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Influence of triadimefon on the growth and development of banana cultivars

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Triazole fungicide triadimefon (bayletone) is a broad systemic fungicide used in agriculture as screening agent. Triadimefon interferes with plant sterol biosynthesis leading to a changeable sterol profile, consequently morphological and cytological abnormalities. Its effect on banana cultivars was studied using shoot-tip cultures placed on Murashige and Skoog solid medium supplemented with 5 mg/L of 6-benzylaminopurine (BAP). The growth and proliferation of triadimefon treated shoot-tip cultures of the three-dessert banana cultivars (Hindi, Basrai and Williams) were affected compared to the control. The optimum culture conditions for root formation were obtained in the case of sub-culturing. The excised shoot cultures into Murashige and Skoog solid medium were supplemented with 1 mg/L indole-3-butyric acid (IBA). The efficiency of root system formation decreased as fungicide concentration increased. Many variations were observed among chlorophyll, carotenoids and protein contents of triadimifon (50 mg/L) treated cultures and untreated ones. High decrease was observed among the usual sterol content of triadimifon (50 mg/L) treated shoot buds compared to the control.

Key words: Banana, cultivars, fungicides, proliferation, shoot-tips, sterol biosynthesis, triadimefon, triazoles.

INTRODUCTION

Triazole fungicides could have side effect on the host plant, in some cases undesirable phytotoxic effects can occur which may limit or affect the growth and development (Gopi et al., 2008; Asami et al., 2003). Triazole compounds inhibit the 14-alpha-demethylation reaction in sterols biosynthesis by interacting with the cytochrome-P-450 monooxygenase of the 14-alpha-demethylase complex (Rahier and Taton, 1997), thus cause an accumulation of 14 -alpha-methyl sterols that cannot pack satisfactory with the fatty acylchains of the phospholipids of cell membrane (Piironen et al., 2000; Khalil et al., 1990), the formation of the latter is disrupted and plant growth is adversely affected (Kaspers, 2009; Asami et al., 2003). Plant growth retarding effect of triazole fungicide triadimefon associate with the accumulation of sterol precursors, delaying of seedling emergence and reducing of plant height, length of coleoptiles, primary leaves and roots (Abdul Jaleel et al., 2008;

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Abbreviations: BAP, 6-Benzylaminopurine; **DMIs,** sterol demethylation inhibitors; **EtOH,** ethanol; **GLC,** gas-liquid chromatography; **IBA,** indole-3-butyric acid; **MS,** Murashige and Skoog.

Table 1. Effect of different concentrations of triadimefon (bayleton) on the viability of shoot-tip explants of banana cultivars, Hindi, Basrai and Williams cultured on MS solid medium supplemented with 5 mg/L BAP for four weeks.

Concentration of triadimefon (mg/L)	Number of shoot-tip explants	% of the living shoot-tips mean ±SD		
		Hindi	Basrai	Williams
Control I	25	100 ± 0.0	100 ± 0.0	100 ± 0.0
Control II	25	100 ± 0.0	100 ± 0.0	100 ± 0.0
30	25	80 ± 0.40	72 ± 0.45	68 ±0.47
40	25	68 ±0.47	60 ± 0.50	32 ± 0.48
50	25	20 ± 0.40	12 ± 0.33	12 ± 0.33
60	25	04 ± 0.20	0.0 ± 0.0	0.0 ± 0.0
70	25	00±0.0	0.0 ± 0.0	0.0 ± 0.0

Control I = Shoot-tip explants cultured on MS solid medium + 5 mg/L BAP. Control II = Shoot-tip explants cultured on MS solid medium + 5 mg/L BAP + 1 ml/L EtOH.

Kishoreukmar et al., 2007). Triazoles fuingicides increases the ratio of Chl a to b in the treated plants (Gopi et al., 2008), affect the protein contents and the rate of photosynthetic rate of treated plant (Gomathinayagam et al., 2008; Lu et al., 2000). Triazoles fuingicides affect mitosis by a direct rather than indirect action on the build up or on the function of the mitotic apparatus (Al Mansouri and Kurup, 2009; Wetzstein et al., 2002). Based on this, the present study attempt to study the *in vitro* effect of triazole fungicide triadimefon on the growth and development of some banana cultivars and shed some light on its phytotoxic effect.

