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Allelopathic potential of weed *Neanotis lancifolia* (Hook.f.) W.H. Lewis on seed germination and metabolism of mungbean and rice

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ABSTRACT

In Laboratory and Field bioassays, the higher concentrations of shoot aqueous extracts of *Neanotis lancifolia* (Hook. f.) W.H. Lewis proved inhibitory to mungbean and rice. The highest concentration (10 %) of aqueous extract reduced the plumule and radicle length of both test crops. From the *N. Lancifolia* extracts, 15-allelochemicals (Phenols, alkaloids, flavonoids, flavonols and glycerol) were identified by GC-MS. Of these 5 were major allelochemicals [Oleic acid, Glycidyl oleate, Linoleic acid, Palmitic acid and 18-Nonadecenoic acid]. These compounds at 5 DAS (days after sowing) significantly inhibited the seed germination in mungbean (58.38 %) and rice (57.48 %) at 5 DAS. The inhibitory effects of allelochemicals on seeds germination followed the order: Oleic acid> Glycidyl oleate >Linoleic acid > Palmitic acid > 18-Nonadecenoic acid. The radicle and plumule growth at 11 DAS also followed the same trend. The metabolic changes in both mungbean and rice crops revealed that the aqueous extract reduced the protein, carbohydrates, phenols, tannins and flavonoids contents and the reductions were concentration dependent. We found that the allelopathic potential of *N. lancifolia* (Hook.f.) W.H. Lewis was due to the presence of 15 inhibitory compounds identified in its extract.

Keywords: Allelochemicals, allelopathic effects, aqueous extract, inhibition, metabolism, mungbean, *Neanotis lancifolia* plumule, radicle, rice, seed germination, seedling growth.

INTRODUCTION

In crop fields, weeds are main competitors of crops for growth resources (water, nutrients, light, etc.). Their allelopathic effects reduce the crop yields. The term allelopathy refers to biochemical interactions between various plants including microorganisms (19). Allelopathy is biological phenomenon in which an organism produces one or more biochemical that influence the germination, growth, metabolism of another plant or organism through the release of allelochemicals in the environment. Hence, biological control of weeds through understanding the weed-weed and crop-weed interactions is an important research area. Crop-weed interactions studies may help to reduce the adverse effects of various weeds on crops. Weeds infestation in crop fields decreases the crop growth, yield and quality. Besides the allelochemicals leached from various parts of weeds and their root exudates adversely affects the beneficial soil micro flora and crop yield, hence, weeds must be controlled in crop fields. Thus we studied the interaction between *N. lancifolia* weed and test crops.

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Plant Inflorescence

Photoplate 1. Plant and Inflorescence of *N. lancifolia*

The tests weed *Neanotis lancifolia* (Hook.f.) W.H. Lewis [synonym *Hedyotis lancifolia* Dalzell, *Anotis lancifolia* (Hook.f.) (Family- Rubiaceae)] is common weed in Western Maharashtra and Konkan, India (2,5,8,26). The genus *Neanotis* has 33 species, widely distributed in India, China and Malaysia. In Maharashtra, its ten species are reported including the *N. lancifolia* (Photoplate1). It is major weed in field crops (Groundnut, maize, sorghum, pearl millet, grapes, tomato, rice, mungbean, etc.) (29) and it reduces the growth and yield of these crops. Mungbean (*Vigna radiata* L.) is main legume crop and rice (*Oryza sativa* L.) is staple food in many regions of India. In the fields, this weed inhibited the crops growth and reduces their yields, may be due to allelopathic impact. There are no previous reports on allelopathic effects of this weed on crops, hence, we did this investigation (26). Hence, this study aimed to (i). *N. lancifolia* weed effects on seeds germination and metabolism in rice and mungbean, (ii). Identify the allelochemicals present in this weed and to (iii). Determine this weed's allelochemicals phytotoxic effects on test crops.

