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Phytotoxic effect of 2-benzoxazolinone (BOA) against some vegetable crops

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Abstract

Benzoxazolin-2(3H)-one (BOA) is a well known allelochemical that is being explored for its herbicidal activity. However, not much is known about its effect on crop plants. The present study investigated the effect of BOA on germination and early growth of four vegetable crops *viz. Pisum sativum* L., *Raphanus sativus* L., *Brassica oleracea* L. var. botrytis and *Brassica oleracea* L. var. capitata. At 1000 µM, germination of *P. sativum*, *R. sativus* and *B. oleracea* var. botrytis was reduced by more than 50%, whereas that of *B. oleracea* var. capitata was completely suppressed. Further, BOA reduced the root and shoot length of the test plants by ~ 40-82% and ~55-85%, respectively. In general, the effect was more pronounced on the root (~82% in *B. oleracea* var. botrytis) than on the shoot growth (~73% *B. oleracea* var. botrytis). 2-Benzoxazolinone significantly enhanced the contents of proteins (by 6-28%) and carbohydrates (by 61-189%) in *B. oleracea* var. capitata and decreased the activities of related enzymes like proteases (by 13-36%), α-amylases (19-60%) and β-amylase (25-70%). The observed decline in the activities of hydrolytic enzymes amylases suggest that BOA interferes with the vital metabolic processes in the germinating seedlings leading to growth reduction. The study reveals that BOA interferes with the germination and early seedling growth of vegetable crops and induces biochemical alterations.

Key words

2-Benzoxazolinone, Germination, Phytotoxicity, Seedling growth, Vegetable crops

Introduction

Allelopathy is a process involving secondary plant metabolites that negatively influence the growth and development of other plants (Rice, 1984). Chemicals that bring about the phenomenon of allelopathy are known as allelochemicals and are involved in practically every aspect of plant growth acting from stimulants to suppressants (Singh *et al*., 2001). In modern sustainable agricultural system, the use of these allelochemicals is being encouraged to utilise this untapped resource for weed control thereby reducing the concerns of ecological, environmental and health problems associated with synthetic pesticides (Singh *et al*., 2003). Natural plant products with biological activity are the ideal leads for new chemical structures useful in the development of molecules with potential utilization in agronomy.

Unlike traditionally thought of, crops are now known to possess greater potential in managing pests and weeds in a number of ways owing to their allelopathic nature. As many as 35 crops with allelopathic tendencies have been enlisted (Batish *et al*., 2001) and can be utilized for sustainable weed management. Allelopathic property of cover, smother and green manure crops grown in rotation has proved as a strategy worth exploiting for weed management (Singh *et al*., 2001; Batish *et al*., 2002). Further, the residues of allelopathic crops like rye, sunflower, wheat and barley also bear a great potential in suppressing weeds (Batish *et al*., 2001; Singh *et al*., 2003).

Wheat (*Triticum aestivum* L.) is a major cereal crop of Northern India and exhibits allelopathic tendency that has been

extensively examined for its weed suppressing potential (Bertholdsson, 2005; Wu *et al*., 2002, 2003). Such an activity of wheat has been attributed mainly to benzoxazolinones (cyclic hydroxamic acids) formed as a result of secondary metabolism. 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), a major hydroxamic acid found in wheat and its degradation products 6-methoxy-2-benzoxazolinone (MBOA) and BOA (2-benzoxazolinone) exhibit phytotoxicity against the weeds and other crop plants. Amongst these, BOA has been extensively studied for its herbicidal potential (Burgos and Talbert, 2000; Singh *et al*., 2005). However, there is no report available pertaining to the effect of BOA on the growth and establishment of other crops such as *Pisum sativum*, *Brassica* spp. or *Raphanus sativus*. Therefore, the present study was undertaken to assess the phytotoxic effect of BOA on the early growth of four vegetable crops, namely, *Pisum sativum* L., *Raphanus sativus* L., *Brassica oleracea* L. var. botrytis and *Brassica oleracea* L. var. capitata commonly grown during the wheat growing season in the northern India. Further, to assess whether BOA-induced growth inhibition involved any biochemical changes, we assessed the alterations in protein and biochemical contents and changes in activities of related hydrolytic enzymes (proteases, α- and β-amylases, polyphenol oxidases).