MATERIALS AND METHODS

Chemicals

Triazole fungicides, triadimefon (bayleton) from Bayer AG, Lever kuse, Germany, were kindly provided by Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Triadimefon was solubilized in EtOH (Ethanol), and then it was added to the medium after autoclaving.

Plant material preparation

To determine the effect of triazole fungicide, triadimefon on the viability of shoot -tip explants of banana cultivars, Hindi, Basrai and Williams, shoot-tip explants (about 0.5 cm length) were cultured on MS solid medium (Murashige and Skoog, 1962) supplemented with 5 mg L^{-1} BAP and different concentrations (30, 40, 50, 60 and 70 mg/L separately) of triadimefon for four weeks. All cultures were incubated at the standard culture conditions of temperature (25 ± 2°C) and light regime (16 h/day) for 4 weeks. Absence of further growth was an indicator of lethality. To determine the effect of fungicide triadimfon on the growth and proliferation, rate of the excised lateral buds were cultured on the same solid medium (MS) supplemented with 5 mg/L BAP and at different concentrations (30, 40, 50 mg/L separately) of triadimefon. To determine the effect of fungicide triadimfon on the root formation shoots approximately 3 to 5 cm long were cultured on MS solid medium supplemented with 1 mg/L IBA and at different concentrations (l0, 20, 30, 40 and 50 mg/L separately) of triazole fungicide, triadimefon, then were incubated in the standard culture conditions as mentioned before. For all treatments two controls were used, the first one (control I) lacked the selective agent and the second one (Control II) lacked the selective agent but supplied with the dissolving agent as shown in the tables.

Chemical analysis of contents

Photosynthetic pigments (chlorophyll a chlorophyll b and carotenoids) were determined using spectrophotometric method as described by Metzner et al. (1965), the protein contents were determined colorimetrically according to the study of Lowery et al. (1951) and 4-demethyl sterols were determined according to AOAC (1984). Data were compared with the control ones.

Statistical analysis of data

Data were statistically analyzed by counting the means and standard deviation using the statistical package program for the social sciences (SPSS).

RESULTS

Banana cultivar shoot-tip explants of Williams were more sensitive to the fungicidal toxicity of triadimefon compared to the others (Table 1). Most of the shoot-tip explants failed to grow at the concentration of 60 mg/L triazole fungicide triadimefon, while two explants of Hindi and one of Basrai observed were growing at this concentration, but these shoot-tips seemed brown and weak in their phenotype. Results of the effect of different concentrations of triadimefon (Table 2), on the growth and proliferation rate of shoot-tip cultures of banana cultivars, showed that the number of shoots in all banana cultivars (Hindi, Basrai and Williams) decreased as well as triadimefon concentration increased, while the lengths of shoots decreased. The number of leaves per shoot showed a lower variation with the increase of triadimefon concentrations. The leaf lengths of hindi, basrai and Williams cultivars showed a lower decrease with the increase of triadimefon concentration compared to the control (Table 2). The results of the effect of different concentrations of triazole fungicide triadimefon on the

Table 2. Effect of different concentrations of triadimefon on the growth and proliferation rate of shoot-bud explants of banana cultivars, Hindi, Basrai and Williams cultured on MS solid medium supplemented with 50 mg/l BAP for four weeks. Values are mean of 25 replicates per treatment in five jars ± SD.

