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Short Communication

Triadimefon induced physiological and ultra structural changes for moisture stress protection in Bougainvillea (*Bougainvillea spectabilis* Willd) at nursery stage

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Abstract: Hardwood stem cuttings of bougainvillea (*Bougainvillea spectabilis* Willd) consisting of two to four leaves were treated with triadimefon (triazole derivative) in the form of foliar spray at 50 ppm and 100 ppm, while the control plants were treated with water spray. The (treatment) plants were irrigated once in two days, once in four days and the control plants were irrigated daily. The experiment was conducted at the Al Maqam campus of the U.A.E. University, Al Ain. The experimental design was completely randomized with the two factors being irrigation and chemical treatment, consisting of nine treatment combinations, replicated three times. The relative water content (RWC) in both concentrations was similar, while the control plants showed high plant-water content. Photosynthetic pigments viz., Chlorophyll *a*, increased with increasing concentration of the chemical, while the Chlorophyll *b* and Total Chlorophyll showed significant differences with different treatment combinations of irrigation frequencies and triadimefon treatment. Combination of triadimefon with irrigation frequencies produced sun-type chloroplasts and increased chlorophyll accumulation. Compared with the control, the stomata from the abaxial epidermal peels in the triadimefon treated leaves were sunken, narrow and appeared partially closed. Both triadimefon treatments increased the number of trichomes compared to the leaf samples of control plants. Formation of epicuticular wax in treated plants was noticed, which was probably formed to act as a barrier against water loss. The results of the present study may add to the plant growth regulatory effects data published previously on the synthesis of photosynthetic pigments, and other growth responses conducive for protection against moisture stress.

Keywords: Triadimefon, moisture stress, chloroplast, trichomes, sunken stomates.

تحفيز التغيرات الفسيولوجية والبنائية الخلوية في نبات الجهنمية (*Bougainvillea spectabilis* Willd) عن طريق معاملتها بمادة Triadimefon لحمايتها من الجفاف في مرحلة التشتيل

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الملخص: أجريت تجربة حقلية في منطقة المقام في الحرم الجامعي في جامعة الإمارات العربية المتحدة بمدينة العين لدراسة تأثير مادة Triadimefon على التغيرات الفسيولوجية والبنائية الخلوية في نبات الجهنمية لحمايتها من الجفاف في مرحلة التشتيل . تم معاملة عقل متخشبة من الجهنمية (*Bougainvillea spectabilis* Willd) تحتوي على ورقتين إلى أربعة أوراق بمادة triadimefon (من مشتقات Triazole) على شكل رذاذ و بتركيز 50 و 100 جزء بالمليون بينما تم معاملة الشاهد بالرش بالماء. النباتات المعاملة كانت تروى مره كل يومين ، مرة كل أربعة أيام بينما الشاهد كان يروى يوميا. تصميم الكامل العشوائية تم استخدامه مع عاملي الري والمعاملة الكيميائية، وتتألف من تسع توليفات و تم تكرارها ثلاث مرات. محتوى الماء النسبي (RWC) في كل من التركيزين متماثلة في حين أظهر الشاهد نسبة اعلي من المحتوى المائي . أصباغ التمثيل الضوئي، الكلوروفيل أ، ارتفع مع زيادة تركيز المواد الكيميائية، في حين أن الكلوروفيل (ب) والكلوروفيل الكلي اظهر اختلافات معنوية باختلاف معاملات تكرار الري والمعاملة ب triadimefon . توليفة المعاملة ب triadimefon مع الري أنتجت اليخضور الضوئي وزيادة تراكم الكلوروفيل. بالمقارنة مع الشاهد فان الثغور في الجزء العلوي من الورقة والمعاملة ب triadimefon كانت غائرة وضيقة، وبدا جزئيا أنها مغلقة. المعاملة ب triadimefon أدى إلى زيادة عدد الأهداب مقارنة بأوراق نبات الشاهد. لوحظ تشكل طبقة من الشمع في النباتات المعاملة، والتي ربما شكلت لتكون بمثابة حاجز ضد فقدان المياه . نتائج الدراسة الحالية

يمكن أن تضاف إلى البيانات تاثير منظمات نمو النبات و التي نشرت سابقا على تصنيع أصباغ التمثيل الضوئي ، وغيرها من الاستجابات للمساعدة في حماية النبات من إجهاد فقدان الرطوبة .

