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COMPARATIVE PHYTOTOXICITY OF SOME BENZOXAZINOIDS ON THE EARLY GROWTH OF SELECTED WEEDS

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ABSTRACT

Benzoxazinoids (or hydroxamic acids), the naturally occurring secondary plant metabolites, mainly in graminceous crops exhibit great structural diversity. The present study explored the phytotoxicity of five selected benzoxazinoids *viz.* 2-benzoxazolinone (BOA), 3-Methyl-2-benzoxazolinone (Me-BOA), 2-H-1, 4-benzoxazin-3(4H)-one (H-BOA), 6-Methoxy-2-benzoxazolinone (M-BOA) and 6-Hydroxy-2-benzoxazolinone (Hy-BOA) on the early growth (in terms of root shoot length) of three weeds namely *Cassia occidentalis* L., *Echinochloa crus-galli* (L.) Beauv. and *Phalaris minor* Retz. The results clearly indicate the negative influence of benzoxazinoids on weed growth. The effect in all cases was a function of concentration. In all test plants maximum effect was observed with the treatment of Me-BOA and M-BOA, followed by Hy-BOA, H-BOA and least incase of BOA. Seeds of *E. crus-galli* and *P. minor* were found to be more sensitive to benzoxazinoid treatment as compared to *C. occidentalis*.

INTRODUCTION

Nature is full of bioactive compounds with unexploited properties. Some of these compounds have a potential to be used directly as pest or weed control agents (Vyvyan, 2002). The knowledge of chemical relationships between plants may allow the development of new herbicides (Macias *et al*., 2001). Natural plant products offer virtually infinite source of chemical structures which could serve as ideal leads for new herbicide discovery (Macias *et al*., 2008). Macias *et al*. (2002) isolated 125 natural allelopathic compounds from different cultivars of sunflower which exhibited phytotoxicity towards many weeds. Haig *et al*., (2005) screened over 130 plant species in search for the plant extracts as an alternative control option for annual ryegrass (*Lolium rigidum*). Reduced reliance on traditional herbicides via the use of allelopathy has frequently been mentioned as environmentally favorable (Macias, 1995; Narwal *et al*., 1998). Analogous to the synthetic herbicides, the structural diversity among the allelochemicals is indicative of the diversity in their mode of action. Natural plant products with biological activity are the main source for new chemical structures useful in the development of molecules with potential utilization in agronomy (Macias *et al*., 2008; Macias *et al*., 2010).

Benzoxazinoids (or hydroxamic acids) are the naturally occurring secondary plant metabolites reported mainly in the family Poaceae (*Triticum aestivum*, *Zea mays*, *Secale cereale* etc.) (Barnes *et al*., 1987; Niemeyer, 1988; Niemeyer *et al*., 1990). These secondary metabolites are bound as glucosides in plant vacuoles and released by α-glucosidases upon disruption of cellular integrity (Yue *et al*., 1998). Major agricultural crops such as wheat (*Triticum aestivum*) and rye (*Secale cereale*) produce benzoxazolin-2-one (BOA) and 6-methoxybenzoxazolin-2-one (M-BOA), respectively, from their corresponding hydroxamic acids 2, 4-dihydroxy-1, 4-(2H)-benzoxazin-3-one (DIBOA) and 2, 4-dihydroxy-7-methoxy-1, 4-(2H)-benzoxazin-3-one (DIMBOA). These benzoxazinoids get leached into soil where they are degraded rapidly into a number of other compounds wit greater bioeffiicacy. Among the various degradation products released, 2-benzoxazolinone (BOA), a relatively stable compound is known to exhibit potential phytotoxicity against a number of plants such as *Avena sativa* L., *Cucumis sativus* L., *Lactuca sativa* L., *Lepidium sativum* L. etc. (Chase *et al*., 1991; Friebe *et al*., 1996; Kato-Noguchi and Macias, 2005).

