



Allelopathic Effect of Neem (*Azadirachta indica*) Leaf Extracts on Germination and Seedling Growth of Some Vegetable Crops

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Authors' contributions

This work was carried out in collaboration among all authors. Authors HK, MSR and MJI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RF and MBH managed the analyses of the study. Author RSNR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aqueous leaves extract of different concentrations of *Azadirachta indica* or Neem was used to study the influence of their allelopathic effects on germination and growth of some vegetable crops namely cucumber, chilli and brinjal during January-July 2019 at the research laboratory of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. There were four treatments including control viz. T₀=control, T₁=3% aqueous extracts, T₂=6% aqueous extracts, T₃=9% aqueous extracts and T₄=12% aqueous extracts of neem. Results showed that 12% aqueous leaf extracts greatly inhibited the germination of the tested crops compared to other concentrations and showed the germination percentage was decreased with the increasing concentrations of leaf extracts. The highest seed germination percentage of cucumber,

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chilli and brinjal were 82.22%, 91.11% and 71.11% respectively in control treatment whereas the lowest germination was observed 55.55%, 66.67% and 40% at 12% aqueous leaf extract at cucumber, chilli and brinjal, respectively. Other germination parameters like germination speed, mean daily germination, peak value and germination value were also influenced by the leaf extracts. The highest root and shoot length were found at 3%, 6% and 9% leaf extract over control. Similar results were recorded for biomass allocation like root and shoot dry weight. The findings of the study showed that stimulatory and inhibitory effect of *Azadirachta indica* aqueous leaf extract may be due to the presence of secondary metabolites (allelochemicals) and the inhibitory effect was more pronounced at higher concentrations.

Keywords: *Azadirachta indica*; leaf extracts; germination; vegetables; allelochemicals.

1. INTRODUCTION

The Neem tree (*Azadirachta indica*) has been known as the wonder tree for centuries in the Indian subcontinent especially in Bangladesh. Today it has become important in the global context because it offers answers to the major concerns that mankind is facing. It has many uses; the most important use of neem products is to fight against crop pests and diseases without any harmful effects on environment. Neem plants are reservoir of different types of natural occurring bio-organic compounds having a wide range of biological activities. Different parts of plants and their extracts have been used for various purposes since long time ago due to their chemical properties, availability, and simple use without side effects. Certain plant extracts found to have cytotoxic effects; some showed antioxidant properties, while a group of plant species effectively showed antimicrobial activities and reduced plant diseases like damping-off and wilt [1].

In an area of land where different plants species grows together in close relationship, interaction is bound to occur. This interaction may range from inhibition, competition, domination, overshadowing, and growth promotion. Researchers have often ignored allelopathy as a possible mechanism in their tree-crop interaction studies [2]. The effect of these allelochemicals has traditionally been considered as negative on other organisms. Allelochemicals inhibit seed germination by blocking hydrolysis of nutrients reserve and cell division and cause significant reductions in the growth of plumule and radical of various crops [3]. Some studies focus that allelochemicals are present in many organs, like leaves, flowers, fruits, and buds [4,5].

The aqueous leaf extract of *Azadirachta indica* showed both inhibitory and stimulatory effects on

seed germination, root length, shoot length, vigour index, percent phytotoxicity, fresh weight, dry weight, total chlorophyll, carbohydrate, protein and phenol content in *Vigna radiate* (green gram) seedlings [6]. The leaves have been reported to produce much allelochemicals compare to the roots, stem bark and fruits [7].