MATERIAL AND METHODS

The fresh shoots samples of *N. lancifolia* (Hook.f.) W.H. Lewis were collected during flowering (August-September 2019) from the agricultural fields at Bamnoli (17°50'48.4"N, 73º52'49.7"E) Maharashtra India. It was identified by Botanical Survey of India, Pune (E) and the sample has been deposited. About 5 Kg freshly collected shoot sample was brought to the laboratory in airtight polythene bags. The shoot samples were cleaned with distilled water and spread on filter paper for shade drying at room temperature for one week (27±2 ºC). The dry plant material was grounded into fine powder using Wiley Mill and passed through 2 mm sieve. From this 100 g powder was

soaked in 1000 ml distilled water for 24 h at 25 ºC and the leachates was filtered through Buchner funnel using Whatman filter paper no. 1 (Barenstein, Germany). The filtered extract was stored in refrigerator in amber coloured bottle to avoid degradation by sunlight. Further desired concentrations (1.5, 2.5, 5.0, 7.5 and 10 %) of extract for the treatments were prepared by diluting the stock solution of extract with distilled water. T_1 was prepared by taking 1.5 ml from the extract stock solution and final volume 100 ml was made with distilled water. Similar procedure was followed to make concentrations of T_2 (2.5 ml), T_3 (5 ml), T_4 (7.5 ml), T_5 (10 ml) and T_6 (12.5 ml).

Laboratory Bioassay

The experimental treatments consisted of two factors: (i) Test crops 2 (Mungbean, rice) and (i) *N. lancifolia* extract concentrations 6 (1,2,3.5,5,7.5 and 10 %). The treatments were replicated thrice in complete randomised design.The seeds of Mungbean (*Vigna radiata* L.*)* variety '*Vaibhav*' and rice (*Oryza sativa* L.) variety '*Phule samrudhi*' were obtained from M.P. Agricultural University, Rahuri, Maharashtra.

(**i) Aqueous extracts:** The test crops seeds were surface sterilized with 0.02 % $HgCl_2$ for 3-4 secs and then rinsed (3-4 times) with distilled water. Twenty seeds of mungbean and rice were uniformly placed on filter paper in each sterilized Petri dish (10 dia) lined with one layer of filter paper and moistened with 5 ml aqueous extracts of different concentrations (as per treatment). The distilled water was used as control. Petri dish were then sealed with Parafilm (Neenah, Wisconsin, USA) to prevent evaporation and placed in germination cabinet at 22 ºC in dark. Five ml aqueous extracts were added to each Petri plate at alternate day. The germination (%), length of plumule and radicle were recorded at 3,5,7,9 and 11 days after sowing.

(ii) Allelopathic compounds: A similar method was used for bioassay of 5-major allelopathic compounds (Oleic acid, Glycidyl oleate, Linoleic acid, Palmitic acid and 18-Nonadecenoic acid) found in extract. Germination was monitored daily for 11- days, by counting the number of germinated seeds. The growing ambient conditions were same for both target species. The root length and shoot length were measured 7 days after sowing.The germination (%) was calculated as under:

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

Field studies

Field experiments were done in Botanical Garden, Department of Botany, SPPU during June-September 2019. Mungbean seedlings were grown in polythene nursery bags (35 cm x 40 cm x 30 cm), filled with 5.0 Kg soil. While rice seedlings were grown on raised beds (1 m x 1 m) (Photoplate- 2). The sowing was done on16 June 2019 and harvesting was done on 18 September 2019. The treatments were replicated thrice in Randomized Block Design. Five seeds of mungbean were sown in each polythene nursery bags at equal distance (6 cm) and bags were kept in natural environment conditions and 15

rice seedlings were transplanted in each row and distance between two seedlings was 2 cm and distance between two rows was 30 cm. The highest and lowest temperatures during the experimental periods were 26.7-35.7 °C and 14.5-22.5 °C, respectively. Relative humidity was 29 to 61 %. The required agronomic practices were followed to grow these crops. Treatments were applied at 15 DAS to mungbean and rice. Two subsequent doses of extracts of 15 ml were applied to per bag and per row of bed at weekly interval. All pots were irrigated with tap water whenever required.

Photoplate 2

Mungbean seedlings 30 days after treatment in Pot culture

Rice seedlings 30 days after treatment on seed beds

Biochemical analyses

Randomly selected leaves samples of field grown mungbean and rice were collected at 30, 60 and 90 days and used for the biochemical parameters: (i) Carbohydrates determined by Anthrone method (24), (ii) Proteins by Lowry's method (15), (iii) Tannins by Ferric chloride method (24), (iv) Phenols by Trease and Evans method (15), (v) Flavonoids by Aluminium chloride colorimetric method (18).