الكلمات المفتاحية : Triadimefon، الجفاف، اليخضور، الأهداب، الثغور الغائرة.

Introduction

Bougainvillea is a very popular and extensively grown shrub meant for landscaping in the public and home gardens of the UAE. It has wide adaptability to different hostile abiotic factors like lack of water, high temperature and salinity. The United Arab Emirates lies in the area of the hot desert climate, characterized by two main seasons, a long dry summer with temperature rising above 48ºC between May and September, and the other - a short, moderate winter between December and March during which the temperature rarely drops below 6ºC (UAE Agriculture Information Center). Arid region soils are characterized by lack of organic matter, very high porosity, which makes the water percolate to the deeper layers beyond the root zone, therefore limiting absorption.

The nursery stage of the crop is critical with respect to the rooting of the cuttings and further field establishment. Experimental evidence (Abdul Jaleel et al., 2008) is available on the use of triazole derivatives to induce combative mechanism in plants to drought, salinity, heat and air pollutants that are unrelated stresses to make the plants hardier. Fletcher et al. (2000) reported that a large number of physiologically important compounds viz., gibberllic acid, abscisic acid, cytokinins, carotenes are products of the isoprenoid pathway, and the triazole compound acts on the pathway to regulate the growth and development of the plant. It also brings about dynamic equilibrium which is considered as a balance in the synthesis of major plant hormones such as ABA, cytokinins and gibberllic acid to induce combative mechanism in plants to unrelated stresses. (Asare-Boamah et al., 1986). This study examines the effect of Tridimefon and irrigation frequencies on the ability of bougainvillea cuttings to withstand moisture stress; and the associated changes in the leaf ultra structure, RWC and chlorophyll content to induce stress protection.

Materials and Methods

The experiment was laid out with bougainvillea as the principal test material at the Al Maqam campus of UAE University, Al Ain, to investigate the response of bougainvillea cuttings to foliar application of triadimefon and varied irrigation frequencies.

Plant material

Hardwood cuttings of bougainvillea were planted in potting mixture consisting of one-part sand, peat moss and cultivation soil each, in pots 9 inches in diameter. The cuttings developed fully opened new leaves in a span of one month, although the older leaves retained at the time of obtaining the cuttings withered off subsequently. The emergence of new leaves did not vary drastically as the location of the cuttings was uniform from the previous season’s growth and identical regions on the stem. The tips and basal portions of the stems were avoided while performing the cuts.

Treatments

The plants were subjected to two sets of treatments varying the frequencies of irrigation in combination with different concentrations of triadimefon (Triazole derivative). The plants were irrigated with 192 ml of water per pot, calculated based on the volume of the soil contained in the pots, and the saturation point of the soil.

Chemical treatment was done in the mode of foliar application. The initial foliar spray at the desired concentration was applied followed by two booster sprays at an interval of two weeks. The chemical formulation was prepared by weighing and dissolving the appropriate quantity of the chemical in a small quantity of water and raised to the desired volume to maintain the fixed concentration.

Irrigation treatments

Variable frequencies of irrigation was supplied to the plants in the form of daily irrigation (I0), irrigation once in two days (I1), irrigation once in four days (I2).

Chemical treatments

Control plants received water spray (T0), tridimefon at 50 ppm concentration (T1), triadimefon at 100 ppm concentration (T2). The experiment was laid out in the field during November, 2006 when the ambient day temperature recorded as 24ºC and the relative humidity as 65%. The ambient conditions changed with the progress of the experiment with the temperature recorded at 20ºC and the relative humidity as ± 5 %.

