

ALLELOPATHIC POTENTIAL OF *CARICA PAPAYA* AGAINST SELECTED WEEDS OF WHEAT CROP

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Abstract

The present study was aimed at screening allelopathic potential of papaya (*Carica papaya* L.) leaf solvent extracts and by using sandwich method (dried powder) against some major weeds of wheat viz. *Phalaris minor*, *Avena fatua*, *Chenopodium album*, *Euphorbia helioscopia* and *Rumex dentatus* on filter paper and soil. Amongst them, methanolic extracts showed highest seed germination inhibition of *E. helioscopia* (50%), followed by *P. minor* (45%) and *A. fatua* (41%). Similarly, radical length of *R. dentatus* was inhibited by 50%, followed by 49% in *P. minor*, while the plumule length of *C. album* and *P. minor* was inhibited by 49% and 48%, respectively. Sandwich bioassay also exhibited inhibitory effects on soil medium having germination inhibition of 57% for *E. helioscopia*, followed by 49% for *P. minor* and 39% for *A. fatua*. The radical length was inhibited best for *R. dentatus* and *P. minor* with 41% and 35%, respectively. On the other hand, plumule length was inhibited by 45% in *C. album* and 33% in *P. minor*. Based on the results, it is concluded that *C. papaya* showed good allelopathic activity that can be used in herbicide screening program for weed control.

Key words: Allelopathic potential, Papaya leaf, Wheat, Seed germination, Radical length, Plumule length, Herbicide screening, Weeds.

Introduction

Allelopathy is a sub-discipline of chemical ecology that refers to the effects of chemicals produced by plants, microorganisms on the growth, development of plants in agriculture systems or in natural communities. Allelochemicals come from the class of secondary metabolites that include alkaloids, flavonoids phenoloids, and glucosionates. Like many other natural compounds, these chemicals have the capacity of producing wide array of biological effects and can be quite useful for agriculture systems as well as weed control processes (Anwar *et al.*, 2013). Weeds can be used as mulch for weed management in wheat due to their allelopathy potential towards noxious weeds of wheat (Gul *et al.*, 2017).

Allelochemicals offer great potential for weed control, directly or their chemistry could be used as a template to develop new herbicides. These are prepared by the plants during their growth and developmental and have been found acting as an agent in the formation and disintegration of a few of the plant hormones, for instance their role in the activation of ABA synthesis via the action of ferulic acid (Zhou *et al.*, 2004). Some useful secondary metabolites such as protocatechuic acid, p-coumaric acid, caffeic acid, kaempferol, quercetin, and chlorogenic acid have identified in different parts of *C. papaya* (Miean *et al.*, 2001; Canini *et al.*, 2007). The main purpose of this study was to evaluate papaya leaf (*C. papaya* L.) for its allelopathic potential against some problematic weeds of wheat such as *Phalaris minor*, *Avena fatua*, *Chenopodium album*, *Euphorbia helioscopia* and *Rumex dentatus*.

Materials and Methods

Leaves of *C. papaya* were collected from district Rawalpindi (73°02' E longitude and 33°36' N latitude, 508 m above sea level), Punjab, Pakistan. Leaves were washed under running tap water and then shade dried (25°C) in bloating paper for four weeks. The dried leaves were crushed using heavy duty blender (waring Lab.), to make fine powder, passed through 2 mm mesh size and kept in air tight plastic zip lock bags at 4°C (Anwar *et al.*, 2013) in Plant Physiology Laboratory, Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan. Seeds of the most densely populated weeds of wheat crop (Qureshi & Bhatti, 2001; Qureshi *et al.*, 2009) such as *Phalaris minor*, *Avena fatua*, *Chenopodium album*, *Euphorbia helioscopia*, *Rumex dentatus* were procured from the Barani Agricultural Research Institute (BARI), Chakwal, Punjab, Pakistan. These were surface sterilized according to the methodology of Biljana & Kragujevac (2015) and Anwar *et al.*, (2016). The germination (%), radicle length and plumule length were used as parameters for screening allelopathic potential.

An aliquot (10g) of dried leaf powder of *C. papaya* was soaked in 100 ml distilled water in a flask and agitated at room temperature for 24 hours on an orbital shaker (160 rpm). The extract was strained through muslin cloth and finally filtered through Whatman filter paper No. 1. Aqueous extract was obtained as filtrate of the mixture and final volume was attuned to 100 ml, this gave 10% water extract (Maharjan *et al.*, 2007). This stock solution was then diluted with distilled water to prepare the different concentration of the extract i.e. T₁ (100%), T₂ (75%), T₃ (50%) (Sahu & Devkota, 2013). The control was distilled water (0%), indicated as T₄. The extract was stored at 4°C

