Volume 2 Nomor 1
Januari - April 2009

ISSN : 1979-0228

JERAMI

Jurnal Agronomi Indonesia

Diterbitkan Oleh :
PERHIMPUNAN AGRONOMI INDONESIA
Komisariat Daerah Sumatera Barat
Sekretariat : Jl. Bandar Damar No10. Padang 25112
Sumatera Barat

JERAMI

Jurnal Agronomi Indonesia
(Indonesian Journal of Agronomy)

Volume 2 • Nomor 1• Januari – April 2009

Jurnal JERAMI (Jurnal Agronomi Indonesia), terbit sejak Januari 2008, adalah majalah ilmiah resmi Perhimpunan Agronomi Indonesia (PERAGI) Komisariat Daerah Sumatera Barat sebagai salah satu bentuk sumbangannya kepada pengembangan ilmu pertanian, khususnya agronomi, yang diterbitkan dalam bahasa Indonesia dan Inggris. Jurnal ini diterbitkan tiga kali dalam setahun, bulan Januari, Mei dan September.

ISSN : 1979-0228

Alamat Redaksi

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ISSN 1979-0228

Daftar Isi

PRELIMINARY STUDY ON ALLELOPATHIC EFFECTS OF SEVERAL WEED SPECIES ON THE GROWTH OF SOYBEAN (*Glycine max* (L.) Merr.): THE EFFECT OF OSMOTIC POTENTIAL ON GERMINATION

(Kajian awal allelopati beberapa jenis gulma terhadap pertumbuhan kedelai (Glycine max (L.) Merr.) : Pengaruh tekanan osmotik terhadap perkecambahan)

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ABSTRAK

Percobaan telah dilaksanakan untuk mempelajari pengaruh potensial osmotik larutan ekstrak berbagai jenis gulma terhadap perkecambahan benih kedelai varietas Melrose. Perlakuan adalah berbagai potensial osmotik larutan D-mannitol (0.112, 0.126, 0.131, 0.141, 0.194 and 0.201 Os/kg air) denngan 10 ulangan dalam Rancangan Acak Lengkap (RAL). Konsentrasi larutan D-mannitol tersebut setara dengan konsentrasi larutan ekstrak gulma teki (*Cyperus rotundus*), bayaman (*Amaranthus powellii*) dan paspalum (*Paspalum dilatatum*) yang diekstarksi dengan cara perendaman dan penggilingan. Benih kedelai yang telah disterilisasi-permukaan dikecambahkan pada Petri dish pada suhu 25°C yang diletakkan dalam ruang perkecambahan tanpa cahaya. Jumlah benih berkecambah (dengan panjang radikula ≥5 mm) diamati setiap 12 jam selama 84 jam. Pada akhir percobaan dilakukan pengukuran panjang radikula. Hasil percobaan menunjukkan bahwa perbedaan potensial osmotik larutan ekstrak gulma yang berbeda tidak berpengaruh terhadap perkecambahan benih kedelai.

Key words: allelopathy, osmotic potential, soybean, germination

INTRODUCTION

About 10% loss of agricultural production could be attributed to the competitive effects of weeds, in spite of intensive weed control in most agricultural systems (Zimdahl, 1980). Soybean yield losses of 50 to 90% are common for soybean grown in natural weed populations (Coble, 1981), and this yield loss has become serious due to the slow growth of the crop at early stages (Andrade, 1995), highlighting the significance of weed-free competition in soybean. However, knowledge concerning the allelopathic interaction between soybean and some major weeds such as Powell's amaranth (*Amaranthus powellii*), nutgrass (*Cyperus rotundus*), and paspalum (*Paspalum dilatatum*) is also lacking.

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.

Allelochemicals can interfere with germination and radicle elongation through different mechanisms. For example, phenolic acids reduced soybean germination through respiratory enzyme inhibition (Abrahim, 2003), mitochondrial function aberration (Rasmussen, 1992), and plant-water relationships interference (Barkosky, 2003), emphasising that germination inhibition may be a secondary effects of the allelochemicals following physiological changes.

To assess the allelopathic effect of weed exudate or leachate on crop species, it is essential that extraction methods mimic the real situation of plant interference. Different extraction methods have demonstrated inconsistent results (either stimulation or inhibition of the target species) (del Moral, 1971; Lovett, 1982; Koch, 1992) causing contention among researchers (Qasem, 1989). For example L-tryptophan, the principal allelopathic compound found in the leachate of mesquite (*Prosopis juliflora*) foliage, inhibits barnyard grass root growth. However, the amount of L-tryptophan extracted from mesquite foliage using the soaking method decreased significantly with time, whereas the amount of L-tryptophan obtained using a leachate-drip method increased over time (Nakano, 2003).

The work reported here examined the effect osmotic potential and whether different osmotic potential of three weed species (*Amaranthus powellii*, *Cyperus rotundus* and *Paspalum dilatatum*) extracts resulted from different extraction methods contribute to allelopathy. It is anticipated that this experiment would provide a better understanding of the allelopathic mechanisms and a possible mode of action of three weeds on soybean.