Azadirachta indica could have allelochemicals that are useful natural resources for developing biological agrochemicals for farming activities with no detrimental effects to the soil and environment as in the case of synthetic agrochemicals [2]. Large number of studies has been undertaken in the laboratory on germination and growth of different vegetable crops. However, the effectiveness of the neem extracts on individual crops in lab condition has not been investigated much. Hence the objective of the study was to determine the efficacy of aqueous leaf extract of neem on germination and growth of some vegetable crops.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material and Seed Samples

Fresh and healthy leaves of *Azadirachta indica* were collected from the botanical garden of Hajee Mohammad Danesh Science and Technology University, Dinajpur. Then the leaves were detached and washed with distilled water to remove the adherent dust particles. Aqueous extract of *Azadirachta indica* leaves was prepared as under 500 g of fresh leaves chopped in small pieces and crushed in the mixture grinder after grinding the material of leaf were filtered through Whatman No. 3 filter papers and then some of the extract was diluted to make the concentrations to 3% (T₁), 6% (T₂), 9% (T₃), 12% (T₄) (on the basis of volume) and distilled water as a control (T₀) treatment. Seeds of the

vegetables were collected from Horticulture Centre, Dinajpur. Here *Azadirindica indica* was considered as the donor plant and the receptor vegetable crops selected were cucumber, chilli and brinjal.

2.2 Bioassay of Germination Studies

The germination test was carried out in sterile Petridishes of 12 cm in size placing a whatman number 3 filterpaper on petridishes. The extract of each concentration was added to each petridish of respective treatment daily in such an amount just enough to wet the seeds. The controls were treated similarly with distilled water. The seeds of test crops were surface-sterilized with 0.5% Mercuric Chloride solution and repeatedly rinsed in distilled water. Twenty seeds were spread in containing Whatman's filter paper petri dish. The petridish were set in the three replications. The treatments were applied in a Complete Randomized Design at room temperature ranging from 25-30°C. The experiment was extended over a period of 7days to allow the last seed germination. The germination was recorded on daily basis. Data were recorded on counting the number of germinated seeds and lengths of root and shoot. The length of primary roots and shoots were measured at 5, 10 and 15 days after germination (DAG).

Seed germination parameters were calculated using the following formula:

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

$$\text{Speed of Germination} = \frac{n1/d1+n2/d2+n3/d3+\dots}{\dots} \text{ Where, } n = \text{number of germinated seeds, } d = \text{number of days [8].}$$

$$\text{Mean Germination Time (MGT)} = \frac{n1 \times d1 + n2 \times d2 + n3 \times d3 + \dots}{\text{Total number of seeds}}$$

Where, n= number of germinated seed d = number of days [9].

$$\text{Mean Daily Germination (MDG)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of days (Czabator, 1962).}}$$

$$\text{Peak Value (PV)} = \frac{\text{Highest seed germinated}}{\text{Number of days (Czabator, 1962).}}$$

$$\text{Germination Value (GV)} = \text{PV} \times \text{MDG [10]}$$

Shoot and root length was measured as described by [11,12]. Chemical analysis of the plant shoot was done as per procedure.

2.3 Statistical Analysis

The obtained data were subjected to statistical analysis using Statistix10 software and Microsoft Excel 2013.

3. RESULTS AND DISCUSSION

In present investigation leaf extract of *Azadirachta indica* on selected test crops showed both inhibitory and stimulatory effects on seed germination, root length, shoot length, fresh weight and dry weight as the concentrations of the extract increased. The highest seed germination of Cucumber, chilli and brinjal were found 82.22%, 91.11%, and 71.11% respectively in control treatment. Whereas the lowest germination was observed 55.55%, 66.67% and 40% at 12% aqueous leaf extract of neem in cucumber, chilli and brinjal respectively (Table 1). The other germination parameters like germination speed, mean daily germination, peak value and germination value were also influenced with the neem leaf extracts. The results showed that the inhibitory effect increased with increasing concentrations. This result was coincided with the result of [2] who reported that, the response of the *Hibiscus cannabinus* L. and *Amaranthus cruentus* L. grown within different composition of *Azadirachta indica* leaves were concentration dependent with respect to the germination and growth parameters studied at 2nd WAP. But at 4th-10th WAP, it was observed that all treatments grew well above the control. This indicates that *Azadirachta indica* L. has allelopathic effect on the studied vegetables. He also reported that *Azadirachta indica* possesses certain phytochemicals (allechemicals) that inhibits seed germination and also the growth parameters. The result was also supported by [13] who conducted a research to investigate the effect of aqueous extract from *Calotropis procera* on the growth of *Brassica oleraceavar botrytis*. They found that higher concentrations of extract (60% and 80%) significantly reduced germination percentage, radicle length, plumule length, dry matter accumulation, and relative water content of the brassica seedlings as compared to control. The retardatory effect increases with the increase in the concentration of three types of extract used,

