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SEARCH FOR ALLELOPATHIC POTENTIAL OF LEAVES OF *SHOREA ROBUSTA* GAERTN.F

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ABSTRACT

Sal forests generally have poor undergrowth. This might be due to release of certain allelochemicals from plant parts of *Shorea robusta* by leaching, exudation, volatilization and decomposition. The present study is maiden attempt to evaluate the nature of allelopathy. For this, leaf leachet of *Shorea robusta* was analysed to find out its effect on seed germination of *Eupatorium odoratum* (associated weed) and *Brassica campestris* (crop).Initial result indicates sharp decrease in seed germination of both plants. Moreover,a characteristic inverse correlation between mitotic index and leachate concentration. was observed The above findings point to the strong inhibitory effect of phytochemicals of *Shorea robusta* leaf leachet. An overall growth parameters of its neighbouring plant species in sal forest ecosystem in both dry (laentic) and biomass rich location.

Keywords: allelopathy. *Shorea robusta*(Sal), leaf leachet

Introduction

Allelopathy is the study of the various chemical compounds produced by certain plants to help improve their chance of survival by negatively affecting the survival reproduction of other plant species and microorganism. Molisch described the field as "Biochemicals interaction between all types of plant including microorganism (Rice,1974)

Allelopathy is defined as a direct or indirect harmful or beneficial effect of one plant on another through the production of chemical compounds released to the environment (Rice, 1984). Many of the phytotoxic substance suspected of causing germination and growth inhibition have been identified from plant tissues and soil. These substances are termed allelochemics (Whittaker and Feeny, 1977). In 1996 The International Allelopathy Society defined allelopathy as follows: "Any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects" (Torres *et al*. 1996). Plant response to allelochemicals

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depends on the dose like stimulatory at low concentration but becomes inhibitory as dose increase (Sinkkonen, 2001).

The effects of allelopathy have been noticed from as early as 300 BC when Greek philosopher Theophrastus realized that the chick pea exhausted the land, making it unsuitable for the growth of crop plants.

Allelopathy denotes biochemical interactions among all types of plants and microorganisms. The action of allelochemicals is diverse and affects a large number of physiological functions and biochemical reactions eg.: seed germination, cell division, cell elongation, membrane Most of the known allelochmicals like phenols, steroids, saponins & alkaloids impart growth inhibiting effect but certain brassinosteroids promote growth.

Allelochemicals are classified into into five different categories: phenylpropanes, acetogenins, terpenoids, steroids, and alkaloids (Rice, 1974). All allelopathic agents require different modes of dispersion into the environment to be effective. Certain chemicals are released via evaporation, like volatile oils from plant organs; few others by root exudation, where the chemicals are leached into the ground and remaining others by decomposition.

Some plant leaf leachets inhibits the germination of seed, seedlings growth, or blocking the metabolism of other plants. This is responsible for some secondary metabolites or organic chemicals release from plant leachets. The effects of leaf leachets on seed germination and seedling growth of weeds and crop have been studied. Generally seed germination is influenced by various internal and external factors. Plant leaf leachets can suppress the growth of seedlings.

Material and Methods

Preparation of Leaf Leachate

Freshly shed leaves of *Shorea robusta* were collected from Joipur Forest Range in Bankura District (Location-I), and Lataguri Forest Range in Jalpaiguri district (Location-II), of West Bengal, India. Collected leaves were washed thoroughly with tap water and then distilled water to remove the dust and then dried in sunlight 3 to 4 days. The dried materials were then chopped in to small pieces and 25gm chopped leaves were allowed to leach for 24hrs.in 250ml of distilled water. The concentrated leachates were obtained after filtration and the volume was made up to 250m with distilled water and this solution was considered as 100% for subsequent use to make working concentrations (like 25%, 50%, and 75%). (modified Seerjana *et al*, 2007)

Cytological Study

Completely dried & healthy bulbs of onion (*Allium cepa L*.) of were selected. and sown on sandbed in our laboratory for 2 to 3 days to develop roots. Such sprouting onion bulbs are then placed on suitable test tubes filled with working concentration of leaf leachates (in quadruplicate) for 24 hrs. Distilled water was used as control set along with different treated set. (Padhy *et al*, 2002)

After treatment, the root squash preparations were made by standard aceto-orcein method (for mitotic index study) and haematoxylin method (for nucleolar frequencies study). Prepared slide were studied using Leica, DM 3000, Trinocular microscope and photographs were taken by Leica DFC295 digital camera with Leica Q-Win Plus image analyzing software.

