

Effects of green manure cover crops (*Canavalia ensiformis* L. and *Mucuna pruriens* L.) on seed germination and seedling growth of maize and *Eleusine indica* L. and *Bidens pilosa* L. weeds

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ABSTRACT

In Lab bioassays and Pot culture, we studied the allelopathic potential of aqueous extracts (0, 1.25, 2.5, 3.75 and 5 % wv⁻¹) of green manure cover crops [jack bean (*Canavalia ensiformis* L.) and velvet bean (*Mucuna pruriens* L.)], on the germination and seedling development of weeds [goosegrass (*Eleusine indica* L.), blackjack (*Bidens pilosa* L.)] and maize (*Zea mays* L.) crop. 25 seeds of each weed or 10 maize seeds were separately sown in pots (soil + powdered green manure of jack bean and velvet bean mixed at 1%). Germination of both weeds was inhibited by the aqueous extracts in the order: leaf extract > stem extract > root extract. Soil amended with the green manure of jack bean and velvet bean reduced the emergence and growth of weed seedlings but had little adverse effect on maize. LC-MS revealed the presence of phenolics such as kaempferol in the tissues of both cover crops. Most of the phenolics demonstrated allelopathic activity on blackjack and goosegrass seeds. The jack bean and velvet bean extracts were phytotoxic to weeds (goose grass and black jack) but not to maize.

Key words: Allelopathy, *Bidens pilosa*, blackjack, *Canavalia ensiformis*, *Eleusine indica*, goose grass, jack bean, bioassay, maize, *Mucuna pruriens*, pot culture, seeds germination, seedlings growth, weed, *Zea mays*

INTRODUCTION

Weeds drastically reduce maize (*Zea mays* L.) yields in Zimbabwe (22). The Zimbabwean farmers know the immediate benefits of application of synthetic herbicides for weed control in maize, but, they think that herbicides “kill the soil” as their residues persist in soil for long periods (18). On the other hand, environmentalists are lobbying to reduce the use of synthetic herbicides due to their negative effects on non-target sites and non-target organisms in the ecosystem (9,28). The synthetic herbicides have led to the many problems including the development of herbicide resistant weed biotypes (1), these necessitate the search for alternate environmentally friendly weed control strategies, with low risk of herbicide resistance development and for organic farming systems.

Green manure cover crops are grown in conservation agriculture and organic agriculture systems as intercrops or rotational crops to reduce soil erosion, improve water infiltration, improve soil physical and biological properties, improve soil fertility through nitrogen fixation and suppress weeds (23). They smother the weeds due to their abundant foliage. Moreover, some crops are sources of phytotoxic secondary compounds called allelochemicals (17), which suppress the weeds. For example, allelopathy of velvet bean (*Mucuna pruriens* (L.) DC var *utilis*) to control weed is due to the presence of L-3, 4-

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dihydroxyphenylalanine (L-DOPA) (19). Jack bean (*Canavalia ensiformis* (L.) DC) inhibits the germination of weeds as it contains several phytotoxic compounds which have also been reported (24).

The past researches on cover crops did not exploit their allelopathic potential in integrated weed management. The allelopathic cover crops can be used in weed control by (i) use of allelopathic cover crop residues as mulch to inhibit weed seed germination and seedling growth (15) and (ii) use of aqueous extracts of allelopathic plants as post emergence sprays to control weeds (26).

Research on weed suppression through allelopathy using the green manure crops is minimal in Zimbabwe. Ten green manure cover crops jack bean (*Canavalia ensiformis* (L.) DC), velvet bean (*Mucuna pruriens* (L.) DC), hyacinth bean (*Lablab purpureus* L.), red sunnhemp (*Crotalaria ochroleuca* G. Don), showy rattlebox (*Crotalaria grahamiana* Wight & Arn.), common bean (*Phaseolus vulgaris* L.), common rattlepod (*Crotalaria spectabilis* Roth.), radish (*Raphanus sativus* L.), tephrosia (*Tephrosia vogelii* L.) and black sunnhemp (*Crotalaria juncea* L.) were selected to study their allelopathic potential for weed control. In this study the allelopathic potential of only jack bean and velvet bean crops was evaluated on test weeds: black jack (*Bidens pilosa* L.) and goosegrass (*Eleusine indica* (L.) Gaertn). This study aimed (i). to evaluate the allelopathic potential of these two crops hypothesizing that the aqueous extracts and soil incorporated green manure incorporated in the soil from jack bean and velvet bean could suppress the germination, emergence and growth of blackjack (*Bidens pilosa* L.) and goosegrass (*Eleusine indica* (L.) Gaertn) but with no effect on maize and (ii). to ascertain their allelopathic effects in maize based crop rotations.

MATERIALS AND METHODS

The study was done at the University of Zimbabwe, Mount Pleasant, Harare, Zimbabwe (17.78°S; 31.05°E, altitude: 1523 m above sea level). Mean rainfall: 716.8mm, mean summer temperature: 15.5 °C to 30 °C. Petri dish bioassays and the pot experiments were conducted in the laboratory and greenhouse, respectively between August 2015 and June 2017. Liquid Chromatography - Mass Spectrometry (LC-MS) was done at the Central Analytical Facilities, Stellenbosch University, South Africa to determine the phenolic content of the cover crop extracts.

Experiment 1. Laboratory Petri plate bioassay

Extract preparation: The green manure crops velvet and jack bean used in the study were grown in an irrigated field, Department of Crop Science, University of Zimbabwe, Harare. These were harvested at 120 days (flowering stage, the concentration of allelochemicals is higher than at maturity), besides plants are succulent, hence, decompose quickly (16). After harvesting, the plants were separated into leaves, roots and stems and cut into 2 cm pieces, air dried in the glasshouse for 3- weeks and oven dried at 70 °C for 48 h. Thereafter, different plant parts were powdered in a hammer mill grinder. The powder was stored in bags for 3-days at room temperature (23-25 °C) till used in the experiments. Seeds of test weeds (goose grass and blackjack) were collected at maturity from fields of Henderson Weed Research Station, Zimbabwe. The seeds were stored in paper envelopes at room temperature. Maize variety 'SC403' seeds were purchased from SEEDCO. Fifty g powdered materials of jack bean or velvet bean were soaked in 1.0L distilled water and stirred on an orbital shaker at

100 rpm for 24 h at 25 °C. The extracts were then strained through 4-layers of cheese cloth before centrifuging (Model Dynac II Centrifuge, Clay Adams) at 4000 rpm for 15 min. The clear solution was further filtered through Whatman # 1 Filter paper and was considered as 5% concentration and stored at 4 °C in bottles sealed with parafilm for 24 h before use. Each stock extract was then diluted with distilled water to get 1.25 %, 2.5 % and 3.75 % concentrations.

The conductivity of stock extracts of jack bean and velvet bean was measured using a conductivity meter Model SX713 ver. 2.0. The values obtained were used to calculate their osmotic potential using the formula:

$$\text{Osmotic potential (in Mpa)} = \text{conductivity (in mS)} * -0.036$$

Germination bioassays were done using solutions of polyethylene glycol (PEG) 6000 at concentrations (-0.05 and -0.30 MPa) to check the potential influence of the osmotic pressure of plant extracts. A water control was also included.

