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Allelopathic potential of *Chromolaena odorata* leaf extracts on seed germination and seedling growth of selected crop and pasture species in Serengeti, Tanzania

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Abstract
The study assessed the allelopathic potential of *C. odorata* aqueous leaf extract on seed germination and seedling growth of crops selected maize and beans (yellow and red), cowpea and finger millet, and pasture species *Clitoria ternatea* and *Macroptilium lathyroides*. Leaf extract concentrations of 0, 25, 50, 75 and 100% were prepared and experiments set in three replicates under completely randomized design. Data on seed germination were collected daily, but shoot and root length were measured on 10th day of the experiment. Germination Index (GI), Rate of germination (RG), Germination percentage (GP), Inhibitory percentage (IP), Seedling Vigor Index (SVI), relative elongation ratio of shoot (RERS) and relative elongation ratio of root (RERR) and were calibrated. *C. odorata* leaves extract significantly effected ($p < 0.05$) GI, GP, SVI and IP for all crops and pasture. The GI and GP reduced with increased concentration on selected species. Nevertheless, cowpea, finger millet and *Clitoria ternatea*, were much affected compared with other species. At 100 %, RERRS and SVI for finger millet, Yellow maize, Red maize, Cowpea, red beans, Yellow beans, *Clitoria ternatea* and *Macroptilium lathyroides* were 68.39, 53.92, 71.43, 3.18, 14.82, 5.90, 34.44 and 200.21, 338.43, 273.82, 0.47, 421.84, 344.53, 14.41, 42.35. The SVI, RERR and RERS were highly impacted at concentration ≥ 75% though GP, GI, and RG were inhibited at 100%. Generally, *C. odorata* leaf extract inhibits seed germination and seedling growth of selected crop and pasture species. Further study suggestions are on inhibitory mechanisms involved and allelopathic behaviour under field conditions.

Keywords: Tanzania, allelopathy, invasive weed, *Chromolaena odorat*a, serengeti

Introduction

In the last two centuries, invasive weeds have been exponentially increasing and invading the most preferred and utilized cereal crops and pasture species in the world (Muzzo *et al*., 2018) [25]. Among them is *Chromolaena odorata* (L.). The weed is noxious invasive species commonly called Siam weed. Alternative common names include Kings Weed in South Africa, Devil Weed in Western Australia, and "Amachabogho" in Serengeti district, Tanzania. *C. odorata* is also a shrub listed among the 100 worst invasive species in the world (IUCN, 2001) [13]. The weed has a deep and massive taproot, growing from 3 m to 7 m and with pungent odorous leaves when crushed. (Muzzo *et al*., 2018) [25]. Sahid and Yusoff (2014) [36] reported being propagated via seed dispersal, but seed germination begins after the rainy seasons where seedlings require sunlight with partial shade to survive. Nevertheless, the invasiveness of the weed to crops and pasture differs with geographical location (Muzzo *et al*., 2018) [25]. Tropical and Sub-tropical areas like Tanzania, Zachariades *et al*. (1999) [50] revealed to be the most vulnerable areas for *C. odorata* invasions.

Muzzo *et al*. (2018) [25] observed the weed in Tanzania, Serengeti, and described it as the most aggressive weed with fast spreading invading grassland habitats, cultivated and arid bushland, as well as in crop and grazing lands. *C. odorata* out competes most with other species in terms of space and nutrient access, reducing crop and pasture yields and quality and general natural ecosystem degradation. Putnam and Tang (1986) [31] stated *C. Odorata* to possess allelopathic properties that support allelochemicals production in all plant tissues present, but mostly on leaves. Though, the composition and amount of allelochemicals for *C. odorata* leaf extracts differ with soil chemical composition, locality, and stage of maturity (Kato-Noguchi and Kurniadie, 2022) [15]. Hence, the study aimed at investigating the allelopathic potential of *Chromolaena odorata* aqueous leaf extracts on seed germination and early growth of the most dominant and preferred cereal crops and pasture species found in Serengeti district, North Western, Tanzania.

Materials and methods

1. Study site

The sample leaves of *C. odorata* and selected crops and pasture species were collected in Kanyemonta Ward in Serengeti district, Tanzania (Figure 1). The study area is 19013.2 ha, located at 35 0 E Long, 20 S Lat, 1416 m altitude and adjacent to Serengeti National Park. The ward vegetation type is grassed woodland with semi-arid climate conditions receiving an annual rainfall of about 500 mm to 1100 mm.

