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Allelopathic effects of invasive plants (*Lantana camara* and *Broussonetia papyrifera*) extracts: implications for agriculture and environmental management

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ABSTRACT

We investigated the allelopathic effects of methanol and chloroform extracts from two invasive plant species, (*Lantana camara* and *Broussonetia papyrifera*), on radish seed germination and lettuce seedling development. The experiments employed radish seed germination assays, seedling growth measurements and the sandwich technique to evaluate the phytotoxicity of the extracts. The results revealed a concentration-dependent inhibitory impact on radish seed germination, with *L. camara* methanol extract exhibiting the highest suppression (72.85 ± 2.69 %) at 10,000 ppm. Comparative analyses between *L. camara* and *B. papyrifera* extracts demonstrated variations in their effects on root and hypocotyl development. Furthermore, the study explored the impact of these extracts on the germination index of radish seeds, indicating a progressive decline in seed germination rates with increasing extract concentrations. *B. papyrifera* methanol extract at 10,000 ppm showed the highest germination inhibition (60.48 ± 1.77 %). The sandwich technique, applied to lettuce seedlings, highlighted significant root and hypocotyl inhibition with increasing concentrations of *L. camara* and *B. papyrifera* extracts. Phytochemical analyses identified various compounds, including alkaloids, tannins, glycosides, flavonoids and phenols in both methanol and chloroform extracts. The presence of these compounds, especially phenolic chemicals, alkaloids and saponins is implicated in the observed phytotoxic effects. The study concludes that extracts from these invasive plants possess potent allelopathic properties, with implications for agricultural and environmental management. The findings emphasize the importance of understanding allelopathic interactions in developing sustainable strategies for crop protection and invasive species control.

Key Words: allelopathic cover crops, allelopathic mulches, integrated weed management, allelochemicals as bioherbicides, weed control

INTRODUCTION

In pursuit of sustainable and environmentally friendly alternatives to conventional weed management practices, the need for ecologically sound farming practices has become increasingly imperative (12). The prevalent use of synthetic herbicides and pesticides in agriculture has raised concerns due to documented health issues, both direct and indirect. Agricultural scientists are currently redirecting their research focus towards various plant species that hold promising potential for the development of innovative agricultural products, such as bio-herbicides and biopesticides (17). Allelopathy, a widespread biological phenomenon, involves the release of biochemicals by one organism that significantly impacts the growth, survival, development and reproduction of other organisms. These biochemicals, termed allelochemicals, can exert either beneficial or detrimental effects on

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target organisms. Harnessing the stimulatory or inhibitory influence of allelopathic plants is crucial for sustainable agricultural development enabling the regulation of plant growth and development while avoiding allelopathic autotoxicity. A key area of ongoing research is an exploration of phytotoxins, with their ability for direct application or models for creating novel herbicides marking a significant stride in weed management innovation (18).

The abundance of weeds in farming systems poses a significant challenge to the crops productivity (24). Traditional approaches to weed management involve mechanical techniques or the application of synthetic herbicides. However, mechanical methods demand considerable time and labor while the use of synthetic herbicides not only contributes to environmental pollution but is also associated with perceived detrimental effects on crop production (5). Additional drawbacks include the emergence of weed tolerance to these chemicals, escalating costs and an unfavorable cost-benefit analysis ratio (20). As a response, there is a global quest for agricultural chemical products that not only serve crop protection needs but are also environmentally beneficial.

The phytotoxic properties inherent in plants present an efficient and eco-friendly technique for weed and infection reduction (45). Studies have indicated that phytotoxins exhibit reduced danger to ecosystems, require fewer resources, are less toxic and possess biodegradable capacities offering a potential solution to the issues associated with synthetic chemicals (27). Phytotoxins are ubiquitous in almost every plant, found in various plant sections such as foliage, stems, blossoms, fruits, seedlings and spores. Foliage consistently synthesizes these compounds across different plant components (28,41). Plants release phytotoxins into the surrounding environment through processes related to ecology, including root emission, leaching, vaporization and damage to plant debris (35). The "phytotoxic potential" of these substances is defined by their effects on target plants, while a plant's "toxic capacity" is characterized by its ability to hinder or delay the development and propagation of seeds from other cultivars (36).