in pre-disinfected flasks. To evade adulteration and forthcoming chemical modifications, the extracts were ensured to be used within 3-4 days (Anwar *et al.*, 2017 a). An aliquot (300 g) of dried leaf powder of *C. papaya* was mixed with hexane (2 L) repeated three times for 6 hours on shaker. This extract was filtered, concentrated and dried over a rotary evaporator (Buchi Rotavapor R-200) in pre-weighed flask. The obtained residue was 6.14 g. The stock solution was then diluted with hexane to prepare three concentrations i.e. T₁ (100%), T₂ (75%), T₃ (50%) and T₄ (0% control). These solutions were stored at 4°C in pre-sterilized flasks (Sahu & Devkota, 2013). The defatted marc (after hexane extraction), was again placed on shaker with methanol (2 L) repeated three time for 6 hours on shaker. This extract was filtered, concentrated, and dried over a rotary evaporator in pre-weighed flask. The obtained residue was 25.29 g. The stock solution was than diluted with methanol to prepare three concentrations i.e. T₁ (100%), T₂ (75%), T₃ (50%) and T₄ (0% control). These solutions were stored at 4°C in pre-sterilized flasks (Sahu & Devkota, 2013; Anwar *et al.*, 2017a & b). An aliquot (15 ml) of *C. papaya* extract added on 25g soil per Petri dish and 5ml extract was added on filter paper per Petri dish while distilled water, hexane and methanol were used as control in aqueous, hexane and methanolic extraction respectively. Ten seeds of selected test species were used per Petri dish. Each treatment was replicated five times. The Petri dishes were wrapped with squash tape, enclosed with Aluminum foil and incubated in the growth chamber (NTS Model MI-25S) at room temperature (15°C) for 15 days. The germination (%), lengths of radical and plumule of each test species was calculated and compared with control (Khan *et al.*, 2008).

The sandwich method was followed after Fujii *et al.*, (2003 & 2004). Five ml of 0.75% (w/v) agar (Nalge Nunc Intl., Roskilde, Denmark, gelling temperature 30-31°C) was poured in each of the six-well (10 cm² area /well) into multi-dish plastic plate. The agar solution was left for solidification. *C. papaya* leaf powder@10 and 50 mg were placed in wells of the plate and were roofed by a thin layer of 0.75% (w/v) agar. After solidification, 10 seeds of each test species were placed on agar gel in each well of the plate. The multi-well plastic plates were then wrapped with the plastic tape and incubated in the growth chamber (NTS Model MI-25S) at room temperature for 15 days. In the control treatment, only agar gel without dried leaves powder was used as a seed bed. Each treatment was replicated five times. The germination (%), lengths of radical and plumule for each test species were recorded afterwards. An aliquot (10 mg) of dried leaf powder of *C. papaya* added on filter paper along with 5ml distilled water per Petri dish while an aliquot (50 mg) of dried leaf powder of *C. papaya* added on 25g soil along with 15ml distilled water per Petri dish (Raana *et al.*, 2012). Ten surface sterilized seeds of test species were placed to each sterilized Petri plate. The Petri dishes were wrapped with squash tape, enclosed with aluminum foil and incubated in the growth chamber (NTS Model MI-25S) at room temperature (25°C) for 15 days. The germination (%), lengths of radical and plumule of each test species were recorded (Anwar *et al.*, 2017 b). A completely randomized design (CRD) was used for the experiments. The statistical analysis was carried out by using STATISTIX 9 and means were separated using Fisher's protected LSD test (Nekonom *et al.*, 2014).

Results

Allelopathic potential of *C. papaya* aqueous extract

Germination (%): The results are shown under Fig. 1A. It revealed that except *R. dentatus* and *C. album*, *C. papaya* aqueous extract highly inhibited seed germination percentage of *E. helioscopia* (35%), followed by *P. minor* (33%) and *A. fatua* (32%) on filter paper; while, *C. papaya* aqueous extract inhibited seed germination of *P. minor* (40%), followed by *A. fatua* (39%) and *E. helioscopia* (38%) on soil as compared to control. However maximum (96%) germination was observed for *R. dentatus* and *C. album* and minimum germination (%) was noted for *E. helioscopia* (65%) and *P. minor* (60%) on filter paper and soil, respectively. The germination (%) reduction of the *E. helioscopia*, *P. minor* and *A. fatua* was concomitant with increase of concentration (Fig. 1A).

Radical length: There was highest radical length reduction observed in filter paper extract of *P. minor* (36%), followed by *R. dentatus* (34%) as compared to control, while the same was inhibited best for *P. minor* (42%) and *R. dentatus* (40%) in *C. papaya* aqueous extract on soil. interestingly the radical length of *A. fatua*, *C. album* and *E. helioscopia* remained unaffected. The results also illustrated minimum radical length reduction in *P. minor* i.e. 64% and 58% on filter paper and soil, respectively; however, maximum radical length (97%) was noted for *A. fatua*, *C. album* and *E. helioscopia* (Fig. 1B).