Cultivar	Treatment (mg/L)	Number of shoots/explant (cm)	Length of shoots (cm)	Number of leaves /shoot	Length of leaves (cm)
	Control I	5 ± 0.3	1.01 ± 0.07	4.00 ± 0.40	0.80 ± 0.07
	Control II	4 ± 0.4	1.12 ± 0.08	2.00 ± 0.28	0.90 ± 0.07
Hindi	30	3 ±0.5	0.98 ± 0.04	2.00 ± 0.47	0.73 ± 0.03
	40	2 ± 0.4	0.93 ± 0.02	2.00 ± 0.51	0.70 ± 0.03
	50	1 ± 0.3	0.91 ± 0.06	1.00 ± 0.48	0.62 ± 0.04
	Control I	4 ± 0.4	1.00 ± 0.04	3.00 ± 0.33	0.72 ± 0.03
	Control II	3 ± 0.4	1.34 ± 0.05	2.00 ± 0.20	1.10 ± 0.04
Basrai	30	3 ± 0.6	1.03 ± 0.08	2.00 ± 0.51	0.76 ± 0.05
	40	3 ± 0.5	0.90 ± 0.04	2.00 ± 0.36	0.70 ± 0.03
	50	1 ± 0.4	0.80 ± 0.04	1.00 ± 0.38	0.60 ± 0.06
	Control I	5 ± 0.5	1.14 ± 0.05	3.00 ± 0.37	0.90 ± 0.02
	Control II	4 ± 0.5	1.10 ± 0.05	2.00 ± 0.43	0.80 ± 0.05
Williams	30	3 ± 0.3	1.10 ± 0.03	2.00 ± 0.29	0.80 ± 0.06
	40	3 ± 0.5	0.90 ± 0.03	2.00 ± 0.50	0.70 ± 0.04
	50	1 ± 0.0	0.80 ± 0.05	1.00 ± 0.48	0.60 ± 0.03

Control I = MS solid medium + 5 mg**/l** BAP. Control II = MS solid medium + 5 mg**/l** BAP + 1 ml**/l** EtOH..

excised shoots rooting of the three-dessert banana cultivars showed decrease in the number of roots per shoot, also decrease in the average lengths of roots with the increase of fungicide concentration (Table 3). The content of various pigment fractions (Chl a, Chl b and carotenoids) of Hindi and Williams, triadimefon treated cultivars showed a little differences compared to the control. The ratio of Chl a / Chl b and the total content showed a lower decrease in the case of Hindi cultivar, and lower increase in the case of Williams cultivar. An increase in various pigment fractions (Chl a, Chl b and carotenoids) contents and the total content of Basrai cultivar was observed compared to control I, but showed a lower decrease in the ratio of Chl a / Chl b (Table 4). Littlie variation in the three cultivar pigment fragments contents in the case of control II were recorded in comparison with each others. The total pigment content of control II showed increase in the case of Basrai cultivar compared to other cultivars and control I. The same result was obtained with control II of Williams cultivar compared to control I of the same cultivar. In the case of Hindi, it was showed that Chl a, carotenoids and the total content of control II showed lower decrease compared to those of other ones and those of control I (Table 4). However, there is no relationship between the effect of the fungicide and its dissolving agent on the pigmentation when the effect of fungicides on the pigments is well obvious phenotypically and analytically compared with those of control I or control II.

GLC analysis for the unsaponifiable matter of banana cultivars developed shoots under the effect of triadimefon (50 mg/L), revealed that the relative percentage of Δ^5 sterols (sitosterol, stigmasterol and campesterol) decreased compared to the control (Table 5), since dramatic quantitative reduction in the total percentage of sterols was observed compared with the control.

The results of protein chemical analysis showed that the total protein content of triadimefon (50 mg/L) treated shoots of Hindi showed little decrease compared to the control (Figure 1a). The soluble protein content of Hindi treated shoots was approximately equal to the control II content. The insoluble proteins content of triadimefon-treated shoots of Hindi cultivar showed a lower decrease compared to those of control I and control II respectively. The total protein content of Hindi cultivar triadimefon (50 mg/L) treated shoots showed a relatively high decreasing compared to control ones. While, in the case of Basrai cultivar showed a highly increase compared to the control (Figure 1b). The total protein content showed a highly increase compared to control I and control II, the same results were obtained in the case of Williams. In the case of Basrai cultivar, the insoluble proteins content of the triadimefon-treated shoots showed an increase in comparison to those of the control. The results show that the total proteins content of Williams cultivar (Figure 1c) treated shoots was higher than those of the control. High increase in the insoluble proteins content of triadimefon treated shoots of Williams cultivar compared to those of

Table 3. Effect of different concentration of tiazole fungicide triadimefon on the rooting of the excised shoots of the three banana cultivars, Hindi, Basrai and Williams cultured on Ms solid medium supplemented with 1mg/l IBA for three weeks.

