



Allelopathic Impacts of an Agroforestry Tree Species (*Streblus asper* Lour.) on Seed Germination and Seedling Growth of Chickpea

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ABSTRACT

Background: Agroforestry might be a better strategy for sustainable land use and crop production. Allelopathic effect of *Streblus asper* Lour. (Moraceae) an agroforestry tree species was documented on chickpea (*Cicer arietinum* L.) seed germination and seedling growth.

Methods: The present study was conducted to evaluate the allelopathic potentialities of different concentrations (0.125%, 0.25%, 0.5%, 1.0%, 2.5% and 5%) of aqueous leaf extracts of *S. asper* on seed germination and seedling growth in laboratory based experiments. The allelopathic potentialities were studied based on seed germination, germination rate, root length, shoot length, biomass, seed vigor index etc.

Result: The inhibitory effect was more pronounced with the increasing concentrations of aqueous extracts. 5% aqueous leaf extracts showed a maximum inhibitory effect on seed germination, root length, shoot length, dry weight of root and shoot as compared to control. Based on the allelopathic index, concentrations of 5% aqueous extract showed a strong inhibitory effect. Aqueous leaf extracts contain water-soluble allelochemicals which affect the seed germination and seedling growth. The inhibitory allelopathic effect can be taken as serious consideration before plantation of chickpea in an association of this tree species because of its inhibitory effect on seed germination and early stages of development.

Key words: Agroforestry, Allelopathic effect, Allelopathic index, *Cicer arietinum*.

INTRODUCTION

Today's world is facing some severe problems like climatic change, extensive growth of population, urbanization, deforestation, yield losses in crops which are some major threats and denoted that the food security and conservation of biodiversity are required as extreme urgency for sustainable development of our ecosystem (FAO, 2009). In most recent decades rapid changes in climatic conditions had a great impact on crop yield and soil fertility of agricultural lands (Zimmermann *et al.*, 2017).

Agroforestry trees maintain soil fertility as hypothesized that they are efficient to transfer nutrients as a form of litter to the intercropped species (Vitousek and Sanford, 1986). In recent decades it is one of the most suitable strategies for land management because woody perennials and crops are deliberately planted on the same land which provides peoples with multiple benefits (Nair, 1991) and encourages them to use forest lands without deforestation. The agroforestry system is crucial for rural peoples for enhancement of their food source and socio-ecological benefits which support different conservational strategies for ecologically fragile areas (Tscharntke *et al.*, 2012).

In agroforestry systems, tree and crops were grown together and interact with each other in different environmental processes which results in an inhibitory or stimulatory effect on the growth of crops. Neighboring trees release allelochemicals into the soil which generally negatively affect seed germination and growth of understory

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or surrounded crop plants (Harborne, 1977). The allelopathic effect of tree species varies from one crop to another (Stowe, 1979), such allelopathic interactions between trees and crops help to understand the allelopathy for developing sustainable agroforestry practices. Agroforestry system focused on a new aspect of modern sustainable agricultural practices to secure our food production but also understanding the disadvantages of farming practices in the association of tree species can serve as an effective key for preventing unwanted outcomes. Tree species are grown with crops and determination of their allelopathic compatibility is very much important for the agroforestry system for sustainable crop productivity (Rizvi *et al.*, 1999).

Streblus asper Lour. is a tree species belonging to the family Moraceae and indigenous to tropical Asian countries such as India, Malaysia, Thailand, Sri Lanka and Philippines.

The allelopathic potentiality of *S. asper* is not well documented whether they influenced the growth and development of crops or not. This study was performed to evaluate the allelopathic potential of *S. asper* on chickpea (*Cicer arietinum*) seed germination and seedling growth in laboratory conditions.