Plumule length: High reduction was observed in the plumule length of *P. minor* (34%) and *C. album* (33%) in filter paper; while, the aqueous extract also inhibited the plumule length of *C. album* (43%) and *P. minor* (42%) as compared to control on soil (Fig. 1C). Interestingly, there was no significant effect on plumule elongation of *A. fatua*, *R. dentatus* and *E. helioscopia*. The statistical data concluded that least plumule length was noted for *P. minor* (64%) and *C. album* (57%) on filter paper and soil, respectively. The statistical Figures also proposed that maximum plumule length (96%) was recorded for *A. fatua*, *R. dentatus* and *E. helioscopia* (Fig. 1C).

Allelopathic potential of *C. papaya* hexane extract

Germination (%): It has been observed from the results that there was significant germination reduction in *E. helioscopia* (35%) followed by *P. minor* (33%) and *A. fatua* (32%) as compared to control on filter paper, whereas, no significant effect was observed on the germination of *R. dentatus* and *C. album*, showing resistance to the allelopathic *C. papaya* hexane extract. Similarly, the highest seed germination inhibition was noted for *E. helioscopia* (50%), followed by *P. minor* (45%) and *A. fatua* (41%) by incorporation of *C. papaya* hexane extract into soil. The maximum (97%) germination noted for *R. dentatus* and *C. album*. In the present study, minimum germination was noted for *E. helioscopia* i.e. 65% and 50% on filter paper and soil, respectively. The statistical analysis revealed that allelopathic inhibitory effect was concentration dependent for *E. helioscopia*, *P. minor* and *A. fatua*, increased concentration, induced more pronounced reduction (Fig. 2A).

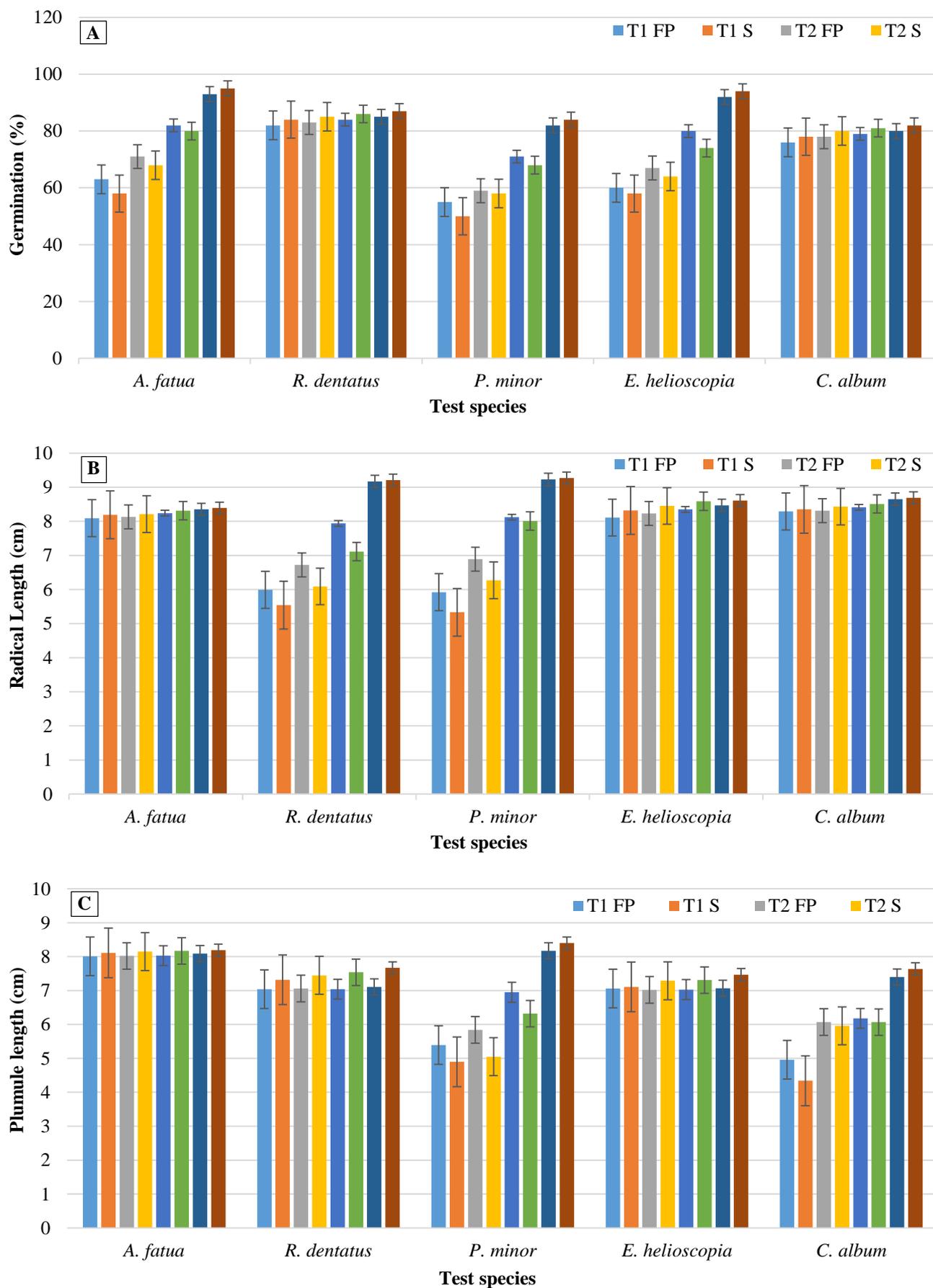


Fig. 1. Allelopathic potential of *C. papaya* aqueous extract against test species on filter paper (FP) and soil (S) on: (A) germination (%) (B) radical length (C) plumule length.

