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Is Combination Ratio an Important Factor to Determine Synergistic Activity of Allelopathic Crop Extract and Herbicide?

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Abstract

Use of allelopathic crop water extracts in combination with herbicide for weed control in crop fields is well recognized. However, studies on joint action of allelopathic crop water extracts and herbicides using established models are still scanty. Addressing this need, the present study investigates the joint action of extracts of sunflower leaves and selected herbicides on shoot emergence and seedling growth of barnyardgrass (*Echinochloa crus-galli*) under laboratory conditions using the Additive Dose Model (ADM). ED_{50} (rate that causes 50% inhibition) from the dose-response curves for the sunflower leaf extracts or herbicides applied alone or in mixtures of fixed ratios were determined. The results of ADM analysis demonstrated synergism for both combinations, but the synergistic effects of pretilachlor and sunflower leaf extracts mixture was 10% stronger than mixture of thiobencarb and sunflower leaf extracts. The degree of synergism increased progressively with the increased ratio of sunflower leaf extracts: herbicide, but antagonism was evident at the lowest ratio of sunflower leaf extracts: herbicide. The results of this study have revealed that, the ratio of allelopathic crop water extracts in combination with herbicide is an important factor in influencing the potency of phytotoxic activity.

Keywords: Joint action; Allelopathy; Additive dose model; Pre-emergent herbicide; Sunflower leaf extracts

Introduction

Herbicide application has become the most widely adopted method for controlling weeds for successful crop production, but their non-judicious use also registers negative effects on soil, water, air, humans and animal health (Vizantinopoulos and Lolos, 1994; Jamal, 2011). Besides, herbicide resistance in weeds due to continuous use of the same herbicide for several years is also an emerging problem. Therefore, it is always required to develop new strategies for getting efficient weed control. Allelopathic suppression of weeds is receiving greater attention in recent years as an alternate weed management tool in sustainable intensive crop production (Farooq *et al*., 2011). Numerous crops have been reported to exhibit allelopathic effects on the associated weeds. These include sunflower (Dilipkumar *et al*., 2012), sorghum (Cheema *et al*., 2005a), rice (Rehman *et al.*, 2010), brassica (Khan *et al*., 2012) and mustard (Jabran *et al*., 2010). Allelopathic crops are used in different ways such as surface mulch (Cheema *et al*., 2000), incorporation into the soil (Chuah *et al.*, 2011), spraying of water extracts (Dilipkumar *et al.*, 2012), rotation (Narwal, 2000), smothering (Singh *et al.*, 2003) or mixed cropping and intercropping (Hatcher and Melander, 2003) to inhibit weeds.

Abundant studies conducted by Cheema and his associates (Cheema *et al.*, 2002, 2003, 2005a, b) have demonstrated allelopathic crops extract mixed with herbicide is an economical method for weed management and a substitute for heavy use of herbicides. This possibility was further explored by researchers in certain recent studies. An experiment conducted by Sharif *et al.* (2005) showed that reduced rate of metsulfuron-methyl at 6.25 g a.i. ha^{-1} combined with sorghum water extract at 12 L ha^{-1} reduced total weed density and weed dry weight by 76 and 79% respectively in wheat field. Furthermore, the economic analysis of this study has revealed that reduced rate of metsulfuron-methyl at 6.25 g a.i. ha^{-1} combined with sorghum water extract at 12 L ha^{-1} was the cost-effective treatment as compared to the full rate of metsulfuron-methyl or sorghum water extract alone. Jamil *et al.* (2005) have found that one foliar spray of sorghum water extract at 12 L ha^{-1} combined with isoproturon at 600 g a.i. ha^{-1} appeared as the best treatment combinations with maximum reduction of total weed density (89–97%) and dry weight (91–99%) of wheat weeds and this decrease was statistically equal to the standard rate of isoproturon at 1000 g a.i. ha^{-1}. Jabran *et al.* (2008) conducted a field study to investigate the allelopathic effects of sorghum, sunflower, brassica, and rice combined with low rates of pendimethalin for weed management in

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canola field. They found that, sorghum and rice water extracts each at 15 L ha^{-1} combined with 600 g a.i. ha^{-1} pendimethalin showed maximum (68%) reduction in total weed density, which was as effective as full rate of pendimethalin at 1200 g a.i. ha^{-1}. Jabran *et al.* (2010) have also revealed that pendimethalin rates for successful weed control in canola can be reduced up to 67% when tank-mixed with sorghum, sunflower, mustard, and rice water extracts each at 15 L ha^{-1} with pendimethalin at 400 g a.i. ha^{-1}. In addition, Rehman *et al.* (2010) stated that rates of butachlor, pretilachlor, and ethoxysulfuron can be reduced by 27 to 67%, when combined with sunflower, rice, and sorghum water extracts in order to control weeds in transplanted rice fields.

