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Allelopathic effects of sunflower leaf extract and selected pre-emergence herbicides on barnyardgrass

(Kesan alelopati ekstrak daun bunga matahari dan herbisid pracambah terpilih terhadap rumput sambau)

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Keywords: allelopathy, pre-emergence bio-herbicide, *Echinochloa crus-galli*, rice, phytotoxicity

Abstract

Barnyardgrass has been the most frequently reported troublesome weed in rice fields because it is an aggressive invader that is difficult to control and reduces rice yields significantly. A laboratory study was conducted to determine the effects of sunflower leaf extract and pre-emergence herbicides, pretilachlor and thiobencarb, on shoot emergence and seedling growth of barnyardgrass. The sunflower leaf extract and the pre-emergence herbicides exhibited phytotoxic effects on barnyardgrass at different degrees of potency. It was found that sunflower leaf extract and thiobencarb are root inhibitors and strong shoot inhibitors respectively. Whereas, pretilachlor is a strong root and shoot inhibitor. The emergence and seedling growth of barnyardgrass were inhibited by 80 – 100% with increasing concentration of sunflower leaf extract from 10 – 15% (w/v). Comparatively, rice seedlings were more tolerant to sunflower leaf extract where rice emergence and growth were inhibited by 10 – 65% at the same concentrations. The present findings suggest the possibility of using sunflower leaf extract as a pre-emergence bio-herbicide for inhibiting seedling growth of barnyardgrass without injuring the rice seedlings.

Introduction

Echinochloa crus-galli (L.) Beauv, commonly known as barnyardgrass, is an annual grass native to Eurasia that was formerly classified as a type of panicum grass (USDA 2011). It is an invasive species of the family Poaceae that was first spotted in the Great Lakes region of USA in 1843 (Rutledge et al. 2000). It is considered to be the most cosmopolitan and economically important member of the genus *Echinochloa* with a wide distribution as a weed all over the world (Holm et al. 1991). A recent survey by the Department of Agriculture of the United States ranked this invasive weed as the fourth worst weed occurring in many crops (USDA 2011).

Barnyardgrass is an important weed in 36 crops in 61 countries (Holm et al. 1991). It can reduce considerably yields of several major field crops such as rice, corn, cotton, sugarbeets, tobacco and potatoes as well as bananas, cassava, citrus, coffee, groundnuts, jute, millet, sorghum, sugarcane, taro, tea

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and vegetables (Holm et al. 1991). Among these crops, barnyardgrass is particularly problematic in rice and the second most competitive weed with rice after weedy rice (Azmi 1992; Ioannis et al. 2000; Rao et al. 2007).

Herbicides constitute the principal component of barnyardgrass management in rice fields. Pre-emergence herbicides play an important role in early elimination of weeds and protect rice during the most critical period (Azmi and Lim 1986). Pre-emergence herbicides are applied to the soil surface after rice emerge for direct seeded rice and before weeds emerge for transplanted rice.

Thiobencarb and pretilachlor are widely used pre-emergence herbicides to control barnyardgrass in rice fields. Thiobencarb is a selective herbicide of thiocarbamate group, mainly used as a pre- or early post-emergence herbicide (Matsuo and Shibayama 2002). It is commonly used in rice fields to control certain grasses, rushes, sedges and broadleaf weeds. In general, thiobencarb is much more effective against annual grass weeds than against annual broadleaf weeds (Ishikawa et al. 1998). On the other hand, pretilachlor is a member of the chloroacetanilide group, mainly used as pre-emergence herbicide to control certain broadleaf weed species and annual grassy weeds (Shim et al. 1990). The importance of pre-emergence herbicides cannot be denied, but the excessive use of herbicides caused various problems such as environmental pollution (Vizantinopoulos and Lolos 1994), public health (Jamal 2011) and increase of herbicide-resistant weed species (Heap 2012).

