Allelopathic effect of C*hromolaena odorata* extracts on the growth and development of C*elosia argentea*

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ABSTRACT

Plant species avoiding or excluding other plants species from growing in its environment is likely to contain allelopathic substances that are responsible for the reaction. *C.odorata* has been observed to possess allelopathic characteristics. Therefore, the allelopathic potentials of chemical substances present in *C. odorata* was assessed on the growth performances of *Celosia argentea* in a pot experiment. Plant extracts from *Chromolaena odorata* were analysed to determine the active chemical ingredients. Results of physico-chemical analyses of the soil indicated that it was moderately suitable for agricultural purpose, as all the nutrients needed for proper performance were contained in the soil such as %Nitrogen(20.55), %Organic carbon(18.60), Organic matter content (28.40), P(µg/g=5.68), pH(6.86) K (mg/100g=1.20), Ca (mg/100g=25.90), Mg(mg/100g=3.16),Na(mg/100g=1.20), Cu(mg/100g=0.64), and Mn(mg/100g=0.05). *Chromolaena* extracts contained Saponins, tannins, alkaloids, flavonoids and terpenoids as active chemical ingredients. Tanins has the highest mean concentration of 52.6mg/100g of the extract while flavonoids recorded the lowest mean concentration of 15.33mg/100g in the leaves. There is significant difference at p= 0.05 in the growth performance of *Celosia* in control and experiments. The results are t α 0.05= 2.8, 3.0, 1.8, 2.7 and 2.3 for the plant height, stem diameter, mid-rib, number of leaves, and number of branches respectively. *Chromolaena* extracts boost the performance of *Celosia argentea,* instead of affecting the growth and performance negatively.
Keywords: *Celosia argentea*, *Chromolaena odorata*, allelopathic, flavonoids, terpenoids, alkaloids, tannins, saponins.

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INTRODUCTION

Allelopathy is a biological process involving interactions between two plants through the production of chemical compounds (allelochemicals) that are released by leaching, volatilization, decomposition, or root exudation which may induce antagonistic and/or synergistic response from the interacting partners (Whittaker and Feeney, 1971). Hence, allelopathy together with competition is a promising environment-friendly tool for weed management. However, detailed knowledge of this phenomenon is necessary for its successful application due to very limited available knowledge (Cheng, 1992, Roger *et al.,* 2006). Suitable use of allelopathic crops in agriculture could reduce the pesticide application and thereby reduce the environmental and food pollution, decrease cost in agriculture, improve food security in poor regions, soil productivity, and increase diversity and sustainability in the agro-ecosystem (Daizy *et al.,* 2006). Weed management in organic agriculture is one of the most difficult aspects of organic farming and uses especially preventive methods that include ways such as cover crops, mulches, green manure, and intercropping in which allelopathy could play an important role. Roots of allelopathic plants such as cover crops/ green manure (smothering crops) decomposing residues release compounds in the soil that are toxic to weeds (Daizy *et al.,* 2006). The weed-suppressive effect is influenced by species, planting date, seeding rate, method, weather and other factors. Decomposition time of plant residues and amounts of biomass are important factors of weed control by mulching. Annual, biennial, or perennial herbaceous plants in a pure or mixed stand can be grown for these purposes.

Chromolaena odorata is an herbaceous perennial that forms dense tangled bushes. 1.5-2.0m in height. It occasionally reaches its maximum height of 6m (as a climber on other plants). Its stems branch freely, with lateral branches developing in pairs from the auxillary buds (Eze and Gill, 1992). The older stems are brown and woody near the base; tips and young shoots are green succulent. The root system is fibrous and does not penetrate beyond 20-30cm in most soils. The flower heads are borne in terminal corymbs of 20 to 60

heads on all stems and branches. The flowers are white or pale bluish-lilac, and form masses covering the whole surface of the bush (Cruttwell, *et al.,* 1989).

C. Odorata is a big bushy herb with long rambling (but not twining) branches; stems terete, pubescent, leave opposite, flaccid-membranous, velvety-pubescent, deltoid-ovate, acute, 3-nerved, very coarsely toothed, each margin with 1-5 teeth, or entire in youngest leaves; base obtuse or subtruncate but shortly decurrent; petiole slender, 1-1.5cm long; blade mostly 5-12cm long, 3-6cm wide, capitula in sub-corymbose axillary and terminal clusters; peduncles 1-3cm long, bracteates; bracts slender, 10-12mm long, upper ones 8-9mm long, all acute, distally ciliate, flat, appressed except the extreme divergent tip; florets all alike (disc-florets), pale purple to dull off-white, the styles extending about 4mm beyond the apex of the involucres, spreading radiately; receptacle very narrow; florets about 20-30 or a few more, 10-12mm long; ovarian portion 4mm long; corolla slender trumpet form; pappus of dull white hairs 5mm long; achenes glaborous or nearly so (Rice, 1984).