Benzoxazinoids have particularly gained the attention of researchers for its great structural diversity and differential phytotoxicity of the transformation products. There are several structurally modified derivatives of benzoxazinoids available naturally as well as in synthetic form. Further, efforts are being made for enhancing the phytotoxicity of benzoxazinoids through modifications of steric and electronic features of the basic benzoxazinoid skeleton (Macias *et al*., 2010). This structural activity relationship has been extensively explored by several researchers earlier but with regards to chemical structure in particular (Macias *et al*., 2006; Villagrasa *et al*., 2009; Macias *et al*., 2010). However, there is no report available pertaining to the comparative phytotoxicity of benzoxazinoids on the growth and establishment of commonly growing weeds. Therefore, the present study was undertaken to assess the phytotoxicity of selected benzoxazinoids *viz*. 2-benzoxazolinone (BOA), 3-Methyl-2-benzoxazolinone (Me-BOA), 2-H-1, 4-benzoxazin-3(4H)-one (H-BOA), 6-Methoxy-2-benzoxazolinone (M-BOA) and 6-Hydroxy-2-benzoxazolinone (Hy-BOA) on the early growth of three weeds. The phytotoxicity was assessed by studying their effect on the radicle and plumule length of three test weeds *viz*. *Cassia occidentalis* L., *Echinochloa crus-galli* (L.) Beauv. and *Phalaris minor* Retz.

MATERIALS AND METHODS

Source of Chemicals and Seeds

2-Benzoxazolinone (BOA, purity 98%), 6-Methoxy-2-Benzoxazolinone (M-BOA, purity 97%) and 6-Hydroxy-2-benzoxazolinone (Hy-BOA, purity 97%), were purchased from Sigma Aldrich U.S.A. Sigma Chemicals Ltd (St Louis, MO, USA). 3-Methyl-2-benzoxazolinone (Me-BOA, purity 98%) and 2-H-1, 4-Benzoxazin-3(4H)-one (H-BOA, purity 99%) were purchased from Acros Organics U.S.A. Seeds of various weeds like coffee weed (*Cassia occidentalis* L.) were collected from wildly growing strands in Panjab University Campus, Chandigarh whereas seeds of barnyard grass (*Echinochloa crus-galli* [L.] Beauv.), and little seed canary grass (*Phalaris minor* Retz.) were collected from the agricultural fields in and around Chandigarh.

Preparation of solutions

A stock solution (1000 μM) of BOA and other test compounds was prepared by dissolving the requisite amount in ethanol and the final volume made with distilled water. The final concentration of alcohol in the stock solution was 0.2% and the same proportion was added to distilled water control. The stock solution was further diluted to get working solutions of 1, 10 and 100 μM and along with the stock solution of 1000 μM were used for the dose response study.

Germination Bioassay

Seeds of test plants were surface sterilized with sodium hypochlorite (0.1% w/v) for 1-2 min and washed with distilled water three times. Seeds of *C. occidentalis* and *P. minor* were treated with concentrated sulphuric acid (H_2SO_4) for 1 and 2 min, respectively and washed several times with distilled water to remove traces of acid completely. Scarified seeds of *C. occidentalis* and *P. minor* and sterilized seeds of *E. crus-galli*, were dipped in distilled water for 48 hr for imbibition prior to germination trials. The imbibed seeds (25 in number) were then equidistantly placed on single layer of Whatman No. 1 filter circle moistened with 7 mL of the respective treatment solution and distilled water as control in a 15 cm diameter Petri dish. At least five replicates were maintained per treatment and all the Petri dishes were placed in a seed germinator at 25 $\pm$ 2°C, 75$\pm$3% relative humidity and a 16:8 hr light: dark photoperiod with a photon flux density of approximately 150 μmoL $m^{-2}s^{-1}$. After 7th day of germination root and shoot length of the emerged seedlings was measured in all the treatments including control.

Statistical Analysis

For each treatment including control there were five replicates. All experiments were repeated twice. The data was subjected to one-way analysis of variance followed by separation of means using post hoc Tukey's test.