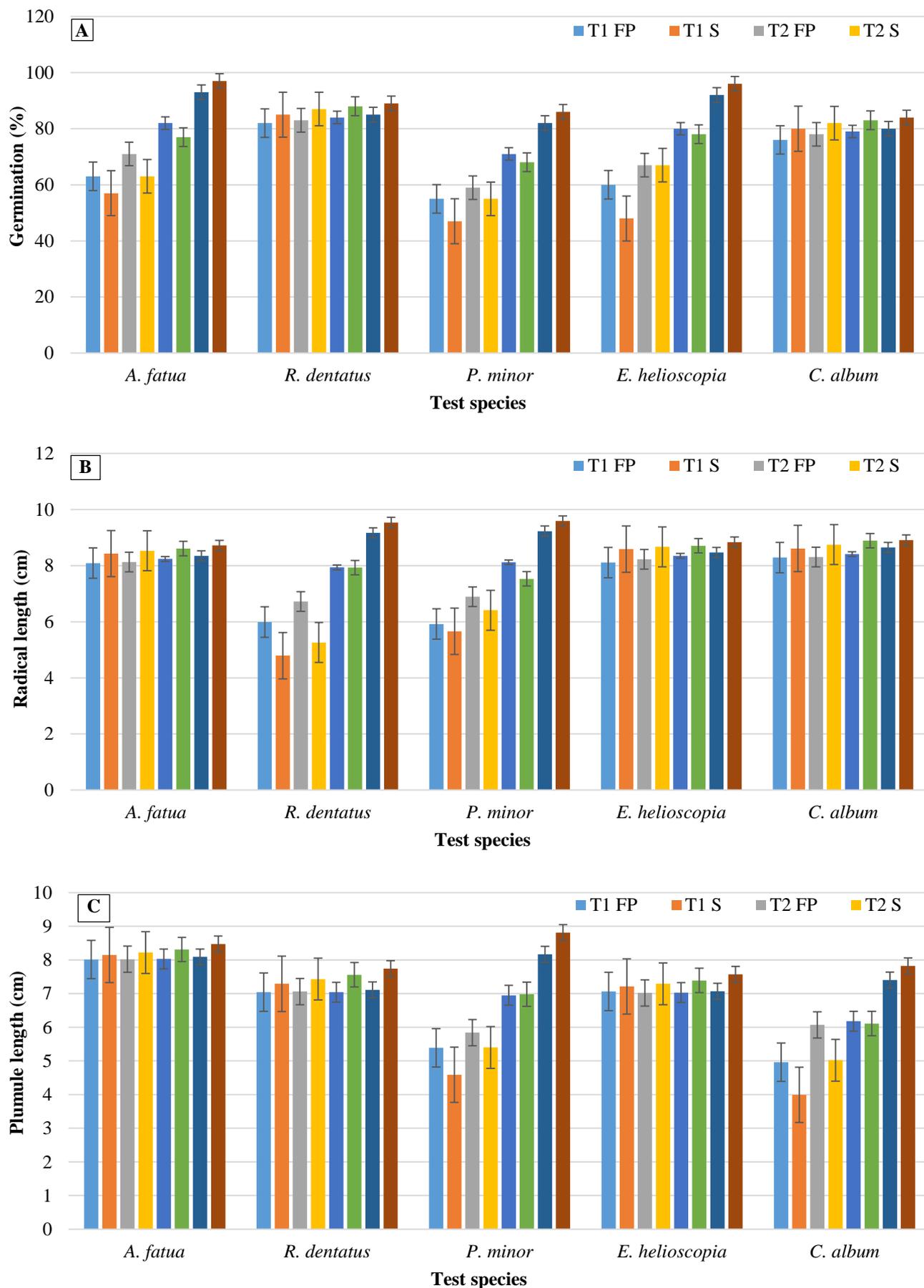


Fig. 2. Allelopathic potential of *C. papaya* hexane extract against test species on filter paper (FP) and soil (S) on: (A) germination (%) (B) radical length (C) plumule length.