Materials and Methods

2-Benzoxazolinone (BOA) (>98% purity) of technical grade was purchased from Sigma-Aldrich, Inc. (St. Louis, USA). Certified and pure-line seeds of crop plants *viz*. *Pisum sativum* L. (pea), *Raphanus sativus* L. (radish), *Brassica oleracea* L. var. botrytis (cauliflower) and *Brassica oleracea* var. capitata (cabbage) were purchased from the local seed store. A stock solution (1000 µM = 135.12 µg ml^{-1}) of BOA was prepared by dissolving the requisite amount in ethanol and the final volume made in 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 concentrations of 1, 10 and 100 µM and used for the dose response study.

Germination studies: Seeds of test plants were surface sterilized with sodium hypochlorite (0.1% w/v) followed by washing with distilled water three times. These were imbibed in respective treatment solutions or distilled water (as control) for 8 hr. The imbibed seeds (25 in number) were then equidistantly placed on a single layer of Whatman No. 1 filter circle moistened with 7 ml of the respective treatment solution in a 15 cm diameter petri dish. At least five replicates were maintained per treatment including control and all the petri dishes were placed in a seed germinator at 25 ± 2ºC, 75 ± 3% relative humidity, and a 16:8 hr light: dark photoperiod with a photon flux density of ~150 µmol $m^{-2}s^{-1}$. Germination was recorded after 3rd day whereas root and shoot length of the emerged seedlings were measured after 3, 5 and 7th day in all the treatments.

Biochemical estimations and enzyme activities: To evaluate the biochemical changes in response to BOA, we measured the contents of proteins, carbohydrates and phenolics and the activities of enzymes–proteases, α- and β-amylases, and polyphenol oxidases– in the 7-day-old roots of one of the test crop, *Brassica oleracea* var. capitata.

Root tissue (200 mg) was crushed in 10 ml of distilled water and the contents were passed through double layer of muslin cloth followed by centrifugation at 15,000 × g for 15 min. The supernatant so obtained was used for determination of protein, carbohydrate and phenolic content. The amount of proteins was determined by the method of Lowry *et al.* (1951) against a calibration standard of bovine serum albumin. The content of carbohydrates was determined by the method of Loewus (1952) using Anthrone reagent. The phenolic content was determined using Folin-cioclateu reagent as per Batish *et al*. (2007).

The activities of enzymes were determined in crude enzyme extract prepared by homogenizing root tissue (100 mg) in 5 ml of 0.1M phosphate buffer (pH 7.0) in a pre-chilled pestle and mortar. The homogenate was centrifuged at 18,000 × *g* for 15 min and the supernatant was stored at 4ºC until further use for assaying enzyme activities (Batish *et al*., 2006a). Proteases were assayed using casein (1% in 0.1 M phosphate buffer, pH 6.0) as substrate solution by the method of Basha and Beevers (1975). Activity of α- and β-amylases was determined using starch as a substrate following the methods of Muentz (1977) and Bernfeld (1951), respectively. Polyphenol oxidases were assayed using catechol (0.01 M in 0.1 M phosphate buffer, pH 6) as a substrate (Van Lelyvold and Pretorius, 1973).

Statistical analysis: For each treatment including control, there were five replicates. All the experiments were repeated once. The data were subjected to one - way analysis of variance (ANOVA) followed by separation of means using post hoc Tukey's test using SPSS package ver. 10 (SPSS, 1999).

Results and Discussion

The results indicate that BOA inhibit the germination of all the test crops *viz*. *Pisum sativum*, *Raphanus sativus*, *Brassica oleracea* var. botrytis and *Brassica oleracea* var. capitata particularly

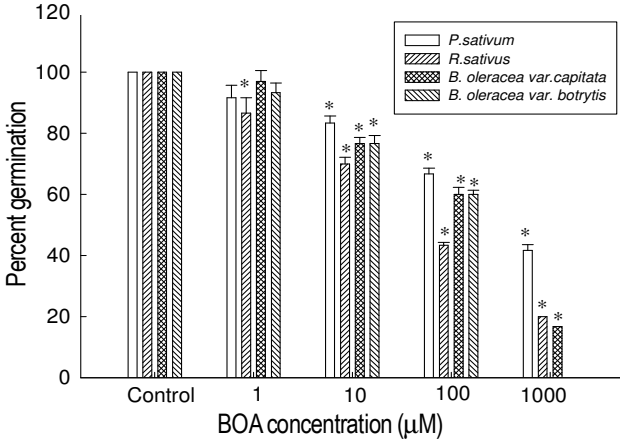

Fig. 1: Effect of 2-Benzoxazolinone (BOA) on % germination of the test crops measured on the 3rd day of germination. *indicates significance over to control at P< 0.05

Table - 1: Effect of BOA on root and shoot length of *Pisum sativum* L.