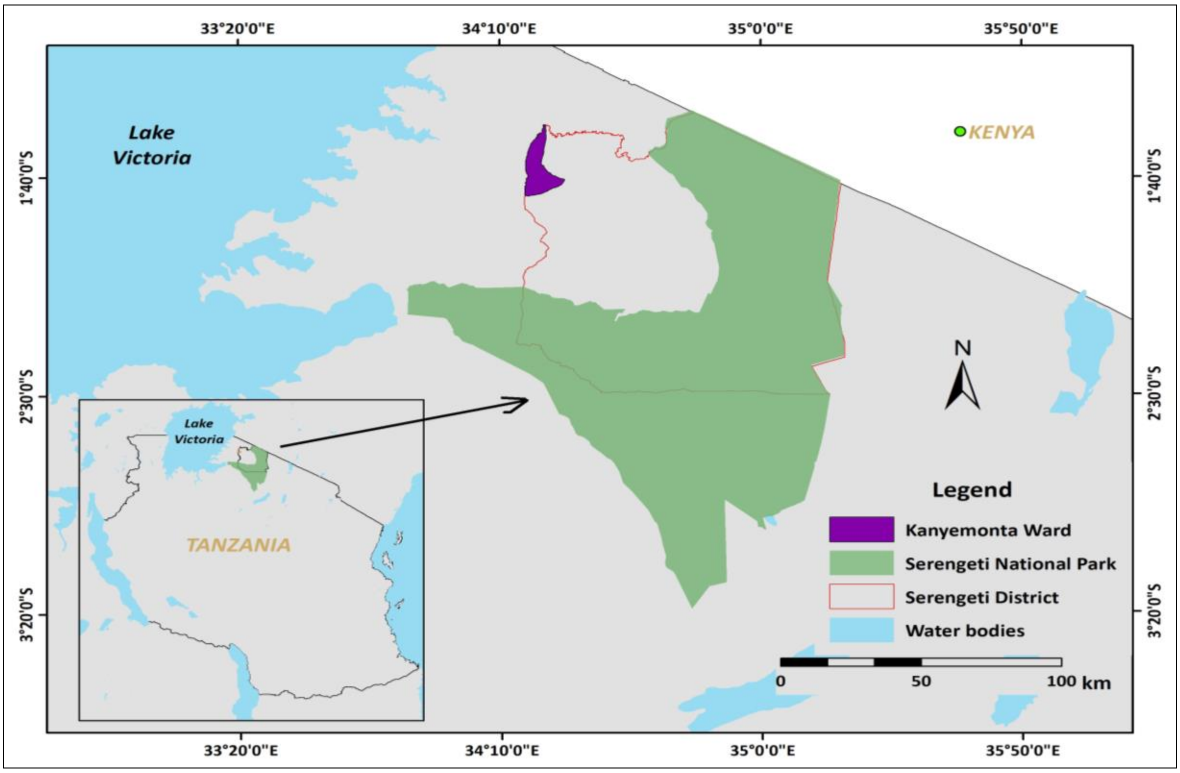

Fig 1: The map of Kanyemonta Ward in Serengeti district, Tanzania

2. Preparation of *Chromolaena odorata* aqueous leaf extract

The collected *C. odorata* leaf samples were analysed at the department of Animal, Aquaculture and Range sciences laboratory, Sokoine University of Agriculture (SUA), Tanzania. The *C. odorata* aqueous leaves preparation was performed according to Muzzo *et al*. (2018) [25]. The *C. odorata* leaves collected were initially air dried at room temperature (30ºC ± 4) for seven days and then in an oven at 70 ºC for 48 hr. The dried leaves of *C. odorata* were milled through a 2 mm sieve. A hundred (100) g of powder extracts was added to 1 litre of distilled water in plastic buckets, vigorously stirred and kept for 24 hrs at room temperature and then filtered through double-layered muslin cloth. The filtrates served as a stock solution of 100 % concentration. By subsequent dilution with distilled water leaf extracts of 25, 50 and 75 % concentrations were prepared and stored in conical flasks until required. However, distilled water was used as control (0%) during the experiment.

3. Preparation of selected crops and pasture species for laboratory study

The collected seeds crops were Finger millet (red variety), *Zea mays* (Red and Yellow), and *Phaseolus vulgaris* (Red and Yellow), *Vigna unguiculata* (Cow pea) while the pasture was *Macroptilium lythrorides and Clitoria ternatea.* The laboratory experimental procedures were undertaken according to Muzzo *et al*. (2018) [25]. The seeds surface sterilization by dipping them into 0.5 % aqueous solution of sodium hypochlorite was first performed thereafter, rinsed several times with distilled water. Fifteen petri dishes of 10 cm double layered with Whatman Number 1 filter papers per specie per 5 concentrations levels from the leaf extracts was used while control in each species is maintained with distilled water. In each selected species, 10 uniform seeds per specie were sequentially placed in separate petri dishes and watered with 10 ml of each prepared concertation and labelled. The petri dishes were kept in a growth chamber at room temperature (28ºC) until the final germination count. The seeds were considered germinated upon the emergence of the root.

4. Experimental design and data collection

The experiment was set with three replicates under a completely randomized design (CRD). The seed germination data were daily recorded while shoot and root lengths were measured 10 days using a 30 cm ruler post sowing. Moreover, calculation of seed germination and seedling growth indices was done as described by Muzzo *et al*., (2018) [25] as below.

Germination indices

$$\text{Germination percentage (GP)} = \frac{\text{Total number of seed germinated}}{\text{Total number of seed sown}} \times 100 \quad (1)$$

$$\text{Germination Index (GI)} = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \ldots\ldots + \frac{\text{No. of germinated seeds}}{\text{Days of final}} \quad (2)$$

Rate of germination (RG**):** was estimated using modified Timpson's index of Khan and Ungar (1984) [17] by firstly calculating Mean Germination Time

$$\text{(MGT)} = \text{MGT} = \Sigma\,(N \times D)/n. \quad (3)$$

Where N is the number of seeds which in D-day grow, n is the total number of seeds grown and D is the number of days from the date of germination. The reversing of MGT at the end of this period RG was obtained

Seedling growth indices

Relative elongation ratio of root (RERR) and shoot (RERS):

$$\text{RER of shoot} = \frac{\text{Mean shoot length of tested plant}}{\text{Mean shoot length of control}} \times 100 \quad (4)$$

$$\text{RER of root} = \frac{\text{Mean root length of tested plant}}{\text{Mean root length of control}} \times 100 \quad (5)$$

$$\text{Inhibitory percentage (IP)} = 100 - \frac{\text{\% FG with } C.\ odorata \text{ aqueous leaf extract}}{\text{\%FG without } C.\ odorata \text{ aqueous leaf extract}} \times 100 \quad (6)$$

Where, FG is final germination percentage.

$$\text{Seedling Vigor Index (SVI)} = \text{Germination \%} \times \text{Root length (cm)} \quad (7)$$

Statistical Analysis

The data for germination and seedling growth indices were analyzed with a one-way of variance under GLM using SAS software version 9.4. The indices mean differences were tested using Least Significant Difference (LSD) fitted at 5% confidence.

Results

1. Allelopathic effects of different concentrations of *Chromolaena odorata* aqueous leaf extract on seed germination and seedling growth of selected cereal grain crops

Finger Millet

The average values of finger millet germination and seedling growth indices are shown in Table 1. Finger millet was affected significantly (P < 0.05) by *Chromolaena odorata* in all indices except RG. The IP increased while the GP, GI, SVI, RERR and RERS decreased significantly (P < 0.05) as concentration increased. Nevertheless, RG did not change significantly (P < 0.05) when *C. odorata* aqueous leaf extract concentration was increasing. Germination and seedling growth parameters of finger millet were highly reduced at 100% concentration level. With regard to seed germination, the RG remained similar but GP and GI decreased with increased concentration levels of aqueous leaf extract of *C. odorata*. The seedling vigour index was more affected by aqueous leaf extract of *C. odorata* than IP, RERR and RERS. The IP increased gradually with increased *C. odorata* concentration and therefore at 75% and 100% concentrations the SVI was twice lower than the control. Additionally, RERS decreased much faster with increased *C. odorata* aqueous leaf extract as compared to RERR.

