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# Allelopathic Effects of the Leaf Extract of *Hevea Brasiliensis* (Muell. Arg). and *Quisqualis Indica* L. on Seed Germination and Early Seedling Growth of *Vigna Radiata* (L.) R. Wilcz.

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***ABSTRACT:*** *Allelopathy is the inhibition of the growth of a plant by the allelochemicals, produced by a nearby plant, into their shared environment. The present study was designed to examine the allelopathic inhibition of aqueous leachate of Heavea brasiliensis and Quisqualis indica leaves on the germination and early seedling growth of test plant material, Vigna radiata. Present results indicated profound allelopathic inhibition of aqueous leaf extract of H. brasiliensis and Q. indica leaf extract on seed germination and early seedling growth of V. radiata. The statistical analysis showed that the inhibition shown by the two species are statistically highly significant (at 1% level) in terms of morphological parameters viz., shoot length, leaf length and leaf breadth when compared to the control seedlings. This is the first report on the allelopathic effect of both species on a crop plant.*

***Key words:*** *allelopathy, allelochemicals, Hevea brasiliensis, Quisqualis indica*

## INTRODUCTION

Allelopathy is a phenomenon in which the plant through leaching, volatility, remnant body decomposition and root system secretion, releases chemical substances to the environment which have harmful or advantageous functions to own or periphery plants including microorganisms indirectly. The research on allelopathy is propitious to guide about the scheme of plantation, system of cultivation, measure of cultivation in the economy of complex growth and it is also a way to use the advantageous effects and avoid the disadvantageous effects among plants. This aids in keeping the diversity of biology and the sustainable agricultural development (Zhi-hui *et al.* 2011). Recent studies present compelling evidence that allelopathic interactions between invasive and native species can be one of the mechanisms underlying the remarkable success of some of the most aggressive plant invaders (Gomez-Aparicio & Canham 2008).

Many native forests have been destroyed by the introduction and large-scale planting of fast growing exotic tree species. Consequently, some native species have become endangered and the ecosystem services provided by native forests are diminishing. Continuous planting of eucalyptus in monoculture may cause accumulation of phytotoxins in soil which results in soil degradation and loss of productivity (Zhang *et al.* 2009). Agricultural plantations have a number of socioeconomic and ecological advantages and disadvantages when compared with smallholder agricultural systems. Plantations are areas that are typically monocropped with perennials. Monoculture plantation forestry may affect soil chemical properties in two important ways. First, there is a nutrient depletion from the soil into the tree components (leaves, twigs, branches and stem log). Secondly, change could take place in the chemical status of the soil surface as the litter layer and organic matter becomes dominated by one species. Forest management practices may have a profound impact on soil nutrient status as well. Therefore, the study of the allelopathic relation between different crops reveals important significance to the aspect of building a harmonious ecological environment.

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The introduction of *Hevea brasiliensis* or 'para rubber' at the beginning of the 20th century caused a dramatic change in land use patterns in India, especially Kerala. India is the third largest producer of 'para rubber' in the world. Kerala with a total area of 3.84 lakh hectares under rubber cultivation produces over 90% of India's natural rubber. Hence the present study was designed to examine the allelopathic inhibition of aqueous leachate of *Heavea brasiliensis* leaves on the germination and early seedling growth of test plant material, *Vigna radiata.* The allelopathic effect of *H. brasiliensis* was compared with that of a cultivated ornamental *Quisqualis indica*.

## MATERIALS AND METHODS

The plant materials used for the study include seeds of *Vigna radiata* and aqueous leaf extracts of *Hevea brasiliensis* and *Quisqualis indica*.

### Preparation of leaf extract

Green leaves of *Hevea brasiliensis* and *Quisqualis indica* were collected from local places, and then shade dried and powdered. Aqueous leaf extract was prepared by the leaf powder using autoclaved distilled water. Series of concentrations (1%, 5% & 10%w/v) were prepared by adding 100ml of autoclaved distilled water and kept under laboratory conditions.

