KATCHEN JULLIANY PEREIRA SILVA

MORPHOPHYSIOLOGICAL CHANGES IN MELON AND CUCUMBER PLANTS, QUALITY OF MELON FRUITS AND PROTECTION OF CUCUMBER AGAINST *Colletotrichum lagenarium* CAUSED BY PACLOBUTRAZOL

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitotecnia, para obtenção do título de *Magister Scientiae*.

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APROVADA EM: 28 de junho de 2011.

Prof.: José Antônio Saraiva Grossi (Co-orientador)	Prof.: Paulo Roberto Cecon (Co-orientador)
Dra.: Maria Aparecida N. Sediyama (Examinadora)	Prof.: Mário Puiatti (Orientador)

Dedico aos meus pais, Júlio e Sônia, e
ao meu esposo André.

..."Em algum lugar, a distância de tempo imensa
divergiam em um bosque duas estradas
eu escolhi a menos viajada
e esta fez toda a diferença."

(Robet Frost)

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SUMÁRIO

RESUMO

SILVA, Katchen Julliany Pereira, M. Sc. Universidade Federal de Viçosa, Junho, 2011. **Alterações morfofisiológicas em plantas de melão e pepino, qualidade de frutos de melão e proteção de pepino contra *Colletotrichum lagenarium*, ocasionadas pelo Paclobutrazol.** Orientador: Mário Puiatti. Co-orientadores: José Antonio Saraiva Grossi e Paulo Roberto Cecon.

Apesar do potencial do Paclobutrazol (PBZ), tanto como fitorregulador e agente elicitor, pesquisas sobre sua utilização em cucurbitáceas ainda são escassas. Este trabalho consistiu de quatro experimentos e objetivou: avaliar o efeito do PBZ sobre as características morfofisiológicas de plantas e sobre a qualidade de frutos de meloeiro; detectar e quantificar a possível presença de resíduo de PBZ na polpa de melão por meio da Cromatografia Líquida de Alta Eficiência (HPLC); e verificar as alterações morfofisiológicas e o potencial elicitor do PBZ contra *Colletotrichum lagenarium* em plantas de pepino, agente causal da antracnose nesta cultura. No experimento 1, os tratamentos consistiram de 11 doses de PBZ (2; 4; 8; 12; 16; 20; 30; 40; 50; 60 e 70 mg PBZ planta^{-1}) e o controle (plantas não tratadas). No experimento 2, foram utilizadas quatro doses de PBZ (0,4; 0,8; 1,2 e 1,6 mg PBZ planta^{-1}) e o controle. No experimento 3, oito frutos por tratamento foram coletados para a detecção e quantificação de resíduo de PBZ na polpa do melão. No experimento 4, foram testados dois isolados de *C. lagenarium* (CLKJ11 e CLKJ25) em 7 tratamentos: meio BDA puro (testemunha) e acrescidos com concentrações dos compostos PBZ (50, 100, 200 e 400 mg PBZ L^{-1}), FTT (Tebuconazole + Trifloxystrobin) (100 mg L^{-1}) e FTB (Tebuconazole) (200 mg L^{-1}) para o bioensaio. Para a avaliação das alterações morfofisiológicas e o potencial elicitor do PBZ, as plantas de pepino foram tratadas com 4 doses de PBZ (50, 100, 200 e 400 mg PBZ planta^{-1}) e suas folhas inoculadas com a suspensão de conídios do isolado CLKJ25, na concentração 1x10^{-8} conídios mL^{-1}. O PBZ é extremamente efetivo na redução do porte de plantas de melão e de pepino, demonstrando ser uma alternativa eficaz no manejo dessas plantas no cultivo em casa de vegetação. Além disso, o PBZ não afetou a produtividade, a aparência e os atributos de qualidade dos frutos do meloeiro e não foram detectados resíduos deste fitorregulador na polpa dos melões. O PBZ também revelou-se eficaz na inibição do crescimento micelial de *C. lagenarium* e na redução da área foliar lesionada por este patógeno. Todavia, este fitorregulador não pode ser considerado um agente indutor de resistência de plantas devido ao efeito tóxico sobre o patógeno avaliado.

ABSTRACT

SILVA, Katchen Julliany Pereira, M. Sc. Universidade Federal de Viçosa, June, 2011. **Morphophysiological changes in melon and cucumber plants, quality of melon fruits and protection of cucumber against *Colletotrichum lagenarium*, caused by Paclobutrazol.** Adviser: Mário Puiatti. Co-advisers: José Antonio Saraiva Grossi and Paulo Roberto Cecon.

