PHYTOTOXIC INFLUENCE OF AQUEOUS LEAF EXTRACTS OF PSIDIUM GUAJAVA L. AGAINST CAPSICUM FRUTESCENS L. AND SOLANUM LYCOPERSICUM L.

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ABSTRACT

Phytotoxic interactions in tree crop associations in agroforestry greatly influence crop production. When trees and crops are grown together, they interact with each other, either through overt or indirect allelopathic interaction, inhibiting or stimulating their growth or yield. The present research aimed to test the phytotoxic influence of Psidium guajava aqueous leaf extracts on seed germination, seedling growth and some biochemical changes of Capsicum frutescens L. and Solanum lycopersicum L. Leaf extracts of P. guajava constrained the germination and seedling development, affected pigment content such as chlorophylls, carotenoids and stimulated the activity of antioxidant enzymes of C. frutescens and S. lycopersicum vegetable crops. C. frutescens was more effective than S. lycopersicum treated with different concentrations of P. guajava leaf extracts.This study showed that the inhibitory effect was concentration-dependent and was more pronounced at higher concentrations. P. guajava has a unique allelochemical that inhibits germination and growth of both seedlings. The present study proves that P. guajava extract could be proficiently exploited as a phytotoxic effect against common crops grown in crop fields.

Keywords: *Phytotoxic; Capsicum frutescens; Solanum lycopersicum; Psidium guajava; allelochemicals.*

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INTRODUCTION

Agroforestry is the interaction of agriculture and trees. Interactions between trees and other agriculture components may be important at various scales: in fields, on farms, and in landscapes. Most agroforestry species produce a good amount of leaf, litter, and debris rich in allelochemical content. In exchange, these allelochemicals provide agroforestry plants with different pest control properties. Therefore, their allelopathic materials may be used as mulch, and their leachates and distilled compounds may be eco-friendly alternatives to synthetic pesticides. Allelopathy refers to the adverse effect of one plant by releasing such chemicals on another crop. These interactions are mediated by allelopathic substances which affect others and are secreted from the species. (Gniazdowska *et al*. 2004). In various species, allelopathic interactions have been identified between individuals of the same species called 'Intraspecific

Toxicity' or 'Autotoxicity' or 'Autoallelopathy' (Kumar,1991). In comparison, when accused organisms are taxonomically distinct from donor or agent species, the term 'Teletoxicity' is used (Kushal 1987). Allelochemicals released from plants are useful in many agricultural settings for weed control options to minimize reliance on commercial herbicides. (Putnam 1988, Weston 1996, Narwal 1999). The most secondary metabolites are allelopathic compounds produced by higher plants. The most active compounds include phenolics, cyanogenic glycosides, quinones, lactones, organic acids, and volatile terpenes. Via volatilization, root exudation, leaching, and decomposition of plants, these substances are released into neighbouring habitats and prevent the growth of neighbouring plants. (Belz, 2007).

Psidium guajava (guava) belongs to the family Myrtaceae and certain bioactive compounds, such as avicularin, quercetin, and guaijaverin, are present in its leaves [Morant *et al*., 2008]. Potential allelopathic metabolites [Monteiro *et al*., 2002], such as flavonoids, terpenoids and cyanogenic acids, are also present in guava leaves.[Gutierrez *et al*., 2019]. Only a few studies have tested guava's allelopathic effects against other plant species [Chapla and Campos, 2010]. *Psidium guajava* is a well-known tropical tree that is widely cultivated for fruit. For medicinal purposes, many nations have a long tradition of using guava. This plant finds uses for diarrhoea, dysentery, gastroenteritis, hypertension, diabetes, caries, and pain relief treatment and improvement of coordination of locomotors. Its leaf extract treats cough, diarrhoea, oral ulcers, and swollen gum injuries. Fruits are rich in vitamins A, C, iron, calcium, phosphorus and minerals. It contains large amounts of organic and inorganic compounds such as secondary metabolites, antioxidants, polyphenols, antiviral and anti-inflammatory compounds. The phenolic compounds found in guava help heal cancer cells and prevent premature aging.Terpenes, caryophyllene oxide and *p*-selinene create relaxation effects. There are several compounds in guava leaves that serve as fungistatic agents and bacteriostatic agents. Guava is rich in significant antioxidants and has a radio-protective ability.Quercetin is the most active antioxidant found in guava leaves and is responsible for its antispasmodic properties. Ethyl acetate extract inhibits bacterial infection and thymus formation. Guava has antiviral, anti-inflammatory, anti-plaque and anti-mutagenic effects. Guava extract exhibits antinociceptive effects and is also effective against liver injury, inflammation, and serum production.

