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Effects of A*zadirachta indica* leaf extract on growth and development of *Oryza sativa* (L) and *Zea mays* (L)

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ABSTRACT

We investigated the effects of *Azadirachta indica* Juss. agroforestry tree aqueous leaf extract on seed germination and seedling growth of *Oryza sativa* L. and *Zea mays* L. The allelopathic effects of 0 (control),10,20,30,40 and 50 % concentrations of *A. indica* aqueous leaf extracts were determined on germination, growth, synthesis of photosynthetic pigments and biochemical constituents (starch, protein and aminoacid) of maize and rice seedlings. These extracts had both inhibitory and stimulatory effects on *O. sativa* and *Z. mays* at different stages of plant growth and development. Seed germination decreased with increasing concentration of leaf extracts from 20 % to 50 %. At lower concentration of 0 % (control) the shoot length, vigour index, and total chlorophyll were significantly increased. The inhibitory and stimulatory activity depended on the extract concentrations, higher concentration strongly inhibited the pigments, vigour index, seedling length, starch, protein and aminoacid. We found that the *A. indica* extract had water-soluble allelochemicals which significantly reduced the growth of *O. sativa* and *Z. mays*.

Keywords: Allelochemicals, allelopathy, *Azadirachta indica*, germination, inhibitory, *Oryza sativa*, stimulatory, *Zea mays*.

INTRODUCTION

Agroforestry is the interaction of crops and trees. It can provide wide range of benefits in times of deepening sustainability and climate crisis (22). Higher plants mediate plant-to-plant eco-physiological interactions by synthesizing secondary metabolites referred as allelochemicals. When released into the host plant's environment, they influence its neighbours' development and growth (5) and are found in all plant tissues. These biochemicals have useful or negative effects on target organisms i.e. can stimulate or inhibit plant germination and growth (24,25). The climate crisis is also making agroforestry ever more relevant as agroforestry is seen by many as the best solution for carbon sequestration after native forests (1). In plant-plant interactions, a plant first observes and recognizes its neighbouring plant before initiating an allelopathic response to control the intra-specific or inter-specific relationships (9). Germination (%) is an excellent indicator to determine allelopathic potential (7) and used to assess the effects of phytochemicals in laboratory or field (34). The process of seed germination includes sequential changes in biochemistry, physiology and morphology of seedlings. Disruption of these changes hampers germination and growth. The allelopathy is the result of tree crop interaction and is considered as biological phenomenon in which an organism produces biochemical that influences the growth, survival, and reproduction of other organisms (17).

Allelochemicals are categorized into 14 classes based on chemical similarity (30). Plant allelopathy is interaction among receptor and donor plants and can exert both positive

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and negative results. The use of allelopathic crops as cover crops, mulch, smother crops, intercrops or green manures, or grown in crop rotation, can fight biotic stresses (weeds infestation, insect pests, disease pathogens) and also increases organic matter/ soil fertility and thereby enhancing farm yields.

The Neem (*Azadirachta indica* A. Juss. family Meliaceae) is found in tropical and temperate regions and native to India (4,31). Its parts are used medicinally since ancient times to treat malaria, diabetes, AIDS, cancer, heart diseases etc. (14). Its active chemical components are: Azadirachtin, Nimbidin, Mibolides, Gedunin, Salanin, Nimbin, and Valassin. It possess allelopathic activities due to the presence of various allelopathic compounds: Cyclohexanol, Butanoic acid, E-11-Methyl-12-tetradecen-1-ol acetate), 3,7-Dimethyl-6-nonen-1-ol, Cyclohexanol, 5-methyl-2-(1-methylethyl)-, 3,7-Dimethyl-6-nonen-1-ol acetate, Oleyl Alcohol, 8-Hexadecenal, 14-methyl-, cis-9,10-Epoxyoctadecan-1-ol, etc.

Zea mays and *Oryza sativa* are major food crop (10) and are the principal source of carbohydrates, proteins and calories. *Zea mays* (family Poaceae) is a vital cereal crop contains provitamin A carotenoids. It is diuretic agent, modifies blood sugar levels, reduces inflammation, reduces hypertension and prevents defects at birth. It also a good source of vitamins A, B, E, and many minerals. It is also good source of minerals, vitamins, fibre and oil, oil is used for cooking and soap-making companies. Maize starch is diluent and used in cosmetics. Its stem is used for paper manufacturing.

Oryza sativa is an edible starchy cereal grain and belongs to the grass family Poaceae. The only two varieties of cultivated rice are African rice and Asian rice. Rice contains fiber, protein, vitamin B, iron, and manganese. Rice is boiled in traditional medicine to make eye lotion, treat stomach, and skin conditions. Oil for use in food production and industries is extracted from bran. The entire plant is utilised as cooking fuel or as animal fodder. Husk and straw from it are used to make paper, brooms, mats, garments, packing supplies, bedding for animals, feed, and thatch for roofing. This study aimed to assess the allelopathic potential of *Azadirachta indica* on growth and developmental of *Zea mays* and *Oryza sativa* and to determine the influence of its aqueous leaf extract on photosynthetic pigments and biochemical changes in test crops.

