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Screening of Alleopathic Activity of the Leaves of *Nicotiana plumbaginifolia* Viv. on Some Selected Crops in Aligarh, Uttar Pradesh, India

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Abstract: *Nicotiana plumbaginifolia* Viv. is one of the fastest growing weed. It belongs to family Solanaceae. Sesquiterpenoid phytoalexins have been reported to be the chief components of secondary metabolites this family produces which are responsible for allelopathy. Every year due to its lush growth in the crop fields, *N. plumbaginifolia* as a weed leads to the destruction of thousand tons of crops around the globe. In the present study allelopathic effect of its leaf extract (2%, 6% and 10 %) on percent seed germination of 4 selected local crops of Aligarh (*Pisum sativum* L, *Raphanus sativus* L, *Triticum aestivum* L and *Oryza sativa* L), on its own seeds and its effects on the soil (control soil, rhizosphere soil of *Nicotiana* and leached soils) was studied. After eight days of incubation at room temperature, it was found that among the four plants *Oryza sativa* was most affected with zero percent seed germination with 2, 6 and 10 % of the leaf extract. Maximum percent of seed germination (80±10) was recorded with *Pisum sativum* at 2%. *Nicotiana* leaf extract was found autotoxic to its own seeds at 6 and 8% respectively. After 10 days of treatment, an increment of pH from control soil (pH: 7.10) to rhizosphere soil of the *Nicotiana* (pH: 7.28) was reported. Organic carbon, potassium and nitrogen also follow the same trend whereas the EC (0.44%) and P (13Kg/hectare) were found the maximum in leaf leached soil.

Keywords: Allelopathy, Leaf Extract, *Nicotiana*, Seed Germination

1. Introduction

The term allelopathy was first described by Molisch [1], as a mean of beneficial and detrimental chemical interaction among plants, including microorganisms. Based on the Molisch concept, Rice [2] defined allelopathy as any direct or indirect positive or negative effect of one plant on the other (including the microbes) through the release of chemicals (allelochemicals) into the environment. Allelochemicals are nothing but secondary metabolites that are primarily produced from therapeutic and aromatic plants [3]; the following have been recognized-alkaloids, glycosides, phenolic acids, flavonoids, terpenoids, glucosinolates and coumarins. These chemical substances (Phytotoxic) are known to be exuded by plants to decrease sprouting or growth of the other plants; allelopathic effects of these compounds are often noted to happen early in the life cycle, causing inhibition of seed germination and seedling development. These compounds present a wide extent of mechanisms of action and interpretations of mechanisms of performance are complicated by the fact that particular compounds can have numerous phytotoxic effects [4].

Nicotiana plumbaginifolia Viv. is one of the most abundant species of the genus *Nicotiana* of the family Solanaceae [5]. Waseem and Siddiqui [6] have reported detailed allelopathic potential of Solanaceae plants. Common name Tex-Mex Tobacco is an annual herb grows 1-3 ft tall and the stem is slender. The leaves at the base are obvate or oblanceolate, 15-23 cm long; the lower ones are round-ovate, without a stalk, the margin is wavy, pointed, usually twisted, with the base clutching the stem. The leaves of upper stem are reduced, lance-like to linear-lanceolate with margin

intensely wavy. The inflorescence is a false raceme possessing a 3-7 mm long stalk. Sepals are unequal, 4-8 mm long, linear. Flowers bear a long thin flower-tube, sharply splaying open into petals which are ovate, acute. The tube is pale green or purplish, long and slender, 3.5-4.5 cm long, 1.5-2 mm wide. Tex-Mex Tobacco grows worldwide, however, is native to Mexico, parts of the Caribbean, and western and southern South America, and has been naturalized in the state of Florida [5].

A survey of Aligarh district of Uttar Pradesh, India confirms that the plant is mainly found in moist soil along the roadside, shades of buildings and in crop fields as a weed.