Capsicum frutescens belongs to the Solanaceae family, and it is a much-branched, erect, perennial plant growing from 1 - 2 meters tall. The stem can become more or less woody near

the base. The species is widely cultivated around the world, especially in temperate to tropical climates, for its edible fruits are used for flavoring foods and for medicinal purposes. Eggplant (*Solanum lycopersicum*) is an annual plant of the Solanaceae family. Commonly called tomato, it is an erect or spreading annual plant that ranges in size from 30 cm to over 2 meters. Tomatoes are a famous fruit and are grown all over the world. In addition to the edible fruit, an oil is also obtained, used as an insect repellent, and has many traditional medicinal uses. The present study was designed to test the allelopathic effects of aqueous leaf extracts of *P. guajava* on seed germination, seedling growth, and specific biochemical parameters of *S. lycopersicum* and *frutescens.*

MATERIALS AND METHODS

The present work was performed in the Department of Botany, Annamalai University, Annamalai Nagar, India. For this experiment, *P. guajava* fresh matured leaves were harvested in the surrounding areas of the University campus. After washing the leaves with tap water, the leaves were also washed with distilled water. The leaves were shadow-dried and ground to powder using a mortar and pestle at room temperature. The extracts were developed in 1000 mL of sterilized water by mixing 100 g of powdered leaves and stored at room temperature for 12 hours. Leaf extracts were then purified and the crude extract was diluted to obtain various concentrations (Whatman No. 1), i.e., 10%, 20%, 30%, 40% and 50% (w/v) solutions. The control represents plants treated with distilled water.

The viable *C. S. and Frutescence seeds* were sown in 10 cm diameter, 10 cm depth mud pots filled with garden soil. 10%, 20%, 30%, 40%, and 50% freshly prepared aqueous foliar concentrates were sprayed on the surface of *C. Frutescen*s and *S. lycopersicum* seedlings evenly cover the entire seedling surface. The control pots were sprayed with distilled water. Each process was repeated three times. One month after sowing, the seedlings were uprooted and washed with tap water to remove root soil. The biophysical and biochemical parameters of seedlings were then examined.

Pigment Analysis

Chlorophyll Content: Using a mortar and a pestle, 1 g of fresh leaves was crushed with 3 ml of 80% acetone. Then, the homogenised content was centrifuged at 10,000g for 20 min at 4 C. At 645 and 663 nm, the supernatant absorbance was collected, and pigments were quantified using a UV-visible double-beam spectrophotometer, according to Arnon (1949).

Carotenoid content: 1 g of fresh leaves was homogenized using a mortar and a pestle with 4 mL of 80% acetone. Then, the homogenised content was centrifuged at 10,000g for 20 min at 4 °C. At 480 and 510 nm, the supernatant absorbance was collected, and the carotenoid content was quantified by Maclachlan and Zalik (1963).

Proline Content: The approach used by Bates *et al*. (1973) was used for proline estimation. A 0.5 g aliquot of fresh leaves was homogenized in a sulfosalicylic acid solution (3% by volume) using a mortar and pestle and centrifuged at 10,000 g for 10 minutes at 4°C. 2 ml of the supernatant was then mixed with 2 ml of ninhydrin and glacial acetic acid and the mixture was incubated in a boiling water bath for 1 hour. Absorption was measured at 520 nm.

Catalase Activity: The catalase activity was determined using the method described by Aebi (1984). In the reaction mixture comprising 1.2 mL of hydrogen peroxide (15 mM), 300 µL of enzyme extract, and 1.5 mL of phosphate buffer, the decomposition rate of hydrogen peroxide was followed by a decrease in absorbance at 240 nm (50 mM; pH 7.0).

Superoxide dismutase (SOD) activity: Superoxide dismutase activity was calculated according to the Kono dismutase process (1978). A mixture of 1.3 mL of sodium carbonate buffer (50 mM, pH 10.2), 500 µL of 24 µM of nitroblue tetrazolium (NBT) and 100 µL of Triton X-100 (0.03 per cent v/v) was prepared in the test cuvettes. After the addition of 100 µL hydroxylamine hydrochloride, the reaction began. 70 µL of the enzyme extract was added after two minutes and the rate of NBT reduction was observed following an increase in absorbance at 540 nm.

