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Effect of Crude Extracts of Six Plants on Vegetative Growth of Soybean (*Glycine max*Merr.)

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Abstract: Boosting production by applying natural products derived from readily available plant sources to increase vegetative growth of soybean could help ensure food sovereignty, security and affordability of this important source of dietary protein. A preliminary trial using crude extracts from six potential growth stimulating sources suggested while treatment with C. asiatica, M.esculenta (cassava) and G. linearis leaf extract increased soybean plant height and leaf area, A.scholaris stem bark, G.mangostana pericarp and Zea mays silk reduced growth. Promising crude extracts from the first trial were retested at five dilute concentrationson14-day old soybean plants using nested design with four replications. Analysis of variance and Duncan's Multiple Range Test ($p \leq 0.05$) showed that crude extracts of cassava leaves, C.asiatica and G. linearis increased plant height, while only C. asiatica increased leaf area. The highest plant height and widest leaf area were obtained by applying 25mg/l crude extract of C. asiatica.

Keywords: soybean, vegetative growth, crude extract, growth stimulating.

Introduction

A green revolution program usingchemical fertilizers does not contribute to productivity enhancements after a certain saturation point. An alternative solution for increasing the growth of soybeans can be done using growth promoting substances from natural products. Up until now, the plant growth promoting proprieties of secondary metabolites of higher plants have been studied and used on a limited number of crops. Some plant crude extracts have, in fact, been reported to increase the plant growth. An extract of garlic, aloe and henna increased the fresh weight of *Schefflera arboricola* (**Hanafy *et al.*,**

2012). The application of red grape skin extract and alfalfa hydrolysate to pepper plants resulted in increased leaf biomass and weight of green pepperswhile increasing the growth and number of red peppers **(Ertani *et al*., 2015)**. Treatingmaize (*Zea mays* L.) with an extract derived from red grape skin (*Vitis vinifera* L.), blueberry fruit (*Vaccinium vitis-ideae* L.) and hawthorn leaves (*Crataegus monogina* Jacq.) resulted in increased root and leaf biomass, chlorophyll and sugar content **(Ertani *et al*., 2016)**. The application of *Moringa oleifera* leaf extract increased growth and yield of tomato **(Culver *et al*., 2012)**, maize and common beans **(Mvumi *et al*., 2012)**, some growth parameters and photosynthesis rate of rocket (*Eruca vesicaria* subsp.*sativa*) **(Abdalla, 2014)**, germination and growth of wheat (Sarmin, 2014) and yield of sesame (*Sesamum indicum*) **(Muhamman and Mohammed, 2014).**

Aqueous extract of arjun tree leaves (*Terminalia arjuna*) increased germination and growth of turnip and okra. Treatment of aqueous extract of *T. belerica* increased germination and growth of spinach, while aqueous extract of neem (*Azadiracta indica*) inhibited a percentage of germination **(Talukder *et al*., 2015)**.

Secondary metabolite compounds including terpenoids, flavonoids, alkaloids and phenolic compounds have a potential as stimulants or inhibitors of plant growth. Secondary metabolites of some plants have been reported to have a wide range of bioactivity. *Centella asiatica* contains triterpenoids (terpenoids), flavonoids, and steroids **(Biradar and Rachetti, 2013; James and Dubery. 2011; Singh *et al*., 2012)**. Corn (*Zea mays*) silk contains steroids, flavonoids, and phenolic compounds **(Hasanudin*et al*., 2012; Bhaigyabati *et al*., 2011)**. Cassava leaves contain flavonoids, terpenoids, alkaloids, steroids and saponins and phenolic compounds **(Siyumbawe *et al*., 2014; Anbuselvi and Balamurugan, 2014; Nuwamanya*et al*., 2014)**. *Gleichenia linearis* fern leaves contain flavonoids and kaempferol **(Yusuf *et al*., 2003; Jubahar, *et al*., 2006; Dey, 2011)**.The pericarp of mangosteen (*Garcinia mangostana*) fruit contains flavonoids, phenolic compounds and xhantons **(Zarena and Sakar, 2012)**. Stem bark of *Alstonia scholaris* contains alkaloids, flavonoids,steroids, saponins and phenolic compounds **(Pankti *et al*., 2012; Pratap *et al*., 2013).**

Many of these plants that have been discovered to contain secondary metabolites are readily available in a tropical context. However, as far as the author is aware, none have been tested on the growth of soybeans (*Glycine max* Merr.). This study investigates the effects of secondary metabolites of some commonly available plants on the growth of this strategic crop.