Broussonetia papyrifera, commonly known as Paper mulberry, is a deciduous, dioecious tree native to East Asia, prevalent in China and Japan and widespread in tropical and subtropical regions. Despite being intentionally introduced in Pakistan during the 1960s for avenue tree purposes, it has become highly invasive, listed among the six worst plant invaders in Pakistan (8, 9). The adverse effects of *B. papyrifera* on native vegetation include damaged ecosystem services, reduced natural biodiversity, negative impacts on human health and increased crow population acting as seed dispersal vectors (11,12,13). Its adaptability to diverse habitats, rapid growth, vegetative regeneration strategy, effective bird dispersal and allelopathic characteristics contribute to its invasive nature, making it one of the worst-known weeds of global importance (Figure 1).

Lantana camara, hereafter referred to as Lantana, belongs to the Verbenaceae family and manifests in two main forms: a cultivated compact variety and a weedy shrubby variety with a thorny stem. Indigenous to tropical and subtropical regions of South and Central America, Lantana has spread to various countries, including Pakistan (8). While initial seedling growth is slow, established roots lead to the formation of thickets through intertwining stems (Figure 2). Growing invasively in open, unshaded areas like wastelands, roadsides, railway tracks, canals, fence lines and agricultural fields, Lantana poses a serious threat to biodiversity (9). Allelopathy, non-palatability and competition for soil nutrients contribute to its successful invasion. Studies report the presence of aromatic phenols and

alkaloids in Lantana, inhibiting seed germination and growth in many plant species, further emphasizing its allelopathic nature (10,11,12,13,14).

In light of the escalating concerns surrounding the environmental and health impacts of synthetic herbicides and pesticides in agriculture, this study aimed to develop sustainable and eco-friendly alternatives for weed management, the phytotoxic properties of two allelopathic invasive plant species, *B. papyrifera* (Paper mulberry) and *L. camara* (Lantana). The objectives include investigating the allelopathic effects of methanol and chloroform extracts from these species on radish seed germination and lettuce seedling development. The study further seeks to evaluate the concentration-dependent impact of these extracts on seed germination rates, root and hypocotyl development and the germination index. Through comprehensive phytochemical analyses, the research aims to identify the key compounds responsible for the observed phytotoxic effects. The ultimate goal is to enhance our understanding of allelopathic interactions, providing valuable insights for sustainable agricultural practices, crop protection and invasive species control.

MATERIALS AND METHODS

Collection of plant material and Preparation of extracts

Fresh and healthy aerial parts of both *Lantana camara* and *Broussonetia papyrifera* were harvested from Rawalpindi (latitude 32°10-34°9N, longitude 71°10-73°55E) (Figure 1). After thorough cleaning under running water, the harvested specimens underwent a shade drying process. The desiccated materials were finely pulverized and each plant's powder underwent individual maceration in methanol and chloroform for an extended period of seven days. Following this maceration, the plant extracts were subjected to filtration. The resultant extracts underwent a concentration process utilizing a rotary evaporator set at 40°C. Subsequently, the concentrated extracts were labeled and then stored at 4 °C for further use. The healthy, viable seeds of radish (*Raphanus sativus* var. *longipinnatus*) and lettuce (*Lactuca sativa*) were purchased locally from the seed store.

(i) (ii)

Figure 1 (a). *Broussonetia papyrifera* (i) Single plant (ii) Population

(i) (ii)

Figure 1(b). *Lantana camara* (i) Single plant (ii) Population

Radish Seed Germination Assay

Following the methodology outlined by Turker & Camper (40), a radish seed germination experiment was conducted. Plant extracts were applied to Whatman No. 1 filter-lined petri plates at different concentrations (10, 100, 500, 1000, and 10000 ppm), with 10 radish seeds per plate. The growth suppress percentage was calculated using root measurements taken on the first, third, and fifth days. In a separate experiment, 100 radish seeds were subjected to three distinct concentrations (100 parts per million) for five consecutive days, and the sprouting rate was monitored each day.