Dehydroascorbate Reductase Activity: The DHAR activity was estimated according to the method of Dalton *et al*. (1986). The mixture included 1.5 mM of reduced (reduced) glutathione and 50 mM of phosphate buffer. A dehydroascorbate of 0.2 mM and a crude extract. The absorbance was then collected at 265 nm. The enzyme's function was determined using an extinction coefficient of 14 mM-1 cm-1.

Ascorbate Peroxidase Activity: In 3 mL of phosphate buffer (50 mM, pH 7.0), an aliquot of 0.5 g of leaves was collected and centrifuged for 20 min at 5000g. A blend of 1.5 mL of phosphate buffer (50 mM, pH 7.0), 300 µL of ascorbate (0.05 mM), 600 µL of H_2O_2 (1 mM) and 600 µL of plant extract was prepared and 290 nm of absorbance reduction was controlled. (Nakano and Asada 1981).

Statistical Analysis

All the experiments were carried out in triplicates and carried out separately three times with consistent findings. Data is defined as the mean of replicates ± SD. Differences between treatments were then tested using one-way-variance analysis (ANOVA) for each parameter under review and followed by Duncan's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Seed Germination: The aqueous leaf extracts of *P. guajava* reduced the germination percentage of *C. frutescence* and *S. lycopersicum* seeds. An increased *P. guajava* extract concentration of 50 % showed a substantial decrease in seed germination, while 10 % and 20 % induced a similar seed germination inhibition. (Fig -1).

