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RESEARCH ARTICLE

ALLELOPATHIC POTENTIAL OF *CARICA PAPAYA*LEAF EXTRACTON GROWTH AND BIOCHEMICAL CONSTITUENTS OF *PHASEOLUS AUREUS*

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ABSTRACT

Carica papaya is known to influence the growth of plants in its vicinity by the release of the allelochemicals during the decomposition of its litter. The purpose of this study is to assess the allelopathic effects of *Carica papaya* aqueous leaf extraction seedgermination characteristics, primary growth and biochemical changes associated with *Phaseolus aureus*. For the study, C papaya aqueous leaf extract of dry mature leaves of concentrations viz., 0. 1%, 0.2%, 0.3%, 0.4% and0.5% were made, while tap water served as control. Green gram seeds were incubated in different concentrations of leaf extracts and germination data was recorded.After 15th, 30th&45th days of exposure to the extract, seedlings were harvested and measurements for root and shoot length, fresh weights of root, shoot, and seed was taken and biochemical characteristics such as carbohydrates, protein, chlorophyll, proline and phenol content of the seedlings wereassayed. Germination and phenotypic results showed no negative affect by the extractin T1, T2, T3 treatments. The amount of total carbohydrate, chlorophyll and protein content were found to be stimulated in T3. Lower concentration (T3) which appear to be suitable to green gram, showed a decrease in stress preventing compounds such as total phenol and free proline. Optimum and healthy growth were observed in T3 treatment. The results indicate the possible application of *C.papaya* aqueous leaf extract for growth improvement without pest attackof *P. aureus.*

INTRODUCTION

Allelopathy in agricultural practices has become more important in biological control of weeds and pests (Piyatida and Noguchi, 2010). The inhibition of the plant depends on the concentrations of the allelochemicals (Ashrafi *et al.,* 2009; Batlang and Shushu, 2007).Many plants including medicinal plants were reported to interact chemically with other plant species. The chemical interference of donor plants on another receptor plants thereby affecting them negatively or positively had been established which is referred to as allelopathy (Hierro *et al*., 2003). Allelopathic influence can have positive effect on the growth of other plants thereby increasing their growth positively and inhibitory effect by suppression of neighboring plant growth by the release of toxic compound (Elijarrat and Barcelo, 2001).Allelopathy plays important role in agro ecosystem leading to the interaction crop to crop, crop to weed, weed to crop, and trees to crop (Narwaland Haouala, 2011) through the production of chemical compounds (allelochemicals) that escape into the environment. These allelochemicals are released from plant parts such as leaves, flowers, seeds, stems, and roots rhizomes (Ahmad *et al*., 2011) from where they are released into the environment by leaching from above ground parts, root exudation, volatilization, and decomposition of plant residues in both natural and agricultural systems (Ferrugson and Rathinasabapathi, 2003).Out of these plant parts, leaves seem to be the most consistent producers of these allelochemicals (Gulzar and Siddiqui, 2014).These allelopathic compounds can also be used as natural herbicides and other pesticides (Einhelling, 1995). Allelochemicals are low molecular weight compounds excreted from plants during the process of secondary metabolism (Rice, 1984)**.**Allelopathy inhibition is complex and can involve the interaction of different classes of chemicals such as phenolic compounds, flavonoids, terpenoids, alkaloids, coumarins, glycosides, and glucosinolates. These chemicals called secondary metabolites are known to be exuded by plants to suppress emergence or growth of other plants (Cespedes *et al*., 2006).The growing use of synthetic pesticides is considered practical by the farmers and lovers of plants to prevent the plants from pest attack; it brings negative impact big enough for humans and the environment. High enough the negative impact of the use of synthetic pesticides, encouraging efforts to pursue empowerment / utilization of natural pesticides as an alternative to synthetic pesticides is of great importance. One of the natural pesticides that can be used is papaya leaf extract.

