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Alamat Redaksi
PERAGI Komda Sumatera Barat
Jl. Bandar Damar No. 10, Padang 25112
Telp. 0812 6610 087, 0813 6302 7898
e-mail: jurnaljerami@yahoo.com
irfan_s@telkom.net

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Daftar Isi

IN VITRO GROWTH RESPONSE OF SOYBEAN (*Glycine max* (L.) Merr.) TO WEED EXTRACTS

(Respon Pertumbuhan Kedelai (Glycine max (L.) Merr.) yang Ditumbuhkan Secara In Vitro terhadap Beberapa Ekstrak Gulma)

Irawati Chaniago

Program Studi Agroekoteknologi, Fakultas Pertanian, Universitas Andalas, Padang
Kampus UNAND Limau Manis, Padang 25163
e-mail: ichaniago@faperta.unand.ac.id

ABSTRAK

Percobaan faktorial dua arah tentang pengaruh konsentrasi ekstrak beberapa jenis gulma terhadap pertumbuhan kedelai secara *in vitro* telah dilaksanakan dengan tujuan untuk mempelajari pengaruh allelopathy ekstrak gulma sekaligus mempelajari pemanfaatan kultur *in vitro* dalam studi allelopati. Perlakuan adalah varietas kedelai (Banjalong, Melrose, dan Valiant) dan konsentrasi ekstrak gulma (*Amaranthus powellii, Cyperus rotundus,* dan *Paspalum dilatatum,* masing-masing 0, 1 dan 10%). Semua unit perlakuan disusun secara Rancangan Acak Lengkap (RAL) dengan 5 ulangan. Media Murashige and Skoog (MS) digunakan untuk menumbuhkan hipokotil dan tunas kedelai. Pengamatan meliputi bobot segar plantlet, kandungan klorofil total daun, dan bobot segar kalus. Hasil memunjukkan bahwa kultur *in vitro* dapat digunakan untuk percobaan allelopati. Species gulma yang diuji mempengaruhi pertumbuhan kedelai, terutama gulma teki. Varietas Melrose menunjukkan pertumbuhan yang melebihi dua varietas lainnya. Bagian atas hipokotil menghasilkan lebih banyak kalus dibandingkan bagian tengah dan dasar hipokotil.

Key wods: allelopathy, soybean, in vitro, chlorophyll content, callusion

INTRODUCTION

Allelopathy refers to any process involving secondary metabolites produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems (excluding animals) (Narwal, 1999). Studies have demonstrated that *Amaranthus powellii, Cyperus rotundus* and *Paspalum dilatatum* interfere with the growth of different soybean cultivars. These weed species had both competitive and allelopathic effects under *in vitro* and *in vivo* experimental conditions (Chaniago *et al.*, 2001; 2002; 2003a; 2003b). The inhibition in soybean growth as indicated by total biomass decline might be one of the complex physiological responses of soybean towards weed interference.

Plant allelochemicals are secondary metabolites (Swain, 1977) and are identified as non-nutritional compounds, synthesised by a plant species that are able to affect growth, health, and behaviour or population biology of another species either as a stimulator or an inhibitor (Whittaker, 1971).

Hundreds of different compounds released from plants and microbes are known to have allelopathic potential, and to interfere with the target species. Recently, many new compounds with allelopathic properties, known as allelochemicals, have been identified and studied (Einhellig, 1995).

Adequate *in vitro* studies of allelopathy on soybean have not been intensively reported. To further investigate the interaction between soybean and weeds, the growth of soybean under the influence of the weed extracts was studied. *In vitro* technique was used to study the influence of the weed extracts on callus formation from soybean hypocotyls as well as shoot growth. This culture was applied to control the environmental factors. In addition, the suitability of using *in vitro* culture in the allelopathy study was sought.

MATERIALS AND METHODS

A two-way factorial experiment with five replicates was designed to study weed extract effects on soybean growth and callus formation. The experimental factors included soybean cultivar (Banjalong, Melrose, and Valiant), and different concentration of weed extracts (amaranth, nutgrass and paspalum each at 1 or 10% and weed-extract free as a control). The weed extract pH was adjusted to 5.8 ± 0.02 to match the pH of the Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) prior to filter sterilisation under aseptic conditions through a Nalgene™ microfilter with a 20 µm pore size. The sterile extracts were kept refrigerated for later use.