MATERIALS AND METHODS

Plant materials

Mature leaves of *S. asper* were collected from topical dry deciduous forests around Santiniketan (23.68°N 87.68°E) of Birbhum district, West Bengal, India. Collections of leaves were done in flowering stages because during flowering stages plants release more allelochemicals than vegetative stages (Ahmed and Wardle, 1994). Leaf samples were brought into the laboratory and washed with tap water for several times to remove the dust particles and cut into small pieces. Leaves were shade dried at room temperature (20 ± 3°C) in dark conditions, then grounded into a fine powder with mortar and pestle. Samples were stored in 4°C refrigeration conditions for further study. Healthy seeds of chickpea were collected from Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, West Bengal, India. Seeds were surface sterilized with 4% sodium hypochlorite then rinsed with distilled water several times to remove excess chemicals. The experiment was conducted during November to February (2017-2020) in the Department of Botany, Visva-Bharati, Santiniketan.

Preparation of aqueous leaf extract

5 gm (w/v) of dried leaf powder of *S. asper* were mixed properly in 100ml of double distilled water for 24 hours at room temperature (20±3°C) and kept in dark condition. Afterward, the extracts filtered through two-layer filter paper to remove the debris and then centrifuged at 5000 rpm for 20-25 minutes. The supernatant was filtered through two-layer Whatman no. 1 filter paper. The resultant solution was considered as a 5% stock solution. The stock solution was diluted with double distilled water for preparing concentrations of 0.125%, 0.25%, 0.50%, 1%, 2.5% (Mutlu and Atici, 2009). To avoid non-relevant results of concentrations due to pH imbalance, pH was neutralized by adding 1(N) NaOH or 1(N) HCL solution.

In-vitro bioassay

Chickpea seeds were presoaked in double-distilled water for 2 hours. Twenty-five seeds were randomly placed on two-layer filter papers in sterilized Petri dishes. In each treatment, 10 ml extract was applied and distilled water was taken as a control and kept at room temperature (20 ± 3°C). Treatments were arranged with three replicates. Seeds were considered as germinated upon the root length of 2 mm. Germination index was determined by counting the number of germinated seeds at 24 hr intervals over 10 days. After 10 days of incubation, the seed germination percentage was calculated following the formula (Saxena *et al.*, 1996):

Germination percentage (%) =

$$\frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Root and shoot lengths of seedlings were measured with a centimeter ruler. After that, roots and shoots were separated and dried at 80°C for 24 hours to obtain dry mass of roots and shoots.

The seed vigor index (SVI) was calculated using the formula of Abdul-Baki and Anderson (1973):

$$\text{SVI} = \text{Germination percentage} \times (\text{Shoot length} + \text{Root length})$$

The germination index (GI) was calculated using the following formula (Chiapusio *et al.*, 1997):

$$\text{GI} = (N_1) \times 1 + (N_2 - N_1) \times 1/2 + (N_3 - N_2) \times 1/3 + (N_4 - N_3) \times 1/4 + \dots + (N_n - N_{n-1}) \times 1/n$$

Whereas,

$N_1, N_2, N_3, N_4, \dots, N_n$: numbers of germinated seeds were observed after 1, 2, 3, 4, ..., n-1, n days. The germination index (GI) denotes the delay in seed germination (Ahmed and Wardle, 1994). Germination index was obtained by dividing the germination of each extract treatment by GI of control multiplied by 100 (Zribi *et al.*, 2014).

The percentage of inhibition or stimulation of seed germination and growth parameters was calculated using the equation of Chung *et al.* (2001);

Inhibition [I] (-)/ Stimulation [S] (+) (%) =

$$\frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$$

Allelopathic index

Allelopathic index (AI) was calculated using the formula of Far and Bagherzadeh (2018):

$$\text{AI} = \frac{(\text{Sx} + \text{Rx} + \text{Gx})}{(\text{Sdw} + \text{Rdw} + \text{Gdw})}$$

Where,

Sx= Average shoot lengths of treated seeds with each extract concentration.

Rx= Average root lengths of treated seeds with each extract concentration.

Gx= Average germination percentage of treated seeds with each extract concentration.