Germination bioassay: The experimental treatments consisted of two factors: (i). Donor plant parts 3 (leaf, stem, root) and (ii). Aqueous extract concentrations 5 (0, 1.25, 2.50, 3.75 and 5 %) and distilled water was used as the control. The treatments were replicated 4-times in Completely Randomised Design (CRD). Each weed specie was considered as separate experiment. To break seed dormancy, goosegrass seeds were soaked in 32 % Hydrochloric acid (HCl) for 20 min and washed twice in distilled water. For blackjack dimorphic seeds (4), only non-dormant long achenes were used in the bioassay. The recipient weeds and maize seeds were sterilised in 1% sodium hypochlorite for 10-min and rinsed 4- times with distilled water. Twenty-five seeds of weeds and 10- maize seeds were sown equidistant in separate 90 mm diameter Petri dishes lined with Whatman No. 2 filter paper. In each Petri dish, 10 mL aqueous extract were added as per treatments, sealed with parafilm and placed in the laboratory at room temperature (25 °C). Data on germination, plumule and radicle length were collected from 5- randomly selected plants on day 7, 10 and day 14 in maize, blackjack and goosegrass, respectively. Seeds were considered germinated, when 2 mm long radicle protruded through the seed coat.

Germination percentage (G %) and Seedling vigour indices (SVI) were calculated as under:

$$G \% = (a/b) \times 100,$$

Where, a: Number of germinated seeds and b: Total number of seeds sown in each Petri dish.

$$SVI = G \% * (RL + PL) (2).$$

Where, SVI is seedling vigour index, RL – Radicle length, PL- Plumule length

Experiment 2. Pot Culture: The pot experiments were done in CRD and replicated four times; only goosegrass experiments were repeated once. Each weed specie was considered

a separate experiment. The effects of cover crop plant parts (leaves, stems and roots) on the emergence and seedling growth of goose grass and black jack were evaluated, separately. No cover crop residues were applied in control pots. The soil used in the goosegrass bioassays was granite derived (Clay 7 %, Sand 5%, Silt 88%, pH= 0.05, CEC= 2.81 milliequivalents percent (me %) collected from Domboshava (17° 37' S, 31° 10' E and 1560 m above sea level), whereas University of Zimbabwe red soils (Clay 18%, Sand 16%, Silt 66%, pH= 5.2, CEC= 12.32 milliequivalents (%)) were used in the blackjack bioassay to mimic the edaphic factors under which the two weeds were dominant (10).

The Pot culture study was done in the green house as per (20). Pots (200mm diameter and 175 mm depth) were three-quarter filled with soil in which 2 g NPK (7% N, 14% P₂O₅, 7% K₂O) fertilizer were added. Each green manure cover crop tissue powder was thoroughly mixed with soil at 1% concentration to provide the concentration similar to green manure cover crop residues in nutrient depleted dry land soils. Thereafter, 25 seeds of respective weed species were counted and placed on the soil surface in the pots, afterwards seeds were covered with 1 cm thin soil layer. In the maize emergence experiment, ten seeds were sown per pot. Initially the pots were watered with tap water to field capacity with watering can fitted with a fine nose. Thereafter, 150 ml tap water was applied daily. The number of daily emerged seedlings of goosegrass, blackjack and maize in the pots were recorded until no further emergence was noted. Final emergence (%) was calculated at the end of the experiment. At 21 days after sowing, weed seedlings were uprooted and washed gently with tap water to remove soil from the roots. The weed plants were oven dried at 70 °C for 72 h to get dry weight.

Seedling vigour index (SVII) was calculated as under (2):

$$SVII = \text{Seedling emergence (\%)} \times \text{seedling dry weight (g)}$$

Experiment 3. Allelopathic compounds in green manure crops and their bioactivity **Liquid chromatography-mass spectrometry (LC-MS)**

LC-MS analyses of jack bean and velvet bean tissue samples were done at Stellenbosch University's Central Analytical Facilities (CAF) as per method briefly described below. The phenolic compounds were identified using LC-MS by comparing the retention times of unknown with standard compounds (L-Dopa, kaempferol, ferulic acid, chlorogenic acid, naringenin, rutin, atropine, genistein, p-anisic acid and hesperidin). All the standards used were purchased from Sigma Aldrich, South Africa. A Waters Synapt G2 quadrupole Time-of-Flight Mass Spectrometer was used for LC-MS analysis. It was fitted with Waters Ultra pressure liquid chromatograph and photo diode array detection. Separation was achieved on a Waters HSS T3, 150 x 2.1 mm column. A gradient was applied using 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid (solvent B). The gradient started at 100% solvent A for one min and changed to 28% B over 22 min in a linear way. It then went to 40% B over 50 seconds and a wash step of 1.5 min at 100% B, followed by re-equilibration to initial conditions for 4- min. The flow rate was 0.3 ml min⁻¹ and the column was kept at 55 °C. The injection volume was 2 µL. Data was acquired in MS^E mode which consisted of a low collision energy scan (6V) from *m/z* 150 to 1500 and a high collision energy scan from *m/z* 40 to 1500. The high collision energy scan was done using a collision energy ramp of 30-60V. The photo diode array detector was set

to scan from 220-600 nm. The mass spectrometer was optimized for best sensitivity, desolation gas was nitrogen at 650 L h⁻¹ and desolation temperature 275 °C, at cone voltage of 15. The instrument was operated with electro spray ionization probe in the negative mode. Sodium formate was used for calibration and leucine encephalin was infused in the background as lock mass for accurate mass determinations.

Weed seeds germination bioassays with standards allelochemicals

There were 6- treatments [control (Distilled water), kaempferol, rutin, genistein, atropine and naringenin]. These were replicated 5- times in CRD. The experiments were done separately for blackjack and goosegrass weeds with standards of compounds detected and quantified by LC-MS analysis. Standards of allelochemicals were dissolved in methanol at 10 mg L⁻¹. The weed seeds were sterilised in 1% sodium hypochlorite for 10-min, rinsed 4- times with distilled water. Five mL of different solutions were applied to double disks of Munktell, Ahlstrom filter paper (Supplied by Lassec, South Africa) kept in 30 mm diameter Petri dishes. Control discs were treated only with pure methanol. These Petri dishes were left open in laboratory for 36 h to evaporate methanol, then 2.5 ml distilled water was added in each Petri dish.

Blackjack assay: Its 10-seeds were sown equidistant in 90 mm diameter Petri dishes lined with Whatman No. 2 filter paper. In each Petri dish, 10 mL aqueous extract was added as per treatments, sealed with parafilm and placed in glasshouse (25 °C). Seeds were considered germinated, when 2 mm long radicle protruded through the seed coat. Germination of blackjack seeds was evaluated after 7- days.

Goosegrass assay: Goose grass showed erratic germination, hence, its uniform pre-germinated seeds were used in this bioassay. Sterile goosegrass seeds were sown in 90 mm diameter Petri dishes lined with Whatman No. 2 filter paper and 10 ml sterile distilled water was added per Petri dish. The Petri dishes were placed in the glasshouse (25 °C). After 5- days, seeds with 1 mm long radicles were selected and used in the germination bioassay with different standards. Ten pre-germinated seeds of goosegrass were put in 30 mm diameter Petri dishes lined with double filter paper treated with different solutions as done in the blackjack bioassay. The seedlings were then placed in the glasshouse at 25 °C for 10- days.

