Bangladesh J. Bot. **42**(1): 65-71, 2013 (June)

EFFECTS OF GROWTH REGULATORS ON SEED GERMINATION, SEEDLING GROWTH AND SOME ASPECTS OF METABOLISM OF WHEAT UNDER ALLELOCHEMICAL STRESS

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Key words: Allelochemical stress, Growth regulators, Seed germination, Metabolism

Abstract

Effects of growth regulators on seed germination, seedling growth and some aspects of metabolism of wheat under allelochemical stress due to oleander and walnut leaf (extract and powder) were investigated. All parameters e.g. seed germination, seedling growth, leaf pigments except the proline content reduced significantly under stress. On the contrary, GA_3 and IBA showed alleviating activities. The alleviation effect of GA_3 was higher than that of IBA .

Introduction

The chemical interactions that occur among living organisms including plants, insects and microorganisms are called allelopathy which induces direct or indirect harmful or beneficial effects through the production of allelochemicals (Putnam 1985, Whittaker and Feeny 1971). Allelochemicals are known to affect seed germination, growth, distribution and reproduction of a number of plant species (Inderjit and Malik 2002).

Allelopathic effects have been reported in many plant species including crops and weeds (Kazincki *et al*. 1997, Rice 1984, Turk and Tawaha 2002, Uygur and Iskenderoglu 1997). Oleanders (*Nerium oleander* L.) and walnuts (*Juglans nigra* L.) are common plants in Mediterranean and the Aegean region of Turkey. They produce a large number of allelochemicals (Matok *et al.* 2009, Rajyalakshmi *et al*. 2011).

Plant growth regulators are used to ameliorate stress impact due to allelochemicals (Terzi and Kocaçalışkan 2010). However, reports on ameliorating effect of growth regulators on plant growth and metabolism under stress due to allelochemicals is scanty. In the present study, ameliorating effects of growth regulators e.g., GA_3 and IBA on seed germination, seedling growth, total chlorophyll, carotenoids and proline contents of wheat seedling under allelochemical stress due to oleander and walnut leaf (extract and powder) were investigated.

Materials and Methods

Seed of wheat (*Triticum aestivum* L.) were obtained from the suppliers TASACO, Antalya, Turkey. One gram of air-dried plant leaf litter was separately soaked in one litter of double distilled water for 12 h at room temperature. Then, the mixtures were filtered through Whatman No.1 filter paper to obtain the oleander and walnut leaf extracts. Also, 10 ppm GA_3, 20 ppm GA_3, 10 ppm IBA and 20 ppm IBA solutions in distilled water were prepared. Seeds were surface sterilised with 5% sodium hypochloride. Twenty five wheat seeds were given in each Petri dish (10 cm dia) provided with two layers filter paper saturated with 5 ml of distilled water (control),

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with 2.5 ml of leaf extract (oleander-O, walnut-W) and 2.5 ml distilled water or 2.5 ml of leaf extract and 2.5 ml growth regulators. The Petri dishes were placed in a growth chamber at 25 ± 1ºC and light/dark regime of 16/8 hrs. Seeds were considered germinated after emergence of root from the caryopses and germination count was recorded up to one week. After one week, shoot and root lengths and fresh weight of the seedlings were measured. Dry weights were recorded after two days oven-drying at 65ºC. Experiment was replicated four times.

In another experiment, wheat seeds were sown in pots (20 × 30 cm^2, 25 seeds for each) filled with perlite and 3 g leaf powder (oleander or walnut) except control. Seedlings grown in a growth chamber (light/dark regime of 16/8 hrs at 25 ± 1ºC, relative humudity of 60 - 70% and light intensity 350 $\mu M/m^2/s$). Seedlings were irrigated (Hoagland and Arnon 1950) with 200 ml distilled water every other day in all groups, with 200 ml Hoagland solution and 200 ml distilled water in control group or with 200 ml Hoagland solution and 200 ml growth regulator solution (10 and 20 ppm GA_3, 10 and 20 ppm IBA) in treatment groups once a week for four weeks. After harvesting, plant samples were analyzed for growth parameters, leaf pigments and proline contents. Vigour index was determined according to Abdul-Baki and Anderson (1973) as vigour index: per cent germination × seedling length. For leaf pigments, 0.5 g fresh leaf samples of four-week-old-seedlings of wheat were extracted with 5 ml 80% acetone and absorbance of supernatants were measured spectrophotometrically at 652, 665 nm for chlorophylls and at 450 nm for carotenoids (Lichtenthaler 1987). Proline was determined in fresh leaf sample according to the modified method of Bates *et al*. (1973). Data were analyzed using one-way ANOVA followed by Fisher's LSD post-hoc test. The criteria for statistical significance were set at $p < 0.05$ and 0.01.