One of the strategies that can be adopted to control barnyardgrass is the use of allelopathic crop water extracts. According to Rice (1984), allelopathy is the influence of one plant on the growth of another one, including microorganisms, by the release of chemical compounds known as allelochemicals into the environment. Allelopathic potential of sunflower (*Helianthus annus* L.) leaf extract has been demonstrated in many recent studies. Sunflower has been reported to have phytotoxic effects to many weed species comparable to herbicide treatments (Weston and Duke 2003). More than 200 natural allelopathic compounds from different cultivars of sunflower have been isolated (Macias et al. 2002). Most of the known allelochemicals inhibit or delay seed germination of many weed species (Inderjit and Duke 2003; Weston and Duke 2003). These allelochemicals offer great potential as natural herbicides and can be used for weed control directly or for development of new herbicides. However, barnyardgrass growth response to sunflower leaf extract and the potential use of sunflower leaf extract as pre-emergence bio-herbicide is yet to be explored. The present study was therefore designed to assess the growth response of barnyardgrass towards sunflower leaf extract in comparison to pretilachlor and thiobencarb.

Materials and methods

Plants and herbicides

Sunflower leaves (*Helianthus annuus* L., var. Sunreach) were collected from several sunflower farms in Cameron Highlands, Malaysia during the harvesting stage. Rice variety MR 219 and barnyardgrass seeds (*Echinochloa crus-galli*) were collected from MARDI rice experimental plots (Seberang Perai Station) and Herbiseed (England) respectively. The herbicides used were commercially available formulations: pretilachlor 300 g/litre and thiobencarb 500 g/litre.

Aqueous extracts of sunflower leaves

Sunflower leaves were chopped into 2 cm long pieces and gently washed with distilled water. The leaves were dried under full sunlight for 4 days. The dried sunflower leaves were ground in a micro-fine grinder to pass a 1 mm screen and then stored in a refrigerator at 5 °C until use. Aqueous extracts were prepared by mixing 200 g of sunflower leaves powder with one litre distilled water in an orbital shaker for 3 days at 200 rpm. The solutions were filtered through two layers of cheesecloth to remove plant debris. The extracts were boiled at 100 °C to reduce its volume by two times (Dilipkumar et al. 2012) to give a final concentration of 40% (w/v). The extracts were then centrifuged at 14,000 rpm at 4 °C for 15 min and filtered through one layer of filter paper. The leaf extracts were stored at 5 °C until use.

Single application of sunflower leaf extracts or herbicides

The sunflower leaf extracts were diluted with distilled water to give final concentrations of 0.5, 1.5, 3.5, 4.5, 6.75, 10 and 15% (w/v). A total of 25 healthy barnyardgrass seeds were placed evenly on two pieces of filter papers lined in 9 cm petri dishes. Six millilitres of the aqueous extract from each concentration were applied to each petri dish. The same steps were repeated with the treatment of thiobencarb at five different concentrations namely, 50, 150, 450, 1,350 and 4,050 ppm (w/v) while pretilachlor at 30, 51, 87, 147 and 251 ppm (w/v). Distilled water was used as the control. All petri dishes were then sealed with laboratory film and placed in a growth chamber at 20 – 30 °C with 12 h photoperiod for 7 days. All treatments were arranged in a completely randomized design with four replications and repeated twice in time.

After 7 days, emerged seeds were counted and shoots and root lengths of each seedling were measured. Shoots of seeds were considered emerged when the plumule visibly protruded from the seed coat and its length reached more than or equal to 2 mm (Gulden et al. 2003). Seed viability was determined by physically pinching the seeds with forceps (Gulden et al. 2003). Non-viable seeds collapsed when pinched with the forceps. Shoot emergence rate, shoot and root lengths data were expressed as percentages of their respective controls as follows:

$$y = (xT/xC) \text{ x } 100\%$$

where y is shoot emergence rate/shoot length/root length, xT is number of seeds with emerged shoots/shoot length/root length in treatment, and xC is number of seeds with emerged shoots/shoot length/root length in control. The morphological changes of shoot and root growth at all treatments were also observed using a video microscope system at 2,400x magnification.