At the end of the bioassay, the data of plumule and radicle length were collected from 5- randomly selected plants. The stimulatory or inhibitory (%) of the analytical standards was calculated as under:

$$\% \text{ Inhibition (-) / Stimulation (+)} = [(Extracts - Control)/Control] \times 100$$

Where, Extract: Plant extract and control: Distilled water.

Statistical analysis

The data were tested for Normality using the Shapiro-Wilk Test. Germination vigour index data for the weeds did not meet the assumptions for analysis of variance (ANOVA) and were ln (x) transformed before analysis using Genstat version 14th edition. The goodness of fit of the models was evaluated using the coefficient of determination (R²). Trend lines

were omitted where there were no significant differences amongst concentrations within the same extract tissue. Mean separation was done using Fischer's protected least significance difference (LSD) at 5% significance level.

RESULTS AND DISCUSSION

Germination and early seedling growth with PEG 6000

The high osmotic potential of some plant extracts is inhibitory to seed germination and seedling growth of receptor plants, due to water stress rather than allelopathic effects of allelochemicals. Hence, we calculated the osmotic potential of leaf, stem and root extracts of both green manure crops. The jack bean extracts showed osmotic potential values (-0.18 to -0.06), while those of velvet bean were: -0.16 and -0.08. Based on these values, we applied blackjack and goosegrass solutions of PEG 6000 with the osmotic potentials indicated in Table 1. The seeds of blackjack that were germinated in water showed the same germination (%) as those placed in PEG solutions. While in goosegrass, osmotic potentials of -0.10 and -0.05 stimulated the germination of weed seeds than water (control). The osmotic potential did not affect the radicle and plumule lengths of both weeds. In this study, the osmotic potentials of different cover crop aqueous extracts that were equivalent to those of PEG solutions used in the study, hence, did not affect the seed germination and seedling development. The osmotic potentials observed did not inhibit the germination and early seedling growth of plants (3). Therefore, the inhibitory activity of extracts observed in this present study could be attributed to the presence of phytotoxic allelochemicals in aqueous extracts of cover crops.

Table 1. Effects of osmotic potentials of PEG 6000 solutions on the germination and seedling growth of blackjack and goosegrass.

Osmotic potentials (Mpa)	Blackjack			Goosegrass		
	Germination (%)	Radicle length (mm)	Plumule length (mm)	Germination (%)	Radicle length (mm)	Plumule length (mm)
0.00	97.60 ^a	43.00 ^a	16.52 ^a	62.40 ^a	15.60 ^a	9.88 ^a
-0.05	98.40 ^a	46.48 ^a	15.44 ^a	81.60 ^c	19.36 ^a	10.80 ^a
-0.10	100.00 ^a	42.12 ^a	14.12 ^a	80.00 ^{bc}	19.68 ^a	10.64 ^a
-0.15	97.60 ^a	46.68 ^a	15.32 ^a	68.00 ^{ab}	15.48 ^a	10.08 ^a
-0.20	98.40 ^a	44.76 ^a	15.52 ^a	62.40 ^a	14.96 ^a	9.68 ^a

*Means followed by different letters in the same column are significantly different at $p < 0.05$.

JACK BEAN AQUEOUS EXTRACTS

(i). Goosegrass germination and seedling growth: The extract concentrations significantly ($p < 0.05$) affected the germination and seedling growth of goosegrass (Fig 1). Leaf extracts of jack bean at all concentrations reduced the germination of goosegrass, with complete inhibition at 4.2 % concentration. Stem extract had hormetic effects on germination at most concentrations assayed except the reduction at 5%. Jack bean aqueous extracts inhibited the goosegrass radicle length in the order leaf > stem > root. All the leaf extract concentrations inhibited the radicle growth of goosegrass. Plumule elongation was inhibited ($p < 0.05$) by

all leaf aqueous extracts with complete inhibition at 4.3% concentration. Consequently, the seedling vigour index of goosegrass seeds treated with jack bean leaf extracts decreased linearly at the tested concentrations. Overall, seedling vigour index of goosegrass was suppressed in the order leaf > root > stem aqueous extracts.

(ii). Blackjack germination and seedling growth: There were significant ($p < 0.05$) interactions between the jack bean extract tissue and concentration on all the germination parameters of blackjack (Fig. 2). Blackjack seeds treated with leaf and stem extracts of jack bean showed reduced germination with increasing extract concentration. In both leaf and stem extracts, inhibition was observed at 3.75 % and 5 % concentration with maximal reduction at 5 %. All extracts decreased the radicle length of blackjack at 5% concentration. The leaf and stem extracts were more inhibitory to radicle growth than root extracts. On the other hand, stem aqueous extracts were inhibitory to plumule growth than leaf and root extracts. Root extracts stimulated the plumule elongation at 1.25 % concentration but was similar to stem extracts at high concentration. Leaf and stem aqueous extracts linearly reduced the plumule elongation at the test concentrations. Concomitantly, all the tissue extracts linearly reduced the seedling vigour index of blackjack. These findings corroborate the earlier findings of (24), who reported weed germination, plumule and radicle growth inhibition of > 90 % by whole plant extracts of jack bean. These results also agree with previous findings (5) who reported allelopathic activity of jack bean on barnyard grass [*Echinochloa crus-galli* (L.) Beauv.]. These results also corroborate the work of (13) who reported that phenolic compounds found in jack bean tissues inhibited the broad leaved weeds [*Cassia* spp (*Mimosa pudica* L), morning glory (*Ipomea plebia* L.) and wandering Jew (*Commelina bengalensis* L.)].

VELVET BEAN AQUEOUS EXTRACTS

(i). Goosegrass germination and seedling growth; The interaction of velvet bean extract tissue and concentration significantly ($p < 0.05$) affected all the germination parameters. Leaf extracts were more inhibitory to seed germination of goosegrass than stem and root extracts, which were statistically similar (Fig 2). The leaf extracts exhibited concentration dependent inhibition of goosegrass radicle growth with maximal reduction at 5% concentration. Root extracts of velvet bean caused hormetic effects on goosegrass radicle and plumule growth. Overall, the velvet bean leaf extracts were more harmful to vigour index of goosegrass than extracts of other tissues. Stem, leaf and root extracts linearly reduced the seedling vigour indices of goosegrass (Fig 2).

Table 2. Effects of aqueous extracts of jack bean and velvet bean on germination and seedlings growth of maize

Aqueous Extract	Germination %		Radicle length (mm)		Plumule length (mm)		Germination vigour index (SVI)	
	Jack bean	Velvet bean	Jack bean	Velvet bean	Jack bean	Velvet bean	Jack bean	Velvet bean
Control	87.00 ^a	89.00	34.30 ^b	27.72 ^b	13.98 ^a	37.38	7.92 ^a	8.60 ^b
Leaf	90.00 ^a	91.00	18.82 ^a	16.90 ^a	10.44 ^a	24.54	7.78 ^a	8.17 ^a
Stem	75.00 ^b	86.00	24.14 ^a	15.36 ^a	11.14 ^a	15.28	7.81 ^a	7.82 ^a
Root	91.00 ^a	91.00	43.86 ^b	18.03 ^a	17.46 ^b	27.55	8.59 ^b	8.20 ^a

Mean values indicated by different letters within a column differ significantly from each other at the $p=0.05$ level.

