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POTENTIAL OF NAPIER GRASS (*PENNISETUM PURPUREUM*) EXTRACTS AS A NATURAL HERBICIDE

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Abstract

The present study was undertaken to investigate the herbicidal potential of aqueous and methanolic extracts of culm plus leaves and root of *Pennisetum purpureum* against two selected weed species; *Hedyotis verticillata* and *Leptochloa chinensis* under laboratory and glasshouse conditions. Extracts in different solvents and plant tissues exhibited markedly variable herbicidal activities against the target weed species. Methanolic and aqueous extracts of culm plus leaves inhibited germination of *L. chinensis* by 50% at a concentration of as low as 0.07 and 0.47g/L, respectively. Radicle growth of *L. chinensis* was strongly suppressed by aqueous root extract. Methanolic extract of culm plus leaves were proven highly phytotoxic to *H. verticillata* where green colour of the leaf disc was reduced by 50% at a concentration less than 0.1g/L. Aqueous root extracts at 150g/L concentration strongly inhibited seedling growth *H. verticillata* but less inhibition was provided by methanolic root extracts at this concentration. The results of this study suggest that *P. purpureum* extracts can be used as natural herbicide for weed management.

Introduction

Nowadays there is much emphasis on search for new methods of weed control which are safe, harmless, less expensive and use crop produced material. Allelopathy has emerged as an important area of weed research and has been accepted very recently as important ecological phenomena. The viable of weed management strategies through allelopathy is receiving increased national and international attention and needs to be extensively studied under laboratory as well as in the field conditions. There are many weed species that are allelopathic in nature. Some potential candidates with strong allelopathic properties have been found out and have shown promising prospect for natural herbicides development (Batish *et al*., 2007b). Several phytotoxic substances causing germination and/or growth inhibitions have been isolated from plant tissues (Turk & Tawaha, 2003; Soyler *et al*., 2012). It has been reported that phytotoxicity assays is an important approach for identifying plants that are likely to be a source of herbicidal compounds of interest (Ma *et al*., 2011). Several studies conducted by Saeed *et al*., (2010) have demonstrated the phytotoxity of organic solvent such as: methanol, ethanol, hexane and dichloromethane can be used to extract these herbicidal compounds. In addition, Chon & Kim (2002) have documented that the phytotoxicity of various plant parts may vary in their allelopathic potential. It is found that allelochemicals that inhibit the growth of some species at certain concentrations might in fact stimulate the growth of the same or different species at different concentrations (Narwal, 1994).

Pennisetum purpureum also known as Napier grass is a perennial grass species found in tropical and subtropical areas throughout the world. Napier grass is considered a noxious weed in sugarcane production and an invasive weed to natural areas in south Florida (Anon., 2005). It contains a high amount of morphological variation within the species and noted as being the fastest-growing plant in the world (Mannetje & Jones 1992). According to Hanna *et al*., (2004), Napier Grass, however, is a major forage crop in the wet tropics of the world. Although this has been the primary use of napier grass, it has potential to produce greater dry-matter biomass yields than other *Pennisetum* grasses, which makes it a potential feedstock for cellulosic biofuel production (Hanna *et al*., 1999). Besides, Khan *et al*., (2006) has exploited the potential of napier grass varieties which provide an acceptable level of protection against stem borer, *C. partellus* in maize and sorghum in the 'push–pull' system. In Malaysia, Napier grass occurs widely along the roadsides, on wastelands and sometimes invades housing areas. The widespread occurrence of this weed may be attributed to its aggressive behaviour, very high seed production potential and suppressive effects on neighbouring plants through allelopathic interactions. It is suspected to release phytotoxins that inhibit the growth of the plant species nearby. Thus, the aim of this research is to get an understanding of herbicidal potential of napier grass (*Pennisetum purpureum*) extracts on 2 selected weed species of *Leptochloa chinensis* and *Hedyotis verticillata* under laboratory and glasshouse conditions.

Materials and Methods

Plant materials: Aboveground (culm plus leaves) and underground (root) tissues of *P. purpureum* were collected at a wasteland of Gong Badak, Kuala Terengganu. Plant materials were cleaned and cut into a length of 1cm, dried for 2 weeks under glasshouse conditions and stored at 4˚C prior to use.