MATERIALS AND METHODS

Treatments were different osmolality of D-Mannitol (0.112, 0.126, 0.131, 0.141, 0.194 and 0.201 Os/kg water, and purified deionised water as the control treatment), and were replicated 10 times. These ranges of osmolality (Table 1) represent weed extract osmolality of different extraction methods. D-mannitol was used in this study because it has been previously demonstrated to act as an inert osmotic medium for such studies (Wardle, 1992). The pH of each solution and water as the control was adjusted to 7.5 ± 0.02 to meet the average pH of the weed extracts, thus eliminating the effect of different pH on soybean germination.

Table 1. Concentrations and osmolalities of weed extracts at different extraction methods.

Weeds	Extraction methods	Concentrations[1]	Osmolalities[2] (Os/kg water)
Water	N/A	0.0%	0.000
Amaranth	Soaking	3.4%	0.194
	Grinding	3.74%	0.201
Nutgrass	Soaking	2.25%	0.126
	Grinding	2.5%	0.141
Paspalum	Soaking	2.0%	0.112
	Grinding	2.32%	0.131

1: concentrations of each extract solutions obtained from the standard curve of standard D-mannitol solutions.
2: osmolality values obtained by calculating from known concentrations of D-mannitol.

Surface-sterilised soybean seeds were placed in Petri dishes, and supplemented with 10 mL of the D-mannitol solution according to treatments or sterile deionised water. The experiment was conducted in a thermostatically controlled growth cabinet at 25°C in total darkness. The number of germinating seeds (with radicles at least 5 mm long) was recorded every 12 hours for 84 hours, until no further seeds germinated. The radicle length was measured at the end of the experiment. The germination indices were calculated following the formulae of Chiapusio *et al.* (1997).

RESULTS AND DISCUSSION

A general trend was observed in which total germination index was not affected by an increase in the osmolality (P_{slope}=0.5377,

Figure). The lack of relationship between osmolality level and total germination was also supported by the results of orthogonal contrast between the treatments ($P \geq 0.1491$).

Soybean radicle length and radicle dry weight were not affected by different osmotic potentials (P_{slope}=0.9206 and P_{slope}=0.8635,

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respectively). In addition, the reduction in radicle length and dry matter accumulation following an increase in osmolality were not significant (P_{slope}=0.2672 and P_{slope}=0.2188, respectively; data not shown).

Results from this experiment demonstrated that weed extract osmolality did not affect the germination of soybean. Therefore, adjusting the osmotic potential of weed extracts was not considered necessary in further experiments.

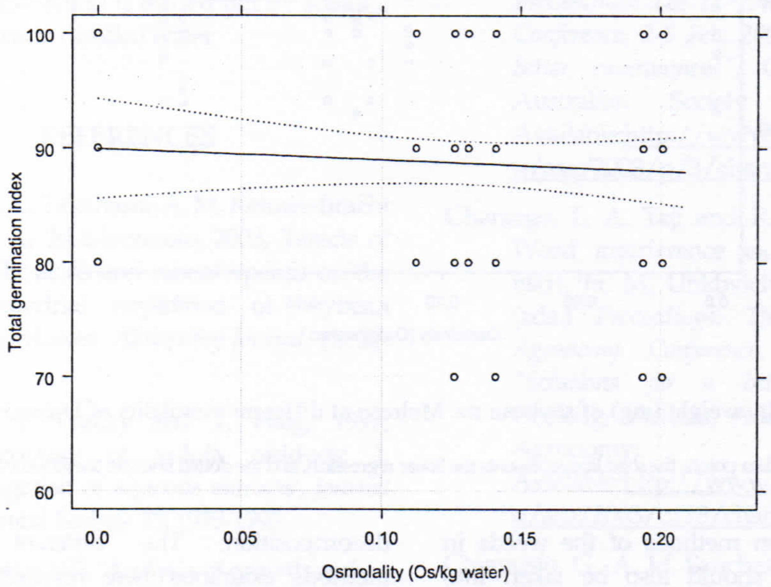

Figure 1. Total germination (G_T) index of soybean cv. Melrose at different osmolality of D-mannitol.

The circles indicate the data points, the solid line represents the linear regression, and the dotted lines are the 95% confidence limits.