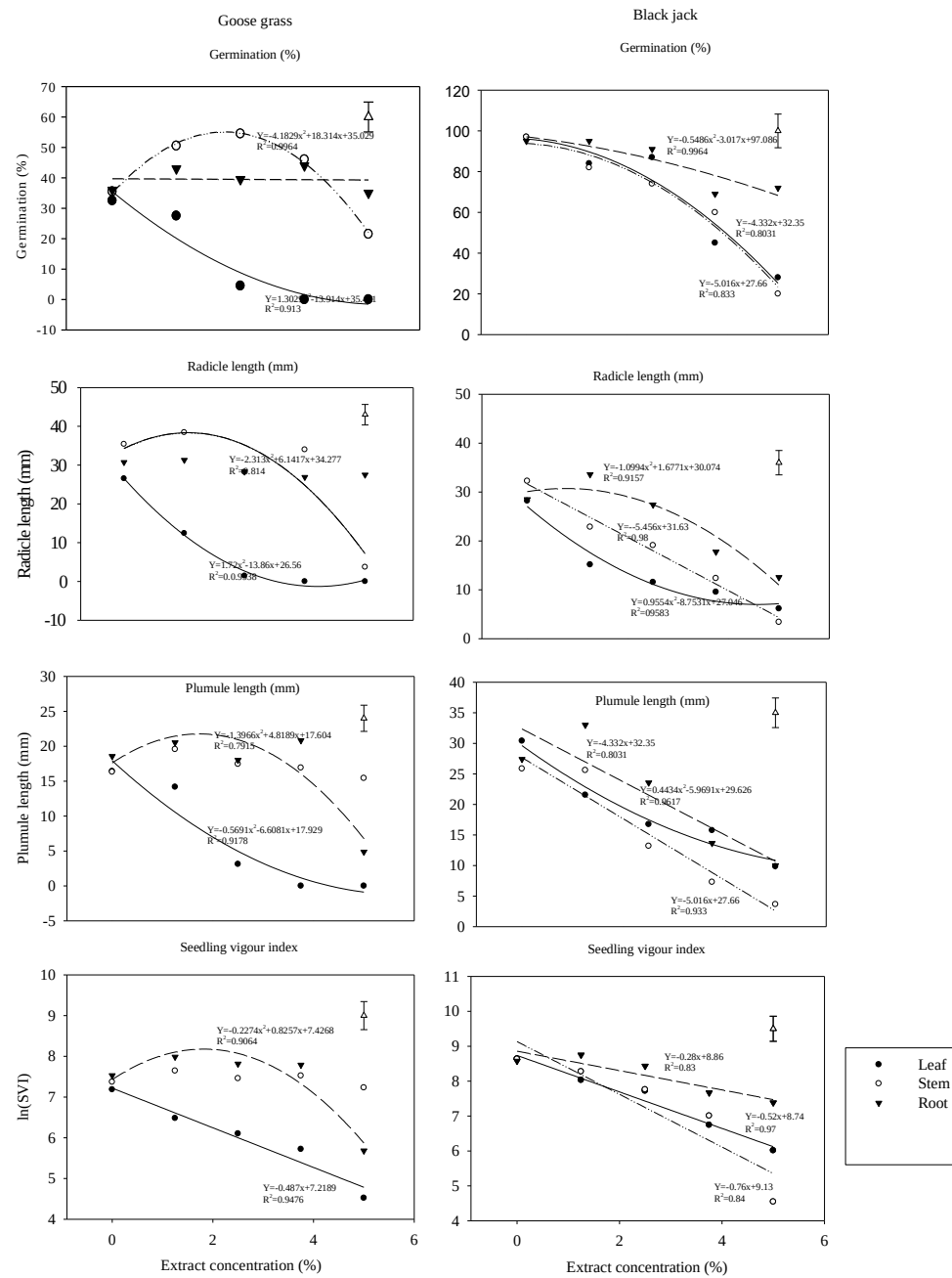


Figure 1. Effects of jack bean aqueous extracts on germination and seedlings growth of blackjack and goose grass weeds

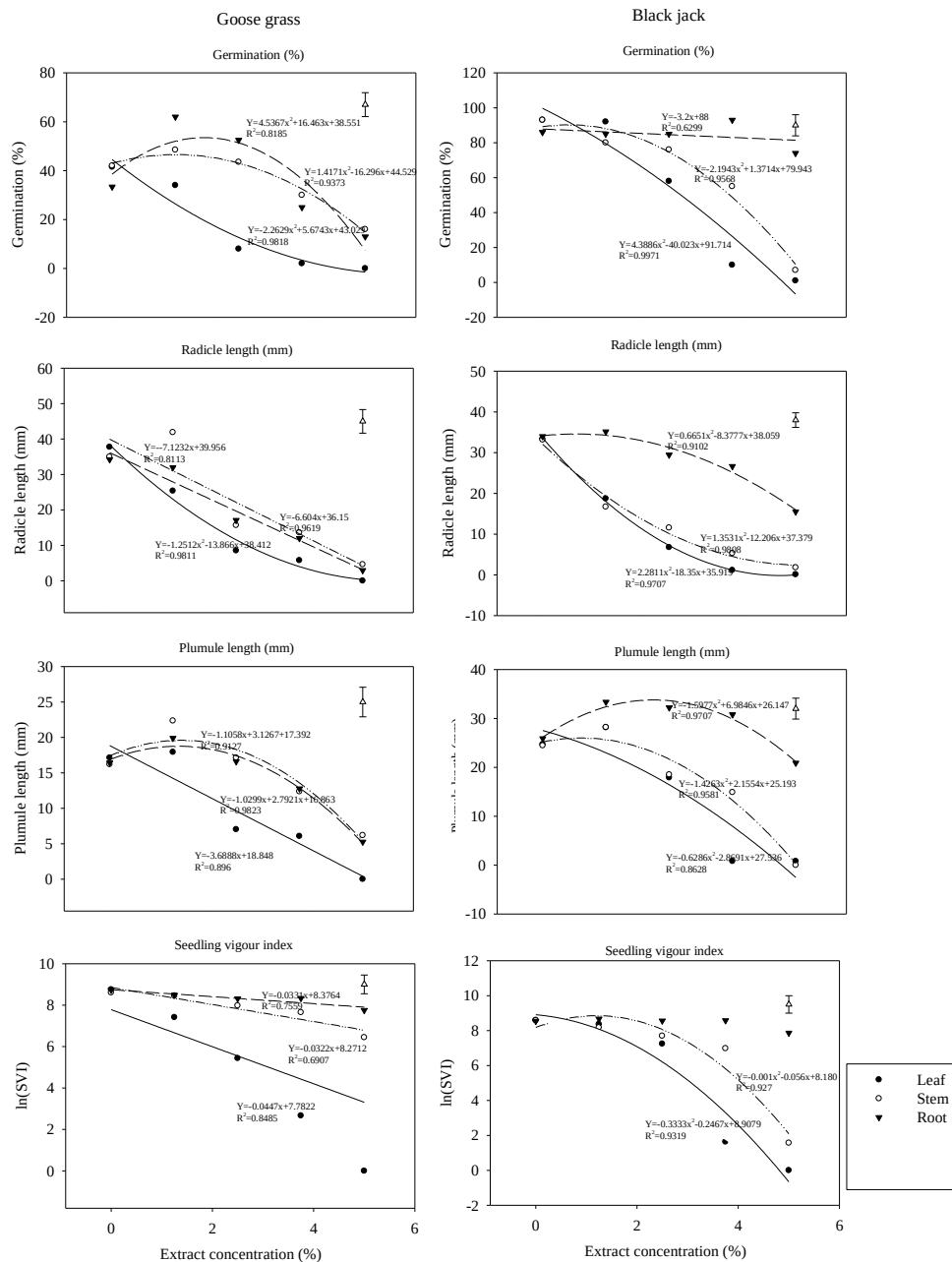


Figure 2. Effects of velvet bean aqueous extracts on germination and seedlings growth of blackjack and goose grass weeds