All the studies discussed above have shown that a mixture of herbicide and allelopathic crop extracts act synergistically at one-third or half the recommended herbicide rates. Despite these successful field trials, the need still exists to maximize possible reduction in herbicide rate without compromising effective weed control. One of the promising methods to achieve this need is through the study of joint action. Understanding the joint action of herbicide and allelopathic crop extracts using an established model is of prime importance to reveal the optimum ratio that provides high degree of synergistic effect. Unfortunately, a joint action of herbicide with allelopathic crop water extracts has yet not been explored. Various reference models can be used to study the joint action of phytotoxins (Kudsk and Mathiassen, 2004), but additive dose model (ADM) has been employed in the present study. This model can apply to almost all chemicals and for mixtures of phytotoxins with similar modes of action or a common toxicological response (Faust *et al.*, 2003; Kudsk and Mathiassen, 2004; Cedergreen *et al.*, 2007). In this study, barnyardgrass was used as bioassay species and joint action study was conducted based on binary mixture of aqueous sunflower leaf extract and pretilachlor or thiobencarb at different ratios to address how a combination ratio affecting degree of synergism. Barnyardgrass 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). Thus, it is a suitable candidate for bioassay study.

Materials and Methods

Plant and Herbicide Materials

Sunflower leaves (*Helianthus annuus* L., var. sunreach) were collected from several sunflower farms in Cameron Highland, Malaysia during the harvesting stage, while barnyardgrass seeds (*Echinochloa crus-galli* L. Beauv) were purchased from Seed Bank (Herbiseed), England with the germination rate more than 90%. The herbicides used were commercially available formulations: pretilachlor 300 g L^{-1} (Sofit N 300 EC, Syngenta Corporation Pte. Ltd., Malaysia) and thiobencarb 500 g L^{-1} (Saturn 50 EC, Agricultural Chemicals (M) Pte. Ltd., Malaysia).

Aqueous Extraction of Sunflower Leaf

Sunflower leaves were chopped into 2 cm long pieces and gently washed with distilled water, and dried under full sunlight for four days. These were then ground in a micro-fine grinder to pass a 1 mm screen and stored in a refrigerator at 5°C until use. Aqueous extracts were prepared by extracting 200 g of sunflower leaves powder with 1 L of distilled water in an orbital shaker for three days at 200 rpm. The solutions were filtered through two layers of cheesecloth to remove plant debris. The extracts were then centrifuged at 14,000 rpm at 4°C for 15 min and filtered through one layer of filter paper to give a final concentration of 2.0 x 10^5 ppm or 20% (w/v). The leaf extracts were stored at 5°C until use.

Single Application of Sunflower Leaf Extracts or Herbicides

Sunflower leaf extracts were diluted with distilled water to give final concentrations of 3 x 10^4, 4.5 x 10^4, 6.8 x 10^4, 10.1 x 10^4, and 15.2 x 10^4 ppm (w/v). A total of 25 healthy barnyardgrass seeds were placed evenly on two pieces of filter papers lined in 9 cm Petri dishes. 6 mL of extract solution from each concentration was added to each petri dish. The same steps were repeated with the treatment of thiobencarb at four different concentrations namely, 50, 150, 450, 1350, and 4050 ppm (w/v), while pretilachlor concentrations were 30, 51, 87, 147, and 251 ppm (w/v). Distilled water was used as a control. All petri dishes were then sealed with laboratory film and placed in a growth chamber at 20/30°C with 12 hours photoperiod for seven days. All treatments were arranged in a completely randomized design with four replications. After seven days, shoot and root lengths of each seedling were measured and number of emerged seeds was counted. 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. Seed viability was determined by physically pinching the seeds with forceps (Gulden *et al.*, 2003). Non-viable seeds collapsed when being pinched with forceps. Shoot emergence rate, shoot and root lengths data were expressed as percentages of their respective controls as follows:

$$y = (xT/xC) \times 100\%$$

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.