Fig :1

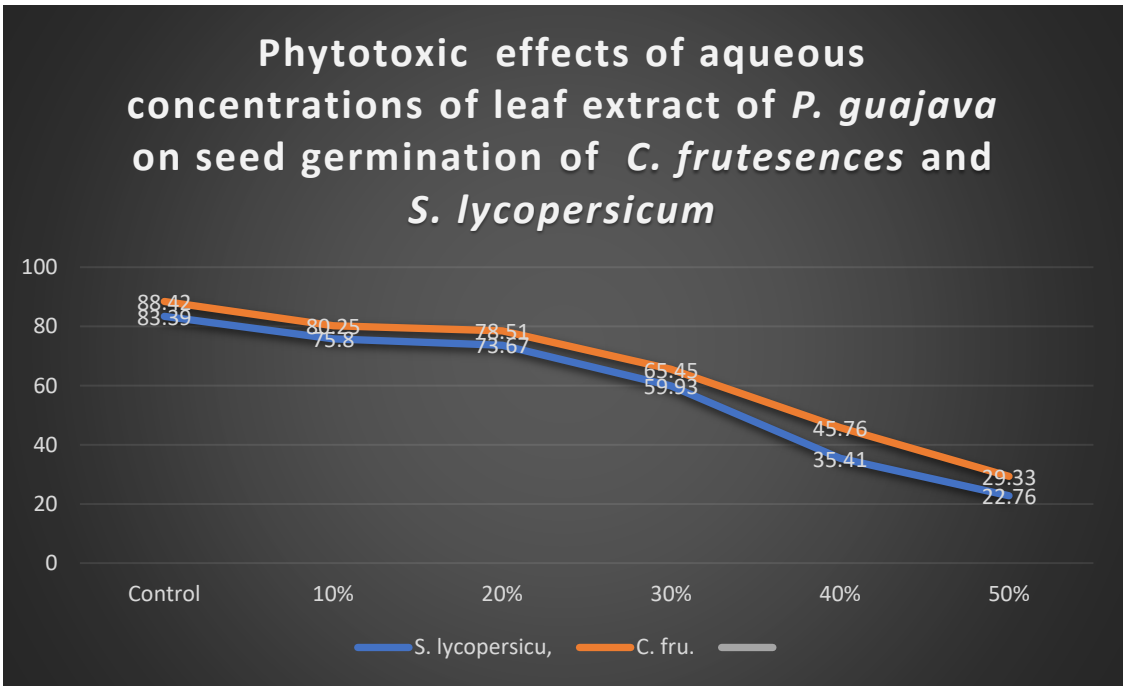


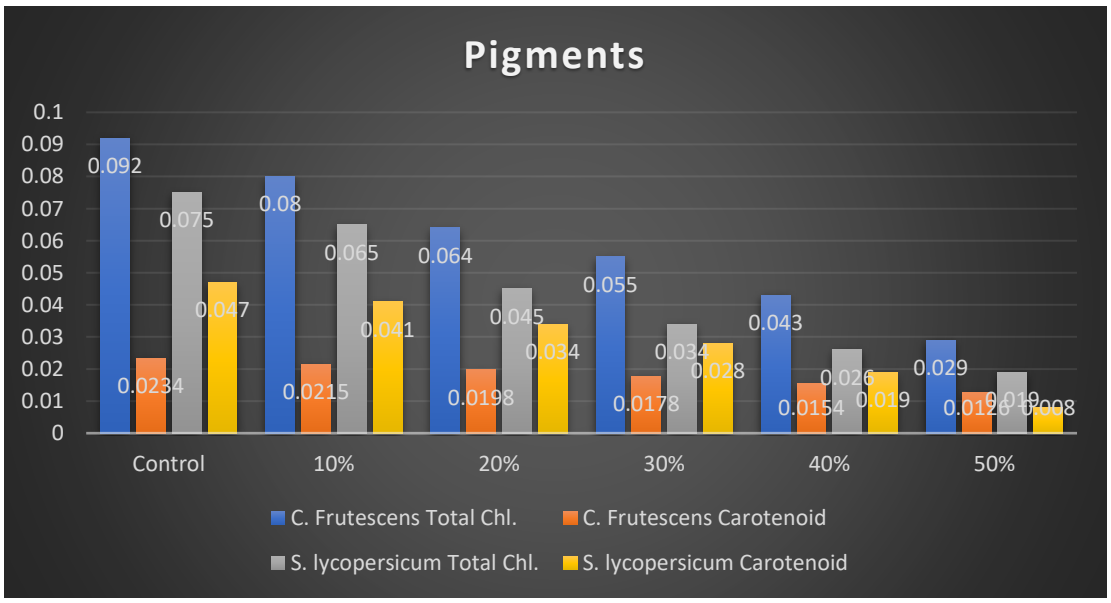
Table – 1:Allelopathic effects of various concentrations of leaf extract of *Psidium guajava* on root and shoot length of *C.fruteseces* and *S. lycopersicum* seedlings

Extract Concentration	*C. frutesences*		*S. lycopersicum*	
	Root length	Shoot length	Root length	Shoot length
Control	13.46	22.4	12.25	20.8
10%	8.34 (-38.0)	18.13 (-19.1)	8.32 (-32.1)	17.78 (-14.5)

20%	7.98 (-40.7)	15.9 (-29.0)	7.36 (-39.9)	16.56 (-20.4)
30%	5.68 (-57.8)	12.89 (-42.5)	4.56 (-62.8)	11.34 (-45.9)
40%	4.32 (-67.9)	8.77 (-60.8)	3.67 (-70.0)	7.88 (-62.1)
50%	3.14 (-76.7)	5.56 (-75.2)	2.75 (-77.5)	4.76 (-77.1)

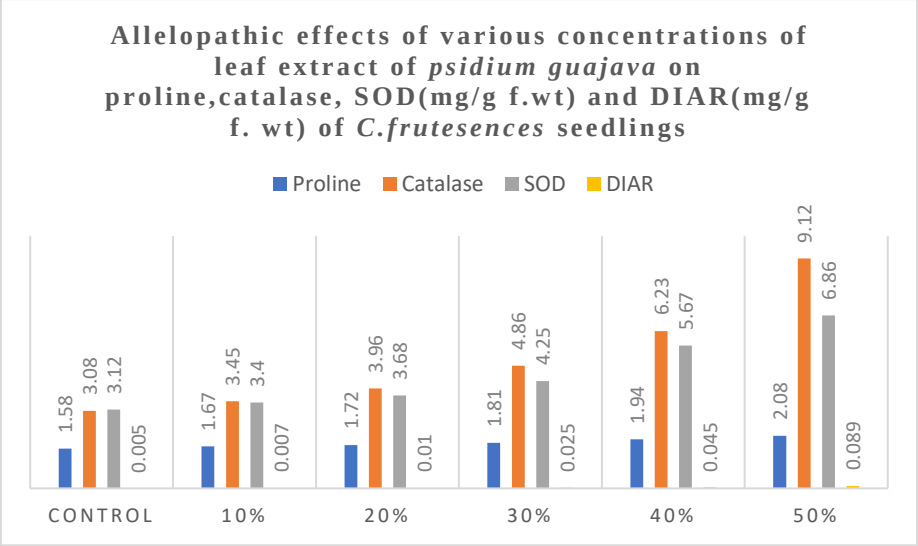
The extract of *P. guajava* inhibited the shoot and root length of *C. frutesences* and *S. lycopersicum* seedlings. *P. guajava* extract was effective even at the lowest concentration applied (10%) inhibited the root development significantly. *P. guajava* extract showed a higher reduction in both seedlings' shoot length and root length, which started to be effective even at the lowest dose. In seedlings treated with a higher level (50 %) of *P. guajava* extract, minimum root and shoot lengths were reported. (Table 1).