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Papaya leaves contain the active ingredient "Papain"and phenolic compounds so effective for controlling pests (Aziz*et al*., 2014). *Carica papaya L.* is a large perennial tree-like but herbaceous fruiting plant from the *Caricaceae* family and it is thought to be native to the tropical Americas (Pierson *et al*., 2012). The Papaya is a short-lived, evergreen plant that can grow up to 25 feet high. Its hollow, fleshy, green or purplish trunk is marked with leaf scars. The Papaya rarely branches. The leaves grow in a spiraled cluster directly from the upper part of the stem on horizontal petioles (leaf stalks) 1 to 31/2 feet long. The leaves are deeply divided and range in width from 1 to 2 feet. The life of a leaf is 4 to 6 months. Papayafruit, also called paw paw, is the major product from the tree and it is well known for its excellent taste and nutritive value when eaten ripe, whereas other parts of the tree such as trunk, stems and leaves are considered as waste (Carvalho and Renner, 2012). Although the leaves are generally discarded, they have been used as a folk remedy for a variety of ailments in many parts of the world, especially in Asia(Ayoola and Adeyeye, 2010).Numerous studies have revealed a wide range of beneficial effects of *Carica papaya* leaf extract on animals, microbes and humans. Currently, no published information exists relating to the optimized use of aqueous leaf extract of *Carica papaya* on plants to promote the growth by evaluating the beneficiary plant biochemical constituents. The present study represents the first concerted attempt to assess the relative efficiency of different concentrations of papaya leaf aqueous extract on the germination, growth and biochemical constituents of *Phaseolus aureus*.

MATERIALS AND METHODS

Plant Materials and Cultivation

Green gram seeds (*Phaseolus aureus*) were obtained from Local market, Virudhunagar, Tamil Nadu, India and surface sterilized with 0.1% $HgCl_2$ solution for1 min with frequent shaking, and then was thoroughly washed with deionized water. The experiments were conducted between January and April 2014.

Preparation of Plant Extract

Fresh Leaves of *Carica papaya* were collected from male tree, Virudhunagar. The leaves were shade dried for 10days and made into fine powder using the laboratory mill. The powder was stored in an airtight container for further use. 0.1g(T1), 0.2g(T2), 0.3g(T3), 0.4g(T4), 0.5g(T5) of *Carica papaya* leaves were dissolved in 100 ml of water after 24 hrs boiled at 70^{o}C ,cooled , filtered and the filtrate was used for further treatment.Tap water was used as control(C).

Seedling Germinationand Growth

Clean and sterilized petri dishes were taken, and a piece of filter paper is placed over it. The filter paper in the petri dishes was moistened by the different concentrations of the*Carica papaya* filtrate and 15 seeds of Green gram of similar size were counted and positioned on the filter papers of each dish, ensuring that the seeds do not touch each other. Each petri dish was then wrapped with parafilm so that the dishes don't dry out and also to prevent infection. Plants weregrown in pots treatedwith control (tap water) and *Carica papaya* leaf filtrate. 20 seeds were sown. The pots (T1-T5) were treated with the filtrate on alternate days to field capacity. The plant samples were collected on 15^{th}, 30th and 45^{th}day for the measurement of various morphological and biochemical characteristics. Each treatment, including the control, was replicated 3 times including germination.

Morphological Parameters

Morphological parameters, including number of germinated seeds, shoot and root length, shoot and root weight were measured in the samples.

Biochemical Analysis

Biochemical analysis to quantify Carbohydrates, Chlorophyll, Protein, Phenol and Proline were carried out in fresh samples ofseeds and leaves collected during germination and pot culture for the period of 15^{th}, 30th and 45^{th} day of growth.

Determination of carbohydrates

Estimation of carbohydrate contents was carried out by Anthrone and Sulphuric acid. A sample of 0.5 g dry weight was hydrolyzed in $4NHCl_2$ for 2 hours in a boiling water bath for total carbohydrates. Also, 0.5 g of dry sample was extracted by distilled water for two hours. Both extracts were filtered and completed to a definite volume and determined by the Anthrone sulphuric acid method (Fales, 1951). A calibration curve using pure glucose was made from which the data were calculated.