GC-MS analysis

To identify the different bioactive compounds in the extracts of *N. Lancifolia* weed, the fine powder (10 g) was mixed in 100 ml acetone. The analysis was repeated thrice and kept in shaker overnight at 25 °C at 60 rpm. The sample was filtered using Whatman filter paper no. 1 and the solvent filtrate was evaporated on rotary evaporator at 35-40 °C (10). For GC/MS analysis, 10 µl crude extract was dissolved in 1 ml acetone. Each chemical analysis experiment was done in triplicate.

GC-MS analysis was done with Shimadzu TQ 8030 GC system. The HP-5 MS capillary column (30 m×0.25 mm; Film thickness- 25 µm; I.D- 0.2 mm) was used for the sample analysis. As a carrier gas helium (99.999 %) was used at a flow rate of 1 ml/min. The temperature programme for GC-MS analysis was as follows: the initial temperature was 50 ºC and was heated for 5 min and then it was heated up to 240 ºC at the rate of 3 ºC per min and was increased at a rate of 3 ºC per minute until 178 ºC. This temperature was maintained for 2 min, with a total time of analysis of 30 min. The information generated in GC/MS was used for quantification of compounds. For this purpose, an external standard method was used (30). MS data were recorded at 70 eV with a mass range of m/z of 45-600 amu. The identification of the compounds was carried by the comparison of retention

indices and mass spectra of most of the compounds with those of authentic compounds available in the database of National Institute Standard and Technology (NIST) and Wiley libraries.

The identification was further supported by the calculation of their retention indices (RI) under identical experimental conditions using *n*-alkanes (C_{10}-C_{40}) and the calculated indices were then compared to those reported in literature (1). The analysis was further confirmed by Co-injection of authentic samples (Sigma-Aldrich) of the identified compounds, wherever possible.

HPLC analysis

The major compounds detected in GCMS were further isolated by HPLC analysis to know their allelopathic impacts on crops. Hence to know such compounds, we did the HPLC analysis. 0.5 g dried power sample was added to 20 ml methanol in a conical flask, place on a rotary shaker for 12 h at 100 rpm, followed by ultra-sonication for 15 min at 60 °C, cooling to room temperature and centrifuging at 5000 rpm for 10 min. The supernatants were filtered using Whatman (No. 1) filter paper. The filtrates were concentrated under vacuum using a rotary evaporator at 30 °C. The residue was dissolved in 50 ml of 50 % v/v of n-hexane and ether and filtered using 0.22 µm cellulose acetate filters. The final samples were dispensed into HPLC vials and stored at -20 °C till use.

Separation of compounds was done withHPLC-1200 infinity series system (Agilent Technologies, Waldbronn, Germany), on a Symmetry C18 column (0.25 m x 4.6 mm x 5 pm). These compounds were eluted with an isocratic system of 2- Propanol R1, Hexane R (1:99 v/v), (A: acetonitrile: 0.05% H_3PO_4 (99:1); and B: water: H_3PO_4 (99:1) in gradient elution timetable) Flow rate: 1 ml/minute. Detection: Spectrophotometer at 254 nm., Injection: 50 µl and total running time 35 min. Compounds were detected using a UV-Vis detector (LC 1110; GBC Scientific Equipment, Braeside, Australia) at 254 nm (30). The different compounds in the plant samples were identified by comparing their retention times with those of known pure authentic compounds (all five) all of which were obtained from Sigma-Aldrich (Steinheim Louis, MO, USA).

Statistical analysis

All data were statistically analyzed using ANOVA and Duncan's Multiple Range Test (DMRT). The data were analyzed by using (SYSTAT) SPSS Statistics software.

RESULTS AND DISCUSSION

I. Aqueous extracts

(i). Seed Germination: The inhibitory effects of aqueous extracts of donor *N. lancifolia* on seed germination and seedling growth of mungbean and rice were concentration dependent (Tables1 and 2). The lower concentrations treatments i.e. 1 to 5 % delayed seed germination over control. Whilst at higher concentration the inhibitory effect was much more pronounced in 7.5 and 10 % treatments at 3 and 5 DAS respectively. Similar was the outcomes of Akter and Sultana (3) had reported in cowpea (*Vigna unguiculata* L.) and mungbean (*Vigna radiata* L.). The inhibition in seed germination might be due to imbibed allelochemicals present in extracts of *Cyperus rotundus* L., *Marselia quadrifolia* L.,

Ludwigia hyssopifolia (G. Don) Exell and *Colocasia esculenta* L. Similarly, Lalita *et al.* (12) also reported reduction in germination and radical growth of mungbean (*Vigna radiata* L.) due to *Parthenium* (*Parthenium hysterophorus* L.) aqueous extracts. Our results demonstrated the similar phenomenon with above findings.