Seed Germination Study

Surface sterilized (0.01$HgCl_2$ for 2-3 minutes followed by repeated washing in distilled water) seed *Eupatorium odoratum* and *Brassica campestris* (25 seeds /set in quadruplicate) were emerged in different working concentration of leaf leachate (25%, 50%, 75% &100% for 3hrs. at 25^{0}C.)These treated seeds were directly sown in Petridish containing moist filter paper in seed germinator under standard conditions (light/dark: 16/8 hrs. at 70% RH at 25^{0}C). The percentages of seed germination were calculated in different treatment sets.

Result & Discussion

Cytological Study

Different concentration of leaf leachates of *Shorea robusta* were tested for their effect on division potential of roots of *Allium cepa* the results indicates that 25% leachate concentration can reduce mitotic potential by 50%(10.1%) with respect to the control (Location-1) and this reciprocal trends (higher leachate concentration with less mitotic index) indicates that the chemical composition of leaf leachate is strongly anti-mitotic. (Table 1) The observed data shows an inverse correlation between leachate concentration & nucleolar frequency as evidenced by that diminishing nucleolar frequencies from 81.32% at control (without leaf leachate), to 33% & 40% at 100% leachet concentration in both Location-I & II, respectively. It is important to note that, the array of different chemicals present in the Sal leaf leachate significantly decreases the nucleolar frequencies. This trend points to the general suppression effect of leachets particularly on chromatin activity i.e. transcription & on overall metabolism, of the cell. In depth study is required to focus the issue of allelochemical-mediated growth suppression by sal leaf leachets on competing weed seeds, to unravel the exact mechanism involved, which might operate in natural Sal forest ecosystem. (Table:2)

TABLE: 1 EFFECT OF DIFFERENT CONCENTRATIONS OF LEAF LEACHATES OF *SHOREA ROBUSTA* ON MITOTIC INDEX OF *ALLIUM CEPA*

Treatment	Mitotic Index (in %)	
Control(0%)	19.87	19.87
25%	10.10	15.16
50%	8.08	12.27
75%	8.02	7.38
100%	5.37	4.25

TABLE: 2 EFFECT OF DIFFERENT CONCENTRATIONS OF LEAF LEACHATES OF *SHOREA ROBUSTA* ON NUCLEOLAR FREQUENCIES OF *ALLIUM CEPA*

Treatment	Nucleolar frequencies (in %)	
Control(0%)	81.32	81.32
25%	58.08	68.70
50%	43.74	60.08
75%	41.14	49.36
100%	33.52	40.20

Germination Study

The inverse relation between leaf leachate concentration and seed germination percentage of a competing weed species i.e., *Eupatorium odoratum* and cultivated crop i.e., *Brassica campestris* indicates growth retarding impact of sal

leaf leachate. It is remarkable to note that in comparison to the *Brassica campestris*, seed of *Eupatorium odoratum* are more vulnerable to increasing concentration of leaf leachate. Almost around 50%reduction in germination percentage is associated with *Eupatorium odoratum* seeds.(81% in control set & 43% in 100% leachate concentration) in comparison to approximately 30% reduction of the same for *Brassica campestris* seeds. Such differential sensitivity of two target species towards sal leaf leachate is expected. The weed *Eupatorium odoratum* is a natural associate of sal forest ecosystem in both South and North Bengal hence a competitor. Thus while germination the natural leaf leachate released by decaying Sal leaf raise the soil allelochmicals level to such a level so that seeds of *Eupatorium odoratum* fail to germinates or die early. Though in North Bengal Sal forest ecosystem, we could find patch of *Eupatorium odoratum* undergrowth within the Sal forest ecosystem. This may be due to high biomass and organic matter content in North Bengal forest that interfere and reduce the negative impact of allelochemicals on seed germination. (Table: 3, Table: 4 , Table: 5 & Fig: 1)

TABLE: 3 EFFECT OF DIFFERENT CONCENTRATIONS OF LEAF LEACHATES OF *SHOREA ROBUSTA* ON SEED GERMINATION OF *EUPATORIUM ODORATUM*

Treatment	% of seed germination	
Control(0%)	81	81
25%	69	71
50%	61	58
75%	56	52
100%	48	43

TABLE: 4 EFFECT OF DIFFERENT CONCENTRATIONS OF LEAF LEACHATES OF *SHOREA ROBUSTA* ON SEED GERMINATION OF *BRASSICA CAMPESTRIS*

Treatment	% of seed germination	
Control(0%)	97	97
25%	91	90
50%	84	87
75%	79	72
100%	76	71

In our study, we find differential expression of allelochemical on its neighbouring weed *Eupatorium odoratum*. Studies regarding allelopathic effect of plant on other plant of probable mechanisms are still scanty and is difficult to explain at

TABLE: 5 COMPOSITE TABLE SHOWING EFFECT OF LEAF LEACHATE OF *SHOREA ROBUSTA* ON KARYOMORPHOLOGY AND SEED GERMINABILITY.

