

## SOME MORPHOLOGICAL AND PHYSIOLOGICAL ABNORMALITIES IN GROWTH AND DEVELOPMENT OF BANANA TRIADIMEFON-TREATED CULTIVARS

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**SUMMARY.** Triadimefon (Bayleton) is a systemic fungicide in the triazole family of chemicals. Triadimefon treatment affected the growth and development of shoot-tip explants of three desert banana cultivars Hindi, Basrai and Williams. The viability and number of lateral bud induction in the three banana cultivars decreased with increasing triadimefon concentration. Most of the shoot-tip explants failed to grow at the lethal triadimefon concentration (60 mg/l). At the sub-lethal concentration (50 mg/l), the fresh weights of shoot clusters of cvs. Hindi, Basrai and Williams were 0.11, 0.11 and 0.10 g/cluster, respectively. The number and length of leaves per shoot decreased with increasing triadimefon concentration. The efficiency of root system formation decreased as the concentration of the fungicide tested increased. Triadimefon treatment increased the photosynthetic pigments (Chl a, Chl b and carotenoids) and affected protein content. GLC analysis of non-saponifiable matter of shoots treated with triadimefon (50 mg/l) showed a decrease in the content of  $\Delta 5$  sterols (sitosterol, stigmasterol and campesterol). It can be concluded that the triazole fungicide triadimefon inhibited the growth and development of the three desert banana cvs. Hindi, Basrai and Williams.

**Key words:** Banana; Cultivars; Fungicides; Growth; Shoot-tips; Sterol Biosynthesis; Triadimefon; Triazoles.

**Abbreviations:** BAP – N<sup>6</sup>-benzylaminopurine; DMIs – Sterol demethylation inhibitors; EtOH – Ethanol; GLC – Gas-liquid chromatography; IBA – indole-3-butyric acid; MS – Murashige and Skoog.

## INTRODUCTION

Triadimefon (Bayleton) belongs to the triazole group of fungicides possessing plant growth regulating properties. Triadimefon displays a cytokinin-like activity by exhibiting antisenescence

properties (Fletcher and Nath, 1984). The retardation of growth is linked to the inhibition of gibberellin biosynthesis in higher plants (Fletcher and Hofstra, 1985). Triadimefon protects plants from stress-

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related injuries due to drought, heat, chilling, ozone and SO (Fletcher and Nath, 1984; Fletcher and Hofstra, 1985). Triadimefon interferes with plant sterol biosynthesis leading to a changeable sterol profile (Hartmann 1998), and consequently to morphological and cytological abnormalities (Kaspers, 2009). Triadimefon inhibits the 14- $\alpha$ -demethylation reaction in sterol biosynthesis by interacting with the cytochrome-P-450 monooxygenase of the 14- $\alpha$ -demethylase complex (Rahier and Taton, 1997), thus causing the accumulation of 14 - $\alpha$ -methyl sterols that cannot pack satisfactorily with the fatty acyl chains of the phospholipids of the cell membrane (Hartmann, 1998). The formation of the latter is disrupted and plant growth is adversely affected (Hartmann, 2004). Triadimefon affects the Chl a:b ratio in the treated plants (Khalil et al., 1990), the protein content (Asami et al., 2003) and the photosynthetic rate of treated plants (Gomathinayagam et al., 2008 , Lu et al., 2000). Triazoles fungicides affect mitosis by a direct rather than indirect action on the build up or on the function of the mitotic apparatus (Al Mansouti and Kurup, 2009). Plant growth retarding effect of triazole fungicide triadimefon is associated with the accumulation of sterol precursors, the delay of seedling emergence and reduction of plant height, length of coleoptiles, primary leaves and roots (Khalil et al., 1990; Kishoreukmar et al., 2007). Triadimefon has also side effects on plants. It can cause undesirable phytotoxic effects which may limit or affect the growth and development (Lu et al., 2000; Khalil et al., 1990). The present investigation aimed to study the

growth-regulating effect of triadimefon on three desert banana cultivars Hindi, Basrai and Williams.

## MATERIALS AND METHODS

### Chemicals

The triazole fungicide triadimefon (Bayleton) from Bayer AG, Leverkusen, Germany, was kindly provided by the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Triadimefon solubilized in EtOH (ethanol), was added thereafter to the sterile media.