(ii). Seedlings Growth: The reduced rate of germination and seedling growth of test recipient crops was attributed to the presence of allelochemicals in the donor extracts (16). The aqueous extracts at all concentrations were inhibitory to the plumule and radicle length over the control and the reduction was concentration dependent in both test crops. Treatments 1 to 5 % decreased the plumule and radicle length at 3, 5, 7 and 9 DAS respectively. While in treatments 7.5 and 10 % the seed germination was completely inhibited at 3 DAS onwards (Table 1 and 2).

Shafiq *et al.* (25) and Pushpa *et al.* (20) had made analogous observations. They revealed significant reduction in seed germination of various crops due to higher concentration of leaf aqueous extract of *Parthenium hysterophorus* L. and *Cicer arietinum* L. Melakhessou *et al.* (17) also reported inhibitory effects of higher concentrations of aqueous extract of *Cynodon dactylon* (L.) Pers. weed on the germination and growth of durum wheat (*Triticum durum* Desf.). The delay in seed germination can have important biological and ecological implications because it can affect the ability of the seedlings to establish in natural conditions (33). The adverse impact of allelochemicals of different weeds on seed germination and seedling growth of various crops had been documented (6,13,28). The delay in seed germination may be due to the different allelochemicals present in leachates of respective weeds. Rawat *et al.* (21) explained that the allelochemicals released by weeds through different modes enters into the plant cells and allelochemicals drastically changed the normal metabolism of associated plants and crops. The change in morphology and physiology of associated plants are due to variations in the type and concentration of allelochemicals present in weeds.

II. Allelochemicals

(i). Seed germination: The application of 5- allelochemicals (Oleic acid, Glycidyl oleate, Linoleic acid, Palmitic acid and 18-Nonadecenoic acid) isolated from the *N. lancifolia* weed inhibited the seed germination at 5 DAS. Mungbean: 61.44 % (Oleic acid), 56.70 % (Glycidyl oleate), 45.52 % (Linoleic acid), 43.58 % (Palmitic acid) and 41.61 % (18-Nonadecenoic acid). The seed germination (%) inhibition in rice was: 57.37 % (Oleic acid), 47.63 % (Glycidyl oleate), 42.74 % (Linoleic acid), 41.61 % (Palmitic acid) and 42.51 % (18-Nonadecenoic acid) (Fig. 1). The order of seed germination inhibition (%) in both test crops followed the order: Oleic acid > Glycidyl oleate > Linoleic acid > Palmitic acid > 18-Nonadecenoic acid (Fig. 1). The inhibitory trend of radicle and plumule growth at 3, 7 and 11 DAS was similar (Fig. 1). The seed germination, radicle and plumule length were inhibited due to non-utilization of reserved food material in the seeds of test crops. Gulzar *et al.* (11) also investigated allelopathic potential of dominant *Calotropis procera* (Aiton) W.T. Ait on phenolic compounds (caffeic acid, gentisic acid, catechol, gallic acid, syringic acid, ellagic acid, resorcinol, p-coumaric acid and p-hydroxy benzoic acid on

Table 1. Effects of *N. lancifolia* extract concentration on Mungbean seedling length

Extract conc. (%)	Germination (%)		Seedling length (cm)* 3 DAS		Seedling length (cm)* 5 DAS		Seedling length (cm)* 7 DAS		Seedling length (cm)* 9 DAS		Seedling length (cm)* 11 DAS	
	3 DAS	5 DAS	PL	RL	PL	RL	PL	RL	PL	RL	PL	RL
C (0)	98.66	100	2.5± 0.14b	3.55± 0.13c	7.92± 0.76ef	4.82± 0.23e	11.71± 1.0c	4.82± 0.28b	14.5± 0.6c	4.82± 0.15b	18.8± 0.61b	4.46± 0.31c
1%	97.55	100	2.19± 0.16d	2.915± 0.32d	8.915± 0.62c	4.6± 0.32ef	11.6± 0.32c	3.88± 0.3ab	13.8 0.64cd	3.97± 0.53c	17.44± 0.23b	3.68± 0.25c
2%	96.66	100	1.9± 0.01de	2.87± 0.05e	8.55± 0.44d	4.32± 0.4efg	11.05± 0.51c	3.87± 0.11e	12.8± 0.32d	3.5± 0.41d	10.06± 0.65c	3.58± 0.67c
3.5%	82.33	95.55	0.7± 0.21 g	2.02± 0.41g	3.25± 0.29f	3.31± 0.51gh	6.21± 0.41f	4.45± 0.57gh	7.38± 0.51f	2.86± 0.17g	00	00
5%	72.45	94.66	0.7± 0.33f	2.02± 0.22h	2.55± 0.39f	2.5± 0.42h	4.19± 0.39g	2.59± 0.27h	6.26± 0.47f	3.2± 1.8gh	00	00
7.5%	00	00	00	00	00	00	00	00	00	00	00	00
10%	00	00	00	00	00	00	00	00	00	00	00	00