with more pronounced effect noticed by leaf extract followed by fruit and flower extract. [14] who found the percentage germination, plumule and radicle length of rice and cowpea decreased with increasing concentration of *Acacia auriculiformis* leaf leachates. The result was partially agreed with the findings of [15] who concluded that the aqueous leaf extract of *A. indica* was found to have inhibitory effect on germination and root and shoot elongation of receptor plants.

Root length and shoot length of the three selected crops were also influenced by the aqueous extract of neem leaf extracts. The highest shoot and root length (cm) were found at

6% concentration of neem leaf extract over control and 12% aqueous extract of neem leaf (Table 2). This concluded that the neem leaf extract has some stimulatory effect on the growth of three selected vegetables up to a certain limit and it was concentration dependent. Similar results were recorded for biomass allocation like root and shoot dry weight (g). The results showed that the inhibitory effect increased with increasing concentrations. The result was made confirm by [16] who reported that the water extracts of sorghum and sunflower when applied alone or in combination increased the root, shoot length and the seedling biomass of rice over control.

Table 1. Influence of neem leaf extracts on the germination parameters of Cucumber, Chilli and Brinjal

Treatments	Germination Percentage	Speed of Germination	Mean germination time	Peak Value	Mean Daily Germination	Germination Value
Cucumber						
T ₀	82.220a	10.329a	5.279a	1.762a	7.190a	12.694a
T ₁	73.330b	9.338ab	5.298a	1.571b	6.428ab	10.102b
T ₂	66.670c	8.745bc	5.215a	1.428c	5.999b	8.571b
T ₃	57.777d	7.388cd	5.254a	1.238d	5.095c	6.320c
T ₄	55.553d	6.968d	5.252a	1.190d	4.809c	5.748c
SE(±)	2.43	0.68	0.07	0.05	0.39	0.79
CV	4.44	9.82	1.65	4.44	8.17	11.12
LSD	5.42	1.52	0.16	0.12	0.87	1.75
Chilli						
T ₀	91.107a	4.600a	8.803a	1.366a	3.966a	5.433a
T ₁	88.883a	4.462a	8.833a	1.333a	3.867a	5.160a
T ₂	75.557b	3.762b	8.850a	1.133b	3.267b	3.733b
T ₃	73.330b	3.783b	8.853a	1.100b	3.300b	3.630b
T ₄	66.667b	3.578b	8.807a	1.000b	3.100b	3.093b
SE(±)	4.21	0.29	0.06	0.06	0.23	0.43
CV	6.53	8.71	0.93	6.53	8.08	12.71
LSD	9.39	0.63	0.15	0.14	0.51	0.97
Brinjal						
T ₀	71.107a	2.945a	8.235a	1.185a	2.667a	3.168a
T ₁	62.220ab	2.719a	8.206a	1.037ab	2.444a	2.609ab
T ₂	57.777b	2.252ab	8.249a	0.963b	2.037ab	1.963bc
T ₃	44.440c	1.838b	8.258a	0.740c	1.667b	1.243c
T ₄	40.000c	1.706b	8.146a	0.667c	1.518b	1.012c
SE(±)	4.44	0.36	0.13	0.07	0.30	0.46
CV	9.88	19.69	1.92	9.88	18.10	28.52
LSD	9.90	0.82	0.28	0.16	0.68	1.03

T₀=control, T₁=3% aqueous extracts, T₂=6% aqueous extracts, T₃= 9% aqueous extracts and T₄=12% aqueous extracts of neem. In a column, different letter (s) show statistically significant at P≤0.05 by LSD

Table 2. Influence of neem leaf extracts on root and shoot length of Cucumber, Chilli and Brinjal