MATERIALS AND METHODS

The experiment was done in the Botanical garden, Annamalai University, Annamalai Nagar, Tamil Nadu, India (11.3918°N latitude, 79.7132°E longitude, altitude: 5.79 m, annual rainfall : 1235 mm, temperature : 26.5 ºC (minimum) to 34.9 ºC (maximum).

Extracts preparation: Fresh mature leaves from actively growing *A. indica* plants were collected from the social forestry, Cuddalore District, Tamil Nadu, India. The leaves were washed several times with sterile distilled water to remove the adherent dust particles, and then shade-dried for 10 days. They were powdered with blender and sieved through cheesecloth. Cold-water extract of leaves powder was prepared separately by adding 10, 20, 30, 40, and 50 g leaves powdered in 1,000 ml sterile distilled water. After 24 h, the extract was filtered through cheese cloth and filter paper and considered these as 10,20,30,40,50 and 100 % stock solutions further analysis. The plant extracts were stored in sterile McCartney bottles and labelled accordingly. Distilled water without the plant extract was used as the control.

Pot culture: Experimental treatments consisted of two factors: (i). Test crops 2 (Rice, Maize) and Neem leaf aqueous concentrations: 6 (0,10,20,30,40,50 %). The treatments were replicated thrice in complete randomised Design. Earthen pots (10 ×10 cm) were filled with five kg of garden soil (silt, FYM and sand). The seeds of *Oryza sativ*a L. - cv.TKM-9 and *Z. mays* Baby corn Co (Bc) 1. The seeds were pre-soaked in distilled water for 3.0 h, then surface sterilized with 0.1 % $HgCl_2$ solution for 30 sec and washed with distilled water several times to remove excess of chemical. Then the crop seeds were sown @ 25/pot uniformly and irrigated. The experiment started on 15th November, 2022 and completed on 15th December, 2023. Freshly prepared *A. indica* aqueous leaf extract of 10 % to 50 % were sprayed on the surface of *O. sativa* and *Z. mays* seeds from first day of the experiment. The control pots were sprayed with distilled water. Seeds germination (%) was recorded 10 days after sowing as under:

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

Crops seedlings were rooted out at 15 days after sowing, washed with tap water to remove soil attached with roots. Five seedlings/pot shoot and root length was measured (cms). The vigour index of seedlings was calculated as under:

SVI= Germination (%) × [shoot length (cm) + root length (cm).

Biochemical parameters determined in test crops seedlings were : chlorophyll a and b (using Arnon's method) (5), carotenoid (using Krik and Allen's method) (18), protein (using Lowery et al.'s method)(20), and amino acid (using Moore and Stein's method) (24).

Statistical analysis: Data are defined as the mean of replicates ± SD. Differences between treatments were then tested using a one-way analysis of variance for each parameter under review, following Duncan's multiple range test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Seed germination

Results revealed that 50 % aqueous extract of *A. indica* inhibited seed germination in both test seeds, *i.e.* in *O. sativa* (67.71 %) and *Z. mays* (34.02 %), 40% of extract recorded 30.21 % in rice and 16.49 % in maize, 30 % recorded 16.67 % in rice and 2.06 % in maize, 20% recorded (-3.12 %) in rice and (+3.09 %) in maize and at 10 % of extract treatment both seeds noticed stimulated on germination as compared to the control (Fig. 1 and 2). Leaf extracts of various concentrations of *A. indica* influenced germination percentage (%). However, maximum germination was observed with the application of 10% leaf extract of *A. indica* in the seeds of both test crops and lowest with 50% conc. as compared to the control. This allelopathic potential may be attributed to the presence of some secondary metabolites such as Phthalic acid, cyclobutyl dodecyl ester, Decanamide, N-(3-cyano-4-ethyl-5-methyl-2-thienyl)-,Silane,[[(16.beta.)-estra-1,3,5(10)-triene-3,16-diyl]bis(oxy)]bis [trimethyl, Benzeneethanamine, N-[(3,4-dimethoxyphenyl)methyl]-3,4-dimethoxy, l-Alanine, N-(p-toluoyl)-, pentadecyl ester, 2,6-dimethoxyphenyl nonyl ester, Imidazole, 4-pentadecafluoroheptyl, 3,4-Dimethoxy-N-methylbenzylamine, Benzo[c]cinnoline-2-carboxylic acid, methyl ester, 4-[(4-Iodo-2-methyl-phenylamino)-methylene]-5-methyl-2-phenyl-2,4-dihy dro-pyrazol-3-one, Cholesta-7,14-dien-3-ol, 4,4-dimethyl-, (3.beta., 5.alpha.)- etc (GCMS study). Water being polar can dissolve maximum allelochemicals such as phenolics from plant parts and through imbibition may cause the death of the embryo of the seeds (27). Hussain *et al*. also confirmed that Indian lilac *(Melia azedarach* L.) and neem (*Azadirachta indica* L.) inhibited the germination in maize by when the aqueous extract was applied at 2 % concentration (18).