DAS: Days after sowing. PL: Plumule Length (shoot), RL: Radicle Length (root). C: Control (distilled water)1 %: 1.5 ml stock solution diluted to 100 ml, 2 %: 2.5 ml stock solution diluted to 100 ml, 3.5 %: 5 ml stock solution diluted to 100 ml, 5 %: 7.5 ml stock solution diluted to 100 ml, 7.5 %: 10 ml stock solution diluted to 100 ml, 10 %: 12.5 ml stock solution diluted to 100 ml and, PL: Plumule Length (shoot), RL: Radicle Length (root)

Table 2. Effects of *N. lancifolia* extract concentration on Rice seedling length

Extract conc. (%)	Germination (%)		Seedling length (cm)* 3 DAS		Seedling length (cm)* 5 DAS		Seedling length (cm)* 7 DAS		Seedling length (cm)* 9 DAS		Seedling length (cm)* 11 DAS	
	3 DAS	5 DAS	PL	RL	PL	RL	PL	RL	PL	RL	PL	RL
C (0)	98.66	100	2.25± 0.06a	3.05± 0.06a	8.55± 0.05a	4.15± 0.06a	11.0± 0.06a	5.75± 0.06a	13.75± 0.05a	5.85± 0.06a	17.17± 0.08a	6.22± 0.06a
1%	97.45	100	2.02± 0.0ab	2.65± 0.6ab	6.5± 0.04b	3.75± 0.06b	10.7± 0.064b	5.05± 0.06b	13.17± 0.08b	5.25± 0.06b	16.17± 0.08b	5.75± 0.06ab
2%	96.35	100	1.25± 0.06d	1.95± 0.06d	4.35± 0.11d	3.05± 0.06d	9.15± 0.06f	4.17± 0.11d	11.95± 0.06d	4.55± 0.06d	14.25± 0.06c	4.75± 0.054c
3.5%	84.65	96.45	0.3± 0.05f	1.12± 0.05g	2.6± 0.10g	2.15± 0.06g	7.55± 0.06f	3.05± 0.06g	10.45± 0.06g	3.15± 0.44g	00	00
5%	72.66	94.55	0.2± 0.04g	1.18± 0.04f	2.17± 0.08g	1.75± 0.06h	6.52± 0.13g	2.7± 0.65h	10.1± 0.04h	3.15± 0.054h	00	00
7.5%	00	00	00	00	00	00	00	00	00	00	00	00
10%	00	00	00	00	00	00	00	00	00	00	00	00

DAS: Days after sowing. PL: Plumule Length (shoot), RL: Radicle Length (root). C: Control (distilled water)1 %: 1.5 ml stock solution diluted to 100 ml, 2 %: 2.5 ml stock solution diluted to 100 ml, 3.5 %: 5 ml stock solution diluted to 100 ml, 5 %: 7.5 ml stock solution diluted to 100 ml, 7.5 %: 10 ml stock solution diluted to 100 ml, 10 %: 12.5 ml stock solution diluted to 100 ml and, PL: Plumule Length (shoot), RL: Radicle Length (root)

