacting with the other crops growing for rotation to suppress the growth. A number of crops have been known to exhibit allelopathic interaction with other crops growing in succession or simultaneously, or may even exhibit autotoxicity. The principal cause of crop autotoxicity includes the deliberate leaving of crop residue or old roots in soil that release phytotoxin. These phytotoxins may directly affect the succeeding crops causing microbial imbalance, change in organic matter of soil, increase in ion leakage, disturbed nutrient uptake and immobilization [1]. The residues of preceding crops affect the performance of succeeding crops through release of allelochemicals [2]. Kalburtji and Gagianas [3] reported the poor performance of cotton following the sugar beet crop due to allelochemicals released from the residue of sugar beet leading to serious repression of the quality and quantity of crop yields.

Spinach (*Spinacia oleracea* L.) is an edible flowering plant in the family of Amaranthaceae. It is native to central and southwestern Asia. Spinach has a high nutritional value and is extremely rich in antioxidants, especially when fresh, steamed, or quickly boiled. It is a rich source of vitamin A (and especially high in lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, betaine, iron, vitamin B_2, calcium, potassium, vitamin B_6, folic acid, copper, protein, phosphorus, zinc, niacin, selenium and omega-3 fatty acids. Recently, opioid peptides called rubiscolins have also been found in spinach. Polyglutamyl folate (Vitamin B_9 or folic acid) is a vital constituent of cells and spinach is a good source of folic acid, but boiling spinach can more than halve the level of folate left in the spinach, though microwaving does not affect folate content [4]. Vitamin B_9 was first isolated from spinach in 1941 [5]. The *Spinacia oleracea* L. interacts with *Trigonella foenum-graecum* and *Coriandrum sativum* equally and there is growth inhibition in spinach in the presence of these two crops. These crops are raised together in several fields. Besides, all the *Spinacea* and *Coriandrum* are reported to produce allelochemicals namely- polyphenolics, quinolizidine alkaloids and linalool or α-pinene, etc, respectively [6, 7]. Coriander and Trigonella are reported to have allelopathic interaction with each other in lab as well as in field [7]. In the present study the interaction between the spinach with *Trigonella* and coriander was studied in seedling stage. The seedling growth, total nitrogen and sugar of seedlings and the leachates were studied for analysis of the inhibitory action of these two crops on the initial growth of spinach plant.

Materials and Methods

Plant material

Fresh seeds of *Spinacia oleracea* L., *Trigonella foenum graecum* L. and *Coriandrum sativum* L. were procured from IARI, New Delhi.

Vigour analysis of seeds

The embryo from seeds were taken for vigour analysis and kept in $TzCl_2$ (Tetrazolium chloride) for approx. three hours. The colour of the living material turned pinkish red indicating that seeds to be 100% viable.

Growth study

The seeds of *Spinacia, Trigonella* and *Coriandrum* were imbibed in distilled water after surface sterilization with 0.1% $HgCl_2$, The imbibed seeds of *Spinacia* were germinated in Petri plates on four layers of Whatman filter paper moistened with the imbibed seed extracts of *Spinacia, Trigonella* and *Coriandrum* separately, in three different dilutions 1:10, 1:20 and 1:50, respectively. 1 indicates 1 gram seeds per 100 ml distilled water were used as stock. Distilled water grown seedlings were used as positive control. Seedlings after radicle emergence were used to the study the negative allelopathic interaction. The radicle length and dry weight were recorded on 3rd and 7th day after radicle emergence. The leachates were also collected and the pH was recorded at same day's interval.

Biochemical analysis

Total nitrogen estimation

Total nitrogen was estimated at 3rd and 7th day after radicle emergence in seedlings by the method of Snell and Snell [9]. In this method all nitrogen present in dry matter was converted into ammonium sulphate. This ammonium sulphate gives red colour with Nessler's reagent, which was read at 445 nm on a Shimadzu#1800 spectrophotometer. Nitrogen was estimated with the help of standard curve made by taking different concentrations of standard ammonium sulphate and expressed as mg g^{-1} dw.