### Plant material preparation

The trial was carried out in the Botany Department, Faculty of Science, Sohag University, with shoot-tip explants excised from shoot-tip cuttings collected from different healthy mature banana trees. Shoot-tip cuttings were washed in soap water prior to surface sterilization. Shoot tip explants were prepared by removing all expanded leaves leaving the shoot meristem with 2-3 leaf primordia. The excised shoot-tips were surface sterilized with 20% (v/v) commercial Clorox solution containing 1.05% (w/v) sodium hypochlorite and a drop of Tween 20 for 15 min, then were dipped in 0.1% (w/v) mercuric chloride solution for 3 min followed by three times rinsing in sterile distilled water, and finally transferred to sterile glass jars (250 ml) containing culture media. In all experiments basal solid Murashige and Skoog medium (Murashige and Skoog, 1962) supplemented with 30 g/l sucrose, solidified with 1% Difco Bacto agar was used. The medium was enriched with 50 mg/l ascorbic acid and 1.0 g/l activated charcoal to prevent the browning phenomenon due to exudation

of phenolic, mucilaginous and saponin compounds from the explants. The pH of the medium was adjusted with KOH or HCl to 5.8 before autoclaving. All cultures were incubated in the growth chamber at standard culture conditions: temperature of  $25 \pm 2^\circ\text{C}$ , light regime of 16 h/d and irradiance intensity of  $50 \mu\text{E m}^{-2} \text{s}^{-1}$  for 4 weeks.

#### **Effect of triazole fungicide triadimefon on the viability of shoot-tip explants**

To determine the effect of triadimefon on the viability of shoot-tip explants of the banana cvs. Hindi, Basrai and Williams shoot-tip explants (about 0.5 cm in length) were cultured on MS solid medium (Murashige and Skoog, 1962) supplemented with 5 mg/l BAP and different concentrations (30, 40, 50, 60 and 70 mg/l, separately) of triadimefon. Absence of further growth was indicative of lethality. The experiment was conducted at least twice with 25 replicates per each treatment.

#### **Effect of triadimefon on the growth and development of shoot-tip explants**

To determine the effect of triadimefon on the growth and development of shoot-tip explants MS solid medium supplemented with 5 mg/l BAP and different concentrations (30, 40 and 50 mg/l, separately) of triadimefon were used.

The following parameters were measured:

- 1 – Number of developed lateral bud per each shoot-tip;
- 2 – Length of developed shoots;
- 3 – Number of leaves per shoot;
- 4 – Length of leaves;
- 5 – Fresh weight of shoot clusters.

#### **Effect of triadimefon on root formation**

To determine the effect of triadimefon on root formation of banana cultivars shoots approximately 3-5 cm long were cultured on MS solid medium supplemented with 1 mg/l IBA and different concentrations (10, 20, 30, 40 and 50 mg/l, separately) of triadimefon. The development of roots was recorded after three weeks of culture initiation.

The following parameters were measured:

- 1 – Percentage of rooted shoots;
- 2 – Number of roots per shoot;
- 3 – Length of roots.

#### **Biochemical analyses**

Photosynthetic pigments (Chl a, Chl b and carotenoids) were determined using the spectrophotometric method as described by Metzner et al. (1965). Protein content was determined colorimetrically according to Lowery et al. (1951) and 4-demethyl sterols were determined according to A.O.A.C. (1984).

#### **Statistical analysis**

The obtained data were subjected to statistical analysis performed using standard deviation (SD) according to the method described by Snedecor and Cochran (1980).

## **RESULTS**

The sub-lethal concentration of the shoot-tip explants was 50 mg/l (Table 1). Most of shoot-tip explants failed to grow at the lethal concentration (60 mg/l) of triadimefon. The viability of the shoot-tip explants of the three banana cvs. Hindi, Basrai and Williams decreased

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

Concentration of triadimefon [mg/l]	% of viable shoot-tips mean $\pm$ SD		
	cv. Hindi	cv. Basrai	cv. Williams
Control I	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00
Control II	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00
30	80 $\pm$ 0.40	72 $\pm$ 0.45	68 $\pm$ 0.47
40	68 $\pm$ 0.47	60 $\pm$ 0.50	32 $\pm$ 0.48
50	20 $\pm$ 0.40	12 $\pm$ 0.33	12 $\pm$ 0.33
60	4 $\pm$ 0.20	0	0
70	0	0	0

Experiment was conducted with 25 replicates per each treatment. Values are means  $\pm$  SD. Control I – shoot-tip explants cultured on MS solid medium plus 5 mg/l BAP; Control II – shoot-tip explants cultured on MS solid medium plus 5 mg/l BAP + 1 ml/l EtOH.