Growth suppress % = 100 $(P_C\text{-}P_T)/P_C$

Here P_T or P_C stands for the treatment's and the control's respective root lengths.

In a separate experiment, 100 radish seeds were subjected to three distinct concentrations (100, 1000, and 10000 ppm) for five consecutive days, and the germination rate was monitored each day. The germination index was calculated as:

$$\mathrm{GI} = (N1) + \frac{N2 - N1}{2} + \frac{N3 - N2}{3} + \cdots + \frac{Nn - Nn - 1}{n}$$

Where N1, N2, N3-----Nn = Proportion of seeds which germinated on day 1-----n.

Sandwich method

Agar solution (0.5 % w/v) was prepared and autoclaved at 121°C for 15 minutes. Plant material (10, 30, and 50 mg separately) was tipped into the wells of a six-well multi-well plate. The first layer of agar (5 ml) was applied by pipette which caused dried plant material to rise. After the gelatinization of the first layer, a second layer of agar was applied to it. After the gelatinization of the second layer, in each dish, five seeds of lettuce were placed. Multi dishes were covered with aluminum foil and kept in an incubator at room temperature for 3 days. Length of radicle and hypocotyl was noted for each plant and growth suppression (%) was calculated:

Growth Suppression (%) = 100 $(P_C\text{-}P_T)/P_C$

P_C or P_T stands for the treatment's and the control's corresponding root or hypocotyl lengths.

Qualitative Phytochemical Analysis

Extracts from plants were subjected to plant chemical examination in the following ways:

Test for alkaloids. An aliquot of plant extract was mixed with 8 mL of 1 % HCl, warmed, and filtered. Filtrate was treated separately with Mayer's reagent and Dragendorff's reagent. The appearance of turbidity/precipitation indicated the presence of alkaloids.

Test for flavonoids. An aliquot of plant extract was dissolved in 10 mL of 80 % ethanol and filtered. Filtrate was mixed with 4 mL of 1 % KOH. The appearance of a dark yellow color indicated the presence of flavonoids.

Test for coumarins. An aliquot of plant extract was taken in a test tube and covered with filter paper moistened with 1 N NaOH. The test tube was placed in boiling water. The filter paper was removed after a few minutes and observed in UV light. Yellow fluorescence indicated the presence of coumarins.

Test for phenols. An aliquot of plant extract was treated with drops of $FeCl_3$ solution. The appearance of a bluish-black color indicated the presence of phenols.

Test for saponins. An aliquot of plant extract was dissolved in boiling water in a test tube, allowed to cool, and shaken thoroughly. Froth formation indicated the presence of saponins.

Test for tannins. An aliquot of plant extract was boiled in 10 mL of distilled water and filtered. 0.1 % $FeCl_3$ was added to the filtrate. The appearance of brownish-green or blue-black coloration indicated the presence of tannins.

Test for glycosides. An aliquot of plant extract was dissolved in 2.0 mL of glacial acetic acid containing one drop of 0.1 % $FeCl_3$ solution. The mixture was then underlaid with 1.0 mL of concentrated H_2SO_4. A brown ring at the interface indicated the presence of glycosides.

Statistical analysis

Statistical analysis of the data was conducted through one-way analysis of variance (ANOVA) at a significance level of $p \leq 0.05$, followed by post-hoc Tukey's test. The entire analysis was performed using SPSS/PC software ver. 16.0 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Radish seed germination assay

Each of the five evaluated concentrations of *L. camara* methanol and chloroform extracts demonstrated a suppressive impact on radish seedlings (Figure 2). Multiple scientific studies have reported the inhibitory effect of *L. camara* extracts on various crops (7,15,23). The concentration of the extract played a crucial role in determining the inhibitory effects, with the *L. camara* methanol extract exhibiting the highest inhibition (72.85 ± 2.69 %) at the maximum tested concentration of 10,000 ppm (Figure 3a). At the same concentration, the *L. camara* chloroform extract showed a percentage inhibition of 54.70 ± 2.26 % (Figure 3b). This observation sheds light on the polar nature of the bioactive compounds responsible for germination suppression, aligning with Achhireddy *et al*. who highlighted the polar and somewhat acidic nature of phytotoxic chemicals in *L. camara* (1). Comparative analysis of the inhibitory action of the two extracts at different concentrations revealed no discernible difference among *L. camara* methanol extracts at concentrations of 100 ppm and 500 ppm (37.46 ± 1.49 % and 45.19 ± 1.49 %, respectively). Similar results