Celosia is a small genus of edible and ornamental plants, similar in appearance and uses to the amaranths. They are sometimes called cockscombs or wool flowers for their brightly loured, woolly flower heads which resemble cockscombs. The name “cocks comb” may be restricted to those whose flower heads are crested by fascination. *Celosia* is a broadleaf annual leaf vegetable (family amaranthaceae) that grows widespread across northern South America, tropical Africa, the West Indies, South, East and South, East and Southeast Asia where it is grows as a native or naturalized wildflower, and is cultivated as a nutritious leafy green vegetable (Gruben, 1977). In Nigeria, particurly amongst the Yoruba tribe, it is known as ‘Soko yokoto’. Suitable use of allelopathic crops in agriculture could reduce the pesticide application and thereby reduce the environmental and food pollution, decrease cost in agriculture, improve food security in poor regions and soil productivity, and increase diversity and sustainability in the agro-ecosystem. Allelopathy together with competition is a promising environment-friendly tool for weed management. However, very little effort regarding the application of this phenomenon has been reported in this part of the world due to very limited

available knowledge. Therefore, this paper focuses on the possibilities of using the allelopathic effect of *Chromolaena odorata* aqueous extracts to control and/or inprove the growth performances of *Celosia argentea*.

MATERIALS AND METHODS

The test plants used were *Chromolaena* seeds and *Celosia* seeds collected (at maturity stage) from Teaching farms, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso Premises. Agricultural soil was collected in front of the convocation ground, beside the Department of Pure and Applied Biology, LAUTECH, Ogbomoso.

The seedlings of the *Celosia argentea* were raised in two buckets of 5-litres sized of 25cm height and 9.5cm diameter. 7.5kg of soils were filled into each of the 11-litres bucket of 25cm height and 9.5cm diameter which served as experimental pot. The seeds of *Celosia* were transplanted after two weeks into the experimental pots containing the soil at two weeks per pot. It was later thinned to one seedling per pot.

220g weight of leaves of the whole plants of *Chromolaena odorata* except the root were chopped into small pieces of 4 cm length and later blended with mechanical grater. The ground plant was soaked in 5 litres of water for 24 hours. The extracts were then filtered, the filtrate obtained served as treatment for the raised seedlings in the first set experimental pots, the fresh filtrate served as treatment for the raised seedlings in the second set experimental pots. While the other set experimental pots were wet with water which served as control. Each of these treatments was replicated ten times.

The vegetative growth parameters of *Celosia argentea* were monitored with the following parameters recorded: Height of plant in centimetre using a ruler, width of stem using Vernier caliper, length of leaf mid-rib in centimetre using a ruler measuring from the tip of the leaf to the tip point of the petiole. Number of leaves and Number of branches were counted.

At two weeks after transplanting, height of plant, number of leaves, number of branches, width of stem and length of median-rib were measured

and recorded for each pot and this is done fortnightly for 12 weeks

Four samples of soils used for experiment were taken to Kappa Biotechnology Centre, Ibadan for laboratory analysis. Aqueous extract was collected from the plant shoot (leaves) using homogenizer (blender) mixed with distilled water, it was then filtered. The filterate served as extract. This extract was taken to Kappa Biotechnology for active ingredient analysis. Common anti-nutrients in food and feed include Oxalate, Phytates, Alkaloids, Saponins, Tannins, etc. Each is determined by extracting them into particular solvent systems pre-determined for it and measured either by instrumental method or titration. For instrumental determination, standard graphs are needed, using specific standards related to the anti-nutrient being determined. The unknown is read off from the standards graph.

Oxalates: Sample is extracted with water for 2 hours. The extract is measured at nm in a spectrophotometer. Standard Graph is produced using Oxalic acid. The unknown is read off from the graph.

Phytates: Sample is extracted with Iso-butyl alcohol for 5 hours. The extract is measured at nm in a spectrophotometer. Standard Graph is produced using Phytic Acid. The unknown is read off from the graph.