Sdw= Average shoot lengths of treated seeds with distilled water.

Rdw= Average root lengths of treated seeds with distilled water.

Gdw= Average germination percentage of treated seeds with distilled water.

AI value is above 1, indicated that allelopathic potential is less expressed and weaker inhibitory effect but if the AI value is below 1, the allelopathic potential is more pronounced than the control and considered as strong inhibitory effect.

Statistical analysis

All experimental data were represented as the mean value \pm standard error (S.E) of three replicates. All data of experiments were assessed to one-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) test to determine statistically significant differences among mean values of treatments at the 5% probability level ($p < 0.05$) using IBM SPSS version 26.0 software. Linear regression was expressed to analyze the relationship between extract concentrations and growth parameters using GraphPad prism version 7.03 software. Heat map visualization of the inhibitory effect (%) of leaf extracts on seed germination and growth parameters were determined software 'R' version 1.3.1093 using 'pheatmap' package.

RESULTS AND DISCUSSION

Seed germination

The response of seed germination in bioassay varied among different concentrations of leaf aqueous extracts. Statistically significant differences were observed in seed germination percentage between the treatments and germination percentage decreased significantly with increasing the extract concentrations (Table 1). The maximum seed germination of 96.80% was observed in control whereas 41.60% in 5% of aqueous leaf extract (Table 1) and showed significantly ($p < 0.05$) higher inhibitory effect (I) of 57.82% in seed germination as compared to control (Fig 6). In lower concentrations of 0.125% aqueous extracts showed an inhibitory effect (I) of 6.61% (Fig 6).

Thapaliyal *et al.* (2007) found that leaf extracts of tree species showed a more pronounced significant inhibitory effect than the bark extracts on crop species. Allelopathic effects varied among species and also the source of allelochemicals (plant parts) and concentrations of applied extracts played as major factor in allelopathic study (Bari and Kato-Noguchi, 2017). In most of the *in-vitro* experiments, the concentrations of allelochemicals were applied in experiments generally far higher than natural conditions

(Reigosa *et al.*, 2000). Many of allelopathic studies found that, low concentrations of allelochemicals showed stimulatory effect in seed germination and growth (Lovett *et al.*, 1989). However, present study reveals that low concentrations of aqueous leaf extracts of *S. asper* have allelopathic inhibitory effect on seed germination of chickpea.

Germination index

The germination index also declined as increasing the concentrations of leaf extracts. Lower concentrations (0.125%, 0.25%) of aqueous leaf extracts showed 82.78% and 72.66% of germination index respectively (Fig 1). 5% aqueous leaf extract showed significantly ($p < 0.05$) delayed seed germination which reflected in the germination percentage with lowest germination index of 24.63% (Fig 1). Statistically significant differences were observed on the germination index between the treatments and it was significantly decreased with increasing the extract concentrations.

The germination index was considered as a very responsive method to understand the allelopathic effect of allelochemicals (Ma *et al.*, 2020). Delayed in seed germination leads to delay root and shoot elongation processes and in results accumulation of allelochemicals that are capable of inhibiting seed germination (Lesuffleur *et al.*, 2007). The inhibitory effect on seed germination by allelochemicals might be a consequence of the suppression of plant growth, hormone synthesis, reduction in cell division and impediment of respiratory enzymes (Rice, 1985). The study of germination indices provides a potentially important factor for identifying the negative effect of applied concentrations which provide a wide array for the establishment of allelopathic phenomena (Vidotto *et al.*, 2008).

