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Full Length Research Paper

Herbicidal activity of *Pennisetum purpureum* (Napier grass)

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This study investigates potential herbicidal effects of *Pennisetum purpureum* extracts on two selected weed bioassay species. Ethyl acetate extract of *P. purpureum* was able to suppress germination of bioassay species by 41%. Although, it had no phytotoxic activity on the root or shoot growth of bioassay species, it had caused leaf discs of bioassay species to turn dark brown. Chromatographic separation of ethyl acetate extract yielded nine fractions. The highest suppressive ability was exhibited by fraction five where the shoot and root growth of bioassay species was inhibited markedly, while the colour of leaf discs changed from green to dark brown. The germination of the bioassay species is particularly sensitive to the fraction five, exhibiting approximately 20% reduction in germination, relative to the control. These results imply that *P. purpureum* contains phytotoxic compounds that can be developed as natural herbicide.

Key words: *Pennisetum purpureum* extracts, allelochemicals, phytotoxic, bioassay.

INTRODUCTION

Weeds are enemies to crop plants and have harmful effects on agricultural crops due to several factors such as competition for space, light and nutrients. Organic chemicals released as leaf leachates, affect crop plants. Weeds species are considered as rich source of secondary metabolites (allelochemicals). These chemicals improve a certain kind of environmental system on other plants growing in their vicinity and this phenomenon is known as allelopathy (Nandal et al., 1994). More recently, Weston (2005) defined allelopathy as an important mechanism of plant interference mediated by the addition of plant secondary products to the soil rhizosphere. These secondary metabolites are located throughout the plant and are present in various plant tissues such as stems, leaves, roots and others. In agriculture, the inhibitory effect of weed species on germination and growth of crops has been attributed to phytotoxic chemicals released from the leaf litter and roots. Romero-Romero et al. (2005) reported that the effect of such compounds is harmful to plant growth and development and it may become a biotic or allelochemical stress.

Pennisetum purpureum (napier grass), a native grass species in the tropical grasslands of Africa (Khan and Pickett, 2004), is spreading rapidly in many parts of the world. According to Hanna et al. (2004), napier grass 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 also exploited the potential of napier grass varieties which provide an acceptable level of protection against stem borer, *Chilo partellus* in maize and sorghum in the 'push–pull' system.

In Malaysia, it occurs widely along the roadsides, on wastelands and sometimes invades housing areas. The widespread occurrence of this weed may be attributed to

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its aggressive behaviour, very high seed production potential and suppressive effects on neighbouring plants through allelopathic interactions. Fong (2007) has demonstrated that aboveground tissues of *P. purpereum* debris at a rate as low as 5 g/kg soil can reduce fresh weight of *Hedyotis verticillata*, *Amaranthus caudatus*, *Zea mays* and *Eleusine indica* seedlings by 11, 31, 37 and 54%, respectively under glasshouse conditions. However, limited information is available on phytotoxic compounds of *P. purpureum.* Hence, the present study was conducted to assess the herbicidal activity of *P. purpureum* extracts through isolation of phytotoxic compounds and to examine their phytotoxicity on two selected weed species.

MATERIALS AND METHODS

Aboveground tissues of *P. purpureum* were collected at a wasteland of Gong Badak, Kuala Terengganu. Seeds of two bioassay species, *Leptochloa chinensis* and *Hedyotis verticillata* were collected from rice fields of Pasir Mas, Kelantan, and oil palm plantations of Setiu, Kuala Terengganu, respectively. All the bioassay species were propagated in a glasshouse.

Crude extract preparation

Aboveground tissues of *P. purpureum* were cleaned and cut into a length of 1 cm, dried for 2 weeks under glasshouse conditions. These dried samples were ground to fine powders and underwent successive extraction with hexane, followed by ethyl acetate, methanol and water (Das et al., 2010) by soaking and shaking the plant materials vigorously at 200 rpm for 72 h on orbital shaker at 25°C, then filtered through two layers of cheesecloth and Whatman No.1 filter paper. The filtrate was evaporated by using a rotary evaporator at 40°C to yield crude extract. The resulting yields of each crude extract were weighed, recorded and stored at 4°C until use. The original crude of hexane, ethyl acetate, methanol and water extracts were bioassayed using *L. chinensis* and *H. verticillata* as test plants. Comparison of bioassay results revealed that the greatest phytotoxicity was from ethyl acetate extract.