Table 2. Effect of different concentrations of triadimefon on the growth and development of shoot-tip explants of three banana cultivars cultured on MS solid medium supplemented with 5 mg/l BAP for four weeks.

Cultivar	Triadimefon concentration [mg/l]	Number of lateral buds/shoot-tip [cm]	Length of shoots [cm]	Number of leaves/shoot	Length of leaves [cm]	FW/shoot cluster [g]
Hindi	Control I	5 $\pm$ 0.3	1.01 $\pm$ 0.07	4.00 $\pm$ 0.40	0.80 $\pm$ 0.07	0.30 $\pm$ 0.05
	Control II	4 $\pm$ 0.4	1.12 $\pm$ 0.08	2.00 $\pm$ 0.28	0.90 $\pm$ 0.07	0.26 $\pm$ 0.03
	30	3 $\pm$ 0.5	0.98 $\pm$ 0.04	2.00 $\pm$ 0.47	0.73 $\pm$ 0.03	0.21 $\pm$ 0.01
	40	2 $\pm$ 0.4	0.93 $\pm$ 0.02	2.00 $\pm$ 0.51	0.70 $\pm$ 0.03	0.14 $\pm$ 0.02
	50	1 $\pm$ 0.3	0.91 $\pm$ 0.06	1.00 $\pm$ 0.48	0.62 $\pm$ 0.04	0.11 $\pm$ 0.05
Basrai	Control I	4 $\pm$ 0.4	1.00 $\pm$ 0.04	3.00 $\pm$ 0.33	0.72 $\pm$ 0.03	0.25 $\pm$ 0.05
	Control II	3 $\pm$ 0.4	1.34 $\pm$ 0.05	2.00 $\pm$ 0.20	1.10 $\pm$ 0.04	0.16 $\pm$ 0.04
	30	3 $\pm$ 0.6	1.03 $\pm$ 0.08	2.00 $\pm$ 0.51	0.76 $\pm$ 0.05	0.15 $\pm$ 0.04
	40	3 $\pm$ 0.5	0.90 $\pm$ 0.04	2.00 $\pm$ 0.36	0.70 $\pm$ 0.03	0.15 $\pm$ 0.02
	50	1 $\pm$ 0.4	0.80 $\pm$ 0.04	1.00 $\pm$ 0.38	0.60 $\pm$ 0.06	0.11 $\pm$ 0.02
Williams	Control I	5 $\pm$ 0.5	1.14 $\pm$ 0.05	3.00 $\pm$ 0.37	0.90 $\pm$ 0.02	0.30 $\pm$ 0.05
	Control II	4 $\pm$ 0.5	1.10 $\pm$ 0.05	2.00 $\pm$ 0.43	0.80 $\pm$ 0.05	0.38 $\pm$ 0.02
	30	3 $\pm$ 0.3	1.10 $\pm$ 0.03	2.00 $\pm$ 0.29	0.80 $\pm$ 0.06	0.15 $\pm$ 0.03
	40	3 $\pm$ 0.5	0.90 $\pm$ 0.03	2.00 $\pm$ 0.50	0.70 $\pm$ 0.04	0.12 $\pm$ 0.01
	50	1 $\pm$ 0.0	0.80 $\pm$ 0.05	1.00 $\pm$ 0.48	0.60 $\pm$ 0.03	0.10 $\pm$ 0.01

Experiment was conducted twice with 25 replicates per each treatment. Values are means  $\pm$  SD. Control I – MS solid medium plus 5 mg/l BAP; Control II – MS solid medium plus 5 mg/l BAP plus 1 ml/l EtOH.

with increasing the concentration of triadimefon. Shoot-tip explants of cv. Williams were more sensitive to the toxicity of the fungicide triadimefon compared to the other cultivars (Table 1). One of Hindi explants was observed to grow at the lethal concentration (60 mg/l) of triadimefon. This shoot-tip seemed brown and weak in its phenotype and it died during the subculture into triadimefon-

free medium. The analysis of the effect of different concentrations of triadimefon on the growth and development of shoot-tip explants of banana cultivars showed that the number and length of lateral buds in the three banana cultivars tested (Hindi, Basrai and Williams) was decreased as triadimefon concentration increased. At the sub-lethal concentration (50 mg/l) the fresh weight of shoot clusters of cvrs.