Seedling growth

All the concentrations of aqueous leaf extract significantly ($P < 0.05$) reduced the root and shoot length as compared to control. Extract concentration of 5% showed significantly ($P < 0.05$) maximum inhibitory effect of 70.11% in root length and 75.83% in shoot length (Fig 6). Shoot lengths were also affected greater than root by aqueous leaf extracts. Lower concentrations of 0.125% and 0.25% also showed significant ($p < 0.05$) inhibitory activity of 21.28% and 29.91% in root length (Fig 6). After the application of aqueous leaf extracts on seeds showed significant ($P < 0.05$) reduction in root and shoot length with increasing the extract concentration (Table 1). The linear regression analysis between different concentrations of aqueous leaf extracts of *S. asper* showed 77% ($R^2 = 0.77$) and 79% ($R^2 = 0.79$) of variations in root and shoot length respectively (Fig 2). The aqueous extract in all concentrations have a great allelopathic activity on both root and shoot length. Concentrations dependent activity on seedling length showed that a higher inhibitory effect denoted a higher slope of regression lines.

Roots are a very sensitive organ that first comes in direct contact with the allelochemicals and absorbed directly (da

Table 1: Effect of different concentrations of aqueous leaf extracts of *S. asper* on seed germination, root and shoot elongation of chickpea.

Treatments (%)	Germination percentage (%)	Root length (cm)	Shoot length (cm)
Control	96.80 \pm 1.49 ^a	11.01 \pm 0.43 ^a	4.51 \pm 0.27 ^a
0.125	90.40 \pm 2.03 ^b	9.33 \pm 0.15 ^b	3.55 \pm 0.25 ^b
0.25	87.20 \pm 2.93 ^b	8.22 \pm 0.49 ^c	3.17 \pm 0.04 ^{bc}
0.5	79.20 \pm 1.49 ^c	7.27 \pm 0.12 ^d	3.02 \pm 0.03 ^c
1	68.80 \pm 1.95 ^d	6.02 \pm 0.08 ^e	2.42 \pm 0.11 ^d
2.5	53.60 \pm 1.60 ^e	5.11 \pm 0.18 ^f	1.96 \pm 0.04 ^e
5	41.60 \pm 0.97 ^f	3.29 \pm 0.16 ^g	1.09 \pm 0.04 ^f

Data are represented as means \pm Standard error (SE) of three replicates. The different letters on the columns indicate significant differences between treatments at $p < 0.05$ (LSD test).

Silva *et al.*, 2017). The water-soluble allelochemicals have the potential to decrease mitotic activity of roots and also affecting the synthesis and integrity of DNA-RNA and also hampered in energy production of mitosis (Khan *et al.*, 2011). Gniazdowska and Bogatek (2005) suggested that the inhibitory effect of allelochemicals during seedling growth can alter mitochondrial respiration which leads to decrease ATP production.

Biomass production of root and shoots

Aqueous extract concentrations caused inhibition of root and shoot dry weight in all concentrations than control and ranging 9.11-67.26% of root biomass and 12.72-80% of shoot biomass. Root and shoot biomass were decreased significantly ($p < 0.05$) as extract concentration increased. Allelopathic inhibitory effect was observed in dry biomass of chickpea root (Fig 6). The concentration of 5% aqueous extract showed a maximum inhibitory effect of 80% in shoot dry weight and 67.26% in root dry weight with respect to

control treatments. The linear regression between treatments of different extract concentrations showed 82% ($R^2 = 0.82$) and 78% ($R^2 = 0.78$) variations in root and shoot biomass respectively (Fig 3).

Biomass of root and shoot are also affected by different concentrations of aqueous leaf extracts. Biomass loss can attribute the water and nutrient uptake ability of plants (Chon *et al.*, 2002) which hamper the normal growth and establishment of a plant. Sahoo *et al.* (2007) suggested that aqueous leaf extracts of two tree species showed a reduction of the fresh and dry weight of maize. Similar results were also reported by Oraon and Mondal (2020) that aqueous leaf extracts of *Putranjiva roxburghii* showed that dry weight of chickpea seeds significantly decreased upon increasing concentrations.