Cultivar	Treatment (mg/L)	No of excited shoots	No of rooted shoots	No of roots /shoot	Length of roots
Hindi line	Control I	25	100	6 ± 0.83	8.8 ± 1.60
	Control II	25	100	6 ± 0.57	3.0 ± 0.35
	10	25	93	4 ± 0.53	0.5 ± 0.04
	20	25	73	2 ± 0.51	0.4 ± 0.09
	30	25	60	2 ± 0.50	0.3 ± 0.04
	40	25	20	1 ± 0.50	0.2 ± 0.00
	50	25	00	0 ± 0.00	0.0 ± 0.00
Basrai line	Control	25	100	5 ± 0.57	5.0 ± 1.06
	Control II	25	100	8 ± 0.50	3.0 ± 0.70
	10	25	93	4 ± 0.46	0.5 ± 0.04
	20	25	40	2 ± 0.57	0.5 ± 0.05
	30	25	26	1 ± 0.00	0.3 ± 0.05
	40	25	0	0 ± 0.00	0.0 ± 0.00
	50	25	0	0 ± 0.00	0.0 ± 0.00
Williams line	Control I	25	100	6 ± 0.95	2.5 ± 0.25
	Control II	25	100	5 ± 1.51	4.5 ± 0.70
	10	25	100	4 ± 0.57	0.6 ± 0.20
	20	25	60	3 ± 0.00	0.5 ± 0.05
	30	25	20	1 ± 0.00	0.5 ± 0.00
	40	25	08	1 ± 0.00	0.2 ± 0.00
	50	25	0	0 ± 0.00	0.0 ± 0.00

Control I = MS solid medium + 5 mg/l BAP. Control II = MS solid medium + 5 mg/l BAP + 1 ml/l EtOH..

Table 4. Photosynthetic pigments content of banana cultivars, Hindi, Basrai and Williams cultured on MS solid medium supplemented with 5 mg/l BAP and 50 mg/l triadimefon for four weeks.

Cultivar	Treatment	Pigment content (mg/g fresh weight)				
		Chl a	Chl b	Carotenoids	a/b	Total
Hindi line	Control I	0.27 ± 0.03	0.10 ± 0.00	0.09 ± 0.01	2.70	0.46
	Control II	0.14 ± 0.04	0.11 ± 0.05	0.08 ± 0.02	1.27	0.33
	50 mg/l	0.22 ± 0.11	0.19 ± 0.00	0.07 ± 0.01	2.44	0.38
Basrai line	Control I	028 ±0.01	0.15 ± 0.01	0.06 ± 0.01	1.87	0.49
	Control II	0.34 ± 0.02	0.20 ± 0.01	0.09 ± 0.00	1.70	063
	50 mg/l	0.35 ± 0.04	0.20 ± 0.04	0.10 ± 0.01	1.75	0.65
Williams line	Control I	020±0.01	0.15 ±0.02	0.06 ± 0.00	1.33	0.4l
	Control II	0.32 ± 0.110	0.20 ± 0.00	0.09 ± 0.030	1.60	0.61
	50 mg/l	0.23 ± 0.030	0.16 ± 0.0007	0.07 ± 0.007	1.44	0.46

Control I = Shoot-tip explants cultured on MS solid medium +5 mg/l BAP. Control II = Shoot-tip explants cultured on MS solid medium +5 mg/l BAP + 1 ml/l EtOH. Values are means of three replicates ± SD.

the control and other cultivars contents was observed. The soluble protein contents of untreated shoots of the three cultivars (Hindi, Basrai and Williams) were approximately equal. While the insoluble proteins content of Basrai and Williams cultivars showed a lower decrease compared with those of Hindi cultivar.