N. plumbaginifolia is a common weed rich in allelochemicals [7]. It synthesizes a number of secondary metabolites of phenol, tannins and polyphenol classes, mostly as glycosides The main polyphenols found in the plant are chlorogenic acid (3-caffeoylquinic acid) and rutin (quercitin- 3-rhamnosidoglucoside). Other polyphenols stated are quercitin, isoquerciterin, kaempferol glycosides, quinic acid, shikimic acid and scopoletin (7-hydroxy-6-methoxy coumarin) [8]. It is also rich in alkaloids in the form of pyridyl-pyrrolidines and pyridyl-piperidines; the chief alkaloids are nicotine, nornicotine, N-acetylnornicotine, anabasine and anatabine [9]. Furthermore, the same author has addressed the reports of N-carbethoxynornicotine found in cell-suspension cultures of *N. plumbaginifolia* as a metabolite of nornicotine [9]. As its allelochemicals lead to the destruction of the crops, the current investigation was attempted to assess the allelopathic potential of the leaf extracts of *N. plumbaginifolia* on 4 different selected cultivated crops and on its own plants (auto-toxicity) at *in vitro* followed by its effect via rhizosphere soil experiment *in vivo*.

2. Materials and Methods

2.1. Collection of the Plant Material

The leaves of *Nicotiana plumbaginifolia* Viv. were freshly collected from many sites in Aligarh, Uttar Pradesh (27°, 29°, to 28°, 10° north latitude and 77°, 29° to 78°, 38° east longitude), at a distance of about 126 Km from India's capital New Delhi. Seeds of the 4 different plants; *Pisum sativum*, *Raphanus sativus*, *Triticum aestivum* and *Oryza sativa,* were purchased from NBPGR, New Delhi, India.

2.2. Preparation of Leaf Extract

The leaf extract from the collected leaves of Nicotiana was prepared using the protocol of Netsere and Mendesil [10] with desired modifications. The osmolality of extract was measured with vapour pressure osmometer (VAPRO 5520). It varied between 104–108 m mol kg-1 that did not induce any osmotic effect, i.e. exosmosis in our test plant. The leaf extract was filtered through Buchner funnel with Whatman filter paper no.1 and was stored at room temperature under the dark condition for 24 hours for further use. After the said time different concentrations (2%, 6% and10%) of the leaf extract were prepared by adding double distilled water.

2.3. Assessment of the Allelopathic Effect of the Leaf Extracts of N. plumbaginifolia

To assess the allelopathic effect of the leaf of *N. plumbaginifolia* on the seeds of 4 different plants, following parameters were studied:

1. Effect of the leaf extract of *N. plumbaginifolia* on percent seed germination of the selected plant seeds *in vitro.*
2. Effect of the leaf extract of *N. plumbaginifolia* on the soil *in vivo*.

2.4. Effect of the Leaf Extract of N. plumbaginifolia on Seed Germination of the Selected Plant Seeds (in Vitro)

2.4.1. Germination Assay

Surface sterilization of seeds: Ten seeds of each plant in triplicate were sterilized with 0.1 % $HgCl_2$ solution (0.5g HgClCs powder was dissolved in 500 ml distilled water) for 5 sec [11]. The surface sterilized seeds were washed with sterilized distilled water in laminar air flow (Microfilt, India) 4-5 times before using for the germination assay.

Seeding of the seeds in Petri plates for germination: Three concentrations (2%, 6% and 10%) of the leaf extract were used to check the allelopathic effect of *N. plumbaginifolia* on the sterilized seeds of 4 plants. In control, distilled water was added. The seeds were aseptically planted in the sterilized Petri-plates (Borosil) with germination paper. All the three concentrations of the N. plumbaginifolia leaf extract were applied to the seeds of each plate (triplicate) except the control. All the plates were incubated at room temperature for 8 days and each plate was watered (10ml) regularly under aseptic conditions. After the 8th day, the percent germination of the seeds, average (triplicate) and standard deviations were calculated using one-way analysis of variance (ANOVA).

2.4.2. Effect of the Leaf Extract of N. plumbaginifolia on the Soil in Vivo

The leaf extract of the *N. plumbaginifolia* (10%) were mixed with plant-free soil and its physicochemical analysis was carried out along with the rhizosphere soil of *Nicotiana,* garden soil and normal soil (*N. plumbaginifolia* free soil) to check the level of allelochemical effect of the leaf extract of the *N. plumbaginifolia* on the soil. So, following key physicochemical characteristic of the soil (pH, electrical conductivity, organic carbon percentage; N, P, and K content) were analyzed.