Design of the experiment

The experiment was laid out in completely randomized design with the two factors being irrigation and chemical treatment. There were a total of nine treatment combinations, and were replicated three times with 6 plants per replication.

Statistical analysis

The data was statistically analyzed using Duncan's multiple range and subject to ANOVA to test the significance.

Physiological parameters

Relative water content (RWC)

The fresh weight of leaf samples was taken after punching out ten uniformly sized leaf disc. They were then set afloat in water and incubated at room temperature for 24 hours. The turgid weight was recorded after blotting to remove surface moisture. The samples were the oven dried at 65^0 C for 48 hours to determine the dry weight.

The RWC was worked out using the formula:

Fresh weight – Dry weight/Turgid weight – Dry weight x 100 (Fletcher et al., 1988).

Photosynthetic pigments

The photosynthetic pigments Chlorophyll *a*, Chlorophyll *b* and Total Chlorophyll were estimated after homogenizing 0.5 g of the fresh leaf sample in 80% chilled acetone. The chlorophyll content was estimated after reading the absorbance using a visible UV spectrophotometer PU 8625 series, at 663 and 665 nm and (Asare – Boamah et al., 1986).

Chlorophyll a (mg/g)= 12.7(OD663)-2.69(OD645) x (vol./wt.)

Chlorophyll b (mg/g)= 22.9(OD645)-4.68(OD663) x (vol./wt.)

Total Chlorophyll (mg/g)= 20.2(OD645) +8.02(OD663) x (vol./wt)

Scanning electron microscopy (SEM)

The leaf blade segments from the central region 5 mm in size were prepared as per the procedure of Hayat (1978). The leaf tissues were fixed with 2.5% gluteraldehyde in 0.07 M Sorensens phosphate buffer at pH 6.8, followed by post fixation in 1% Osmium Tetroxide in 0.07 M Sorensens phosphate buffer at pH 6.8 for 4 hr. They were then washed in 0.1 M phosphate buffer (pH 6.8) 5 to 6 times, for 15 min each time. This was done until the smell of glutaraldehyde completely disappeared which took approximately two hours. The specimens were then passed through a series of 25 to 100% ethanol in order to dehydrate the samples. There was a 15 min interval between each washing and they were repeated twice in 100% ethanol. The specimens were then coated with 20-30 nm of 60% gold in a Technics Hummer V sputter before being observed in the scanning electron microscope (Joel Model JSM – 5600 and (EDX) fully computerized) at a resolution of 18,000 and a magnification of 30,000.

Results and Discussion

Photosynthetic pigments

The plants that received 100 ppm (T2) triadimefon showed a significantly high amount of chlorophyll *a* pigment, 0.2711 mg/g, compared to the control, 0.1667 mg/g; while plants that received 50 ppm (T1) triadimefon contained 0.2467 mg/g of chlorophyll *a*, irrespective of irrigation treatment (Table 1). The effectiveness of the triazole derivative in enhancing the synthesis of the chlorophyll pigment was evident from the presence of dark green leaves in the triadimefon treated plants, while the control plants had light green leaves. These results were similar to those reported in the triadimefon treated bean seedlings (Asare-Boamah et al., 1986). The synthesis of the photosynthetic pigment by the action of the triazole derivative is confirmed with the stimulation of chlorophyll levels in cucumber plants after application of Triadimefon (Fletcher and Arnold, 1986).

A similar stimulation of chlorophyll synthesis in cucumbers after the application of triadimefon and uniconazole was reported as being mediated through the effect of cytokinins (Fletcher and McCullagh, 1971). The decline in Chlorophyll level is indicative of oxidative stress damages and was prevented by treatment with a triazole derivative (Asare-Boamah and Fletcher, 1986).

Table 1. Effect of triadimefon treatment on the content of chlorophyll *a* irrespective of irrigation.

Treatments (ppm)	Chlorophyll *a* (mg /g fresh wt.)
0	0.1667 b
50	0.2467 a
100	0.2711 a

Triadimefon was applied as foliar spray after the emergence of leaves in the cuttings. Each figure is a mean of six plants .Means within columns followed by the same letter are not significantly different at $P<0.05$ according to Duncan's Multiple Range test.