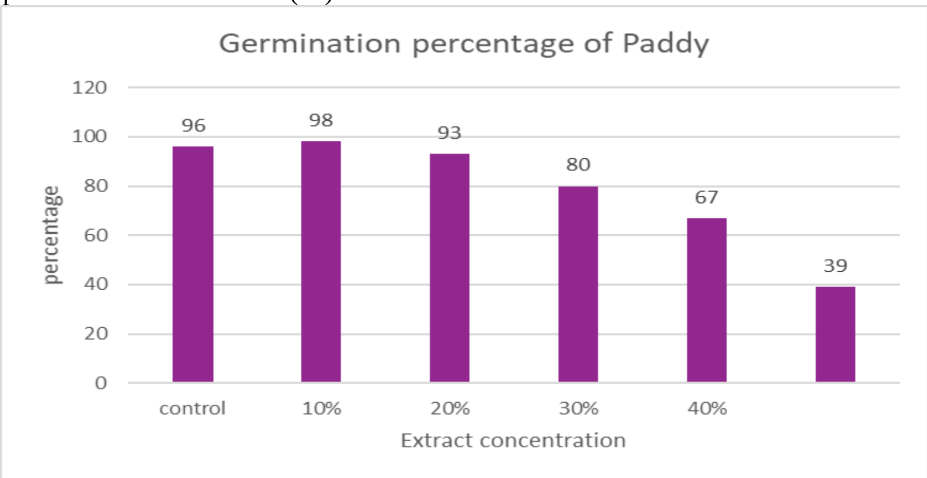

Figure 1. Allelopathic effects of *A. indica* leaf extracts on seeds germination (%) of *O. sativa*

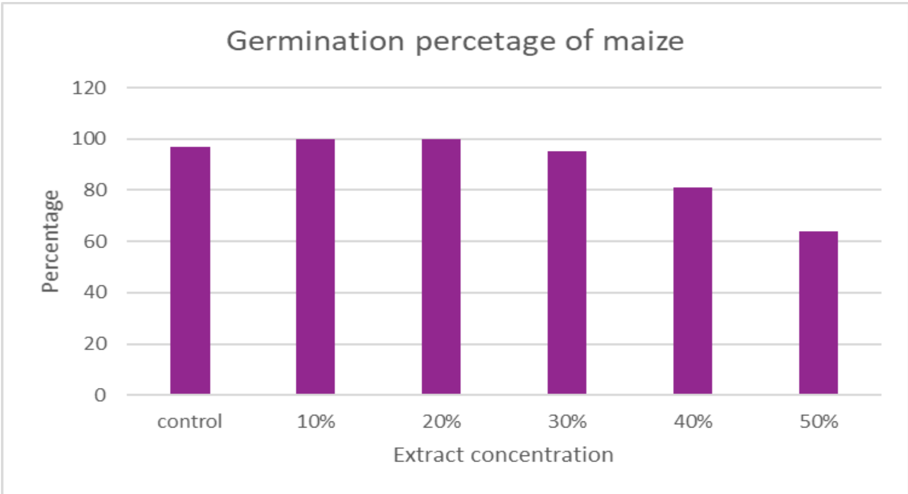

Figure 2. Allelopathic effects of *A. indica* leaf extracts on seeds germination (%) of *Z. mays*

Seedling root and shoot length

It was revealed that the root and shoot length of seedlings of both tested crops were significantly influenced by different concentrations of leaf extract of *A. indica* (Fig. 3,4). It was noted that higher concentration of *A. indica* leaf extract (50 %) greatly reduced the root length (1.3 cm and 1.8 cm) and shoot length (4.9 cm and 6.9 cm) of *O. sativa* and *Z. mays* crops. Maximum reduction in root and shoot length of *O.* sativa (51.85 & 60.48 %, respectively) and *Z. mays* (47.06 & 52.41 %, respectively) was recorded with the application of 50 %. *A. indica* leaves extract over control. While at lowest concentration (10 %) stimulatory effects were noticed in both test crops. At 10% neem leaves extract treatment stimulatory (root length in rice 3.7 % and in maize 5.9 %; shoot length in rice 1.3 % in rice and 1.6 % in maize respectively) effect was observed over control, Between the two crops, more stimulatory effect was noticed in *Z. mays* than *O. sativa* seedlings. Based upon the concentration of phytochemicals such as Methoxyacetic acid, 4-hexadecyl ester, 1-Hexadecanol, 3,7,11,15-tetramethyl, Behenyl chloride, Nimbin, Sulfurous acid, 2-ethylhexyl pentadecyl ester, Sulfurous acid, pentyl tridecyl ester, Hexadecane, 2,6,10,14-tetramethyl, 5,8,11-Heptadecatrien-1-ol, Cholestan-3-ol, 2-methylene-, (3.beta.,5.alpha.)-Carbonic acid, heptadecyl isobutyl ester, 1-Chloroeicosane, 1-Bromodocosane, Triacontane, Heptadecanoic acid, 16-methy, 7-desacetyl-7-benzolgedunin, Octadecanoic acid, Cyclopropaneoctanoic acid, etc. that are present in *A. indica* might have stimulated or inhibited the said characters (Fig. 3 and 4). From the present study the root and shoot length of *Oryza sativa* and *Zea mays* increased at 10% extract treatment of *A. indica.* The length decreased with the increased concentration of both test seedlings.