Preparation of crude aqueous and methanolic extracts: The conical flasks containing plant materials of *P. purpureum* were filled with distilled water or methanol and agitated vigorously for 24 hours at 200 rpm at 25^{o}C on an orbital shaker (Lab Companion SK-300). The aqueous or methanolic extracts were filtered through two layers of cheesecloth and centrifuged (Hitachi himac CR

22GII) at 9,000 rpm (15,300 x g) for 15 minutes. The supernatants were filter-sterilized through 0.22µm membrane filter to ensure that the extracts were free from microorganisms. For methanolic extracts, the filtrate was evaporated by using a rotary evaporator at 40°C to yield crude residues and the resulting yields of methanolic crude extract were weighed and recorded. All extracts were stored at 4°C before use.

pH and simulated moisture stress medium preparation: The pH and osmotic potential of crude extracts were determined using a pH meter (WTW inoLab® pH 720) and osmometer (Wescor Vapro® 5520), respectively, before being applied on the bioassay species. The pH medium was prepared by MES (2-(N-morpholino) ethansulfonic acid) and HEPES (4-2-hydroxyethyl-1-piperazineethansulfonic acid) (Reddy & Singh, 1992) while the moisture stress was simulated with solutions of polyethylene glycol (PEG) 8000 (Mitchel, 1983).

Germination test: The seeds of *Hedyotis verticillata* (25 seeds) and *Leptochloa chinensis* (50 seeds) were placed separately in 9cm diameter Petri dishes lined with two layers of filter papers Whatman No. 1 and moistened with 5ml of pH solutions (pH 5 – pH 8), osmostic potential solution (-0.20 MPa) or filtered crude extracts at 5, 10, 20, 50 and 100 g/L. Petri dishes moistened with distilled water were treated as controls. The Petri dishes were kept in a growth chamber at 30/20°C with 12 hours photoperiod for 14 days. Seeds are considered germinated when attained a length of 1mm. At the end of the incubation period, the germinated seeds were recorded as a percentage of the total number of viable seeds used in each replication. The radicle length of germinated seeds were measured and recorded. The data were expressed as percentage of control.

Leaf disc test: Leaf discs with 5mm diameter of selected bioassay species were punched from fully developed leaves. Then, 5 leaf discs were dipped into each Petri dish containing methanolic or aqueous extracts of *P. purpureum* at a concentration of 50, 100 and 150 g/L in the growth chamber at 30/20°C with 12 hours photoperiod. Distilled water was applied to the controls. After 48 or 72 hours, the degree retention of green coloration (*a* value) of leaf disc was measured by using a Minolta chromameter (model CR-400X Minolta Camera Co. Ltd., Japan). The data were expressed as percentage of control.

Seedling growth test: Aqueous and methanolic extracts from both aboveground and underground tissues with concentrations at 50, 100 and 150 g/L were prepared. Homogenous seedlings from each bioassay species were selected and transplanted into 6 cm diameter cups with 100g of soil (pH 4.5; composition: sand 30%, silt 61% and clay 9%). Extracts were applied on the soil surface for 28 days under glasshouse conditions. Seedlings that applied with distilled water were treated as controls. The aboveground parts of the plant tissues were harvested. Fresh weight and shoot height of the seedlings were determined and the data were expressed as percentages of their respective controls.

Statistical analysis: Bioassays of each treatment were conducted in 5 replicates and arranged in completely randomized design. All the percentage data of germination and leaf disc tests were fitted to a logistic regression model, as follows (Kuk *et al*., 2002):

$$Y = d/ (1 + [x/x0] b)$$

where Y is percentage of germination/root length/green color retention, d is the coefficients corresponding to the upper asymptotes, b is the slope of the line, x0 is crude methanolic/aqueous extract concentration required to inhibit the germination/root length/ to reduce green color retention by 50% relative to untreated seeds/leaf discs, and x is the crude methanolic/aqueous extract concentration.

Results and Discussions

Effect of moisture stress and pH on germination of bioassay species: Water stress and pH may limits plant survival and early seedling growth by delaying its beginning or decreasing the final germinability (Kaydan & Yagmur, 2008). The effects of moisture stress at -0.20 MPa and pH at 5 to 8 were tested on bioassay species based on the osmostic potential and pH values of crude extracts, respectively. It is found that germination, shoot and root growth of the bioassay species were not affected by these environmental stresses, implying that moisture and pH of extracts do not play a key role for suppressing seed germination and growth of the bioassay species.