The MS basal medium containing 30 g/L sucrose and 8 g/L BiTek™ agar at pH 5.8 was prepared. The medium was solidified by adding the agar, which was heated and dissolved, then sterilised at 1.1 kg/cm (103 kPa) at 120°C for 20 minutes. The medium was allowed to cool to approximately 50 to 60°C before the sterile weed extracts were added according to treatments. The medium was dispensed into tubes or Petri dishes as required.

Four surface-sterilised soybean seeds were aseptically germinated on 20 mL of 1/10th strength MS basal medium for 1 week. One shoot tip of the soybean seedling, approximately 1 cm long, was transferred to 20 mL MS basal medium with or without the weed extracts in a 125 mL tube for evaluating shoot growth. The seedling hypocotyl sections were grown in 9 cm diameter sterile plastic Petri dishes on 20 mL of medium similar to that for shoot growth. The hypocotyls were cut into 5 mm segments designated as basal, middle and top sections, and were longitudinally halved before being placed onto the medium, with the cut surface in contact with the agar. Each Petri dish contained six sections of each hypocotyls (three segments, each longitudinally halved). After transferring hypocotyls to the medium, Petri dishes were sealed with Parafilm to prevent contamination and drying of the medium. The shoots and hypocotyls were cultured at temperatures of 25 ± 2°C with a 16 hour photoperiod. Light was provided with cool white fluorescent tubes at 30 µmol/m^2/s.

At the end of a 4 week period, callus fresh weight, including the hypocotyl was recorded. Both longitudinally-halved sections of each hypocotyl segment (basal, medium, or top) were weighed at the same time. Plant fresh weight and total leaf chlorophyll content were determined simultaneously from the plantlets grown in the tubes. Chlorophyll content was determined from the unifoliate leaves of cultured soybean following the procedures from Arnon (1949).

RESULTS AND DISCUSSION

The interaction between soybean cultivar, weed species and weed extract concentration did not significantly affect plantlet fresh weight ($P>0.05$). However, all weed extracts significantly reduced the plantlet fresh weight (Figure 1). Nutgrass demonstrated the strongest inhibition of plantlet growth, and the effect was markedly lower than that of the amaranth or paspalum extracts, whereas the latter two weed extracts showed similar effects.

Differences were observed between the cultivars ($P<0.001$) in plantlet fresh weight. Melrose had the highest plantlet weight in all extracts at all concentrations and showed little variation between weed species or extract concentration (Figure 1). Valiant was affected most by nutgrass extract at both concentrations, and the 1 and 10% extracts reduced plantlet fresh weight by 16 and 21% of the control, respectively. All weed extracts reduced plantlet fresh weight relative to the control, but nutgrass extract resulted in the greatest reduction compared to other weed extracts. Overall, increased extract concentrations were followed by reduced plantlet fresh weight, except in Melrose.

Total chlorophyll content was not affected by the interaction effects of soybean cultivar, weed species, and the extract concentration ($P>0.05$) although all weed extracts reduced chlorophyll content in all soybean cultivars (Figure 2). Nutgrass extract reduced the chlorophyll content more than did other weed extracts especially at the higher concentration. Minor cultivar differences were also observed in the total chlorophyll content ($P<0.001$) with Melrose and Banjalong being similar (mean = 0.34 µg/mg fresh leaf), and Valiant being slightly higher (0.36 µg/mg fresh leaf).