Seed vigor index (SVI)

The seed vigor index decreased with increase of the extract concentrations while in the control treatment, the vigor index was 1502.33 (Fig 4). At 5% aqueous leaf extract the seed

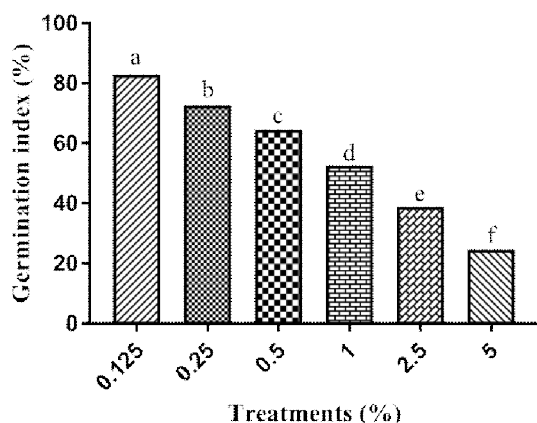


Fig 1: Effect of different concentrations of aqueous leaf extracts of *S. asper* on the germination rate of *C. arietinum*. The different letters on the column bar indicate significant differences between treatments at $p < 0.05$ (LSD test).

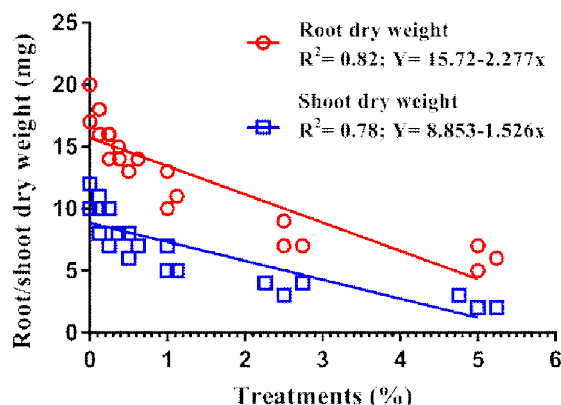


Fig 3: Linear regression analysis of root and shoot dry weight of *C. arietinum* in different concentrations of *S. asper* aqueous leaf extracts.

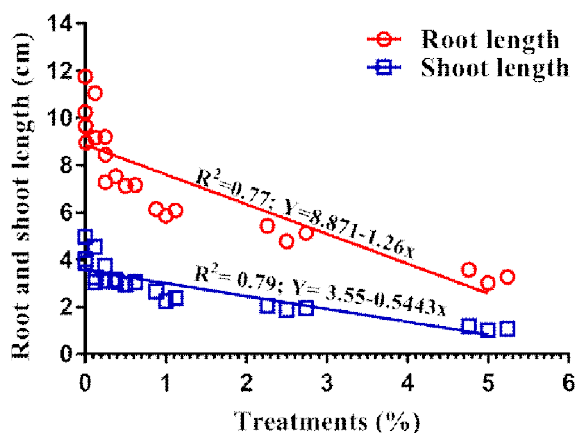


Fig 2: Linear regression analysis of root and shoot length of *C. arietinum* in different concentrations of *S. asper* aqueous leaf extracts.

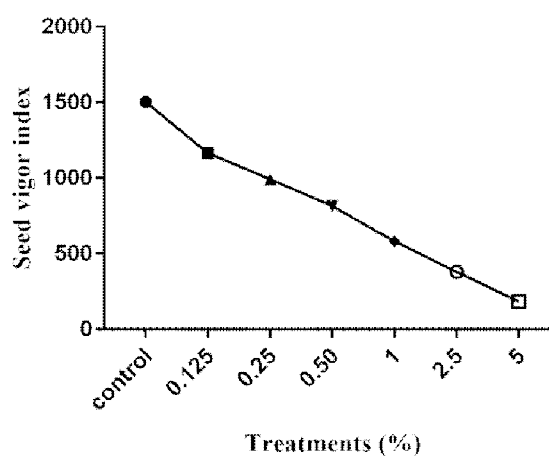


Fig 4: Effect of different concentrations of aqueous leaf extracts of *S. asper* on seed vigor index of *C. arietinum*.