Effect of *P. purpureum* extracts on germination and radicle growth of bioassay species: The concentration of methanolic and aqueous extracts of *Pennisetum purpureum* culm plus leaves and root that gives 50% inhibition in germination (GR_{50}) of two bioassay species is presented in Table 1. *Leptochloa chinensis* was found to be very sensitive to culm plus leaves extracts because of low GR_{50} value ranging from 0.06–0.50 g/L. The inhibitory effect of methanolic culm plus leaves extracts was markedly stronger than that of aqueous culm plus leaves extracts on the seed germination of *L. chinensis*. In the case of root extract, *L. chinensis* was more tolerant and aqueous extracts recorded a greater allelophatic stress against germination as compared to that of methanolic extracts. Similarly, root aqueous extract had greater inhibitory effects on *Hedyotis verticillata* than aqueous extracts of aerial portions. On the other hand, roots of both biosassay species were more susceptible to root extracts than culm plus leaves extracts regardless of any solvent used (Table 2). However, the inhibition of radicle growth in the bioassay species was greater in aqueous extract as compared to methanolic extracts. Both *L. chinensis* and *H. verticillata* showed great sensitivity to aqueous root extract where concentration that gave 50% inhibition of radicle growth ranged from 3 to 14 g/L (Table 2).

Table 1. GR_{50} values of *Hedyotis verticilata* and *Leptochloa chinensis* in relation to crude extracts of *P. purpereum*.

Plant tissue	# GR_{50} (g/L)			
	Aqueous extracts		Methanolic extracts	
	H. verticillata	*L. chinensis*	*H. verticillata*	*L. chinensis*
Culm plus leaves	38.50 (7.53)	0.47 (0.01)	*	0.07 (0.01)
Root	24.14 (4.34)	24.28 (2.49)	*	50.26 (7.68)

GR_{50} is the crude extract concentration required to reduce germination by 50%. The values in parentheses are the standard error of the mean

* GR_{50} cannot be determined because the highest concentration tested has no or weak phytotoxic activity

Table 2. RL_{50} values of *Hedyotis verticilata* and *Leptochloa chinensis* in relation to crude extracts of *P. purpereum.*

Plant tissue	# RL_{50} (g/L)			
	Aqueous extracts		Methanolic extracts	
	H. verticillata	*L. chinensis*	*H. verticillata*	*L. chinensis*
Culm plus leaves	38.62(3.80)	20.61(1.00)	68.85(4.19)	*
Root	13.36(1.66)	3.94(0.64)	42.30(4.53)	23.45(4.08)

RL_{50} is the crude extract concentration required to reduce radicle growth by 50%. The values in parentheses are the standard error of the mean

* RL_{50} cannot be determined because the highest concentration tested has no or weak phytotoxic activity

The relative phytoxicity of plant tissue on seed germination and radicle growth vary with bioassay species and solvent used for extraction (Tables 1 & 2). Alagesaboopathi & Thamilazhagan (2010) reported that aqueous leaves and stem extracts of *Andrographis lineata* significantly decreased germination and radicle growth of balckgram (*Vigna mungo*) and greengram (*Vigna radiata*) greater when compared to root extracts. This may be due to the presence of more water soluble compounds in plants and the presence of more active substances in leaves and stem than root to affect the germination and radicle growth (Turk & Tawaha, 2003). However, Marwat *et al*., (2008) reported that Parthenium aqueous leaves extract application slightly affected the seed germination of several weed species such as *Cyperus rotundus, Echinochloa curus-galli* and *Xanthium strumariam* at the same or higher concentrations. Some plants such as millet, chickling pea, cotton and alfalfa, have more phytotoxic effects in root extracts than in leaf and stem extracts (Miri, 2011). Similarly, Okwulehie & Amazu (2004) demonstrated that the root aqueous extract of *C. odorata* had the most inhibitory effect on germination and radicle growth of cowpea and maize than leaves and stem extracts. In the present study, the root extract gives great reductions in the root elongation of both weed species as compared to those of culm plus leaves (Table 2). However, Ebana *et al*., (2001) reported that leaves and stem of aqueous extracts from rice plants showed greater inhibition on root growth of ducksalad than root extract. Root growth of rice Basmati Pak variety showed the most susceptible response to aqueous fresh sunflower leaf and stem extracts than root extracts at higher concentration of 15% (Bashir *et al*., 2011). These findings are also supported by earlier work of (Ashrafi *et al*., 2007), who investigated the effects of aqueous extracts concentration from various Barley plant parts on the radicle length of 7-d old wild barley seedlings. It is found that more inhibition was obtained at a higher extract concentration where the degree of phytoxicity of leaves and stem parts was stronger than root part.