MATERIALS AND METHODS

Preparation of crude extracts

Extracts from six plants were used in this research:cassava leaves (*M.esculenta*), *C.asiatica* stems and leaves, *G.linearis* leaves, corn silk, *A.scholaris* stem bark, and the pericarp of mangosteen fruit. Crude extracts of plants were prepared by maceration and boiling. Dry powder of *C. asiatica*, *A.scholaris* stem bark and pericarp of mangosteen fruits were macerated in methanol 70% (2 days), the extracts were filtered and concentrated in a rotary evaporator (45^{o}C) and the final result was a solid crude extract **(Demissie and Lele, 2013)**. Fresh cassava leaves and *G. linearis* were boiled in water (2 hours) then pressed and the extracts were filtered. After 3 days the crude extract, a yellow sediment was collected **(Jubahar, *et al*., 2006).**

Phytochemical Analysis

The secondary metabolite contents inthe crude extracts were analyzed using phytochemical analysis. For analyzing flavonoids, crude extract was diluted in 5 ml methanol and some drops of water, 10 mg of Mg powder, and two drops of HCl 5N added. A red color indicates the presence of flavonoids **(Shahid-Ud-Duaula and Anwarul, 2009)**.

For analyzing steroids/terpenoids the Liebermann–Burchard reaction was used, crude extract was diluted in 5 ml chloroform and 5 ml water. The chloroform mixture/solution was passed through a small column containing active charcoal and then collected on a plate. After drying, 2 drops of anhydride acetate and 2 drops H_2SO_4were added, formation of a reddish brown ring confirms the presence of terpenes **(Obianime and Uche. 2008)**. A Change of colour from violet to blue confirms the presence of steroids **(Boxi *et al*., 2010).**

For analyzing polar/semipolar phenolic compounds and saponin, crude extract was diluted in 5ml chloroform and 5ml water. Then one drop of the aqueous phase was placed on a plate and mixed with 2 drops of 1% $FeCl_3$ for polar phenolic compounds and mixed with some drops of methanol and 2 drops of 1% $FeCl_3$ for semipolar phenolic compounds. A red coloured solution with acetic acid indicates the presence of tannins and phenolic compounds **(Boxi *et al.*, 2010).**

For analyzing alkaloid, crude extract was diluted in 5ml chloroform ammonia 0.05N and 5ml water. 5-10 drops of H_2SO_4 2N was added to the chloroform phase and shaken slowly, then the acid phase was transferred into a reaction tube and 2 drops of Meyer's reagent were added **(Adegoke *et al.*, 2010)**.

For saponin, some drops of the aqueous phase were transferred into a reaction tube and shaken quickly (1 minute). If the foam was stable up to 1 minute it indicated that the extract contained saponin **(Adegoke *et al.*, 2010)**.

Preliminary Trial

Seeds of soybean var. Anjasmoro were planted in black polythene bags containing 8 kg of alluvial soil and placed in an area surrounded with screen cloth walls and a netting roof for insect protection. 7 days after sprouting the seedlings were fertilized with 0.5g Urea, 1.2g TSP dan 1.2g KCl per bag. Dimethyl sulphoxide was added to crude extract to make it soluble in water. Three concentration levels (25 mg/l, 50 mg/l, 100 mg/l) and a plain water control was then sprayed onto the leaves14 days after planting at a rate of 25 ml per plant. Each solution was sprayed onto three plants **(Kalaivanan *et al.*, 2012; Grabowska *et al.*,2012).**

The Second Trial

The crude extracts that proved most promising in terms of growth enhancement from the preliminary trial were then trialed again. *C.asiatica*, cassava and *G.linearis* leaf extracts were trailed at four concentration levels (25 mg/l, 50 mg/l, 100 mg/l and 200 mg/l) along with a plain water control. Nested design was used with four replications.