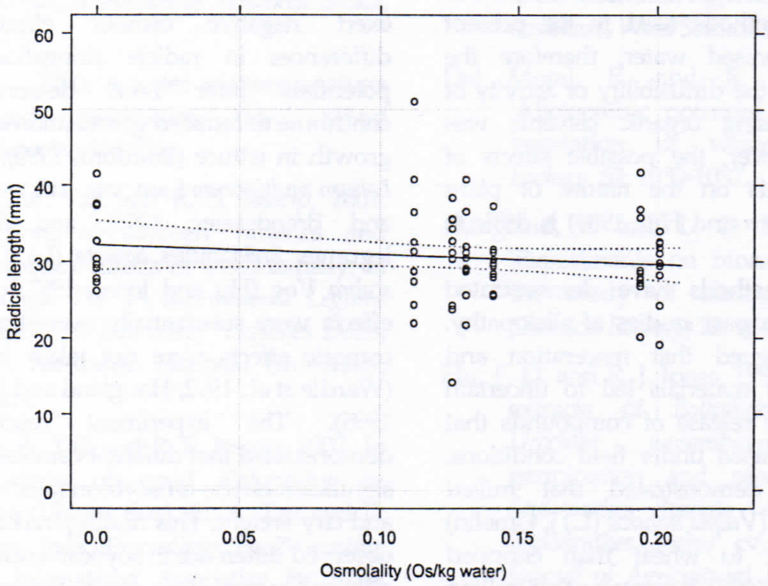

Figure 2. Radicle length (mm) of soybean cv. Melrose at different osmolality of D-mannitol.

The circles indicate the data points, the solid line represents the linear regression, and the dotted lines are the 95% confidence limits.

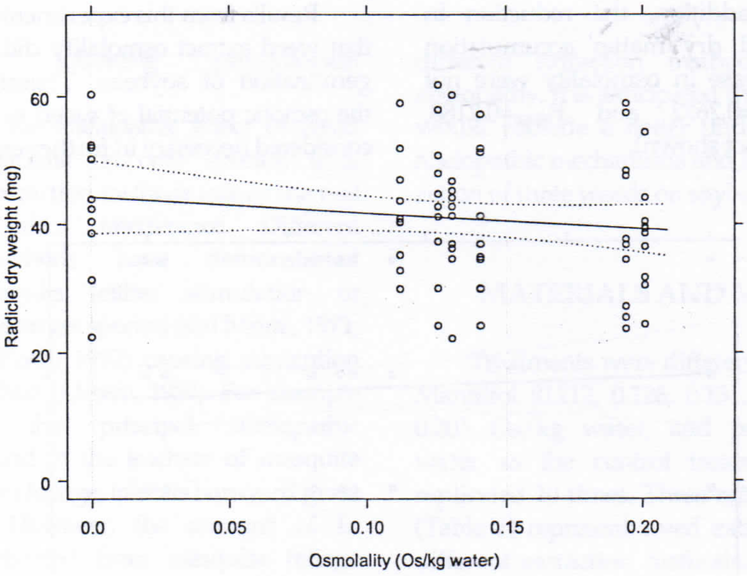

Figure 3. Radicle dry weight (mg) of soybean cv. Melrose at different osmolality of D-mannitol.

The circles indicate the data points, the solid line represents the linear regression, and the dotted lines are the 95% confidence limits.

The extraction methods of the weeds in bioassay studies should also be taken into consideration (Inderjit, 1996). In other trials (data not reported), different extraction methods resulted in similar soybean seed germination responses. However, water extraction neglects the possibility that some organic solvents may enhance the diffusion and activity of soluble compounds (Putnam, 1983). The extraction methods used in the present study used deionised water; therefore the possible effect on the diffusibility or activity of allelochemicals using organic solvents was eliminated. However, the possible effects of extraction methods on the nature of plant compounds (Qasem and Hill, 1989) is difficult to overcome.

Extraction methods have demonstrated different results in past studies of allelopathy. Lovett (1982) argued that maceration and grinding of plant materials led to uncertain results due to the release of compounds that might not be released under field conditions. Another report demonstrated that milled residues of vulpia (*Vulpia myuros* (L.) J. Gmelin) were more toxic to wheat than chopped residues (An *et al.*, 1997), suggesting that milling plant residues may accelerate the release of free phytotoxins inherent in the material by rupturing cells and increasing the surface area in contact with water. Milling can also provide microorganisms with increased surface area activity which in turn would accelerate the decomposition. The different extraction methods examined here resulted in similar germination in the soybean. The grinding method resulted in a more viscous solution. However, the extract centrifugation separated the supernatant and the aliquot from the extract solution. This may reduce the extract osmolality.

It is possible that weed extracts may also exert negative osmotic effects causing differences in radicle elongation. Osmotic potentials have been demonstrated to contribute to reduced germination and seedling growth in lettuce (Bradford, 1990), radish and *Lolium multiflorum* Lam. var. *italicum* (Haugland and Brandsaeter, 1996), and the tropical legumes *Stylosanthes hamata* (L.) Taub and *S. scabra* Vog (Hu and Jones, 1997). Allelopathic effects were substantially overestimated when osmotic effects were not taken into account (Wardle *et al.*, 1992; Haugland and Brandsaeter, 1996). The experiment reported here demonstrated that different osmolality had non significant effects on soybean radicle elongation and dry weight. This finding indicates that the observed difference in soybean radicle length in all weed extracts was due to factors other than the extraction methods and the extract osmolality. Reduced radicle growth may therefore have resulted from the allelochemicals derived from the weed extracts.

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CONCLUSION

The osmotic potentials of the weed extracts did not affect soybean germination. Therefore, adjusting the osmotic potentials of the weed extracts was considered unnecessary for other trials when extraction is carried out by soaking the weed parts in distilled water.

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ISSN 1979-0228