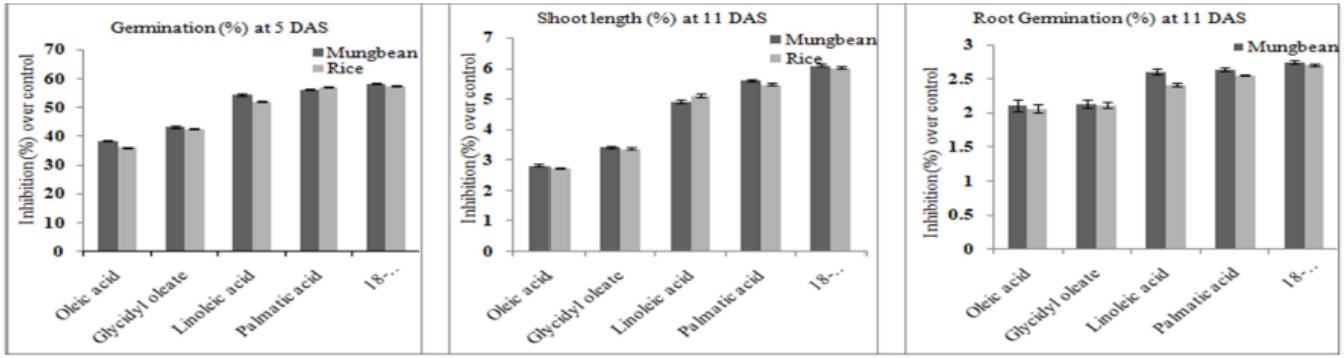

Figure 3: Inhibitory effects of major allelochemicals applied at 1 µg/100 ml on seed germination and seedling growth of mungbean and rice

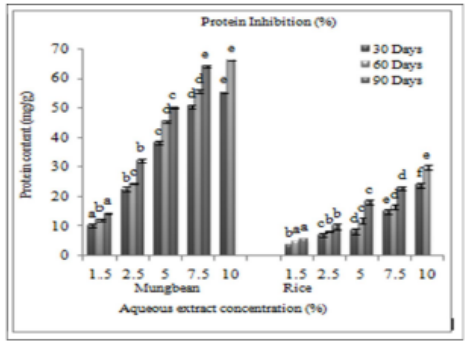

Figure 4. Effects of *N. lancifolia* aqueous extract (%) on Protein content of mungbean and rice. Vertical bars show standard errors. Bars with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

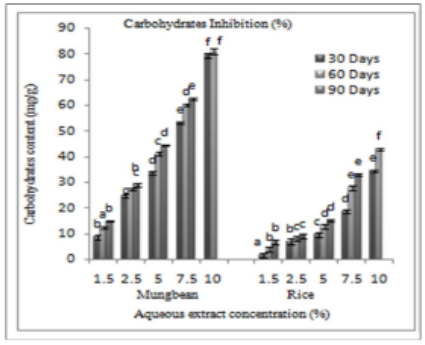

Figure 5. Effects of *N. lancifolia* aqueous extract (%) on carbohydrates content of mungbean and rice. Vertical bars show standard errors. Bars with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test

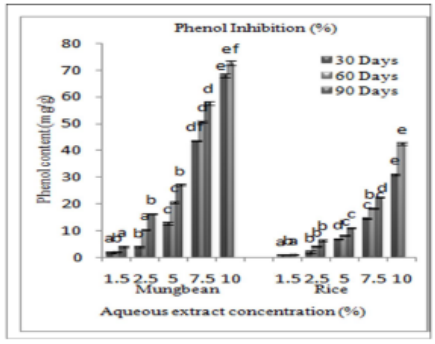

Figure 6. Effects of *N. lancifolia* aqueous extract (%) on Phenol content of mungbean and rice. Vertical bars show standard errors. Bars with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

associated crop *Brassica oleracea* L. The changes in germination process results from the membrane permeability, protein translation, and function of the secondary messengers, respiration, enzymes confirmation and receptors in the membranes or joint action of these changes (23).

Metabolic changes in test crops: The weed aqueous extract (10 %) drastically reduced protein (66 %) and Carbohydrates (80 %) in mungbean. However, in 10 % aqueous extract concentration at 90 DAS; there was complete wilting of both field grown crops (mungbean and rice) the trend was similar in rice at 60 DAS over the control (Fig. 2 and 3). After 30 days the foliar applications of aqueous extracts of *N. lancifolia* at low concentration (1.5 %) was slightly on field crops inhibitory to protein (10 %) and carbohydrates (9 %) in mungbean (Fig. 2 and 3). While in rice, the inhibition was 4 and 2 % respectively. Siyar *et al*. (27) and Al-Hawas *et al*. (4) also quantified. The high concentrations of leaf extract/ leachate of *Avena fatua* L., *Melilotus officinalis* (L.) Pall., *Polypogon hissaricus* (L.) Desf., *Artemisia monosperma* L. and *Cassia uniflora* L. (27) decrease in carbohydrates and protein contents in wheat, mungbean and rice. The higher concentrations of bioactive molecules present in the leachates might be decreasing the protein, starch, carbohydrates, metabolism and photosynthesis (7,9). The allelochemicals, when enter in to the recipient plants, generate new events and changes all the physiological, biochemical, enzymological and hormonal processes in a synergistic and integrated manner, leading to positive (at lower conc.) or negative (at higher conc.) changes in plants development and growth. Leela *et al*. (14) reported adverse effects the leachates of *Echinochloa crus-galli* (L.) on protein and carbohydrates content in germinating seeds of *Abelmoschus esculentu*s (L.) and *Solanum lycopersicum* (L.).