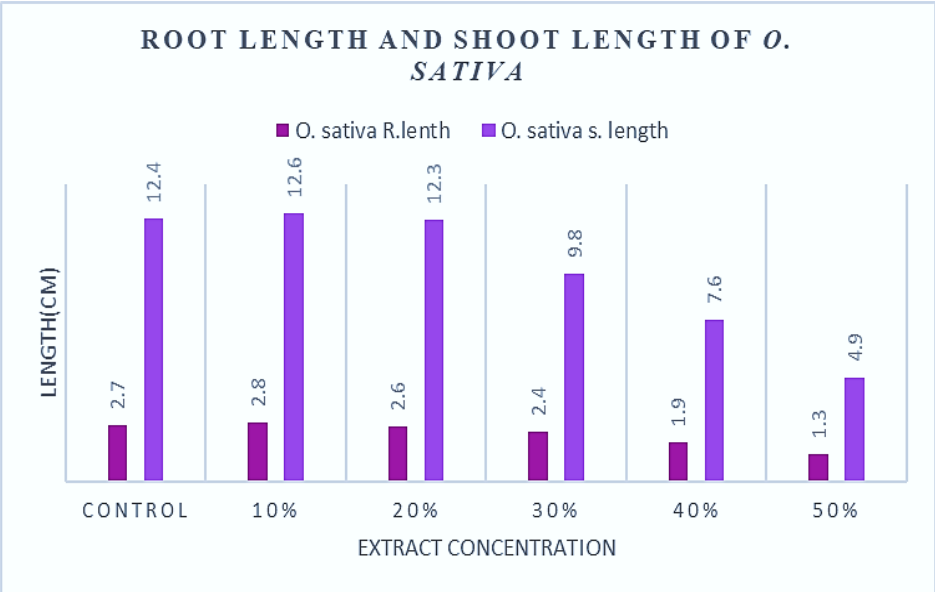

Figure 3. Allelopathic potentials of *A. indica* leaf extracts on root length and shoot length (cm/plant) of *O. sativa* seedlings

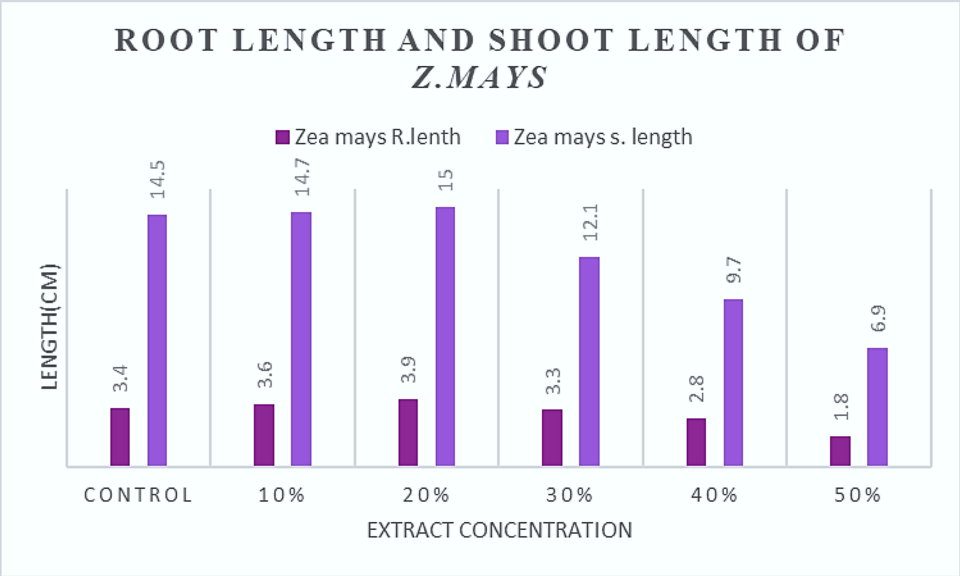

Figure. 4. Allelopathic potentials of *A. indica* leaf extracts on root length and shoot length (cm/plant) of *O. sativa* seedlings

The leaves of the treated plants with 10% conc. of A. indica leaf extract appeared to be more healthy and green in comparison to the control plants. The present study result showed that in lower concentrations (10 % and 20 %), *A. indica* leaf extract has very little harm in *O. sativa* and *Z. mays* as compared to control. Same way the Vigour index of the seedlings was also dependent on the concentration of the leaf extract (Fig 5 and 6).