Mixture of Sunflower Leaf Extracts and Herbicides

Shoot emergence tests were conducted to determine the joint activity of sunflower leaf extracts with herbicides on barnyardgrass as described above. The combinations of sunflower leaf extracts and herbicides were prepared based on the ratios of 0:100, 10:90, 30:70, 50:50, 70:30, and 90:10, 100:0. Distilled water was used as control. After seven days, number of seeds with emerged shoots was counted. Shoot emergence rate data was expressed as percentages of controls as described above.

Statistical Analysis

All the percentage data in phytotoxicity test were 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 difference between two treatments in b values at 5% of significant level. Combined phytotoxic effects of sunflower leaf extracts and herbicides were evaluated in relation to the ADM model using shoot emergence data. Isobolographic method was used to present the data. Isobolograms are used to show the chemical combinations needed to produce a 50% effect level in a test system. Data were analyzed by using the method described by Sorensen *et al.* (2007). The first model is the sigmoid log-logistic dose-response model as follows:

$$y_i = d/1 + \exp\{b_i[\ln(x_i) - \ln(e_i)]\}$$

Where, y_i denotes the response for the ith herbicide or sunflower leaf extracts concentration x_i ($i = 1\ldots., 5$), and d is the common upper limit of the response of all mixture ratios when the sunflower leaf extracts or herbicide concentration x_i is zero. The parameter e_i is the rate of sunflower leaf extracts and herbicide mixture i giving a response of 50% of d, and b_i is proportional to the slope around e_i.

In the second model, the e_i values of the first model were constrained to follow an isobole model proposed by Volund (1992), which includes two parameters (η_1 and η_2) to describe the isobole curvature. This model was chosen because it allows a larger degree of antagonism and able to describe asymmetric isoboles:

$$\left(\frac{x_1}{ED_1}\right)^{\eta_1}\left(\frac{x_1}{ED_1}+\frac{x_2}{ED_2}\right)^{1-\eta_1} + \left(\frac{x_2}{ED_2}\right)^{\eta_2}\left(\frac{x_1}{ED_1}+\frac{x_2}{ED_2}\right)^{1-\eta_2} = 1$$

In this model, x is the concentration of sunflower leaf extracts and herbicides mixture at a predefined effect level, and ED is the concentration of the same sunflower leaf extracts or herbicides giving that effect, when tested alone. The subscription 1 and 2 denote the sunflower leaf extracts and herbicides in the mixture. A η value larger than 1 indicate antagonism, whereas η values that are smaller than 1 indicate synergism. Different η values describe asymmetric isoboles.

The third model, which replaces e_i with an isobole model, was equal to the second model with η_1 and η_2 fixed at the value 1. One-sample T test was used to determine deviation from ADM based on third model values at 5% of significant level. The sum of toxic units (ΣTU) signifies the relative amount of chemical in a mixture that will give a certain effect. This study works with a 50% effect level. If the ΣTU is 1, the mixture effect follows ADM. If it is 0.8, only 80% of the chemicals are needed to reduce the response to 50% compared with that expected from ADM. The ΣTU can be calculated for all mixture ratios. The ΣTU of the 50:50% effect ratio ($\Sigma TU_{50:50}$) is used as a measure of the size of synergism or antagonism across the two isobole models, as it is this mixture ratio at which the deviation from ADM is largest for symmetric isoboles. For the Volund isobole model, the $\Sigma TU_{50:50}$ is calculated as $1/(2^{-\eta 1}+ 2^{-\eta 2})$ (Sorensen *et al*., 2007).

Results

Phytotoxicity of Sunflower Leaf Extracts or Herbicides

Aqueous extracts of sunflower leaf showed strong inhibitory effects on shoot emergence and seedling growth of barnyardgrass at all tested concentrations. A 50% reduction in shoot emergence with respect to control (ED_{50}) was observed at the concentration of 7.67×10^4 ppm (Table 1). Besides, ED_{50} value for root length was observed at 1.87×10^4 ppm, whereas shoot length needed 7.64×10^4 ppm to attain the same percentage of reduction (Table 1). On the other hand, dose-response experiments of both tested herbicides showed high potential to inhibit shoot emergence and seedling growth of barnyardgrass (Fig. 1 and 2). ED_{50} values for shoot emergence, shoot length, and root length of barnyardgrass were found to be 1000, 19, and 294 ppm, respectively, when treated with thiobencarb (Table 1). Almost similar severe damages and inhibition degrees of both shoot and root growth were observed after seeds of barnyardgrass were treated with pretilachlor (Fig. 2C). The ED_{50} values were recorded at the concentrations as low as 18 and 21 ppm for shoot length and root length, respectively, while for shoot emergence it was observed at 63 ppm (Table 1).