Rice tolerance test

Shoot emergence test was conducted to determine the phytotoxicity of sunflower leaf extracts on rice as described above. Six different concentrations of sunflower leaf extracts namely, 1.25, 2.5, 5, 10, 20 and 40% (w/v) were examined in this study. Distilled water treatment was used as control. All treatments were arranged in a completely randomized design with four replications and repeated twice. Seven days after treatment, number of seeds with emerged shoots was counted. Shoot emergence rate, shoot lengths, and root lengths data were expressed as percentages of controls as described above.

Statistical analysis

All percentage data in phytotoxicity test were pooled from the two repetitions of the experiments because no significant differences between experiments were observed after conducting the analysis of variance at $p = 0.05$ level. The data were than fitted to a logistic regression model, as follows (Kuk et al. 2002):

$$Y = d/(1 + [x/x_0]^b)$$

where Y is percentage of shoot emergence/root length/shoot length, d is the coefficients corresponding to the upper asymptotes, x is herbicide/sunflower leaf extracts concentration, x_0 is herbicide/sunflower leaf extracts concentration required to inhibit the shoot emergence/root length/shoot length by 50% relative to untreated seeds, and *b* is the slope of the line. Regression analyses were conducted and x_0 were calculated from the regression equations. T test was conducted to compare the differences between the two treatments in *b* values at 5% level of significance.

Results and discussion

Phytotoxicity of sunflower leaf extracts or herbicides on barnyardgrass

Aqueous extracts of sunflower leaf showed both stimulatory and inhibitory effects on shoot emergence and seedling growth of barnyardgrass. The stimulation responses of barnyardgrass shoot emergence and shoot length ranged from 9 – 22% with the increase of sunflower leaf extract concentrations from 0.5 – 1.5% (*Figure 1A*). However, root length of barnyardgrass declined to 74 – 84% at this concentration, implying that sunflower leaf extracts acts as a root inhibitor. This statement was further confirmed when 50% reduction in root length with respect to control (ED_{50}) was observed at the concentration of 0.2% whereas shoot length needed 7.6% to attain the same percentage of reduction (*Table 1*).

These results are in agreement with other findings which indicated that water extracts of allelopathic crops generally have more pronounced effects on root rather than shoot growth (Ashrafi et al. 2007; Ashraf et al. 2008). Such an outcome might be expected because plant root is often the first tissue to be in contact with allelochemicals

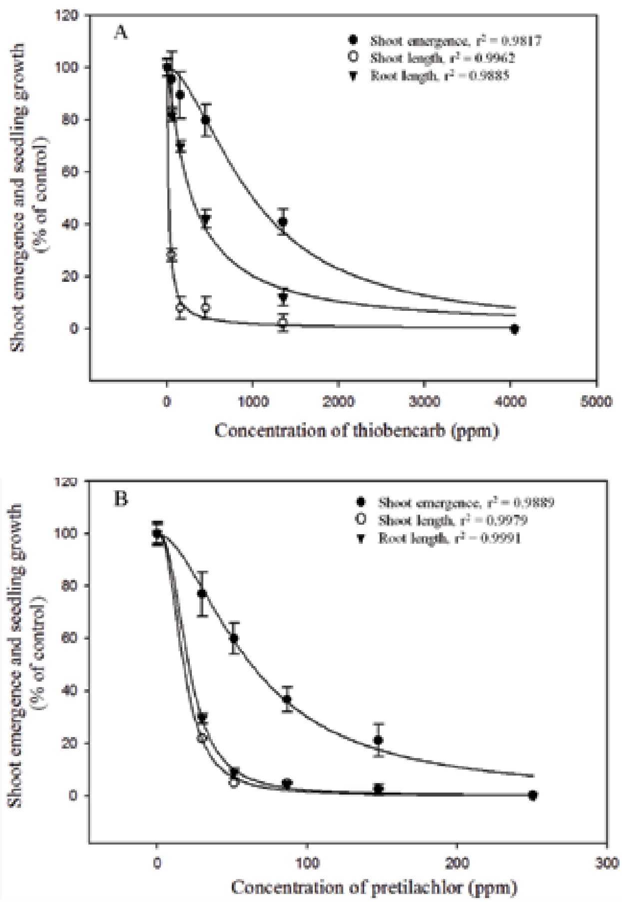

Figure 1. Effects of sunflower leaf extracts on shoot emergence and seedling growth of barnyardgrass (A) and rice (B). Vertical bars represent ± standard error of the mean; r2 indicates the coefficient of determination at p < 0.01