Radical length: Results revealed that the highest radical length inhibition activity was exhibited on *P. minor* (36%) and *R. dentatus* (35%) in *C. papaya* hexane extract at filter paper. The data also suggested that hexane extract on soil caused significant radical length reduction of *R. dentatus* (50%) and *P. minor* (41%) as compared to control. The statistical findings of current study revealed that radical length of *A. fatua*, *C. album* and *E. helioscopia* remained unaffected. The results also illustrated that minimum radical length was noted for *P. minor* and *R. dentatus* i.e. 64% and 50% on filter paper and soil, respectively. The maximum radical length (96%) was noted for *C. album* and *E. helioscopia* (Fig. 2B).

Plumule length: The data obtained exhibited that *C. papaya* hexane extract significantly inhibited the plumule length of *P. minor* (34%) and *C. album* (33%) as compared to control on filter paper. The statistical data exposed that there was no significant effect on plumule elongation of *A. fatua*, *R. dentatus* and *C. album*. Likewise, *C. papaya* hexane extract significantly inhibited plumule length of *P. minor* (49%) and *C. album* (48%) in soil. The statistical data concluded that minimum plumule length was noted for *P. minor* i.e. 66% and 51% on filter paper and soil, respectively. The statistical data also proposed that maximum plumule length (95%) was noted for *A. fatua*, *R. dentatus* and *E. helioscopia* (Fig. 2C).

Allelopathic potential of *C. papaya* methanolic extract

Germination (%): The data illustrated that *C. papaya* methanolic extract significantly inhibited germination of *P. minor* (41%), *E. helioscopia* (37%) and *A. fatua* (36%) on filter paper, whereas, no significant effect was observed on germination of *R. dentatus* and *C. album* showing resistance against extract. Similarly, *C. papaya* methanolic extract on soil significantly suppressed seed germination of *E. helioscopia* (50%), *P. minor* (45%) and *A. fatua* (41%). It was noted that maximum (95%) germination (%) was observed for *R. dentatus* and *C. album*. In the present study, minimum germination was noted for *P. minor* (59%) and *E. helioscopia* (50%) on filter paper and soil, respectively. The results revealed that germination reduction of the *P. minor*, *E. helioscopia* and *A. fatua* were concentration dependent (Fig. 3A).

Radical length: The data revealed that *C. papaya* methanolic extract significantly inhibited radical length of *P. minor* (47%) and *R. dentatus* (45%) on filter paper, whereas, no significant effect was noted for *C. album* and *E. helioscopia* showing resistance against extract. Similarly, the applications of extract into soil significantly suppressed radical length of *R. dentatus* (50%) and *P. minor* (41%) as compared to control. The maximum (96%) radical length was observed for *A. fatua*, *C. album* and *E. helioscopia*. The final data concluded that minimum radical length was exhibited for *P. minor* and *R. dentatus* i.e. 53% and 50% on filter paper and soil, respectively (Fig. 3B).

Plumule length: The data obtained exhibited that *C. papaya* methanolic extract significantly suppressed the plumule length of *P. minor* (41%) and *C. album* (36%) as compared to control on filter paper. Interestingly, there was no significant effect on plumule elongation of *A. fatua*, *R.*

dentatus and *C. album*. Likewise, *C. papaya* methanolic extract significantly inhibited plumule length of *C. album* (49%) and *P. minor* (48%) in soil. The statistical data revealed minimum plumule length for *P. minor* (59%) and *C. album* (51%) on filter paper and soil, respectively. The statistical data also proposed maximum plumule length (96%) for *A. fatua*, *R. dentatus* and *E. helioscopia* (Fig. 3C).

Allelopathic potential of *C. papaya* in sandwich method

Germination (%): The data revealed *A. fatua*, *P. minor* and *E. helioscopia* exhibiting 39%, 38% and 33% germination inhibition respectively as compared to control in *C. papaya* leaf powder on filter paper, whereas, no significant effect on germination of *R. dentatus* and *C. album* was observed showing resistance to dry powder. It was also found from the results that *E. helioscopia*, *P. minor* and *A. fatua* showed 51%, 49% and 47% germination inhibition respectively as compared to control when powder was applied into soil. The results also declared that maximum (98%) germination for *R. dentatus* and *C. album*. In the present study, minimum germination was demonstrated for *A. fatua* (61%) and *E. helioscopia* (49%) on filter paper and soil, respectively. The experimental results of the current study indicated highest germination reduction for *P. minor* (41%), followed by *A. fatua* (39%) and *E. helioscopia* (36%) at 10 mg conc on agar. Similarly, the highest germination reduction was noted for *A. fatua* (51%), followed by *P. minor* (49%) and *E. helioscopia* (47%) at 50 mg conc. The statistical data concluded that minimum germination for *P. minor* (59%) and *A. fatua* (49%) at 10 mg and at 50 mg conc., respectively. The statistics also recommended that with the increase of concentration, the inhibitory effect was progressively increased for *A. fatua*, *P. minor* and *E. helioscopia*. The statistical results recommended that *R. dentatus* and *C. album* were completely resistant to dry powder in terms of germination (Fig. 4A).