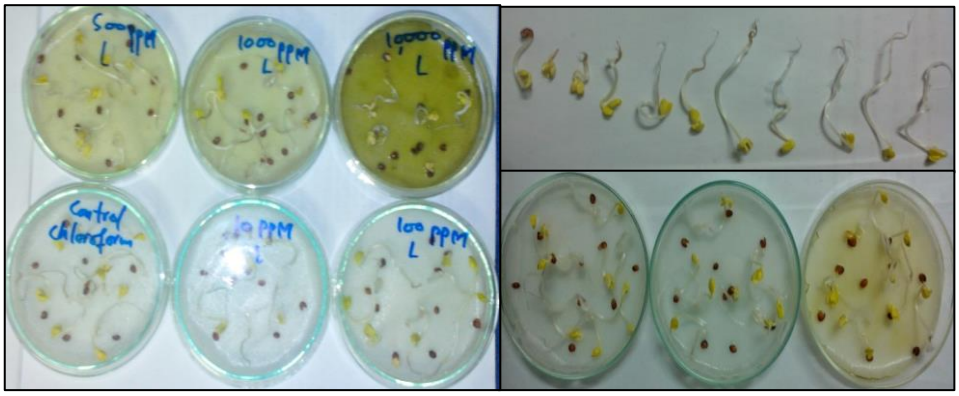

Figure 2. Radish seed germination and early seedling development at various concentrations

were observed for 1000 ppm and 10,000 ppm methanol extracts (64.86 ± 7.89 % and 72.85 ± 2.69 %, respectively). The percentage of root length suppression among *L. camara* chloroform extracts at concentrations of 500 ppm and 1000 ppm (33.65 ± 8.13 % and 43.24 ± 5.37 %, respectively) and 1000 ppm and 10,000 ppm (43.24 ± 5.37 % and 54.70 ± 2.26 %, respectively) were noted. Significant variations in root length suppression were observed among different doses in radish seedlings (Figure 3c).

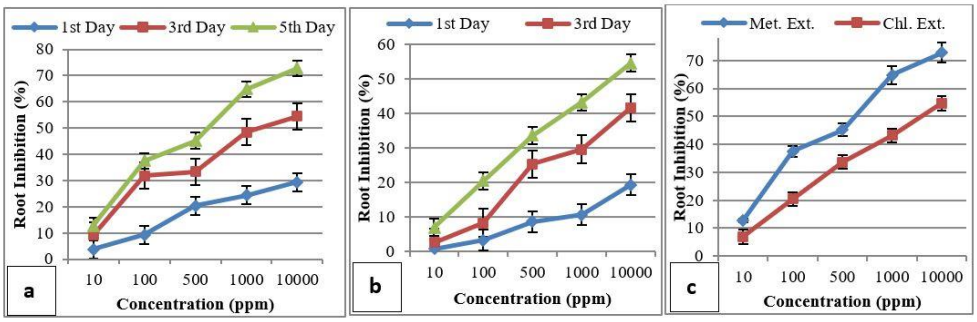

Figure 3. Radish seedling root growth suppression (%) at varying concentrations of *L. camara* (a): methanol extract; (b): chloroform extract; (c): radish seedling root growth suppression (%) observed at various levels of *L. camara* extracts on the 5th day of experiment.

The subsequent stage of the experiment examined the implications of three distinct concentrations of extracts on the germination index. On the fifth day of incubation, a progressive decline in the seed germination rate for all samples was seen (Figure 4). A prominent outcome observed in the biological assays involving the allelopathy is a reduction in seed germination (30,38). The chloroform extract of *L. camara* had the greatest germination inhibition in terms of the germination rate (63.53±4.73 %) at a 10,000 ppm concentration. The germination percentage for *L. camara*'s extract of methanol was 76.80±3.79 %. The cell division and elongation may be interfered with by phytotoxins (2). Charoenying *et al*. (6) presented identical findings. *Zanthoxylum limonella* chloroform

extract suppressed the germination of seeds in *Raphanus sativus* while the methanol extract delayed root development. Aquatic extracts of *Thymus kotschyanus* did not significantly affect the germination rate of *Bromus tomentellus* among 5 % extracts, but at 25 % and 50 % levels, there was a significant decrease (29).