Despite the potential of Paclobutrazol (PBZ) as a plant growth regulator and elicitor agent, researches about its use in cucurbits are still scarce. This study was divided in 4 experiments and aimed: to evaluate the PBZ effects on development of melon plants; to evaluate the PBZ effect on morphophysiological features and quality of melon fruits; to detect and quantify PBZ residue in melon pulp using High Performance Liquid Chromatography (HPLC); and finally, verify morphophysiological changes and its elicitor potential against *C. lagenarium* in cucumber plants, causal agent of anthracnose in this culture. At first, treatments consisted of 11 PBZ doses (2, 4, 8, 12, 16, 20, 30, 40, 50, 60 and 70 mg PBZ $plant^{-1}$) and the control (untreated plants). In the second experiment 4 PBZ doses were used (0.4, 0.8, 1.2 and 1.6 mg PBZ $plant^{-1}$) and the control. To the third experiment, 8 fruits per treatment were collected to detect and quantify PBZ residues on melon pulp. In the fourth experiment, two *C. lagenarium* isolates (CLKJ11 and CLKJ25) were tested in 7 treatments: pure BDA medium (control) and plus with PBZ concentrations (50, 100, 200 and 400 mg L^{-1}), FTT (Tebuconazole + Trifloxystrobin) (100 mg L^{-1}) and FTB (Tebuconazole) (200 mg L^{-1}) to the bioassay. To evaluate the morphophysiological changes and elicitor potential of PBZ, cucumber plants were treated with 4 PBZ doses (50, 100, 200 and 400 mg PBZ $plant^{-1}$) and its leaves inoculated with suspension of isolate CLKJ25 at concentration of $1x10^{-8}$ conidia mL^{-1}. PBZ is extremely efficient in reducing the size of melon and cucumber plants proving to be an effective alternative for management in greenhouse crop. In addition, PBZ did not affect the productivity, appearance and quality attributes of melon fruits and no residue was found in melon pulp. The PBZ also proved to be effective in inhibiting the *C. lagenarium* mycelial growth and in the reduction of lesioned leaf area by this pathogen. Even so, this phytoregulator can not be considered a plant resistance inducer due to the toxic effect on the tested pathogen.

GENERAL INTRODUCTION

Melon (*Cucumis melo* L.) is an herbaceous plant of *Cucurbitaceae* family, demanding in soil and special climatic conditions. It is an annual cycle plant that has a very strong creeping stems, variable number of tendrils and well branched root system (Joly, 1991; Fontes & Puiatti, 2009). Due to the climatic conditions of central-south region of Brazil, the cultivation can only be done during the summer (Fontes & Puiatti, 2009). However, this period is characterized by intense rainfall, which, besides promoting the plants death and crop delaying; contributes to pests and diseases emergence, and cause a reduction in leaf area resulting in small fruits, poorly reticulation, low sugar content and low quality of fruits and yield, requiring the crop to be grown at greenhouse conditions (Coelho et al., 2003). However, a common problem encountered in this type of cultivation is the excessive plants growth. Thus, it is necessary to use techniques that reduce plant size in height, without reducing productivity and fruit quality.

The plant growth retardants act as chemical signals in the regulation of growth and development of plants. Among them, Paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol], known as PBZ, is a gibberellin biosynthesis inhibitor, which has proven to be an effective plant growth regulator and a plant protector with fungicidal activity of low toxicity, and so, shows a large potential use as a plant growth retardant of ample aspect (Tongumpai, 1991). Its use has as main purpose of promote the reduction of plant growth due to its action mechanism by blocking the oxidation reactions in the passage of kaurene to kaurenoic acid synthesis in the way of gibberellins substance (Salisbury & Ross, 1992), promoting a series of physiological changes in plants, including the partitioning of carbohydrates, reduction of internodes length and reduction in the length and width of leaves, which could increase flowers and fruits production (Basra, 2000).

In general, this compound is not considered phytotoxic and has no neurotoxicity or carcinogenic effect, however, exposure of female rats to the product during the reproductive stage can lead to fetal abnormalities, both physical and behavioral, the occurrence of critical periods in animal development during pregnancy and immediately after birth (Silva & Fay, 2003). In saturated environment, can cause irritation of the eyes and respiratory system by inhalation and irritation when in contact with skin.

