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Allelopathic effects of *Calotropis procera* (Aiton) W.T. Aiton extracts on crops and fungi

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ABSTRACT

The allelopathic potential of *Calotropis procera* (Aiton) W.T. Aiton was investigated on germination, growth and minerals uptake of 5-Agricultural crops : gram (*Cicer arietinum* L.), tomato (*Solanum lycopersicum* L., mungbean [*Vigna radiata* (L.) Wilczek], fenugreek (*Trigonella foenum-graecum* L.) and lettuce (*Lactuca sativa* L.). For this purpose, we treated seeds with 0 %, 10 %, 20 % and 30 % concentrations of the entire *C. procera* plant aqueous extract. The effect on germination was examined for six days, while the root and shoot length of the seedling were measured after 10 days of growth. The germination parameters, including germination (%) and mean germination time (MGT), were recorded at each concentration, including control. The results showed that these parameters except MGT were significantly reduced at higher concentrations. Likewise, biomass, minerals uptake and chlorophyll contents were markedly reduced. With the *C. procera* extract treatment, the fungal species (i.e. *Fusarium solani, Macrophomina phasiolina, Sclerotinia sclerotium, Fusarium semitectum, Phompsos* sp., *Lasiodiplodia theobromae and Rhizoctonia solani*) exhibited severe inhibition effects with the methanolic and lesser inhibition by the aqueous extract. Allelochemicals like *p*-hydroxybenzoic acid, coumaric acid, syringic acid, ferulic acid, quercetin and caffeic acid were identified using HPLC and showed their antifungal activities. These results suggested that *C. procera* is harmful to crops under field conditions, but its extract may be used as a fungicide to control pathogenic fungi.

Keywords: Allelochemicals, aqueous extract, *Calotropis procera,* fenugreek (*Trigonella foenum-graecum*), fungicidal activity, *Fusarium semitectum, Fusarium solani,* gram (*Cicer arietinum*), HPLC, *Lasiodiplodia theobromae,* lettuce (*Lactuca sativa*), *Macrophomina phasiolina,* mungbean (*Vigna radiata*), physiological attributes, *Phompsos* sp., *Rhizoctonia solani*, tomato (*Solanum lycopersicum*).

INTRODUCTION

Chemical pesticides have made significant contributions to global agricultural development and saved countless people from hunger. However in the recent past, the overuse of chemical pesticides has resulted in excessive pesticide residues in agricultural products, posing severe environmental and human health risks (56). Consequently, two major concepts, including "ecological farming" and "organic products," were developed to reduce use of chemical fertilizers and pesticides and find novel, non-toxic, effective and environmentally friendly substitutes.

Allelopathy is a biochemical interaction between plants caused by secondary metabolites or allelochemicals released from plants (4). Allelopathy is a biological

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phenomenon characterized by the production of diverse secondary metabolites by native or non-native plants, which affects the vegetative growth, reproductive ability and development of associated species (10,50). Plants release various chemical substances such as phenolic acids and terpenoid compounds (35,36). Nevertheless, several environmental factors such as climate, edaphic and microbes under natural circumstances significantly impact these allelochemical (8). But due to the complex interactions between these abiotic and biotic components, determining the allelopathic effects in the natural environment is difficult.

Giant Milkweed [(*Calotropis procera* (Aiton) W.T. Aiton) family Asclepiadaceae] is a well-known evergreen perennial shrub (42) growing within or outside of the agricultural fields (18) between an altitudinal range from 200 to 1500 m above sea level (22). *C. procera* is a characteristic species of the arid and semi-arid environment and is abundantly found in Pakistan. It is a multi-purpose plant, hence, studied due to its phytopathogenic properties (28) and bio-control agent for plants and animals (11). This plant contains cardiac glycosides, flavonoids, sterols, triterpenes and several other allelochemicals or secondary metabolites such as Gigantin, calotropin, catotoxin and calcilin (19), which have negative impacts on the germination, plumule and radicle development of several plant species. Furthermore, a feasible option for biological weed and insect control has been recommended due to its strong allelopathic potential. A few studies suggested that this plant causes acute toxicity as a weed in cropping systems, with considerable effects on cereal crops and vegetables (55). Minerals intake, photosynthetic activity, respiration rate, pigments, hormone production, membrane stability and enzyme activities are examples of plants' morphological, molecular and physiological characteristics affected by its allelochemicals intervention (38). Generally, allelochemicals immediately affect surrounding plants by accumulating in soil and have a long-term deleterious effect on many species' growth, especially on agronomic crops (16). Likewise, allelochemicals are highly adaptable to unfavourable environmental conditions and their allelopathic qualities augment their invasive potential (55). In this context, the phytotoxicity of *C. procera* has been tested against various crops and weed species besides possessing significant pesticidal and fungicidal properties in its extracts. Some researchers have also proposed natural products as a viable solution for controlling fungal diseases (24,25,27,29,31), as these offers a potentially stable and environmentally sustainable alternative to synthetic pestsicides. It has been observed that several plant species, including *Senna occidentalis*, *Chenopodium quinoa* and *Ageratum conyzoides* contains antifungal compounds that significantly reduce *Macrophomina phaseolina* growth (5,25,30). In another study, the methanolic extracts of *C. oxycantha* leaf, stem, root and inflorescence inhibited *Rhizoctonia solani* growth (45). However, these aspects are not extensively researched and more studies are required to validate the fungicidal prospects of *C. procera*.

C. procera grows in crop fields, but its allelochemical interference mechanism with these plants has remained unexplored. Hence, this *in-vivo* and *in-vitro* study aimed to investigate (i) the phytotoxic effects of *C. procera* aqueous extracts on the morphological and physiological attributes of selected agronomic crops during early growth stages and (ii) to test the antifungal efficacy against pathogenic fungus and (iii) to identify its active phenolic components using HPLC.