Table 1. ED_{50} and b values of barnyardgrass and rice in relation to sunflower leaf extracts, thiobencarb or pretilachlor treatment

	Barnyardgrass			Rice
	Sunflower leaf extracts	Thiobencarb	Pretilachlor	Sunflower leaf extracts
Shoot emergence	7.6 (0.6)*	1000 (155.5)*	63 (6.1)*	19 (2.3)*
Shoot length	7.6 (0.4)*	19 (6.1)*	18 (2.8)*	17 (1.7)*
Root length	0.2 (0.05)*	294 (53.9)*	21 (1.3)*	11 (0.9)*
Shoot emergence	5.9 (0.9)**	1.7 (0.4)**	1.8 (0.3)**	3.6 (1.6)**
Shoot length	6.5 (0.7)**	1.0 (0.2)**	2.4 (0.6)**	3.0 (0.8)**
Root length	1.0 (0.2)**	1.1 (0.2)**	2.5 (0.3)**	2.1 (0.3)**

*ED_{50} is the rate of sunflower leaf extracts/thiobencarb/pretilachor that cause 50% inhibition of shoot emergence/shoot length/root length of barnyardgrass or rice.
**b is slope of the regression line.
Sunflower leaf extracts rate is expressed as percentage (w/v) and herbicides rate is expressed as parts per million. The values in parentheses are the standard error of the mean.

(Zhou and Yu 2006). Besides the inhibition of shoot and root lengths, sunflower leaf extracts also altered shoot and root morphology, displayed severe interveinal chlorosis with leaf dieback (*Plate 1C*) compared to the control, which was long, flattened, pale green, with parallel hyaline veins (*Plates 1A* and *1B*). The roots were severely twisted, turned hard and reddish brown with retarded root hairs (*Plate 2C*), as compared to the control seedlings, which were long and soft with creamy whitish smooth root hairs (*Plates 2A* and *2B*).

Sunflower leaves are rich in sources of terpenoids (sesquiterpene lactones, annuionon, heliannuols), phenolic compounds such as phenolic acids (cinnamic, benzoic, p-coumaric and ferulic acids) and flavonoids (Macias et al. 2002; Anjum and Bajwa 2005). These allelochemicals inhibit shoot emergence and seedling growth probably by affecting cell division and elongation processes or by interfering with enzymes involved in mobilization of nutrients necessary for shoot emergence (Batlang and Shushu 2007).

On the other hand, dose-response experiments of both tested herbicides showed high potential to inhibit shoot emergence and seedling growth of barnyardgrass. ED_{50} values for shoot emergence, shoot length and root length of barnyardgrass were found to be 1,000, 19 and 294 ppm respectively, when treated with thiobencarb (*Table 1*), implying that thiobencarb acts as an inhibitor of shoot on barnyardgrass seedlings (*Figure 2A*). Similarly, Al-Mamun and Shimizu (1978) reported that thiobencarb causes leaf dieback and abnormal emergence of panicle with fewer spikelets on barnyardgrass. In addition, a severe abnormal morphology of shoot and root were observed, where the leaf deformities occurred with leaf necrosis and dense covering of unexpanded cells on leaf tip (*Plate 1D*) while the root was short and hard in reddish brown colour coupled with blunted root tip (*Plate 2D*). Besides, almost similar severe damages and inhibition degrees of both shoot and root growth were observed after seeds of barnyardgrass were treated with pretilachlor (*Figure 2B*).