Total carbohydrate estimation

Total carbohydrate was estimated by the method of Hedge and Hofriter [10]. The dry organic matter was reacted with 2.5 N HCl on water bath for 3 hours to convert into 5 hydroxy methyl furfural. This gives green colour with Anthrone reagent. The carbohydrate was estimated by the standard curve prepared by different concentrations of glucose and expressed as mg g^{-1} dw.

Results and Discussion

Growth Analysis

Growth of spinach plants in their own seed extract and in the seed extracts of *Trigonella* and *Coriandrum* showed growth inhibition at 3rd day and at 7th day after radicle emergence (Fig. 1). The inhibition in the radicle length in the spinach extract was higher in 1:10 dilution, while it gradually decreased in 1:20 dilution and in 1:50 dilution there was no growth inhibition, the value was almost same as in distilled water grown control. This indicates that the spinach seed extract has negative interaction with the germination of their own seed extract but when concentrated and at higher dilution (1:50) their inhibitory action was reduced to zero (Fig. 2). In *Trigonella* seed extract the spinach radicle growth showed inhibition in all dilutions and gradually decreased in 1:10 to 1:50 dilution at both 3rd and 7th day. *Trigonella* showed negative interaction with spinach plant and inhibited the growth of radicle (Fig. 4). In coriander seed extract the growth of spinach seedlings greatly reduced from 3rd day to 7th day. The growth inhibition of radicle was more in 1:10 dilution of coriander seed extract and gradually decreased but at 1:50 dilution the growth inhibition was higher as compared to 1:50 dilution of *Trigonella* and spinach seed extracts (Fig. 6). The growth inhibition of spinach plants in the presence of seed extracts of *Trigonella*, Spinach and *Coriandrum* was clearly recorded in present study, thereby indicating that all the plants negatively interact with spinach. However, these plants were not analysed for the production of any allelochemicals. The dry weight of the above crop was also analysed and it followed the same pattern as the length of the radicle. The reduction in dry weight of spinach seedlings was more in coriander seed extracts as compared to spinach and *Trigonella* seed extracts from 3rd to 7th day after radicle emergence (Fig. 3, 5 and 7). In spinach seed extracts however, the dry weight was almost the same as in distilled water grown control at 1:50 dilution. The reduction in dry weights also confirmed the allelopathic potential of the crop.

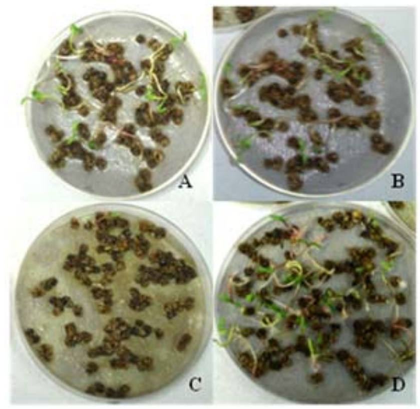

Fig: 1 Seventh day old spinach seedlings growing in the (at 1:10 dilution) seed extract of a) spinach, b) trigonella, c) coriander and d) seedlings grown in distilled water (control)