Determination of Chlorophyll

Chlorophyll from leaves were extracted with 80% acetone and quantified following the procedure of Arnon, 1949.

Determination of Protein

Protein content was measured according to Lowry *et al*., 1951.

Determination of total Phenolic content

Total water-soluble phenolics were estimated as per the method using Folin-Ciocalteau reagent. Their amounts were determined spectrophotometrically at 700 nm against the standard of Catechol. (Bray and Thorpe, 1954)

Determination of Proline

Free proline content in leaves was measured using the Ninhydrin method (Bates *et al*., 1973).

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT). The values are presented as mean ± SD for 3 samples in each group. P values ≤0.05 were considered significant.

RESULTS AND DISCUSSION

In the present study, leaf extracts at different concentration of *Carica papaya* were tested for their allelopathy activity against *Phaseolus aureus*. The allelopathy effect was tested both at seed germination and seedling growth stage.Polyphenols in the papaya leaf extracts were found to have a close correlation with scavenging and total antioxidant activities(Vuong *et al.,* 2013). There was a variation observed in seed germination rate. The seed germination rate decreased with increase in extract concentration (Table 1). Similar to our results, inhibition of seed germination observed in charlock with *Glaucium* species (Ghorbanli *et al*., 2011), in soybean and chive with ginger (Chun-Mei *et al*., 2008), in sesame, corn, sorghum and sunflower with mungbean (Letmongkol *et al*., 2011).

Table 1.Effect of aqueous *C.papaya* leaf extract on Seed Germination of *P.aureus*

Treatment (n=30)	Seeds germinated	Seeds not germinated
Control	24	6
T1	23	7
T2	23	7
T3	26	4
T4	22	8
T5	21	9

The lowest germination rate compared to control was noted in T4 and T5, and the result was found to be significant at $p < 0.001$.Reigosa*et al.,* 1999 assumed that allelopathic compounds are affecting many different physiological processes simultaneously and these effects are concentration dependent. The speed of seed germination was also decreased at high concentrations of plant extract. This is evident from the results that number of seeds germinated incontrol, T2 and T3 were 100% on 2nd day itself but in treatments, seed germination delayed significantly with increase in plant extract concentration. (Table 1). This result correlates with the results of Khaliq *et al.,* 2011 where delayed seed germination observed in rice treated with crop residues of sorghum, sunflower and brassica. These findings are also in accordance with previous reports of which concluded that sometimes seed germination is not inhibited, but the process may be delayed, cotyledon and root size are diminished, or radicle or seedling development is abnormal (Chaves *et al*., 1997, SoumitraNath*et al*., 2016). It was reported that, seed characteristics such as seed size and seed coat permeability can influence the uptake and effects of allelochemicals in seeds and interference of the allelochemicals varies accordingly (Marianne *et al*., 2000).

Root and Shoot length and weight

The treatment of aqueous *Carica papaya* leaf extract at lower rate of (0.3%) induced a stimulatory effect in growth parameters of both root and shoot of *Phaseolus aureus*. The magnitude of promotion in all growth parameters was more pronounced in shoot than root as compared with their respective control at each growth stage (Table 2). However, increasing the level of up to 0.5% (T5) induced a pronounced reduction in shoot and root length but slightly stimulated the length, fresh and dry weights of shoot of 15, 30 and 45-day-old plants relative to the control (Table 2). This finding is in agreement with the results of Jayakumar *et al.,*1990 who showed that the irrigation of groundnut and maize with 5, 10, 15 and 20% water extract of abscised *Eucalyptus globulus* leaf greatly reduced plant height. Abu El-Soud*et al*., 2001 demonstrated stimulation in pea growth parameters by increasing the rate of the incorporated *Acacia nilotica* leaf residue from 0.25 – 0.5% (w/w), but gradual suppression at 1.5 to 2% (w/w). Reigosa *et al.,*1999 assumed that allelopathic compounds are affecting many different physiological processes simultaneously and these effects are concentration dependent.The allelopathy treatments significantly influenced the carbohydrate content at T3 concentration (Figure 1). Maximum increase in carbohydrate contents was observed at T3.