RESULTS

Root Length

The results (as evidenced from the Fig. 1- 6) clearly indicate the negative influence of benzoxazinoids on the early growth of the test weeds. In all respects the effect was concentration dependent *i.e.* more decrease was observed at highest concentration. The root length in *C. occidentalis* was measured to be 4.28 $\pm$ 0.09 cm in control (Fig. 1). At the same time at 1 μM concentration of benzoxazinoids the root length was measured to be in the range varying from 2.90 to 3.50 cm, exhibiting a reduction between 18 to 33%. At still higher concentration of 10 μM a further decline in the root length was observed. At highest concentration of 1000 μM maximum reduction (more than 92%) was observed in Me-BOA and H-BOA treatment. In other benzoxazinoids the reduction was statistically significant compared to control (Fig. 1).

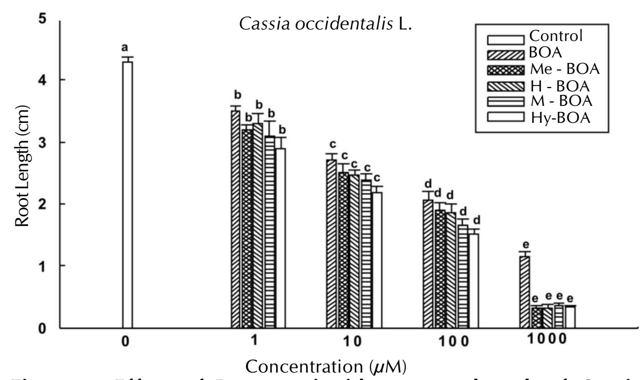

Figure 1: Effect of Benzoxazinoids on root length of *Cassia occidentalis* L.

In case of *E. crus-galli* root length in control was 7.62 $\pm$ 0.10 cm (Fig. 3). At lower benzoxazinoid concentrations of 1 and 10 μM the reduction in root length was not very significant. At higher concentrations of 100 and 1000 μM though same trend of inhibition was observed, yet there was variability w.r.t. activity of different benzoxazinoids. At the highest concentration (*i.e.* 1000 μM) maximum effect was observed

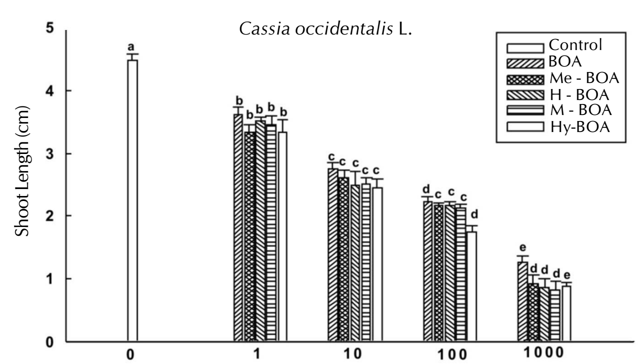

Figure 2: Effect of Benzoxazinoids on shoot length of *Cassia occidentalis* L.

Different alphabets on the bars of different concentrations of a particular treatment in each Fig. represent significant difference applying Tukey's test at pd"0.05

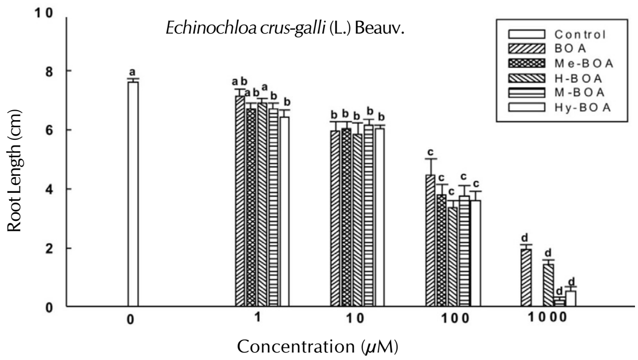

Figure 3: Effect of Benzoxazinoids on root length of *Echinochloa crus-galli* (L.) Beauv.