Data Analysis

The parameters of vegetative growth, plant height, number of leaves, leaf area, dry weight of crown and root and total dry weight of plant, of the second trial were analyzed using ANOVA and Duncan's Multiple Range Test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The results of phytochemical analysis are shown in Table 1. Each extract contained at least three different classes of secondary metabolites that could have a potential impact on soybean plant growth. It was decided that all six plant extracts warranted further investigation. **Ismail *et al*., (2016)** reported that both the methanolic and aqueous extracts of *Faidherbia albida* legumes contain terpenes, while alkaloids, saponins and phenolic compounds were found in the aqueous extract, and steroids were found in methanolic extracts only.

Table 1.Results of phytochemical analysis to contents of secondary metabolites in crude extract of six plants

The source of crude extract	Flavonoid	Steroid	Terpenoid	Alkaloid	Polar phenolic compound	Semipolar phenolic compound	Saponin
Cassava leaf	+	-	+	-	+	+	-
G. linearis leaf	+	-	+	-	-	+	+
C. asiatica	-	+	+	-	+	-	-
Stigma of corn female fllower	+	+	-	-	+	+	-
Bark of *A. scholaris*stem	-	+	+	+	+	+	+
Pericarpof mangosteen fruit	-	-	+	-	+	+	+

Note : (+) = present, (-) = absent

Results of the preliminary trial showed,just as **Talukder *et al*., (2015)** reported,that some plant crude extracts inhibited and others promoted growth. Figures 1 and Figure 2 (a) and 2 (b) shows that the treatment with crude extracts of *C.asiatica*, cassava and *G.linearis* leaves with low concentration showed a tendency to increase plant height and leaf area at 28

days after planting, while crude extracts of stem bark of *A.scholaris* and pericarp of mangosteen fruits and corn silk showed a tendency to decrease the growth parameters.

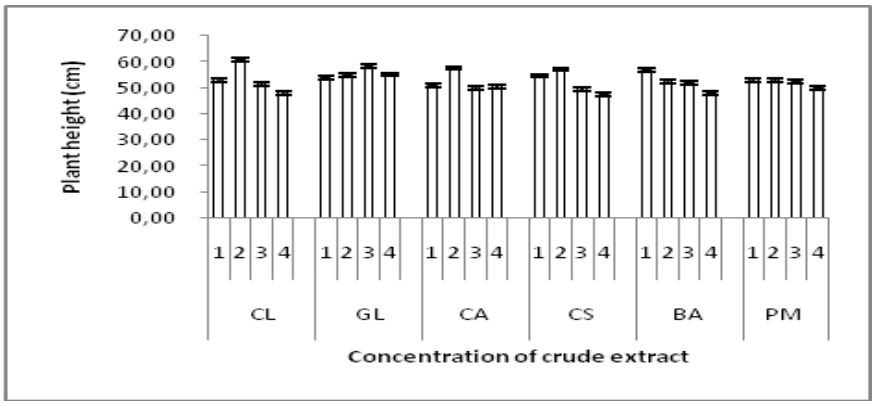
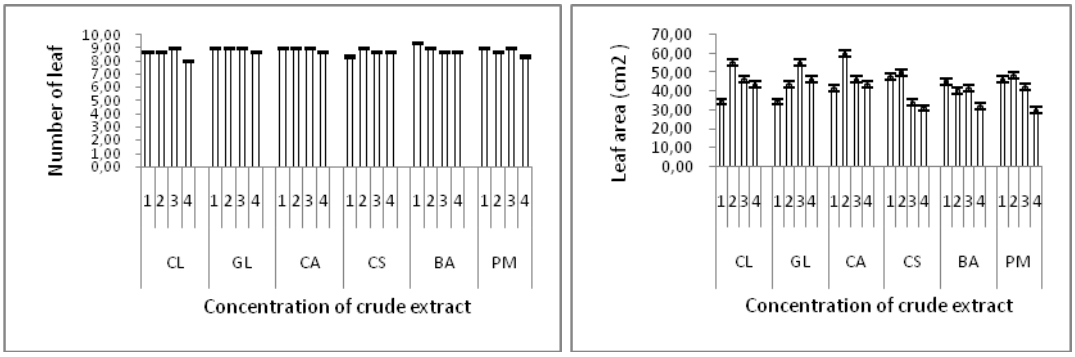


Figure 1. Plant height of soybean by crude extract treatment of six plants with four concentration levels at 28 days after planting.