Chlorophyll *b* and Total Chlorophyll also showed a significant difference with different treatment combinations of irrigation frequencies and triadimefon treatment. The highest amount of Chlorophyll *b* and Total Chlorophyll was observed in plants irrigated once in two days with 100 ppm triadimefon (Table 2). However, it was observed in this study that Chlorophyll *b* and Total Chlorophyll levels were unaffected by wider frequency of irrigation, carried out once in four days (I2) in combination with triadimefon at 50 ppm (T1). Enhanced synthesis of the chlorophyll pigment by triadimefon treatment has previously been reported, and is attributed to the increased cytokinin activity (Davis et al., 1988; Sankhla et al., 1997).

Hence, it is visualized that irrigating once in four days with the application of 50 ppm triadimefon is the optimum level for bougainvillea to maintain the highest chlorophyll content for better growth and stress protection.

Table 2. Effect of triadimefon treatment on the content of chlorophyll *b* and total chlorophyll.

Treatments		Chl *b* (mg/g)	Total Chl. (mg/g)
Irrigation	**Triadimefon (ppm)**		
Daily (I0)	0	0.14 abc	0.353 bcd
	50	0.137 abc	0.41 ab
	100	0.127 bc	0.36 bc
Once in two days (I1)	0	0.09 c	0.226 d
	50	0.12 bc	0.33 bcd
	100	0.19 a	0.53 a
Once in four days (I2)	0	0.11 bc	0.367 cd
	50	0.163 ab	0.4267 ab
	100	0.143 abc	0.383 bc

Values followed by the same letter are not significantly different at $P<0.05$ according to Duncan's Multiple Range test.

Scanning electron microscopy (SEM)

Chloroplast

Scanning electron micrographs showed densely packed and larger-sized chloroplasts (sun-type), in triadimefon treatment compared to those in daily irrigated plants without the application of triadimefon (Fig. 1). Similar results were reported in wheat seedlings (Gao et al., 1988). In a number of previous studies, triadimefon treated plants were dark green with significantly higher levels of chlorophyll than in the control plants. Cytokinins are known to have the property to induce greening of the plants, whereas triadimefon has shown to enhance cytokinin levels in plants (Fletcher, 1985). The increase in the number of chloroplasts, and production of sun-type chloroplasts with increased size in triadimefon treated plants could be the result of triadimefon–enhanced cytokinin levels (Fletcher and Arnold, 1986)

Sun-type chloroplasts induce tolerance against water and temperature stresses. Lichtenthaler (1979) reported that triadimefon caused the formation of sun-type chloroplasts and increased chlorophyll accumulation in radish cotyledons, similar to those produced in the treated bougainvillea cuttings in the present study. Increased frequency and production of sun-type chloroplasts was also noticed in cucumber leaves upon induction of moisture stress after the application of triadimefon (Shyam and Nair, 1999).