In the present study, the slope of regression line is represented by b value (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.

Table 1: [a]ED_{50} and [b]b values of barnyardgrass in relation to sunflower leaf extracts, thiobencarb, or pretilachlor treatment

Parameters	Sunflower leaf extracts	Thiobencarb	Pretilachlor
		ED_{50} (ppm)	
Shoot emergence	76700 (2425)	1000 (155)	63 (6)
Shoot length	76400 (1682)	19 (6)	18 (2)
Root length	18700 (1748)	294 (54)	21 (1)
		b	
Shoot emergence	5.8 (0.9)	1.7 (0.4)	1.8 (0.3)
Shoot length	6.5 (0.7)	1.0 (0.2)	2.4 (0.6)
Root length	5.4 (1.0)	1.1 (0.2)	2.5 (0.3)

[a]ED_{50} is the concentration required to inhibit the shoot emergence/root length/shoot length by 50% relative to untreated seeds. [b]b is slope of the regression line. The values in parentheses are the standard deviations of the means (n = 4)

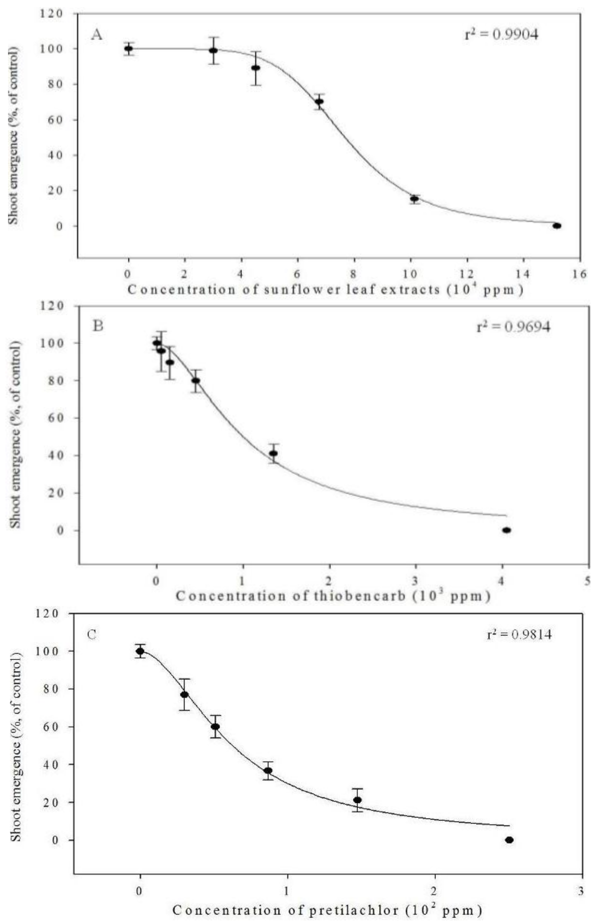

Fig. 1: Effects of sunflower leaf extracts (A), thiobencarb (B), and pretilachlor (C) on shoot emergence of barnyardgrass. Vertical bars represent standard deviations of the mean (n = 4). r^2 indicates regression model significance of the coefficient of determination at the 0.01 level of probability

This finding is in line with the results of previous studies, which have demonstrated that both pretilachlor and thiobencarb are inhibitors of fatty acid biosynthesis. 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 mode of action on barnyardgrass.

Table 2: [a]ED_{50} values of barnyardgrass in relation to mixtures of pretilachlor and sunflower leaf extracts at different ratios

Ratio	Pretilachlor (A)	Sunflower leaf extracts (B)
	ED_{50} (ppm)	
100% A + 0% B	63	-
90% A + 10% B	132 (7.0)	17947 (946)
70% A + 30% B	27 (1.3)	14153 (675)
50% A + 50% B	17 (0.8)	20416 (915)
30% A + 70% B	9 (0.4)	25754 (1229)
10% A + 90% B	2 (0.1)	20764 (876)
0% A + 100% B	-	76700