Table 1: Germination and seedling growth indices of Finger millet

Conc (%)	GP (%)	GI	RG (day^{-1})	IP (%)	SVI	RERR (%)	RERS (%)
0	76.67^{a}	29.35^{a}	0.35^{a}	0.00^{e}	480.59^{a}	105.95^{a}	100.00^{a}
25	68.34^{b}	27.89^{ab}	0.32^{a}	8.26^{d}	378.66^{b}	100.00^{ab}	94.78^{a}
50	65.00^{c}	27.88^{ab}	0.29^{a}	11.77^{c}	320.82^{c}	90.10^{ab}	83.18^{a}
75	63.00^{cd}	26.61^{b}	0.11^{a}	12.96^{b}	235.72^{d}	87.39^{ab}	78.78^{a}
100	61.67^{d}	19.75^{c}	0.07^{a}	18.51^{a}	200.21^{e}	71.43^{b}	68.39^{a}
SEM	0.895	0.756	0.103	0.005	0.145	10.088	12.857
P-Value	0.0001	0.0001	0.2158	0.0001	0.0001	0.0001	0.0001

Values in the same column followed by different values are significantly different at P < 0.05. GP= Germination Percent; GI= Germination Index; RG = Rate of germination; IP= Inhibitory percentage; SVI = Seedling Vigour index; RERR =Relative Elongation Ratio of Root; RERS =Relative Elongation Ratio of Shoot

Yellow maize

The mean values for germination and seedling growth indicators of yellow maize are presented in Table 2. Yellow maize was affected significantly (P < 0.05) by *Chromolaena odorata* in all indices except on RERS. The GP, GI, SVI, and RERR decreased significantly (P<0.05) with increased *C. odorata* aqueous leaf extract concentration from 0 to 100%. On the other hand, IP increased and RERS did not change significantly (P > 0.05). Germination of yellow maize was highly affected at 100% concentration where GP, RG, GI were reduced by 11.11%, 1.98 day $^{-1}$ and 7.32 respectively. Seedling growth was highly retarded (IP and SVI) at 100 % concentration. Nevertheless, RERR and RERS were severely inhibited at 75% and 100% concentrations as compared to the control. Generally, germination of yellow maize was highly inhibited as concentration increased from 50% but germination capacity and speed were reduced at comparable RG. Inhibitory percentage increased causing a rapid decline in seedling vigour leading to higher inhibition of root elongation than shoot.

Table 2: Germination and seedling growth indices of Yellow maize

Conc (%)	GP (%)	RG (day-1)	GI	IP (%)	SVI	RERR (%)	RERS (%)
0	100.00^{a}	2.11^{a}	11.92^{a}	0.00^{c}	2380.79^{a}	107.64^{a}	153.22^{a}
25	100.00^{a}	2.08^{a}	9.70^{b}	0.00^{c}	2240.79^{b}	100.00^{a}	100.00^{a}
50	98.90^{a}	0.79^{a}	8.11$^{b\,c}$	0.00^{c}	1166.94^{c}	97.05^{a}	93.32^{a}
75	95.56^{b}	0.22^{a}	7.30^{c}	3.33^{b}	438.84^{d}	49.83^{b}	81.82^{a}
100	88.89^{c}	0.13^{a}	4.60^{d}	10.02^{a}	338.43^{e}	40.74^{b}	53.92^{a}
SEM	0.861	0.947	0.646	0.012	0.181	10.865	40.797
P-value	0.0001	0.010	0.0001	0.0001	0.0001	0.0001	0.5567

Values in the same column followed by different values are significantly different at P < 0.05. GP= Germination Percent; GI= Germination Index; RG = Rate of germination; IP= Inhibitory percentage; SVI = Seedling Vigour index; RERR =Relative Elongation Ratio of Root; RERS =Relative Elongation Ratio of Shoot

Red maize

The mean values for germination and seedling growth indices of red maize are shown in Table 3. Red maize was significantly (P <0.05) affected by *Chromolaena odorata* in all indices with the exception of RG, RERR and RERS. Increased concentration from 0 to 100%, did not affect significantly (P < 0.05) RG and RERS but increased significantly (P<0.05) the IP and decreased significantly (P < 0.05) the GP, GI, and SVI. The seedling growth of red maize was highly affected at 75% and 100% concentrations. Moreover, IP, GI and RERR at 25% and 50 % concentrations did not change significantly (P >0.05). At the highest concentration (100 %) there was highest growth inhibition (10.02 %) that decreased GP, RG, GI, SVI, RERR, and RERS by 11.11%, 0.52 day $^{-1}$, 7.82, 1489.08, 41.94% and 28.57%, respectively. Consequently, seed germination rate for red maize did not vary with decreasing germination capacity and speed as concentration of *C. odorata* leaf extract increased. With increasing C. odorata concentration inhibitory percentage increased gradually and weakened seedling vigour that affected root elongation while shoot length remained unchanged.

Table 3: Germination and seedling growth indices of red maize

Conc. (%)	GP (%)	RG (day-1)	GI	IP (%)	SVI	RERR (%)	RERS (%)
0	100.00 ^{a}	1.15 ^{a}	14.47 ^{a}	0.00 ^{d}	1762.09^{a}	100.00^{a}	100.00 ^{a}
25	97.78 $^{a\ b}$	1.2 ^{a}	11.48 ^{b}	3.33 ^{c}	874.71^{b}	83.17 ^{ab}	91.37 ^{a}
50	95.56 ^{b}	1.13 ^{a}	10.06 ^{b}	3.33 ^{c}	810.37^{c}	71.76 ^{ab}	86.01 ^{a}
75	92.22 ^{c}	1.12 ^{a}	7.36 ^{c}	6.67 ^{b}	520.45^{c}	60.54 ^{ab}	82.00 ^{a}
100	88.89 ^{d}	0.63 ^{a}	6.65 ^{c}	10.02 ^{a}	273.82^{d}	58.06 ^{b}	71.43 ^{a}
SEM	0.994	0.468	0.70	0.0154	0.916	13.217	19.277
P-value	0.0001	0.3119	0.0001	0.0001	0.0001	0.2229	0.8699

Values in the same column followed by different values are significantly different at P < 0.05. GP= Germination Percent; GI= Germination Index; RG = Rate of germination; IP= Inhibitory percentage; SVI = Seedling Vigour index; RERR =Relative Elongation Ratio of Root; RERS =Relative Elongation Ratio of Shoot

2. Allelopathic effects of different concentrations of *Chromolaena odorata* aqueous leaf extract on seed germination and seedling growth of selected legume crops

Cowpea

The mean values for germination and seedling growth indices of cowpea are summarized in Table 4. Generally, all measured parameters of cowpea were affected significantly (P < 0.05) by *C. odorata* aqueous leaf extract except RG. Inhibitory percentage increased significantly (P<0.05) while GP, GI, and SVI decreased with increased concentration. Seed germination of cowpea was highly inhibited at 100% concentration with GP and GI decreased to 3.33 % and 0.05, respectively. Seedling growth was highly inhibited at highest concentration and thus the SVI, RERR, and RERS were highly decreased to 0.47, 3.17 % and 3.18 %, respectively. Seed germination capacity and speed decreased while the rate of germination did not change as the concentration increased. Inhibitory percentage increased with concentration levels and therefore, seedling vigour deteriorated leading to reduced shoot and root elongation.