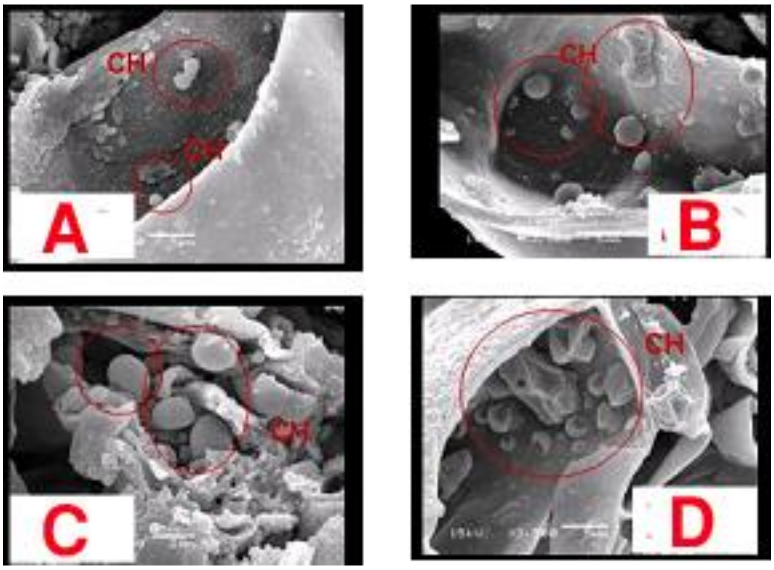

Figure 1. Scanning electron micrograph of bougainvillea leaves showing chloroplasts (CH) inside circles. Compare control (A) irrigation daily without triadimefon, (B) irrigation once in two days without triadimefon, (C) irrigation once in two days with 50 ppm triadimefon and (D) irrigation once in four days with 100 ppm triadimefon.Note that triadimefon produced densely packed and sun-type chloroplasts. (x 1000).

Stomates

Scanning electron micrographs of the stomata from abaxial epidermal peels of the bougainvillea leaves. (Fig. 2) showed that in the triadimefon-treated leaf samples, the stomates were sunken and narrow with the stomatal pores partially closed. The subsidiary cells were wider than the controls because they were expanded. These changes are probably due to the fact that triadimefon increased epidermal cell width, that subsidiary cells are specialized epidermal cells .The control leaf samples showed the stomata wide open with the possibility of increased transpiration rate, which was compensated by regular irrigation to maintain high plant-water content. Triadimefon treatments also increased the stomatal number per unit area compared to the untreated controls (Fig. 2). The increase in stomatal number increases the transpiration rate, which explains how the plants with T2 treatments have lower RWC (Table 3). However T1 also had more stomates compared to that in T0, but they were partially closed and sunken, which helped in preserving higher plant-water content by reducing the transpiration rate, and continuing to allow carbon influx for photosynthesis. The effect of triazole derivatives in enhancing the photosynthetic efficiency in plants under moisture stress has been reported with "greening effect" mediated through the effect of cytokinin induced chlorophyll synthesis in cucumber (Fletcher and McCullagh, 1971). Also, a 17 to 26% increase in chlorophyll fluorescence Fv/Fm ratio was seen related to the efficiency of leaf photosynthesis in corn under moisture and heat stress by the application of triazole derivative (Pinhero and Fletcher, 1994).

These changes in stomata could be induced as a result of the effect of triadimefon on ABA and GA hormone balance as suggested by Fletcher and Hofstra (1985). There is many fold increase in the synthesis of ABA in roots which causes a single transient rise in endogenous concentration of the hormone resulting in high stomatal resistance thus regulating stomatal mechanism (Asare-Boamah et al., 1986). The reduction in stomatal aperture as a result of triadimefon-induced hormonal changes and formation of sunken stomates might be the cause of the reduced transpiration rate (Zeiger, 1983).

Trichomes

Trichomes are epidermal hairs found on the aerial surfaces of most plants. They assume many shapes and form and some function as glandular secretor organs, while others act as protection against environmental stresses and insect predators (Fig. 3).

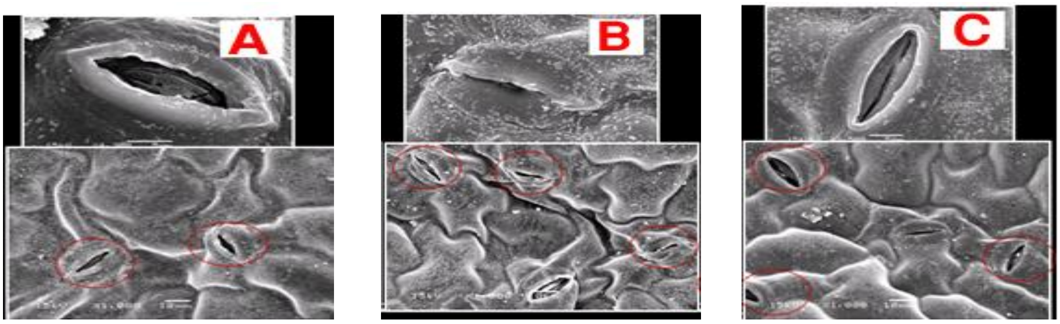

Figure 2. Scanning electron micrographs of the abaxial surface of bougainvillea leaves showing structural changes in stomates denoted inside circles. Compare control (A) without triadimefon, (B) 50 ppm triadimefon and (C) 100 ppm triadimefon.Note that triadimefon treatments made the stomates sunken and narrow while wide open in the control (x 1000).