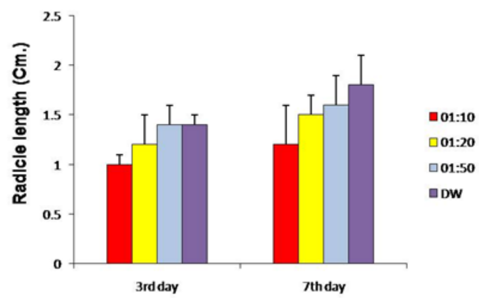

Fig: 2 Spinach seedling growth in spinach seed extracts

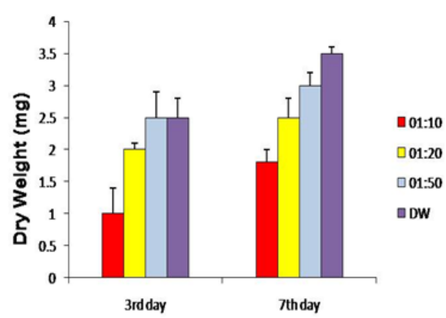

Fig:3 Dry weight of spinach seedling in spinach seed extracts

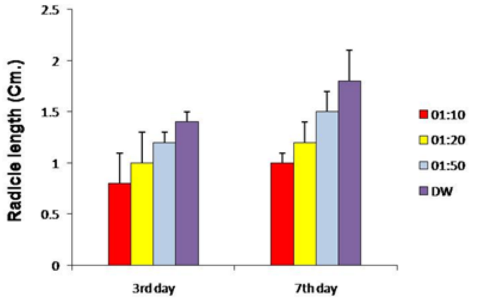

Fig: 4 Spinach seedling growth in trigonella seed extracts

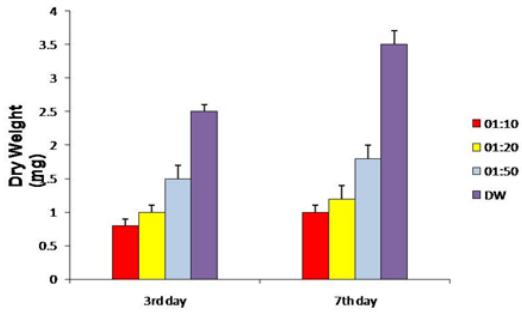

Fig: 5 Dry weight of spinach seedling in trigonella seed extracts

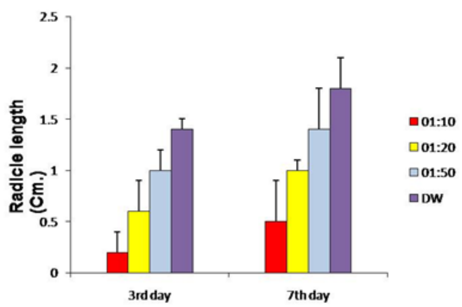

Fig: 6 Spinach seedling growth in coriander seed extracts

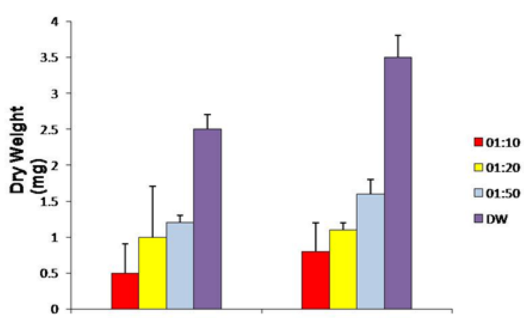

Fig: 7 Dry weight of spinach seedling in coriander seed extracts

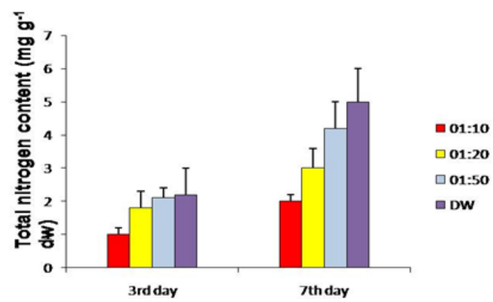

Fig: 8 Total nitrogen content of spinach seedling grown in spinach seed extracts

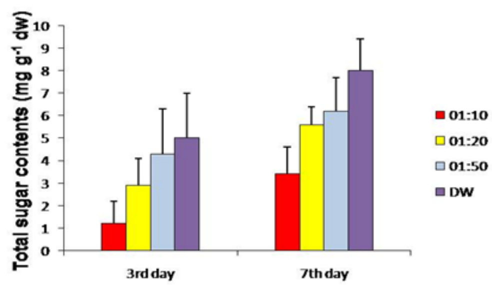

Fig: 9 Total sugar contents of spinach seedling grown in spinach seed extracts

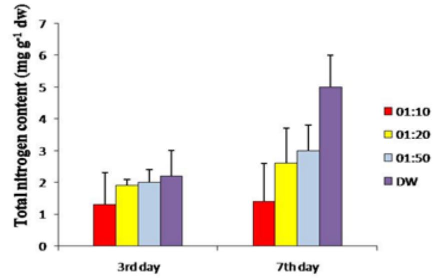

Fig:10 Total nitrogen content of spinach seedling grown in trigonella seed extracts