Table 3. Effect of different concentrations of triazole fungicide triadimefon on the rooting of excised shoots of three banana cultivars cultured on MS solid medium supplemented with 1mg/l IBA for three weeks.

Cultivar	Triadimefon concentration [mg/l]	% of rooted shoots	Number of roots/shoot	Length of roots [cm]
Hindi	Control I	100	6±0.83	8.8±1.60
	Control II	100	6±0.57	3.0±0.35
	10	93	4±0.53	0.5±0.04
	20	73	2±0.51	0.4±0.09
	30	60	2±0.50	0.3±0.04
	40	20	1±0.50	0.2±0.00
	50	00	0	0
	Basrai	Control	100	5±0.57
Control II		100	8±0.50	3.0±0.70
10		93	4±0.46	0.5±0.04
20		40	2±0.57	0.5±0.05
30		26	1±0.00	0.3±0.05
40		0	0	0
50		0	0	0
Williams		Control I	100	6±0.95
	Control II	100	5±1.51	4.5±0.70
	10	100	4±0.57	0.6±0.20
	20	60	3±0.00	0.5±0.05
	30	20	1±0.00	0.5±0.00
	40	08	1±0.00	0.2± 0.00
	50	0	0	0

Experiment was conducted with 25 replicates per each treatment. Control I – excised shoots cultured on MS solid medium plus 1 mg/l IBA; Control II – excised shoots cultured on MS solid medium plus 1 mg/l IBA plus 1 ml/l EtOH.

Hindi, Basrai and Williams were 0.11, 0.11 and 0.10 g/cluster, respectively. The leaf length of cvs. Hindi, Basrai and Williams decreased with increasing triadimefon concentration compared to controls (Table 2). At the sub-lethal concentration (50 mg/l) the leaf lengths of cvs. Hindi, Basrai and Williams were 0.62, 0.60 and 0.60 cm, respectively (Table 2). The analysis of the effect of different concentrations of triadimefon on the rooting of the excised shoots of the three banana cultivars (Table 3) showed that the number of roots per shoot and the average length of roots decreased as triadimefon concentration increased. The sub-lethal concentration for the rooting of cvs. Hindi and Williams was 40 mg/l while the sub-lethal concentration for the rooting of cv. Basrai was 30 mg/l. At these concentrations the percentage of shoots that developed roots for cvs. Hindi, Williams and Basrai were 20%, 8% and 26%, respectively.

These roots appeared black and thick in phenotype. Pigment content (Chl a, Chl b and carotenoids) in triadimefon (50 mg/l) treated shoots increased compared to control I. The Chl a/b ratio showed a less decrease in cv. Hindi and a less increase in cv. Williams as compared to control I. An increase in the pigment content (Chl a, Chl b and carotenoids) of cv. Basrai was observed compared to control I, but the decrease in the Chl a/b ratio was less (Table 4). The obtained results of control II showed increases in the pigments tested in the three cultivars. No variations were recorded in the tested pigments of the three cultivars in control II. In cv. Basrai Chl a/b ratio of control II was increased as compared to the other cultivars and control I. The same result was obtained with control II of cv. Williams when compared to control I. Carotenoids were less decreased in cv. Hindi compared to control I. Chl a measured in cv. Hindi

Table 4. Photosynthetic pigment content in three banana cultivars cultured on MS solid medium supplemented with 5 mg/l BAP and 50 mg/l triadimefon for four weeks.