Saponins: Sample is extracted with Petroleum Ether for 6 hours. The extract is measured at nm in a spectrophotometer. Standard Graph is produced using Saponic Acid. The unknown is read off from the graph.

Tannins: Sample is extracted with a mixture of 20% of 10% Glacial Acetic Acid and 80% Acetone for 5 hours. The extract is measured at nm in a spectrophotometer. Standard graph is produced using Tannic Acid. The unknown is read off from the graph.

Alkaloids: The sample is extracted by heating under reflux with Ethanol and water mixture this is done to eliminate Non-alkaloid Nitrogen. The residue is digested in a Kjeldahl flask. The digested is distilled and the Percentage Nitrogen is determined by titration. The Percentage Nitrogen is multiplied by 3.26 to obtain the amount of Alkaloids in part per million.

Oxalate: Tile extraction is done by weighing 1g of each sample into 250m/s conical flask and soaked with 100m/s of distilled water. These are allowed to

stand 3 hours and each is filtered through a double layer of filter paper. 10ppm, 20ppm, 30ppm, 40ppm and 50ppm standard solution of Oxalic Acid are prepared and read on the spectrophotometer at 420nm for the absorbance. The absorbances of filtrate from each sample are also read on the spectronic 20.

Phytic acid (Phytate): 2g of each sample is weighed into 250m/s conical flask 100m/s of 2% concentrated Hydrochloric Acid is used to soak each sample into conical flask for 3 hours. This is filtered through a double layer of hardened filter paper. 50m/s of each filtrate is placed in 250m/s beaker and 107m/s of distilled water is added in each case to give proper acidity. 10m/s of 0.3% Ammonium Thiocyanate solution is added into each colourless solution to determine the percentage of Ammonia (NH_3) by Kjeldahl distillation method Percentage Nitrogen got is converted to percentage total Alkaloid by multiplying by a factor of 3.26.

Saponin: 2g of sample was weighed into a 250ml beaker and 100ml of Isobutylalcohol was added left for 5 hours on a UDY shaker for uniform mixing to obtain uniform solution. The mixture was then filtered through a No1 Whatman filter paper. The filtrate is then transferred to another 100ml beaker and was saturated with magnesium carbonate solution. The mixture obtained here was then filtered to obtain a clear colourless to be read on a spectrophotometer at 380nm. 0ppm to 10ppm of standard saponin solution were prepared from 1000ppm saponin stock standard solution and saturated with magnesium carbonate above and also filtered. The absorbances of the saponin standard solution (that is 0-10ppm) were also read at 380ppm to obtain the gradient of the plotted curve.

Tannic acid (Tannin): 1g of each sample was weighed into a beaker. Each was soaked with solvent mixture (80m/s of acetone and 29m/s of glacial acetic acid) for 5 hours to extract tannin. The filtrates were removed; the samples were filtered through a double layer filter paper to obtain the filtrate. A standard solution of Tannin acid was prepared ranging from 10ppm to 30ppm. The absorbances of the standard solution as well as that of the filtrate were read at 500nm on a spectronic 20 solution as indicator. This is titrated with standard Iron (III) chloride solution which contained 0.00195g iron per ml. The end

point is slightly brownish yellow which persisted for 5 minutes.

Total alkaloids: 2g of sample is weighed into a 100ml conical flask and 20ml of 80% of Alcohol added to give a smooth paste. The mixture is transferred to a 250ml flask and more alcohol is added to give up to 100ml of magnesium oxide is added. The mixture is digested in a boiling water bath for 1.5 hours under a reflux air condenser with occasional shaking. The mixture is filtered white hot through a small Buchner Funnel. The residue was returned to the flask and re-digested for 30 minutes with 50ml alcohol alter which the alcohol will be evaporated, adding hot water to replace the alcohol lost. When all the alcohol has been removed 2 to 3 drops of 10% HCl is added. The whole solution was transferred into a 150ml volumetric flask. 5ml of Zinc Acetate solution and 5ml of potassium ferrocyanide solution is added, thoroughly mixed to give a homogenous solution. The flask is allowed to stand for few minutes, filtered through a dry filter paper and 10ml of filtrate is transferred into a separating funnel and the alkaloids present are extracted vigorously by shaking with five successive 30ml portions of chloroform. The residue obtained is dissolved in hot water and transferred into a Kjeldahl flask with the solution. 0.2g sucrose and 10ml concentrated H_2SO_4 and 0.02g selenium for digestion.