The ED_{50} values were recorded at concentrations as low as 18 and 21 ppm for shoot length and root length respectively, while shoot emergence was observed at 63 ppm (*Table 1*). These results suggests that pretilachlor acts as shoot and root inhibitors. Furthermore, leaf necrosis and chlorosis and malformed leaf with covering

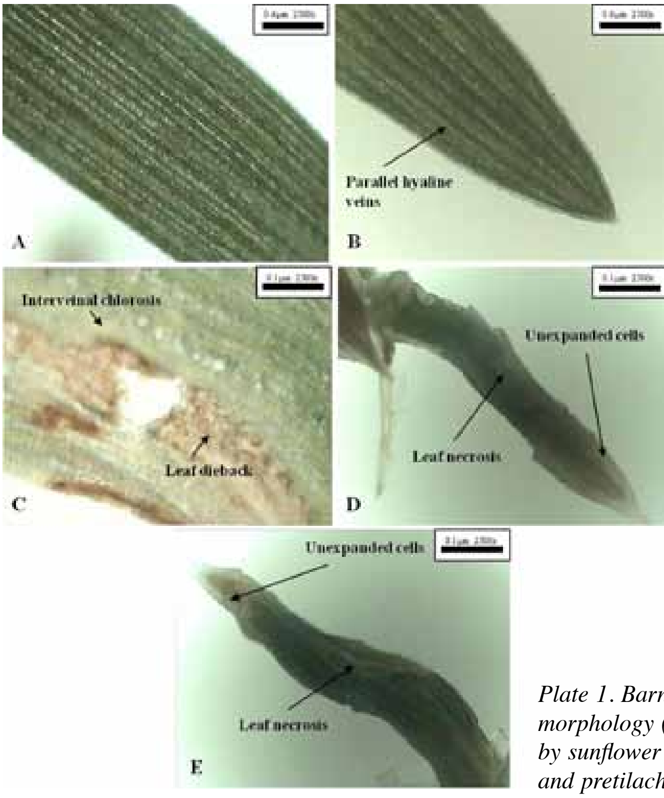

Plate 1. Barnyardgrass normal shoot morphology (A) and (B), shoot injuries caused by sunflower leaf extract (C), thiobencarb (D) and pretilachlor (E)

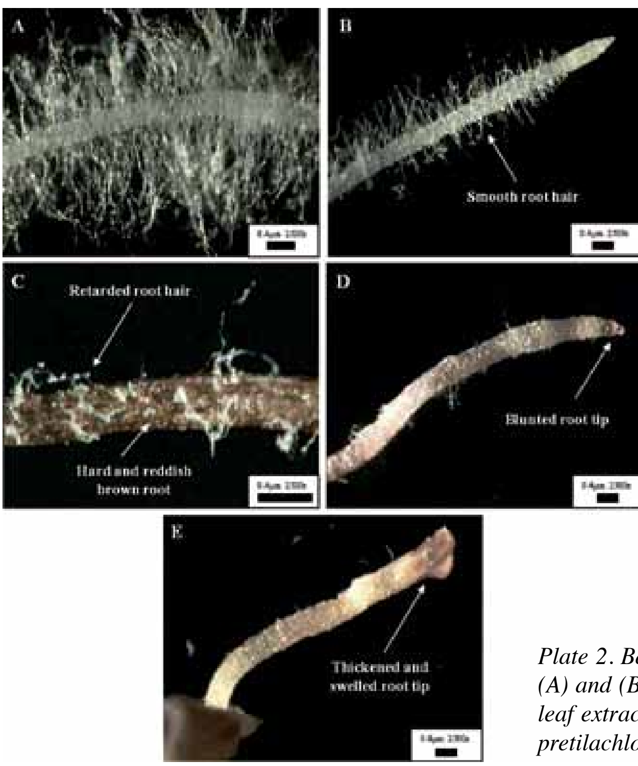

Plate 2. Barnyardgrass normal root morphology (A) and (B), root injuries caused by sunflower leaf extract (C), thiobencarb (D) and pretilachlor (E)

of unexpanded cells on leaf tip were evident (*Plate 1E*) and the root became thickened, shortened with swollen root tip which leads to stunting (*Plate 2E*). Monaco et al. (2002) documented that a common symptom caused by pretilachlor was distortion of the first foliar leaf and restriction of coleoptile emergence in grass seedlings. In treated fields, susceptible grasses often emerged but remained very small, with severely distorted young leaves and roots, and eventually the plant died.