Isolation of phytotoxic compounds

The ethyl acetate extract was subsequently directed to column chromatographic in a regular Merck silica gel (0.063 to 0.200 mm) by using column (50 × 3cm) and eluted with a gradient of chloroform and acetone (0→100% acetone). A total of 65 fractions were assembled according to their thin-layer chromatography (TLC) profiles which was visualized by anesaldehyde spraying reagent and UV light to yield nine fractions. These fractions were further evaporated to dryness at 40°C and diluted to 500 ppm with chloroform. The phytotoxicity of each fraction was examined through germination test and leaf disc test as described below.

pH and simulated moisture stress medium preparation

The pH and osmotic potential of the extracts was determined with pH meter and osmometer, respectively. The pH medium was prepared by MES (2-(N-morpholino) ethansulfonic acid) and HEPES (4-2-hydroxyethyl-1-piperazineethansulfonic acid) (Reddy and Singh, 1992), while the moisture stress was simulated with solutions of polyethylene glycol (PEG) 8000 (Mitchel, 1983).

Germination test

A total of 25 seeds of *L. chinensis* were placed separately in 9-cm diameter Petri dishes lined with two layers of filter papers Whatman No. 1 and moistened with 5 ml of pH solutions (pH 5 - 7), osmotic potential of -0.20 MPa, filtered crude extracts or column fractions at 500 ppm. 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 h photoperiod for 14 days. Seeds are considered to have germinated when they attained a length of 1 mm. 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 and shoot length of germinated seeds were measured and recorded. The data were expressed as percentage of the control.

Leaf disc test

A total of 5 leaf discs with 5 mm diameter of *H. verticillata* were punched from fully developed leaves. Then, five leaf discs were dipped into each Petri dish containing water, methanol, ethyl acetate, hexane or column fractions extract of *P. purpureum* at a concentration of 500 ppm in a growth chamber at 30/20°C with 12 h photoperiod. Distilled water was applied to the controls. After 7 days, green colour retention of leaf disc was scored, recorded and captured using a digital camera. The visual assessment on colour changes of leaf disc was determined based on a modified method of Itoh et al. (1990) (Table 1).

Statistical analysis

Each experiment was arranged as a completely randomized design (CRD) with three replications. The data were subjected to one-way analysis of variance (ANOVA). Tukey HSD was used to compare the mean among the treatments. Differences are regarded as significant when the p-values are less than 0.05 ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of moisture stress and pH on germination of bioassay species

Water stress and pH may limit plant survival and early seedling growth by delaying its onset or decreasing the final germinability (Gamze, 2005; Kaydan and Yagmur, 2008; Silva et al., 2001). The effects of moisture stress at -0.20 MPa and pH 5 to 7 were tested on bioassay species based on the osmotic potential and pH values of crude extracts, respectively. It was 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 in suppressing seed germination and growth of the bioassay species.

Effect of *P. purpureum* extracts on germination and leaf disc of bioassay species

Ethyl acetate extract of *P. purpureum* was highly toxic to

Table 1. The visual assessment on colour changes of leaf disc.

Score/color	Leaf disc appearance	Phytotoxic Effect
Green	The leaf disc surface is completely green	No
Yellow greenish	60 to 90% of the leaf disc surface is green in color	Weak
Green yellowish	10 to 30% of the leaf disc surface is green in color	Moderate
Dark brown	More than 90% of the leaf disc surface, in aggregate, is dark brown	Strong

Table 2. Color changes of *H. verticillata* leaf disc 7 days after treatment.

Score	Color of leaf disc	Type of crude extract	Phytotoxic effect
1	Green	Control	No
2	Yellow greenish	Water, hexane	Weak
3	Green yellowish	Methanol	Moderate
4	Dark brown	Ethyl acetate	Strong

Table 3. Phytotoxic activity of *P. purpureum* crude extract on germination, root and shoot growth of *L. chinensis* 14 days after treatment.

