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Assessment of the Potential Allelopathic Effects of *Pennisetum purpureum* Schumach. on the Germination and Growth of *Eleusine indica* (L.) Gaertn.

(Penilaian Potensi Kesan Alelopati *Pennisetum purpureum* Schumach. ke atas Percambahan dan Pertumbuhan *Eleusine indica* (L.) Gaertn.)

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ABSTRACT

Pennisetum purpureum *Schumach. is a weed that is currently spreading rapidly to many parts of the world particularly tropical countries. The abundance of* P. purpureum *in Malaysia is presently a serious problem. A study was conducted to investigate and evaluate the potential allelopathic effects of* P. purpureum *on* Eleusine indica *L. Gaertn. using the aqueous leaf extract and plant debris incorporated into the soil. Low concentrations of the* P. purpureum *aqueous extract (2%) and debris incorporated into the soil (25/500 g) inhibited germination and seedling growth of the bioassay species (*E. indica*) by >80%. The responses of the bioassay species to the aqueous extract and debris-incorporated soil were concentration dependent. The aqueous extract had higher total phenolic content compared to that from the debris incorporated soil, indicating the presence of certain phytotoxic compounds in the leaf debris and leaf extracts.*

Keywords: Allelopathy; Eleusine indica*;* Pennisetum purpureum*; phenolics*

ABSTRAK

Pennisetum purpureum *Schumach. adalah rumpai yang sedang merebak dengan cepat ke kebanyakan bahagian dunia terutamanya negara-negara tropika. Lambakan* P. purpureum *di Malaysia kini telah menjadi masalah yang serius. Satu kajian telah dijalankan untuk mengkaji dan menilai potensi kesan alelopati* P. purpureum *terhadap* Eleusine indica *L. Gaertn. dengan menggunakan ekstrak akues dan sarap yang digaul ke dalam tanah. Kepekatan akues ekstrak yang rendah (2%) dan sarap* P. purpureum *dalam tanah (25/500 g) didapati merencat percambahan dan pertumbuhan anak benih spesies bioasai (*E. indica*) pada >80%. Respon spesies bioasai untuk ekstrak akueus dan sarap yang digaul dalam tanah adalah bergantung kepada kepekatan. Ekstrak akues menunjukkan jumlah kandungan fenolik yang lebih tinggi berbanding dengan sarap yang digaul dalam tanah dan ini menunjukkan kehadiran sebatian fitotoksik tertentu dalam sarap dan ekstrak daun.*

Kata kunci: Alelopati; Eleusine indica*; fenolik;* Pennisetum purpureum

INTRODUCTION

Weeds not only compete for moisture, nutrients and light but can also affect crop growth through the exudation of allelochemicals into the surrounding habitat (Kadioglue et al. 2005; Rice 1984). The parts of a plant such as the leaf, stem, root and fruit, possibly have the potential to cause allelopathic actions (Mahmood et al. 1999). Recent studies have indicated that exotic invasive plants might possess allelopathic chemicals which are possibly the main cause of their phytotoxic effects on the native and non-adapted plants (Callaway & Aschehong 2000; Tantiado & Saylo 2012). Scientists have named this phenomenon as the 'Novel Weapon Hypothesis', an approach for these invasive species to spread and grow fast (Callaway & Ridenour 2004), by exerting allelopathic effects on crops and other weed seed germination and growth, by releasing water-soluble compounds into the soil (Batish et al. 2007).

Pennisetum purpureum Schumach. is a common weed in Malaysia. It is a fast-growing, perennial weed belonging to the family Poaceae and is widespread throughout Australia and the tropical mainland of Africa and Asia. This plant has become a dominant species in many regions of the world where it is present in several plantations. In Malaysia, the amount spent on herbicides to combat this species and other noxious weed species range from RM220-230 million/year, amounting to 76-79% of the total expenditure on pesticides (Baki 2004). However, excessive and persistent use of herbicides can cause various problems such as environmental pollution, public health hazards (Qasem 2011) and an increase in the number of herbicide-resistant weed species e.g. the persistent use of glufosinate and paraquat have resulted in the development of multiple resistance in a biotype of goosegrass (*Eleusine indica* (L.) Gaertn.) in Malaysia (Chuah et al. 2010).