In the present study, the slopes of regression lines are represented by b values (*Table 1*). There were significant differences between pretilachlor and thiobencarb in their respective b values of root length (p = 0.047) and shoot length (p = 0.002). However, there was no significant difference between pretilachlor and thiobencarb in their respective b values of shoot emergence (p = 0.845), suggesting that these two herbicides may share some degree of similarity in mode of action on barnyardgrass. This finding is in line with the results of previous studies which demonstrated that both pretilachlor and thiobencarb are inhibitors of fatty acid biosynthesis (Tomlin 1994; Boger 2003). On the other hand, b values for shoot emergence, shoot length, and root length of barnyardgrass treated with sunflower leaf extracts showed significant difference (p <0.05) as compared to those subjected to pretilachlor or thiobencarb treatment. This indicates that sunflower leaf extracts and pretilachlor or thiobencarb are most likely to have different modes of action on barnyardgrass.

Tolerance of rice to sunflower leaf extracts

Based on the results of this study, the marked reduction (80 – 100% inhibition) of barnyardgrass emergence and seedling growth were observed when being treated with 10 – 15% sunflower leaf extract concentrations (*Figure 1A*). The phytotoxicity test was subsequently carried out to examine the susceptibility of rice seedlings towards sunflower leaf extracts. Interestingly, the shoot emergence and seedling growth of rice were reduced by 10 – 65% when treated with sunflower leaf extracts at the same concentrations (*Figure 1B*). This result clearly shows that the rice plants are more tolerant than barnyardgrass when subjected to sunflower leaf extracts. In contrast to the present results, Bashir et al. (2011) found that sunflower leaf extracts at 15% (w/v) greatly inhibit emergence and seedling growth of rice variety Basmati Pak. This unequal susceptibility of rice varieties to the sunflower extracts may be due to inherent differences in physiological and morphological characteristics of rice genotypes.

ED_{50} values for shoot emergence, shoot length, and root length of rice seedlings were found to be 19, 17, and 11% respectively, when treated with sunflower leaf extracts (*Table 1*). This again confirms that root is the target site for the action of sunflower leaf extracts. In conjunction with the phytotoxicity test of the sunflower leaf extracts on barnyardgrass, the present results also showed both stimulatory and inhibitory effects on shoot emergence and seedling growth of rice when subjected to sunflower leaf extracts (*Figure 1B*). Reports have shown abundant evidence that the response of a plant to a toxin is stimulation at low concentration (Inderjit and Duke 2003). However, as the concentration increases, the stimulation gradually turns to inhibition (An et al. 1993).

Conclusion

Phytotoxic studies on activities of sunflower leaf extracts and selected pre-emergence herbicides clearly showed that sunflower leaf extracts had the potential to be applied as pre-emergence bio-herbicide for inhibiting emergence and growth of barnyardgrass. Although rice plant has shown acceptable tolerance to sunflower leaf extracts, substantial injury or inhibition of