Statistical Analysis: Analysis of variation was employed to analyse the fresh shoot extracts of *Chromolaena odorata* to show variation in the active ingredients.

RESULTS AND DISCUSSION

The agricultural soil was analysed before the experiment Iron (Fe) was the highest in quantity. Organic Matter Content was very high and it shows that the soil can accommodate plant growth conveniently (Table 1).

Table 1: Physico-chemical properties of the agricultural soil before the experiment.

Physical/ Chemical properties	*Quantity*
pH	7.0
Organic carbon (%)	18.6
Organic matter (%)	23.4
Phosphorus (ug/g)	5.63
Nitrogen (%)	20.5
Calcium (mg/100g)	25.89
Magnesium (mg/100g)	13.16
Potassium (mg/100g)	11.15
Sodium (mg/100g)	1.54
Manganese (mg/100g)	0.05
Iron (PPM)	32.63
Copper (PPM)	11.01
Zinc (PPM)	91.01

Table 2: Active ingredients in *Chromolaena* plant extract.

Active ingredients	*Alkaloids*	*Saponins*	*Tannins*	*Flavovoids*	*Terpenoids*	*Cyanogenetic Glycosides*
Values in mg/100g	44.1667	35.83333	52.6	7.5	30.66667	ND
Standard Error	0.83333	0.166667	1.307669	0	0.666667	ND
Standard Variation	1.44338	0.288675	2.264950	0	1.154701	ND
Sample Variation	2.08333	0.083333	5.13	0	1.333333	ND