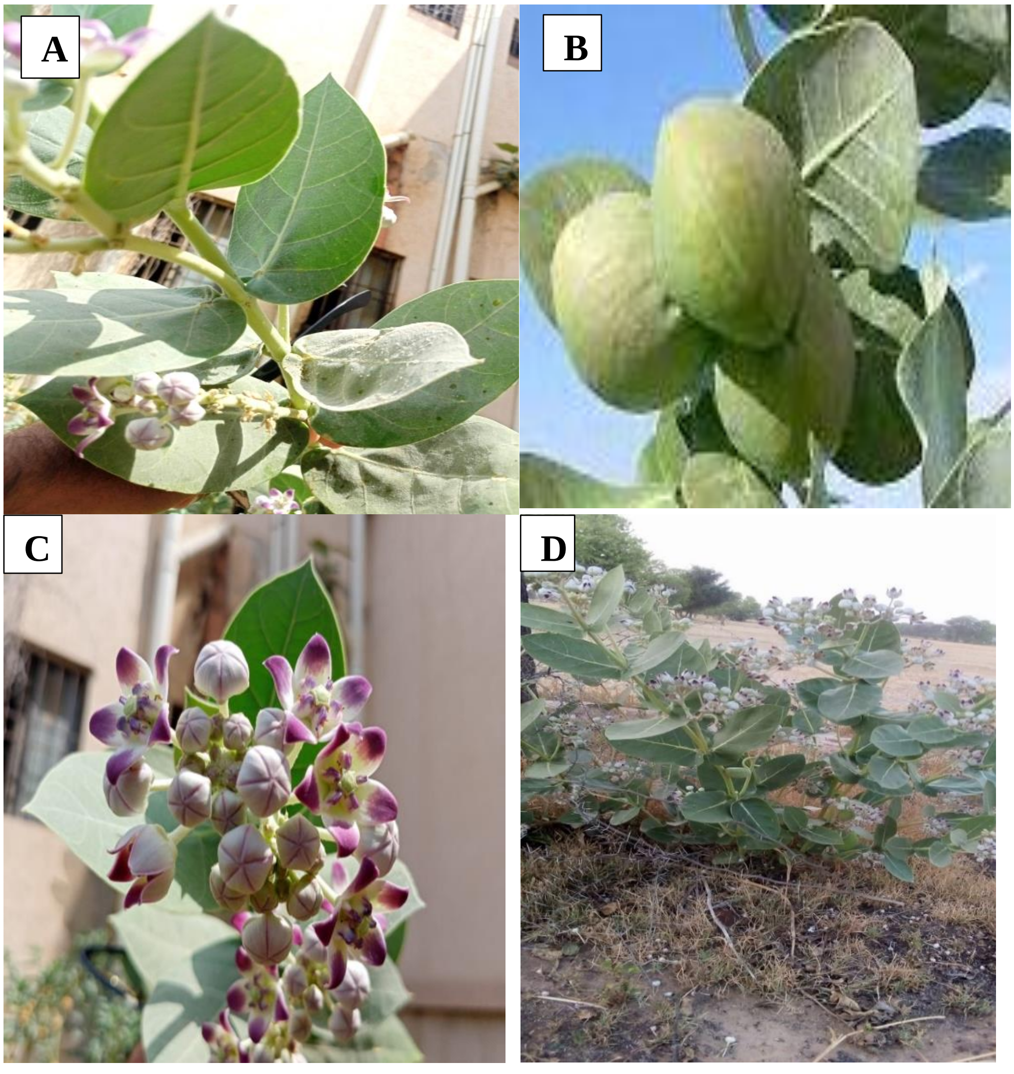

Figure 1. *Calotropis procera* (A) Leaves, (B) fruits, (C) flowers and (D) whole plant

MATERIAL AND METHODS

Plant aqueous extract preparation

Calotropis procera at the flowering stage was collected from May to September 2021 from open patches in the Karachi University (Latitude: 24° 53' 59.99" N, Longitude: 67° 04' 60.00" E, mean annual temperature: 26.1 °C, annual rainfall: 145 mm) and surrounding fields for laboratory analysis.

Plant parts were cut into 5-cm pieces, thoroughly washed with distilled water and air-dried (15 days) at room temperature in the laboratory. The dried samples were powdered using a TFCFL 110V Electric Grain Mill and were soaked (100 g) in 1000 mL distilled water. After an hour of shaking, the extracts were stored at room temperature (25 °C) for 24 h and then filtered using Whatman filter paper. Dilutions of 10 %, 20 % and 30 % of the stock solution were made with distilled water and used in the allelopathic experiments (52).

Lab Bioassay, Pot Culture Experiments

We obtained seeds of 5-agronomic crops (mungbean, lettuce, gram, tomato and fenugreek) from the local market. These crops seeds were surface sterilized with 5 % sodium hypochlorite (for 10-12 min), followed by sterilization with 70 % alcohol (for 1 min) and washed 3-4 times with distilled water.

I. Petri plate Bioassay: In *in-vitro* studies, pre-autoclaved Petri-dishes lined with 2-filter papers were used. We placed 10 seeds of each test specie on filter papers and added 5-10 ml of *C. procera* extract of each concentration (0 %, 10 %, 20 % and 30 %) as per treatments. All treatments were replicated five times in complete randomised design. After 7 days, germination was recorded and calculated different germination parameters as under:

(i). Percent germination: $Percent\ Germination = \frac{Total\ numbers\ of\ seeds\ germinated}{Total\ seeds} \times 100$

(ii). Mean Germination Rate: $MGR = \frac{Total\ number\ of\ seeds\ germinated}{Days\ till-7\ days}$

(iii). Germination index (GI) was calculated as $GI = \sum \frac{Gt}{Dt}$.

Gt : Number of germinated seeds on day t and Dt : Total number of days (7 d in this experiment).

(iv). Coefficient of germination velocity: $CVG = \frac{Ni}{NiTi} \times 100$.

Where, G: Number of germinated seeds per day (7 days in this experiment). Ti : Number of days from the start of experiment, Ni : Number of seeds germinated every day.

(v). Mean germination time: = $MGT = \frac{\Sigma(nd)}{\Sigma n}$.

Where n: number of germinated seeds on day d and Σn: total seeds germinated during the experimental period.

(vi). Median germination time: It is computed as $t50 = \frac{Ti + [(N / 2) - Ni)(Tj - Ti))}{(Nj - Ni)]}$

Where T_{50}: Median germination time, N: Final number of germinated seeds and N_i and N_j are total numbers of seeds germinated at time T_i and T_j respectively when $N_i < N/2 < N$.

II. Pot culture : We also conducted an *in-vivo* experiment in plastic pots (15-cm dia and 10-cm depth). The sterilized seeds were sown 2-3 cm deep in soil in pots, containing 500 g soils (soil: manure = 3:1). The soil was irrigated with 25 ml of 0 %, 10 %, 20 % and 30 % extracts as per treatments. For control, distilled water was used. Another 30-ml was added after every fifth day (150-ml total, 5 times during the experiment). The soil was irrigated with distilled water as needed. After 30 days, the seedlings were harvested. In all test crops, germination traits, root-shoot length, fresh weight and dry weight were determined in all replicates to calculate root-shoot ratios and seedling vigour index (SVI) as under:

Seedling vigour index: $SVI = RL + SL \times GP$

Where, RL: Root length (cm), SL: Shoot length (cm) and GP: Germination percentage.