Conc. (µM)	Root length			Shoot length		
	3rd day	5th day	7th day	3rd day	5th day	7th day
Control	3.12±0.56	4.76±0.86	6.30±0.46 a	NM	1.22±0.19	1.40±0.19
1	2.74±0.32 (–12.18)	4.22±0.39(–11.34)	5.18±0.46(–17.78)	NM	0.50±0.32(–59.02)	1.10±0.11*(–21.43)
10	2.64±0.27 (–15.38)	4.00±0.53(–15.97)	4.92±0.50*(–21.90)	NM	0.34±0.24*(–72.13)	1.02±0.34*(–27.14)
100	2.42±0.51*(–22.44)	3.60±0.55*(–24.37)	4.18±0.18*(–33.65)	NM	0.14±0.14*(–88.52)	0.52±0.12*(–62.86)
1000	1.98±0.23*(–36.54)	2.98±0.20*(–37.39)	3.78±0.23*(–40.00)	NM	0(–100)	0.22±0.14*(–84.29)

NM = not measurable, Values are mean of five replicates ± SE, Value in parenthesis is percent decrease in growth over that in control, * indicates significance over control at P<0.05

Table - 2: Effect of BOA on root and shoot length of *Raphanus sativus* L.

Conc. (µM)	Root length			Shoot length		
	3rd day	5th day	7th day	3rd day	5th day	7th day
Control	3.84±0.08	6.84±0.97	9.90±0.82	2.10±0.03	3.96±0.19	5.24±0.04
1	3.42±0.23(–10.94)	5.70±0.22(–16.67)	8.92±0.78(–9.90)	2.02±0.17(–3.81)	3.38±0.06(–14.65)	4.92±0.34(–6.11)
10	3.22±0.12(–16.15)	4.82±0.39*(–29.53)	8.56±0.91(–13.54)	1.56±0.14*(–25.71)	3.10±0.04*(–21.72)	4.68±0.22(–10.69)
100	2.74±0.31*(–28.65)	3.86±0.13*(–43.57)	7.06±0.83*(–28.69)	1.38±0.07*(–34.29)	3.06±0.12*(–22.73)	3.96±0.23*(–24.43)
1000	0.82±0.26*(–78.65)	2.00±0.55*(–70.76)	3.08±0.17*(–68.89)	0.18±0.12*(–91.43)	1.46±0.27*(–63.13)	1.98±0.36*(–62.21)

Values are mean of five replicates ± SE, Value in parenthesis is percent decrease in growth over that in control, * indicates significance over control at P<0.05

Table - 3: Effect of BOA on root and shoot length of *Brassica oleracea* L. var. botrytis

Conc. (µM)	Root length			Shoot length		
	3rd day	5th day	7th day	3rd day	5th day	7th day
Control	2.30±0.20	5.64±0.15	6.60±0.16	0.60±0.05	1.58±0.09	2.62±0.06
1	2.16±0.14(–6.09)	4.64±0.18*(–17.73)	5.84±0.30(–11.52)	0.60±0.01(–0.00)	1.42±0.08(–10.13)	2.56±0.09(–2.29)
10	2.02±0.12*(–12.17)	3.86±0.21*(–31.56)	5.64±0.16*(–14.55)	0.58±0.02 (–3.33)	1.20±0.03*(–24.05)	2.34±0.07*(–10.69)
100	1.94±0.11*(–15.65)	3.68±0.12*(–34.75)	4.80±0.27*(–27.27)	0.52±0.02*(–13.33)	1.20±0.06*(–24.05)	2.28±0.17*(–12.98)
1000	NM*	NM*	1.22±0.12*(–81.52)	NM*	NM*	0.70±0.00*(–73.28)