(a) (b)

Figure 2. Number (a) and Leaf area (b) of soybean by crude extract treatment of six plants with four concentration levels at 28 days after planting.
Notes: CL = cassava leaf; GL = *G. linearis* leaf; CA = *C. asiatica*; CS = stigma of corn female flower; BA = bark of *A.scholaris* stem; PM = pericarp of mangosteen fruit; 1 = control; 2 = 25 mg/l; 3 = 50 mg/l; and 4 = 100 mg/l

A tendency of a crude extract to increase and decrease vegetative growth appears to be related to the secondary metabolites it contains. The crude extracts of *C.asiatica*, cassava and *G.linearis* leaves, that showed the increase of vegetative growth, all contained terpenoids. It is known that at least gibberellin phytohormones, a common class of

diterpenoid, has growth-promoting bioactivity and other terpenoids can promote the action of gibberellins **(Zi *et al*.,2014).**

The crude extract of *A.scholaris* stem bark and pericarp of mangosteen fruits decreased vegetative growth. Alkaloid and both polar and semipolar phenolic compounds are known to be inhibitors of plant growth. No sign of alkaloids was found in the three extracts that appeared to be growth promoting while all three extracts that appeared to be growth inhibiting contained detectable polar and semipolar phenolic compounds. In general, alkaloid compounds have an allelopathic effect that inhibits growth of both monocotyl and dicotyl plants **(Agbagwa, 2014).**

Polar phenolic compounds have a phytotoxic effect and inhibit cell division **(Zhao-Hui *et al*., 2010)**. While each of the three growth promoting extracts contained either polar or semi-polar phenolic compounds, it is possible that these compounds were present in small enough concentrations that these effects were not as apparent as in the other extracts or were modified by the presence of other metabolites.

Saponin is present in some, but not all of the growth enhancing extracts but it is also present in some of the growth inhibiting ones also. Sometimes saponin has a toxic effect on plants and it inhibits seedling growth **(Prokofeva *et al*., 2003; Kohata *et al*., 2004)**, but saponin triterpenoid shows a growth stimulant activity on corn **(Saha *et al*., 2010)**.

Some concentrations of the crude extracts of the three plants that appeared to promote growth (*C. asiatica*, cassava leaves and leavesof *G. linearis*) were tested again on new soybean plants. The results indicated that some concentrations of these extracts had a statistically significant influence on growth as shown in Table 2.

The only treatment that had a significant effect on leaf area was 25 mg/l crude extract of *C. asiatica*. Other studies have discovered leaf area increases with use of crude extracts. **Agbagwa *et al*., (2003)** reported seedlings of *Celosia argentea* sprayed with the higher concentrations of the crude extract (75%and 100%) of *Senna alata* showed significant promotion effects on leaflet enlargement. **Fathima *et al*., (2011)** reported that application of the crude brassinosteroid extract resulted in increase of shoot length, fresh weight, dry weight along with leaf area of seedlings of *Gossypium hirsutum* and *Vigna mungo*. Agbagwa

reported that the growth promoting substances in a crude extract of *Senna alata* caused a general increase in leaf area of *Celosia argentea*L. as well as its height **(Agbagwa, 2014**).

Table 2.Vegetative growth of soybean resulted by crude extract treatment of cassava leaf, *C. asiatica* and *G. linearis* leaf with five concentration levels at 28 days after planting