Table 4: Germination and seedling growth indices of cowpea

Conc (%)	GP (%)	GI	RG (day-1	IP (%)	SVI	RERR (%)	RERS (%)
0	86.67^{a}	10.45^{a}	0.84^{a}	0.00^{e}	731.46^{a}	151.19^{a}	112.89^{a}
25	78.89^{a}	6.75^{b}	2.49^{a}	16.35^{d}	332.93^{b}	100.00^{ab}	105.87^{a}
50	68.89^{b}	6.28^{b}	2.48^{a}	24.82^{c}	247.15^{c}	85.52^{ab}	100.00^{a}
75	56.67^{c}	3.75^{b}	0.79^{a}	36.83^{b}	196.43^{d}	79.66^{ab}	75.45^{ab}
100	3.33^{d}	0.05^{c}	0.31^{a}	96.34^{a}	0.47^{e}	3.17^{b}	3.18^{b}
SEM	2.676	1.145	1.013	0.012	0.050	35.353	29.480
P-Value	0.0001	0.0001	0.4131	0.0001	0.0001	0.1342	0.1277

Values in the same column followed by different values are significantly different at P < 0.05. GP= Germination Percent; GI= Germination Index; RG = Rate of germination; IP= Inhibitory percentage; SVI = Seedling Vigour index; RERR =Relative Elongation Ratio of Root; RERS =Relative Elongation Ratio of Shoot

Red beans

The mean values for germination and seedling growth indices of red beans are summarized in Table 5. Red beans germination and seedling growth indices were significantly (P < 0.05) affected by *C. odorata* leaf extract. Increased *C. odorata* aqueous leaf extract concentration, increased IP significantly (P<0.05) while GP, GI, SVI, RERR, and RERS (P<0.05) decreased but RG, did not change significantly (P>0.05). Seed germination measurements were highly affected at 75% and 100% concentration. Seedling growth was persistently inhibited from 50 % aqueous leaf extract concentration and therefore SVI was highly weakened as the root and shoot growth declined.

Table 5: Germination and seedling growth indices of Red beans

Conc (%)	GP (%)	GI	RG (day-1)	IP (%)	SVI	RERR (%)	RERS (%)
0	100.00^{a}	7.45^{a}	1.02 ^{a}	0.00 ^{b}	1043.33 ^{a}	106.13 ^{a}	147.47^{a}
25	100.00 ^{a}	6.22 $^{a\ b}$	0.84 ^{a}	0.00 ^{b}	880.00 ^{b}	100.00 ^{b}	100.00 ^{b}
50	100.00 ^{a}	5.22 ^{ab}	0.53 ^{a}	6.67 ^{a}	609.14 ^{c}	93.85 ^{c}	79.65 ^{c}
75	94.44 ^{b}	4.20 ^{b}	0.33 ^{a}	6.67 ^{a}	438.07 ^{d}	90.64 ^{d}	57.76 ^{d}
100	81.11 ^{a}	1.67 ^{c}	0.29 ^{a}	6.67 ^{a}	421.84 ^{e}	71.24 ^{e}	14.82 ^{e}
SEM	0.703	0.724	0.528	0.004	0.027	0.151	7.282
P-Value	0.0001	0.0001	0.8280	0.0001	0.0001	0.0171	0.0265

Values in the same column followed by different values are significantly different at P < 0.05 Germination Percent; GI= Germination Index; RG = Rate of germination; IP= Inhibitory percentage; SVI = Seedling Vigour index; RERR =Relative Elongation Ratio of Root; RERS =Relative Elongation Ratio of Shoot.

Yellow beans

The mean values for germination and seedling growth indices of yellow beans are summarized in Table 6. Yellow beans were affected significantly by *Chromolaena odorata* at (P<0.05) in all indices except on RG. As concentration increased from 0 to 100%, IP increased; but GP, GI, and SVI decreased while RERS, RG and RERR did not change (P> 0.05). Among the germination parameters, GP was affected above 50% concentration level while GI was affected at 100% concentration though RG did not change as concentration varies. Inhibitory percentage of seedling growth of yellow beans was higher at 100% than 75% concentration level i.e. 20.01% and 6.67%, respectively. On the other hand, SVI and RERS were more affected at 100% concentration and were reduced to 662.09 and 95.1% as compared to control.

Table 6: Germination and seedling growth indices of yellow beans

Conc (%)	GP (%)	GI	RG (day^{-1})	IP (%)	SVI	RERR (%)	RERS (%)
0	100.00 ^{a}	19.23 ^{a}	0.38 ^{a}	0.00 ^{c}	1006.62 ^{a}	123.79 ^{a}	100.00 ^{a}
25	100.00 ^{a}	18.08 ^{a}	0.37 ^{a}	0.00 ^{c}	918.02 ^{b}	100.00 ^{a}	90.95 ^{a}
50	98.89 ^{a}	17.92 ^{a}	0.31 ^{a}	0.00 ^{c}	812.65 ^{c}	99.46 ^{a}	80.67 ^{a}
75	94.44 ^{b}	15.95 ^{a}	0.26^{a}	6.67 ^{b}	726.75 ^{d}	77.15 ^{a}	75.75 ^{a}
100	81.11 ^{c}	3.94 ^{b}	0.05 ^{a}	20.01 ^{a}	344.53 ^{e}	52.42 ^{a}	5.90 ^{b}
SEM	0.861	1.364	0.160	0.005	0.018	23.383	9.820
P-Value	0.0001	0.0001	0.0818	0.0001	0.0001	0.2372	0.0004

Values in the same column followed by different values are significantly different at P < 0.05 Germination Percent; GI= Germination Index; RG = Rate of germination; IP= Inhibitory percentage; SVI = Seedling Vigour index; RERR =Relative Elongation Ratio of Root; RERS =Relative Elongation Ratio of Shoot.