LeachateConc.	MI (%)		NF (%)		% of seed germination			
					Weed		Crop	
	L-I	L-II	L-I	L-II	L-I	L-II	L-I	L-II
Control(0%)	19.87	19.87	81.32	81.32	81	81	97	97
25%	10.10	15.16	58.08	68.70	69	71	91	90
50%	8.08	12.27	43.74	60.08	61	58	84	87
75%	8.02	7.38	51.14	49.36	56	52	79	72
100%	5.37	4.25	33.52	40.20	48	43	76	71

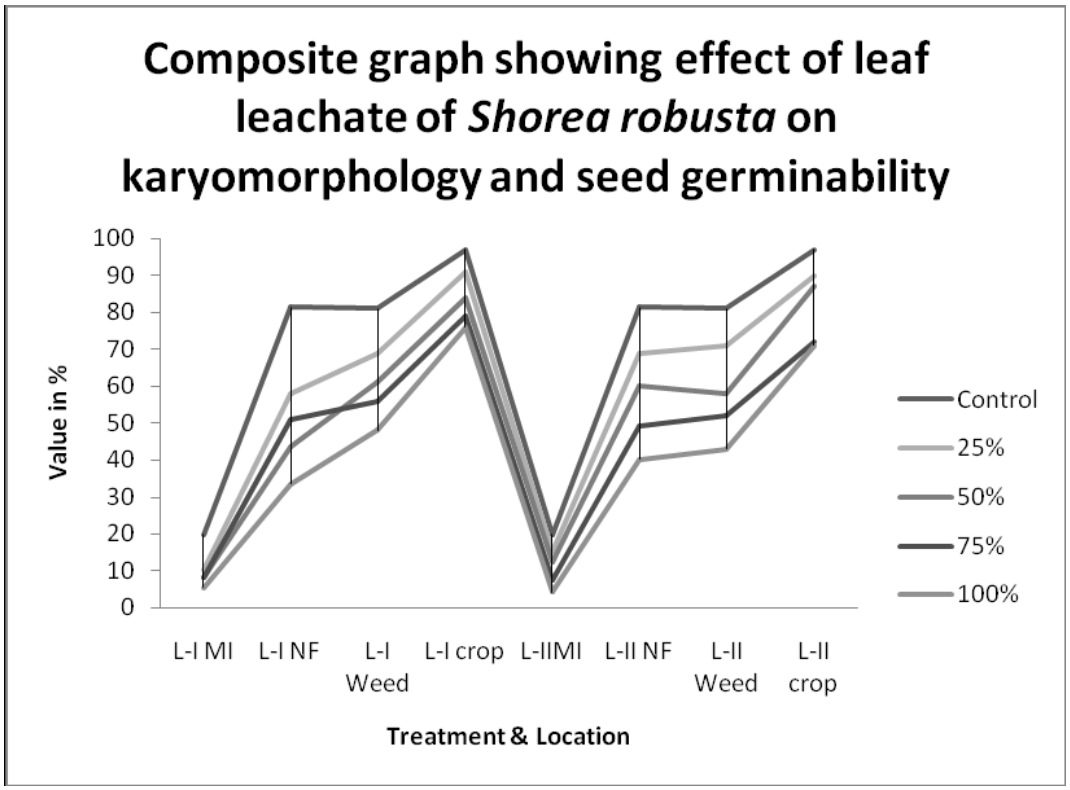
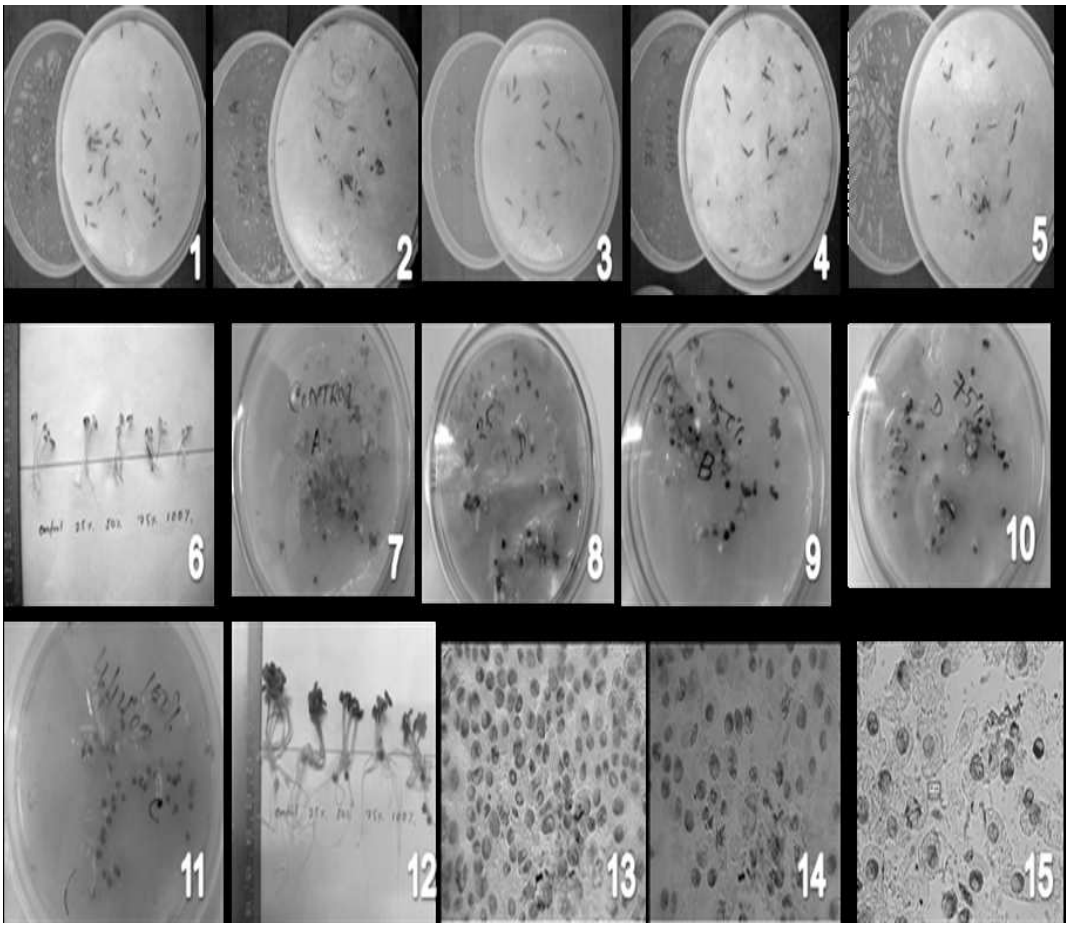