Chlorophyll Content: The chlorophyll content of fresh leaves was extracted and estimated using the standard method (3). We macerated 0.1 g of fresh green leaf tissues in 3 ml of 80 % acetone and centrifuged at 1000 rpm for 5 min at low temperature (15-20 °C). The supernatant was decanted and the debris was centrifuged again with 80 % acetone until the material became pale (white). The supernatant was collected in a separate tube and acetone was added to bring the total volume to 10 ml. The chlorophyll content was estimated by recording the extracts absorbance at 480 nm, 510 nm, 645 nm and 663 nm using a spectrophotometer (JENWAY 6305). The calculations were made by computing the formulas below;

$$\text{Chl a (mg/g)} = \frac{12.7\,D663 - 2.69\,D645}{W} \times V$$

$$\text{Chl b (mg/g)} = \frac{22.9\,D645 - 4.68\,D663}{W} \times V$$

$$\text{Total Chlorophyll (mg/g)} = \frac{20.2\text{ D645} + 8.02\text{ D663}}{W} \times V$$

Minerals profile

The oxidation method was used to determine the amount of N, P and K in test plants. 0.3 g of plant material was placed in a digestive tube with 2.5 ml of salicylic acid, sulfuric acid, selenium and hydrogen peroxide. This mixture was then agitated for 2 h, then heated for another 2 h at 100 °C and cooled. One ml of H_2O_2 was added to this sample and heated at 330 °C until the extract became colourless. After drying, 48.3 ml of distilled water was added, thoroughly mixed and left for 24 h.

The amount of nitrogen (N^{3-}), phosphorus (P^{3+}) and potassium (K^{1+}) in dry plant material were then evaluated for minerals uptake. The total nitrogen was determined using the Kjeldahl method (Gerhardt 9801/Ac) and the available phosphorus and potassium were determined using a flame photometer (46).

Chemical composition of methanolic extract for antifungal activities

An electric grinder was used to process dried plant material, of which 10 g of powdered material was soaked in 100 mL ethanol, shaken and kept at room temperature for 7 days. The extract was filtered with filter paper after 7 days. A rotary evaporator was used to remove the solvent from the extract. We prepared a stalk solution by adding 100 ml of distilled water to the final volume. make 10 %, 20 % and 30 % PDA. (Potato Dextrose Agar).

In *Calotropis procera* methanolic extract, we identified and quantified compounds present using high-performance liquid chromatography. The material was then centrifuged and filtered into 2 mL HPLC vials using a Polytetrafluoroethylene (PFTE) syringe filter to detect and quantify potential allelochemicals (58). Because of its great effectiveness for hydrophilic molecules, pure methanol recovers free phenolic acids from the soil. Moreover, methanol is the ideal solvent for phenolic acid extraction because it may reduce phenolic compound oxidation caused by phenoloxidase activity (44). HPLC standards are denoted in bold (Table 5).

Statistical analysis

We ensured statistical validity of the unbiased results obtained from the laboratory (*in-vitro*) and pots (*in-vivo*) experiments by adopting a complete randomization procedure, which is often recommended in biological assays for robust quantification or modulation of any desirable physiological signal (43). Experiments conducted under high-stress conditions tend to have more evident geographical patterns and variation in yielding ability, making randomization a critical component of the design process (40). We used 2-way ANOVA to analyze the data for all the treatments of five agronomic crops to determine any statistically significant differences between the means. Since ANOVA is an omnibus statistical test (9). Thereafter, Duncan's multiple range test was used as a Post hoc test for determining specific group differences (37). We performed all the analyses in Microsoft Excel and GraphPad ver. 8.0 respectively.

RESULTS AND DISCUSSION

GROWTH PARAMETERS

I. Germination : The *Calotropis procera* aqueous extract (CPAE) showed inhibitory effects on the seeds germination of test crops (Fig. 1). In all test crops, increased the CPAE concentrations (10 %, 20 %, 30 %) reduced the seed germination percentage (GP). Mung bean (4.1, 6.25, 10.14 %), tomato (5.55, 11.11, 22.22 %) had smaller reductions in germination than gram (35.55, 40, 52.9 %) and fenugreek (35.55, 40, 52.9 %) (13.75, 31.25, 43 %). However the maximum reductions in germination (%) were in lettuce : 20.25, 42.88 and 61.11 %, respectively. Germination inhibition followed the order : mung bean < tomato < fenugreek < gram < lettuce. The germination parameters, such as germination rate, germination index, coefficient of germination velocity and duration to 50 % germination (Table 1), showed the similar decreasing trend as above. The higher concentrations of aqueous extract caused maximum delays in germination of lettuce and fenugreek and delay in germination of mung bean was least. Allelochemical stress inhibits the germination rate by decreasing water intake and modifying the activity of gibberellic acid, which influences the de novo amylase production during the seedling stage (51). However, low leachate concentrations of *Nicotiana* boosted amylase activity in maize seedlings, perhaps due to

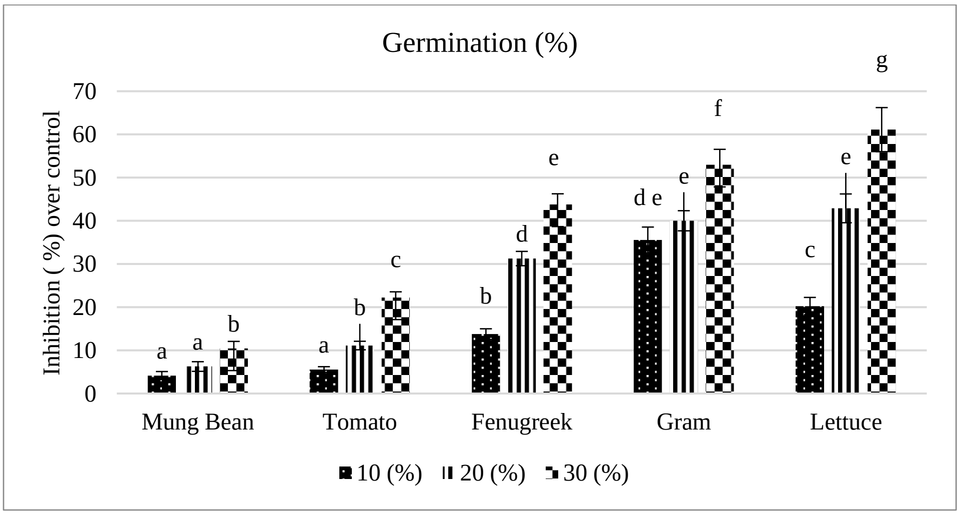

Figure 1. Inhibitory effects of *C. procera* aqueous extracts on Germination of test crops. Means not followed by the SME letter are significantly different at $p< 0.05$.