Beyond this level the carbohydrate contents regularly decreased (Figure 1).The same findings were obtained by El-Darier*et al*., 2002. Minimum carbohydrate content was observed at T5 lower as compared to control. In the present study a remarkable increment was observed in the chlorophyll contents at lower applied doses of aqueous leaf extract of *Carica papaya* (Figure 2). The chlorophyll contents in the leaf showed similar trend as that of carbohydrate.Johnson *et al.,* 2009 reported a significant increment in photosynthetic pigment especially chlorophyll a and carotenoids with the application of minerals in barley. The chlorophyll content was reduced by 41% and 48%, when *Phaseolus aureus*seedlings were treated withT4 and T5concentration of Papaya aqueous leaf extract, respectively (Fig. 2).

Table 2. The effect of aqueous *C.papaya* leaf extract on morphological parameters of *P.aureus*.

ROOT & SHOOT GROWTH							
Age/ (days)	Treatment (w/w)	Root Length (cm)	Shoot Length (cm)	Root Weight(g)		Shoot Weight (g)	
				Fresh	Dry	Fresh	Dry
15	Control	13.4±0.57	10.7±0.28	0.08±0.39	0.007±0.003	0.16±0.02	0.02±0.03
	0.1%	13.1±0.54	10.1±0.21	0.095±0.70	0.005±0.004	0.17±0.04	0.03±0.05
	0.2%	13.2±0.53	10.4±0.32	0.12±0.45	0.007±0.07	0.17±0.06	0.03±0.02
	0.3%	15.4±0.75	11.5±0.18	0.15±0.041	0.01±0.09	0.2±0.04	0.04±0.04
	0.4%	12.3±0.23	10.8±0.27	0.13±0.043	0.008±0.002	0.16±0.09	0.02±0.04
	0.5%	12.1±0.22	10.6±0.23	0.11±0.32	0.006±0.06	0.14±0.08	0.02±0.04
30	Control	4.9±0.30	16.6±0.23	0.38±0.04	0.17±0.02	0.4±0.06	0.08±0.04
	0.1%	4.8±0.28	16.8±0.34	0.41±0.09	0.18±0.05	0.43±0.12	0.085±0.05
	0.2%	5.3±0.26	17.1±0.31	0.44±0.08	0.18±0.02	0.44±0.04	0.089±0.1
	0.3%	5.8±0.29	17.9±0.29	0.5±0.02	0.2±0.04	0.5±0.04	0.11±0.07
	0.4%	5.2±0.38	17.2±0.26	0.46±0.09	0.16±0.01	0.39±0.11	0.078±0.11
	0.5%	5.1±0.34	16.9±0.22	0.42±0.03	0.14±0.02	0.37±0.07	0.07±0..09
45	Control	5.7±0.37	25.6±0.21	0.71±0.09	0.24±0.02	1.07±0.19	0.26±0.03
	0.1%	5.1±0.29	26.2±0.25	0.72±0.08	0.26±0.07	1.09±0.22	0.27±0.05
	0.2%	5.6±0.27	26.6±0.32	0.76±0.09	0.27±0.05	1.16±0.31	0.27±0.08
	0.3%	6.8±0.32	27.0±0.26	0.8±0.11	0.3±0.04	1.2±0.14	0.31±0.03
	0.4%	5.4±0.43	26.4±0.31	0.75±0.18	0.25±0.09	1.07±0.21	0.25±0.14
	0.5%	5.3±0.31	26.2±0.34	0.73±0.21	0.23±0.10	1.05±0.27	0.23±0.11