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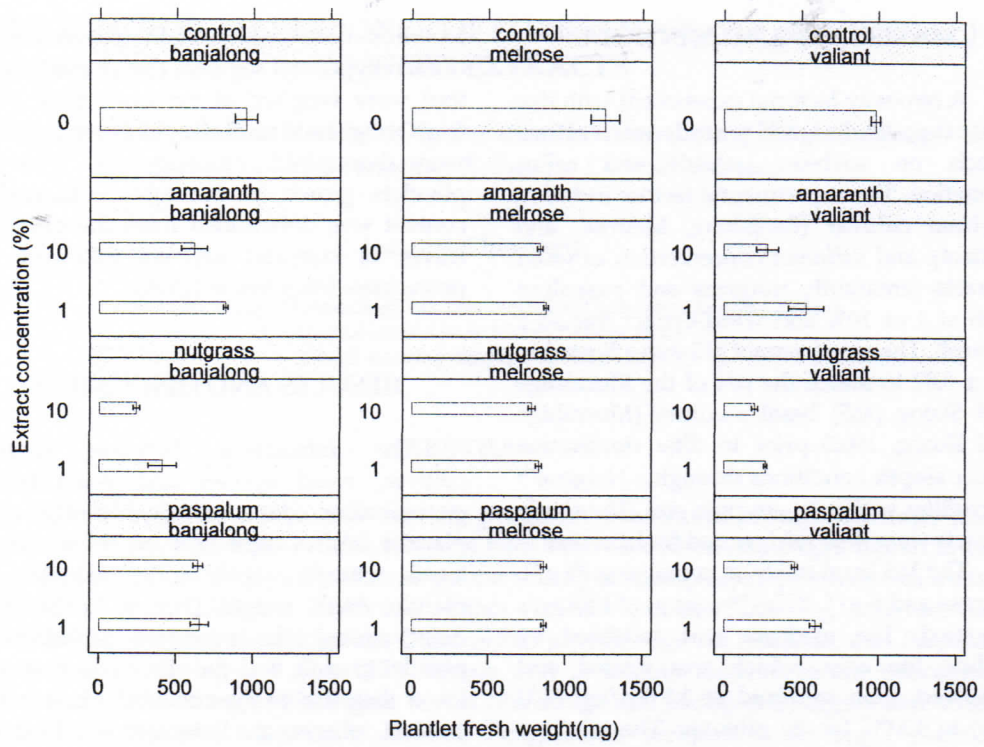

Figure 1. Plantlet fresh weight (mg/plantlet) of soybean grown *in vitro* with different concentration of weed extracts

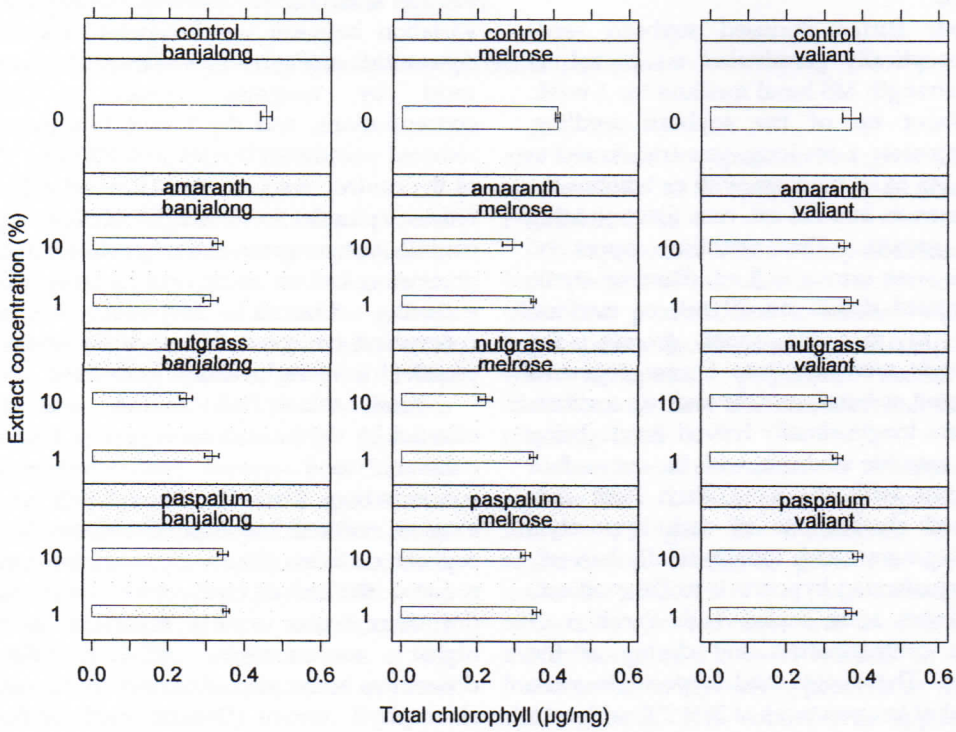

Figure 2. Total chlorophyll content (µg/mg fresh leaf) of soybean grown *in vitro* with different concentration of weed extracts

An increase in nutgrass extract concentrations reduced chlorophyll content with a P_{slope}<0.001, but amaranth and paspalum extracts did not cause a significant reduction in chlorophyll.

Callusing of the soybean hypocotyl was not affected by the interactions of soybean cultivar, weed species and weed extract concentrations (P>0.05). While all weed extracts significantly reduced callus weight compared with the control (P<0.001, (Figure 3), there was no diference between weeds (P>0.05). Increasing the extract concentrations in the medium resulted in reduced callus fresh weight in all soybean cultivars in all weed extracts, except Melrose.