Table 3. Relative Water Content (RWC) in bougainvillea leaves as influenced by Triadimefon.

Treatments (ppm)	RWC (%)
0	87.12a
50	82.53ab
100	81.68b

Means within columns followed by the same letter are not significantly different at P<0.05 according to Duncan's Multiple Range test.

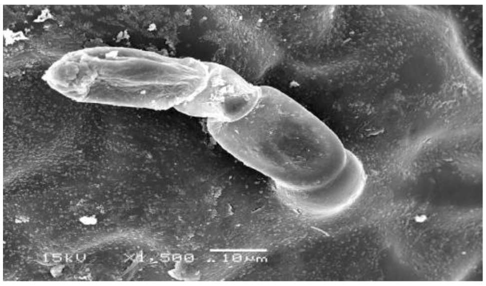

Figure 3. Scanning electron micrographs of the adaxial surface of bougainvillea leaves showing trichomes (x 500).

Scanning electron micrographs from the adaxial leaf surface (Fig. 4) of treated and untreated plants could explain the increased frequency of trichomes in leaves by the application of the triadimefon. T1 had significantly higher number of trichomes which provide a physical barrier to water loss. It was also reported that the trichomes may be considered as an adaptive advantage that enables leaves to develop and function in habitats marked by strong variations of solar radiation, air temperature and humidity (María Guadalupe Klich, 2000).

T1 has a higher frequency of trichomes per leaf area compared to T0 and T2, which explains how the bougainvillea have the optimum triadimefon concentration as 50 ppm in order to induce tolerance to drought. Similar behavioral changes in trichome formation in response to triazole derivatives have been reported by Gao et al. (1988).

