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Allelopathic effects of *Dioscorea hispida* Dennst. (Dioscoreaceae) tuber from Terengganu, Peninsular Malaysia

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The allelopathic effects of water extract and methanol crude extract from *Dioscorea hispida* Dennst. (*Ubi gadung*) tuber was tested on the seeds of selected crops in petri dishes. Experiments were conducted to determine the effects of water extract and methanol crude extract on the germination, radicle length and fresh weight of bioassay test species; namely maize (*Zea mays*), mustard (*Brassica* sp.), cucumber (*Cucumis* sp.), spinach (*Amaranthus* sp.) and radish (*Raphanus* sp.). The results showed that the water extracts gave significant effects on germination rate and radicle length of these bioassay species. The water extract strongly inhibited the germination and radicle length of mustard (*Brassica* sp.) at 12.5 g/L and 50.0 g/L concentrations. Besides, the concentration of water extract at 25.0 g/L inhibited the germination and radicle length of *Cucumis* sp. However, the concentration at 12.5 g/L stimulated the germination and radicle length of maize (*Zea mays)*. These results also showed that the methanol crude extracts gave significant effects on radicle length of bioassay species. It significantly inhibited the radicle length of all bioassay species; mustard (*Brassica* sp.), cucumber (*Cucumis* sp.), spinach (*Amaranthus* sp.) and radish (*Raphanus* sp.) at all concentrations.

Key words: *Dioscorea hispida*, water extract, methanol extract, bioassay species, inhibition, stimulation

Introduction

Allelopathy naturally exists and plays the important roles in the reasonable arrangements of crop cultivation systems, controlling weeds, preventing crop from diseases and insect infections, and also reducing the succession cultivation on crops (Regina *et al.*, 2007). It can be described as the negative or positive effects of chemicals that are released by one plant species

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on the growth of another plant species. Allelopathic characteristic can be defined as the biological properties of the allelochemicals, as opposed to its physical properties, in virtually all plant tissues, including leaves, flowers, fruits, stems, roots, rhizomes, seeds and pollens (Putman and Tang, 1986). This characteristic can interfere with the growth of other plants or microorganisms by allelochemicals that are released through volatilization, leaching and root exudation during growth, and decomposition of plant or root residues (Putman and Tang, 1986). Besides, they can also indirectly affect plants through the inhibition of microorganisms, including nitrogen-fixing and nitrifying bacteria (Ibrahim *et al.,* 1999). Each plant species can produce the toxicant that can enter other plants through diffusive process, but the amount of allelopathic production is different between plants (Khan *et al.,* 2008).

The development of weed management strategies that make use of allelopathic crop plants is receiving a great interest in national and international levels (Ko *et al.,* 2005). Recently, many researchers have focused on the exploration of plant allelopathy as there were many weed species that are allelopathic in nature. It is a viable weed management strategy but need to be studied under laboratory and field conditions. Allelochemicals that are derived from plants can be used directly in weed management or their chemistry could be used to develop new herbicides. These allelochemicals have good potential to be developed as they are environmentally safe (Khan *et al*., 2008).

Based on several studies, there are trees that have negative effects on seed germination and they can contribute to pesticide industry if the studies are fully explored (Weston, 1996). *Parthenium hysterophorus* L. had been reported to have inhibitory effects on many crops (Khan *et al*., 2005). With the increasing concentration of *Parthenium* extracts, they can decrease the seed germination and growth of *Eragrostis* sp. significantly (Narwal, 1994). This was associated with the release of parthenin through aqueous extraction of fresh leaf materials of *P. hysterophorus,* where it will provide significant phytotoxicity, and the relative role of parthenin to overall phytotoxic effects of the crude extracts are approximately 16 to 100% (Rice, 1964).