Results and Discussion

Germination of wheat seeds was inhibited slightly by oleander and walnut compared to control (Table 1). However, treatments of wheat seeds with GA_3 and IBA significantly alleviated this inhibition. GA_3 was found more effective than IBA to alleviate the inhibitory effect on seed germination (Table 1). Alleviation of inhibitory effect by GA_3 was 4.3% in oleander and 6.5 % in walnut. Seedling vigour index decreased with oleander and walnut leaf extract and increased with extract+10 ppm GA_3 and extract + 20 ppm GA_3 applications compared to the control (Table 1). Both root and shoot elongation of seven-day-old-seedlings of wheat decreased with oleander and walnut leaf extract compared to the control. This inhibition of root and shoot elongation was alleviated by GA_3 reatments. IBA treatments decreased root elongation but increased shoot elongation. On the other hand, generally growth regulators alleviation of oleander and walnut stress was higher on shoot elongation than on root elongation (Table 1). Similarly, fresh and dry weights of roots and shoots of seven-day-old-seedlings of wheat were also reduced by oleander and walnut leaf extract and the effect was alleviated by growth regulators. The greatest alleviation was achieved with oleander extract + 10 ppm IBA treatment 50% in shoot fresh and shoot dry weight, in root fresh weight and root dry weight and walnut extract + 10 ppm IBA with treatment alleviation of 29.23% in shoot fresh weight, 22.22% in shoot dry weight,16.24% in root fresh weight and 21.74% in root dry weight (Table 2).

Previous reports showed that allelochemicals of walnut and oleander inhibited germination and seedling growth of several plant species (Matok *et al*. 2009, Qian *et al*. 2010, Rajyalakshmi *et al*. 2011) and growth regulators can significantly overcome allelochemical stress on seed germination and seedling growth (Terzi and Kocaçalışkan 2010). It was indicated that oleander and walnut leaf extracts inhibited germination and seedling growth of wheat, and growth regulators overcome this allelochemical stress (Tables 1, 2).

Oleander and walnut extracts caused a reduction in seedling vigour index of wheat compared to the seedlings grown with distilled water only (Table 1). Djanaguiraman *et al*. (2005) found a similar type of result in rice, sorghum and blackgram due to *Eucalyptus globulus*. The reduction in

Table 1. Effects of growth regulators on seed germination, root and shoot growth of seven-day-old-seedlings of wheat grain under allelochemical stress due to oleander and walnut leaf extracts.

Treatments	Germination (%)	Vigour index	Root length (cm)	Shoot length (cm)
Extract + 0	93 ± 1.26	1780,95	9.95 ± 1.20	9.20 ± 1.00
Extract +10 ppm GA_3	97 ± 0.98**	2716,97	12.50 ± 0.97	15.51 ± 2.80**
Extract + 20 " GA_3	96 ± 0.82**	2271,36	12.33 ± 0.65	11.33 ± 1.23*
Extract + 10 " IBA	96 ± 0.82**	1909,44	8.82 ± 1.90	11.07 ± 0.82
Extract + 20 " IBA	95 ± 0.50*	1673,90	6.60 ± 1.00**	11.02 ± 0.66
Extract + 0	92 ± 0.82	1920,04	10.37 ± 2.02	10.50 ± 1.14
Extract + 10 ppm GA_3	98 ± 0.58**	2779,28	12.35 ± 0.81	16.01 ± 2.27**
Extract + 20 " GA_3	98 ± 1.00**	2398,06	11.78 ± 0.97	12.69 ± 0.63*
Extract + 10 " IBA	96 ± 0.82**	2069,76	9.59 ± 1.66	11.97 ± 1.02
Extract + 20 " IBA	95 ± 0.96**	1775,55	6.86 ± 1.64**	11.83 ± 0.93
Control (Seeds soakd with distilled water only)	96 ± 0.82**	2230.08	11.31 ± 3.09	11.92 ± 0.67*
$LSD_{0.01}$	2.245	-	3.355	2.781
$LSD_{0.05}$	1.667	-	2.491	2.065

LSD test was applied. * and ** indicate at significant $p < 0.05$ and 0.01, respectively.

Table 2. Effects of growth regulators on root and shoot fresh and dry weight of seven-day-old-seedlings of wheat grain under allelochemical stress due to oleander and walnut leaf extracts.