ND= Not Detected

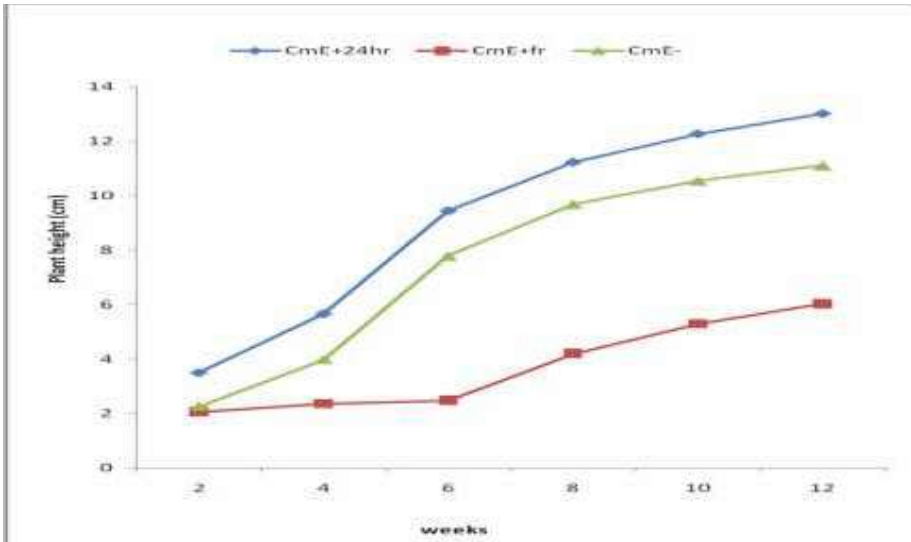

Fig. 1: Plant height of *Celosia argentea*

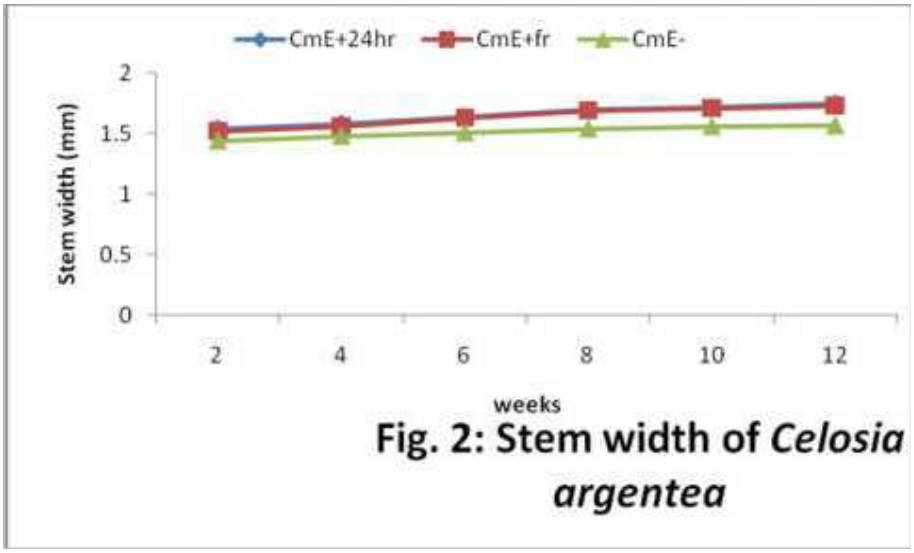

Fig. 2: Stem width of *Celosia argentea*

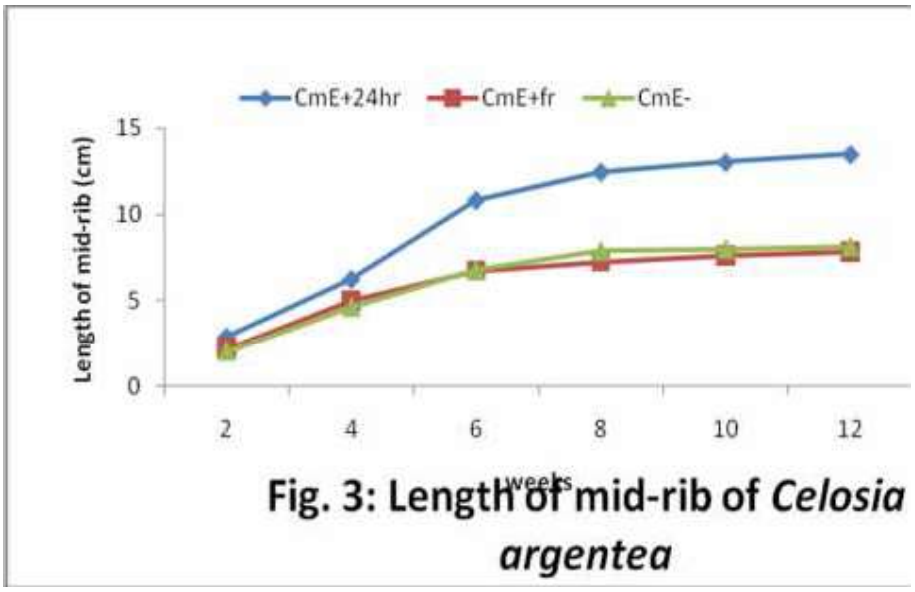

Fig. 3: Length of mid-rib of *Celosia argentea*

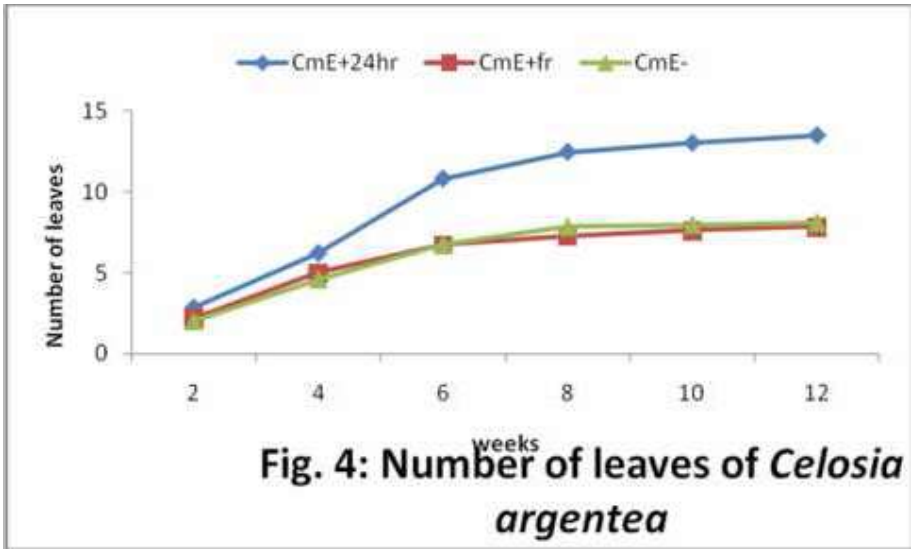

Fig. 4: Number of leaves of *Celosia argentea*

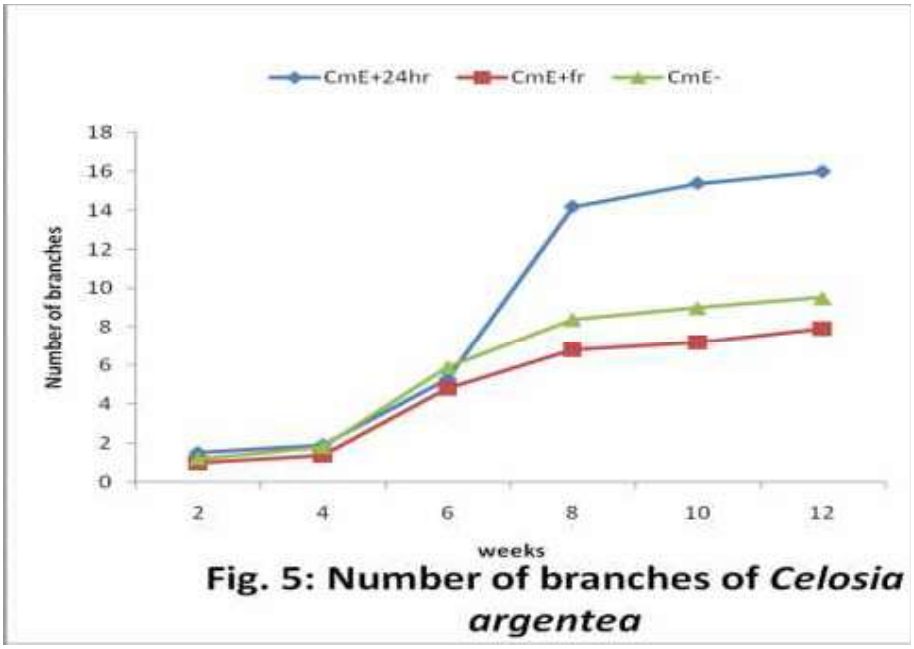

Fig. 5: Number of branches of *Celosia argentea*

It was observed that there was accelerated growth on the general growth of *Celosia argentea* been treated with the 24 hr shoot aqueous extract of *Chromolaena odorata* compared to the growth of *Celosia argentea* been treated with fresh shoot aqueous extract (FSAE) of *Chromolaena odorata* and compared to the steady but slow growth rate of *Celosia argentea* treated with water as the control, as been shown in the graphs of growth parameters in Figures 1-5

The results obtained from this study indicated that the shoot height, leaf area, fresh and dry weights of the plants treated with the aqueous were higher than those of the plants in the control regime. These showed that the aqueous extract of *Chromolaena odorata* enhanced these growth parameters of *Celosia argentea*. This shows that *Chromolaena* extract have beneficial role on *Celosia argentea.* This result agrees with the work of Rice, 1984 that allelochemicals have been reported to have a beneficial or harmful effect on the growth and development of plants. The result was contrary with the finding of Chengrong *et al*., (2005), who stated that allelochemicals from *Wedelia triblabata* reduced germination, plants height; fresh and dry weights root and