Besides, studies done in the effects of the husk extracts of seven rice varieties on growth of barnyard grass (*Echinochloa cruss-galli* L.) are also meaningful, where the inhibitory effects had been reported (Uremis *et al.,* 2005). There are also adverse effects of water extracts of different *Brassica* sp. against germination and growth of cutleaf ground cherry weed (*Physalis angulata* L.), that have also been reported (Darwin and Watson, 2002). *Prosopis juliflora* significantly inhibits the seed germination in Pearl millet (Mostafa *et al.,* 2008) while Ibrahim *et al*. (1999) reported the allelopathic effects of *Eucalyptus camaldulensis* on crops.

Dioscorea hispida is a climber plant with a bulky tubers, that are often lobed or compound, and these tubers are produced at the surface of soil. The tubers are large and can be very easily dug-up. It is widely distributed from India and southern China to New Guinea. It grows wild in the lowlands up to 50 m altitude, especially in secondary forests, village grooves, brushwoods, and occasionally cultivated in the garden. The whole plant of *D. hispida* is poisonous, and need to be washed-out with water prior to be eaten. The poison that had been identified is the alkaloid dioscorine, and the second being dioscoricine. The poisonous properties of *D. hispida* are useful. It can be used for poisoning and also for medicinal uses. Medicinally, it can be used for external application to treat sores of puru and chronic rheumatism (Xinxiang *et al.*, 2009).

This study aimed to determine the allelopathic effects of *D. hispida* tuber on the germination and growth of *Zea mays* L. (maize), *Brassica nigra* (L.) Koch (mustard), *Cucumis sativus* L. (cucumber), *Amaranthus* sp. (spinach) and *Raphanus sativus* L. (radish) seeds, by using water and methanol extracts.

Materials and methods

Plant Materials

Five kg of plant materials each of tubers of *Dioscorea hispida* were collected separately from Kampung Bari, Setiu; located at (N 05^{o} 32.778', E 102^{o} 51.025'), and 16 feet from sea level in Terengganu, Peninsular Malaysia. The plants were collected in October until December 2009 and December 2010.

Crop Seeds

The crop seeds for bioassay tests were purchased from Koperasi Peladang Terengganu. *Zea mays* (maize), *Brassica nigra* (mustard) and *Cucumis sativus* (cucumber) were used as bioassay test species using water extract. However for methanol extract tests; *Brassica nigra* (mustard), *Cucumis sativus* (cucumber), *Amaranthus* sp. (spinach) and *Raphanus sativus* (radish) were used.

Extraction

Approximately 500 g of *Dioscorea hispida* tuber was cut thinly into 2 cm to 4 cm wide slices before extraction. Plant materials were pre-soaked and blended in 2500 mL distilled water or methanol by using the laboratory blender (Model HGBTWT83; Warring Commercial, Torrington Connecticut, USA). Then, they were kept in several flasks and agitated for one hour on an orbital

shaker (120 r.p.m; Firstek Scientific Model S102, Hsin Chuang, Taiwan) at room temperature (28 ± 3 ^{o}C). The water filtrate was left standing overnight in order to observe for any development of precipitation.

The methanol and water extracts were separately-strained through one layer of plastic filter and further filtered by one layer of 0.2 µm nylon membrane filter (Whatman International Ltd., Maidstone, England) by using a filter pump. After filtration, the methanol filtrates were concentrated to dryness by a rotary evaporator at 45 °C until 21 g of total crude extract was obtained. The water extract and methanol crude extract were kept separately in a refrigerator at 4 °C until used (modified from Ismail and Chong, 2002).

Laboratory Bioassay

Ten seeds of each bioassay plant species were placed in separate petri dishes which had been lined with 9 cm Whatman No. 2 filter paper. Petri dishes were then placed on benches in laboratory room and arranged in Completely Randomized Design (CRD). Exactly 10 mL of *D. hispida* water or methanol extracts for the treatments, or distilled water for control, were used to wet the filter paper. The water extract was diluted and applied at three concentrations, which were 12.5 g/L, 25.0 g/L and 50.0 g/L. On the other hand, the methanol crude extract was used after dilution with distilled water at concentrations of 1.88 g/L, 3.75 g/L and 7.50 g/L. The percentages of germination, radicle lengths and fresh weights of bioassay plants were observed and recorded after seven days (modified from Ismail and Chong, 2002).