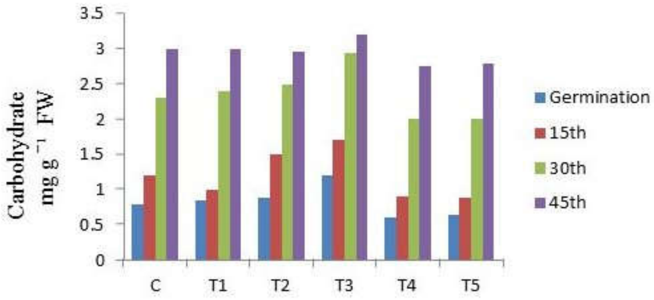

FIG.NO.1: The effect of *Carica papaya* leaf extract on Carbohydate of *Phaseolus aureus.*

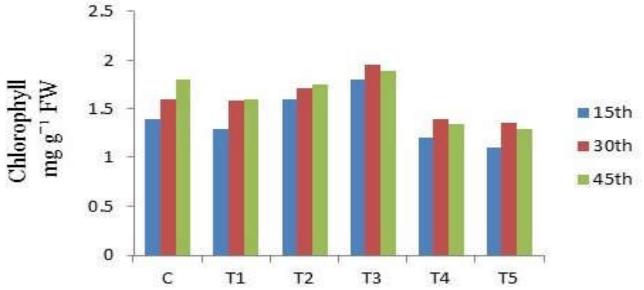

FIG.NO.2 : The effect of *Carica papaya* leaf extract on Chlorophyll of *Phaseolus aureus.*

T4 and T5 concentration also decreased protein content of the green gram seedlings. More than the control T3 treatment gave significantly the highest protein content as compared to other treatments. Protein content of *P.aureus*increased to reach 1.2 fold high at T3treatment (Fig. 3).

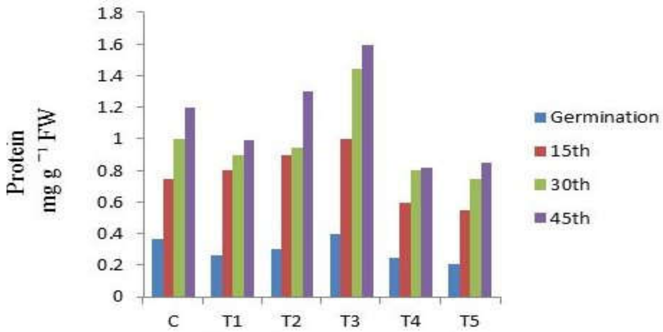

FIG.NO.3 : The effect of *Carica papaya* leaf extract on Protein of *Phaseolus aureus.*

These results are supported by the findings of Corsato *et al*(2010)and Salama*et al* (2015) who stated that the allelopathic effects is a natural interference in which the plant produces substances and metabolites that may benefit other plants when released. Based on the results obtained in this study, at T2 and T3 we can postulate that root and shoot length, fresh and dry weight of both tested species were all stimulated due to the plant's capacity to accumulate chlorophyll which is an essential component of food manufacturing process; the photosynthesis.

In the present study lower applied doses of *C.papaya* leaf extract (0.3%) which appears to be suitable to *P.aureus* showed a decrease in stress preventing compounds such as total phenol and free proline at optimum concentration (T3).Metabolic changes that lead to the increased biosynthesis of phenolic compounds are well-known response of the acceptor plants under allelochemicals stress (Politycka*et al*., 1998). Phenol content in T1, T2, and T3were found to be relatively similar and quite higher than control but lower than T4 and T5 (Figure 4). Results of the present studies indicate that effect of *Carica papaya* leaf extracts on phenolic compound accumulation depends on the species and the applied extract (Magdalena Szwed*et al*., 2014). Phenolic compounds, in addition to antioxidant properties, may also display prooxidant effect (Sakihama *et al*., 2002). *Carica papaya*leaf extract are a rich source of those substances, which is acknowledged as a basis of allelopathic properties of that species (Golisz *et al*. 2007). Inhibition of the growth of acceptor plant (*Phaseolus aureus*) under the effect of *Carica papaya*leaf extract maytherefore be related to disturbance in oxidoreductiontransformation in their tissues (M. Szwed *et al*., 2014). This may lead to the oxidation damage of proteins, lipids, and nucleic acids (Sharma *et al*., 2012).