Table 3: [a]ED_{50} values of barnyardgrass in relation to mixtures of thiobencarb and sunflower leaf extracts at different ratios

Ratio	Thiobencarb (A)	Sunflower leaf extracts (B)
	ED_{50} (ppm)	
100% A + 0% B	1000	-
90% A + 10% B	1495 (99)	12740 (843)
70% A + 30% B	641 (11)	21073 (359)
50% A + 50% B	306 (17)	23454 (1300)
30% A + 70% B	157 (7)	28045 (1200)
10% A + 90% B	41 (3)	28408 (1896)
0% A + 100% B	-	76700

[a]ED_{50} is the concentrations of thiobencarb or sunflower leaf extracts that cause 50% emergence of barnyardgrass. The values in parentheses are the standard deviations of the means (n = 4)

Joint Action of Sunflower Leaf Extracts and Herbicides

ED_{50} values of barnyardgrass treated with pretilachlor, thiobencarb and sunflower leaf extracts alone were reduced drastically by 2 to 30-fold when sunflower leaf extracts were mixed with thiobencarb or pretilachlor at all ratios except at a combination of 90% herbicides and 10% sunflower leaf extracts that exhibited antagonistic activity (Table 2 and 3). The greatest synergistic effect was found with 10% herbicides plus 90% sunflower leaf extracts but the synergistic effect was reduced when the concentrations or ratios of tested herbicides increased (Fig. 3). Based on one sample T-test, it was shown that both combinations deviated from ADM and exhibited synergism. Both η1 and η2 values for the combination of sunflower leaf extracts and thiobencarb or pretilachlor are less than 1, respectively, indicating synergistic responses (Table 4). The $\Sigma TU_{50:50}$ for sunflower leaf extracts plus pretilachlor is 0.614, implying that about 61% of the chemicals are needed to reduce the response to 50% compared with that expected from ADM. On the other hand, $\Sigma TU_{50:50}$ for combination of thiobencarb and sunflower leaf extracts is 0.679. This suggests that

Table 4: $^{a}\eta_1$, $^{b}\eta_2$ and $^{c}\Sigma TU_{50:50}$ values of barnyardgrass in relation to mixture of sunflower leaf extracts plus thiobencarb or pretilachlor

Combination	η_1	η_2	$\Sigma TU_{50:50}$
sunflower leaf extracts + thiobencarb	0.041 (0.005)	0.995 (0.058)	0.679 (0.011)
sunflower leaf extracts + pretilachlor	0.030 (0.002)	0.623 (0.023)	0.614 (0.003)

$^{a}\eta_1$ and $^{b}\eta_2$ describe the isobole curvature. $^{c}\Sigma TU_{50:50}$ is the sum of toxic unit that signifies the relative amount of chemical in a mixture that gives 50% effect. The values in parentheses are the standard deviations of the means (n = 4)

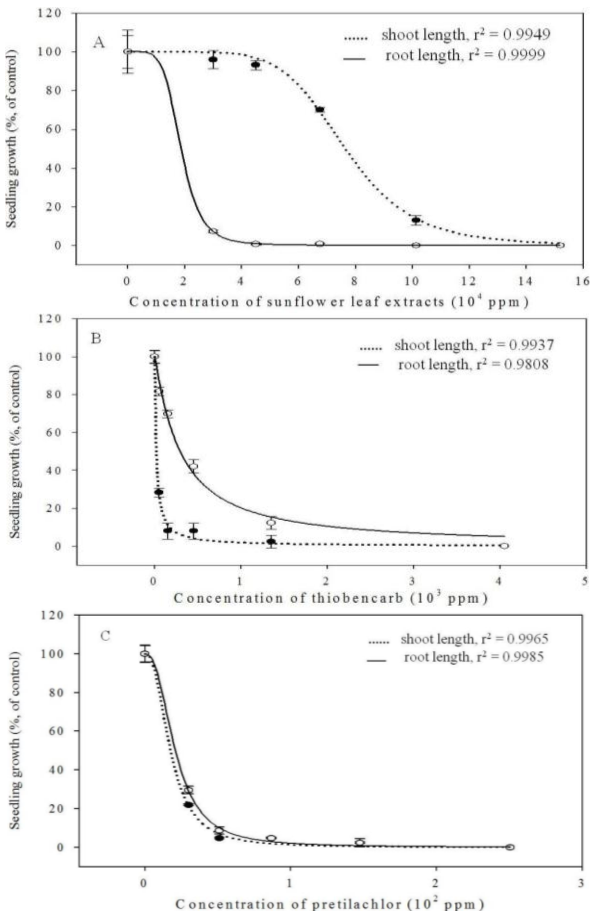

Fig. 2: Effects of sunflower leaf extracts (A), thiobencarb (B), and pretilachlor (C) on shoot (····) and root length (—) of barnyardgrass. Vertical bars represent ± standard deviations of the mean (n = 4). r^2 indicates regression model significance of the coefficient of determination at the 0.01 level of probability