Results and discussion

Effects on Germination Rate

The water extract of tuber significantly suppressed the germination of *Cucumis* sp. and *Brassica* sp. by 3% and 20% of control, respectively, at 50.0 g/L concentration. However, the tuber extract at this concentration significantly stimulated germination of *Zea mays* by 20% of control. The largest percentages of germination were reduced at 25.0 g/L water extract of tuber for *Cucumis* sp. and *Brassica* sp. which were at 13% and 37% of control, respectively (Table 1).

However, the germination rate for *Brassica* sp. at that concentration (25.0 g/L) did not show significant difference from the control. In contrast, the germination rate for *Zea mays* was higher than the control, with the highest germination at 73% when treated with water extract of tuber at the concentration of 12.5 g/L (Figure 1). This was not expected since other concentrations for *Brassica* sp. showed significant differences from the control.

So, this difference was probably contributed by different allelochemical quantities presence in plant tissues. In addition, the allelopathic interaction depended on the chemical stability of bioactive compounds and the concentration of extract used in the experiment (Ismail and Kumar, 1996).

The methanol crude extract of tuber did not show significant effects on germination rate of test species. There were stimulatory effects at all concentrations tested for *Brassica* sp. but no effect was seen at 7.5 g/L concentration for *Cucumis* sp. and *Amaranthus* sp. (Figure 2). The highest percentage of germination was stimulated at 3.75 g/L methanol crude extract of tuber for *Brassica* sp. which was at 15% of control (Table 2).

Table 1. The effects of tuber water extracts on germination of bioassay plant species (% of control)

Concentration (g/L)	***Zea mays***	***Brassica* sp.**	***Cucumis* sp.**
12.5	27*[a]	7*[b]	0*
25.0	23*[a]	37[b]	13*[b]
50.0	20*[a]	20*[b]	3*[b]

Note: * denotes significant when 0 is outside 95% Confidence Interval or $\rho < \alpha$ (0.05)
[a] denotes stimulatory effect of *D. hispida*, [b] denotes inhibitory effect of *D. hispida*

Table 2. The effects of tuber methanol crude extracts on germination of bioassay plant species (% of control)

Concentration (g/L)	***Brassica* sp.**	***Cucumis* sp.**	***Amaranthus* sp.**	***Raphanus* sp.**
1.88	8[a]	2[b]	10[b]	7[b]
3.75	15[a]	3[a]	5[b]	2[a]
7.50	12[a]	0	0	2[a]

Note: * denotes significant when 0 is outside 95% Confidence Interval or $\rho < \alpha$ (0.05)
[a] denotes stimulatory effect of *D. hispida*, [b] denotes inhibitory effect of *D. hispida*

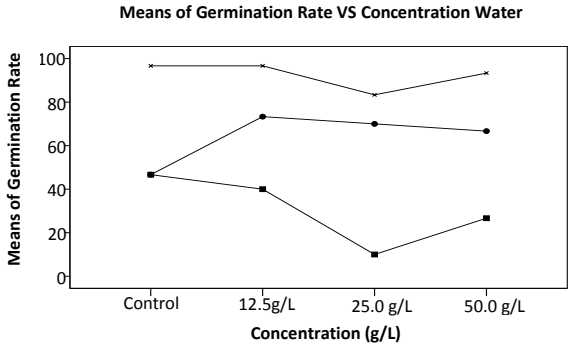

Fig. 1. The allelopathic effects of tuber water extracts on germination rate of bioassay species (●) *Zea mays*, (■) *Brassica* sp. and (**X**) *Cucumis* sp.