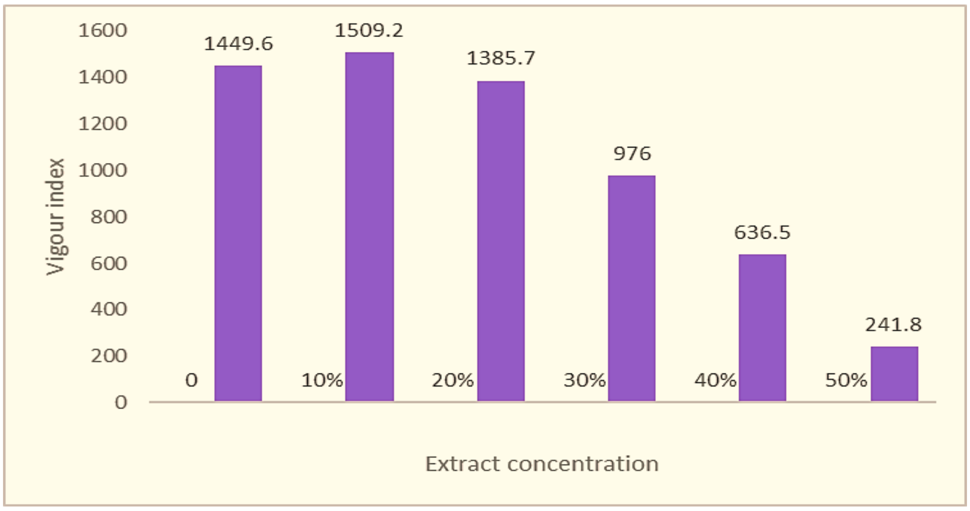

Figure. 5. Allelopathic potentials of *A. indica* leaf extracts on Vigour Index of *O. sativa* seedlings

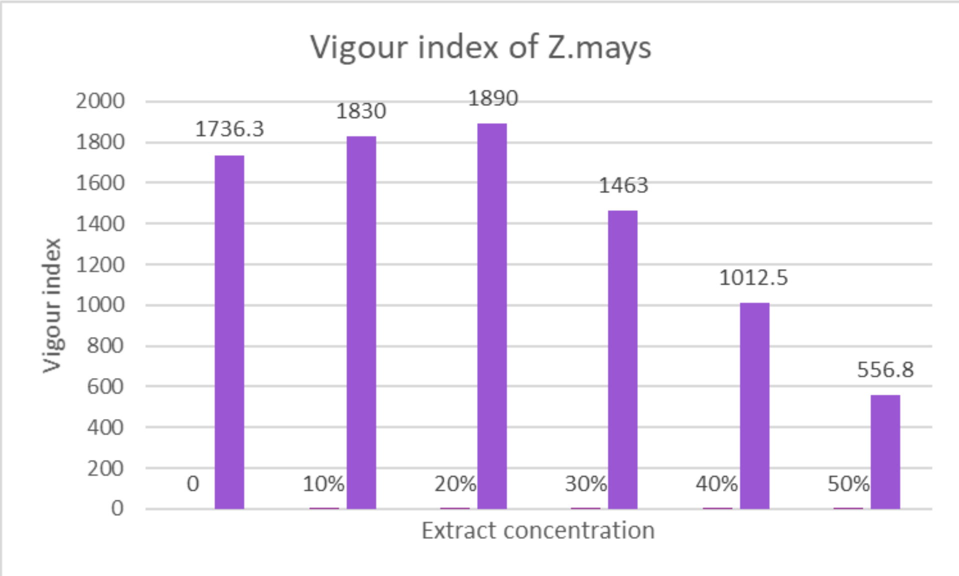

Figure. 6. Allelopathic potentials of *A. indica* leaf extracts on Vigour Index of *O. sativa* seedlings

Phytochemicals involved in allelopathy in some plants may decrease the permeability of cell membrane, which cause a reduction in root length and shoot length (cm). These results are confirmed by Shinwari *et al*. (33). Hussain and Shah also reported from their study that allelochemicals disturbed the normal permeability and plant water uptake where the treated pulse seedlings possessed characteristic variations in their root length compared to the control plants. The presence of volatile allelochemical myrcene might have rapidly induced ROS production and significantly increased the activity of lipoxygenase (LOX) in rice roots (16) which has an inhibitory effect.