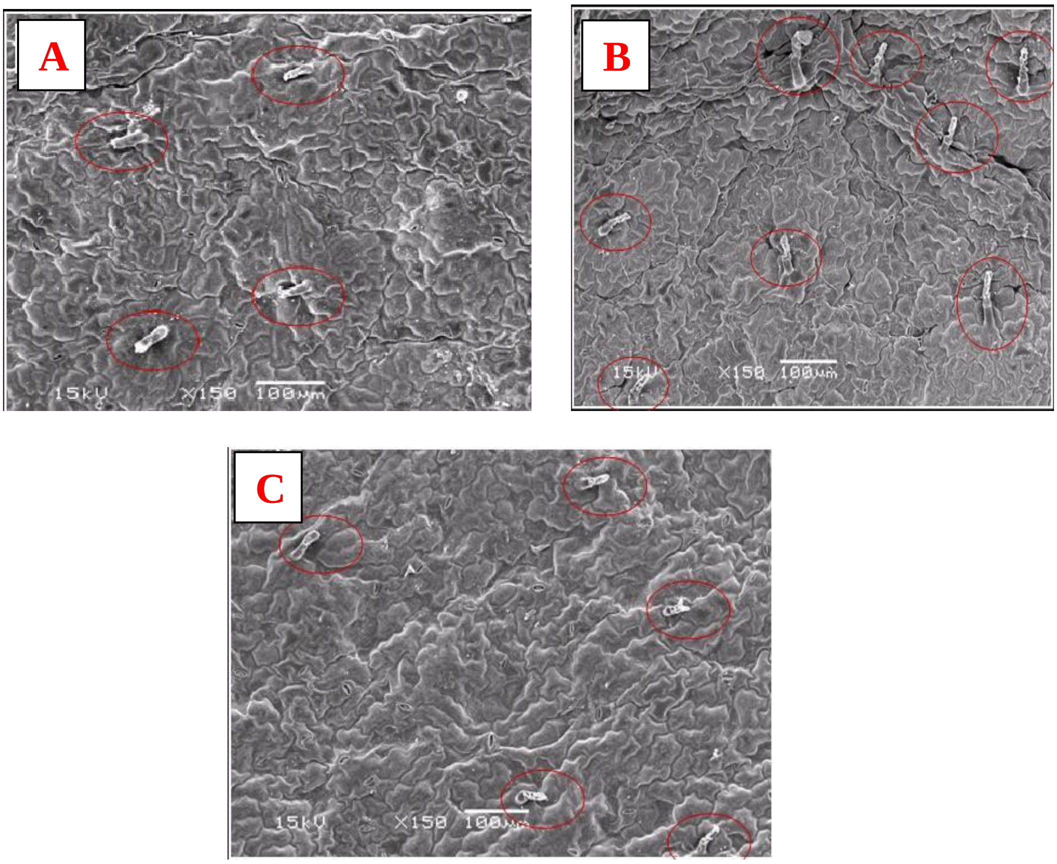

Figure 4. Scanning electron micrographs of the adaxial surface of bougainvillea leaves showing trichomes denoted in circles. Compare control (A) without triadimefon, (B) 50 ppm triadimefon and (C) 100 ppm triadimefon. Note that the triadimefon increased the frequency of trichomes (x 150).

Epicuticular wax layer

In bougainvillea leaves, epicuticular wax forms a thick layer on the outer most cuticle layer of the plant treated with triadimefon, representing an interface between leaf surface and environment (Fig. 5). A major function of this is to serve as a barrier against uncontrolled water loss. This effect may be a mechanism by which triazole application functions as an anti-transpirant and thereby protect plants from water stress (Fletcher and Nath, 1984). In

T0, the cells on the outer surface line could be clearly seen, but in triadimefon treated samples the cells were not visible due to the thick wax layer coating the cells. In some cases, waxes cause an increase in the reflection of solar radiation. In fungi, it has been shown that triadimefon interferes with lipid metabolism and increases free fatty acid, sterols, and total lipid content. These target effects of triadimefon on lipid metabolism in fungi could also occur in the non-target higher plants, as evidenced by enhanced epicuticular wax in bougainvillea leaves after triadimefon treatment (Zeiger, 1983).

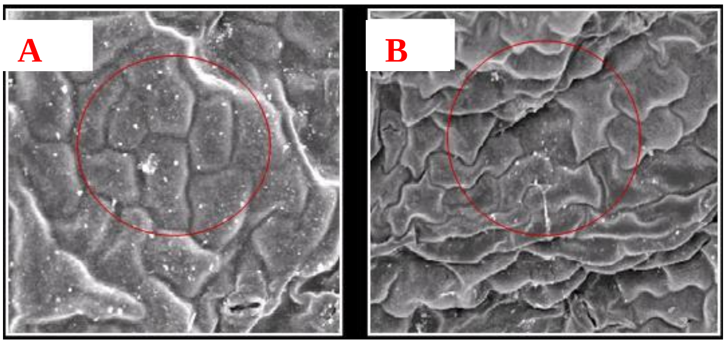

Figure 5. Scanning electron micrographs of the abaxial surface of bougainvillea leaves showing epicuticular wax formation.Compare the control (A), and triadimefon treated (B). Note that the triadimefon increased the amount of wax (x 150).

Conclusions

The present study indicates that the foliar application of triadimefon induces changes in certain aspects of physiology and ultra structure of leaves in bougainvillea cuttings to protect them from moisture stress. These changes could be mediated through an effect on the balance of major hormones like GA, ABA and CK as suggested by Fletcher and Hofstra (1985). The induced changes are in the areas of relative water content, increased photosynthetic pigments, epicuticular wax, frequency and formation of sun-type chloroplasts and sunken and partially closed stomates. It was noticed that Trichomes were very prominent and large in number. These changes could account for the plant growth regulatory activities of triadimefon at optimum concentration, and the protective role against water stress induced by increasing the frequency of irrigation.