Cultivar	Variations	Pigment content [mg/g fresh weight]			
	Triadimefon concentration [mg/l]	Chl a	Chl b	Carotenoids	Chl a/b
Hindi	Control I	0.27±0.03	0.10±0.00	0.09±0.01	2.70
	Control II	0.34±0.04	0.11±0.05	0.08±0.02	1.27
	50 mg/l	0.22±0.11	0.19±0.00	0.07±0.01	2.44
Basrai	Control I	0.28±0.01	0.15±0.01	0.06±0.01	1.87
	Control II	0.34±0.02	0.20±0.01	0.09±0.00	1.70
	50 mg/l	0.35±0.04	0.20±0.04	0.10±0.01	1.75
Williams	Control I	0.20±0.01	0.15±0.02	0.06±0.00	1.33
	Control II	0.32±0.11	0.20±0.00	0.09±0.030	1.60
	50 mg/l	0.23±0.03	0.16±0.0007	0.07±0.007	1.44

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



decreased less compared to control I (Table 4). The surface area of some leaves of the three banana cultivars was a little small than that of the controls. These leaves appeared intense green and thick in

their phenotype.

GLC analysis (Table 5) of the non-saponifiable matter of banana cultivars developing shoots upon treatment with triadimefon (50 mg/l) revealed that the

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

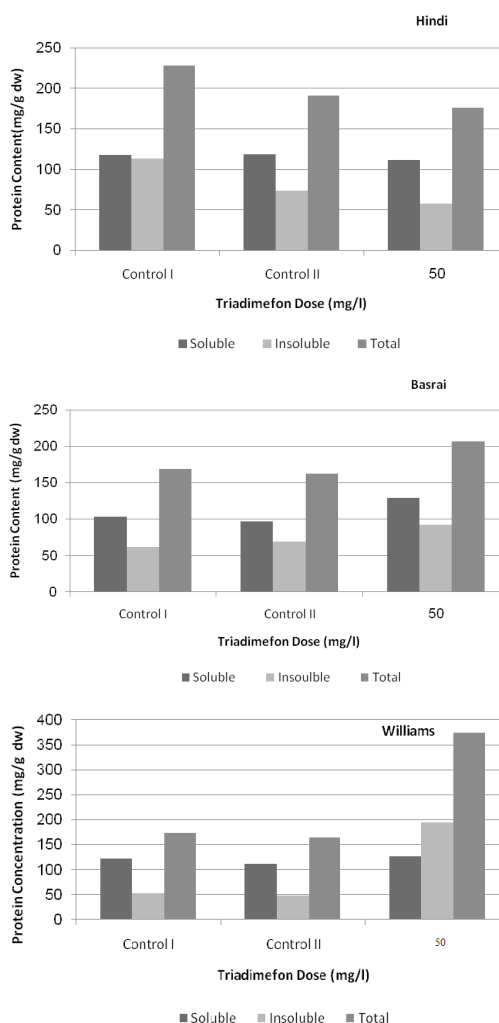
Treatment	cv. Hindi				cv. Basrai				cv. Willimas			
	Sito	Stigma	Camp	Tot	Sito	Stigma	Camp	Tot	Sito	Stigma	Camp	Tot
Control	2.1	20.6	5.1	27.8	6.7	2.5	3.9	13.1	11.9	5.7	12.5	30.1
50 mg/l	1.1	16.9	3.4	21.4	0.3	2.1	0.3	2.7	6.7	2.8	2.4	11.9

% was calculated as relative to the total percentage of non-saponifiable matter. Control – MS basal medium plus 5 mg/l BAP. Sito – sitosterol; Stigma – stigmasterol; Camp – Campesterol; Tot – total.

Figure 1. A). Effect of triadimefon on soluble, insoluble and total protein contents (mg/g DW) in cv. Hindi shoots cultured on MS solid medium supplemented with 5mg/l BAP plus 50 mg/l triadimefon for four weeks. Control I - shoot-tip explants cultured on MS solid medium plus 5 mg/l BAP; Control II - shoot-tip explants cultured on MS solid medium plus 5 mg/l BAP plus 1 ml/l EtOH. Values are means of three replicates.

B). Effect of triadimefon on soluble, insoluble and total protein contents (mg/g DW) in cv. Basrai shoots cultured on MS solid medium supplemented with 5 mg/l BAP plus 50 mg/l triadimefon for four weeks. Control I - shoot-tip explants cultured on MS solid medium plus 5 mg/l BAP; Control II - shoot-tip explants cultured on MS solid medium plus 5 mg/l BAP plus 1 ml/l EtOH. Values are means of three replicates.