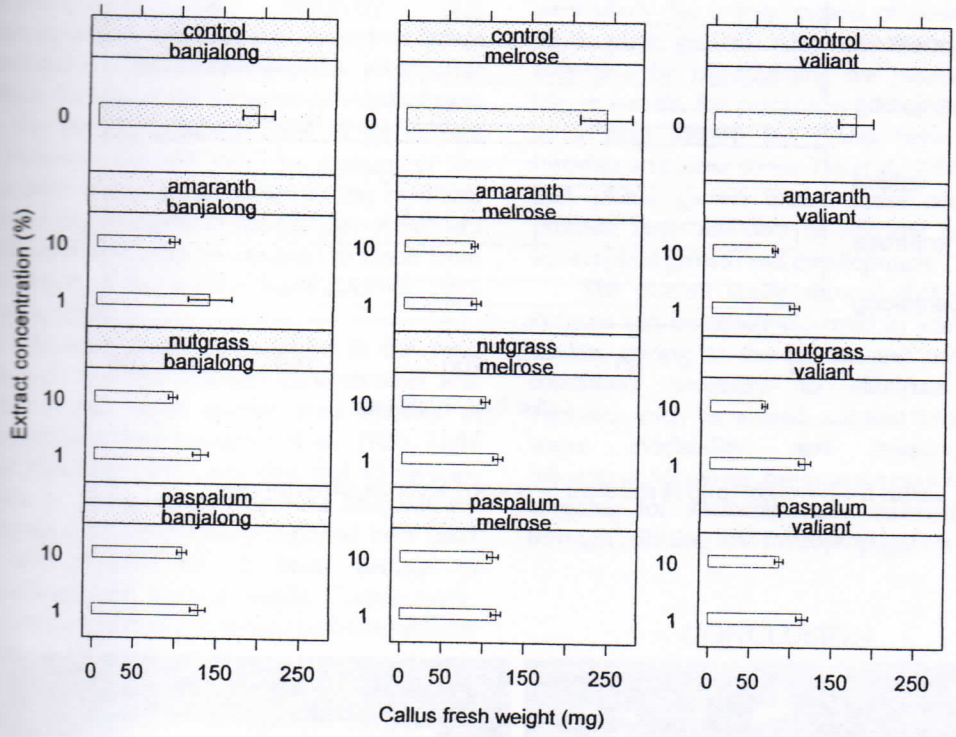

Figure 3. Hypocotyl callus fresh weight (mg) of different soybean cultivars grown *in vitro* with different concentrations of weed extracts

Callus fresh weight varied between hypocotyl segments (P<0.001) although the interaction effects between the hypocotyl, soybean cultivars and the weed extracts were not significant (P>0.05). Top segments produced higher callus weights than either basal or middle segments (Figure 4). in all cultivars. Within each cultivar, the hypocotyl top segment produced significantly higher callus weight than other segments, but there was no difference in callus weight between the basal and middle segment within each cultivar. The difference in callus weight may reflect the different callusing potential of various parts of the soybean hypocotyl. All hypocotyl sections remained dark green and enlarged from their original size, producing light green and friable callus (Figure 5). Figure 6 illustrates differences between soybean plants grown in medium with amaranth extract and in the control medium.

The weed extracts reduced soybean callus production and growth *in vitro*, indicating reduced cell division. Other research has reported reduced cell division due to allelochemicals such as caffeine (Vaughan and Vaughn, 1988) and artemissin (Dayan *et al.*, 1999) in onion root cells. Various mechanisms of inhibition of cell division have been reported such as interference with the function and formation of microtubules (Vaughan and Vaughn, 1988) and chromosome separation (Devine *et al.*, 1993). However, cell division is