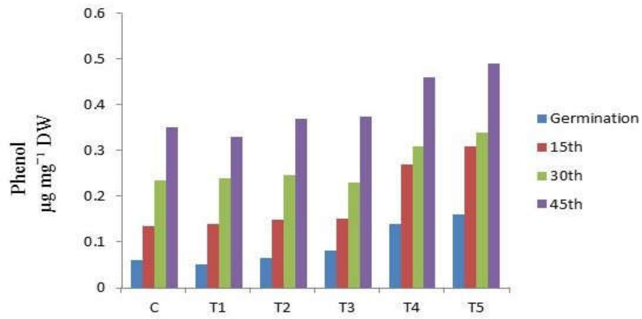

FIG.NO.4 : The effect of *Carica papaya* leaf extract on Phenol of *Phaseolus aureus.*

But this concept was slightlydifferent in our results obtained which clearly state that application of extract at lower concentration might neutralize the effect of allelochemicals thus exerting beneficial effect. In corroboration with our results, Bido*et al*., 2010 & Mewis *et al*., 2012 stated that some biotic factors may also cause a decrease in the content of antioxidants. Similarly free proline contents of *P.aureus* were the lowest at T3 and showed a decrease of 13.15% and 71.92%, respectively, over the control indicating that the beneficiary plant is not under stress. Higher application of extract (0.4 &0.5%) resulted in increased free proline contents (Figure 5).

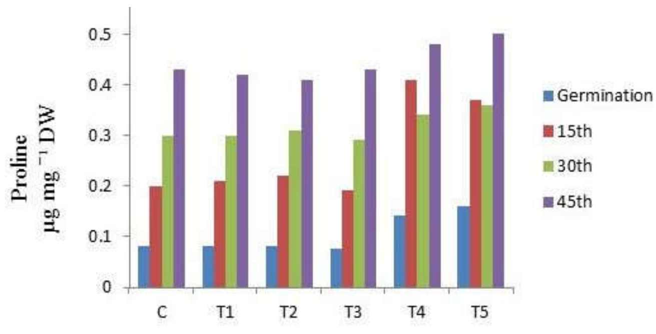

FIG.NO.5 : The effect *of Carica papaya* leaf extract on Proline of *Phaseolus aureus.*

Tolulope *et al*., 2016observed a significant increment in proline contents in *V.unguiculata* with the treatment of *Tithonia diversifolia* shoot extract at higher concentration. The induction of phenolic compounds biosynthesis was also observed in rice plant in response to *T.diversifolia*leaf extract (Ilori*et al*, 2007).

Biosynthesis, as well as the release and effectiveness of allelochemicals may undergo changes under the effect of environmental factors (Inderjit *et al*. 2011).

Understanding the mechanisms may contribute to the use of some plant compounds as natural pesticide, use of plant residue of allelopathic properties or cultivation with under plant, which will make it possible to limit the use of synthetic pesticides and herbicides (Khanh *et al*., 2005). Insecticidal activity of *C. papaya*leaf extract against mustard aphid was already studied in detail by Aziz Ahmed Ujjan *et al*., 2014. Results of the present work clearly indicate that *Phaseolus aureus* plant seedlings have different sensitivity at varying concentration of *Carica papaya* leaf extract .But at lower concentration T3, the plant exhibited excellent growth as evidenced by the morphological and biochemical characteristics. The amount of phenol content in T3 though slightly higher than control it was found to be healthy without pest attack which might be due to the positive allelochemicals stress attributed by *Carica papaya* leaf extract on *Phaseolus aureus* seedling.

Conclusion

The allelochemicals in *Carica papaya* leaf extract had significant effects on seed germination, seedling growth, and the biochemical parameters and growth of *Phaseolusaureus.*Moreover the plants were found to be pest resistant which may be a basis for further research on the use of *Carica papaya* leaf extract as natural pesticides without affecting the beneficiary plant growthand this is an economic procedure.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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