Fig: 2. Comparison of total chlorophyll (mg/g f.wt) and carotenoid (mg/g f.wt) content in seedlings of *C. frutesences* and *S. lycopersicum* treated with leaf extract of *Psidium guajava*.



Chlorophyll content decreased when the seedlings were treated with various concentrations of leaf extract of *P. guajava*. In seedlings of S, the most significant decrease in chlorophyll content was re*ported. Lycopersicum* treated with *P. guajava* extract at the maximum concentration (50 %) relative to *C.frutesences*. Seedlings treated with a lower concentration (10%) of P. guajava showed a smaller reduction in chlorophyll content (Fig: 2). Simultaneously, *C. frutesences* seedlings showed a decrease in carotenoid content when treated with the highest dose of *P. guajava* extract (50%). The *S. lycopersicum* seedlings also exhibited significant inhibition of pigments.

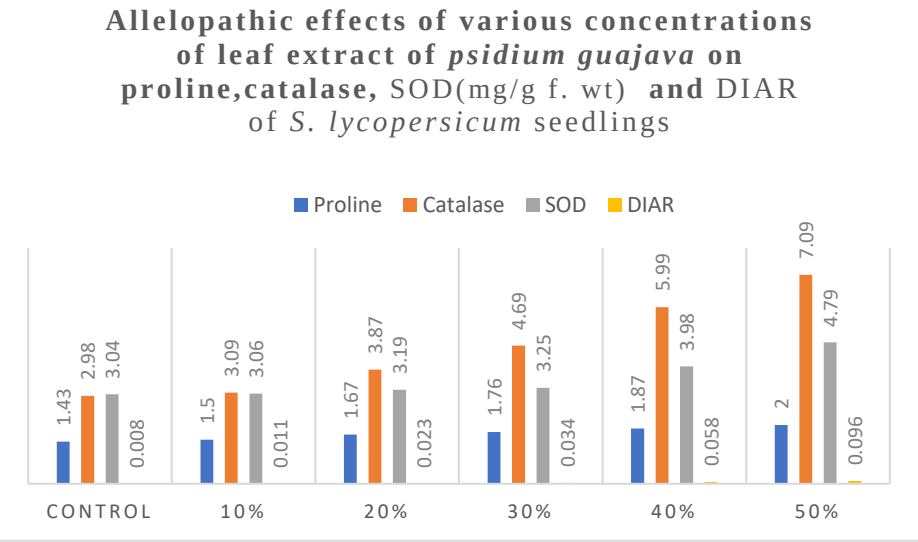
Fig : 3



The highest Super oxide dimutase activity (Fig: 3&4) was observed in both test crops treated with different concentrations of *P. guajava* aqueous leaf extract. Maximum activity of Super oxide dimutase was observed in 40 and 50% extract treatments in the seedlings.

Dehydroascorbate Reductase activities were stimulated in the seedlings of *C. frutesences*, and *S.lycopersicum* treated with *P. guajava* leaf extracts. The influence was noticed from lower concentration to higher concentrations of the extract. When the concentration of the extract increased, the stimulus also increased in both test seedlings.

Fig: 4



Minimum proline and catalase content were observed in seedlings treated with the lowest concentration of aqueous leaf extract of *P. guajava* (10%). The maximum levels of proline and

catalase contents were observed when the seedlings were treated with 50%. No other differences were detected between treated seedlings and control plants of *C. frutesences* and *S.lycopersicum* (Fig:3 &4). The highest activity of Super oxide dimutase was observed in both test crops treated with different concentrations of aqueous leaf extract of *P. guajava*. Maximum activity of Superoxide dimutase was observed in 40 and 50% extract treatments in the seedlings. Dehydroascorbate Reductase activities were stimulated in the seedlings of *C. frutesences* and *S.lycopersicum* treated with *P. guajava* leaf extracts. The influence was noticed from lower concentrations to higher concentrations of the extract. When the concentration of the extract increased, the stimulus also increased in both test seedlings.