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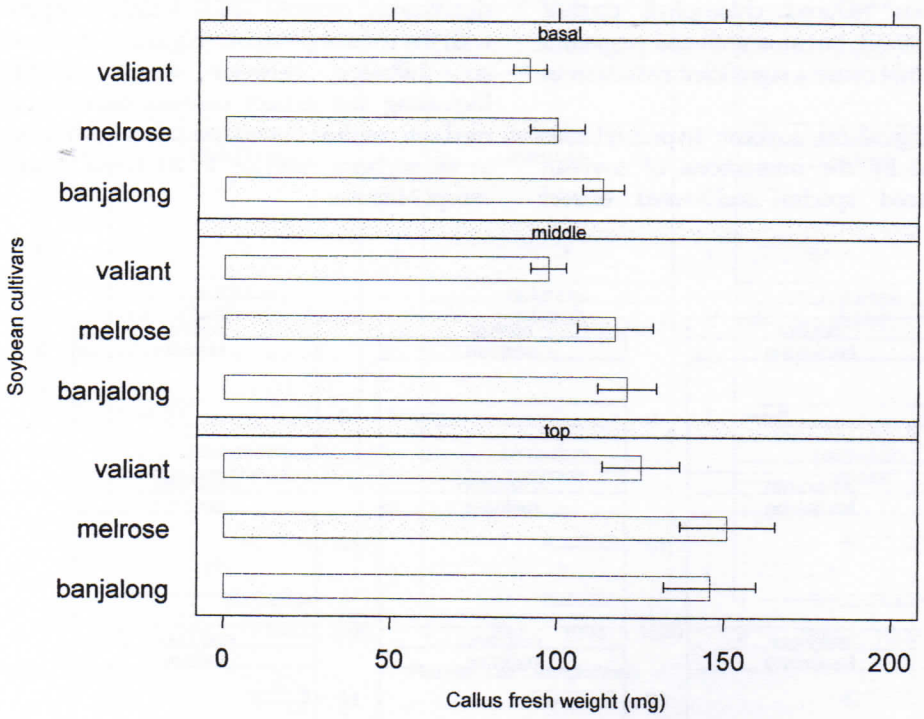

Figure 4. Callus fresh weight (mg) of different hypocotyl sections grown *in vitro*

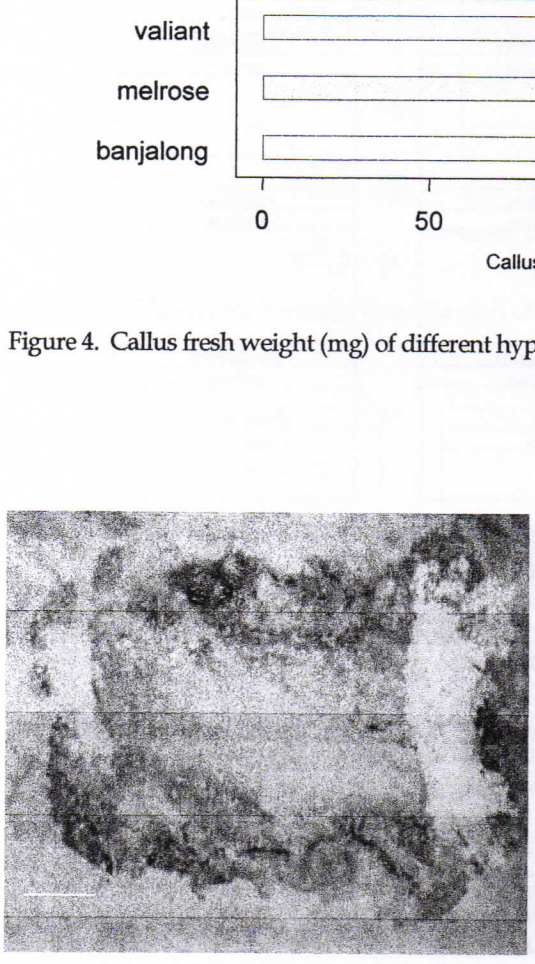

Figure 5. Callus of top-segment of soybean hypocotyl cv. Melrose at 10% of nutgrass extract. Bar = 3.75 mm.