Biochemical constituents

After 15 days of application of aqueous leaf extracts of *A. indica*, depending upon the concentration both increase and decrease in the contents of starch, protein and amino acids of *O. sativa* and *Z. Mays* seedlings were recorded over control (Fig.7,8). When aqueous leaf extracts of 40 % (rice; 30.91 % starch; 38.71 % protein; and 41.77 % amino acid; and maize; 22.22 % starch; and 50 % (rice; 60 % starch; 67.44 % protein; and 70.89 % amino acid; and maize; 51.85 % starch; and 56.25 % protein; and 66.67 % amino acid) were used, a significant reduction in these constituents was found in the seedlings of both test crops. The highest inhibition was noticed in *O. sativa* as compared to *Z. mays* seedlings

When treated with a lower concentration of A. indica leaf extract, phytochemicals viz. 4,4,5,8-Tetramethylchroman-2, 7-Octadecyne, 2-methyl- 2-Pentadecanone, 6,10,14-tri, 3,7,11,15-Tetramethyl-2-hexa Phthalic acid, Hexadecanoic acid, Tetrapentacontane, Tritetracontane, etc. might have stimulated the sugar, protein and amino acid contents of the test crops over control, while at higher concentration had reduced the contents of said biochemical constituents. Das et al. (10), also observed a reduction in the dry weight of root and shoot, sugar, protein, and amino acid contents of gram seeds at various levels of leaf

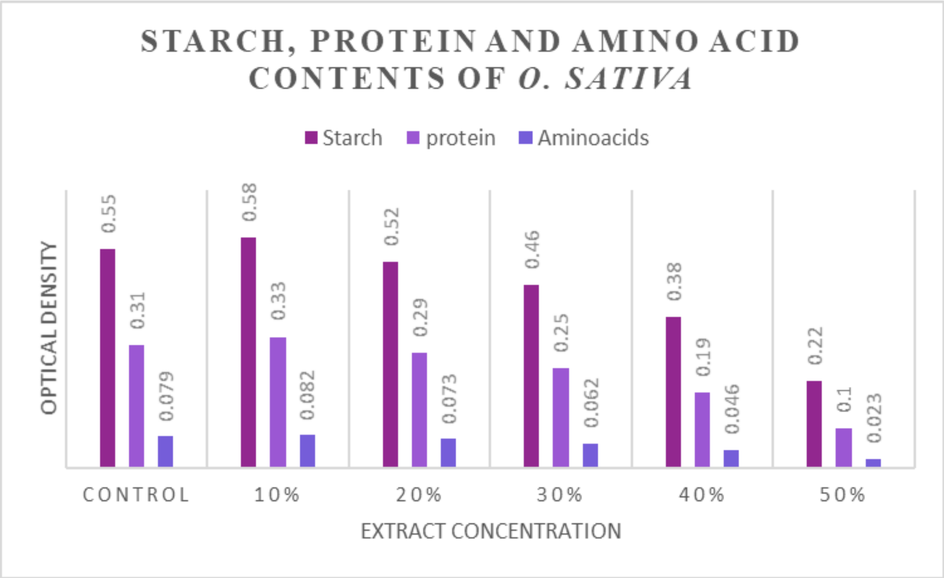

Figure.7. Allelopathic effects of *A. indica* leaf extracts on starch, protein, and amino acids (mg/g fr.wt) contents of *O. sativa* seedlings

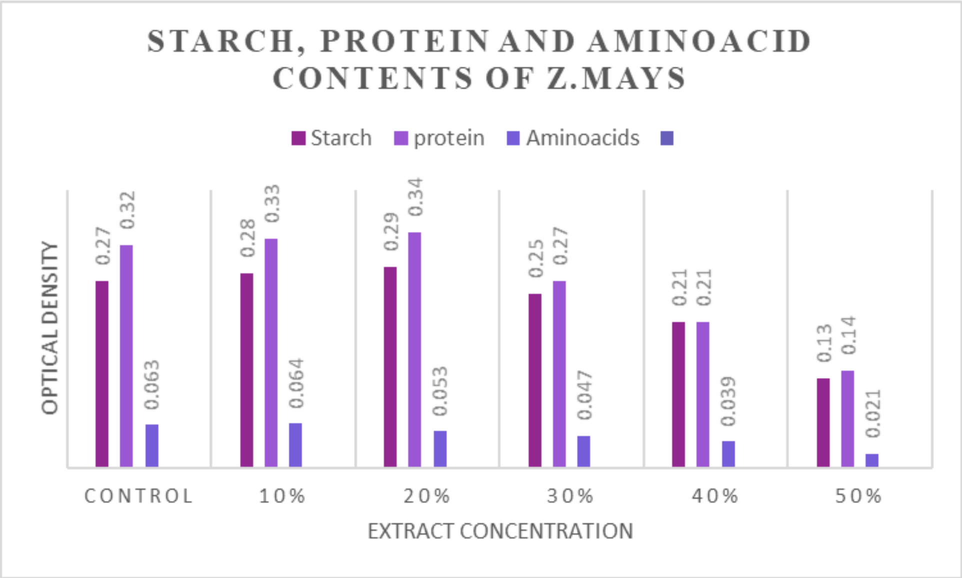

Figure 8. Allelopathic effects of *A. indica* leaf extracts on starch , protein and amino acids (mg/g fr.wt) contents of *Z. mays* seedlings