Radical length: The data showed radical length inhibition of *P. minor* and *R. dentatus* by 41% and 30% respectively as compared to control in *C. papaya* leaf powder on filter paper, whereas, no significant effect on radical length of *A. fatua*, *C. album* and *E. helioscopia* was found indicating resistance to dry powder. *R. dentatus*, and *P. minor* showed 41% and 35% radical length inhibition respectively as compared to control in powder applied into soil. The results also declared maximum (98%) radical length for *A. fatua*, *C. album* and *E. helioscopia*. In the present study, minimum radical length was demonstrated for *P. minor* and *R. dentatus* i.e. 59% on filter paper and soil, respectively. The experimental results of the current study showed highest radical length reduction for *P. minor* (35%) and *R. dentatus* (31%) at 10 mg conc on agar. Similarly, the highest radical length reduction was noted for *P. minor* (45%) and *R. dentatus* (42%) at 50 mg conc. The statistical data revealed minimum radical length for *P. minor* i.e. 65% and 55% at 10 mg and at 50 mg conc., respectively (Fig. 4B).

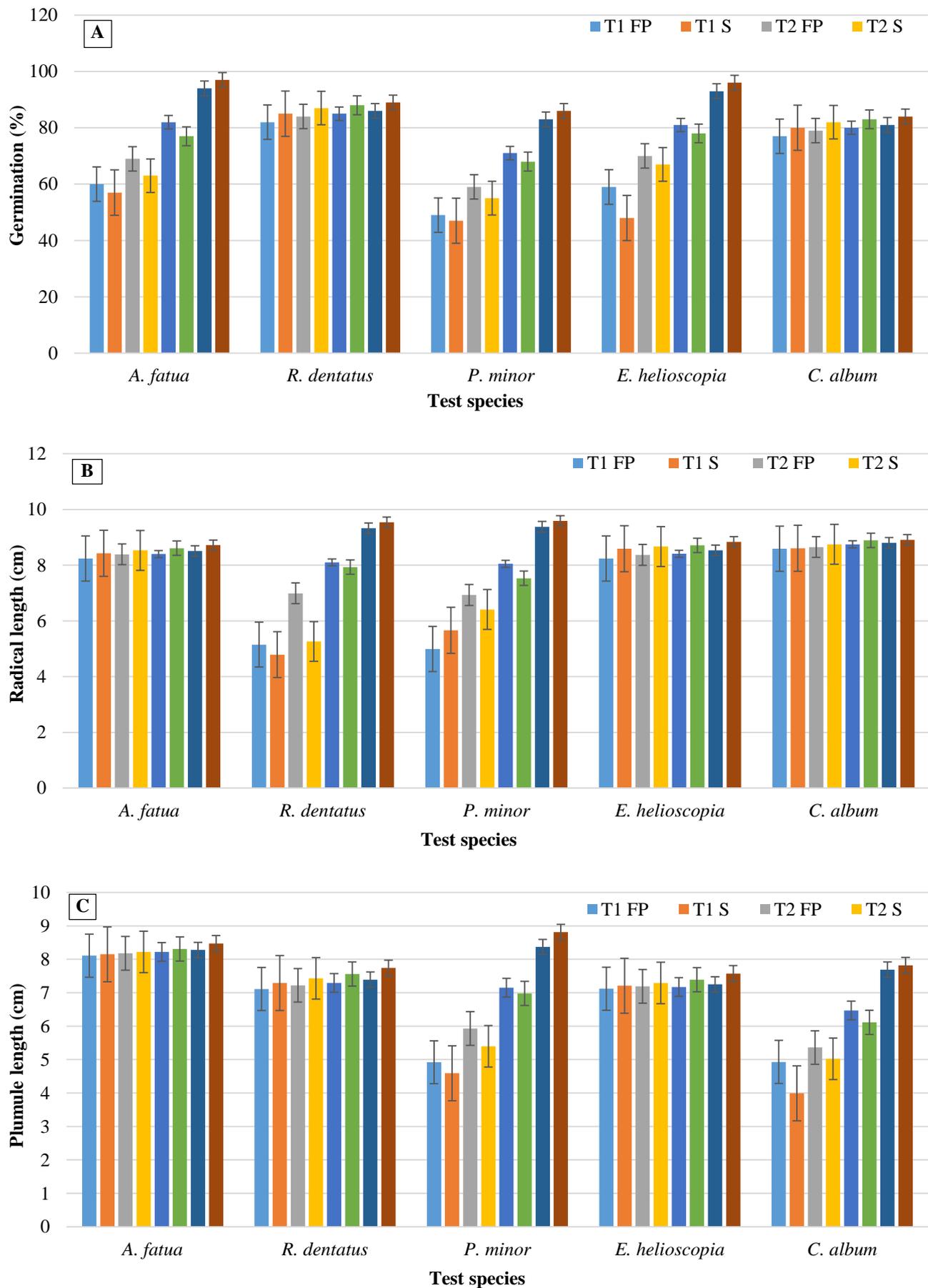


Fig. 3. Allelopathic potential of *C. papaya* methanolic extract against test species on filter paper (FP) and soil (S) on: (A) germination (%) (B) radical length (C) plumule length.