Treatment		Average of					
The source of crude extract	Concentration of crude extract (mg/l)	Plant height(cm)	Number of leaf	Leaf area (cm^2)	Dry weight of crown (g)	Dry weight of roots (g)	Total dry weight of plant (g)
Cassava leaf	0 (control)	0.3563^{ab}	7.00^{ns}	22.93^{a}	1.88^{ns}	0.51^{ns}	2.40^{ns}
	25	0.3700^{c}	7.00^{ns}	26.51^{a}	2.08^{ns}	0.54^{ns}	2.62^{ns}
	50	0.3700^{c}	7.25^{ns}	27.31^{a}	2.23^{ns}	0.53^{ns}	2.76^{ns}
	100	0.3600^{b}	7.00^{ns}	23.50^{a}	2.04^{ns}	0.50^{ns}	2.55^{ns}
	200	0.3550^{a}	6.75^{ns}	22.55^{a}	1.93^{ns}	0.54^{ns}	2.47^{ns}
C. asiatica	0 (control)	0.3725^{c}	7.00^{ns}	26.53^{a}	2.08^{ns}	0.53^{ns}	2.61^{ns}
	25	0.4573^{d}	7.25^{ns}	33.01^{b}	2.46^{ns}	0.61^{ns}	3.07^{ns}
	50	0.3575^{b}	7.00^{ns}	27.33^{a}	2.03^{ns}	0.50^{ns}	2.53^{ns}
	100	0.3538^{a}	6.75^{ns}	24.01^{a}	1.75^{ns}	0.36^{ns}	2.11^{ns}
	200	0.3738^{c}	6.75^{ns}	25.59^{a}	1.58^{ns}	0.56^{ns}	2.15^{ns}
G. llinearis leaf	0 (control)	0.3588^{bc}	6.75^{ns}	25.38^{a}	1.87^{ns}	0.56^{ns}	2.43^{ns}
	25	0.3425^{a}	6.50^{ns}	25.87^{a}	2.03^{ns}	0.50^{ns}	2.53^{ns}
	50	0.3563^{b}	7.00^{ns}	26.28^{a}	2.06^{ns}	0.54^{ns}	2.60^{ns}
	100	0.3638^{d}	6.75^{ns}	27.72^{a}	1.92^{ns}	0.56^{ns}	2.48^{ns}
	200	0.3563^{b}	7.25^{ns}	22.86^{a}	1.75^{ns}	0.57^{ns}	2.31^{ns}

Note: values with common letter in the same column are not significantly different at 5% level as per DMRT; ns = non significant

Treatment using 25 mg/l crude extract of *C. asiatica* resulted in significantly higher plant height than the control and the other concentrations of *C. asiatica*. This extract increase in plant height was observed between 14 to 28 days after planting, while the plant height at other concentrations was relatively similar to the control treatment at every week as can been seen from Fig. 4. Table 2 also shows that use of 25 mg/l and 50 mg/l concentrations of cassava leaf and 100 mg/l of *G.linearis* leaf extracts also produced higher plants although the growth enhancement compared to the control was not as great as with *C. asiatica*.

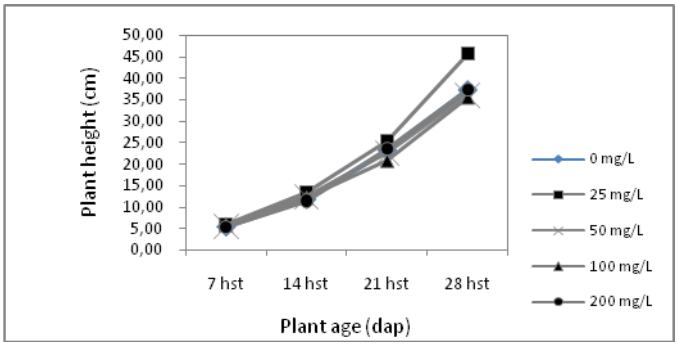


Figure 4. Plant height of soybean resulted treatment of *C. asiatica* crude extract with five concentration levels from 7 up to 28 day after planting. Note: dap = days after planting

Interestingly, *C. asiatica* crude extract with low concentration (25 mg/l) increased plant height and leaf area while high concentration decreased these parameters. The concentration of crude extracts of plant material has been shown to be an influential factor in studies using much higher concentrations. **Emongor (2014)** reported that *Moringa* leaf extract at 11% up to 50% concentration increased vegetative growth of snap beans (*Phaseolus vulgaris*) but concentrations outside this range did not. **Baeshen (2014)** reported that aqueous extract of *Mentha peperina* which could enhance the seed germination and other growth parameters of *Lens culinary* with a lower concentration of crude extracts, but these were reduced at a higher one.

CONCLUSIONS

The results showed that low concentrationcrude extract of *C.asiatica* (25 mg/l) significantly increased both height and leaf areaof soybean plants. The height was 22.77% higher than the control. Low concentration of crude extract is a potential growth stimulant for soybean plants. Further investigations will determine the effect of steroids and terpenoids from purified extracts of *C. asiatica* on the growth and production of soybean. If these secondary metabolites are found to be effective growth stimulants, this will help in the search for an environmentally friendly, commercially viable and cost effective way to improve soybean production in a tropical context.

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