(ii). Blackjack germination and seedling growth: The velvet bean extract tissue and concentration interaction was significant ($p < 0.05$) on all germination parameters (Fig. 2). Velvet bean leaf and stem extracts were more phytotoxic to blackjack seeds germination than root extracts. Stem extracts reduced radicle growth and were concentration dependent. Root extracts however, stimulated the plumule elongation at 1.25% to 3.75% concentrations but were inhibitory at 5% concentration. In contrast, leaf extracts were highly phytotoxic to radicle elongation with complete inhibition at 4.4 % concentration. Leaf extracts decreased the vigour index linearly within the tested range of extract concentration. Stem extracts reduced the seedling vigour index only at 5% concentration (Fig. 2). Thereby, germination vigour index was reduced in the order leaf > stem aqueous extracts. The allelopathic activity of velvet bean on both weeds may be attributed to the presence of allelochemicals found in this cover crop. (6) identified an amino acid L-3, 4- Dihydroxy phenylalanine (L-DOPA) and cyanamide as the allelochemicals in velvet bean that inhibit plant growth. Fujii (7) suggested that the growth inhibitory activity of velvet bean that was observed on cucumber was caused by reactive quinones generated from melanin during the metabolism of L-DOPA, which resulted in quinoprotein formation and mitochondrial impairment. This study indicated that all tissues of velvet bean used in this study, possessed phytotoxic allelochemicals. The goosegrass was less affected by velvet bean extracts than by blackjack extracts. Because the morphological differences in these weed species, Gramineae and Leguminosae species are less affected by jack bean extracts, than species of Brassicaceae, Compositae, Cucurbitaceae and Hydrophyllaceae (27).

(iii). Maize germination and seedling growth: Jack bean stem extracts significantly ($p < 0.05$) reduced the germination of maize seeds (Table 2) but the velvet bean extracts did not affect the germination of maize seeds. Jack bean leaf and stem aqueous extracts significantly reduced the maize seedling radicle and plumule length than root extracts, which did not affect the germination. Aqueous extracts of all velvet bean tissues reduced the maize radicle growth but had no effect on plumule length. Overall, stem and leaf extracts significantly reduced the maize seedlings vigour. Similar results were obtained using velvet bean extracts. These findings corroborate the findings of (5) who reported that green manure cover crops did not affect the germination of large seeded crops like maize.

POT CULTURE

Effects of soil added green manure of Jack bean and velvet bean

(i). Goosegrass and blackjack : Leaf, stem and root biomass of both green manure crops were significantly ($p < 0.05$) phytotoxic to the seedling emergence of goosegrass (Fig. 3). Jack bean and velvet bean leaf residues also inhibited the blackjack seedling emergence by 91 and 95.5 %, respectively. The green manure crops leaf residues significantly ($p < 0.05$) influenced the dry weight pot^{-1} of both test weeds (goosegrass and blackjack). Jack bean leaf residues were more inhibitory (>80 %) to goosegrass dry weight than velvet bean. On the other hand, leaf residues of velvet bean stimulated the weed growth by 28.9%. Consequently, green manure of jack bean stem and root biomass incorporated into soil significantly ($p < 0.05$) reduced the seedling vigour indices of both test weeds except velvet bean residues, which caused slight stimulation of 11.6% (Fig.3). These findings further confirm the presence of non-selective allelochemicals in jack bean and velvet bean reported earlier (27).

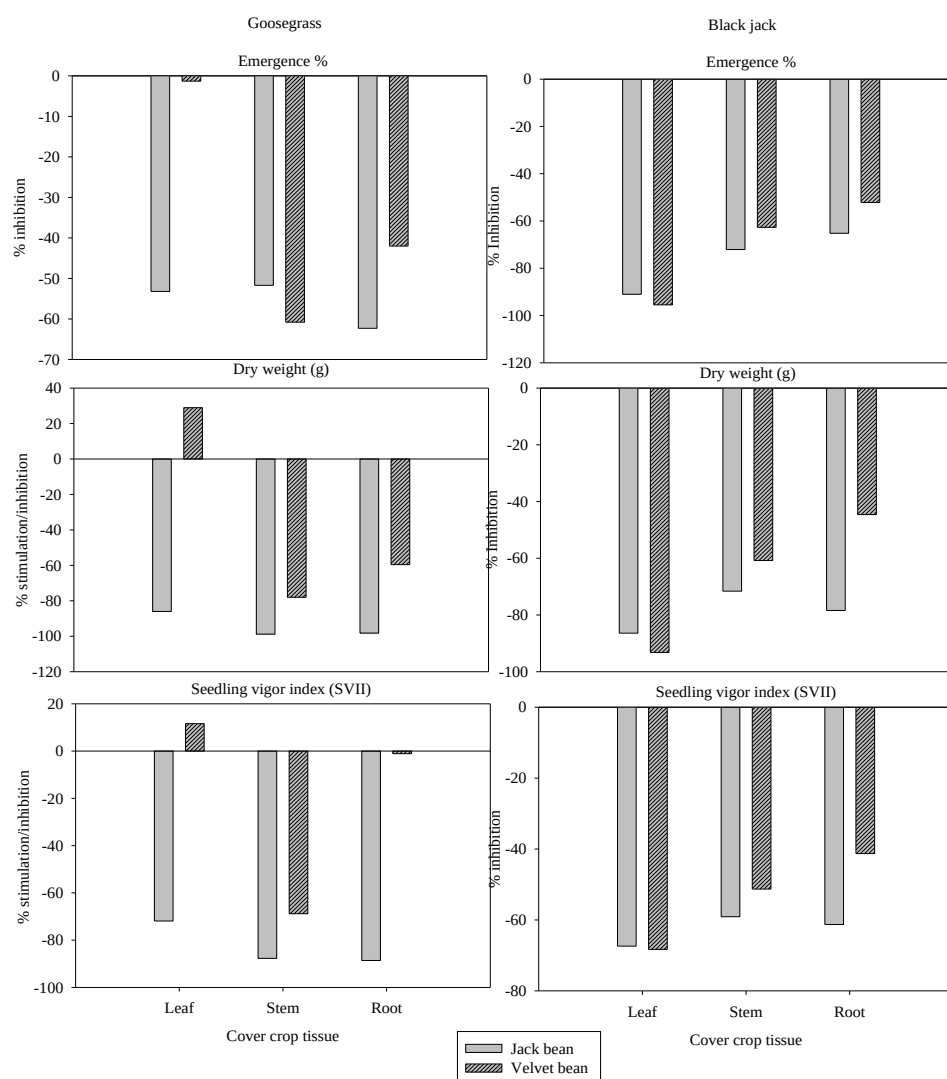


Figure 3. Effects of soil incorporated biomass of leaves, stems and roots of green manure crops on seedlings emergence and growth of goosegrass and blackjack weeds