Table 1. Effects of *C. procera* aqueous extracts (CPAE) concentrations on germination traits of test crops

CPAE (%)	GR	GI	CGV	MGT	T50
Gram					
0	0.41 **a**	1.38 **a**	40.9 **a**	2.46 **a**	1.94 **a**
10	0.38 **(7.3) a**	0.88 **(36) b**	38.3 **(6.3) a**	2.63 **(+6.4) a**	2.13 **(+8.9) a**
20	0.34 **(17) b**	1.1 **(20) a**	34.4 **(15.8) b**	2.92 **(+15.7) b**	2.44 **(+20.4) b**
30	0.32 **(21.9) c**	0.67 **(51.5) c**	32.1 **(21.5) c**	3.13 **(+21.4) c**	2.63 **(+26.2) c**
Mean	0.346 **(15.4)**	0.88 **(35.8)**	34.9 **(14.5)**	2.89 **(+14.1)**	**2.4 (+4.65)**
Mung Bean					
0	0.5 **a**	4.8 **a**	50 **a**	2 **a**	1.5 **a**
10	0.5 **(0) a**	4.6 **(4) a**	50 **(0) a**	2 **(0) a**	1.5 **(0) a**
20	0.43 **(14) b**	4 **(16.6) b**	43.3 **(13) b**	2.32 **(+13.8) b**	1.78 **(+15.7) b**
30	0.41 **(18) c**	3.87 **(19) b**	43.38 **(13) b**	2.29 **(+12.7) b**	1.74 **(+13.7) b**
Mean	0.446 **(10.66)**	4.15 **(13.4)**	45.56 **(8.88)**	2.2 **(+10.16)**	1.67 **(+9.84)**
Fenugreek					
0	0.39 **a**	6.88 **a**	31.2 **a**	1.32 **a**	2.75 **a**
10	0.31 **(20.5) b**	5.75 **(16.4) b**	30 **(3.8) a**	3.21 **(+58.8) b**	2.9 **(+5.2) a**
20	0.29 **(25.6) c**	5.1 **(25.8) c**	29.28 **(6.1) b**	3.42 **(+61.4) b**	3.01 **(+8.6) a**
30	0.29 **(25.6) c**	4.07 **(40.8) d**	28.9 **(7.3) b**	3.47 **(+61.9) b**	3.63 **(+24.2) b**
Mean	0.296 **(23.93)**	4.97 **(27.7)**	29.39 **(5.79)**	3.3 **(+60.7)**	3.18 **(+12.66)**
Lettuce					
0	0.45 **a**	1.47 **a**	43 **a**	2.25 **a**	1.73 **a**
10	0.38 **(15.5) b**	1.1 **(25) b**	38 **(11.6) b**	2.78 **(+19.1) b**	2.27 **(+23.7) b**
20	0.33 **(26.6) c**	0.85 **(42) c**	32.88 **(23.5) c**	3.13 **(+28.0) c**	2.55 **(+32.0) c**
30	0.33 **(26.6) c**	0.5 **(65.9) d**	32.67 **(24) c**	3.2 **(+29.6) c**	2.7 **(+35.9) c**
Mean	0.34 **(22.96)**	0.816 **(44.44)**	34.5 **(19.7)**	3.03 **(+34.9)**	2.5 **(+30.6)**
Tomato					
0	0.49 **a**	2.21 **a**	48.9 **a**	2.05 **a**	1.53 **a**
10	0.38 **(22) b**	1.67 **(24.4) b**	38.1 **(22) b**	2.64 **(+22.3) b**	2.15 **(+28.8) b**
20	0.38 **(22) b**	1.54 **(30) c**	37.8 **(22.6) b**	2.65 **(+22.6) b**	2.19 **(+30.0) b**
30	0.31 **(36.5) c**	1.29 **(41.6) d**	35.42 **(27.5) c**	2.85 **(+28.7) c**	2.33 **(+34.3) c**
Mean	0.356 **(27.2)**	1.5 **(32.12)**	37.10 **(24.11)**	2.71 **(+32.35)**	2.25 **(+30.6)**

Mean values of 10-Replications. GR: Germination Rate, GI: Germination Index, CGV: Coefficient of Germination Velocity, MGT: Mean Germination Time (days), T 50: Time to 50 % germination. The data in Parentheses/ Brackets indicate Inhibition (%) over control for GR, GI and CVG; whereas, Increase (%) over control of MGT and t50. Means not followed by the same letter are significantly different at ($p < 0.05$).

elevated gibberellic acid levels (48). Allelochemicals prevents cell division and elongation, which are key mechanisms for growth and are concentration-dependent (59). Similarly, essential oils of *Achillea biebersteinii* inhibited the seed germination of *Crisium arvense, Amaranthus retroflexus* and *Lactuca serriola* (33).

The CPAE had variable inhibitory effects on the seed's vigour index (SID), depending on the concentration of the extract (Table 2). The 10, 20, 30 % CPAE extracts caused greatest (SID) reduction (15.62, 19.73, 75.7 %) in lettuce, while mung bean was least affected, with reduction of 16.48, 31 and 38.74 %, respectively. The 30 % CPAE caused greatest decrease in SVI in all crops. Gram and fenugreek both showed a similar reduction in SVI. The 10 % and 30 % aqueous extract, identically reduced the SVI in gram (47.77, 61.19 %) and fenugreek (46.39, 60.87 %).