shoot per plants of rice. Also, Daizy *et al*., (2006) finding that aqueous leachate of (*Chenopodium album*) plant parts (root, whole plant and leaf) inhibited the germination, plant height, growth and biomass of *Cassia occidentalis* was inconsistent with the result of this study. However, the result also agreed with the study of Hussain *et al*., (2007), who stated that senna extract promoted the growth of *Avena fatua*, *Dactyloctenium aegyptium* and *Echinochloa colona.* This observation might be due to low concentration of allelochemical present in the aqueous extract. This was consistent with the observation of Einhellig *et al*., (1982) who stated that allelochemicals have to be present above a threshold concentration for impact. He was of the opinion that some plants processes might be stimulated below this threshold. The stimulatory effects on some growth parameter of *Celosia argentea* indicated that the weed could be used as green manure.

CONCLUSION

Allelopathy, through allelochemicals (tannins, saponins, alkaloids) extracted from *Chromolaena odorata* have beneficial effect on the growth and development of potted *Celosia argentea* plants. *Chromolaena odorata extracts promotes growth of potted celosia plants better* than water (control).

Twenty four (24hrs) hour shoot aqueous extract of was more active and effective than fresh shoot aqueous extract (FSAE) on growth of *Celosia argentea* while the control i.e. *Celosia argentea* treated with water had slower growth rate than those with extracts from *Chromolaena odorata*.

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