Treatments	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
Extract + 0)	0.131 ± 0.020	0.058 ± 0.013	0.025 ± 0.005	0.008 ± 0.002
Extract + 10 ppm GA_3	0.134 ± 0.022	0.087 ± 0.017*	0.027 ± 0.004	0.012 ± 0.002*
Extract + 20 " GA_3	0.133 ± 0.024	0.072 ± 0.012	0.026 ± 0.005	0.010 ± 0.002
Extract + 10 " IBA	0.139 ± 0.017	0.073 ± 0.008	0.028 ± 0.004	0.010 ± 0.002
Extract + 20 " IBA	0.135 ± 0.022	0.065 ± 0.013	0.027 ± 0.005	0.009 ± 0.003
Extract + 0	0.117 ± 0.019	0.065 ± 0.014	0.023 ± 0.004	0.009 ± 0.003
Extract + 10 ppm GA_3	0.119 ± 0.017	0.078 ± 0.011	0.024 ± 0.004	0.010 ± 0.001
Extract + 20 " GA_3	0.116 ± 0.021	0.066 ± 0.013	0.023 ± 0.003	0.009 ± 0.002
Extract + 10 " IBA	0.136 ± 0.021	0.084 ± 0.013	0.028 ± 0.005	0.011 ± 0.001
Extract + 20 " IBA	0.131 ± 0.020	0.069 ± 0.012	0.026 ± 0.004	0.010 ± 0.002
Control (Seeds soaked with distilled water only)	0.136 ± 0.017	0.071 ± 0.017	0.027 ± 0.007	0.010 ± 0.003
$LSD_{0.01}$	0.225	0.225	0.007	0.007
$LSD_{0.05}$	0.167	0.167	0.005	0.005

LSD test was applied. * and ** indicate at significant $p < 0.05$ and 0.01, respectively.

Table 3. Effects of growth regulators on root and shoot length, fresh and dry weight of four-week-old-seedlings of wheat grain under allelochemical stress due to oleander and walnut leaf powders.

Treatments	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Extract + 0)	5.20 ± 0.18	0.081 ± 0.012	0.019 ± 0.004	15.43 ± 1.65	0.078 ± 0.010	0.012 ± 0.001
Extract + 10 ppm GA_3	5.48 ± 1.03	0.083 ± 0.017	0.019 ± 0.001	21.55 ± 1.60**	0.088 ± 0.005	0.013 ± 0.001
Extract + 20 " GA_3	4.20 ± 1.06	0.068 ± 0.008	0.015 ± 0.002	20.43 ± 1.30**	0.060 ± 0.016	0.009 ± 0.002
Extract + 10 " IBA	3.38 ± 0.43*	0.035 ± 0.006**	0.007 ± 0.001**	14.90 ± 1.32	0.025 ± 0.006**	0.004 ± 0.002**
Extract + 20 " IBA	3.35 ± 0.83*	0.030 ± 0.008**	0.006 ± 0.009**	14.58 ± 0.62	0.020 ± 0.003**	0.003 ± 0.001**
Extract + 0	4.50 ± 1.60	0.083 ± 0.010	0.020 ± 0.008	17.80 ± 1.72	0.039 ± 0.006	0.006 ± 0.001
Extract + 10 ppm GA_3	6.30 ± 0.76*	0.104 ± 0.020	0.025 ± 0.004	20.70 ± 0.95*	0.059 ± 0.003*	0.009 ± 0.001
Extract + 20 " GA_3	5.38 ± 1.47	0.089 ± 0.003	0.022 ± 0.004	19.34 ± 0.41	0.050 ± 0.008	0.007 ± 0.001
Extract + 10 " IBA	3.90 ± 0.38	0.055 ± 0.010*	0.013 ± 0.002	17.45 ± 2.19	0.053 ± 0.005*	0.008 ± 0.001
Extract + 20 " IBA	3.18 ± 1.31	0.045 ± 0.013**	0.012 ± 0.004*	17.25 ± 2.38	0.051 ± 0.006*	0.007 ± 0.001
Control (Seeds soaked with distilled water only)	6.20 ± 0.88*	0.103 ± 0.021	0.025 ± 0.004*	19.13 ± 1.15**	0.080 ± 0.008**	0.012 ± 0.001*
$LSD_{0.01}$	2.264	0.318	0.007	3.409	0.318	0.007
$LSD_{0.05}$	1.681	0.236	0.005	2.531	0.236	0.005

LSD test was applied. * and ** indicate at significant $p < 0.05$ and 0.01, respectively.

vigour index in wheat may be due to reduced germination and seedling length (Singh and Rao 2003). Pretreatments of *Agropyron elongatum* and *Bromus inermis* seeds with the chemical stimulators e.g., GA_3 before planting could significantly reduce the negative effect of allelopathic compounds in *Thymus kotschyanus* during the germination stage too (Saberi *et al.* 2011).