Table 2. Inhibitory effects of C. *procera* aqueous extracts on seed vigour index of test crops

CPAE (%)	Gram	Mung Bean	Tomato	Fenugreek	Lettuce
Control	2480.3 [a]	2509.44[a]	3376 [a]	2530.08 [a]	1919.5 [a]
10	1413 **(43.03)**[b]	2095.94 **(16.48)** [b]	2335.8 **(30.8)** [b]	1797.45 **(28.9)** [b]	1619.5 **(15.6)** [b]
20	1295.4 **(47.77)**[b]	1730.32 **(31)** [c]	1555.2 **(53.94)** [c]	1356.3 **(46.4)** [c]	1540.7 **(19.7)** [b]
30	962.4 **(61.19)** [c]	1537.2 **(38.74) c**	1206.8 **(64.2)** [d]	990 **(60.8)** [d]	465.92 **(75.7)** [c]
Mean	1223.6 **(50.66)**	1787.82 **(28.74)**	1699.1 **(49.64)**	1381.25 **(45.36)**	1208.7 **(37)**

The data in Parentheses/Brackets indicate Inhibition (%) over control. Means not followed by the same letter are significantly different at (p < 0.05)

II. Seedling growth: The CPAE inhibited the root and shoot lengths of all test crop (Figs 2,3) over the control (p 0.05). In all treatments, the greatest reduction was in lettuce root length (42.6, 60 and 69 %). On the other hand, mung bean proved most resistant, resulting

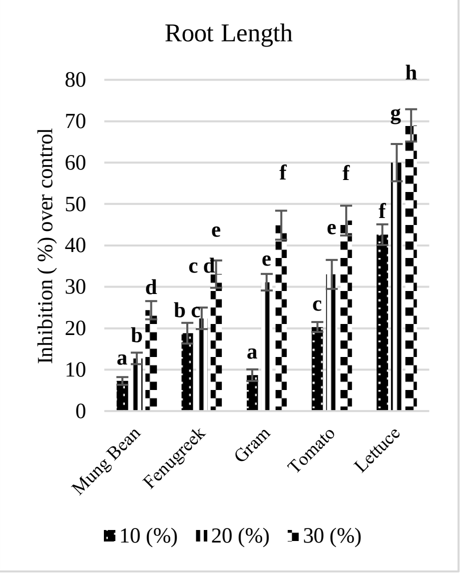

Figure 2. Inhibitory effects of *C. procera* aqueous extracts on root length of test crops. Means not followed by the SME letter are significantly different at $p < 0.05$.

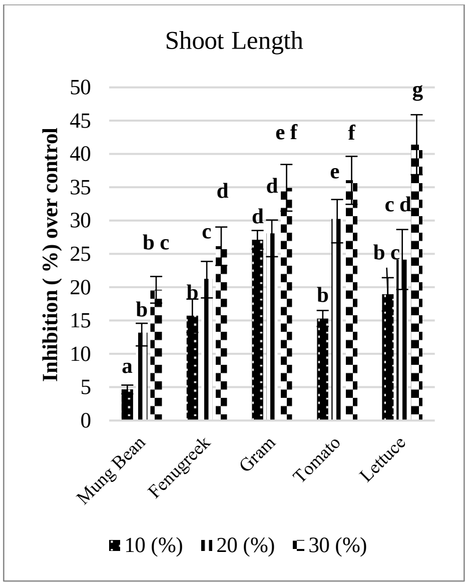

Figure 3. Inhibitory effects of *C. procera* aqueous on shoot length of test crops. Means not followed by the SME letter are significantly different at $p < 0.05$

in the least loss in root length (7.27, 12.72 and 24.36 %). Furthermore, increasing extract concentration significantly reduced the shoot length; the reduction pattern was identical to root, lettuce showed maximum reduction in shoot length (18.94, 24.17 and 41.4 %). While, Mung bean, proved the most resistant crop and showed the least reduction in shoot length (4.66, 13.2 and 19.62 %).

III. Root length: It was more influenced by allelochemicals than shoot length. Because root development is more sensitive to allelochemicals in the rhizosphere, which may alter the root metabolic processes and cell division in root tips, as the root tissues are more permeable to allelochemicals than shoot tissues (14). The higher phytotoxic impact may be due to the direct interaction of allelochemicals and disruption of metabolic processes in the growing regions of the root (15). The root growth reduction due to allelochemical stress limits the water absorption, resulting in a shorter seedlings length (12). Allelochemicals slows down the respiration, mitochondrial activity and ATP production, thus, affecting cellular biochemical processes (7). Due to these physiological changes, plant growth may be impeded.

IV. Biomass: Biomass is the product of plant growth and metabolic processes and plays a significant role in crop yield (34). The CPAE significantly inhibited the dry weight (dry biomass) of test crops seedlings (Figure 4). The CPAE reduced the dry biomass of lettuce, fenugreek and tomato by 51.26 %, 49.23 % and 45.9 %, respectively and the reductions was similar. The CPAE concentration (%) was least harmful to the dry weight of mung bean and gram (29.17 % and 30.8 %, respectively) reduction. The Dry biomass reduction followed the order: Mung bean < gram < tomato = fenugreek < lettuce.

The *C. procera* extract was phytotoxic to proteases, which may be responsible for decrease in the dry biomass. The Eucalyptus extracts reduces *Zea mays* shoot dry biomass (6,49), supporting our findings. On the other hand, several allelochemicals inhibits the macro and micronutrients absorption and IAA oxidase in plant root cells, these decreases the dry and fresh biomass (17).

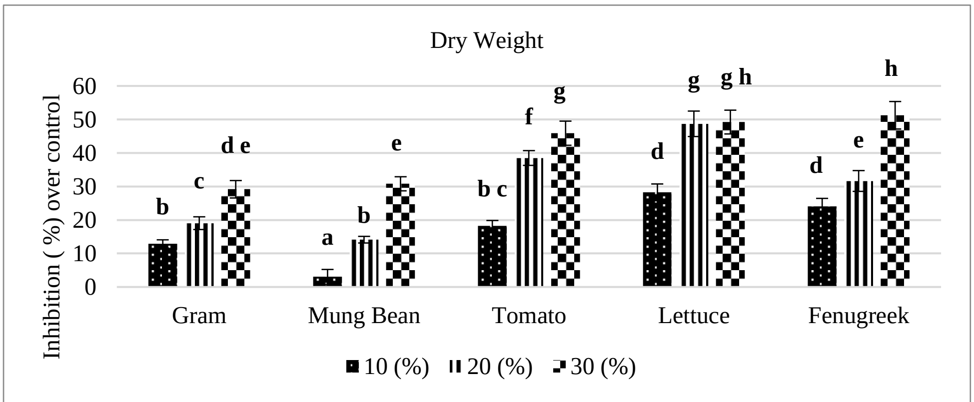

Figure 4. Inhibitory effects of *C. procera* aqueous extracts on seedlings dry matter of test crops. Means not followed by the same letter are significantly different at $p < 0.05$.