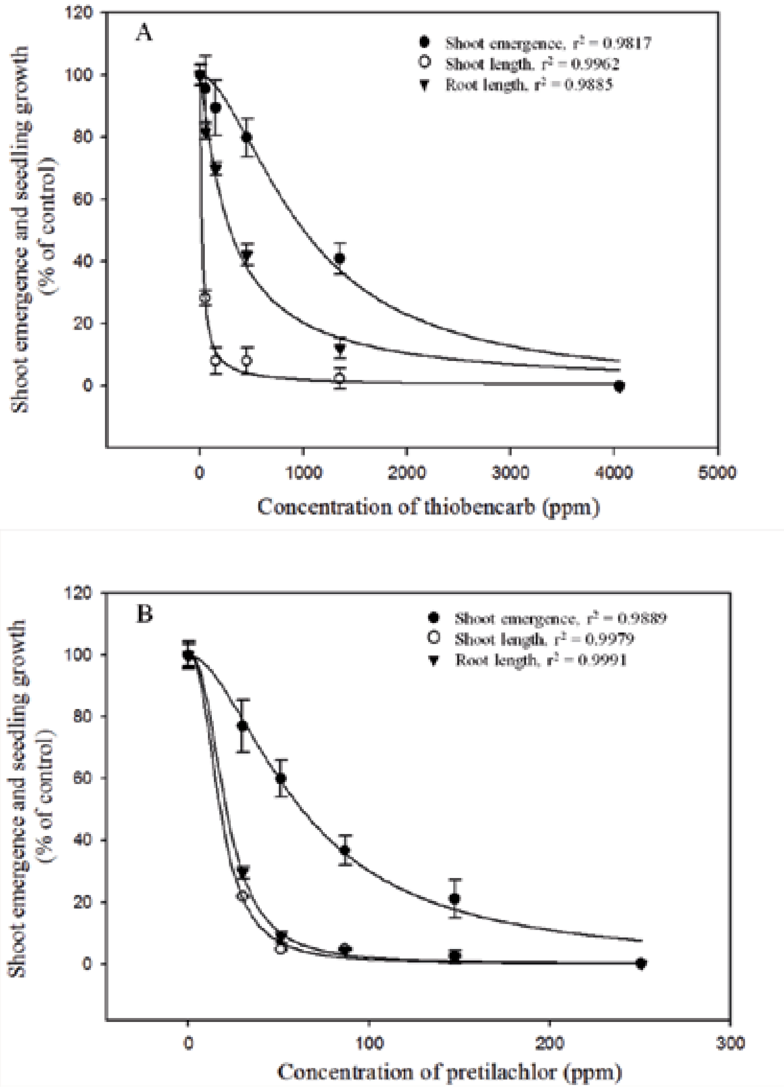

Figure 2. Effects of thiobencarb (A) and pretilachlor (B) on shoot emergence and seedling growth of barnyardgrass. Vertical bars represent ± standard error of the mean; r2 indicates the coefficient of determination at p <0.01

its growth can occur at high concentrations. The present study has also proven that allelopathic extracts of sunflower leaf that inhibit the growth of barnyardgrass at certain concentrations (10 – 15%) might stimulate or has no influence on the growth of rice plants at the same concentration. Therefore, it is essential to identify the concentration at which each specific response occurs if allelopathic interaction is to be used in weed management programmes. Since this study was carried out under laboratory conditions, caution should be taken regarding the ecological implications of the data because phytotoxicity of sunflower leaf extracts is influenced by biotic and abiotic factors in soil.

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Abstrak

Rumput sambau paling kerap dilaporkan sebagai rumpai paling teruk di sawah padi kerana ia merupakan rumpai agresif yang sukar untuk dikawal dan menjejaskan hasil padi secara signifikan. Satu kajian makmal telah dijalankan untuk menentukan kesan ekstrak daun bunga matahari dan herbisid pracambah pretilaklor dan tiobenkab ke atas kemunculan dan pertumbuhan rumput sambau. Ekstrak daun bunga matahari dan herbisid pracambah menunjukkan kesan fitotoksik ke atas rumput sambau pada kadar yang berlainan. Ekstrak daun bunga matahari dan tiobenkab masing-masing didapati merupakan perencat akar dan perencat dedaun yang kuat. Manakala pretilaklor adalah perencat akar dan dedaun yang kuat. Kemunculan dan pertumbuhan anak benih rumput sambau telah terencat sehingga 80 – 100% dengan peningkatan kepekatan ekstrak daun bunga matahari dari 10 – 15% (w/v). Secara perbandingan, anak benih padi didapati lebih toleran terhadap ekstrak daun bunga matahari di mana kemunculan dan pertumbuhan padi terencat sehingga 10 – 65% pada kepekatan yang sama. Kajian ini mencadangkan kemungkinan menggunakan ekstrak daun bunga matahari sebagai bioherbisid pracambah untuk mengawal kemunculan dan pertumbuhan rumput sambau tanpa mencederakan anak benih padi.

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