NM = not measurable, Values are mean of five replicates ± SE, Value in parenthesis is percent decrease in growth over that in control, * indicates significance over control at P<0.05

Table - 4: Effect of BOA on root and shoot length of *Brassica oleracea* L. var. capitata

Conc. (µM)	Root length			Shoot length		
	3rd day	5th day	7th day	3rd day	5th day	7th day
Control	1.90±0.03	4.88±0.25	9.70±0.42	0.50±0.00	1.70±0.16	3.54±0.13
1	1.90±0.10(–0.00)	4.52±0.27(–7.38)	9.12±0.62(–5.98)	NM	1.48±0.07(–12.94)	2.60±0.21(–26.55)
10	1.76±0.10(–7.37)	4.22±0.10*(–13.52)	8.44±0.28*(–12.99)	NM	1.42±0.05*(–16.47)	2.42±0.17*(–31.64)
100	1.60±0.11*(–15.79)	3.98±0.24*(–18.44)	7.58±0.18*(–21.86)	NM	1.32±0.10*(–22.35)	2.32±0.10*(–34.46)
1000	-	0.40±0.24*(–91.80)	2.94±0.04*(–69.69)	-	0.22±0.14*(–87.06)	1.54±0.12*(–56.50)

- = no germination, NM = not measurable, Values are mean of five replicates ± SE, Value in parenthesis is percent decrease in growth over that in control, * indicates significance over control at P<0.05

at ≥100 µM (Fig. 1). At 1000 µM, germination of all the test crops was reduced by more than 50% whereas that of *B. oleracea* var. capitata was completely suppressed (Fig. 1). It clearly indicates that BOA interferes with the germination of test crops. Further, the root and shoot length (measured at three different stages *i.e.* on 3rd, 5th and 7th day of germination) of all the test plants were also measured to be lesser in response to BOA compared to control (Table 1-4). The effect was more pronounced on the root growth than on the shoot elongation. On 7th day of germination, the root length of *P. sativum* at 1000 µM BOA was lesser by over 40% as compared to shoot length which was lesser by over 84% (Table 1). In case of *R. sativus,* the root length on the 7th day of germination was found to be lesser by over 68% and while that of shoot length was lesser by nearly 62% at 1000 µM BOA in comparison to control (Table 2). The root length of *B. oleracea* var. botrytis at the highest concentration of BOA was reduced by nearly 81% after 7th day and

Table - 5: Effect of BOA on the content of proteins, carbohydrates and phenolics in the roots of *B. oleracea* var. capitata

Concentration (μM)	Protein (μg mg^{-1} f. wt.)	Carbohydrates (μg mg^{-1} f. wt.)	Phenolics (μg mg^{-1} f. wt.)
Control	91.36±0.25	13.56±0.07	1.96±0.01
1	90.18±0.43(–1.29)	14.04±0.09(+3.39)	2.81±0.05*(+43.37)
10	97.04±0.52*(+6.22)	21.86±0.03*(+61.21)	3.49±0.03*(+78.06)
100	108.18±0.56*(+18.41)	29.33±0.05*(+116.30)	5.40±0.02*(+179.59)
1000	117.23±0.49*(+28.32)	39.18±0.07*(+188.94)	8.14±0.01*(+315.31)

Values are mean of five replicates ± SE, Value in parenthesis represent percent increase (+) /decrease (–) over control, * indicates significance over control at P<0.05

Table - 6: Effect of BOA on the specific activity of proteases, α–amylases, β–amylases and polyphenol oxidases in the roots of *B. oleracea* var. capitata

Concentration (μM)	Proteases (μg hr^{-1} mg^{-1} protein)	α–amylases (μg min^{-1} mg^{-1} protein)	β–amylases (μg min^{-1} mg^{-1} protein)	Polyphenol oxidases (Kat sec^{-1} mg^{-1} protein)
Control	76.42±2.39	45.45±0.33	33.41±0.29	0.223±0.016
1	72.19±3.42(–5.54)	43.33±0.27(–4.66)	30.99±0.56(–7.24)	0.236±0.013(+5.83)
10	66.31±4.21*(–13.23)	36.89±0.38*(–18.83)	25.19±0.16*(–24.60)	0.281±0.043*(+26.01)
100	60.09±3.60*(–21.37)	29.11±0.25*(–35.95)	18.55±0.13*(–44.48)	0.359±0.021*(+60.99)
1000	49.12±4.16*(–35.72)	18.11±0.32*(–60.15)	9.96±0.25*(–70.19)	0.461±0.009*(+106.73)