Allelopathic effects of different crops on weeds have been reported but report on the allelopathic effects of *P. purpureum* on weeds is limited. Recent studies by Norhafizah et al. (2012) have shown that two bioassay species namely *Hedyotis verticillata* and *Leptochloa chinensis* showed approximately 20% reduction in germination. However, information on the effects of allelopathy under glasshouse conditions is lacking. Therefore, the present study were conducted in order to determine the effects of *Pennisetum* leaf extracts and debris-incorporated soil on *E. indica* germination and seedling growth, and to obtain a quantitative estimate of the water-soluble phenolic compounds in the *P. purpureum* debris-incorporated soil and leaf extracts.

MATERIALS AND METHODS

COLLECTION OF *P. PURPUREUM* PLANTS

The above-ground tissues of *P. purpureum* were collected from a greenhouse wasteland area of the Universiti Kebangsaan Malaysia, Bangi, Selangor. Mature plants were uprooted and after air drying for 4 days, the leaves and stems were separated and cut into small pieces with a pair of scissors. Seeds of the bioassay species, *E. indica,* were collected from the same area. In order to breakdown seed dormancy of the weed seeds of the test species (*E. indica*), the *E. indica* seeds were soaked in 0.1% KNO_3 solution for 72 h. According to Gholinejad et al. (2012), soaking in 0.1% KNO_3 was the best treatment for breaking the dormancy as well as the best treatment for improvement of the seed germination properties.

EFFECTS OF THE WATER-SOLUBLE LEAF EXTRACTS OF *P. PURPURE*UM ON SEEDLING GROWTH OF *E. INDICA*

Dried *P. purpureum* leaves were ground into a fine powder using a commercial grinder so that it could pass through a 1 mm sieve. It was then stored in a refrigerator at 5°C prior to use. Six aqueous extracts of *P. purpureum* were obtained using a modified method of Ismail and Chong (2009). The aqueous extract was prepared by immersing 10 g of ground *P. purpureum* leaves in 100 mL of distilled water, followed by shaking for 24 h on an orbital shaker (150 rpm; Firstek Scientific Model S102; Hsin Chuang, Taiwan, ROC) at room temperature (28±3°C). The extract was then filtered through Whatman no. 2 filter paper (Whatman International Ltd., Maidstone, England) to remove solid materials. Distilled water was used to establish varying dilutions from the original leaf extract, equivalent to 0, 2, 4, 6, 8 and 10 g/mL^{-1}. Fifty non-dormant seeds of *E. indica* were placed in Petri dishes lined with filter paper (no.1; Whatman International, Maidstone, UK) and saturated with 10 mL of the water-soluble extracts of each of the different concentrations. The Petri dishes were kept in an incubator at 30°C for 24 h under 12 h/12 h light/dark photoperiod daily. The experiment was carried out using four replications. After 7 days, seed germination, seedling root/shoot length and fresh/dry weight were measured and recorded.

ALLELOPATHIC EFFECTS OF *P. PURPUREUM* DEBRIS-INCORPORATED SOIL ON SEEDLING GROWTH

Five concentrations of the leaf and stem debris (0, 5, 25 and 50 g dry weight per 500 g soil) were mixed thoroughly with soil and allowed to decompose naturally in bags. Control bags were filled with soil only (without debris) and kept moist by daily watering (watering was stopped when the water started to leach from the holes at the bottom of the bag). Fifty seeds of *E. indica* were placed in each bag and kept under greenhouse conditions (temperature 25±8°C, light intensity: 1783±571 μEm^{-2} s^{-1}, relative humidity: 48%). The bags were watered daily with an appropriate amount of water to maintain the soil moisture at 100% field capacity. The estimation of soil moisture was determined using the soil moisture meter which was calibrated prior to the experiment. A completely randomized design was maintained throughout the experimental period. Four replicates were used for each experimental treatment and the seedlings were maintained for 30 days. The root and shoot lengths as well as the fresh and dry weights were recorded at the end of 30 days.