Our results found noticeable changes in secondary metabolites (phenols (Fig. 4), tannins (Fig. 5) and flavonoids (Fig. 6) in recipient mungbean and rice and confirmed the impact of foliar spraying of weed extracts on test crops. The presence of secondary metabolites (phenols, tannin, flavonoids, etc.) in plants indicates the intensity of stress. They have great effects on the seed germination, growth, development and metabolic functioning of plants and have predominant role in structure and functioning of ecosystems (22,31). Phenolic compounds are the major allelochemicals, through which higher plants interact with each other (32). Hence, it is necessary to record the negative impact of weeds on associated crops through physiological/biochemical parameters. The aqueous extracts at lower concentrations (1.5 and 2.5 %) reduced the secondary metabolites contents over the control. However, at medium and higher concentrations (5, 7.5 and 10 %), there was significant decrease in phenols, tannins and flavonoids at 30, 60 and 90 DAS than control. In mungbean, the decrease in phenols was 68-74%, tannins (67-73 %) and flavonoids (53-58 %) (Fig. 4, 5 and 6). Similar decreasing trend was recorded in these secondary metabolites in rice (Fig. 4, 5 and 6).

The inhibition in germination and growth of recipient crops may be due is the presence of allelochemicals in aqueous extract of *N. lancifolia*. The foliar leaf leachates/aqueous extract of *N. montholonii* and *Trianthema portulacastrum* L. significantly reduces the phenols, tannins and flavonoids in mungbean, rice and maize (2,30). The accumulated phenolic contents may adverbs impact the cell membrane permeability, water absorption and other metabolic functions in germinating seeds leading to inhibition of growth in recipient plants. The high concentration leachate of *Tithonia*

diversifolia (Hemsl.) A. Gray reduces the phenolic, tannin, flavonoids, etc. contents in *Sorghum bicolor* (L.) Moench. The decrease in phenolic contents was correlated with increase in leachate treatments.

In this study, reduced growth and development of receiver plants (mungbean and rice) may be due to the allelochemicals released from the weed *N. lancifolia*. The mungbean was more sensitive to the aqueous extract of *N. lancifolia* weed. The inhibitory effects of aqueous extract of *N. lancifolia* may due to the presence of various classes of secondary metabolites (Linoelaidic acid, Glycidyl oleate, 18-Nonadecenoic acid, Palmitic acid and Glycidyl palmitate) detected by GC-MS (Table 3) and HPLC (Figs.7,8) had strong allelopathic effects on recipient test crops. These allelochemicals might be acting synergistically on seed germination, seedling growth and physiology of mungbean and rice. These allelochemicals were reported for the first time in *N. lancifolia* weed.

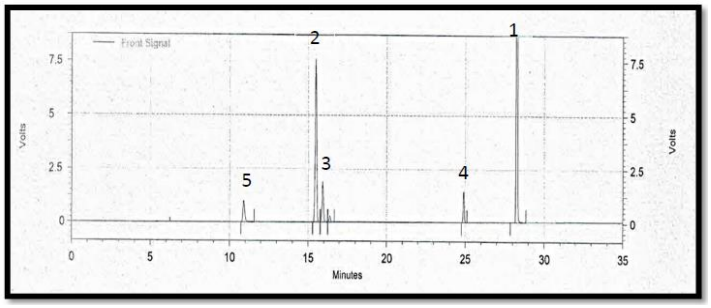

Figure 7. HPLC chromatogram of standards compounds.
(1= Oleic acid, 2= Glycidyl oleate, 3= Linoleic acid, 4= Palmitic acid and 5=18-Nonadecenoic acid)