C). Effect of triadimefon on soluble, insoluble and total protein contents (mg/g DW) in cv. Williams shoots cultured on MS solid medium supplemented with 5mg/l BAP plus 50 mg/l triadimefon for four weeks. Control I - shoot-tip explants cultured on MS solid medium plus 5 mg/l BAP; Control II - shoot-tip explants cultured on MS solid medium plus 5 mg/l BAP plus 1 ml/l EtOH. Values are means of three replicates.



relative percentage of  $\Delta 5$  sterols (sitosterol, stigmasterol and campesterol) decreased compared to controls. The relative percentage of sitosterol, stigmasterol and campesterol was 1.1, 0.3 and 6.7, respectively. A dramatic quantitative reduction in the total percentage of sterols was observed compared with controls. The relative percentage of total sterol content was 21.4, 2.7 and 11.9, respectively.

The total protein content in triadimefon (50 mg/l) treated shoots of cv. Hindi showed a relatively high decrease compared to the control while in cvs. Basrai and Williams it was strongly increased compared to controls (Fig. 1a, b, c). The soluble protein content of Hindi shoots was approximately equal to the control while in cv. Basrai it was slightly increased compared to the control. The results on insoluble protein content of triadimefon-treated shoots showed a decrease in cv. Hindi (Fig. 1a), while in cvs. Basrai and Williams it was slightly increased as compared to controls (Fig. 1b, c).

## DISCUSSION

Plant cell as a dynamic living system responds to systemic fungicides by a complex series of biochemical changes. The results of this study revealed that the viability of shoot-tip explants of three desert banana cultivars Hindi, Basrai and Williams decreased as the concentration of triadimefon increased. These results are in agreement with those obtained by Lu et al. (2000) who reported that triadimefon affected many plant growth properties. The shoot-tip explants of cv. Williams were more sensitive to the fungicide toxicity of triadimefon while

the shoot-tip explants of Hindi were more tolerant. The sub-lethal concentrations for rooting were equal (40 mg/l) for cvs. Hindi and Williams while the sub-lethal concentration for rooting of cv. Basrai was 30 mg/l. These results are in agreement with those reported by Kaspers (2009) and Fletcher et al. (2000) who pointed out that the triadimefon inhibitory effect varied according to plant genotype. Triadimefon affected the number and length of lateral buds, the number and length of leaves, the shoot clusters fresh weight and the efficiency of root formation in the three desert banana cultures. These results are in agreement with those obtained by Khalil et al. (1990) who reported that triazole fungicide triadimefon can be associated with the delay of seedling emergence and reduction of plant height, length of coleoptiles, primary leaves and roots. The results of this study are also in agreement with those obtained by Sivakumar et al. (2009) who reported that the application of triadimefon inhibited the number of leaves, total leaf area, stem length, internode length, leaf and stem dry weight and relative shoot growth rate. Abdul Jaleel et al. (2008), Fletcher and Hofstra (1985) pointed out that triadimefon effected plants including the development of shorter and more compact shoots. Sheena and Sheela (2010) pointed out that the application of triadimefon caused a retarding effect on the height of plants compared to the untreated ones. The triadimefon-treated plants exhibited a high leaf area index for untreated plants compared to treated ones (Fletcher and Hofstra, 1985). Alterations in leaf morphology and cell contents triggered by triadimefon have been widely reported (Fletcher and Nath 1984, Fletcher and Hofstra, 1985). The leaves



become shorter, thicker, sturdier, greener and more erect compared to the untreated seedlings. Specific leaf area and the dry matter content per shoot remained lower in the treated seedlings compared to the control (Fletcher et al., 2000). The results of this study showed that treatment with triadimefon caused a decrease in the number of roots per shoot and the average length of roots. These results are in agreement with those obtained by Khalil et al. (1990) and Sheena and Sheela (2010) who reported that application of triadimefon decreased the number and length of plant roots. The same results were obtained by Sivakumar et al. (2009) who reported that triadimefon treatment decreased the root length and root dry weight of plants. Al Mansouti and Kurup (2009) pointed out that triadimefon affected mitosis by a direct rather than indirect action on the build up or on the function of the mitotic apparatus. Fletcher and Hofstra (1985) pointed out that triadimefon application affected the membrane vesicle which was in close association with microtubules. Our results showed that pigment content (Chl a, Chl b and carotenoids) in the treated shoots of cvs. Hindi, Basrai and Williams was increased compared to control I. These results are in agreement with those obtained by Khalil et al. (1990) and Kishorekumar et al. (2007) who reported that the triazole fungicide triadimefon increased chlorophyll and carotenoid content in the treated plants. Triadimefon treatment increased the leaf area and chlorophyll content in beans, peas and radish over their respective controls (Fletcher and Nath, 1984). The intense greening of the leaves of the treated shoots with the sub-lethal concentration (50 mg/l) of triadimefon may be attributed