about 68% of the chemicals are needed to reduce the response to 50% compared with that expected from ADM (Table 4). These results have demonstrated that the synergistic effects of pretilachlor and sunflower leaf extracts mixture is 10% greater than thiobencarb and sunflower leaf extracts mixture.

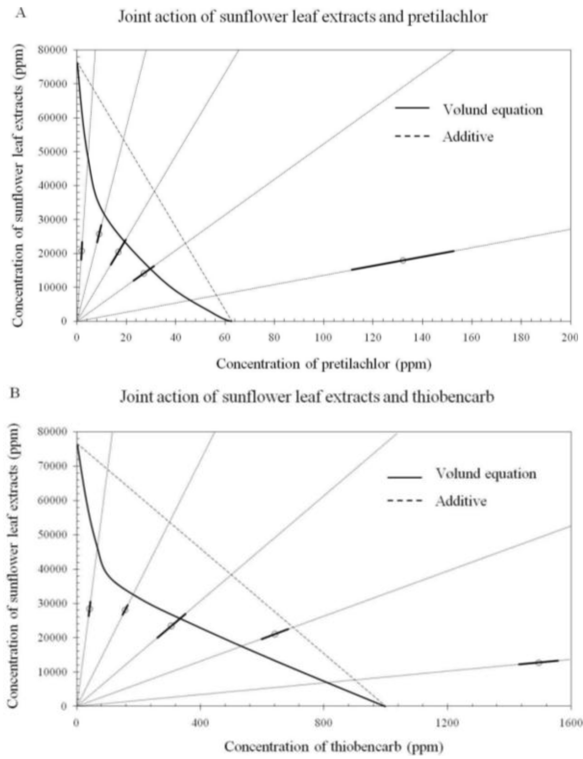

Fig. 3: Isoboles for a combination of sunflower leaf extracts and pretilachlor (A) or thiobencarb (B), at the ED_{50} level, with data given as ED_{50} ± standard deviation

Discussion

The present study has shown that, sunflower leaf extracts affect shoot emergence, shoot growth, and root growth of barnyardgrass. The inhibition of shoot emergence and seedling growth was concentration dependent. These results are similar to those obtained by other researchers who observed that shoot emergence and seedling growth of other crops and weeds were inhibited when subjected to sunflower leaf extracts treatment (Tongma *et al*., 2001; Batish *et al*., 2002; Anjum *et al*., 2005; Bogatek *et al*., 2006). The lowest ED_{50} value observed in root length have demonstrated that sunflower leaf extracts act as a root inhibitor on barnyardgrass seedlings (Table 1). These results are in agreement with the finding that, water extracts of allelopathic crops generally have more pronounced effects on root rather than shoot growth (Ashrafi *et al*., 2007, 2008). Such an outcome might be expected because plant root is often the first tissue to contact an allelochemical (Zhou and Yu, 2006). Macias *et al*. (2002) have isolated more than 200 natural allelopathic compounds from different cultivars of sunflower. Aqueous extracts from 15 varieties of sunflower leaf are rich in sources of terpenoids (sesquiterpene lactones, annuionon, heliannuols), phenolic compounds such as phenolic acids (cinnamic, benzoic, p-coumaric, and ferulic acid) and flavonoids (Macias *et al*., 2002; Anjum and Bajwa, 2005). The phenolic acids inhibit shoot emergence and seedling growth probably by affecting cell division and elongation processes or by interferring with enzymes involved in mobilization of nutrients necessary for shoot emergence (Batlang and Shushu, 2007). Besides, dose-response experiments of herbicides showed that, pretilachlor acts as shoot and root inhibitor, while thiobencarb acts as an inhibitor of shoot on barnyardgrass seedlings (Table 1). Similarly, Monaco *et al*. (2002) documented that, a common symptom caused by pretilachlor was distortion of the first foliar leaf and restriction of coleoptile and radicle emergence in grass seedlings. Furthermore, Al-Mamun and Shimizu (1978) reported that thiobencarb causes leaf deformities, leaf dieback, and abnormal emergence of panicle with fewer spikelets on barnyardgrass.