Variety	Shoot length (cm)			Root length (cm)		
	5 DAG	10 DAG	15 DAG	5 DAG	10 DAG	15 DAG
Cucumber						
T ₀	5.883a	6.733c	9.967c	6.000c	9.783b	14.567ab
T ₁	8.767a	8.617b	9.950c	10.967ab	13.650a	14.967ab
T ₂	8.783a	10.950a	13.717a	13.267a	15.450a	17.783a
T ₃	8.400a	10.850a	13.317a	7.800c	11.017b	17.667a
T ₄	7.500b	9.667ab	11.667b	8.250bc	8.917b	11.867b
CV	10.34	9.37	5.43	16.23	11.02	13.70
SE(±)	0.66	0.71	0.51	1.22	1.06	1.71
LSD	1.48	1.59	1.15	2.73	2.35	3.82
Chilli						
T ₀	4.916ab	6.200bc	7.616a	5.100a	5.616a	7.416a
T ₁	5.650a	7.066ab	7.966a	5.033a	6.300a	7.316a
T ₂	5.900a	7.533a	8.216a	5.317a	6.333a	7.616a
T ₃	5.483a	7.216ab	8.133a	4.867a	6.100a	7.133a
T ₄	4.383b	5.800c	6.500b	5.250a	6.216a	6.966a
CV	10.49	8.52	4.92	14.58	12.58	6.58
SE(±)	0.45	0.47	0.31	0.60	0.62	0.39
LSD	1.00	1.04	0.69	1.35	1.39	0.87
Brinjal						
T ₀	2.250b	4.300a	5.716a	3.716b	4.250b	5.816b
T ₁	3.416a	4.733a	5.800a	3.600c	5.700a	7.033a
T ₂	3.466a	4.750a	5.950a	5.550a	5.916a	7.116a
T ₃	3.183a	4.733a	5.766a	3.450d	3.866b	5.700b
T ₄	2.300b	3.550b	4.250b	2.800e	3.050c	3.667c
CV	5.46	6.72	6.15	1.22	5.52	3.33
SE(±)	0.13	0.24	0.27	0.03	0.20	0.16
LSD	0.29	0.54	0.61	0.08	0.45	0.35

T₀=control, T₁=3% aqueous extracts, T₂=6% aqueous extracts, T₃= 9% aqueous extracts and T₄=12% aqueous extracts of neem. In a column, different letter (s) show statistically significant at P≤0.05 by LSD

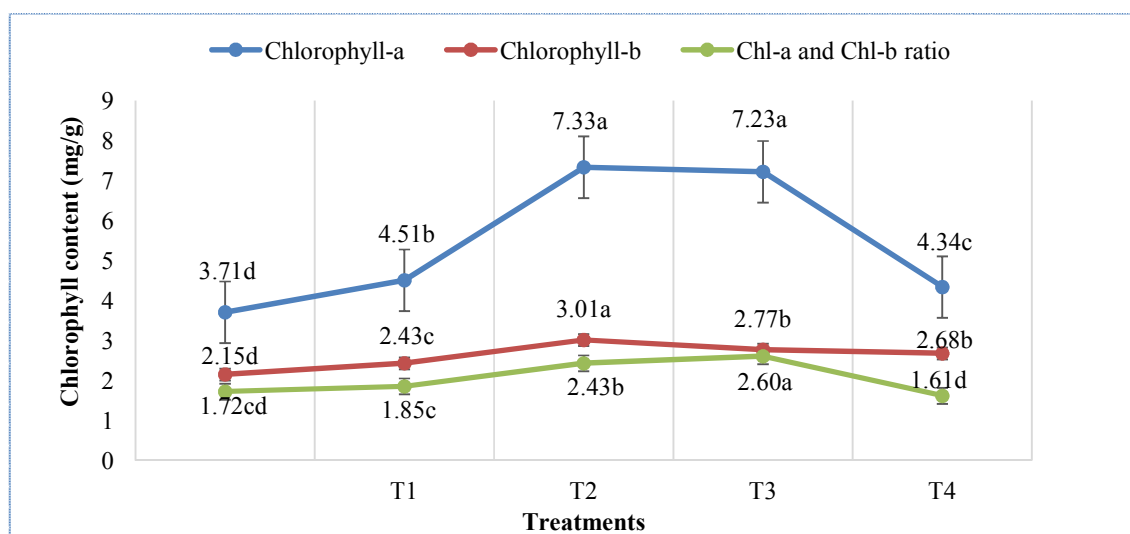


Fig. 1. Effect of neem leaf extracts on chlorophyll content in cucumber

Table 3. Influence of neem leaf extracts on root and shoot dry weight of Cucumber, Chilli and Brinjal