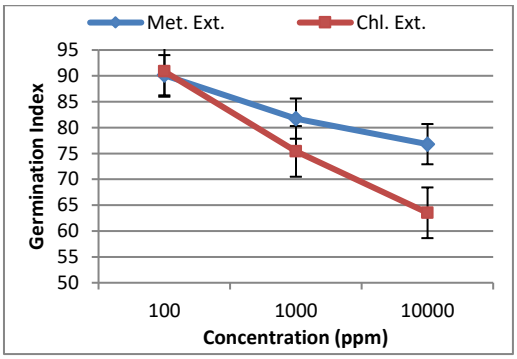

Figure 4. Inhibitory effects of *L. camara* extracts on germination index of radish seeds

B. papyrifera significantly inhibited the root development of radish seedlings when exposed to both polar and non-polar extracts. Notably, the methanol extract demonstrated the highest efficacy in preventing root development at a concentration of 10,000 ppm (60.48 ± 1.77 %). The chloroform solution of *B. papyrifera* exhibited suppression of 49.44±3.25 % at the same concentration (Figure 5).

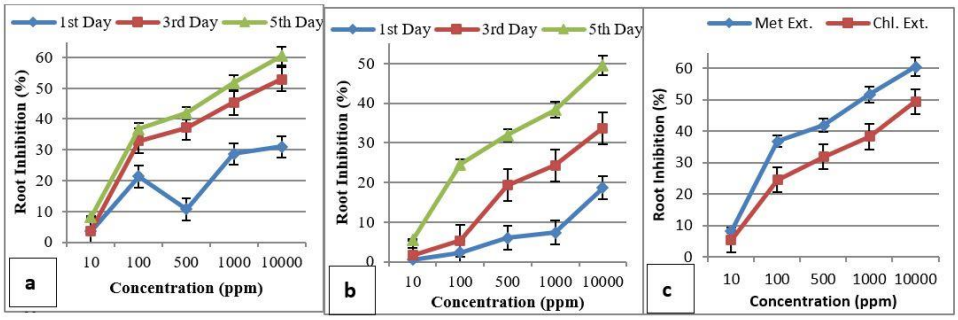

Figure 5. Radish seedlings root suppression at various concentrations of *B. papyrifera* (a): methanol extract; (b): chloroform extract; (c): radish seedlings percent root stop of at various level of *B. papyrifera* extract (5^{th}-day data)

There was no significant difference in root length suppression by *B. papyrifera* methanol extracts at concentrations of 100 ppm and 500 ppm (36.77 ± 7.68 % and 41.93 ± 3.0 %, respectively). Similarly, no significant differences were observed in root length inhibition between 500 ppm and 1000 ppm (41.93 % and 51.67 %, respectively) or between 1000 ppm and 10,000 ppm (51.67 % and 60.48 %, respectively). The chloroform extract showed no significant root length inhibition between 500 ppm and 1000 ppm. The extent of root suppression in radish seedlings varied significantly across all concentrations, indicating

a dose-dependent response. Additionally, the application of varying concentrations of *B. papyrifera* methanol and chloroform extracts had a significant impact on the germination index of radish seeds. The chloroform-based extract at 10,000 ppm demonstrated the highest suppression of germination rate (63.13 ± 5.86) (Figure 6).

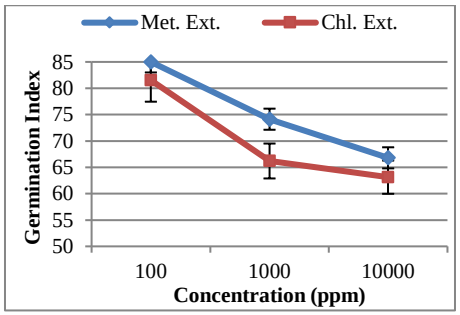

Figure 6. Decrease in the germination index of radish seeds by *B. papyrifera* extracts

The negative impact of *B. papyrifera* was more pronounced during seed germination than seedling development, aligning with previous studies (17). Furthermore, the polar extracts exhibited stronger phytotoxic effects compared to their nonpolar counterparts, consistent with existing research (34).