### Germination and seedling growth test

Ten seeds were used for each treatment. The seeds were treated with different concentrations of *Hevea brasiliensis* and *Quisqualis indica* extract (1%, 5% & 10%w/v) at various time intervals such as 2 hours, 6 hours and 24 hours. The treated seeds were planted per pot of equal size. For every treatment controls were also maintained. Proper watering was done regularly for germination in all the cases and watering was done daily. The seeds were germinated within 3 days. Morphological observations were taken for parameters including length of shoot, number of nodes, length of internodes, number of leaves, length of leaves, breadth of leaves and thickness of stem. The percentage of germination was calculated using the following formula:

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

### Statistical data analysis

Each treatment was carried out in six replications and the mean value and standard error of each observation was calculated. Statistical significance of the treatment effect against the control experiments was compared using Student's t- test.

## RESULTS AND DISCUSSION

Present study examined the allelopathic inhibition of aqueous leaf extract of *Hevea brasiliensis* on seed germination and early seedling growth of *Vigna radiata*. Allelopathic effect shown by *H. brasiliensis* leaf extract was also compared to that shown by the cultivated ornamental *Quisqualis indica*. Seeds of *Vigna radiata* were treated using different concentrations [1%, 5%, 10% (w/v)] of aqueous leaf extract of *H. brasiliensis* and *Q. indica* leaf extract each combined with three different time exposures viz., 2hrs, 6 hrs, 24 hrs. Control experiments were also carried out along with the treatments. Seeds were germinated within three days in both control and treatments and observations were made on the effect of *Hevea* and *Quisqualis* leaf extract on the seed germination percentage after three days. Morphological observations were also made after seven days of germination to study the effect of leaf extract on early seedling growth. Morphological parameters selected include length of shoot, number of nodes, length of internodes, number of leaves, length of leaves, breadth of leaves and thickness of stem. Details of all the treatments on various aspects were tabulated and recorded.

### Effect of leaf extract on seed germination

Treatment of *V. radiata* seeds with aqueous leaf extract of *H. brasiliensis* significantly reduced the seed germination percentage, while 100% germination was observed in the control experiments. Percentage of germination was reduced with increase in the concentration of extract as well as with increase in time exposure (Table 1). The present findings corroborate the earlier report (Bora *et al*. 1999) that, the inhibitory effect of leaf extract on seed germination and seedling growth is proportional to the concentration of the extract.

Seed treatment with aqueous leaf extract of *Q. indica* was different from that of *H. brasiliensis*. Reduction in the percentage of seed germination was noticed with increase in treatment duration. All the three concentrations (1%, 5% and 10%) showed 100% germination at 2hrs exposure. However, an increase in the treatment exposure to 6 or 24 hrs significantly reduced the seed germination in all the three concentrations (Table 2). Treatment with 5% and 10% extract for 24 hrs reduced the seed germination to 80%. A gradual reduction in the percentage of seed germination with increase in treatment exposure was noticed in treatment with 10% extract.

**Effect of leaf extract on early seedling growth**

Two hour treatment with aqueous leaf extract of *H. brasilensis* showed highly significant inhibition of early seedling growth in *V. radiata*. Shoot length, leaf length and leaf breadth were significantly reduced in treated seedlings irrespective of the treatment concentration when compared to that of control seedlings. Shoot length was 16.7 ± 0.78 cm in control plants while it was 10.56 ± 0.55 cm, 9.81 ± 0.32 cm and 6 ± 0.34 cm respectively in treatments using 1%, 5% and 10% extract which was statistically significant at 1% level. Differences in leaf length and leaf breadth between control and treated seedlings were also statistically highly significant (at 1% level) irrespective of the treatment concentrations (Table 3). Significant reduction of morphological parameters was observed in six hour treatment also. Shoot length was significantly reduced in treated seedlings when compared to that of control seedlings irrespective of the treatment concentrations. The difference was statistically significant at 1% level (Table 4). Leaf length also showed statistically significant (at 1% level) reduction in treated seedlings irrespective of the treatment concentrations when compared to that in control seedlings. 5% and 10% extract showed statistically significant reduction of leaf breadth when compared to that of control. Statistically significant reduction in shoot length, leaf length and leaf breadth were also observed in seedlings treated with 1%, 5% and 10% extract for 24 hours when compared to that of control experiment. Shoot lengths were 9.52 ± 9.07 cm, 9.65 ± 0.03 cm and 8.5 ± 0.88 cm in 1%, 5% and 10% treatments respectively as against to 16.7 ± 0.78 cm in control and the difference was highly statistically significant at 1% level(Table 5) .