Treatments	Root			Shoot		
	Fresh weight (g)	Dry weight (g)	Loss of weight (g)	Fresh weight (g)	Dry weight (g)	Loss of weight (g)
Cucumber						
T ₀	0.0948b	0.0074b	0.0874b	0.4131d	0.0298c	0.3832d
T ₁	0.0895b	0.0090b	0.0805b	0.5012c	0.0411b	0.4600c
T ₂	0.1307a	0.0164a	0.1148a	0.7233a	0.0493a	0.6739a
T ₃	0.0892b	0.0086b	0.0807b	0.6957ab	0.0447ab	0.6510ab
T ₄	0.0755c	0.0096b	0.0659c	0.6506b	0.0412b	0.6094b
CV	4.89	13.47	4.47	4.17	7.95	4.29
SE (±)	0.003	0.001	0.003	0.02	0.003	0.019
LSD	0.008	0.002	0.007	0.045	0.006	0.043
Chilli						
T ₀	0.4123b	0.0389b	0.3734b	0.8088b	0.0736b	0.7319b
T ₁	0.4647a	0.0389b	0.4259a	1.0479a	0.0875a	0.9596a
T ₂	0.4902a	0.0439a	0.4463a	1.0531a	0.0935a	0.9604a
T ₃	0.3843b	0.0323c	0.3520b	1.0517a	0.0934a	0.9582a
T ₄	0.2736c	0.0254d	0.2483c	0.6061c	0.0642b	0.5419c
CV	4.17	4.23	4.16	7.55	8.46	7.44
SE (±)	0.014	0.002	0.012	0.056	0.005	0.050
LSD	0.03	0.003	0.03	0.125	0.012	0.112
Brinjal						
T ₀	0.0942ab	0.0083b	0.0860ab	0.4073a	0.0407a	0.3666a
T ₁	0.0923ab	0.0094ab	0.0830ab	0.1953b	0.0190b	0.1763b
T ₂	0.1032a	0.0102a	0.0930a	0.4747a	0.0454a	0.4293a
T ₃	0.0951ab	0.0095ab	0.0857ab	0.2077b	0.0197b	0.1880b
T ₄	0.0868b	0.0087ab	0.0781b	0.1680b	0.0163b	0.1517b
CV	8.91	9.03	8.91	20.27	20.34	20.27
SE (±)	0.0068	0.0007	0.0060	0.0481	0.0046	0.0434
LSD	0.0153	0.0015	0.0138	0.1072	0.0104	0.0968

T₀=control, T₁=3% aqueous extracts, T₂=6% aqueous extracts, T₃= 9% aqueous extracts and T₄=12% aqueous extracts of neem. In a column, different letter (s) show statistically significant at P≤0.05 by LSD