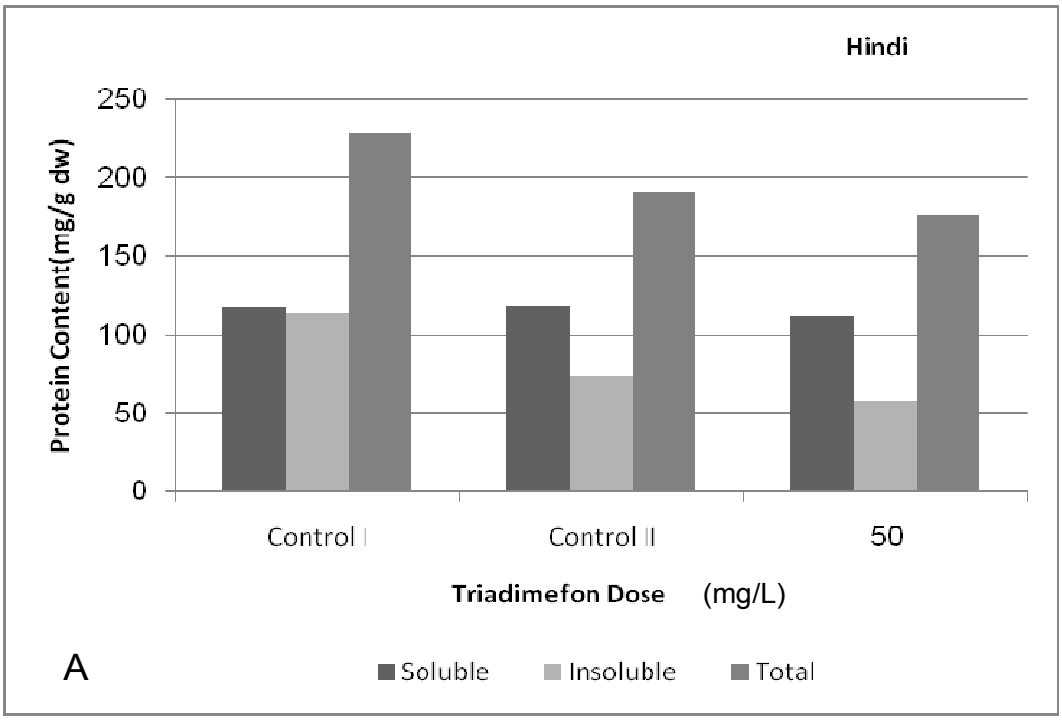
Table 5. Effect of triazole fungicide triadimefon (50 mg/l) on the 4-dementhyl sterols (sitosterol, stigmasterol and campesterol) content of the three banana cultivars shoots cultured on MS solid medium supplemented with 5 mg/l BAP for four weeks.

Treatment	Hindi line				Basrai line				Willimas line			
	Sitosterol	Stigmasterol	Campesterol	Total	Sitosterol	Stigmasterol	Campesterol	Total	Sitosterol	Stigmasterol	Campesterol	Total
Control	2.1	20.6	5.1	27.8	6.7	2.5	3.9	13.1	11.9	5.7	12.5	30.1
50 mg/L	1.1	16.9	3.4	21.4	0.3	2.1	0.3	2.7	6.7	2.8	2.4	11.9

- % was calculated as relative to the total percentage of unsaponifiable matter. - Control = MS basal medium + 5 mg/l BAP.