Chlorophyll content: CPAE decreases the total chlorophyll contents significantly ($p < 0.05$) of the test crop grown in pots (Table 3). The total chlorophyll content of tomato and lettuce was most affected, with reductions (51.8 % and 46.6 %, respectively), whereas, gram, mung bean and fenugreek were equally affected, with reductions of 34.7, 37.6 and 34.4 %. Allelochemical stress reduces the chlorophyll content (17,48) and might be attributed to decrease in chlorophyll biosynthesis and increase in chlorophyll degradation. The allelochemicals present in black pepper leachates reduced the chlorophyll content of *Vigna mungo*, perhaps by targeting the enzymes involved in the conversion of porphyrin precursors (47).

Table 3. Inhibitory effects of *C. procera* aqueous extracts (CPAE) on Chlorophyll contents of test crops.

CPAE (%)	Chl a (mg g^{-1} F.W)	Chl b (mg g^{-1} F.W)	Total Chl (mg g^{-1} F.W)
Gram			
0	1.44 **a**	1.64 **a**	3.125 **a**
10	1 **(30.5) b**	1.62 **(1.2) a**	2.68 **(14.2) b**
20	0.81 **(43.7) c**	1.53 **(5.5) a**	2.38 **(23.8) c**
30	0.71 **(50.6) d**	1.3 **(19.7) b**	2.04 **(34.7) d**
Mean	0.84 **(41.66)**	1.49 **(8.02)**	2.36 **(24.26)**
Mung Bean			
0	0.46 **a**	0.3 **a**	0.77 **a**
10	0.32 **(30.4) b**	0.23 **(23.3) b**	0.54 **(29.8) b**
20	0.29 **(36.9) b**	0.22 **(26.6) b**	0.52 **(32.4) c**
30	0.24 **(47.8) c**	0.21 **(30) c**	0.48 **(37.6) d**
Mean	0.28 **(38.4)**	0.22 **(26.66)**	0.51 **(33.33)**
Fenugreek			
0	0.56 **a**	0.58 **a**	1.15 **a**
10	0.55 **(1.8) a**	0.42 **(27.6) b**	1.01 **(12) a**
20	0.46 **(16.36) b**	0.32 **(44.8) c**	0.79 **(31.1) b**
30	0.44 **(20) b**	0.2 **(65.5) d**	0.66 **(42) c**
Mean	0.48 **(11.5)**	0.31 **(45.97)**	0.82 **(28.69)**
Lettuce			
0	0.54 **a**	0.34 **a**	0.9 **a**
10	0.3 **(44.4) b**	0.33 **(2.9) a**	0.65 **(27.7) b**
20	0.29 **(46.3) b**	0.22 **(35.2) b**	0.52 **(42) c**
30	0.24 **(55.5) c**	0.23 **(32) b**	0.48 **(46.6) c**
Mean	0.27 **(48.7)**	0.26 **(23.5)**	0.55 **(38.88)**
Tomato			
0	0.97 **a**	0.57 **a**	1.58 **a**
10	0.85 **(12.3) b**	0.34 **(40) b**	1.22 **(22.2) b**
20	0.46 **(52.5) c**	0.3 **(47.3) b**	0.77 **(51) c**
30	0.32 **(67) d**	0.21 **(63) c**	0.54 **(65.8) d**
Mean	0.54 **(43.98)**	0.28 **(50.29)**	0.84 **(46.6)**

The data in Parentheses/Brackets indicate Inhibition (%) over control. Means not followed by the same letter are significantly different at ($p < 0.05$)

Minerals uptake: The CPAE decreased the minerals uptake significantly ($p < 0.05$) of test crops grown in pots (Table 4). Mineral absorption in tomato and lettuce was significantly reduced in the presence of 10 %, 20 % and 30 % CPAE. The 30 % CPAE caused maximum reduction in minerals uptake of tomato : N (38.9 %), P (27 %) and K (51.8 %) and lettuce N (27.8 %), P (52.8 %) and K (51.8 %). In contrast, 10 % CPAE reduction was non-significant in minerals absorption in mung beans, but 20 % and 30 % CPAE significantly reduced the minerals uptake. Among the test species, grams showed the least decrease in chlorophyll and minerals contents, with a reduction in N, P and K uptake of (13.7, 19.2 and 17.1 %). Although all 3-doses of CPAE revealed significant allelopathic potential, but the degree of inhibition increases at higher concentrations. Nutrients absorptions are critical components in plant growth, development and proper physiological activities (39). A few studies showed a substantial decrease in N, P and K absorption in bean plants exposed to allelochemicals from Eucalyptus leaves (13). In addition, phenolic activity is the most recognized ways to reduce nutrients absorption (32).

Table 4. Inhibitory effects of *C. procera* aqueous extract s (CPAE) on Minerals uptakes of test crop

CPAE (%)	N (mg g^{-1} F.W)	P (mg g^{-1} F.W)	K (mg g^{-1} F.W)
Gram			
0	8.10 **a**	0.83 **a**	9.33 **a**
10	7.06 **(12.8) b**	0.82 **(1.2) a**	8.75 **(6.2) a**
20	7.86 **(12.9) b**	0.82 **(1.2) a**	8.67 **(7) a**
30	6.99 **(13.7) c**	0.67 **(19.2) b**	7.73 **(17.1) b**
Mean	7.3 **(9.8)**	0.77 **(7.22)**	8.38 **(10.14)**
Mung Bean			
0	11.6 **a**	5.33 **a**	18.66 **a**
10	10.22 **(11.8) ab**	5.233 **(1.8) a**	17.99 **(3.5) a**
20	9.93 **(14.3) b**	4.94 **(7.3) b**	16.84 **(9.7) b**
30	9.77 **(15.7) b**	4.41 **(17.2) c**	15.56 **(16.6) c**
Mean	9.97 **(14.02)**	4.86 **(8.81)**	16.79 **(9.98)**
Fenugreek			
0	4.94 **a**	2.96 **a**	7.7 **a**
10	4.88 **(1.2) a**	2.58 **(12.8) b**	7.10 **(7.8) a**
20	3.3 **(33) b**	2.55 **(13.8) b**	6.6 **(14) b**
30	3.1 **(37) b**	2.39 **(24.6) c**	5.23 **(32) c**
Mean	3.76 **(23.88)**	2.45 **(17.11)**	6.31 **(18.05)**
Lettuce			
0	6.1 **a**	0.7 **a**	16.8 **a**
10	6.1 **(0) a**	0.61 **(12.8) b**	12.4 **(26.19) b**
20	5.8 **(4.9) c**	0.43 **(38.5) c**	12.3 **(26.7) b**
30	4.4 **(27.8) b**	0.33 **(52.8) d**	10.93 **(34.4) c**
Mean	5.46 **(10.38)**	0.45 **(34.76)**	11.87 **(29.3)**
Tomato			
0	10.55 **a**	0.852 **a**	17.65 **a**
10	9.95 **(5.68)** a	0.798 **(6.3) a**	15.94 **(9.6) a**
20	7.70 **(27)** b	0.694 **(18.5) b**	14.24 **(19.3) b**
30	6.44 **(38.9)** c	0.602 **(27)** c	8.5 **(51.8) c**
Mean	8.03 **(23.88)**	0.7 **(17.3)**	12.8 **(26.9)**