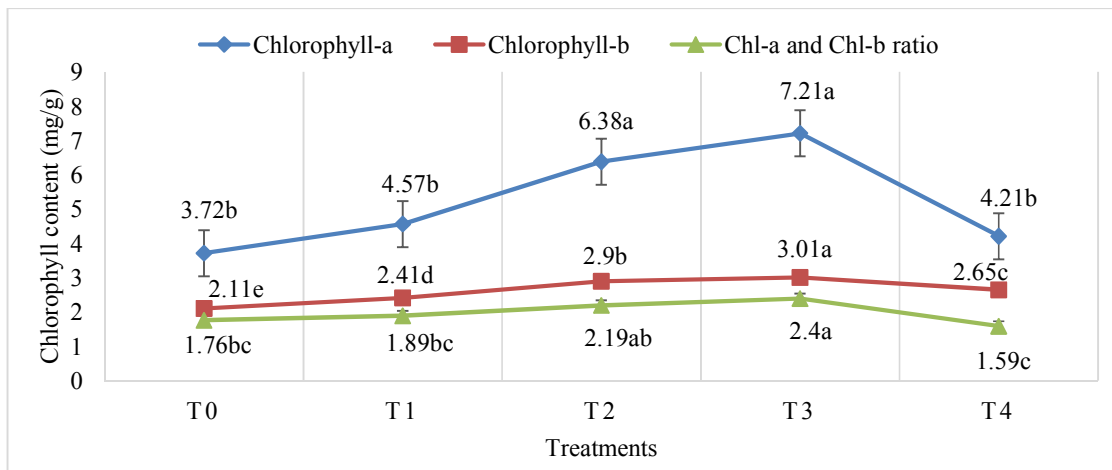


Fig. 2. Effect of neem leaf extracts on chlorophyll content in chilli

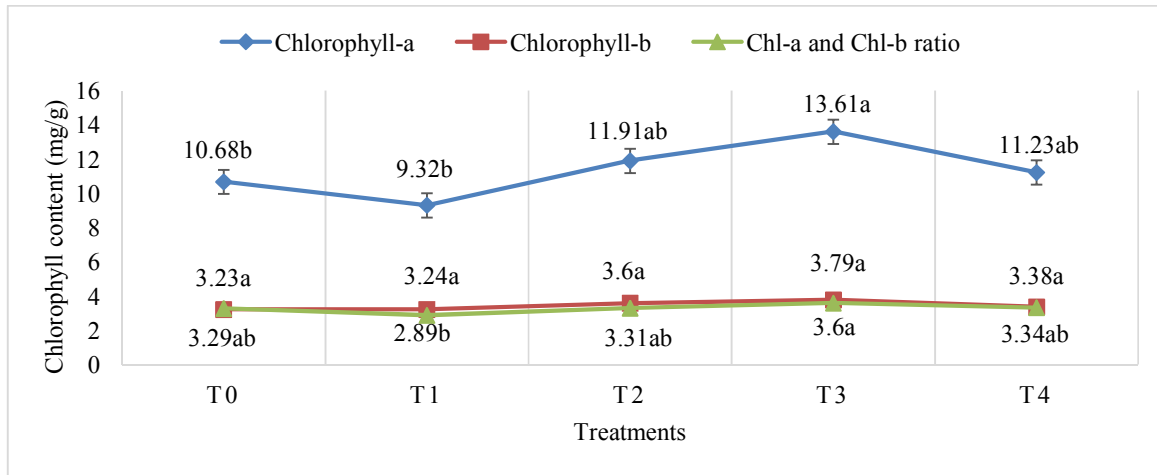


Fig. 3. Effect of neem leaf extracts on chlorophyll content in brinjal
T₀=control, *T₁*=3% aqueous extracts, *T₂*=6% aqueous extracts, *T₃*= 9% aqueous extracts and *T₄*=12% aqueous extracts of neem. In bar diagram, different letter (s) show statistically significant at *P*≤0.05 by LSD

Table 4. Effect of neem leaf extracts on nutrients content of Cucumber, Chilli and Brinjal