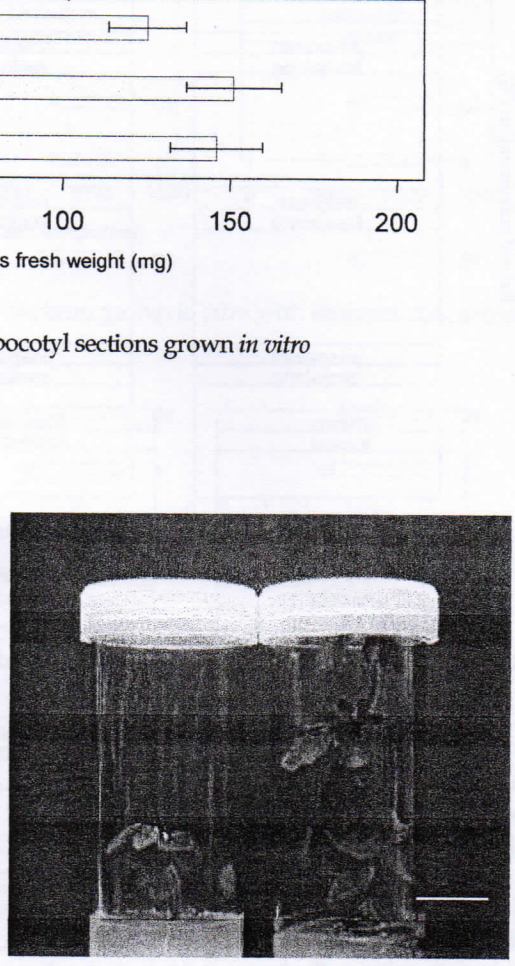

Figure 6. Soybean plantlets cv. Melrose at 4 weeks after transfer to MS basal medium. (Left: in 10% of amaranth extract; Right: in an extract-free medium). Bar = 0.8 cm.

only one of the processes that could be affected by the action of the weed extracts in causing growth reduction. Other mechanisms such as nutrient uptake and respiration may be influenced by weed extracts (Michelsen *et al.*, 1995; Sert *et al.*, 1998; Abrahim *et al.*, 2003).

Qasem and Hill (1989) reported difficulties in studying allelopathy plant residues added to soil. Adding plant material to soil can modify nutrient availability negatively such as immobilisation, or positively (e.g. decomposition and nutrient release) or water availability, confounding possible allelopathic effects. However, the inclusion of weed extracts to the nutrient solutions used in the current experiment did not vary the amount of the available nutrient and water in the medium. Therefore, alteration in the amount of nutrient uptake, if any, may be assumed to result from the action of the weed extracts (Lincoln, 1993; Mallik, 2003).

Another factor to consider is the ratio between the test solution concentration and volume and target species seed number in germination (Weidenhamer *et al.*, 1987). Liebl and Worsham (1983) reported that 10 soybean seeds in 10 mL of solution was adequate for bioassays. The experiment reported here used 20 mL of solidified MS basal medium to germinate four soybean seeds. Consequently, the amount of medium should be favourable for soybean germination.

In vitro culture has often been used for allelopathy studies. For eaxmple Hogan and Manners (1992) used the suspension culture cell method to study the detoxification of hydroquinone in leafy spurge. Veronneau *et al.* (1997) also used this method to study the allelopathic influence of American elm, red maple and pin cherry on lettuce and duckweed owing to its applicability for use with relatively small volumes of samples, and found that the system suited allelopathy studies. These authors argued that phytotoxicity detection using whole plant systems, such as germination and seedling growth, did not directly correlate to the tissue culture system. Therefore, they suggested using several bioassay procedures to get the most conclusive evidence of allelopathic activity. This is in accordance with the work in this thesis, in which different bioassay methods were applied to study different allelopathic mechanisms of the weed extracts on soybean.

Results of the current experiment demonstrated that nutgrass extracts was the most inhibitory and paspalum was the least; Melrose was the most productive cultivar in the control treatment and was also the most tolerant of the weed extracts; and increasing weed extract concentrations usually increased the inhibition of the growth.

However, some limitations were encountered in the *in vitro* study of allelopathy. For instance, the space available for the target species to grow in the culture vessels, particularly for a long period of observation, limits plant growth. Although this could be overcome by transplanting the plantlets into bigger vessels, the practicality of this is limited as it may disturb the plants between the transfers and cause stress. Taji *et al.* (1997) stated that plants grown under stress conditions produce large amounts of ethylene gas, and inhibit plant growth and development.

The present study showed that *in vitro* systems can be effectively used in allelopathy studies, owing to the aseptic and controlled conditions necessary to eliminate other variables such as altered nutrient levels and water availability, and microorganism infestation. Moreover, the systems may facilitate breeding for allelochemically resistant crops through callusing and micropropagation

CONCLUSION

The *in vitro* system can be used effectively to study allelopathy. Under *in vitro* conditions, all weed extracts interfered with soybean growth particularly nutgrass. Melrose was the most superior cultivar in response to all weed extracts, as indicated by its higher plantlet fresh weight than other cultivars. Top segments of soybean hypocotyl were the most productive source of explant for callus formation.

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