The data in Parentheses/Brackets indicate Inhibition (%) over control. Means not followed by the same letter are significantly different at (p< 0.05)

ANTIFUNGAL ACTIVITY

The effect of pure and different concentrations of *C. procera* methanolic extract (CPME) and *C. procera* aqueous extract (CPAE) is presented in (Fig 5 and 6). Pure methanolic extract demonstrated 100 % inhibition of all examined pathogenic fungal species followed by 30 %, 20 % and 10 % CPME. It was observed that 10 % CPME had no inhibitory impact on *Sclerotinia sclerotium*. These findings are consistent with (53), who investigated the bio-control of *Fusarium mangiferae* and floral malformation in mango using methanol: water (70/30 v/v) *C. procera* extract. The extract was an effective, competitive, cost-effective and long-term technique for controlling floral malformation in mango with minimal pollution. It was also reported that *Ageratum conyzoides* methanolic stem extract and its chloroform sub-fraction have strong antifungal activities against *Macrophomina phaseolina* (5).

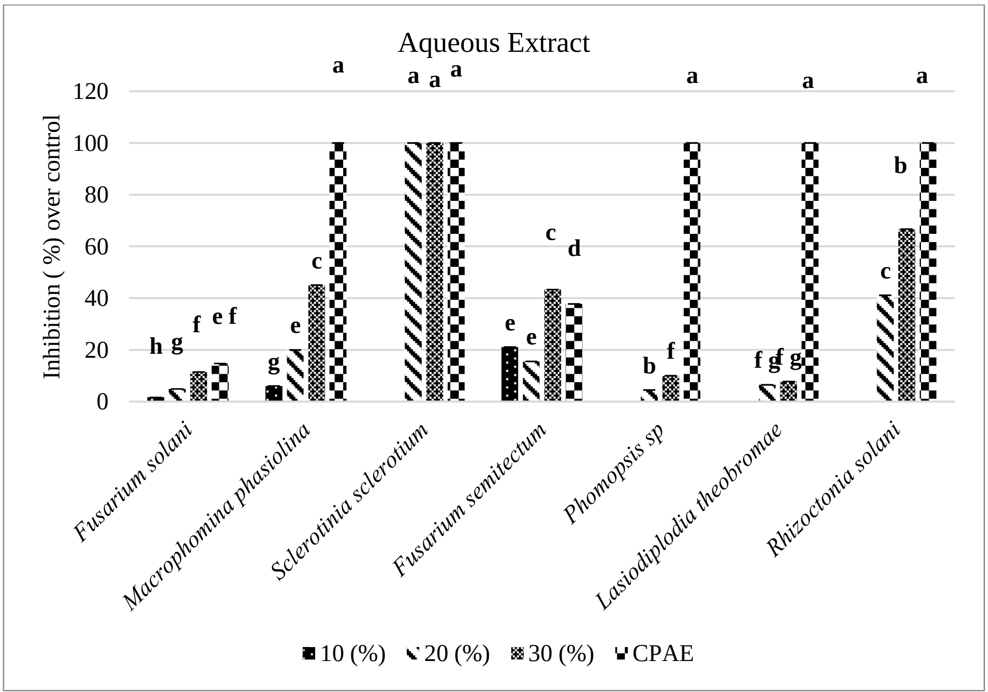

Figure 5. Inhibitory effects of *C. procera* aqueous extract (CPAE) on different pathogenic fungi. Means not followed by the same letter are significantly different at $p < 0.05$.

The aqueous extract suppressed all fungal species investigated; however, it was less effective than methanolic extract. Pure CPAE inhibited *Macrophomina phasiolina, Sclerotinia sclerotium, Phomopsis* sp., *Lasiodiplodia theobromae and Rhizoctonia solani.* In comparison, 30 % and 20 % CPAE were the most effective in reducing *Sclerotinia sclerotium,* whereas, 10 % aqueous extract did not affect fungal species except *Fusarium semitectum*. Water extract was less effective than methanolic extract despite having antifungal activity against all the pathogenic species included in our study. These findings

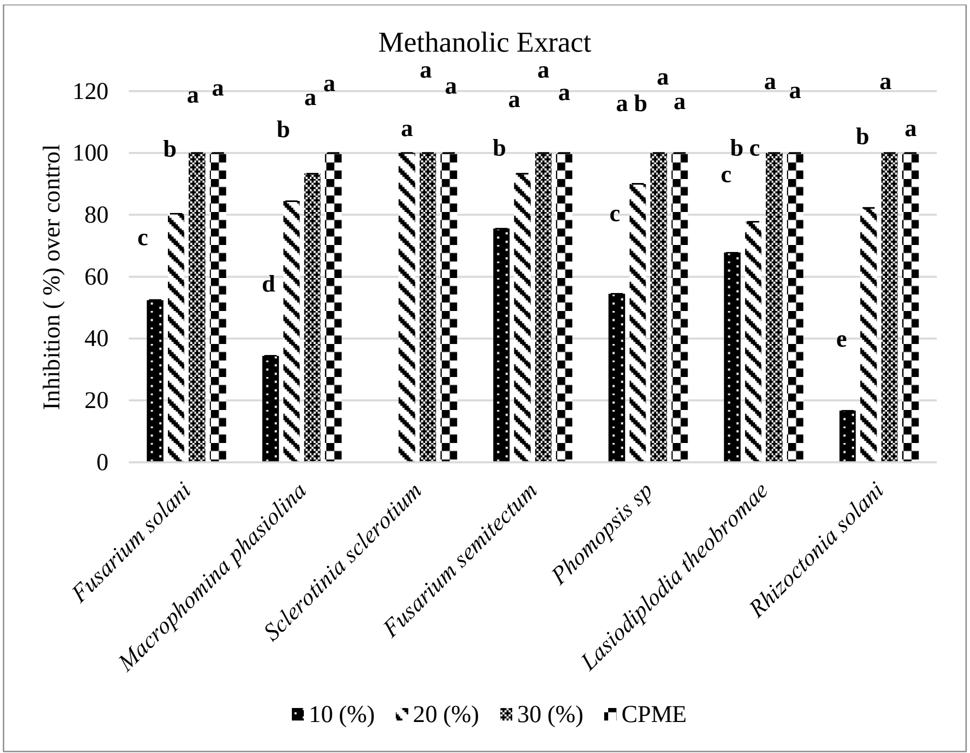

Figure 6. Inhibitory effects of *C. procera* methanolic extract (CPME) on different pathogenic fungi. Means not followed by the same letter are significantly different at $p < 0.05$.