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References

Abdul Jaleel, C., G. M. A., Lakshmanan, M. Gomathinayagam and R. Panneerselvam. 2008. Triadimefon induced salt stress tolerance in

Withania somnifera and its relationship to antioxidant defense system. South African J. of Botany. 74(1):126-132.

Asare-Boamah, N. K., G. Hofstra, R. A. Fletcher and E. B. Dumbroff. 1986. Triadimefon protects bean plants from water stress through its effects on abscisic acid. Plant Cell Physiol. 27: 383-390.

Asare-Boamah, N. K. and R. A. Fletcher. 1986. Protection of bean seedlings against heat and chilling injury by triadimefon. Physiol Plant. 67:353-358.

Davis, T. D., G. L. Steffens and N. Sankhla. 1988.Triazole plant growth regulators. Hort. Rev. 10:63-105.

Fletcher, R. A. 1985. Plant Growth regulation properties of sterol inhibiting fungicides. In hormonal regulation of plant growth and development. Edited by S. S. Purohit. Agro Botanical Publishers. India. pp.103-114.

Fletcher R. A., A. Gilley, N. S. and D. T. Davis. 2000. Triazoles as Plant Growth Regulators and Stress Protectants. Canada: Ed. John Wiley & sons, Inc.

Fletcher, R. A. and G. Hofstra. 1985. Triadimefon a plant multi-protectant. Plant Cell Physiol. 26:775-780.

Fletcher, R. A. and D. McCullagh. 1971. Cytokinin-induced chlorophyll formation in cucumber cotyledons. Planta. 101:88-90.

Fletcher, R. A. and V. Nath. 1984. Triadimefon reduces transpiration and increases yield in water stressed plants. Physiol.Plant. 62:422-426.

Fletcher, R. A., M. Santhakumari and D. P. Murr. 1988. Imposition of water stress in wheat seedlings improves the efficacy of uniconazole-induced thermal resistance. Physiologia Planta.74:360-364.

Fletcher, R. A. and V. Arnold. 1986. Stimulation of cytokinins and chlorophyll synthesis in cucumber cotyledons by triadimefon. Physiol.Plant. 66:197-201.

Gao, J., G. Hofstra and R. A. Fletcher. 1988. Anatomical changes induced by triazoles in wheat seedlings. Can. J. Bot. 66:1178-1185.

Hayat, M. A. 1978. Introduction to biological scanning electron Microscopy. University Park Press, Baltimore, MD.

Lichtenthaler, H. K. 1979. Effect of biocide on the development of the photosynthetic apparatus of radish seedlings grown under strong and weak light conditions. Z. Naturforsch. 34:936-940.

María Guadalupe Klich. 2000. Leaf variations in *Elaeagnus angustifolia* related to environmental heterogeneity. Environmental and Experimental Botany. 44(3):171-183.

Pinhero, R. G. and R. A. Fletcher. 1994. Paclobutrazol and ancymidol protect corn seedlings from high and low temperature stresses. Plant Growth Regulation.15:47-53.

Sankhla, D., N. Sankhla, A. Upadhyaya, S. Kachhwaha, S. Mali, R. Dinesh and S. Joshi. 1997. Effect of uniconazole on growth and activities of active oxygen processing enzymes in Ziziphus seedlings. Proc. Plant Growth Reg.Soc.Am. 24:208-212.

Shyam, S. K. and N. R. Nair. 1999. Anatomical and ultra structural changes induced by triazoles conducive for moisture stress protection in cucumber (*Cucumis melo* L). Proc. Plant Biology 99, American Society for Plant physiologists. pp.150. Baltimore. July 24-28.

Zeiger, E. 1983. The biology of stomatal guard cells. Annual Rev. Plant Physiol. 34:441-475.