DISCUSSION

Plant cell is a dynamic living system response to systemic fungicides by complex series of biochemical changes. Systemic fungicides enter the plant and encounter a variety of physiological and biochemical changes. The results of our work showed that the viability of shoot-tip explants of the three banana cultivars, Hindi, Basrai and Williams decreased as well as the concentration of triadimefon increased. These results are in agreement with those mentioned by Gopi et al. (2008) and Lu and Guo (2000) who reported that triazole fungicides affected many plant growth properties. The obtained results of this study indicated that the phytotoxic effect of triadimefon includes reduce in surface area of leaves and growth retardation of shoots and roots. This conclusion is in agreement with those reported by Abdul Jaleel et al. (2008) and Kishorekumar et al. (2007), who reported that the phytotoxic effect of triadimefon was parallel to the inhibition of plant sterol biosynthesis rather than gibberellins biosynthesis. Many explanations have been given by many investigators regarding the decrease of growth and proliferation rates (Kaspers, 2009; Khalil et al., 1990). The suppression of growth could be attributed to the inhibition of the enzyme (cytochrome P-450-dependent obtusifoliol-14-demethylase) responsible for the removal of the C-14 methyl group, led to the accumulation of 14-alpha-methyl sterols at the expense of Δ5-sterols (sitosterol, stigmasterol and campesterol) this observation is in agreement with the study of Lu et al. (2000) . The plant growth retardant effect of triadimefon may be associated with an inhibition of the biosynthetic pathway of of campesterol (Asami et al., 2003), whereas brassinosteroids has been shown via two pathways from campesterol. Brassinosteroids are plant sterols that cause cell elongation, cell expansion, enhances gravitropism, retard abscission and promote xylem differentiation (Asami et al., 2003; Hartmann, 1998). Δ5-Sterols play an important metabolic role in the cell proliferation process (Piironen et al., 2000) who reported that stigmasterol might be specifically required for cell proliferation. Lower concentrations of stigmasterol were unable to restore growth of celery cells treated with an inhibitor of the obtusifoliol 14-demethylase, but a combination of low concentration of stigmasterol together with a high concentration of cholesterol was effective as a relatively high concentration of stigmasterol alone (Hartmann, 1998; Kisorekumar et al., 2007) pointed out that the triazole fungicides inhibit the 14-demethylation reaction in plant sterols biosynthesis by interacting with the cytochrome -P-450- monooxygenase of the 14-alpha- methyl sterols that cannot pack satisfactory with the fatty acyl chains of the phospholipids of the plant membrane. The formation of the latter is disrupted and the plant growth is adversely affected. Wetzstein et al. (2002) repotted that triazoles fungicides affect mitosis by a direct rather than indirect action on the build up or on the function of the mitotic apparatus so spindle damage could be caused by interference with microtubule polymerization or with the replication of the spindle organizing center or increase the frequency of abnormalities such as chromosome clumping at metaphase and anaphase. Al Mansouti and Kurup (2009) and Kaspers (2009) reported that triazole fungicides might be interfere with the membrane vesicle which in close association with microtubules. The effect of DMIs on the biosynthesis of chloroplast pigments remained unclear. The intense greening of leaves of the treated shoots in the sub-lethal concentration (50 mg L^{-1}) of triadimefon may be attributed to the increase in ChI concentration per unit area of leaf. This observation is in agreement with those of Gomathinayagam et al. (2008) and Kisohrekumar et al. (2007), whereas the greening effect might be associated with the growth retarding activity of the fungicides. Pigments probably condensed into a smaller leaf area of the treated shoots, which appeared darker green than control ones. These observations correspond to those of Abdul Jaleel et al. (2008), Gopi et al. (2008) and Khalil et al. (1990) who reported that triazole fungicides were ineffective in changing the ChI content (per unit fresh weight). It appeared