extract of allelopathic tree species *Acacia auriculiformis L.*, *Anacardium occidentale L.*, *Albizia lebbeck L.*, *Eucalyptus citriodora L.*, *Enblica officinalis L.*, *Shorea robusta L.*, *Tectona grandis L*. These findings support the present findings (8). The percentage of inhibition increased with the increasing concentration of aqueous extract or litter amount of *M. dubia*. Similarly, the findings of the present investigation revealed that biochemical contents were higher in control and lower percentage extract treatment as compared to the different (30 %, 40 % and 50 %) leaf extracts.

Allelochemicals (higher conc.) can inhibit amino acid absorption, in addition to transport, thus interfering with protein synthesis, which affects cell growth (3). All phenolic acids have an impact on the integrity of DNA and RNA. Ferulic acid and cinnamic acid as well as many phenols and alkaloids can also inhibit protein synthesis (23). Based upon the review cited by various workers, it can be inferred that the presence of allelochemicals of *A. indica* such as Phthalic acid, cyclobutyl decyl ester, 6-Bromo-4-methyl-1-phenylanthrapyridone, Imidazole, 2-pentadecafluoroheptyl, 3-[2-(4 Methylphenylthio) ethyl]-4-H-sydnon,Thiocyanato [3-azabicyclo [3.2.2] nonane-3-thiocarboxylic acid, Benzeneethanamine, 3-methoxy-N-[(pentafluorophenyl) methylene]-4-[(trimethylsilyl) oxy]-, Pyrimidine, tetrakis [(trimethylsilyl) oxy], Beryllium, .mu.4-oxohexakis [.mu.-(propanoato-O:O')], 2 2-Chloroethyl oleate etc might have stimulated or inhibited the seedling growth and development of respective crops based upon the applied concentration of leaf extract.

Pigment contents

The data presented in Fig. 9 and 10 elucidated that leaves extracts of *A. indica* caused a highest adverse impact on chlorophyll contents of *O. sativa and Z. mays* by a higher concentration of 50 % leaf extracts of *A. indica*. The pigment contents viz. chlorophyll-*a*, chlorophyll *b*, total chlorophyll and carotenoid reduced in the test crops at higher concentrations of leaf extract treatment. These pigment contents were increased significantly from 15 DAS to 30 DAS in the test crops. A decreasing trend in pigment content was recorded in the test crops with increasing concentrations (10, 20, 30, 40 and 50 %) of neem leaf extracts. The photosynthetic pigments viz. chl-*a* and chl-*b* increased in the test crops treated with 10 % of *A. indica* leaf extracts. However, when the test crops were treated with *A. indica* extract at the same 10% level, the amount of carotenoid in the crops decreased. At the same time, 10% extract treatment on *O. sativa and Z. mays* had positive influence on increasing the content of chlorophyll *a*, (2.9 and 5.13 %, respectively) and chlorophyll *b* (5.13 and 2.32 %, respectively) and reduction was noticed in carotenoid (-2.78 and -2.78 %, respectively) content. The result of our study noticed that in lower concentration, allelochemicals might have acted as Auxin growth regulator, which had a positive effect on growth.

A reduction in chlorophyll content may cause reduction in photosynthesis and also on abiotic activities. Hussain et al. also reported that the chlorophyll content of maize seedlings was adversely affected by different plant extracts (19). Allelochemicals, which are produced by plants, are known to have an impact on the germination, growth, metabolism, distribution, behavior, and reproduction of other species. Allelochemicals frequently give