are consistent with (41), who investigated *C. procera* antibacterial activity of aqueous and organic extracts, with the highest antimicrobial activity found in leaf and latex methanolic extracts. Moreover, *Sisymbrium irio* L. leaf extract includes antifungal substances such as sitosterol, di-n-octyl phthalate and 1,3-benzene dicarboxylic acid, bis(2 ethylhexyl) ester, which are responsible for *Fusarium oxysporum* suppression (2).

Chemical composition of *C. procera*

Phenolic acids can alter the plant growth and various physiological and biochemical processes that may cause stress in plants (54). Plant extract from *C. procera* included the 6-phenolic components : *p*-hydroxybenzoic acid, Coumaric acid, Syringic acid, Ferulic acid, Quercetin and Caffeic acid (Table 5 and Fig 7).

The p-hydroxybenzoic acid one of the phenolic compounds, has been reported as an autotoxin in cucumber (*Cucumis sativus* L.) by (20), causing a decrease in meristematic cell activity due to reduced reactive oxygen species (ROS) formation in root tips. p-Coumaric acid strongly affects the root development and lignin concentration in soybean seedlings (57). According to (21), p-coumaric acid, caffeic acid, vanillic acid, ferulic acid and syringic

Table 5. Phenolic profile of *C. procera* leaf (mg/g)

Peak	Rt (min)	Identity	Absorption Spectra (nm)	Contents (mg/g)
1	1	p-Hydroxybenzoic Acid	256	13.2
2	5.3	*p*-Coumaric acid	308	0.44
3	7.4	Syringic Acid	274	0.42
4	11.2	Ferulic acid	325, 279	0.44
5	11.8	Caffeic Acid	323, 298	0.93
6	18.4	Luteolin-7-glucoside	350, 256	15.6
7	19.3	Kaempferol-3-glucoside	348, 266	17.9
8	19.5	Kaempferol-7-glucoside	367, 254	13.4
9	21.8	Isorhamnetin-3-glucuronide	342, 251	1.28
10	24.5	Quercetin	371, 256	2.66
11	27.9	Kaempferol	338, 266	8.98
Total				**75.3**

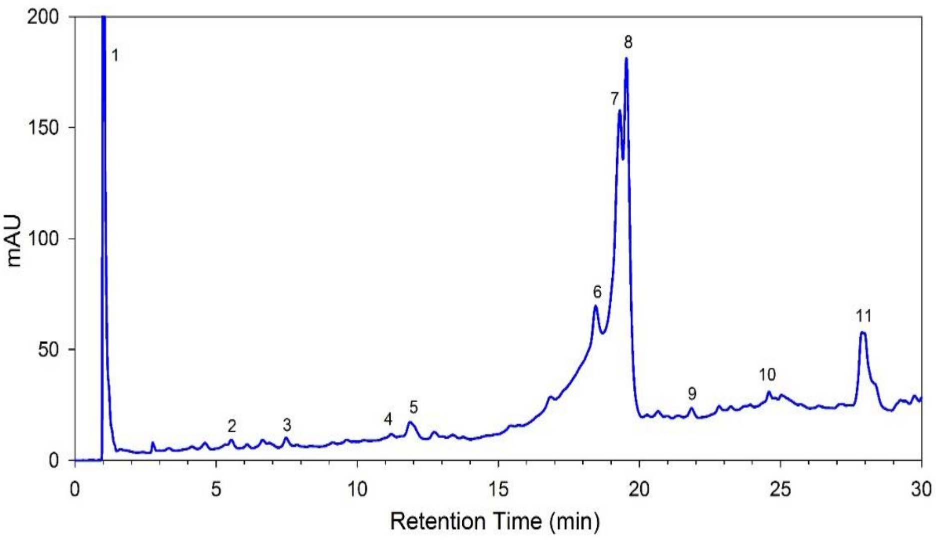

Figure 7. Retention time of Phenolics profile of *C. procera*

acids reduces the plumule and radicle cell sizes in *Allium cepa*. Quercetin and isoquercitrin exhibited weak inhibitory effects on weeds. The p-Hydroxybenzoic acid and Ferulic acid disturb the thylakoid membrane structure, organization and physiology during photosynthesis, causing an imbalance in how electrons are transported, ATP is made and NADPH is generated (23). p-Coumaric acid inhibits the growth of soybean seedlings and root lignin concentration (57). Other acids (Syringic, Ferulic and Caffeic) impede plant development. Plumule and radicle cell diameters were decreased in *Allium cepa* by adding *p*-coumaric acid, vanillic acid, ferulic acid and syringic acid (21).

The phytotoxic effects of *C. procera* extracts may be associated with a number of phenolic compounds present in its plant extracts. These effects significantly reduced the plant growth and fungal activities at higher concentrations of plant extracts. It is apparent from the results that the phytotoxic effects were more pronounced at higher concentrations compared to lower concentrations. Several allelochemicals are responsible for the toxic allelopathic effects of *C. procera* under natural conditions. Phenols are one of the most prevalent water-soluble phytotoxins that significantly impact the development of plants (1).

CONCLUSIONS

The allelochemicals released by *C. procera* were inhibitory to pathogenic soil fungi. Hence, its aqueous and methanolic extract can potentially be used as a fungicide. Whereas allelochemicals released by *C. procera* were inhibitory to germination and growth of test crops, enabling *C. procera* to compete with these species. Such allelopathic plants may also have significant antimicrobial activities that must be tested, popularized and utilized for medicinal purposes at the pace of current health dynamics.

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DECLARATION

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

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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