(% of control)	Types of crude extract			
	Water	Methanol	Hexane	Ethyl acetate
Germination	22±7^{a}	52±7^{b}	37±8^{ab}	41±4^{b}
Root length	105±20^{a}	120±16^{a}	118±23^{a}	153±9^{a}
Shoot length	93±17^{a}	87±12^{a}	93±17^{a}	100±13^{a}

Mean within the same row followed by similar letter has no significant difference at $P<0.05$ as determined by Tukey test.

the bioassay species, *H. verticillata*, where the colour of leaf discs turned dark brown (Table 2). By contrast, water, methanol or hexane extracts had weak or moderate phytotoxic effect on the leaf discs of *H. verticillata*. It is interesting to note that the tested crude extract was found to have strong to moderate inhibitory effects on the seed germination of *L. chinensis*. However, the ethyl acetate extract had less inhibition on the germination of *L. chinensis* as compared to the water extract. On the other hand, the ethyl acetate extract was comparable to water, methanol or hexane extract in stimulating root growth but it had no phytotoxic effect on shoot growth of *L. chinensis* (Table 3).

The selection of extracts is very important because it directly affects the feasibility of the operational process and the quality and quantity of the products, therefore extraction selection must be taken into account in the study of allelochemicals' activity. Kato-Noguchi (2000, 2001) documented that different phytotoxic potentials were observed when n-hexane, acetone and water were used for the extracts of *Evolvulus alsinoides* and *Melissa officinalis*, with water extraction having the strongest phytotoxic activity. Though water is the 'solvent of extraction in nature' and has been shown to remove more compounds from cover crops than methanol, there remains a broad spectrum of chemicals in plant residues that could exhibit phytotoxic properties. Hence, solvents such as ethyl acetate, ethyl ether, hexane and methanol are commonly used to perform plant extractions aimed at examining phytotoxic potential or isolating the liable phytotoxic compounds (Beninger and Hall, 2005)

There is evidence that in some cases, alternative solvent extracts are more potent than those in which water is the solvent (Chon et al., 2005). For example, ethyl acetate extracts of buckwheat (*Fagopyrum esculentum* Moench) have been shown to have more activity than their aqueous extracts (Iqbal et al., 2003). According to Abd El-Moaty and Balah (2009), ethyl acetate crude extract of *Taverniera lappacea* showed the most phytotoxic activity in inhibiting germination and seedling growth of *Convolvulus arvensis, Abutilon thiophrastes* and *Echinochloa crus-galli,* as compared to that caused by methanol and acetone extracts. In laboratory studies, ethyl acetate extract of the aerial part of common buckwheat had the maximum activity in suppressing the root and shoot growth of lettuce seedlings, and plants grown in the presence of the ethyl acetate extract exhibited severe root browning (Zahida et al., 2003). This is also consistent with other works in which ethyl acetate has been used to extract phytotoxins from plant residues

Table 4. Phytotoxic activity on germination, root and shoot growth of *L. chinensis* 14 days after treatment.

(% of control)	Fraction of ethyl acetate extracts of *P. purpureum*								
	1	2	3	4	5	6	7	8	9
Germination	98±3^{ab}	101±7^{ab}	102±8^{ab}	108±16^{b}	78±6^{a}	105±3^{b}	101±18^{ab}	99±5^{ab}	90±4^{ab}
Root length	92±3^{cd}	85±17^{bc}	64±4^{b}	166±11^{f}	38±6^{a}	82±6^{bc}	71±6^{bc}	114±11de	119±10^{e}
Shoot length	90±9^{cd}	85±15^{b}	77±10^{ab}	125±5^{c}	77±5^{ab}	84±16^{b}	57±5^{a}	88±8^{b}	97±16^{cd}

Mean within the same row followed by similar letter has no significant difference at P<0.05 as determined by Tukey test.

Table 5. Color changes of *H. verticillata* leaf disc 7 days after treatment.

Score	Color of leaf disc	Fraction	Phytotoxic Effect
1	Green	Control and 4	No
2	Yellow greenish	1, 2, 7 and 9	Weak
3	Green yellowish	3, 6 and 8	Moderate
4	Dark brown	5	Strong

(Neves and Gaspar, 1990). Similarly, based on the present findings, it is inferred that ethyl acetate extract of *P. purpureum* has more potent herbicidal activity as compared to other extracts.