3. Allelopathic effects for different concentrations of *Chromolaena odorata* aqueous leaf extract on seed germination and seedling growth in selected pasture legume species

Clitoria ternatea

The mean values for germination and seedling growth parameters of *Clitoria ternatea* are presented in Table 6. *Clitoria ternatea* was significantly (P<0.05) affected by *Chromolaena odorata* in all parameters excluding RG and RERR. The IP increased but the GP, GI, SVI, and RERS decreased significantly (P < 0.05) with increased *C. odorata* concentration. The RG and RERR did not change significantly (P>0.05) as *C. odorata* concentration increased from 0 to 100%. Germination indices for *C. ternatea* were highly inhibited at ≥ 50% concentration level. The RG remained unaffected at any concentration. The IP increased significantly (P < 0.05) with C. odorata concentration and therefore decreased the SVI which was reflected by a decrease in RERS.

Table 7: Germination and seedling growth indices of *Clitoria ternatea*

Conc (%)	GP (%)	GI	RG (day-1)	IP (%)	SVI	RERR (%)	RERS (%)
0	68.89^{a}	6.98^{a}	5.01^{a}	0.00^{e}	169.78^{a}	100.00^{a}	115.05^{a}
25	41.67^{b}	5.87^{a}	2.90 ^{a}	38.45^{d}	89.17^{b}	81.53^{a}	100.00^{a}
50	31.67^{c}	3.12^{b}	2.20 ^{a}	53.88^{c}	57.64^{c}	79.47^{a}	63.19^{ab}
75	25.00^{d}	2.06^{b}	1.33 ^{a}	64.14^{b}	32.65^{d}	79.14^{a}	44.17^{b}
100	20.00^{e}	1.94^{b}	0.84 ^{a}	71.71^{a}	14.41^{e}	62.18^{a}	34.44^{b}
SEM	0.993	0.824	2.174	0.011	0.022	14.348	17.405
P-Value	0.0001	0.000	0.6909	0.0001	0.0001	0.5118	0.0333

Values in the same column followed by different values are significantly different at P < 0.05 Germination Percent; GI= Germination Index; RG = Rate of germination; IP= Inhibitory percentage; SVI = Seedling Vigour index; RERR =Relative Elongation Ratio of Root; RERS =Relative Elongation Ratio of Shoot.

Macroptilium lathyroides

The mean values for germination and seedling growth indices of *Macroptilium lathyroides* are presented in Table 7. The specie was significantly affected (P<0.05) by *Chromolaena odorata* in all indices except RG, RERR and RERS. As concentration increased from 0 to 100%, IP increased significantly (P<0.05) while GP, SVI, and RERR decreased significantly (P<0.05) but RG and RERR and RERS changed just slightly. Germination and seedling growth for the specie was highly inhibited at highest concentration in all indices. However, the RG, RERR and RERS were not affected in all tested concentrations. GP, GI, SVI were reduced to 30%, 13.66 and 220.99 respectively while IP was increased to 30.02%.

Table 8: Germination and seedling growth indices of *Macroptilium lathyroides*

Conc (%)	GP (%)	GI	RG (day-1)	IP (%)	SVI	RERR (%)	RERS (%)
0	100.00^{a}	17.31^{a}	0.94^{a}	0.00^{e}	270.01^{a}	108.96^{a}	100.00^{a}
25	93.33^{a}	13.01^{b}	0.53^{a}	6.67^{d}	230.82^{a}	100.00^{a}	92.36^{a}
50	90.65^{a}	7.62^{c}	0.35^{a}	10.02^{c}	230.82^{a}	98.00^{a}	88.01^{a}
75	88.89^{a}	6.46^{cd}	0.30^{a}	20.01^{b}	75.02^{b}	70.75^{ab}	85.90^{a}
100	70.00^{b}	3.65^{d}	0.15^{a}	30.02^{a}	49.02^{b}	42.35^{b}	76.94^{a}
SEM	4.789	1.225	0.282	0.009	16.552	15.026	9.661
P-Value	0.0001	0.0001	0.3508	0.0001	0.0001	0.0562	0.5683

Values in the same column followed by different values are significantly different at P < 0.05 Germination Percent; GI= Germination Index; RG = Rate of germination; IP= Inhibitory percentage; SVI = Seedling Vigour index; RERR =Relative Elongation Ratio of Root; RERS =Relative Elongation Ratio of Shoot.

Discussion

1. Allelopathic effects of different concentrations of *Chromolaena odorata* aqueous leaf extract on seed germination and seedling growth of selected cereal crops

Finger millet

The seed germination indicators of finger millet were significantly affected by *Chromolaena odorata* leaf extract except the rate of germination (RG). Germination percentage (GP) and Germination index (GI) indicators were highly affected at 100% concentration. The conflicting results were noted under the study for allelopathic effect of *lantana camara* L. on finger millet as among investigated crop that, as the concentration increased germination was not significantly influenced except on Tef whose germination was significantly reduced at 75% (Desalegn, 2014) [6]. However, the RG of finger millet was unaffected at varying concentration levels in the present study. This might be due to non-allelochemicals substances which limit water uptake by the seeds of this particular crop. Seedling growth indicators for finger millet were significantly affected by *Chromolaena odorata* leaf extract. With increase in *C. odorta* concentration, inhibitory percentage (IP) increased while seedling vigour index (SVI), relative elongation ratio of root (RERR) and relative elongation ratio of shoot (RERS) decreased. The reason might be due to an increased amount of phenolic compound as main part of allelochemicals as *C. odorata* concentration increased. Kato-Noguchi and Kurniadie (2022) [15] noted that such phenolic compound prevents cell division and inhibit cell elongation. The highest level of inhibition on seedling growth indices was exhibited at highest concentration (100%). The current study also showed that RERS was highly reduced than RERR as compared to control. Similar results were also noted by Wang *et al*. (2022) who reported that, root length was highly stimulated at low concentration rather than highest concentration. This was due to high phenolic compounds in higher concentrations which prevent root cell division (Li *et al*., 2010) [20]. Chon and Nelson (2010) [5] also noted that leaf extract of most allelopathic plants had high toxic which delayed seed germination and mainly reduced root elongation than shoot.