Effect of *P. purpureum* extracts on leaf disc discoloration of bioassay species: The concentration of methanolic and aqueous extracts of aerial portions and roots that retains green color of leaf discs by 50% is shown in Table 3. It is observed that the phytotoxic effects of methanolic extracts of culm plus leaves and root were species dependent. *Hedyotis verticilata* was found to be more sensitive than *Leptochloa chinensis* when the leaf discs were subjected to the extracts. The methanolic culm plus leaves extract was more phytotoxic than the methanolic root extract where it diminished the green color of *H. verticillata* leaf disc by 50% at a concentration as low as 0.06 g/L while *L. chinensis* needed 69.56 g/L extract concentration to exhibit the same phytotoxic activity. It is surprise to note that aqueous extracts did not exhibit apparent reduction of green color of both bioassay species leaf discs irrespective of any plant tissues tested.

It is clear that culm plus leaves extracts appeared to give a higher inhibitory effect by reducing the green color of leaf discs as compared to that of root extracts (Table 3). These results are in agreement with previous findings documented by Reinhardt and Bezuidenhout (2001) where leaves appear to be the most consistent source of chemicals involved in phytotoxicity, while fewer and less potent toxins occur in roots. El-Khatib *et al*., (2004) reported that aquoues shoot extracts of *Chenopodium murale* was more severe in its reduction on the pigment content of all test species than root extracts. According to Reigosa *et al*., (2006), the decrease in chlorophyll pigments is a common response of plants to phytotoxin, and this might be a subsequent response of plant to these chemicals beside cellular damage. Einhellig and Ramussen (1993) stated that allelochemicals cause marked reduction in the chlorophyll content of the test plants through their effect on biosynthesis and denaturation of chlorophyll molecules.

Table 3. DS_{50} values of *Hedyotis verticilata* and *Leptochloa chinensis* in relation to crude extracts of *P. purpereum.*

Plant tissue	# DS_{50} (g/L)			
	Aqueous extracts		Methanolic extracts	
	H. verticillata	*L.chinensis*	*H. verticillata*	*L.chinensis*
Culm plus leaves	*	*	0.06(0.01)	69.56(8.00)
Root	*	*	0.16(0.01)	84.82(9.89)

DS_{50} is the crude extract concentration required to reduce green color retention of leaf disc by 50%. The values in parentheses are the standard error of the mean

* DS_{50} cannot be determined because the highest concentration tested has no or weak phytotoxic activity

Table 4. Effects of *P. purpureum* extracts on fresh weight of bioassay species.

Plant tissue	Extract concentration (g/L)	Aqueous extracts		Methanolic extracts	
		H. verticillata	*L. chinensis*	*H. verticillata*	*L. chinensis*
		Fresh weight (% of control)			
Culm plus leaves	50	149 ± 4 c	338 ± 15 a	121 ± 5 a	83 ± 5 b
	100	121 ± 5 b	455 ± 14 b	120 ± 4 a	66 ± 5 a
	150	95 ± 3 a	466 ± 9 b	104 ± 15 a	82 ± 5 b
Root	50	93 ± 7 c	103 ± 4 b	105 ± 4 b	116 ± 1 c
	100	73 ± 9 b	93 ± 3 a	100 ± 2 ab	110 ± 1 b
	150	39 ± 8 a	90 ± 1 a	97 ± 2 a	96 ± 2 a

Mean within the same column of each plant tissue followed by similar letter has no significant difference at $p<0.05$ as determined by Tukey test

Effects of *P. purpureum* extracts on fresh weight of bioassay species: The effects of methanolic and aqueous extracts on the fresh weight of bioassay species are shown in Table 4. Changes of seedling fresh weight varied with plant tissue extract, concentration and bioassay species. Fresh weight of *Hedyotis verticillata* was greatly reduced when concentration of aqueous root extract increased. Fresh weight of *H. verticillata* was decreased by 61% at 150 g/L concentration of aqueous root extracts but no inhibitory activity was exerted by methanolic root extracts at the same concentration. However, there was slight inhibition or stimulation on seedling growth of *Leptochloa chinensis* when being subjected to the aqueous or methanolic root extracts. It is interesting to note that sensitivity of *L. chinensis* to culm plus leaves extracts were solvent dependent. High stimulatory effect on seedling growth of *L. chinensis* was found when being treated with aqueous extracts. In contrast, growth of *L. chinensis* subjected to methanolic extracts was greatly inhibited. Surprisingly, growth of *H. verticillata* was stimulated and slight inhibited when being subjected to culm plus leaves extracts. These results, however, are not in accordance with Shahrokhi *et al*., (2011), who found that the aqueous leaf and stem extracts of pigweed was more allelopathic on wheat seedling growth than root extract at the highest concentration.