Effect of isolated fractions of *P. purpureum* ethyl acetate extract on germination and leaf disc of bioassay species

Table 4 shows the effect of the isolated fractions (1 to 9) on germination, root and shoot growth of *L. chinensis*. The results show that not all of the isolated compounds inhibited the growth of *L. chinensis*. However, it is apparent that shoot growth of the bioassay species was more sensitive than the root growth when subjected to the respective fractions. The outcome of the bioassay employed is not in line with the previous investigation (An et al., 1997), in that the root growth bioassay in most fractions is more sensitive than the shoot growth. Nevertheless, it is observed that fraction five gave the highest inhibitory effect on the root, while it exhibited moderate herbicidal activity on germination and shoot growth. In comparison, other fractions exhibited lesser inhibition or stimulation on germination, root and shoot growth of the bioassay species.

Inhibition of seed germination and abnormalities of seedling development are common observed actions of allelochemicals. Catabolism of storage reserves and energy production are most important factors ensuring undisturbed seed germination. According to Bogatek et al. (2005), the phytotoxic effect of sunflower allelochemicals on seed germination appears to be mediated through a disruption of cellular metabolism rather than through organelle damage. Reserve mobilization seems to be blocked or delayed during allelopatic stress. Disorder in respiration rate (O_2 uptake) leads to a limitation on the availability of metabolic energy (ATP level) and in consequence may decrease seed germination and seedling growth of the bioassay species in the present study. Fraction five was also biologically active in degradation of leaf pigment of *H. verticilata* where the color of leaf discs changed from green to dark brown. In comparison, other fractions had either no effect, weak or moderate phytotoxic effects on leaf discs (Table 5). This finding indicates that *P. purpureum* has allelochemicals that affect the photosynthesis and plant growth by destroying the chlorophyll. Likewise, a previous study by Ervin and Wetzel (2000) showed that aqueous extracts of above-ground *Juncus dffusus* tissues retarded the *Eleocharis obtuso* seedlings by decreasing the concentration of chlorophyll. On the other hand, it has been demonstrated that the essential oil of cinnamon injures dandelion leaf discs by increasing membrane permeability of the leaf discs (Thomas, 2002).

It is found that inhibitory effect of the ethyl acetate crude extract (41%) was more potent as compared to fraction five (78%) on germination of *L. chinensis*. The lower value of seed germination in ethyl acetate extract suggests that a combination of all isolated fractions (1 to 9) may act synergistically to give a higher inhibitory effect on germination of the bioassay species. On the other hand, fraction five exhibited moderate inhibitory effect on shoot growth as compared to ethyl acetate extract which did not have any herbicidal activity. It may be due to the presence of both growth stimulators and growth inhibitors in a balance ratio which has caused no inhibitory effect on shoot growth in ethyl acetate extract. Ethyl acetate crude extract has displayed stimulatory effect on root growth. However, after chromatographic resolution, a strong inhibitory activity on root growth of the bioassay species was found in fraction five. The root inhibitory

activity of fraction five might be masked by the presence of strong root stimulators exerted by fractions 4, 8 and 9 when these fractions were combined with ethyl acetate extract.

In conclusion, the results presented in this paper have demonstrated that *P. purpureum* contain phytotoxic compounds that inhibit or stimulate germination and growth of *L. chinensis* besides reducing the green colour of *H. verticillata* leaf discs at different degree, thereby showing a selective herbicidal activity. The ethyl acetate extract exhibits the most phytotoxic effect on leaf disc of bioassay species. Therefore, the ethyl acetate extract has been selected for further chromatographic separation. We found that fraction five has strong phytotoxic activity on root growth while having moderate phytotoxic effect on germination and shoot growth. Further study is being conducted to isolate potential phytotoxic compounds of fraction five, followed by identification of the phytotoxic substances through gas chromatography-mass spectrometer (GC-MS). The identification of these substances might provide chemical basis for the development of natural herbicides for environmentally friendly sustainable agricultural system.

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