(ii). **Maize:** The plant part used as green manure had a significant ($p < 0.05$) impact on maize emergence (Fig. 4). Green manures from the different plant parts of jack bean stimulated maize seedlings emergence by 16-32%. Overall, only velvet bean root green manures stimulated the seedling vigour of maize. The other treatments did not significantly affect the seedling vigour index of maize. These findings suggested that maize is not affected by concentrations of these allelochemicals which were phytotoxic to weeds. Our results concur

with findings of (14) who reported that jack bean and velvet bean did not affect maize emergence and productivity under field conditions suggesting that it is not affected by allelochemicals that were phytotoxic to weeds. Lack of phytotoxic activity of GMCC residues could be due to differences in their seed sizes or sensitivity. Sensitivity to allelochemicals is high in small seeds than in large seeds (2), hence, maize was not affected by leaf residues of cover crops and this was attributed to the large amount of food reserves that are present in maize seeds to support the germination and early seedling establishment. Tolerance of large seeded crops to allelochemicals that exhibit phytotoxicity to small seeded weed species could also be attributed to the ability of large seeded plants to enzymatically detoxify the allelochemicals or due to low absorption and translocation of these phytotoxic chemicals in the plants (12).

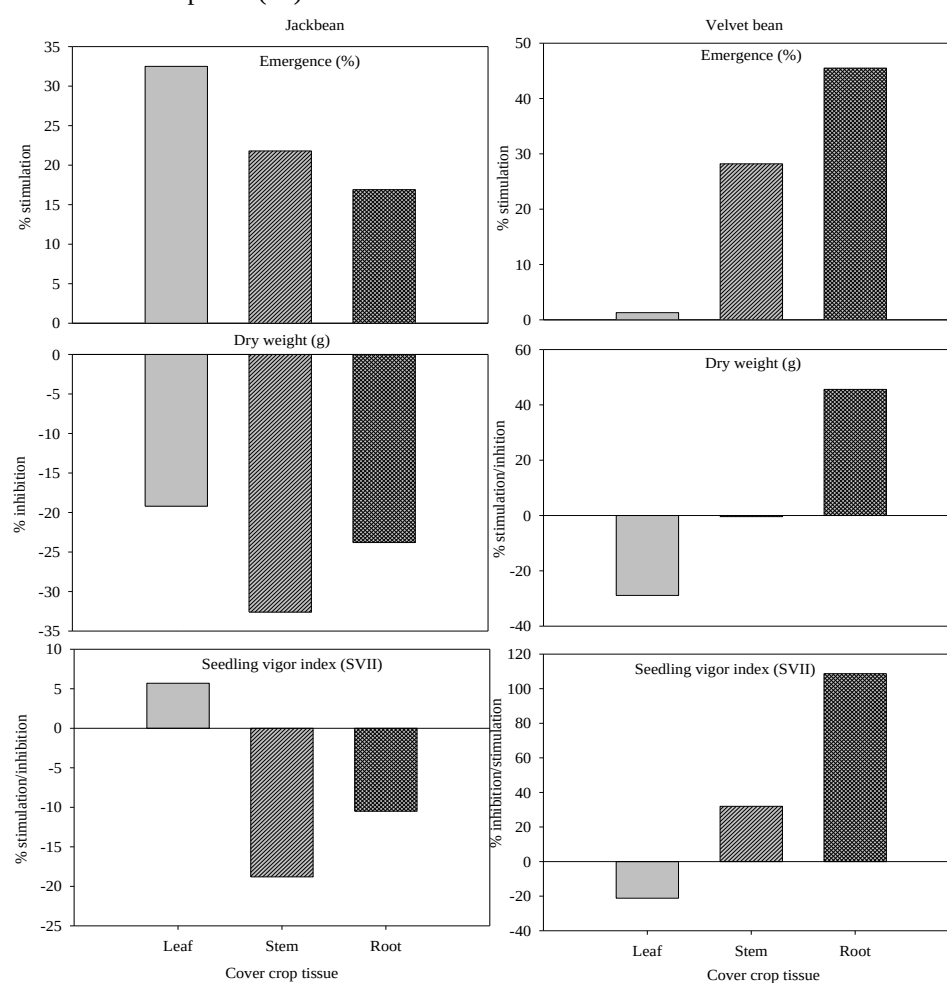


Figure 4. Effects of Jack bean and velvet bean (leaves, stems and roots) soil incorporated residues on emergence, dry weight and seedling vigour index (SVII) of maize

Detection and identification of allelopathic compounds

The masses of several allelochemicals were found in both jack bean and velvet bean leaf, stem and root samples. Mass spectra showed many major molecular peaks at different m/z values and these allelochemicals were confirmed by comparing with literature values. The compound with the peak obtained at m/z 593.1509 and 19.18 min retention time was identified as kaempferol-3-rutinoside (Fig.5 and Fig.6), it was previously reported in pumpkin and zucchini (*Cucurbita pepo* L.) (21). Kaempferol-3-*O*-(6-*O*-rhamnosylglucoside)-7-*O*-rhamnoside was detected in leaf and stem extracts of jack bean. It was eluted at retention time 16.76 min with m/z ion at 739.2099 and presented three product ions as m/z 593, 285, 741. This compound and fragments were previously reported in zucchini and *Viola tricolor* L. commonly known as heartseases (10). The compound with retention time = 15.82 and m/z = 755.2057 was tentatively identified as kaempferol 3-*O*-[6-*O*-(glucosyl) glucoside]-7-*O*-rhamnoside. These findings concur with those of (25) who reported the presence of kaempferol in jack bean root. The LC-MS chromatograms showed the presence of kaempferol-*O*-glycoside and kaempferol-*O*-sabubioside in the stems and roots of velvet bean. The compound with m/z ion = 295.0450- and 14.75-min retention time detected in jack bean leaves was tentatively identified as 2-*O*-caffeoylmalic acid. These findings are in agreement with (8) who reported the presence of a compound with similar parent and fragment ions in zucchini although they could not establish its identity. (25) reported the presence of caffeic acid in jack bean roots but it was not inhibitory to seed germination of several weeds species, suggesting that this compound may not be a very important allelochemical on its own. However, perhaps this allelochemical could be allelopathic, when acting together with other allelochemicals. Hence there is a need to evaluate its synergistic or additive effects when mixed with other compounds isolated from jack bean.