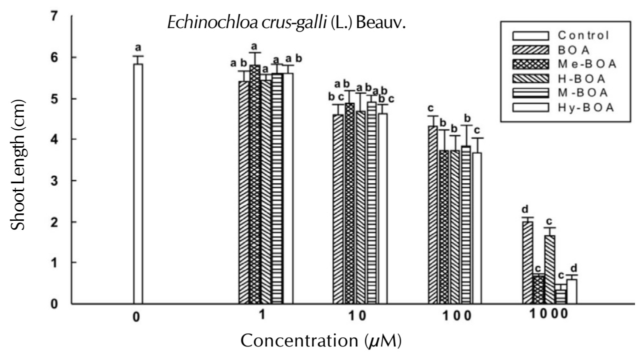

Figure 4: Effect of Benzoxazinoids on shoot length of *Echinochloa crus-galli* (L.) Beauv.

Different alphabets on the bars of different concentrations of a particular treatment in each figure represent significant difference applying Tukey's test at pd"0.05

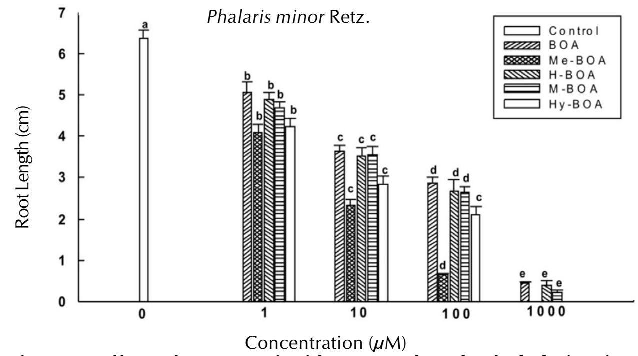

Figure 5: Effect of Benzoxazinoids on root length of *Phalaris minor* Retz.

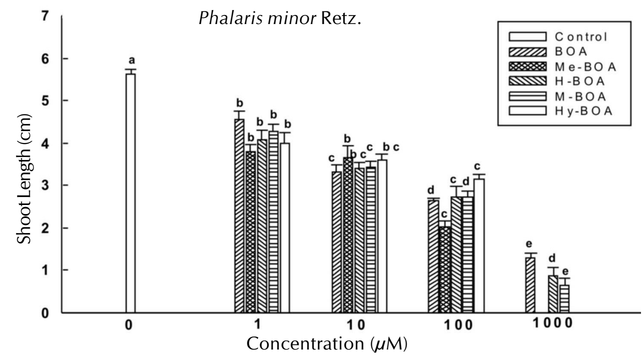

Figure 6: Effect of Benzoxazinoids on shoot length of *Phalaris minor* Retz.

Different alphabets on the bars of different concentrations of a particular treatment in each figure represent significant difference applying Tukey's test at pd"0.05

with the treatment of M-BOA, followed by Me-BOA, Hy-BOA, H-BOA and least effect was incase of BOA (Fig. 3).

In *P. minor* root length of the untreated control was 6.36 ± 0.19 cm (Fig. 5). Compared to this the root length was measured to be shorter in all treatments and the difference was a function of concentration. Consequently in this case also there was maximum effect at highest concentration and minimum at lowest concentration. At 1000 µM the reductions were maximum in all the five benzoxazinoids with complete inhibition of radicle emergence by Me-BOA and Hy-BOA while in other treatments also reduction was more than 90% (Fig. 5).

Shoot Length

As the observation of root length, the shoot length was also significantly and variably reduced upon exposure to different test benzoxazinoids. The percent reduction was however observed to be directly proportional to the increasing concentration of the treating benzoxazinoids. The shoot inhibitory effect of benzoxazinoids was found to be less than their root inhibitory potential. Maximum shoot length *i.e.* 4.48 ± 0.10 cm was observed in the untreated control seedlings of *C. occidentalis* (Fig. 2). Even at 1 µM concentration percent reduction was calculated to be nearly 19, 25, 21, 23 and 25% for BOA, Me-BOA, H-BOA, M-BOA and Hy-BOA, respectively. Further decline in shoot length was observed at higher concentrations. At 1000 µM concentration maximum reduction was induced by M-BOA (82%), followed by Me-BOA (79%), while that for H-BOA and Hy-BOA was nearly same, being nearly 80% respectively and minimum reduction was caused by BOA (72%) (Fig. 2).