to the increase in Chl concentration per unit area. This observation is in agreement with those reported by Khalil et al. (1990) and Sivakumar et al. (2009) who pointed out that pigments probably condensed into a smaller area of the leaf, which appeared dark green than control ones. Kisohrekumar et al. (2007) and Gomathinayagam et al. (2008) attributed the greening effect to the growth-retarding activity of triazole fungicides. Sivakumar et al. (2009) reported that triazole fungicide triadimefon reduced the leaf area but increased the epicuticular, leaf width and thickness of leaves by inducing additional layers of palisade and mesophyll cells. Fletcher and Hofstra (1985) and Abdul Jaleel et al. (2008) reported that triazole fungicide triadimefon effects on plants include thicker and greener leaves and reduced leaf growth with significantly higher content of photosynthetic pigments (chlorophyll, carotenoids, xanthophylls). The results of the present study showed an increase in all tested photosynthetic pigments of the three cultivars in the case of control II. It is well known that ethyl alcohol at a low concentration is an activator of many biochemical processes in higher plants for the photosynthetic pigments biosynthesis.

Inhibition of the biosynthesis of  $\Delta^5$ -sterols (sitosterol, stigmasterol and campesterol) could be attributed to the inhibition of the enzyme cytochrome P-450-dependent obtusifoliol-14 $\alpha$ -demethylase responsible for the removal of the C-14 methyl group, leading to accumulation of 14 $\alpha$ -methyl sterols at the expense of  $\Delta^5$ -sterols (Rahier and Taton, 1997). Fletcher and Hofstra (1985) showed that triadimefon blocked gibberellin biosynthesis by inhibiting C-14

demethylation reactions in higher plants. The growth retardant effect of triadimefon may be associated with an inhibition of the biosynthetic pathway of campesterol (Asami et al., 2003). Brassinosteroids have been shown to be synthesized from campesterol via two pathways (Hartmann, 1998). Brassinosteroids are plant sterols that cause cell elongation, cell expansion, enhance gravitropism, retard abscission and promote xylem differentiation (Hartmann, 2004).  $\Delta 5$ -sterols play an important metabolic role in the cell proliferation process. Stigmasterol might be specifically required for cell proliferation (Hartmann, 2004). Rahier and Taton (1997) have shown that triazole fungicides inhibit the 14-demethylation reaction in plant sterol biosynthesis by interacting with 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. Kaspers (2009) pointed out that the phytotoxic effect of triadimefon was accompanied with the inhibition of plant sterol biosynthesis. Our results showed that the total protein content in cv. Hindi triadimefon-treated shoots decreased compared to controls while in cvs. Basrai and Williams it increased. These results are in agreement with those obtained by Hy et al. (2002) and Schrick et al. (2000) who reported that systemic fungicides reflect a type of particular stress conditions exhibiting alteration of gene expression inducing a changes in plant metabolism resulting in an alteration in the protein synthesis that may vary according to the genotype of plants. The results of the present study

showed that the insoluble protein content of triadimefon-treated shoots decreased in cv. Hindi while in cvs. Basrai and Williams a slight increase was observed. In addition, the results on soluble protein content in cv. Hindi shoots were similar to controls while in cv. Basrai a strong increase was observed compared to controls. These results are in agreement with those obtained by Kaspers (2009) who reported that protein content varied according to the physiological and morphological characters of species.

### Conclusion

The triazole fungicide triadimefon inhibited the growth and development of three desert banana cultivars Hindi, Basrai and Williams. The inhibitory effect of triadimefon was clearly expressed on the phenotype of shoots and roots and the sterol (sitosterol, stigmasterol and campesterol) content. Triadimefon treatment increased the photosynthetic pigments (chlorophyll a chlorophyll b and carotenoids) in the three desert banana cultivars. The inhibitory effect of triadimefon might be attributed to its phytotoxic effect or its accumulation in plant tissues.

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