Yellow maize

Germination indices for yellow maize were significantly affected by *Chromolaena odorata* leaf extract. The results exhibited that germination percentage (GP) and germination index (GI) were highly inhibited at highest concentration. These results agreed with the findings of other workers (Devi and Dutta, 2012 [7]; Usuah *et al*., 2013 [47]). However, GP of 81.11% obtained at 100% concentration under present study was higher than for the previous studies. Among the germination indices, RG was unaffected at any concentration tested. The GP and GI for yellow maize were highly affected by *C. odorata* at highest concentration which might be due to an effective state for its allelochemicals and might act on synthesis of crop germination hormones (Li *et al*., 2022) [19]. Therefore, decline in GP and GI is expected at highest concentration level but the rate of germination (RG) remains unaffected which might be due to particular chemical compounds that might be in *C. odorata* leaves that inhibit plant hormone abscisic acid (ABA) at insignificant difference as concentration increased. This increased seed imbibition ability that might promote cell wall extensibility (Farooq *et al*., 2022**)** [10]**.** through stimulating germination hormones such as gibberellin and auxin or activating essential enzymes such amylases required for seed germination. The observation from the current study showed that, yellow maize seeds had low imbibition period of 2 -3 days with fast priming phase of 4 – 6 days and constant germination for 7-8 days at exponential growth phase. These observations might support the stated reasons above. Seedling growth of yellow maize was also affected significantly by *Chromolaena odorata* leaf extract at varying degree except on relative elongation ratio of shoot (RERS). However, higher inhibitory percentage (IP) was observed from above 25% concentration. This implied that the presence of *C. odorata* leaves extract affected seed germination of yellow maize. However, seedling vigour index (SVI), relative elongation ratio of root (RERR) and RERS were inhibited above 50% concentration level. In contrary, Devi and Dutta (2012) [7] reported opposite results that, highest degree of inhibition in root and shoot growth of *Zea mays L*. was at highest concentration (10%). Moreover, at highest concentration the radicle and plumule length were inhibited to 71.81% and 62.95% while in the present study were 40.74% and 53.92%. The level of concentration leading to highest seedling growth inhibition might vary between maize cultivars. Nonetheless, highest concentration of *Bothriochloa laguroides var* did not only show lower inhibition of RERR and RERS (3.85% and 42.00%) but also higher IP of 80% (Scrivanti, 2010) [38].

Red maize

Among germination indices for red maize, germination percentage (GP) and, germination index (GI) were affected significantly by *Chromolaena odorata* leaf extract. These results are similar with those reported by Adetayo *et al*. (2005) [1], Devi and Dutta (2012) [7] and Usuah *et al*., (2013) [47]. However, the results from this study are contrary to the results reported by Kato-Noguchi and Kurniadie (2022) [15] and Tadele (2014) [42] on the effect of *Lantana camara* and *Tithonia diversifolia* on red maize germination and seedling growth. The present study indicated that, GP, and GI were inhibited by the concentrations of the *C. odorata* aqueous leaf extracts and higher concentration had stronger inhibitory effect. Similar findings were also noted by Devi and Dutta, (2012) [7] and Usuah *et al*., (2013) [47]. The rate of germination (RG) for red maize was significantly unaffected as the concentration of *C. odorata* aqueous leaf extract increased in the study. The seedling growth indices for red maize were significantly affected by *Chromolaena odorata* except RERS. The increase of *C. odorata* aqueous leaf extract concentration resulted to in increased Inhibitory percentage (IP) and therefore decreased significantly seedling vigour index (SIV) and relative elongation ratio of root (RERR) but the relative elongation ratio of shoot (RERS) was not affected significantly. The IP on red maize started just above 50% concentration while other seedling growth indices were inhibited at highest concentration. The present study showed that the presence of aqueous *C. odorata* leaf extract reduced shoot and root elongation which was also supported by Muhammadi (2014) [24]. results. The greater inhibition of RERR than RERS was also in agreement with Masum *et al*. (2012) [22]. It has also been noted that not only root growth in maize is greatly inhibited than shoot by *C. odorata* leaf extract but also in case of *Echinochloa crus-galli* (Tong *et al*., 2022[45]; Suwal *et al*., 2010[41]) and *Cyperus rotundus* (Leela, 1995) [18]. In another study on the allelopathic effect on root growth, Shahrokhi *et al*. (2011) [39] reported that, aqueous extract of *Amaranthus retroflexus* had the greatest inhibitory effect on root length of wheat seedling.

2. Allelopathic effects of different concentrations of *Chromolaena odorata* aqueous leaf extract on seed germination and seedling growth of selected legume crops.

Cowpea

The germination indicators for cowpea were significantly affected by leaf extract of *Chromolaena odorata*. The findings from this study revealed that, cowpea seeds treated with *C. odorata* aqueous leaf extract concentrations had lower germination than seeds in control regime. Thus, as the *C. odorata* leaf extract concentration increased germination percentage (GP) and germination index (GI) decreased. These results are comparable with those of Adetayo *et al*. (2005) [1], Ilori *et al*. (2011) [11] and Popoola *et al*. (2022) [30]. This might be due to an increasing of germination inhibiting allelochemicals substances with the increase in *C. odorata* aqueous leaf extract concentration. Adetayo *et al*. (2005) [1], Ilori *et al*. (2011) [11] and Usuah *et al*. (2013) [47] reported that, the cowpea was highly affected at highest concentration and showed to have lower GP of 14, 45 and 40% respectively than 83.34% of the present study. GP was higher than from previous cited studies. This could be caused by the stage of growth at which sample leaves of *C. odorata* were collected. Phuwiwat *et al*. (2012) [29] pointed that, young weed leaves showed high seed germination inhibition than mature leaves. However, a study carried by Mustapha and Rahimatu (2015) [24] assaying allelopathic effect of leaf and seed extract of nutgrass (*Cyperus tuberosus*) on the germination of cowpea also pointed out that, leaf extract of *Cyperus tuberosus* had high toxicity which had high effect on the germination of cowpea seed especially from the 3rd day after setting the experiment. Also, they stated that, as the concentration increased GP decreased and the highest concentration (in that case 3%) showed no seed germination at all from day 1 to day 13 of the experimental period. This might be due to the difference in the amount and active state of allelochemicals substances between the weeds at pure concentrations. The GI of cowpea in the present study was also inhibited at highest concentration and was reduced to 10.4 compared to control while the rate of germination (RG) was unaffected at varying concentrations. Seedling growth indicators for cowpea were significantly affected by an increase in aqueous *C. odorata* leaf extract concentration levels and this supported results by Ilori *et al*. (2010) [11]. In the present study, inhibitory percentage (IP) increased with concentration levels and agreed with Rafiqul Hoque *et al*. (2003) [32]. Nevertheless, in comparative to control, the IP (96.34%) was higher than 63.3% as reported by Ilori *et al*. (2010) [11] at the highest concentration. Moreover, relative elongation ration of root (RERR), and relative elongation ratio of shoot (RERS) were highly reduced by highest application level of the leaf extract. These results have consisted with the finding of Ilori *et al*. (2010) [11]. However, the reported shoot length (44.28%) and root length (52.02%) at the highest concentration as compared to control by Ilori *et al*. (2010) [11] were lower than 148.02% and 109.71%, of the present study. Seedling vigour index (SVI) showed to be inhibited and was also dependent on concentration levels and decreased to 0.47 at 100 % leaf extract concentration as compared to 731.46 in the control. The seedling growth indicators in the present study revealed to be highly inhibited than other reported findings by Ilori *et al*. (2010) [11] and Rafiqul Hoque *et al*. (2003) [32]. The young stage of the collected leaves sample might be the reason for the difference. The reports show that cowpea seed germination and seedling growth are not only affected by some Crop Residues (Kayode and Ayeni, 2009), but also with other weeds in the family Aesteracea like *Tithonia diversifolia, Helianthus annus, Parthenium hysteropharus and Pichea lanceolata (*Inderjit and Darkshimi, 1994[12]; Javed and Asghari, 2008[14]).