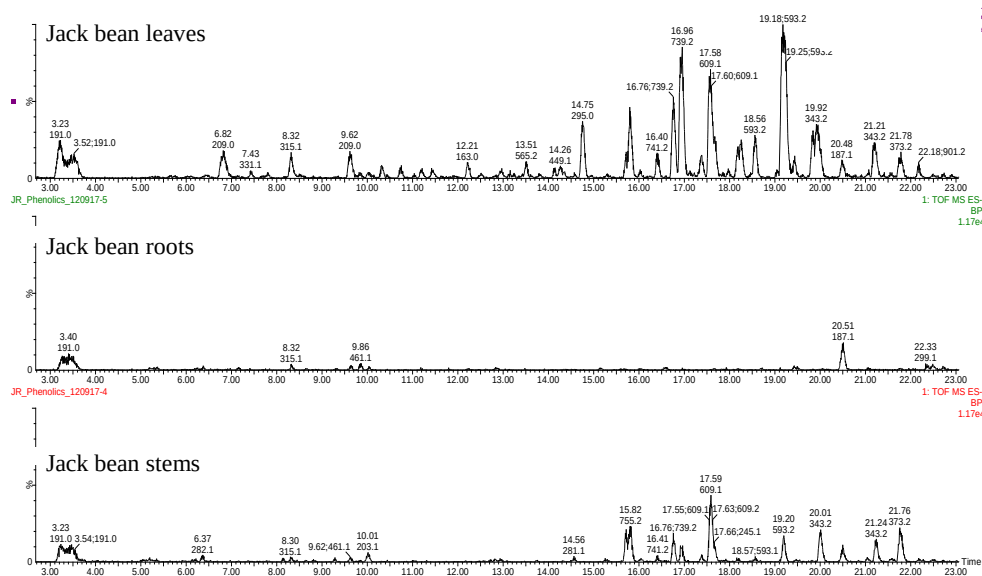


Figure 5. LC-MS chromatograms of leaf, stem and root methanol extracts of jack bean

Similarly, leaf extracts of velvet bean had more compounds than the stem and root extracts as shown by the highest number of major peaks (Fig.7). An unidentified compound with parent m/z ion at 563.1- and 15.82-min retention was found in velvet bean leaves only (Fig8). (11) also reported the presence of a compound with similar properties but could not establish its identity. The compound with m/z ion 593.1501- and 14.42-min retention time was detected in the leaves and stems of velvet bean and was tentatively identified as kaempferol-3-rutinoside (Fig. 8). Another unidentified compound with retention time 17.66 min and molecular mass of 533.1255 and fragments at 245.0936 and 203.0662 was detected in velvet bean leaves and stems but not in the roots (Fig.8). Therefore, the identification of this compound together with another unidentified compound ($m/z = 533.1255$, retention time 17.66) detected in velvet bean require nuclear magnetic resonance (NMR) spectroscopy. Unfortunately, the quantities of these compounds were too little to allow NMR study.

Quantification and detection of polar compounds using LC-MS

Quantification of allelochemicals done using LC-MS indicated differences in the amount of phenolic acids in various tissues of both jack bean and velvet bean (Table 3). Kaempferol concentration (ppm) was 23% and 41% higher in the roots than in the stems and leaves of jack bean, respectively. Similarly, jack bean root extracts had the highest concentration of genistein of 71.6 ppm followed by stems, with the leaves having the lowest amount. Rutin was the most abundant compound in jack bean. The concentration of rutin was 162% more in the leaves than in the stem extracts. Of all the phenolics quantified, only genistein was detected in amounts exceeding 10 ppm in the roots and stems of velvet bean but was not detected in the leaf tissues. Genistein was 7-folds more in velvet bean root extracts compared to the stems. Atropine was only detected in velvet bean stem extracts. Probably kaempferol, naringenin, rutin and genistein detected in the leaves of these cover crops could work synergistically or additively resulting in greater allelopathic activity compared to other tissues. This could explain why leaf extracts were more suppressive to germination and early seedling growth of blackjack and goosegrass.

Table 3. Detection and quantification (ppm) of previously reported compounds in jack bean and velvet bean tissue samples

Compound	Jack bean			Velvet bean		
	Leaf	Stem	Root	Leaf	Stem	Root
Kaempferol	76.9	99.5	130.6	ND	ND	ND
Naringenin	449.1	ND	ND	ND	ND	ND
Chlorogenic acid	ND	ND	ND	ND	ND	ND
Ferulic acid	ND	ND	ND	ND	ND	ND
Rutin	4512.1	1719.8	ND	ND	ND	ND
Genistein	19.0	32.0	71.6	ND	19.7	141.9
Hesperidin	ND	ND	ND	ND	ND	ND
Atropine	ND	ND	ND	ND	0.036	ND

ND: Not detected (concentration < 10 ppm)

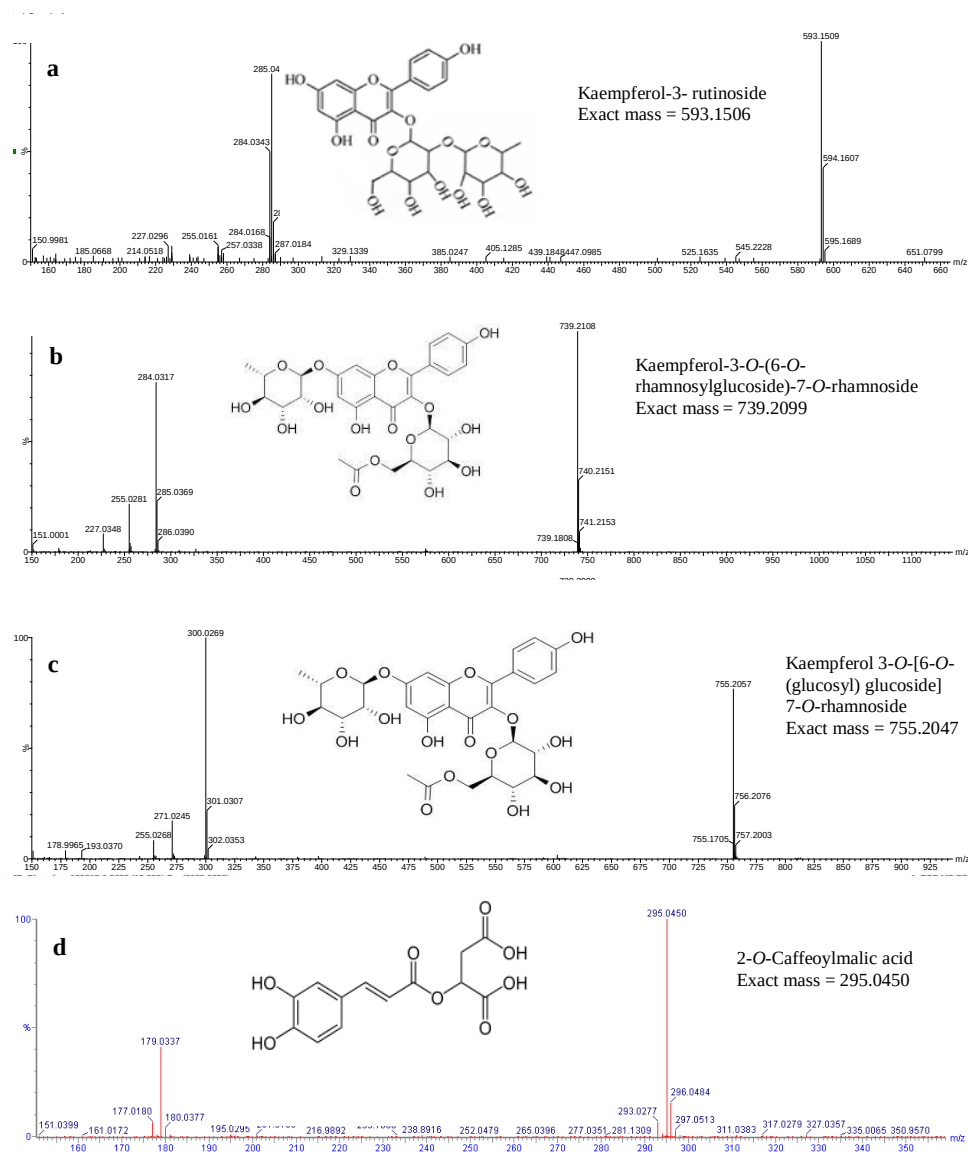


Figure 6. Cumulative LC-MS spectra of major peaks from extracts of different parts of jack bean