ESTIMATION OF PHENOLICS

The total water-soluble phenolic compounds (TPC) in the water-soluble leaf extract and debris-incorporated soil were determined as described by Ismail and Chong (2009) and Swain and Hill (1959) using the Folin-Ciocalteau reagent with minor modifications. The amount of phenolic compounds was determined spectrophotometrically at 750 nm with gallic acid as the standard. For the assay, an aliquot of 1 mL from the water-soluble leaf extract and debris-incorporated soil (both only tested at the highest concentration viz: 10 g/L and 50 g/500 g soil, respectively) was placed into separate test tubes and 5 mL Na_2C0_3 (2% in 0.1 N NaOH) was added to each test tube and left for 5 min. Five mL Folin-Ciocalteu reagent was added, followed by mixing. The absorbance was determined from the reading of the spectrophotometer (Hitachi U- 2000) set at 760 nm, after 2 h. A standard curve was prepared in a similar manner using a series of gallic acid solutions in water. The concentration of phenolic compounds in the water-soluble leaf extract and debris-incorporated soil extract was estimated (gallic acid equivalent) based on the standard curve.

STATISTICAL ANALYSIS

All the statistical analyses were carried out using the SPSS version 20.0 (SPSS IBM, Chicago, IL, USA). The experimental data were subjected to the analysis of variance. The means of fresh/dry weight and root/shoot length of the test species were compared to the control using the LSD test at the 5% level of significance.

Results and Discussion

EFFECTS OF WATER-SOLUBLE LEAF EXTRACTS OF *P. PURPUREUM* ON THE SEEDLING GROWTH OF *E. INDICA*

In general, the *P. purpureum* leaf extracts showed inhibitory effects on the germination, root and shoot length as well as the fresh and dry weight of the test plants compared to that of the control seedlings. Data presented in Table 1 shows that all the test plants were significantly inhibited by the different concentrations of the aqueous extracts (ranging at 2-10%) and this result was unexpected and contrary to those reported by Adler and Chase (2007) where the aqueous foliar extracts of cowpea (*Vigna unguiculata* cv. Iron Clay) had no phytotoxic effect on the *E. indica* seed germination at the lowest concentration (5% aqueous extract of cowpea) 14 days after DAT treatment. The germination percentage of *E. indica* at the treatment with 5% aqueous extract of cowpea was similar to that of the control. Hence, this may reflect the possibility that the *P. purpureum* leaf extracts contained water-soluble inhibitors which could totally inhibit the germination of *E. indica* (Table 1). Another example is caffeic acid that was reported to totally inhibit the germination and growth of goosegrass (*Eleusine coracana* var. *coracana*) and *Dactylis hispanica* (Monica et al. 2012). The 100% inhibition (germination, root length, shoot length, fresh and dry weight) by *P. purpureum* aqueous leaf extracts on goosegrass in the filter paper assay at concentrations ranging from 2 to 5% showed that water-soluble allelochemicals might be present. This suggests that root and shoot growth of the test species is very sensitive to the phytotoxic chemicals in the *P. purpureum* leaf aqueous extracts. The root and shoot growth might have been affected because both were in direct contact with the extract (Meksawat & Pornprom 2010). The results of the study also support the finding of Tanveer et al. (2010) where the same results were obtained with different weed species - water-soluble leaf extracts of *Euphorbia helioscopia* reduced the fresh/dry weight, root/shoot length and completely inhibited the germination of a lentil. The complete inhibition of the germination of goosegrass in the present study might be related to its direct exposure to the allelochemical. Allelochemicals may penetrate the seeds and be phytotoxic to the cell activities. Muscolo et al. (2001) reported that low molecular weight phenolic compounds impair glycolytic enzymes during seed germination, thereby reducing germination rates.