Roots of *L. chinensis* are very susceptible to aqueous root and culm plus leaves extracts in filter paper under laboratory conditions (Table 2). Surprisingly, the seedling fresh weight of *L. chinensis* was increased by approximately 470% in soil even after treated with aqueous culm plus leaves extracts at a concentration as high as 150 g/L. In contrast, susceptibility of *H. verticilata* to aqueous root extracts in the filter paper was also exhibited in the soil where the seedling fresh weight was reduced by 61% when subjected to the same extracts at 150 g/L (Table 4). These results imply that phytotoxic compounds of aqueous root and culm plus leaves extract from *Pennisetum purpureum* may have interacted with organic compounds or microbes in the soil, thereby resulting in stimulatory or inhibitory effects and this response varies with biosasay species. The results of present study are in accordance with findings of Javaid *et al*., (2010) who found that the effect of *Alstonia scholaris* (L.) R. Br. leaf extract on root length of *Parthenium hysterophorus* L. was evident where the low concentration of 0.4g/L extracts application greatly declined the root elongation. However, the phytotoxic effect of the leaf extract on seedling fresh weight of *P. hysterophorus* was highly reduced even at a high concentration of 500g/L in soil.

Effects of *P. purpureum* extracts on shoot height of bioassay species: The effects of methanolic and aqueous extracts on shoot height of two bioassay species are presented in Table 5. Shoot height of *Hedyotis verticillata* was slightly reduced when concentration of aqueous root extract increased. Shoot height of *H. verticillata* was decreased by 20% at 150 g/L concentration of aqueous root extracts but less inhibition was provided by methanolic root extracts at the same concentration (Table 5). Similarly, aqueous root extracts had less inhibitory effect on shoot growth of *Leptochloa chinensis* regardless of any extract concentration. Similar trend was also observed in methanolic root extracts except at a concentration of 50 g/L which gave stimulatory effect. It

is apparent that sensitivity of both bioassay species to culm plus leaves extracts was solvent dependent. *H. verticillata* and *L. chinensis* displayed slight inhibition or stimulation when being treated with methanolic culm plus leaves extracts. However, both bioassay species only registered stimulation when subjected to aqueous culm plus leaves extract, with *L. chinensis* being highly stimulated.

The results has shown that there was slight detectable impact on the shoot height of weed species when extract concentration increased (Table 5). In a new study conducted by Mehmood *et al*., (2011), it was shown that aqueous extracts of bark of *Syzygium cumini* at a concentration ranging from 50 to 200 g/L exhibited an erratic pattern of increase in shoot growth of *Parthenium hysterophorus*. These less herbicidal effects on shoot height are likely to emerge because of different response and sensitivity of allelochemicals on plant growth or influenced by mechanism (mode of action) of allelopathic activity. Caton *et al*., (1999) have documented that residues, exudates and leachates of many plant or weeds can affect the growth of the other plants with a wide range of injurious effect where the plant parts are not equally susceptible to allelochemical.

Table 5. Effects of *P. purpureum* extracts on shoot height of bioassay species.

Plant tissue	Extract concentration (g/L)	Aqueous extracts		Methanolic extracts	
		H. verticillata	*L. chinensis*	*H. verticillata*	*L. chinensis*
		Shoot height (% of control)			
Culm plus leaves	50	117 ± 7 a	170 ± 3 a	101 ± 2 b	90 ± 9 a
	100	118 ± 6 a	201 ± 2 b	94 ± 4 a	90 ± 1 a
	150	111 ± 5 a	205 ± 3 b	93 ± 0 a	105 ± 7 b
Root	50	109 ± 10 b	96 ± 1 a	111 ± 2 c	108 ± 3 b
	100	91 ± 4 a	95 ± 4 a	103 ± 4 b	98 ± 4 a
	150	80 ± 2 a	95 ± 3 a	95 ± 3 a	95 ± 3 a

Mean within the same column of each plant tissue followed by similar letter has no significant difference at $p<0.05$ as determined by Tukey test

Conclusions

Based on the results of this study, it can be concluded that the culm plus leaves extracts of *P. purpureum* posses greater herbicidal activity than the root extracts. The varying degree of inhibition on germination and radicle growth and reduction in green color retention of leaf disc highlights its selective herbicidal activity in *H. verticillata* and *L. chinensis*. On the other hand, culm plus leaves extracts had more allelopathic effect (either negative or positive) than did the root extracts on the seedling growth of bioassay species. *P. purpureum* is plant with proven herbicidal potential, which requires more studies related to the effects of their allelochemicals to other weed plants. Further study on isolation and identification of allelochemicals or compounds from culm plus leaves extracts could provide means to maximize their inhibitory effects for the development of natural herbicides.

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