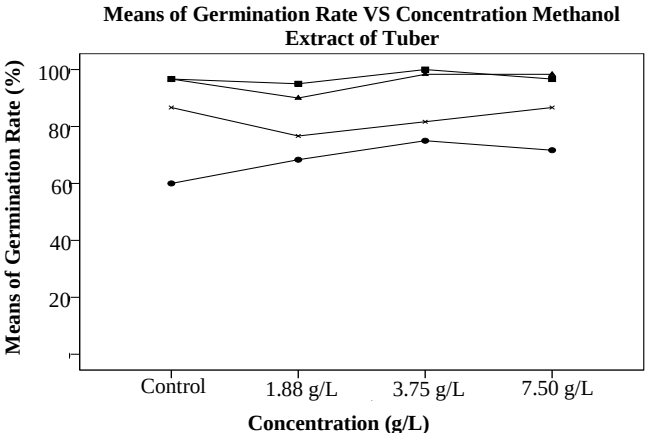

Fig. 2. The allelopathic effects of tuber methanol crude extracts on germination rate of bioassay species (●) *Brassica* sp., (■) *Cucumis* sp., (**X**) *Amaranthus* sp. and (▲) *Raphanus* sp.

Effects on Radicle Length

The water extract of tuber significantly reduced the radicle length of *Zea mays*, *Cucumis* sp. and *Brassica* sp. (Figure 3). The radicle length of *Zea mays* and *Brassica* sp. were most significantly reduced at 50.0 g/L concentration by 1.04 cm and 0.62 cm of control, whilst the radicle length for *Cucumis* sp. was most significantly reduced at concentration of 25.0 g/L by 1.88 cm of control. On the other hand, no significant reduction of radicle lengthwas observed in *Brassica* sp. at concentration of 25.0 g/L (Table 3).

All concentrations of methanol crude extract of tuber significantly reduced the radicle length of all test species. The highest reduction can be seen at 7.50 g/L concentration for all test species whilst the lowest radicle length reduction can be seen at 1.88 g/L (Figure 4). The highest amount of reduction in radicle length was observed in *Cucumis* sp. at 7.50 g/L concentration where the extract reduced the radicle length by 4.63 cm of control which was equivalent to 61% of control. Meanwhile, the concentration at 1.88 g/L contributed to the lowest amount of radicle length reduction of *Amaranthus* sp. by 0.56 cm of control which was equivalent to 50.4% of control (Table 4).

Table 3. The effects of tuber water extracts on radicle length of bioassay plant species (cm of control)

Concentration (g/L)	*Zea mays*	*Brassica* sp.	*Cucumis* sp.
12.5	0.70*[a]	0.06*[b]	0.81*[b]
25.0	0.20*[b]	1.84[b]	1.88*[b]
50.0	1.04*[b]	0.62*[b]	0.00*[a]

Note: * denotes significant when 0 is outside 95% Confidence Interval or ρ< α (0.05)
[a] denotes stimulatory effect of *D. hispida*, [b] denotes inhibitory effect of *D. hispida*

Table 4. The effects of tuber methanol crude extracts on radicle length of bioassay plant species (cm of control)

Concentration (g/L)	*Brassica* sp.	*Cucumis* sp.	*Amaranthus* sp.	*Raphanus* sp.
1.88	0.70*[b]	1.76*[b]	0.56*[b]	2.08*[b]
3.75	1.10*[b]	3.13*[b]	0.64*[b]	2.64*[b]
7.50	2.33*[b]	4.63*[b]	0.67*[b]	3.10*[b]

Note: * denotes significant when 0 is outside 95% Confidence Interval or ρ< α (0.05)
[a] denotes stimulatory effect of *D. hispida*, [b] denotes inhibitory effect of *D. hispida*