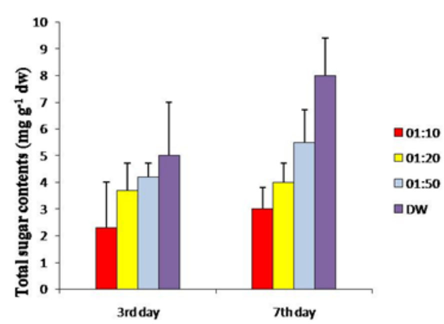

Fig: 11 Total sugar contents of spinach seedling grown in trigonella seed extracts

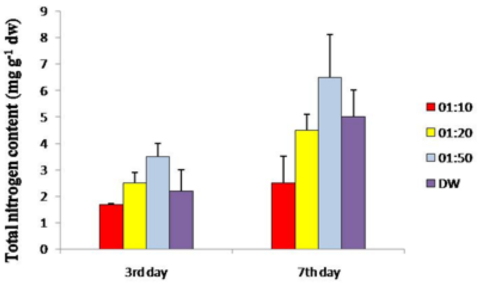

Fig: 12 Total nitrogen content of spinach seedling grown in coriander seed extracts

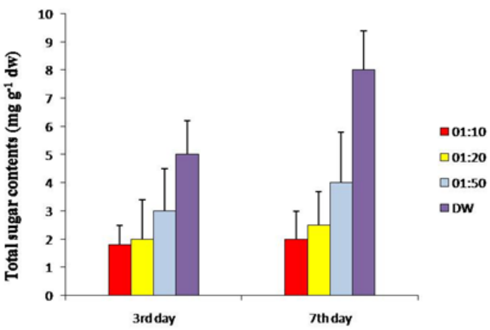

Fig: 13 Total sugar contents of spinach seedling grown in coriander seed extracts

Biochemical Analysis

Total nitrogen content was calculated in spinach seedlings grown in their own seed extract besides fenugreek and coriander seed extracts along with distilled water as control. It increased from 2.2 to 5 mg g^{-1} dw from 3rd to 7th day in distilled water (Fig. 8, 10 and 12). In seedlings grown on spinach and *Trigonella* seed extracts the nitrogen content was lower in 1:10 dilution and gradually increased up to 1:50 dilution, though remained lower than control (Fig. 8 and 10). However, in seedlings grown on coriander seed extracts the total nitrogen content increased as compared to distilled water grown seedlings in almost all dilutions and this suggests presence of nitrogenous compounds in the available form in the extract with growth inhibitory effect. This is in contradiction with earlier reports suggesting non-nitrogenous terpenes to be present in the extracts of coriander as allelochemicals [11, 12]. Higher nitrogen content in coriander extract treated spinach seedlings than in distilled water treated control spinach seedlings, with very poor growth potential, warns for the presence of nitrogenous allelochemicals in this extract.

Total carbohydrate content followed the same pattern of maximum inhibition in 1:10 dilution and minimum inhibition in 1:50 dilution of all the extracts studied compared to distilled water grown control (Fig. 9, 11 and 13).

The inhibition due to *Spinacia* and *Trigonella* seed extracts was comparatively of the same order, whereas due to *Coriandrum* seed extracts, the inhibition was of the highest order under all dilutions. This indicates a potential negative allelopathic interaction of *Spinacia* with studied seed extracts in decreasing order as *Coriandrum*> *Trigonella*> *Spinacia*.

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