Both elongation and fresh-dry weights of four-week-old-seedlings of wheat were also reduced by oleander and walnut leaf powders, and the GA_3 and IBA alleviated these parameters. The most effective treatments in alleviating inhibition are oleander extract + 10 ppm GA and Walhut extract + 10 ppm GA in root length, fresh and dry weights, also in shoot length, fresh and dry weights (Table 3). Total chlorophyll was reduced by 23.22% in oleander and 36.55% in walnut leaf powder. Besides, carotenoid amounts were decreased by 2.84% in oleander and 17.08% in walnut leaf powder compared to the control. The greatest alleviation was achieved with oleander + 10 ppm GA (23.20%) and Walnut + 10 ppm GA (13.93%) in total chlorophyll and in carotenoid amounts (2.07%) (Table 4). Proline contents of wheat were affected by the oleander and walnut leaf powders and maximum proline contents were in walnut extract + 20 ppm IBA and walnut extract (Table 4).

Table 4. Effects of growth regulators on total chlorophylls, carotenoids and proline contents of four-week-old-seedlings of wheat grain under allelochemical stress due to oleander and walnut leaf powders.

Treatments	Total chlorophyll (mg/g)	Carotenoid (mg/g)	Proline (µmol/g FW)
Powder + 0	0.582 ± 0.010	1.303 ± 0.006	8.027 ± 0.147
Powder + 10 ppm GA_3	0.717 ± 0.051**	1.472 ± 0.010**	6.373 ± 0.127**
Powder + 20 " GA_3	0.463 ± 0.034**	1.291 ± 0.004**	8.774 ± 0.136**
Powder + 10 " IBA	0.512 ± 0.012**	1.251 ± 0.029**	8.877 ± 0.100**
Powder + 20 " IBA	0.447 ± 0.050**	1.221 ± 0.071**	12.930 ± 0.227**
Powder + 0	0.481 ± 0.016	1.112 ± 0.032	5.614 ± 0.065
Powder + 10 ppm GA_3	0.548 ± 0.021**	1.135 ± 0.118	4.247 ± 0.098**
Powder + 20 " GA_3	0.247 ± 0.033**	0.899 ± 0.006**	4.293 ± 0.039**
Powder + 10 " IBA	0.385 ± 0.001**	1.088 ± 0.040	4.557 ± 0.051**
Powder + 20 " IBA	0.299 ± 0.007**	0.907 ± 0.056**	4.626 ± 0.014**
Control (Seeds with distilled water only)	0.758 ± 0.027**	1.341 ± 0.007**	4.672 ± 0.082**
$LSD_{0.01}$	0.022	0.123	0.256
$LSD_{0.05}$	0.017	0.091	0.190

LSD test was applied. * and ** indicate at significant $p < 0.05$ and 0.01, respectively.

In general, allelochemicals are known to inhibit plant growth by reducing chlorophyll and protein contents (Kocaçalışkan *et al*. 2009, Singh and Rao 2003). Elongation and fresh dry weights (Table 3) and total chlorophyll, carotenoid contents (Table 4) of four-week-old-seedlings of wheat were reduced by oleander and walnut leaf powder. Besides, the plant growth regulators alleviated this negative effects in this study (Tables 3, 4). These results are supported by the findings of Terzi and Kocaçalışkan (2010). Pretreatement of barley, wheat, cucumber, alfalfa and tomato seeds with GA_3 and Kn or their combination, alleviated the inhibitory effect of juglone on seed germination and seedling growth. Proline increased under stress condition. The leaf powders of oleander and walnut increased proline content (Table 4). Similar results were indicated by Djanaguiraman *et al*. (2005) and Wang *et al.* (2009). This effect was alleviated significantly by

GA_3 (Table 4). The alleviation effect of GA_3 was stronger than that of IBA (Tables 1, 3, 4). Similarly, GA_3 was found more effective than Kn in alleviation of juglone stress (Terzi and Kocaçalışkan 2009).

In conclusion, this study suggests that GA_3 and IBA protect protein and chlorophyll synthesis and prevent proline accumulation against oleander and walnut inhibitory effect. Especially 10 ppm GA_3 treatments induced protection against allelochemical stress due to walnut and oleander leaf (extract and powder) on wheat. GA_3 possesses the antagonistic activity against allelochemicals of plant growth retardant nature and hence can be used to alleviate their detrimental effects. This may be beneficial practically in arragement of intercropping system(s) of the sensitive species between walnut and neriander plants in the same field and useful for agriculture and landscape.

Acknowledgement

The author would like to thank the authority of C.E.V. Alanya Hamdullah Emin Pasa Private Schools for providing funds, materials and lab space.

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(Manuscript reveived on 5 December, 2012; revised on 18 May, 2013)