Interactions in herbicide and allelopathic crop water extracts mixtures can occur prior, during, or after application of the mixture. Thus, herbicides and allelopathic crop water extracts mixtures may interact physically or chemically in the spray solution or biologically in the plant. Pretilachlor is a selective herbicide that results in depletion of very-long-chain fatty acids at the plasma membranes of plant cells (Boger, 2003). On the other hand, thiobencarb is also a selective herbicide that acts by inhibiting biosynthesis of fatty acid (Tomlin, 1994), but the mechanism of action and metabolism pathway of sunflower leaf extracts on plants varies with its allelochemicals (Macias *et al*., 2002; Anjum and Bajwa, 2005). These clearly show that, sunflower leaf extracts exhibit multiple sites of actions, which differ from pretilachlor or thiobencarb which show a single site of action. Since sunflower leaf extracts have different modes of action with pretilachlor or thiobencarb, these extracts and pretilachlor or thiobencarb probably have a greater chance to interfere at their respective sites of action, and thus do not compete for a common binding agent/site. This may explain synergism observed in the combination of sunflower extracts and pretilachlor or thiobencarb (Fig. 3). This is in agreement with a previous study conducted by Kaushik *et al*. (2006) who reported that mixtures of herbicides with different molecular targets exhibited synergistic activity. They found that strong synergism response in a combination of chlorsulfuron and pretilachlor that act as acetolactate synthesis inhibitor and long chain fatty acids inhibitor, respectively. Similarly, Crawford and Jordan (1995) found that combination of thiobencarb (as fatty acid inhibitor) and propanil (as photosynthesis inhibitor) was more effective as compared to single application of propanil in controlling barnyardgrass and providing the greatest economic return on rice yield.

Present results showed that antagonism occurs at a combination of 90% thiobencarb or pretilachlor and 10% sunflower leaf extract (Tables 2 and 3). This phenomenon is commonly found in herbicide mixture, where there is one particular ratio that shows antagonism, while other ratios give synergistic responses (Kudsk and Mathiassen, 2004; Syberg *et al*., 2007). Based on Figure 1A, sunflower leaf extracts at 3×10^4 ppm are only able to inhibit shoot emergence of barnyardgrass by 1%. Meanwhile, concentration of 10% sunflower leaf extracts is less than 2×10^4 ppm when combined with 90% thiobencarb or pretilachlor (Table 2 and 3). Sunflower leaf extracts at this concentration provide a hormetic effect on shoot emergence of barnyardgrass (Dilipkumar *et al*., 2011), thereby reducing the thiobencarb or pretilachlor activity by giving stimulation response on barnyardgrass shoot emergence. Interestingly, it is noted that a combination of 70% pretilachlor and 30% sunflower leaf extracts also shows synergistic response, though concentration of sunflower leaf extracts at this ratio is lower than the concentration at the ratio of 90:10 (Table 2). It is because the hormetic effect of sunflower leaf extracts at 1.8×10^4 ppm is stronger than that at 1.4×10^4 ppm (Dilipkumar *et al*., 2011). As a result, a higher concentration of pretilachlor is needed to overcome the hormetic effect, thereby leading to antagonism. A similar hormetic effect has been observed in a study by Cedergreen *et al*. (2005), who have found that metsulfuron-methyl at low concentration shows hormetic effect on the leaf growth of barley plants. Increasing the concentration of metsulfuron-methyl from 0.01 to 0.1 ppb can increase the leaf length from 190 to 210% as compared to control treatment.

In conclusion, sunflower leaf extracts have potential to reduce rate of tested herbicides for inhibiting emergence and growth of barnyardgrass. However, the results of joint action analysis have revealed that the synergistic action of the allelopathic crop water extracts and the herbicide is combination ratio-dependent. Since this study was carried out under laboratory conditions, caution should be taken regarding the ecological implications of the data, because phytotoxicity of the herbicide and sunflower leaf extracts is influenced by biotic and abiotic factors in soil. Although we expect similar trends in the growth response with receiver species other than barnyardgrass, further research with different receiver plants in the field are desirable.

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