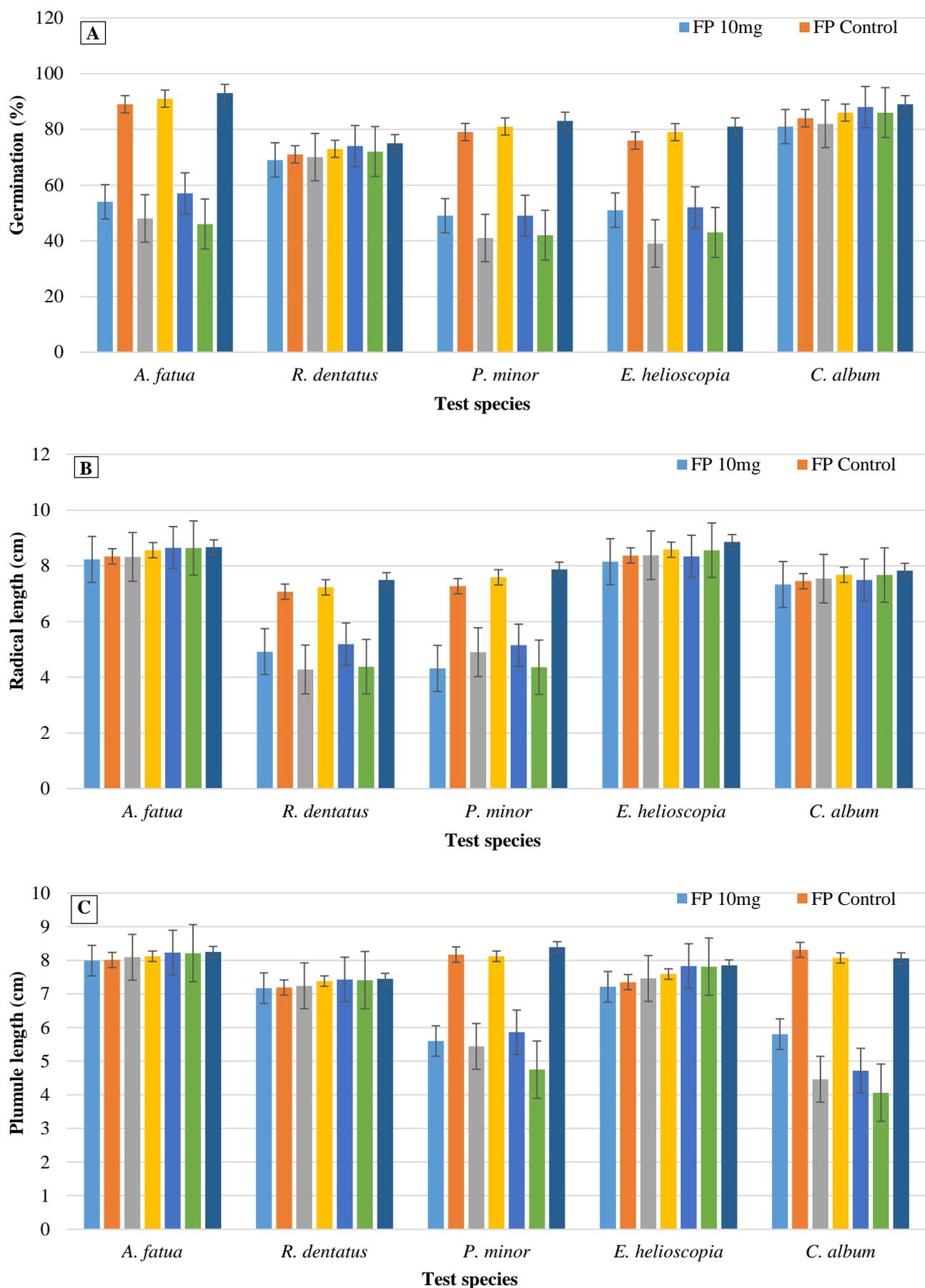


Fig. 4. Allelopathic potential of *C. papaya* leaf powder against test species on filter paper (FP), soil (S) and agar (A) on: (A) germination (%) (B) radical length (C) plumule length.

Plumule length: The data showed plumule length inhibition of *P. minor* by 31% and *C. album* by 30% as compared to control in *C. papaya* leaf powder on filter paper, whereas, no significant effect on plumule length of *A. fatua*, *R. dentatus* and *E. helioscopia* was found indicating resistance to dry powder. It was also clear from the results that *C. album* and *P. minor* showed 45% and 33% plumule length inhibition respectively as compared to control in powder applied into soil. The results also declared that maximum (96%) plumule length was for *A. fatua*, *R. dentatus* and *E. helioscopia*. In the present study, minimum plumule length was demonstrated by *P. minor* (69%) and *C. album* (55%) on filter paper and soil, respectively. The experimental results of the current study indicated highest plumule length reduction for *C. album* (41%), followed by *P. minor* (30%) at 10 mg conc on agar. Similarly, the highest plumule length reduction was noted for *C. album* (50%), followed by *P. minor* (43%) at 50 mg conc. The statistical data concluded that minimum plumule length was observed for *C. album* i.e. 59% and 50% at 10 mg and at 50 mg conc., respectively (Fig. 4C).