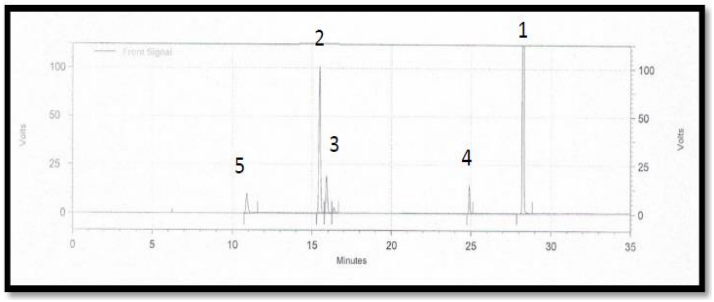

Figure 8. HPLC chromatogram of *N. lancifolia* extract.
(1= Oleic acid, 2= Glycidyl oleate, 3= Linoleic acid, 4= Palmitic acid and 5=18-Nonadecenoic acid)

Table 3. Chemical composition of ethanol extracts of *N. lancifolia* by GC-MS (Concentrations based on calibration curve of Major compounds. Authentic compounds) and the Quantity of Major compounds

Sr. No.	Name of the compounds	Formulae	RT	RI a	RI b	Peak Area % ±	Identification	Total amount (µg/g)
1	n-Decanoic acid (capric acid)	$C_{10}H_{20}O_2$	16.602	1372	1374	0.15	MS, RI	-
2	2,6-Di-Tert-butylphenol	$C_{14}H_{22}O$	18.462	1444	1443	1.3	MS, RI	-
3	Phthalic acid	$C_8H_6O_4$	18.636	1620	1620	3.5	MS, RI	-
4	Octadecane	$C_{18}H_{38}$	20.277	1800	1800	0.64	MS, RI, CI	-
5	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$	20.314	1868	1871	4.02	MS, RI	-
6	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	20.5	1884	1884	3.42	MS, RI	-
7	**Palmitic acid**	**$C_{16}H_{32}O_2$**	**21.503**	**1964**	**1963**	**9.15**	**MS, RI, CI**	**5.14 ± 0.1**
8	Oleyl alcohol, trifluoroacetate	$C_{20}H_{37}F_3O_2$	21.656	2019	2019	1.97	MS, RI	-
9	Stearic acid	$C_{18}H_{36}O_2$	21.779	2128	2130	0.45	MS, RI, CI	-
10	Glycidyl palmitate	$C_{19}H_{36}O_3$	23.304	2170	2175	4.32	MS, RI	-
11	**Glycidyl oleate**	**$C_{21}H_{38}O_3$**	**23.555**	**2168**	**2170**	**19.65**	**MS, RI**	**13.05 ± 0.0**
12	**Oleic acid**	**$C_{18}H_{34}O_2$**	**24.15**	**2175**	**2177**	**22.51**	**MS, RI**	**15.15 ± 0.1**
13	**Linoleic acid**	**$C_{18}H_{32}O_2$**	**24.42**	**2183**	**2180**	**11.36**	**MS, RI**	**6.25 ± 0.0**
14	Nonadecanoic acid	$C_{19}H_{38}O2$	24.554	2236	2236	4.27	MS, RI	-
15	**18-Nonadecenoic acid**	**$C_{19}H_{36}O_2$**	**24.783**	**2256**	**2258**	**5.17**	**MS, RI**	**4.08 ± 0.0**

Bold Text: Major compounds

RT: Retention Time, RI[a] : Retention indices relative to C_{10}-C_{40} *n*-alkanes on HP-5ms Column, RI[b] : Retention indices reported in the literature (Adams, 2007), % : Percentage composition of each compound, MS : Mass spectrum of respective compounds from the NIST and Wiley Library, RI : Reported retention indices, CI : Co-injection with the authentic sample.

CONCLUSIONS

The aqueous extract of weed *N. lancifolia* had strong allelopathic effects on seed germination of mungbean and rice, which were concentration dependent. Fifteen allelochemicals (Phenols, alkaloids, flavonoids, flavonols and glycerol) were identified in its extract. The five major allelopathic compounds (Oleic acid, Glycidyl oleate, Linoleic acid, Palmitic acid and 18-Nonadecenoic acid) bioassays showed negative effects on seeds germination, seedling growth and physiology. In pot culture, the inhibitory nature of the aqueous extract obtained from this weed was confirmed by its foliar applications on recipient plants grown in pots. The aqueous extract was inhibited to the growth and yield of both test crops.

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