Sandwich method

In the sandwich technique, lettuce (*Lactuca sativa*) seedlings were subjected to various concentrations (10 mg, 30 mg, and 50 mg) of pulverized *Lantana camara* aerial parts, and two parameters, root length inhibition and percent hypocotyl inhibition (%) were measured (Figure 7).

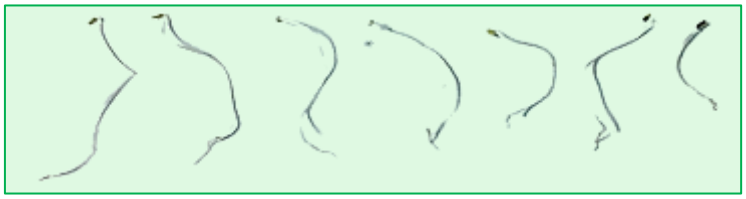

Figure 7. Seedling development of Lettuce in the Sandwich method

Exposure to different levels of *L. camara* aerial parts resulted in a reduction in root length, with the 50 mg concentration showing the most significant root length inhibition (49.26 ± 5.40 %) (Table 1). The inhibitory effects on root length decreased to 39.21 ± 0.87 % at 30 mg and 20.62 ± 1.74 % at 10 mg of *L. camara*. Anjum *et al.* (3) reported that 50 mg concentration had the most noticeable impact on root and hypocotyl development when initially assessing the allelopathic effects of 14 ethnobotanical species using the sandwich method. Different concentrations exhibited significantly diverse inhibitory effects, indicating a strong dose-dependent influence on lettuce seedling development suppression. Consistent with previous studies (16,39), a higher concentration of phytotoxins may be responsible for more profound suppression at higher concentrations.

B. papyrifera, at a concentration of fifty mg, demonstrated a significant root length suppression of 42.94 ± 1.32 %. The suppression decreased to 33.14 ± 3.51 % at a concentration of 30 mg and at 10 mg the rate of root suppression was 24.54 ± 1.72 % (Table 1). The greatest suppression of hypocotyl elongation (32.15 ± 2.18 %) occurred at a concentration of 50 mg *B. papyrifera*. At a concentration of 30 mg, the percentage of hypocotyl elongation suppression was 19.82 ± 1.52 %, while at 10 mg of *B. papyrifera*, it was 9.63 ± 0.79 %. These findings suggest that leaf leachates influence germination and seedling growth in a concentration-dependent manner (10).

Table 1. Inhibitory effects of leaf litter leachates of *L. camara* and *B. papyrifera* root and hypocotyl

Dose (mg)	**Root Inhibition (%)**	**Hypocotyl Inhibition (%)**	**Root Inhibition (%)**	**Hypocotyl Inhibition (%)**
	Lantana camara		*Broussonetia papyrifera*	
10	20.62±1.74[a]	5.44±4.17[a]	24.54±1.72[a]	9.63±0.79[a]
30	39.21±0.87[b]	10.37±1.09[a]	33.14±3.51[b]	19.82±1.52[b]
50	49.26±5.40[c]	25.90±1.04[b]	42.94±1.32[c]	32.15±2.18[c]

Data in the column marked by unique lowercase letters indicate significant differences at a level of $p < 0.05$.