Treatment of *V. radiata* seeds with different concentrations of aqueous leaf extract of *Q. indica* for 2 hrs significantly inhibited the early seedling growth. Shoot length was 15.28 ± 0.47cm in control seedlings while the same was gradually get reduced with increase in the concentration of extract (Table 6). The difference was highly statistically significant (at 1% level). Shoot length was reduced to half when the seeds were treated with 10% extract. Though internodal length was similar in both treated and control seedlings leaf length and leaf breadth showed statistically significant difference (at 1% level). One third reduction in leaf breadth was observed at 10% treatment when compared to that of normal. Leaf length was reduced to half in 10% treatment when compared to that of control. Six hour treatment also exhibited statistically significant (at 1% level) reduction in shoot length, leaf length and leaf breadth when compared to that of control. 10% treatment induced shoots with an average of 6.02 ± 0.28 cm lengths as against 15.28 ± 0.47cm long shoots in control (Table 7). Leaf length and leaf breadth were also significantly reduced in treated seedlings when compared to that of control. The differences were statistically significant at 1% level. Treatment of *V. radiata* seeds with various concentrations of aqueous leaf extract of *Q. indica* for 24 hrs showed significant inhibition on early seedling growth. Shoot length was reduced to one fourth at 5% and 10% treatment when compared to that of normal. Leaf length and leaf breadth were also significantly inhibited irrespective of the concentration of the extract when compared to that of control seedlings. The difference was statistically significant at 1% level (Table 8). Similar allelopathic inhibition has been reported in *Lantana camara* (Ahmed *et al.* 2007) as the leaf extracts of *L. camara* significantly delayed the germination in all the receptor crops compared to the control treatment.

Leaf extracts of *A. ferox* above 4 mg/mL exhibited significant inhibitory effects on seed germination of several vegetable crops, whereas no significant inhibitory effect was recorded for root extract on germination of the crops (**Arowosegbe & Afolayan 2012**). The stronger allelopathic impact of leaf extracts than root extracts on the tested vegetable crops might be connected with the presence of higher concentrations of allelochemicals such as tannins, alkaloids and flavonoids in the leaves than in the roots (Kanchan & Jayachandra 1980). Different tissues of plants (leaves, stems and roots) are capable of

**Table 1**
**Effect of *Hevea brasiliensis* leaf extract on seed germination (%) of *Vigna radiata***

| *Treatment duration* | *Seed germination (%) Concentration of the extract (w/v)* | | |
|---|---|---|---|
| | *1%* | *5%* | *10%* |
| 2 hrs | 90% | 80% | 90% |
| 6 hrs | 100% | 90% | 90% |
| 24 hrs | 90% | 80% | 80% |

**Table 2**
**Effect of *Quisqualis indica* leaf extract on seed germination (%) of *Vigna radiata***

| *Treatment duration* | *Seed germination (%) Concentration of the extract (w/v)* | | |
|---|---|---|---|
| | *1%* | *5%* | *10%* |
| 2 hrs | 100% | 100% | 100% |
| 6 hrs | 90% | 100% | 90% |
| 24 hrs | 90% | 80% | 80% |