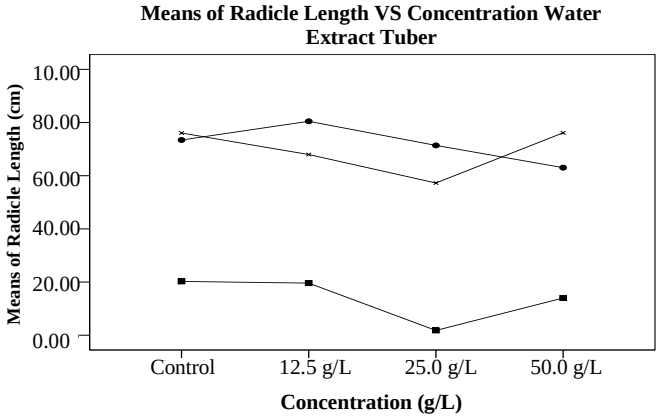

Fig. 3. The allelopathic effects of tuber water extracts on radicle length of bioassay species (●) Z*ea mays*, (■) B*rassica* sp. and (**X**) *Cucumis* sp.

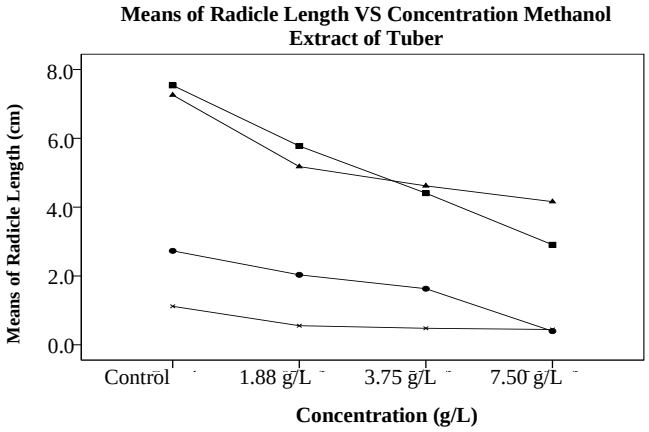

Fig. 4. The allelopathic effects of tuber methanol crude extracts on the radicle length of bioassay species (●) *Brassica* sp., (■) *Cucumis* sp., (**X**) *Amaranthus* sp. and (▲) *Raphanus* sp.

Effects on Fresh Weight

The water extract of tuber did not significantly reduce the fresh weights of all test species (Figure 5). At 50.0 g/L, the fresh weight of *Zea mays* and *Brassica* sp. were reduced by 0.109 g and 0.005 g of control, respectively. However, fresh weight for *Cucumis* sp. was mostly reduced at concentration of 25.0 g/L by 0.006 g of control (Table 5).

The tuber methanol crude extract has no significant effect on the fresh weight of test species. There was no effect for fresh weight of *Brassica* sp. at concentrations of 1.88 g/L and 3.75 g/L (Figure 6). However, the fresh weight of *Brassica* sp. was stimulated by 0.002 g of control which was equivalent to 50% of control at 7.50 g/L concentration. There was also no effect to *Amaranthus* sp. at concentrations of 3.75 g/L and 7.50 g/L but at concentration of 1.88 g/L, the fresh weight was stimulated by 0.001 g of control which was equivalent to 50% of control. The *Raphanus* sp. showed decrement in fresh weight at 3.75 g/L and 7.50 g/L concentrations by 0.002 g of control and 0.008 g of control (Table 6) which were equivalent to 2.82% and 11.27% of control, respectively.

Table 4. The allelopathic effects of tuber water extracts on fresh weight of bioassay plant species (% of control)

Concentration (g/L)	*Zea mays*	*Brassica* sp.	*Cucumis* sp.
12.5	0.037[b]	0.005[b]	0.002[a]
25.0	0.085[b]	0.008[b]	0.006[b]
50.0	0.109[b]	0.005[b]	0.024[a]

Note: * denotes significant when 0 is outside 95% Confidence Interval or $\rho < \alpha$ (0.05)
[a] denotes stimulatory effect of *D. hispida*, [b] denotes inhibitory effect of *D. hispida*

Table 5. The allelopathic effects of tuber methanol crude extracts on fresh weight of bioassay plant species (% of control)

Concentration (g/L)	*Brassica* sp.	*Cucumis* sp.	*Amaranthus* sp.	*Raphanus* sp.
1.88	0	0.001[b]	0.001[a]	0
3.75	0	0.003[a]	0	0.002[b]
7.50	0.002[a]	0.004[b]	0	0.008[b]