2.4.3. Auto Toxicity of the Nicotiana

Allelopathic effect of the *Nicotiana* leaf extract on its own seeds was also checked. Similar to other seeds treatment, *Nicotiana* seeds were also treated with 2%, 6% and 10% of the *Nicotiana* leaf extract and percent germination was determined along with the control.

2.5. Statistical Analysis

All the experiments were performed in triplicates. One way analysis of variance (ANOVA) from SPSS version 16.0

was used to test the significant difference of all the data recorded in the studies. The data are presented in the form of mean with standard deviation and considering p values < 0.05 as significant. All results were statistically analyzed through Duncann Multiple Range Test (DMRT).

3. Results and Discussions

3.1. Assessment of the Allelopathic Effect of the Leaf Extracts of N. plumbaginifolia

After eight days of incubation at room temperature the percent germination was calculated. The details of the percent seed germination plant wise are given below:

1. *Pisum sativum*: The seeds of this plant showed normal germination at all concentrations with respect to control except for the 10% concentration where only 15±5% germination was recorded. (Figure 1).
2. *Raphanus sativus*: This plant shows normal germination percentage in control, but at 6%, 100 percent inhibition (0.00±0.00 %) of seed germination was observed (Figure 1).
3. *Triticum aestivum*: Strong allelopathic effect of *Nicotiana plumbaginifolia* leaf extract was recorded in this plant at 2%, 6% and 10% concentration and 0.00±0.00 % germination was recorded (Figure 1).
4. *Oryza sativa*: In 2%, 6% and 10% concentrations, seeds showed the complete inhibition of seed germination (0.00±0.00 % germination) hence strong allelopathic effect was documented (Figure 1).

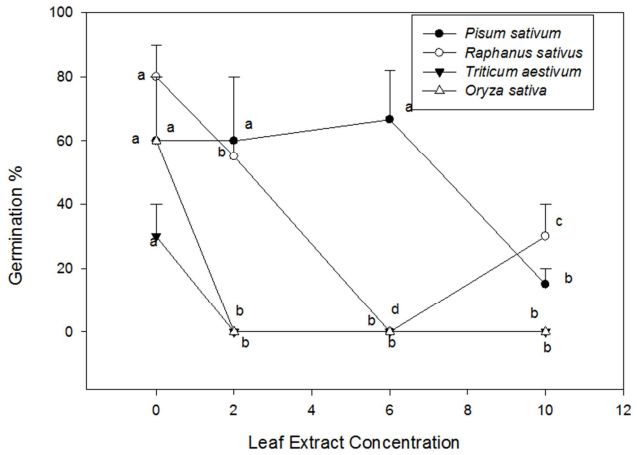

Figure 1. *Effect of leaf extract of N. plumbaginifolia on seed germination of Pisum sativum, Raphanus sativus, Triticum aestivum and Oryza sativa. Significance difference at p<0.05 represented by different subscript symbols along a curve among themselves applying DMRT, vertical bars represent standard deviation.*

3.2. Effect of the Leaf Extract of N. plumbaginifolia on the Soil in Vivo

After 10 days of the treatment the rhizosphere soil of *N. plumbaginifolia* along with garden soil and normal soil were analyzed. The physicochemical properties of a soil sample such as pH, electrical conductivity, organic carbon content, phosphorus (P), potassium (K), and nitrogen (N), (Table 1) of the soils (control soil, leaf leached soil and rhizosphere soil of *Nicotiana*) reported the increment of pH from Control soil (pH: 7.10) to rhizosphere soil of the Nicotiana (pH: 7.28). Organic carbon, P and N also follows the same trend whereas the EC (0.49%) and P (13Kg/hectare) were found the maximum in leaf leached soil.