**Table 3**
**Effect of 2 hour treatment with *Hevea brasiliensis* leaf extract on early seedling growth of *Vigna radiata***

| *Morphological parameters (after 7 days)* | *Concentration of the extract (w/v)* Control (Mean ± SE) | 1% (Mean ± SE) | 5% (Mean ± SE) | 10% (Mean ± SE) |
|---|---|---|---|---|
| Length of shoot (cm) | 16.7 ± 0.78 | 10.56 ± 0.558** | 9.81 ± 0.325** | 6 ± 0.343** |
| Number of nodes | 2 ± 0 | 2 ± 0 | 2 ± 0 | 2 ± 0 |
| Length of internodes (cm) | 0.27 ± 0.0106 | 0.2 ± 0.0304 | 0.24 ± 0.0328 | 0.2 ± 0.0283 |
| Number of leaves | 2 ± 0 | 2 ± 0 | 2 ± 0 | 2 ± 0 |
| Length of first pair of leaves (cm) | 3.05 ± 0.0919 | 2.06 ± 0.015** | 1.5 ± 0** | 1.52 ± 0.0151** |
| | 3.13 ± 0.0141 | 1.9375 ± 0.045** | 1.6142 ± 0.0302** | 1.58 ± 0.0313** |
| Breadth of first leaf pair (cm) | 1.775 ± 0.0152 | 1.05 ± 0.0367* | 1.18 ± 0.034** | 1 ± 0** |
| | 1.725 ± 0.0339 | 1.1 ± 0.0353** | 0.64 ± 0** | 0.4 ± 0.018** |
| Thickness of stem (cm) | 0.6 ± 0.0282 | 0.6 ± 0.0304 | 0.414 ± 0.0302 | 0.457 ± 0.0151 |

*Significant at 5% level

**Significant at 1% level

**Table 4**
**Effect of 6 hour treatment with *Hevea brasiliensis* leaf extract on early seedling growth of *Vigna radiata***

| *Morphological parameters (after 7 days)* | *Concentration of the extract (w/v)* Control (Mean ± SE) | 1% (Mean ± SE) | 5% (Mean ± SE) | 10% (Mean ± SE) |
|---|---|---|---|---|
| Length of shoot (cm) | 16.7 ± 2.7789 | 10 ± 0.8542** | 10.88 ± 0.6425** | 8.06±0.6116** |
| Number of nodes | 2 ± 0 | 2 ± 0 | 2 ± 0 | 2 ± 0 |
| Length of internodes (cm) | 0.27 ± 0.0106 | 0.1714 ± 0.016 | 0.1714 ± 0.016 | 0.1625 ± 0.017 |
| Number of leaves | 2 ±0 | 2 ±0 | 2 ±0 | 2 ±0 |
| Length of first pair of leaves (cm) | 3.05 ± 0.0919 | 2.52 ± 0.0604 | 2.45 ± 0.0944 | 1.8 ± 0.0671** |
| | 3.13 ± 0.0141 | 2.5 ± 0.0264** | 2.5142 ± 0.0453** | 1.8875 ± 0.0597** |
| Breadth of first leaf pair (cm) | 1.775 ± 0.0152 | 1.714 ± 0.037 | 1.242 ± 0.0151** | 0.5 ± 0** |
| | 1.725 ± 0.0339 | 0.8714 ± 0.052 | 1.76 ± 0.0907* | 0.575 ± 0.0176** |
| Thickness of stem (cm) | 0.6 ± 0.0282 | 0.657 ± 0.0151 | 0.55 ± 0.015 | 0.45 ± 0.0176 |

*Significant at 5% level

**Significant at 1% level

**Table 5**
**Effect of 24 hour treatment with *Hevea brasiliensis* leaf extract on early seedling growth of *Vigna radiata***

| *Morphological parameters (after 7 days)* | *Concentration of the extract (w/v)* Control (Mean ± SE) | 1% (Mean ± SE) | 5% (Mean ± SE) | 10% (Mean ± SE) |
|---|---|---|---|---|
| Length of shoot (cm) | 16.7 ± 2.7789 | 9.52 ± 9.0734** | 9.65 ± 0.0318** | 8.5 ± 0.8844** |
| Number of nodes | 2 ± 0 | 2 ± 0 | 2 ± 0 | 2 ± 0 |
| Length of internodes (cm) | 0.27 ± 0.0106 | 0.24 ± 0.0181 | 0.22 ± 0.17 | 0.22 ± 0.17 |
| Number of leaves | 2 ± 0 | 2 ± 0 | 2 ± 0 | 2 ± 0 |
| Length of first pair of leaves (cm) | 3.05 ± 0.0919 | 2.5 ± 0.0566* | 2.37 ± 0.102* | 2.71 ± 0.1035 |
| | 3.13 ± 0.0141 | 1.9142 ± 0.139** | 2.628 ± 0.1133 | 2.57 ± 0.0529** |
| Breadth of first leaf pair (cm) | 1.775 ± 0.0152 | 1.25 ± 0.018** | 1.34 ± 0.015** | 1.08 ± 0.037** |
| | 1.725 ± 0.0339 | 0.6125 ± 0.074** | 1.0142 ± 0.0113** | 0.885± 0.0529** |
| Thickness of stem (cm) | 0.6 ± 0.0282 | 0.61 ± 0.0302 | 0.55 ± 0.018 | 0.45 ± 0.018 |