DISCUSSION

Based on the results, the allelochemicals inherent in *P. guajava* significantly inhibited both plant species' germination, shoot, and root length elongation. These results were in line with the findings of Chapla & Campos (2010), who observed that the success of *P. guajava* in invading various ecosystems is partially attributed to the allelochemical inherent in it, which suppresses germination, growth, and survival of other plant species. Previous studies on the chemical composition of *P. guajava* leaves have identified chemical products belonging to the groups with allelopathic properties such as terpenoids, coumarins, cyanogenic acids and flavonoids, among others (Omeja *et al*., 2004). These allelochemicals inhibit seed germination and growth by blocking the hydrolysis of nutrients reserve and cell division, thus causing a significant reduction in germination percentage and plumule and radical growth of various plant species (Khan *et al*., 2014). The present study correlated with Namkeleja *et al*., 2014 findings. Allelochemicals from Cosgrove are well-known germination and plant growth inhibitors and seedling growth developers (Gniazdowska *et al*.,2015). (1999) stated that endoglucanases, xyloglucan endotransglycosylases, pectinases, pectin esterases, debranching enzymes, and non-enzymatic proteins such as expansins are responsible for the extensibility of the cell wall among cell wall proteins. The results showed that *P. gujava* leaf phytochemicals might change membrane permeability, interfere with chlorophyll formation, inhibit protein synthesis, and inactivate certain hormones and enzymes' activity and functions. As the concentration levels increased, these impacts on the seedlings also increased. Tanveer *et al*. (2014) reported that Seed germination is a complex process involving a set of morphological, physiological, and biochemical changes in a well-defined manner. The results incorporated Tanveer *et al*. (2014) findings.). It may be the reason for the significant reduction in the

germination of seeds in *C. frutescens* and *S. lycopersicum.* The shoot and root length elongations in *C. frutescens* and *S. lycopersicum* were significantly suppressed.

The properties of allelochemicals may inhibit the actions of inducible hormones such as Gibberellin and the activities of specific enzymes such as amylases and Proteinases, which are important for germination (Isfahan & Shariati, 2007). Karuppanapandian *et al*., 2011 found that regulating the concentrations of hormones such as auxins and gibberellins is essential for growth in plant cells and morphogenesis. Most allelochemicals inherent in invasive species have been reported to disrupt hormone equilibrium (Namkeleja *et al*., 2014). This disruption is brought about by inhibiting polar auxin transport leading to a disturbance in normal auxin levels resulting in the induction of lateral shoots and roots and subsequent suppression of growth (Brunn *et al*., 1992). Ashafa *et al*., 2012 reported that. Impaired metabolic activities induced by these allelochemicals also suppress shoot and root length elongation. The present findings also correlated with Ashafa *et al*. findings. Siddiqui *et al*., (2009) and, Yarnia *et al*., (2009). Yarnia *et al*., (2009) reported that the inhibitory effect was concentration-dependent and different species responded differently to the extracts.

This analysis showed that *P. guajava* aqueous leaf extracts decreased chlorophyll and carotenoid levels, indicating possible photosynthetic limitations of both test crop leaves exerted by the extracts. Prasad *et al*. (2004) observed that chlorophylls and carotenoids are important photosynthetic pigments for plants, and that their content and functionality are necessary for photosystems to absorb and direct light.

CONCLUSION

Allelopathy is a vital mechanism through which plants spread toxic compounds as their competitive technique in nature; it is a significant environmental methodology for managing weeds, minimizing the application of herbicides, and growing yields. In environments with positive and negative effects on plants, allelopathy plays a major role. From the cited literature, it can be inferred that allelochemicals in *P. gujava* suppressed the rate of germination, seedling growth, biochemicals, and pigments. Growth and biochemical parameters were affected more in higher concentrations of extracts than in lower concentrations. Based on the findings, it can be inferred that allelopathy is a concentration-dependent phenomenon; its harmful effects often increase receptor plants as the concentration of allelochemicals gradually rises. The phytochemicals such as antioxidants, polyphenols, terpenes, caryophyllene oxide, and *p*-selinene may inhibit seedlings' growth. This study showed that *P. guajava* leaf extracts have inherent allelochemicals, which significantly inhibited germination and growth of *S. lycopersicum* and

C. frutescens seeds. The allelochemicals can be used as lead molecules to synthesize bio-herbicides, benefit farmers in controlling weeds, improving crop production,and enhancing environmental conservation. Between the two test crops, *S. lycopersicum* seedlings had a significant suppressive effect at 50% *P. gujava* extract concentration than the *C. frutescens* seedlings at the same concentration.

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