Discussion

The present study was conducted to understand the effect of different plant extract and leaf powder on seed germination, radical length and plumule lengths of the selected weeds. According to literature, no study *C. papaya* extracts was carried out on the seed germination, radical and plumule length of selected weeds of wheat crop. The aqueous extract of *C. papaya* highly inhibited seed germination percentage of *E. helioscopia*, *P. minor* and *A. fatua* on filter paper as well as on soil, the reduction in germination percentage was concomitant with increase of concentration. These findings are in accordance to the previous findings where it was reported that root and shoot elongation along with germination of *Lactuca sativa* seedling was significantly checked by *C. papaya* aqueous extract (Wabo *et al.*, 2011). This *allelopathic potential* may be attributed to the presence of some beneficial secondary metabolites such as chlorogenic acid, quercetin, kaempferol, caffeic acid, p-coumaric acid and protocatechuic acid reported in various parts of *C. papaya* (Canini *et al.*, 2007). Water being highly polar can extract maximum allelochemicals such as phenolics from a plant samples (Miean *et al.*, 2001).

The weed seeds of *E. helioscopia*, *P. minor*, *A. fatua* were inhibited best by *C. papaya* hexane extract on filter paper. Similarly, radical length inhibition was noted in *A. fatua*, *P. minor* and *R. dentatus*. Besides, there was reasonable inhibition of plumule length of *P. minor* and *E. helioscopia* in aforementioned extract. *C. papaya* is reported to possess benzyl isothiocyanate (BITC) which is known to be germination inhibitors (Wolf, 1984). Some researchers have suggested that a specific dose of allelochemicals may cause growth stimulation; however, the large quantities are mostly found to be phytotoxic effects (Pelinganga & Mashela, 2012). Stimulation was usually due to presence of some growth promoting substances along with many inhibitory substances (Yamada *et al.*, 2010). The allelochemicals may also affect on essential growth parameters such as reduction in

cell division and cell sizes (Einhellig & Leather, 1988; Ortega *et al.*, 1988).

C. papaya methanolic extract significantly inhibited germination of *P. minor*, *E. helioscopia* and *A. fatua* on filter paper as well as soil. Besides, there was significant inhibition of radical length of *P. minor*, *R. dentatus* and *A. fatua*. Likewise, *C. papaya* methanolic extract significantly suppressed the plumule length of *P. minor* and *E. helioscopia*. Anwar *et al.*, (2018) reported the inhibitory activity of methanolic *Rhazya stricta* extract against the weeds of wheat crop for the seed germination, radical and plumule length like the present investigation. Aslani *et al.*, (2014) reported reduction in seed germination rate, radical and plumule length of rice weeds by methanol extracts of *Tinospora tuberculata*. Likewise, observations were also reported by Turk & Tawaha, (2003) who reported seed germination inhibition by *C. papaya* extract on *A. fatua*. According to observations of Bajwa *et al.*, (2013) different types of plant extracts were observed to be completely inhibitory on germination of *A. fatua*. Likewise, some other researchers such as Oudhi (2001) and Maharjan *et al.*, (2007) have given same findings.

C. papaya leaf powder showed germination inhibition of *A. fatua*, *P. minor* and *E. helioscopia* on filter paper as well as soil by using sandwich method. Anwar *et al.*, (2016) reported strong seed germination inhibition of *A. fatua* and *R. dentatus* by applying *Parthenium hysterophorus* powder. Similar results were found in experiment with lettuce seeds (Gherardi & Valio, 1976; Chow & Lin, 1991) and by Reyes *et al.*, (1980). Chow & Lin (1991) used extracts of sarcotesta and papaya seeds to check germination of lettuce seeds. Such kind of results had already been observed by Gherardi & Valio, (1976). According to them, seed germination inhibition may be attributed to the presence of abscisic acid in sarcophagus of papaya seeds.

Conclusions

Based on results, it is concluded that among the various extracts of *C. papaya*, the methanolic extract remarkably inhibited seed germination, radical and plumule length of all the tested weeds by using optimized assays on both filter paper and soil. Besides, extract applied on soil had more promising inhibitory effect than leaf extracts. The allelopathic activity may be attributed due to the presence of promising allelochemicals in *C. papaya* that can be explored in future studies which may either be used directly or in preparing certain herbicides in particular case.

Acknowledgement

This paper is extracted from the Ph.D. thesis of the first author.

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(Received for publication 27 February 2018)