Table 1. *Physiochemical analysis of the soils.*

S. No.	Parameter	Type of soil		
		Control	Leaf-leached	Rhizosphere
1	pH	7.10	7.10	7.28
2	EC(misa cm)	0.26	0.49	0.21
3	Organic carbon	0.24	0.78	1.05
4	N(Kg/hectar)	168.00	546.00	735.00
5	P(Kg/hectar)	11.84	13.00	12.94
6	K(Kg/hectar)	651.65	683.34	1017.08

3.3. Auto Toxicity of the Nicotiana

The leaf extract at 2% do not show much effect on the percentage of the seed germination as compared to control whereas increasing concentration of the leaf extract lead to inhibition of seed growth and no seed germination was recorded at 8% (Figure 2). The results (Figure 2) show that *Nicotiana* does possess the auto-toxic activity.

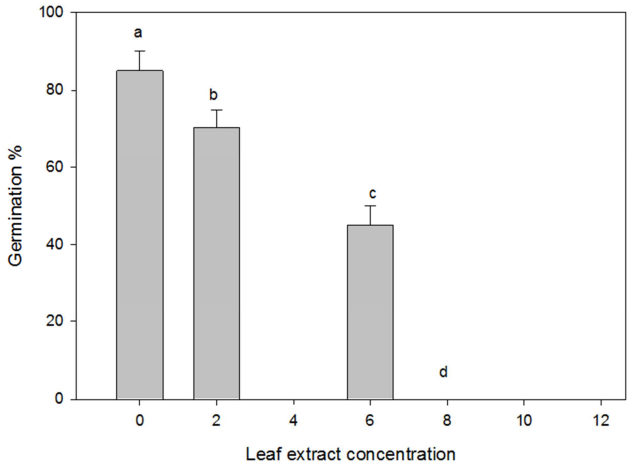

Figure 2. *Autotoxic activity of leaf extract of N. plumbaginifolia Viv. on germination percentage. Significance difference at p<0.05 represented by different subscript symbols along a bar among themselves applying DMRT, vertical bars represent standard deviation.*

The present study documented the allelopathic potential of *Nicotiana* leaf extracts on the percent seed germination of the four selected crops (*Pisum sativum*, *Raphanus sativus*, *Triticum aestivum* and *Oryza sativa*). Further, the physicochemical properties of soil, the process of leaf exudation, leaf leachates of the *Nicotiana* are very important. By this process numerous secondary metabolites are released from the leaf parts, they altered the physicochemical properties of soil, and this altered soil is due to secretion of allelochemicals, which enhance the constituent of soil by using the mechanism of allelopathy. In the previous studies [7], shoot extract of the *Nicotiana plumbaginifolia* Viv. was used to assess the level of allelopathic effect on the seed

germination and seedling growth of *Zea mays*. They reported a delay in seed germination and the delay was proportional to the concentration of leachate [7]. Our result is in agreement with them, we reported zero percent seed germination in case of *Triticum aestivum* and *Oryza sativa* at all the three (2%, 6% and 10%) concentrations. Leachate was inhibitory in its higher concentration as reported by Singh et al. [12] Higher concentrations of allelochemicals influence the physiological processes during germination. Higher concentrations of alkaloids cause slowing down the utilization of food reserves [13]. A possible indirect association between lower germination rate and allelopathic inhibition may be the result of inhibition of water uptake [14] and modification in the synthesis/ activity of gibberellic acid (GA) [15]. Physicochemical properties of soil of *N. plumbaginifolia* is altered during release of some secondary metabolites, allelochemicals by the process of leaf exudation, volatilization, decomposition of residues and rhizosphere soil also shows high rate of physicochemical properties like electrical conductivity, pH; organic carbon, N, P and K content as compared to normal soil and control soil. Allelochemicals are capable of alleviating the physiochemical properties of soil [16].

4. Conclusion

Based on the present study it will be wise to conclude the leaf extract of the *N. plumbaginifolia* does possess allelopathic effect. Besides affecting the other plants, this plant also reported autotoxic effect on its own species. *Nicotiana* leaf produces allelochemical(s) and leads to an increase in the contents of organic carbon and nitrogen in the rhizosphere soil of *Nicotiana* compared to that of control and leaf leached soil. Due to its strong allelopathic potential *Nicotiana* may lead to huge loss of agricultural production per annum if not handled properly.

Declaration

The authors do not have any conflict of interest.

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