*Significant at 5% level

**Significant at 1% level

**Table 6**
**Effect of 2 hour treatment with *Quisqualis indica* leaf extract on early seedling growth of *Vigna radiata***

| *Morphological parameters (after 7 days)* | *Concentration of the extract (w/v)* | | | |
|---|---|---|---|---|
| | *Control (Mean ± SE)* | *1% (Mean ± SE)* | *5% (Mean ± SE)* | *10% (Mean ± SE)* |
| Length of shoot (cm) | 15.28 ± 0.471 | 10.63 ± 0.391** | 9 ± 0.253** | 7.14 ± 0.268** |
| Number of nodes | 2 ± 0 | 2 ± 0 | 2 ± 0 | 2 ± 0 |
| Length of internodes (cm) | 0.27 ± 0.0106 | 0.23 ± 0.0187 | 0.24 ± 0.0181 | 0.22 ± 0.0170 |
| Number of leaves | 2 ± 0 | 2 ± 0 | 2 ± 0 | 2 ± 0 |
| Length of first pair of leaves (cm) | 2.93 ± 0.0742 | 2.1 ± 0.0326** | 1.72 ± 0.0188** | 1.6 ± 0.0264** |
| | 2.91 ± 0.0388 | 1.9 ± 0.0326** | 1.57 ± 0.0566** | 1.52 ± 0.0188** |
| Breadth of first leaf pair (cm) | 1.55 ± 0.0176 | 0.96 ± 0.0489** | 0.61 ± 0.0302** | 0.55 ± 0.0529** |
| | 1.45 ± 0.0424 | 1.1 ± 0.34* | 0.64 ± 0.0453** | 0.4 ± 0.0566** |
| Thickness of stem (cm) | 0.55 ± 0.0176 | 0.45 ± 0.0204 | 0.35 ± 0.0755 | 0.35 ± 0.0755 |

*Significant at 5% level
**Significant at 1% level

**Table 7**
**Effect of 6 hour treatment with *Quisqualis indica* leaf extract on early seedling growth of *Vigna radiata***

| *Morphological parameters (after 7 days)* | *Concentration of the extract (w/v)* | | | |
|---|---|---|---|---|
| | *Control (Mean ± SE)* | *1% (Mean ± SE)* | *5% (Mean ± SE)* | *10% (Mean ± SE)* |
| Length of shoot (cm) | 15.28 ± 0.471 | 10.61 ± 0.895** | 10.41 ± 1.098** | 6.02 ± 0.286** |
| Number of nodes | 2 ± 0 | 2 ± 0 | 2 ± 0 | 2 ± 0 |
| Length of internodes (cm) | 0.27 ± 0.0106 | 0.27 ± 0.0166 | 0.26 ± 0.0187 | 0.26 ± 0.0166 |
| Number of leaves | 2 ± 0 | 2 ± 0 | 2 ± 0 | 2 ± 0 |
| Length of first pair of leaves (cm) | 2.93 ± 0.0742 | 2.5 ± 0.0944 | 2.43 ± 0.1306 | 1.41 ± 0.0565** |
| | 2.91 ± 0.0388 | 2.27 ± 0.0566** | 2.16 ± 0.0163** | 1.57 ± 0.0601** |
| Breadth of first leaf pair (cm) | 1.55 ± 0.0176 | 0.94 ± 0.0453** | 1.5 ± 0.0326 | 0.63 ± 0.0169** |
| | 1.45 ± 0.0424 | 0.87 ± 0.0151 | 1.06 ± 0.0816 | 0.57 ± 0.0318** |
| Thickness of stem (cm) | 0.55 ± 0.0176 | 0.61 ± 0.0302 | 0.45 ± 0.0204 | 0.45 ± 0.0176 |