Values are mean of five replicates ± SE, Value in parenthesis represent percent increase (+) /decrease (–) over control, *indicates significance over control at P< 0.05.

that of shoot was reduced by over 73% (Table 3). At similar concentration, the root length of *B. oleracea* var. capitata on 7th day of germination was lesser by over 69%, whereas the reduction in shoot length was ~ 57% (Table 4). The maximum effect of BOA on radicle length was observed in *B. oleracea* var. botrytis where ~ 82% reduction was observed (Table 3) and the maximum effect on shoot length was observed on *P. sativum* where a reduction of ~85% over that of control was noticed (Table 1).

The observed inhibitory activity of BOA is in agreement with the earlier reports that BOA reduces growth of other plants and is suppressant of root growth (Belz and Hurle, 2004; Burgos *et al*., 2004; Singh *et al*., 2005). Earlier, BOA has been reported to suppress the emergence and growth of weeds (Burgos and Talbert, 2000) and inhibit root growth of *Cucumis sativus* (Burgos *et al*., 2004). It has also been demonstrated to inhibit the formation of adventitious roots in mung bean hypocotyls (Singh *et al*., 2005). In the present study, *P. sativum* (a large seeded crop) was less affected (in terms of germination and root length) compared to *Brassica* species and *R. sativus* (small seeded crops). It is in agreement with an earlier report that BOA possesses species-specificity since it is more phytotoxic towards small-seeded plants than large-seeded plants (Burgos and Talbert, 2000). Such a property can be easily manipulated for the management of small-seeded weeds associated with large seeded crops.

2-Benzoxazolinone is a well-known allelochemical reported from several graminaceous crops (Barnes *et al*., 1987). It is also reported to possess pesticidal properties (Burgos and Talbert, 2000). Efforts have been made to understand its mode of action since it is biomolecule of interest for the agrochemists (Friebe, 2001). We, therefore, estimated the biochemical alteration in response to BOA treatment.

2-Benzoxazolinone (except at 1 μM) significantly enhanced the contents of macromolecules measured in the 7-day old roots of *B. oleracea* var. capitata. Protein content increased by ~28% compared to control at 1000 μM BOA (Table 5). The content of carbohydrates and phenolics was also greater in the BOA-treated roots of *B. oleracea* var. capitata compared to control. At 1000 μM BOA treatment, the carbohydrate content increased by ~189%, whereas phenolic content increased by ~315% over control (Table 5).

Unlike the macromolecular content, the specific activity of enzymes (except PPO) decreased in response to BOA. The activity of proteases decreased (significant at P<0.05, except 1 μM BOA treatment) with the treatment of BOA (Table 6). At 1000 μM, it decreased by ~36% over that in control (significant at P<0.05). Likewise, the activities of amylases decreased in BOA-treated roots (Table 6). At 1000 μM, the activity of α-amylase decreased by nearly 60% whereas that of β-amylase declined by 70% over control. On the contrary, the activity of PPO increased with the BOA treatment. At 100 μM, the activity increased by 61% whereas at 1000 μM ~107% increase was measured (Table 6).

The alterations in the macromolecular content and the activities of enzymes observed in the present study in response to BOA are parallel to an earlier observation that BOA-inhibited root formation involves interference with biochemical processes related to rhizogenesis (Singh *et al*., 2005). The observed decline in the activities of enzymes like proteases and α- and β-amylases suggest that BOA interferes with the vital metabolic processes in the germinating seedlings leading to growth reduction. The increased PPOs activity in response to BOA further indicates a BOA-induced stress in plant tissue as the activity of PPO relates positively to stress in plant tissue and there occurs enhanced induction of PPO to help in plant defence

(Thipyapong *et al*., 1995). In fact, Batish *et al*. (2006b) demonstrated that BOA induces generation of reactive oxygen species leading to oxidative damage.

In conclusion, the present study indicates that BOA affects the germination, early growth and induces biochemical changes in vegetable crops grown along with wheat.

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