EFFECTS OF *P. PURPUREUM* DEBRIS-INCORPORATED SOIL ON SEEDLING GROWTH OF *E. INDICA*

Table 2 shows similar significant reduction of fresh weight (FW), dry weight (DW), root length (RL) and shoot length (SL) of *E. indica* with greater reduction corresponding to higher amounts of *P. purpureum* residue. Approximately >95% reduction was observed for FW, RL, DW and SL compared with the control. However, a low rate of 1% did not affect germination, fresh and dry weights as well as shoot and root lengths. In contrast, there were stimulatory effects at this low concentration. This was not expected since other concentrations of *P. purpureum* debris-incorporated soil showed significant difference compared with the control. Ismail and Kumar (1996) stated that this difference was probably contributed by different quantities of allelochemicals present in plant tissues and the allelopathic interaction depending on the chemical stability of the bioactive compounds and the amount of plants decaying in the soil. In addition, Coder (1998) stated that the variation in the inhibition or stimulation of germination depended upon dosage - too much or too little can damage growth and this is best illustrated by the statement of Paracelsus (1493-1541) – 'All things are poison and not without poison; only the dose makes a thing not a poison'. The decaying of *P. purpureum* leaves and stems which are capable of inhibiting *E. indica* growth indicated that the allelopathic agents may be present or are being formed (Tesio et al. 2012) in the amended soil and they might contain certain water soluble phytotoxic chemicals, which are released into the soil, causing reduced growth in *E. indica* (Faravani et al. 2008). This finding is supported by a study by Fong (2007), where it was reported that the aboveground tissues of *P. purpureum* debris at a concentration of as low as 5 g/kg soil can reduce the fresh weight of *Hedyotis verticillata*, *Amaranthus caudatus, Zea mays* and *E. indica* seedlings by 11, 31, 37 and 54%, respectively, under greenhouse conditions. Batish et al. (2002) and Kumar et al. (2009) also found that the seedling growth of the test species, grown in soil amended with residues of plants with allelopathic potential, was less than that of those grown on unamended soil. Rashid et al.

TABLE 1. Effect of aqueous leaf extracts of *P. purpureum* on early seedling growth of *E. indica*

Conc. (mg/L)	FW	DW	RL	SL	G
0	0.0011±0.0009[b]	0.0002±0.0005[b]	0.854±0.647[b]	0.729±0.298[b]	70%
2	0±0[a]	0±0[a]	0±0[a]	0±0[a]	0%
4	0±0[a]	0±0[a]	0±0[a]	0±0[a]	0%
6	0±0[a]	0±0[a]	0±0[a]	0±0[a]	0%
8	0±0[a]	0±0[a]	0±0[a]	0±0[a]	0%
10	0±0[a]	0±0[a]	0±0[a]	0±0[a]	0%

*FW: Fresh weight, DW: Dry weight, RL: Root length, SL: Shoot length, and G: germination
*Data are mean (±S.D.) of four replicates; different superscripts in a column indicate significant difference at $p<0.05$

TABLE 2. Growth performance of *E. indica* in *P. purpureum* leaf and stem (LSAS) amended soil (g/g) after 30 days

Treatments	FW	DW	RL	SL	G
Control	0.4524±0.4695 [b]	0.0132±0.0098[b]	10.59±2.02[b]	4.036±1.224 [c]	85%
Debris 1%	0.079±0.072[c]	0.0293±0.0203[c]	13.69±11.77[c]	4.972±0.892[d]	36%
Debris 5%	0.0015±0.002[a]	0.0004±0.0007[a]	2.58±2.85[a]	0.576±0.676[b]	21%
Debris 10%	0.0002±0.0005[a]	0.0003±0.0002[a]	0.46±1.40[a]	0.065±0.201[a]	11%

*FW: Fresh weight, DW: Dry weight, RL: Root length, and SL: Shoot length
*Data are mean (±S.D.) of four replicates; different superscripts in a column indicate significant difference at $p<0.05$

(2010) reported that the common symptom of allelopathic compounds is a decrease in photosynthetic efficiency and this could be the reason for the reduction of fresh/dry weight and root/shoot length of *E. indica*.