Fig. 1

present(Kumar & Goutam,2008).Moreover the role of allelopathy has been proved in field but its mechanism difficult to demonstrate without control experiment, due to difficulty in separating resource competition from allelopathy (Bias *et al*, 2003).In general, early stage of allelopathy induced inhibition of seed germination is mainly characterize by loss of seed vigour. such low vigour seed decreasing membrane integrity and thus more cytoplasmic solutes are released in to imbibing medium, which can be verified by conductivity of seed leachets (Tekorny & Spears, 2001). In a study on Echinacea angustifolia, it has been found that the root volatile compound had the greatest allelopathic potential on seedling development of common weed species (Viles & Reese, 1996). Almost similar mechanism might be operative in sal forest ecosystem\which is the next objective of the current research. Moreover scenescing leaves of sal falling on forest floor release allelochemicals into the soil & their subsequent uptake adversely affect the general metabolism & germinability seeds of Eupatorium odoratum. Similar situation operates in *Micromeria fruticosa*, the leaf residue of which inhibits germination of wheat seeds (Dudai *et al*, 2009). Considering the constrains in seed germination of sal & high seedling mortality (may be a case of auto/ self-allelopathy), this initial finding is crucial in understanding the nature of allelopathy in sal forest ecosystem which has not been explored in details, so far.



Plate

1. Set- Control -Seed germination of weed seeds 2. Set -25%-Seed germination of weed seeds
3. Set- 50% -Seed germination of weed seeds 4. Set-75% -Seed germination of weed seeds
5. Set -100% -Seed germination of weed seeds 6. Shoot & root length of weed seedlings in control and deferent concentration. 7. Set -Control-Seed germination of crop seeds (*Brassica sp*)
8. Set -25%-Seed germination of crop seeds (*Brassica sp*) 9. Set -50%-Seed germination of crop seeds (*Brassica sp*) 10.Set -100%-Seed germination of crop seeds (*Brassica sp*) 11. Set -75%-Seed germination of crop seeds (*Brassica sp*) 12. Shoot & root length of crop seedlings in control set and in different concentrations of leachate 13. Chromosomal abnormality (Metaphase with clumped chromosome) at higher concentration (100%) of leachates. 14. Chromosomal abnormality (Laggard chromosome) in higher concentration (100%) of leachates 15. Showing chromosomal abnormality (Anaphase with sticky bridge) in higher concentration (75%) of leachates

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