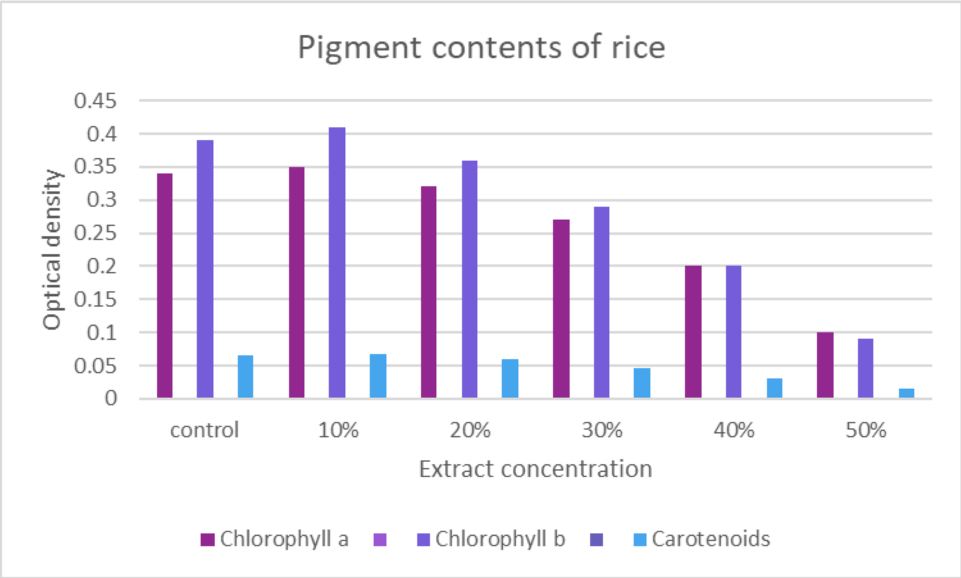

Figure. 9. Allelopathic effects of *A. indica* leaf extracts on Chl. *a,* Chl. *b*, and carotenoid (mg/g fr.wt) contents of *O. sativa* seedlings

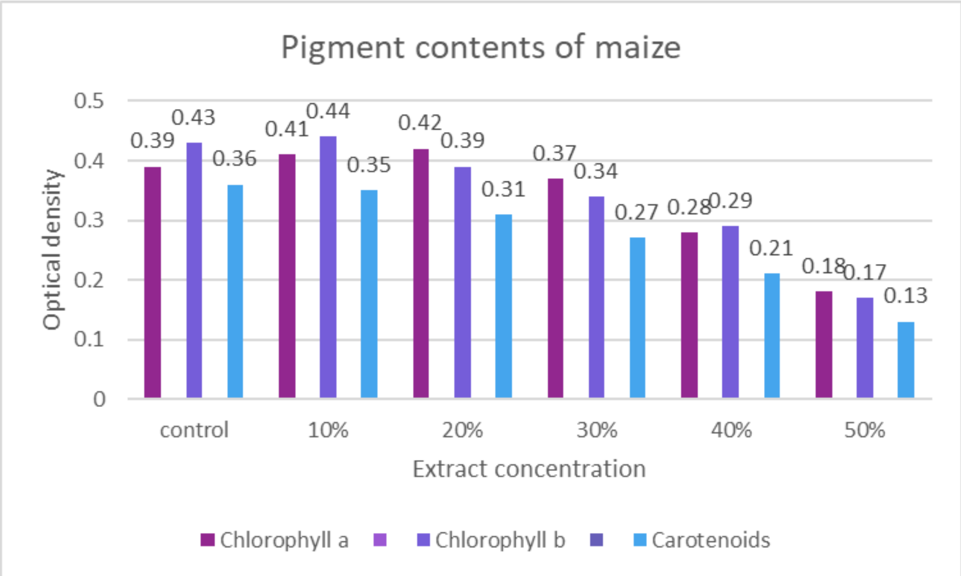

Figure.10. Allelopathic effects of *A. indica* leaf extracts on Chl. *a,* Chl. *b*, and carotenoid (mg/g fr.wt) contents of *Z. mays* seedlings

plants resistance to diseases, insects, and nematodes while also lowering weed growth. Investigating the pest-control capabilities of Agroforestry species is greatly facilitated by agroforestry systems. The majority of agroforestry species produces a good amount of leaf, litter, and debris with high allelochemical content. Agroforestry species receive several types of pest control qualities from these allelochemicals. Thus, their purified chemicals and leachates could serve as eco-friendly substitutes for synthetic pesticides, and their allelopathic materials could be used as mulch. Farooq *et al.* stated that when grown in rotation with tobacco (*Nicotiana tabacum* L.), the stand establishment and growth of *Zea mays* had progressed in comparison to *Vigna radiata*, while mung bean stands establishment and growth had been suppressed (11). Therefore, the allelopathic nature of crops should be taken into consideration in crop rotation, intercropping, and stalk mulching. Abenavoli *et al*. observed that the allelochemicals trans-cinnamic, ferulic acid, and p-coumaric acid inhibited internet nitrate uptake and plasma membrane H+-ATPase activity in maize seedlings, even as umbelliferone and caffeic acid did now no longer have an effect on H+-ATPase activity (2).

CONCLUSIONS

Increased concentrations of *A. indica* leaf extracts stimulated (10 %) and inhibited (20 % to 50 %) the growth, pigment synthesis and biochemical constituents in seedlings of test rice and maize. The 30 % to 50 % concentrations of aqueous extract inhibited these parameters of both crops. While, lower concentrations were stimulatory i.e. concentration dependent. The leaf extracts at 40 % and 50 % concentrations inhibited the seed germination, seedling length, vigour index, chlorophyll, sugar, protein, and amino acid contents in seedlings of *O. sativa* and *Z. mays*. Leaf extracts of *A. indica* had greater effects on *O. sativa* than on *Z. mays*. Further research is needed to apply allelopathy to agricultural production on a large scale.

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