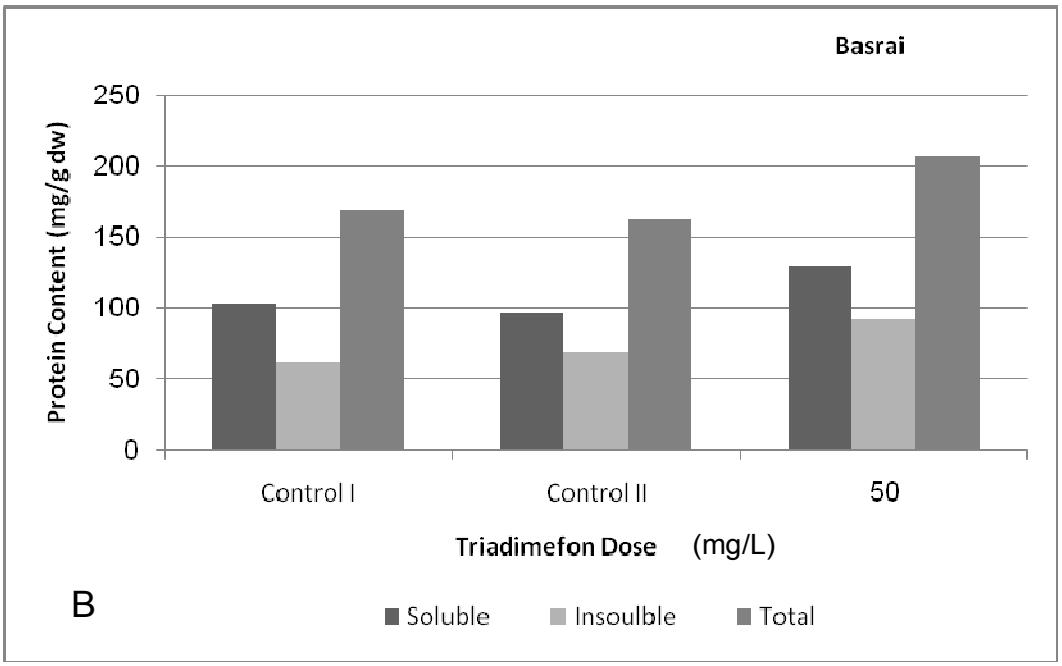


Figure 1. Showing the effect of triadimefon on soluble, insoluble and total protein contents (mg/g dw) of **(A)** Hindi (B) Basrai (C) Williams shoots cultured on Ms solid medium supplemented with 5 mg/L BAP + 50 mg/L triadimefon for four weeks. Control I = Shoot-tip explants cultured on MS solid medium + 5 mg/L BAP. Control II = Shoot-tip explants cultured on MS solid medium + 5 mg/L BAP + 1 ml/L EtOH. Values are means of three replicates.

that changes observed in the Chl and carotenoids content of the treated shoots with triadimefon were related to the growth retardation of the fungicide, but the ratio of Chl a / Chl b was not influenced by it. This showing that the triazole fungicide, triadimefon had no immediate effect on the carotenoids hydroxylation systems in the leaf; this result are in agreement with those obtained by Kishorekumar et al. (2007) and Abdul

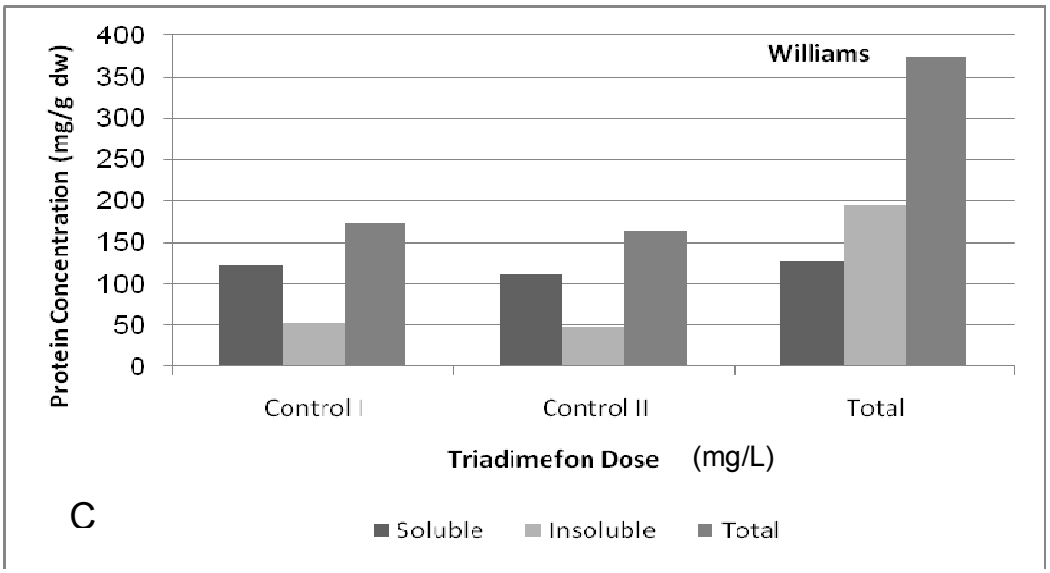


Figure 1. Continued.

Jaleel et al. (2008). There is no evidence that triazole fungicides inhibit all cytochrome P-450 mixed functions oxygenases like those of sterol or GA biosynthesis. Protein synthesis is essential for normal cell proliferation and differentiation. Increasing or decreasing of protein contents could be attributed to the differences in the physiological and morphological characters of each cultivar (Kaspers, 2009). It is well known that fungicides reflect a type of particular stress conditions exhibit alteration of gene expression inducing a change in the plant metabolism resulting in an alteration in the protein synthesis which may vary according to the phenotype of plant (Hy et al., 2002; Schrick et al., 2000). In conclusion this study revealed that the systemic triazole fungicide triadimefon might have an inhibitory effect on the morphology and physiology of higher plants, which appeared *in vitro* on the growth and development of the three desert banana cultivars, Hindi, Basrai and Williams and its inhibitory effect might be attributed to its phytoxic effect or its accumulation in plant tissues.

Conflict of interests

The author(s) have not declared any conflict of interests.

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