*Significant at 5% level
**Significant at 1% level

**Table 8**
**Effect of 24 hour treatment with *Quisqualis indica* leaf extract on early seedling growth of *Vigna radiata***

| *Morphological parameters (after 7 days)* | *Concentration of the extract (w/v)* | | | |
|---|---|---|---|---|
| | *Control (Mean ± SE)* | *1% (Mean ± SE)* | *5% (Mean ± SE)* | *10% (Mean ± SE)* |
| Length of shoot (cm) | 15.28 ± 0.471 | 8.75 ± 0.388** | 4.27 ± 0.306** | 4.27 ± 0.328** |
| Number of nodes | 2 ± 0 | 2 ± 0 | 2 ± 0 | 2 ± 0 |
| Length of internodes (cm) | 0.27 ± 0.0106 | 0.2 ± 0.0212 | 0.15 ± 0.0188 | 0.17 ± 0.0681 |
| Number of leaves | 2 ± 0 | 2 ± 0 | 2 ± 0 | 2 ± 0 |
| Length of first pair of leaves (cm) | 2.93 ± 0.0742 | 2.23 ± 0.0671 | 1.37 ± 0.0151** | 1.15 ± 0.0188** |
| | 2.91 ± 0.0388 | 2.33 ± 0.0459** | 1.12 ± 0.0696** | 1.4 ± 0** |
| Breadth of first leaf pair (cm) | 1.55 ± 0.0176 | 0.5 ± 0** | 1.02 ± 0.0151** | 1.01 ± 0.0491** |
| | 1.45 ± 0.0424 | 0.61 ± 0.0275** | 1.01 ± 0.0302** | 0.88 ± 0.0302** |
| Thickness of stem (cm) | 0.55 ± 0.0176 | 0.45 ± 0.0176 | 0.55 ± 0.0186 | 0.41 ± 0.0302 |

*Significant at 5% level
**Significant at 1% level

releasing different amounts of allelochemicals into the surrounding environment (Scrivanti 2010), thereby inflicting different effects on other plants in that vicinity. Stronger inhibitory potential of leaf extracts than other vegetative parts was also reported for some other plants like *Parthenium hysterophorus* (Tefera 2002) and *Croton bonplandianum* (Sisodia, A., Siddiqui).

Present results indicated profound allelopathic inhibition of aqueous leaf extract of *H. brasiliensis* and *Q. indica* leaf extract on seed germination and early seedling growth of *V. radiata*. The statistical analysis showed that the inhibition shown by the two species are statistically highly significant (at 1% level) in terms of morphological parameters viz., shoot length, leaf length and leaf breadth when compared to the control seedlings. Both species reduced the shoot length (Fig 1), leaf length (Fig 2) and leaf breadth (Fig 3) of *V. radiata* seedlings irrespective of the treatment