Treatments	Ca (g/100 g)	Mg (g/100 g)	S (g/100 g)	P (g/100 g)	K (g/100 g)	Na (g/100 g)
Cucumber						
T ₀	1.3633bc	1.7600b	5.2100ab	0.5733b	0.6867c	0.2267a
T ₁	1.4567b	1.8000b	2.7367ab	0.4633c	0.7500c	0.2600a
T ₂	1.3400c	1.5933c	3.6367ab	0.5767b	0.9367bc	0.2400a
T ₃	1.7167a	2.4400a	5.4000a	0.6967a	2.5700a	0.2600a
T ₄	1.0833d	1.3233d	2.5433b	0.4967c	1.4867b	0.1767b
CV	4.08	3.50	39.90	7.33	24.30	8.74
SE (±)	0.046	0.051	1.272	0.033	0.0255	0.017
LSD	1.033	0.1135	2.8345	0.0749	0.5686	0.370
Chilli						
T ₀	1.4800c	1.9567b	3.4267b	0.5033c	0.6167d	0.1833c
T ₁	1.5300b	1.7800c	2.1367d	0.5800b	0.6800d	0.2967ab
T ₂	1.4267d	1.5800d	3.3200b	0.6067b	0.8233c	0.2700b
T ₃	1.7567a	2.4200a	5.1833a	0.6800a	1.9833a	0.3200a
T ₄	1.0500e	1.3667e	2.3800c	0.4000d	1.2167b	0.2000c
CV	1.62	2.04	1.92	6.98	7.21	10.22
SE (±)	0.0215	0.030	0.052	0.032	0.063	0.012
Brinjal						
T ₀	1.9547c	1.2067c	0.9140d	0.4800c	0.3020e	0.3053d
T ₁	2.1087b	1.2933b	1.1317c	0.4427d	0.5687b	0.5677b
T ₂	1.0827d	1.3033b	0.8903d	0.3747e	0.3360d	0.3347c
T ₃	3.6397a	2.2033a	4.1593a	0.8060a	0.6550a	0.6533a
T ₄	3.6220a	2.1367a	2.3963b	0.6620b	0.5567c	0.5677b
CV	1.8	2.57	1.5	1.41	1.30	1.16
SE (±)	0.0365	0.0342	0.0233	0.0063	0.0051	0.0045

T₀=control, *T₁*=3% aqueous extracts, *T₂*=6% aqueous extracts, *T₃*= 9% aqueous extracts and *T₄*=12% aqueous extracts of neem. In a column, different letter (s) show statistically significant at *P*≤0.05 by LSD

This finding was supported by [17] who carried out a study on allelopathic effect of leaf leachate of four species viz., Custard apple (*Annona squamosa*), Aonla (*Emblica officinalis*), Neem (*Azadirachta indica*) and Subabool (*Leucaena*

leucocephala) on Soybean (*Glycin max*), Maize (*Zea mays*), Okra (*Abelmoschus esculentus*), Sunhemp (*Crotolaria juntia*), Green gram (*Phaseolus aurus*), Pigeon pea (*Cajanus cajan*), Fodder jowar (*Sorgham vulgar*), Sesamum

(*Seasamum indicum*) and Moth bean (*Phaseolusa conitifolius*). Seed germination was suppressed by leaf leachate of all tree species as compared to control (seed treatment with distilled water).

Different concentrations of leaf extract are also influenced the chlorophyll content at tender seedling leaves. 9% aqueous leaf extract performed better stimulatory effect over other treatments and brinjal containing highest amount of chlorophyll a (13.61 mg/g) among the other tested crops (Figs. 1, 2 and 3). The nutrients content namely Ca, Mg, S, P, K and Na (g/100 g) were found at greater amount at 9% aqueous extract of neem leaf three selected crops (Table 4).

From the study it should be concluded that germination of cucumber, chilli and brinjal seeds were affected by different concentrations of leaf extracts of *Azadirachta indica*. Higher concentrations of extracts had strongest inhibitory effect on the tested crops. Among the three tested crops, brinjal was highly affected by leaf extracts compare to other two tested crops. It can also suggest that *Azadirachta indica* could have allelochemicals that are useful natural resources for developing biological agrochemicals for farming activities.

4. CONCLUSIONS

From this research, it can be concluded that *Azadirachta indica* have both stimulatory and inhibitory effect and all the concentrations of leaf extract reduced the germination of cucumber, chilli and brinjal seeds. It also showed that the inhibitory effect was much more pronounced at higher concentration and brinjal was highly affected by *Azadirachta indica* leaf extracts among the tested vegetables. So, it can be suggested that *Azadirachta indica* could be a source of useful natural secondary metabolites (allelochemicals).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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