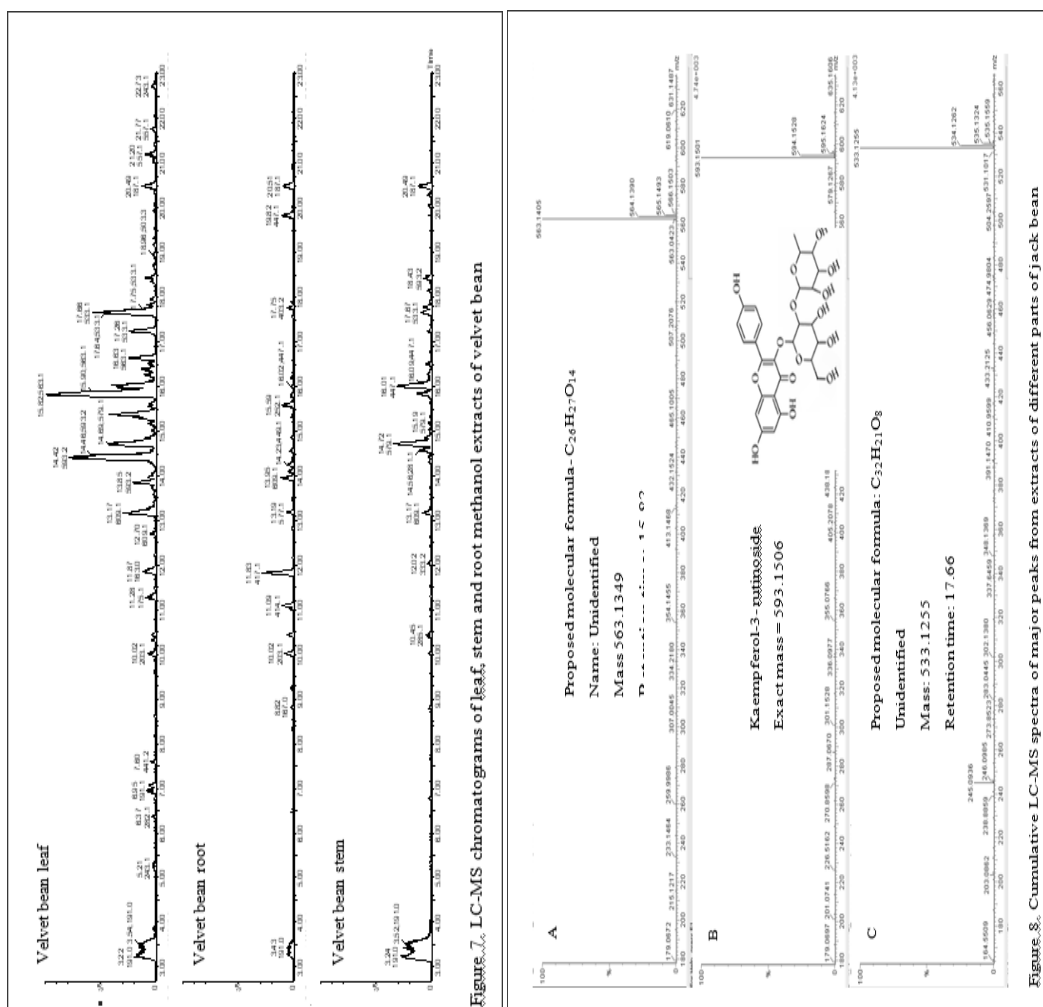


Table 4. Inhibitory effects of different plant secondary compounds on seed germination and seedling growth of blackjack and goosegrass weeds

Treatment	Blackjack			Goosegrass	
	Germination (%)	Plumule (mm)	Radicle (mm)	Radicle (mm)	Plumule (mm)
Control	100.00	17.27 ^a	39.47 ^a	15.07 ^a	7.60 ^a
Atropine	88.00	15.73 ^{ab}	23.53 ^{bc}	9.67 ^b	5.53 ^c
Genistein	88.00	17.40 ^a	34.27 ^{ab}	3.27 ^c	4.00 ^d
Kaempferol	86.00	15.73 ^{ab}	29.80 ^{ab}	3.27 ^c	3.73 ^d
Naringenin	92.00	15.73 ^{ab}	31.53 ^{ab}	14.73 ^a	6.53 ^b
Rutin	78.00	10.87 ^c	14.53 ^c	4.27 ^c	3.73 ^d

Means in columns followed by the same letter are not significantly different as determined by Fisher's protected least significant difference test ($p \leq 0.05$).

(ii). Goosegrass weed: All the secondary compounds significantly ($p < 0.05$) suppressed the plumule elongation of goosegrass (Table 4). Kaempferol and rutin inhibited the plumule growth by $> 50\%$ than control. Similarly, radicle growth was significantly ($p < 0.05$) inhibited by the majority of secondary compounds except naringenin. Genistein and kaempferol caused maximum inhibition (78%) in radicle elongation followed by rutin which also caused $> 70\%$ inhibition. The fact that jack bean root aqueous extracts inhibited the germination parameters of blackjack but not goosegrass suggests the presence of allelochemicals with a narrower spectrum of activity than the other above ground parts of this cover crop. Rutin was the most phytotoxic compounds to blackjack. This suggests that it could be the allelochemical that was mostly responsible for weed germination and growth suppression obtained in this study. In this study kaempferol did not affect the germination of blackjack. These findings confirm the selective activity of kaempferol on weed germination as previously reported by (25) where kaempferol did not affect the germination of *Cassia occidentalis* L. but inhibited the germination of *Mimosa pudica* L. and *Cassia tora* L. by 43% and 40%, respectively. The phenolic compounds are highly phytotoxic to germinating seedlings due to their ability to stimulate production of oxidative enzymes that modify membrane permeability, lignin synthesis and consequently reduce the root growth (4). These results therefore confirm the presence of potent allelochemicals with a wide spectrum of activity in jack bean tissues making this a desirable crop for cultural weed control in integrated weed management programmes.

CONCLUSIONS

In the laboratory bioassay, the leaf extracts of both green manure crops were most inhibitory to the germination and seedling growth of both test weeds. Thus use of aqueous extracts of both jack bean and velvet bean and/or their mulches may control weeds in maize crop. Future studies need to focus on identifying and quantifying the putative allelochemicals in different plant parts of green manure crops and to evaluate their efficacy on weeds and crops, when applied as post emergence. The allelopathic potential of these green manure crops should be studied under field conditions to determine their efficacy in

reducing the weed emergence in crop fields. They reduced the weeds infestation due to their smothering effect as they rapidly produce lot of biomass.

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