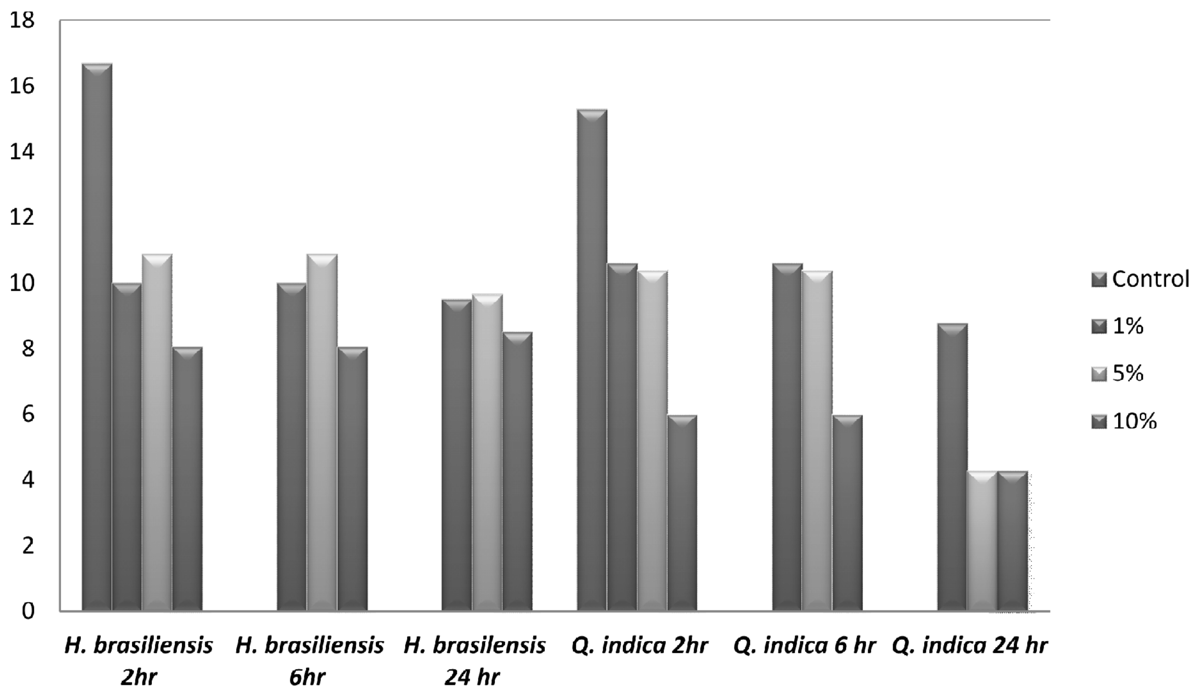


**Figure 1: Effect of *H. brasiliensis* and *Q. indica* leaf extract treatment on shoot length (in cm) of *V. radiata***

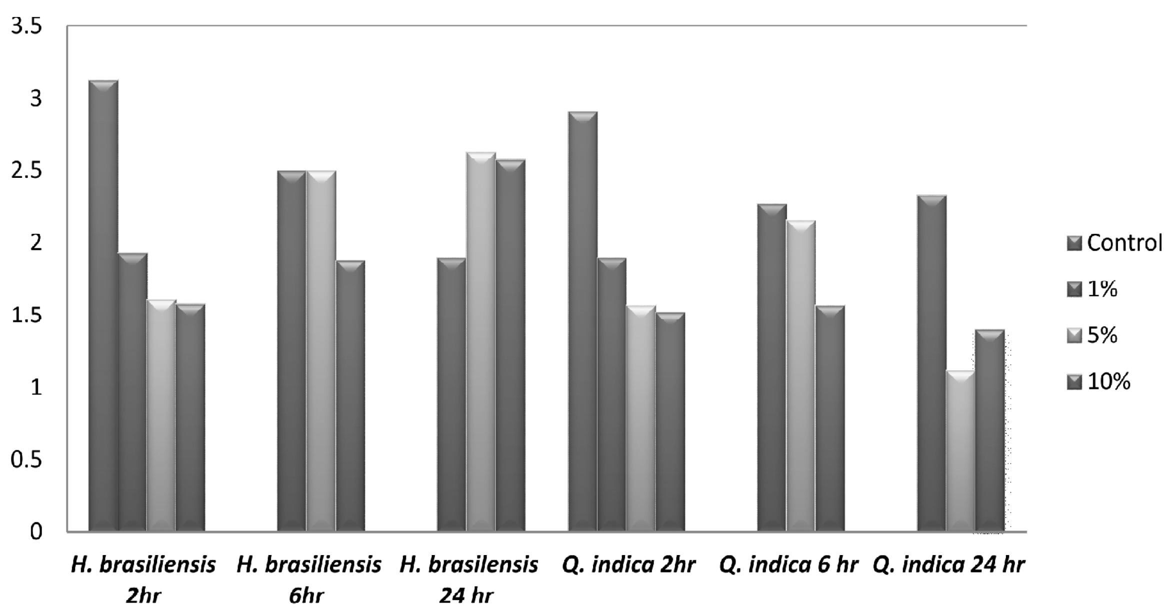


**Figure 2: Effect of *H. brasiliensis* and *Q. indica* leaf extract treatment on leaf length (in cm) of *V. radiata***

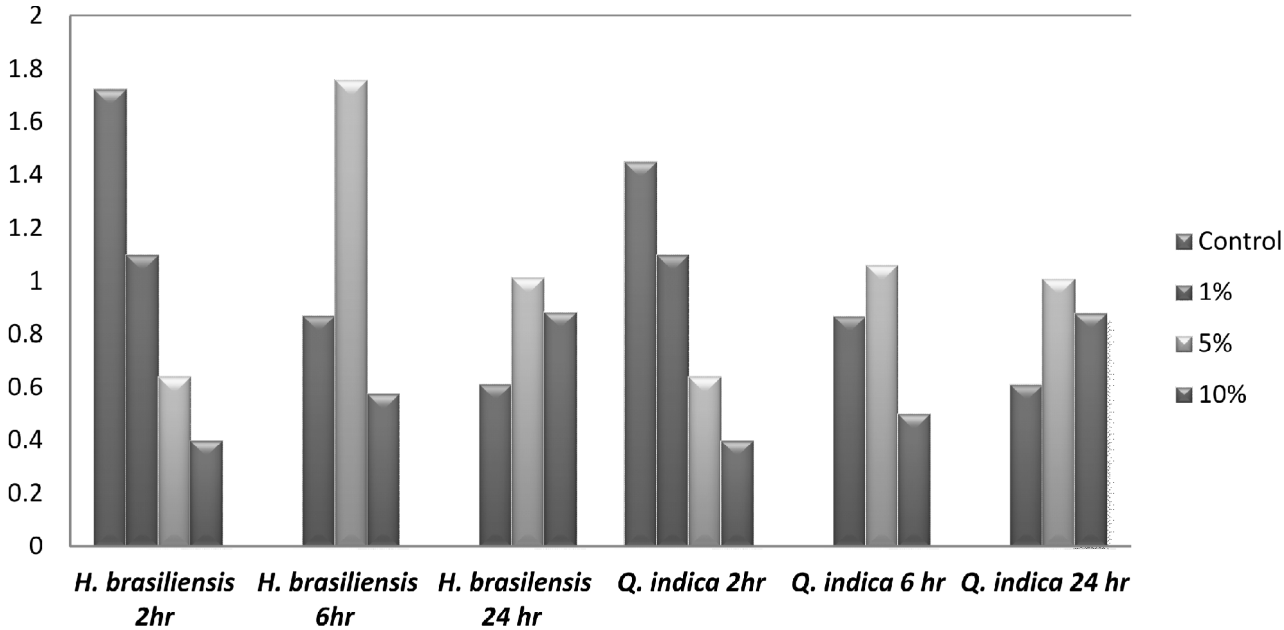


**Figure 3: Effect of *H. brasiliensis* and *Q. indica* leaf extract treatment on leaf breadth (in cm) of *V. radiata***

concentrations and exposures. Our results suggest that aqueous leaf extract of *H. brasiliensis* and *Q. indica* shows significant negative effects on the seed germination and seedling growth of *V. radiata*. To our knowledge this is the first report on the allelopathic effect of both species on a crop plant.

## CONCLUSIONS

Present results indicate profound allelopathic inhibition of aqueous leaf extract of *H. brasiliensis* and *Q. indica* leaf extract on seed germination and early seedling growth of *V. radiata*. Present results suggests that strong allelopathic inhibition exerted by both species may cause extinction of underneath flora in rubber plantations as well as in *Q. indica* cultivated areas respectively, and ultimately lead to loss of biodiversity. Since Kerala is richest in biodiversity and contains many fragile hotspots; caution must be taken against the changing land use patterns and spreading of monoculture plantations.

### Conflict of Interest

Authors declare that there is no conflict of interest

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