Note: * denotes significant when 0 is outside 95% Confidence Interval or $\rho < \alpha$ (0.05)
[a] denotes stimulatory effect of *D. hispida*, [b] denotes inhibitory effect of *D. hispida*

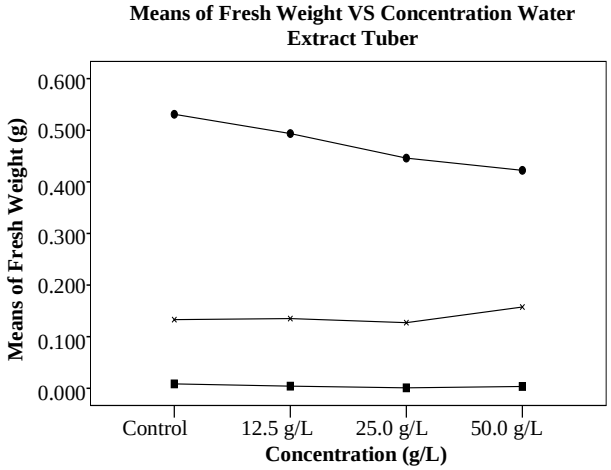

Fig. 5. The allelopathic effects of tuber water extracts on radicle length of bioassay species (●) *Zea mays*, (■) *Brassica* sp., and (**X**) *Cucumis* sp.

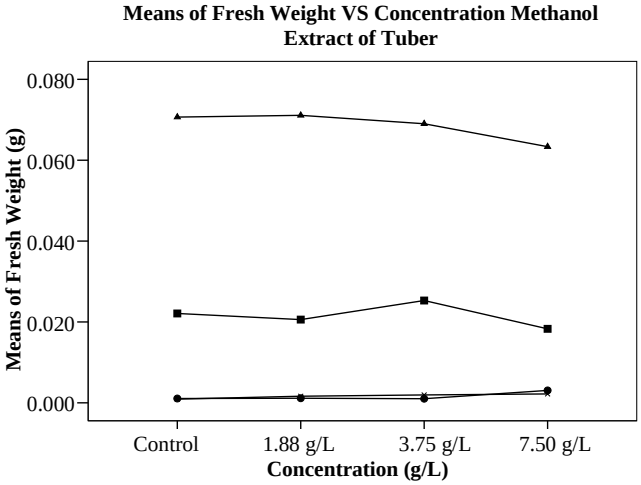

Fig. 6. The allelopathic effects of tuber methanol crude extracts on the fresh weight of bioassay species (●) *Brassica* sp., (■) *Cucumis* sp., (**X**) *Amaranthus* sp. and (▲) *Raphanus* sp.

In this study, the water extract of *Dioscorea hispida* tuber exerted different level of toxicity on bioassay test species. However, better explanation to ascertain these differences can be done by carrying out qualitative and quantitative measurements and analysis of allelochemicals which are present in fresh leaves and tubers of *D. hispida* (Ismail and Kumar, 1996). The interaction might be contributed to the synergistic effects of osmotic potential and allelochemicals in the extract (Ismail and Sugau, 1993).

Conclusion

The water extract of tuber has significant effects on germination rate and radicle length of bioassay test species but the methanol crude extract of tuber has significant effects on radicle length only. The germination and radicle length of *Zea mays* were stimulated by water extract at concentration of 12.5 g/L. However, at concentrations of 12.5 g/L and 50.0 g/L, the germination and radicle length of *Brassica* sp. were inhibited. The germination and radicle length of *Cucumis* sp. were inhibited by water extract at concentration of 25.0 g/L but at concentration of 50.0 g/L, the radicle length of *Cucumis* sp. was stimulated. On the other hand, methanol crude extract has significant effects on radicle length of all bioassay test species at all concentrations. The water extract and methanol crude extracts of *D. hispida* tuber have not significantly affected the fresh weight of all bioassay species.

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