The shoot length of untreated control seedlings in *E. crus-galli* was maximum, measured to be 5.84 ± 0.19 cm (Fig. 4). On treatment with 1 µM concentration of the benzoxazinoids, the shoot length was reduced by ~ 7.5, 0.7, 6.9, 4.1 and 4.1% for BOA, Me-BOA, H-BOA, M-BOA and Hy-BOA respectively. At 1000 µM concentration the reduction in shoot length was nearly 66, 72, 88, 90 and 94% incase of BOA, H-BOA, Me-BOA, Hy-BOA and M-BOA. Thus it is clearly evident that M-BOA exhibited maximum phytotoxicity and BOA the least (Fig. 4).

The shoot length of untreated control seedlings of *P. minor* was measured to be 5.62 ± 0.13 cm (Fig. 6). On treatment with 100 µM BOA, Me-BOA, H-BOA, M-BOA and Hy-BOA the shoot length was reduced by nearly 53, 64, 52, 51 and 44% respectively. The shoot emergence and growth was completely inhibited in response to 1000 µM treatment of Me-BOA and Hy-BOA. While the percent inhibition for 1000 µM BOA, H-BOA and M-BOA was calculated to be 77, 85and 89% respectively (Fig. 6).

DISCUSSION

It is thus clear from the present study that BOA and other benzoxazinoids interfere with the early growth of weeds *viz. Cassia occidentalis* L., *Echinochloa crus-galli* (L.) Beauv. and *Phalaris minor* Retz. Several reports of phytotoxic interference of hydroxamic acids on the growth of other plants are already available (Barnes *et al.*, 1987; Niemeyer, 1988; Perez, 1990;

Burgos and Talbert, 2000; Singh *et al*., 2005; Batish *et al*., 2006; Singh *et al*., 2009). Earlier Perez, (1990) reported inhibition of root growth of wild oat, *Avena fatua* L. by 2, 4-Dihydroxy-7-methoxy- 1, 4-benzoxazin-3-one (DIMBOA), the main hydroxamic acid of wheat, and its decomposition product 6-methoxy-benzoxazolin-2-one (MBOA), at concentrations of 0.7 and 0.5 mM respectively. 6-Methoxy-benzoxazolin-2-one also inhibited seed germination of *A. fatua* at all concentration tested. However, it stimulated root growth in *A. sativa* at concentrations below 1.5 mM and inhibited it at higher concentration (Perez, 1990). Burgos and Talbert (2000) reported that BOA interferes with the germination of some crops and seeds, however its effect is more on small rather than on large seeded crops. In our study also same observation was made as the effect was more pronounced on small seeded weeds (*viz. E. crus-galli* and *P. minor*) as compared to large seeded weed (*viz. C. occidentalis*). The hydroxamic acids are effective not only in pure extracted forms but in the form of their leachates being exuded from the mulch crops as indicated by the study of Gavazzi *et al*. (2010). They explored the weed suppressiveness of rye mulch (known to release benzoxazinoids) on three warm season weeds (*Chenopodium album* L., *Amaranthus retroflexus* L., and *Portulaca oleracea* L.) in greenhouse. The weed suppression thus observed was attributed to the contents of benzoxazinoids, DIBOA and DIBOA-Glucose in rye cultivar tissues. Field study too yielded appreciable results with mulching, which significantly reduced the density of grass and broadleaf weeds by 61% and 96%, respectively (Gavazzi *et al*., 2010).

Though reports are available for the phytotoxicity effect of BOA, however, so far no such reports are available for the other hydroxamic acids (chosen for the present study). Our observations indicate that structurally modified derivatives of BOA are more effective than BOA. Studies of Macias *et al*. (2008, 2010) have shown that structurally modified derivatives of benzoxazinoids are more phytotoxic and thus there is lot of scope for improvement of bioefficacy of benzoxazinoids compounds. Based on our observations the effect of Me-BOA was maximum followed by M-BOA, Hy-BOA, H-BOA and BOA.

Thus based on the observations in the present study it can be concluded that benzoxazinoids possess potential for weed control, provided specific studies are conducted to test their toxicity against crops.

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