ESTIMATION OF PHENOLICS

Rice (1984) reported that phenolics are the most common and widely distributed water-soluble allelochemicals. Thus, their presence was presumed in *P. purpureum* aqueous leaf extracts and in the amended soils. Table 3 shows the amount of phenolics detected in the aqueous leaf extracts and amended soils. The amount of phenolic compounds in the extract was higher (12.42 mg/g tissues) compared with those detected in the debris (2.78 mg/g tissues). The lower phenolic content found in the *P. purpureum* debris incorporated-soil might be due to others environmental factors such as temperature, light density, moisture and microorganisms which could have influenced the direct release of possible toxic substances from plant parts through degradation and decomposition in the soil (Meksawat & Pornprom 2010). However, the present study showed that the amount of phenolics in the *P. purpureum* debris-amended-soil was adequate to reduce the growth of *E. indica*. This could be because phenolics endure various abiotic and biotic processes in the dynamics of the soil system, namely microbial action, sorption to organic matter or clay particles thus causing the available amount to vary greatly (Blum et al. 1999). Several studies have proposed techniques for extracting allelochemicals from the soil. At a given concentration (filter paper assay) the complete reduction (0% goosegrass seed germination) could probably be due to the absence of most of the mineral nutrients that cause the membranes to become much more permeable, possibly resulting in an unnaturally high release or absorption of allelochemicals (Tantiado & Saylo 2012) compared with germination in the soil assay. In the present study although there was no further isolation of allelochemicals responsible for growth inhibition or stimulation (found in the aqueous leaf extracts and debris-amended soil) the results showed that there might be presence of other unknown allelochemicals that could have caused inhibition of growth and germination. Besides, Whitehead et al. (1981) concluded that phenolics extracted with water are more ecologically important. Therefore, the present study has considered only the dissolved (water-soluble) fraction of the phenolics in the soil, which were the product of debris decomposition. During the incubation period, the phenolic content could be altered by microbes and then leveled off over time, thereby causing the phenolic content of the amended soil to be lower compared to that of the aqueous leaf extract. The results of the study are supported by the findings of Singh et al. (2005) where the same results were obtained but with different weeds (*Parthenium hysterophorus*). The total phenolic content reported in the residue extracts was higher than that of the residue-amended soil (475 µg/mL and 11.6 mg/100 g soil, respectively).

CONCLUSION AND RECOMMENDATIONS

On the basis of the results of the present study, it can be stated that *P. purpureum* leaf extracts and debris can cause inhibition in the germination and growth of the bioassay species, *E. indica*. The reduction in germination and growth of the bioassay species may due to the presence of phenolic compounds in the aqueous and debris extracts of *P. purpureum*. In addition, field application, soil microbial communities and the testing of allelopathy through improved methodology/design, the testing of more bioassay species are suggested for further investigations so that the importance of alellopathy can be thoroughly evaluated. Also, it is suggested that a study on the control of the target species could be carried out using a mixture

TABLE 3. Total phenolic content of the leaf debris extract, leaf-stem debris amended soil and P. purpureum infested soil (mg phenolic compound/gram tissues)

Treatment	Amount of phenolics
Extract	12.42±1.76[a]
Soil-amended	2.78±1.27[b]

*Data are mean (±S.D.) of four replicates; different superscripts in a column indicate significant difference at $p<0.05$

of allelochemical compounds with adjuvants. Besides, the potential of *P. purpureum* to suppress weed growth could be utilised for future sustainable weed management programmes.

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