When comparing the results of both the Sandwich technique and the Radish seed growth assay-it is evident that plant extracts significantly hindered the development of the tested species. According to Tefera (37), *Parthenium hysterophorus* leaf extract has a stronger inhibitory effect than any other plant component. The results of two biological assays for phytotoxicity showed that the hazardous effects of aerial components derived from two invasive plants (germination of seeds, length of root in radish and lettuce) depended upon the plant variety and the extract quantity. Worldwide, scientists are exploring the molecular functions of phytotoxins because they offer a substitute for manufactured nematocides, herbicides, and insecticides within agri-environments (26). According to Onocha *et al*. (25), *Lemna minor* was significantly harmed by the methanolic-derived substance of *Acalypha torta, A. wilkesiana,* and *A. hispida* (Euphorbiaceae) foliage. Shanee *et al*. (33) examined the harmful effects on plant growth caused by water-based extracts from different concentrations of *Euphorbia dracunculoides*. Khan *et al*. (19) found that *Euphorbia prostrata* relies on dose suppression to influence crop wheat sapling establishment and growth. *Sageretia thea* contains phytocidal compounds that are harmful to *Lemna minor* (32). These conclusions provide substantial support for the present findings. Additionally, the results of phytotoxicity studies show that plant extracts have a higher potential for suppression than dried plant materials.

Throughout the current experiment, plant-derived substances obtained from methanol and chloroform solvent materials underwent a basic phytochemical examination. A phytochemical analysis of two *L. camara* fractions revealed the presence of alkaloid compounds, tannins, glycosides, flavonoids, and phenols in both polar and non-polar extracts, excluding saponins (Table 2).

Compared to the chloroform extract, the methanol extract showed more evidence of the existence of various botanical constituents. This may be the reason for the polar extract's improved phytotoxicity and cytotoxicity. Similarly, the methanol extract of *B. papyrifera* exhibited the presence of phenols, tannins, glycosides, flavonoids, coumarins, alkaloids, and

Table 2. Qualitative phytochemical analysis of plant extracts.

Plant chemical	***L. camara* extract**		***B. papyrifera* extract**	
	Met.	Chl.	Met.	Chl.
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	-	+	-
Tannins	+	+	+	-
Coumarins	+	+	+	+
Phenols	+	+	+	+

'+' present '-' not-present

saponins. Conversely, the chloroform extract was found to lack tannins and saponins. Phenolic chemicals, including flavonoids, tannins, and phenols, are the most prevalent and broadly distributed hazardous metabolites in crops (4). These phenolic compounds have significant uses in farming as insecticides, herbicides, and fungicides (31). Alkaloids, also naturally toxic, assist vegetation in fending off intruders and act as potential biological weedkillers (11,21). Saponins play a protective role in defending plants against phytopathogenic microbes, insects, and animals due to their toxicity (8,22,42). The presence of these poisonous substances, such as phenolic compounds, alkaloids, and saponins in the extracts may be responsible for the hazardous effects observed in this study.

CONCLUSIONS

The radish seed germination assay revealed that both methanol and chloroform extracts from *Lantana camara* exerted a suppressive impact on radish seedlings, with varying degrees of inhibition observed at different concentrations. The methanol extract exhibited the highest inhibition at the maximum tested concentration of 10,000 ppm, emphasizing the role of concentration in determining inhibitory effects. The germination index assay further demonstrated a decline in seed germination rates for all samples, with the chloroform extract of *L. camara* displaying the highest germination inhibition at 10,000 ppm. Similar inhibitory effects were observed with *Broussonetia papyrifera* extracts, where the methanol extract showed superior efficacy in preventing root development. The findings emphasize the concentration-dependent responses and highlight the potential of plant extracts to influence germination and seedling growth. Phytochemical analyses of the extracts revealed the presence of various compounds, such as alkaloids, tannins, glycosides, flavonoids, and phenols with polar extracts exhibiting enhanced phytotoxicity. The study contributes valuable insights into the allelopathic effects of these plant extracts, emphasizing their potential as sources of bioactive compounds with applications in agriculture and environmental management.

ACKNOWLEDGMENTS

NA

CONFLICT OF INTEREST

The authors announce that they have no conflicts of interest.

DECLARATION

We declare that all authors of this manuscript have made substantial contributions. We have not excluded any author who substantially contributed to this manuscript. We have followed the ethical norms established by our respective institutions.

AUTHORS CONTRIBUTION

1. Qureshi, T. Anwar, and S. Fatima: Conceptualization, Methodology, Data curation, Formal analysis, review, and editing
2. Shirani, S. Riaz, and Z. Liaquat: Investigation, Data curation, Formal analysis, writing

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct.

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