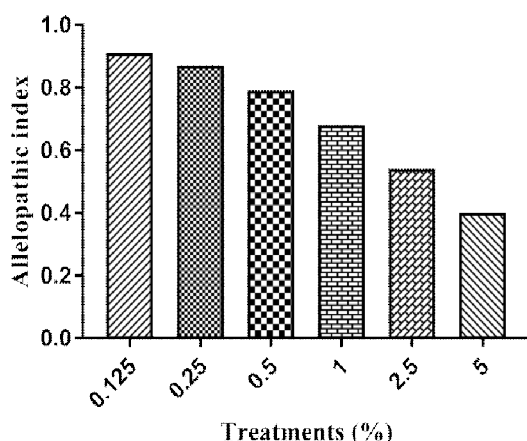


Fig 5: Effect of different concentrations of aqueous leaf extracts of *S. asper* on the allelopathic index of *C. arietinum* as compared to control.

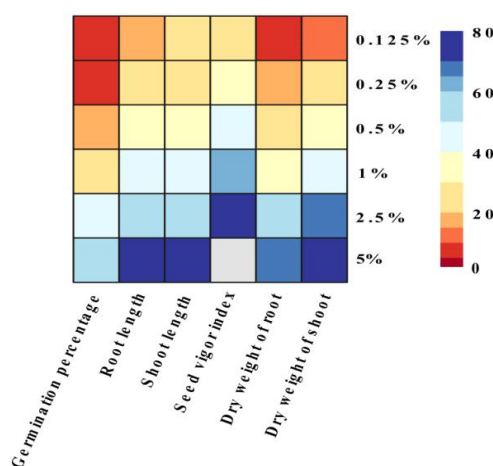


Fig 6: Heat map visualization of the percentage of inhibitory effect of different concentrations of aqueous leaf extracts of *Streblus asper* on seed germination, root length, shoot length, seed vigor index, root and shoot dry weight of chickpea as compared to control.

vigor index was 182.20 (Fig 4) which showed 87.87% of inhibitory effect than the control (Fig 6). Lower concentrations of aqueous leaf extracts of 0.125% and 0.25% also showed 22.49% and 33.88% inhibitory effect respectively than control (Fig 6). The vigor index in chickpea was decreased by all aqueous leaf extract concentrations.

According to Usha and Dadlani (2015), the study of seed vigor is a key parameter for investigating the quality of seeds. Through *in vitro* seed germination test, it was very difficult to evaluate the performance of seeds in field conditions thus; researchers highlighted the study of seed vigor in allelopathy research. Seed vigor index was reduced with increasing concentrations and higher concentration strongly affects the vigor indices (Oraon and Mondal, 2021).

Allelopathic index

The allelopathic index decreased proportionally with the increasing concentrations of aqueous leaf extracts. Allelopathic index < 1, indicated stronger allelopathic potentialities of extract concentrations. Concentrations of 5% leaf extract showed allelopathic index of 0.40, which denoted that it had a strong allelopathic potentiality and lower concentrations (0.125% and 0.25%) showed allelopathic index of 0.91 and 0.87 respectively as compared to control (Fig 5).

According to Far and Bagherzadeh (2018), the allelopathic index was minimum in lower extract concentrations and higher concentrations showed 0.72 allelopathic indexes after treatment of aqueous extract of *Carum copticum* on wheat. The present study revealed that AI was gradually decreased with the concentrations and allelopathic index lower than 1 is considered as strong allelopathic potentiality of higher concentrations which denoted a strong allelopathic effect on chickpea.

CONCLUSION

Aqueous leaf extract of *S. asper* showed allelopathic inhibitory effect on seed germination, biomass production and early growth of chickpea. A pronounced allelopathic inhibitory effect is required a serious consideration before sowing it in the agroforestry system. Further studies are needed to identify the potential allelochemicals and required to understand their stability in the soil environment as it is more complex with the involvement of soil microorganisms.

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