Red beans

Red beans germination indices were significantly affected by *Chromolaena odorata* leaf extract. These findings were similar with studies conducted on other beans cultivars with other weeds such as; *Lyngbya wollei* on *Phaseolus vulgaris* (Bhadha *et al*., 2014) [3], *E. globulus* on kidney- beans (El-khawas and Shehata, 2005) [9] and Silybum *marianum* on Kidney bean (Khan *et al*., 2011) [16]. Seed germination indices were highly affected at 75% and 100% concentration whereas germination percentage (GP) was repressed to 5.56% and 18.89%, germination index (GI) to 3.25 and 5.78 and the rate of germination (RG) was insignificantly reduced to 0.69 day^{-1} and 0.73 day^{-1} compared to control. The results were corresponding with Netsere and Mendesil, (2011) [28] and Khan *et al*. (2011) [16]. Seedling vigour index (SVI) [41] of red bean decreased with increased leaf extract concentration. This was in agreement with Shikha and Jha (2015) [41] and Tanveer *et al*., (2010) [45], and was highly weakened when concentration was ≥ 75%. Yasin *et al*. (2012) [50] claimed SVI of Chickpea and Lentil were reduced when applied with various aqueous extract of *Euphorbia helioscopia* up to 87.23 % due to the phytotoxicity effect of *Calotropis procera*. Moreover, relative elongation ratio of root (RERR) and relative elongation ratio of shoot (RERS) of brown beans were affected significantly by increased leaf extract concentrations levels and were highly decreased to 34.89% and 132.65% as compared to the controls. This similar with the findings of Netsere and Mendesil (2011) [28], Masum *et al*. (2013) [23] and Tahseen *et al*. (2015) [44]

Yellow beans

The results on germination parameters showed that, germination percentage (GP) was affected from above 50% concentration level. This was contrary to Shikha and Jha (2015) [41] and Mersie and Singh (1987) [23], but comparable with Treber *et al*. (2015) [47]. Germination index (GI) was affected at 100% concentration and this was similar to Mersie and Singh (1987) [23]. The RG of yellow beans was unaffected but had insignificant decrease as concentration increased. Conversely, Shikha and Jha (2015) [41] pointed that, RG of *Pisum sativum* was significantly affected by *Parthenium* specie. Seedling growth indices of yellow beans showed that, inhibitory percentage (IP) was higher at 75% and 100% with 6.67% and 20.01%. Yellow bean performed well from concentration less than 50 %. Seedling vigour index (SVI) and relative elongation ratio of shoot (RERS) decreased as concentration increased and was similar with Shikha and Jha (2015) [41] who tested the allelopathic effect of *Parthenium hysteropharus* on leguminous seeds. However, the previous studies carried by Netsere and Mendesil (2011) [28] and Masum *et al*. (2013) [23] showed that, not only SVI and maize was significantly unaffected as the concentration of *C. odorata* aqueous leaf extract increased in the study. The seedling growth indices for red maize were significantly affected by *Chromolaena odorata* except RERS. The increase of *C. odorata* aqueous leaf extract concentration resulted to in increased Inhibitory percentage (IP) and therefore decreased significantly seedling vigour index (SIV) and relative elongation ratio of root (RERR) but the relative elongation ratio of shoot (RERS) was not affected significantly. The IP on red maize started just above 50% concentration while other seedling growth indices were inhibited at highest concentration. The present study showed that the presence of aqueous *C. odorata* leaf extract reduced shoot and root elongation which was also supported by Li *et al*., (2022) [19]. results. The greater inhibition of RERR than RERS was also in agreement with Masum *et al*. (2012) [22]. It has also been noted that not only root growth in maize is greatly inhibited than shoot by *C. odorata* leaf extract but also in case of *Echinochloa crus-galli* (Suwal *et al*., 2010) [41] and *Cyperus rotundus* (Leela, 1995) [18]. In another study on the allelopathic effect on root growth, Shahrokhi *et al*. (2011) [40] reported that, aqueous extract of *Amaranthus retroflexus* had the greatest inhibitory effect on root length of wheat seedling.

3. Allelopathic effects for different concentrations of *Chromolaena odorata* leaf extract on seed germination and growth in selected pasture legume species

Clitoria ternatea

The germination percentage (GP) and germination index (GI) of *Clitoria ternatea* were significantly affected by *C. odorata* leaf extract except the germination rate (RG). However, GP and GI for *Clitoria ternatea* was also reported to be affected by *Argemone mexicana* weed (Namkeleja *et al*., 2013) [27]. In the present study, the GP and GI were significantly affected as concentration of *C. odorata* aqueous leaf increased while the RG decreased insignificantly. The previous studies have reported germination for various crops and pasture legumes were affected by an increasing *C. odorata* aqueous leaf extract concentration **(**Rusdy, 2016[35]; Rusdy *et al*., 2015[36]; Usuah *et al*., 2013[47]; Devi and Dutta, 2012[7]). However, GP and GI were highly inhibited at ≥ 50% concentration level except the RG in the study. These findings are in agreement with those of Treber *et al*. (2015) [47] where the highest GP and GI reduction was recorded at 5% under study carried to assess the allelopathic effect of *pale persicaria* weed on two soya bean cultivars set under 1, 5 and 10 % concentration levels. However, high germination inhibition of *Clitoria ternatea* was reported at the highest concentration of *Argemone mexicana* (Namkeleja *et al*., 2013) [27]. This may be due to high amount and active allelochemicals compounds present at highest concentration that interrupt mitotic activities in young cell (Rice, 1984) [34]. Seedling growth parameters for *Clitoria ternatea* were significantly affected by *Chromolaena odorata* leaf extract except relative elongation ration of root (RERR). This implies that *C. odorata* leaf extract did not affect root elongation of *C. ternatea*. This might be due high amount allelochemicals compound that inhibit stimulation of gibberellin than auxin hormone during lag phase. Moreover, Inhibitory percentage (IP), seedling vigour index (SVI) and relative elongation ratio of shoot (RERS) decreased as concentration of *C. odorata* leaf extract increased in the present study. These results are in contrary with Namkeleja *et al*. (2013) [27] who reported that RERR was among the seedling growth parameter of *C. ternatea* reduced as concentration of *Argemone mexicana* was increased. According to studies carried by Suwal *et al*., (2010) [41]and Adetayo *et al*. (2005) [1] some allelochemicals from *C. odorata* were reported to interfere the process of cell division and elongation in roots and shoots which in turn might reduce SVI and RERS of *C. ternatea* in the current study. Elsewhere, the presence of high concentrations of phydroxybenzoic acid was found to reduce soybean seedlings growth (Barkosky and Einhellig, 2003) [2], vanillin and cinnamic acid decreased eggplant seedling growth (Chen *et al* 2011) [4] cinnamic acid inhibited root growth in cucumber and shoot and root length of cabbage seedlings (Ding *et al*., 2007) [8]. Furthermore, highest inhibition of seedling growth parameters was exhibited at ≥ 50% concentration. The root length is an exceptional indicator of allelopathic effect of plant extracts because it is more sensitive to phytotoxic compounds than shoot growth (Li *et al.,* 2022) [19]. In comparative analysis between control treatment and aqueous leaf extract treatments, the shoot lengths were more inhibited than root lengths. This might be due to high amount of shoot inhibiting phytotoxic compound which are in aqueous that are directly in contact with root in filter paper leading to constant absorption of the extract solution. The root tissues are stated to have greater allelochemicals permeability than shoot (Nishida *et al.,* 2005). Hence, *C. ternatea* roots might have an ability to prevent root inhibiting allelochemicals compounds that resulted to have unaffected RERR and shown to be higher than RERS. However, these findings are opposite to those reported by Sarkar *et al.* (2012) that, leaf extract has more impact on radicle length, because it has direct contact with radicle.

Macroptilium lathyroides

The germination percentage (GP) and germination index (GI) were significantly affected as the concentration of *C. odorata* aqueous leaf extract increased. These findings accord with Rusdy *et al*. (2015) [36] who carried out the study to investigate the allelopathic effect of *C. odorata* on pasture legume that is similar with *M. lathyroides*. However, germination rate (RG) was significantly unaffected in the present study. Additionally, germination indices were highly inhibited at highest concentration level (100%). Similar results were pronounced when *C. odorata* was tested on other species (Rusdy *et al*., 2015[36]; Devi and Dutta, 2012[7]; Sahid and Yusoff, 2014[37]). The opposite results were reported by Treber *et al*. (2015) [47] where highest germination inhibition was recorded at 5% concentration. In comparative to control, GP, GI, and RG were reduced to 30%, 13.66 to 0.79 day $^{-1}$ respectively. The difference in the level of concentration that causes high germination inhibition might be due to specie difference in tolerating stress and preventing ability of germination inhibiting allelochemicals compounds found in *C. odorata*. The effect of *Chromolaena odorata* on *Macroptilium lathyroides* seedling growth was explicated by inhibitory percentage (IP), seedling vigour index (SVI), relative elongation ratio of root (RERR) and relative elongation ratio of shoot

(RERS) parameters. IP and SVI were significantly affected by *Chromolaena odorata* leaf extract and decreased as concentration level increased. These results accord with Rusdy *et al*. (2015) [36] who also carried a study to investigate the allelopathic effect of *Chromolaena odorata* on Germination and Seedling Growth of similar pasture legume. Moreover, physiology on germination and seedling growth clarifies the relation between IP and SVI. This is because, when the seeds are tested with phytotoxic plant (*C. odorata*), tolerance ability of the seed decreased as concentration increases that might result to the decline in seed germination leading to high inhibition of germination. Conversely, RERR and RERS were insignificantly unaffected with anincreased concentration *C. odorata*. However, the opposite results were reported by Namkeleja *et al*. (2013) [27] where RERR and RERS were significantly affected and decreased as concentration of *Argemone mexicana* increased when tested on pasture legume. Moreover, RERR and RERS for numerous cereal crops and pasture species were reported to be significantly affected by *Chromolaena odorata* (Khan *et al*., 2011[16]; Devi and Dutta, 2012[7]; Usuah *et al*., 2013[47]; Sahid and Yusoff, 2014). Seedling growth parameters for *Macroptilium lathyroides* were more inhibited at the highest concentration of *C. odorata* aqueous leaf extract. These results are similar with other reported findings when the weed was tested with other species (Adetayo *et al.*, 2005[1]; Devi and Dutta, 2012[7]; Usuah *et al*., 2013) [47]. Comparatively to control, IP increased to 30.02% while SVI, RERR and RERS were reduced to 220.99, 66.61%, and 20.06% respectively. Moreover, *C. odorata* extract reduced more root growth than shoot.

Conclusion

The early seedling growth of the selected species was more inhibited by *Chromolaena odorata* aqueous leaf extract than germination. High inhibition of germination and seedling growth was highly pronounced at 75 and 100% concentration levels. The effect of *Chromolaena odorata* leaf extracts was revealed in selected cereal and pasture crops. However, yellow maize and finger millet exhibited better performance among the cereal grains, brown beans and cowpea among cereal legumes and *Macroptilium lathyroides* in selected legume pasture species. The effect of *C. odorata* on numerous species was revealed at 75 and 100% concentration. Thus, areas with a high infestation of weed coverage are advised to reduce or remove the weed by advised methods to less than 70 %. Further investigation is needed on the allelopathic behavior under field conditions and the inhibitory mechanism involved. The study was done in the laboratory; hence, it would be interesting to observe the allelopathic effect of the weed on the seed germination and seedling growth of selected cereal and pasture species in field conditions. Moreover, controlling *C. odorota* effect on pasture and crops requires further research for developing environmentally based means opposite to current most regular herbicides.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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