The World Health Organization (WHO) classifies PBZ as moderately toxic (toxicity class III), establishing, by the FAO a value of acceptable daily ingestion (ADI) of 0.1 mg kg^{-1} for body weight (Tonlim, 1997). In case of fruits like pears, some countries of European Union have established maximum residue limits (MRL) for PBZ consumer safety, such as Spain, Germany, France and Switzerland (0.05, 0.05, 0.5 and 0.3 mg $kg^{-1}$for body weight, respectively). Other countries like Japan and organizations such as the Codex (NZFSA, 2010) also set its own values, ranging from 0.2 to 1.0 mg kg^{-1} for body weight. However it should be noted that despite the establishment of tolerance values of PBZ in fruits like pears and apples, their use is not yet registered for these crops. In Brazil, according to ANVISA (2010), the plant regulator also has toxicity class III, however, ADI is 0.068 mg kg^{-1} for body weight, and registered for now, just for mango culture.

Nevertheless, PBZ has been widely studied in agriculture and its effects have been reported in several plant species, emphasizing: the group of ornamental plants, which most benefited from the advances resulting from PBZ use, due to significant reductions in stem elongation (Goulston & Shearing, 1985); the grain crop (Lenton et al., 1994), due to maximize the grain lodging; the horticulture, due positive effects on productivity, fruit quality and in changes on pattern of plants development in relation to stem elongation, flowering and fructification (Siqueira & Salomão, 2002); and the maintenance of grass and margins and/or central beds of highways, where the application of inhibitors of gibberellin synthesis restricts plant growth, avoiding large costs of manual or mechanical pruning (Taiz & Zaiger, 1998).

In vegetables, researches are few and restricted to a small number of species. Promising results have been found in garlic (Resende et al., 1999), potato (Tekalign & Hammes, 2005), tomato (Berova & Zlatev, 2000), onion (Yiu et al., 2008), watermelon (Oh, 2008) and cucumber (Shimotsuma & Jones, 1972). Information about the use of growth regulators and, more specifically, the use of PBZ in melon, are still scarce. Nevertheless, Zhang et al. (2006) found that the use of PBZ controlled the length of the internodes, axis plumule and preventing the overgrowth of melon plants effectively.

Besides being effective growth regulator, this compound has also been used in the activation and regulation of processes involved in the biosynthesis of plant defense, presenting thus potential elicitor. For instance, cucumber plants treated with PBZ had their levels of indole acetic acid reduced, which is unfavorable to the development cucumber scab caused by *C. cucumerinum* (Van Andel, 1968). PBZ also promoted reduction in the incidence of *Fusarium* wilt on melon plants (Cohen et al., 1987) and *Verticillium* Wilt on cotton (Cimen et al., 2004). However, further studies are needed to confirm the efficiency of this growth regulator on disease prevention.

PBZ has been shown to be chemically stable in hydrolysis tests (pH 4-9), with ultraviolet light at pH 7 in aqueous solutions over a period of 10 days. It is considered stable under photolysis studies in surface soils. Despite the lack of studies about PBZ in vegetables metabolism, some researches indicate that this compound leaves residue in treated fruits. Thus, its wide application in different crops needs residue monitoring especially in products for human consumption such as fruits and vegetables (Zamora, 2004). The literature presents some methods for determination of PBZ residue. One of them is the High Performance Liquid Chromatography (HPLC) (Singh & Bhattacherjee, 2005), with the advantage of being relatively inexpensive, rapid, less labor intensive and very accurate, showing to be advantageous in this sense (Witchard, 1997).

This study aimed to evaluate the effect of PBZ doses on morphophysiological features of melon plants, fruit quality and yield; detect and quantification of PBZ content in melon pulp using HPLC; evaluate the *in vitro* sensitivity of *C. gloeosporioides* isolates to PBZ and verify the elicitor potential of PBZ on treated cucumber plants inoculated with *C. lagenarium*.

REFERENCES

ANVISA. Agencia Nacional de Vigilância Sanitária. Agrotóxicos e toxicologia. 2010. Monografias de Produtos Agrotóxicos. http://www.anvisa.gov.br/toxicologia/monografias/index.htm. Acessed 8 June 2011.

BASRA AS. 2000. *Plant Growth Regulators in Agriculture and Horticulture: Their Role and Commercial Uses.* The Haworth Press, Binghamton, NY-USA, 264p.

BEROVA M, ZLATEV Z. 2000. Physiological response and yield of Paclobutrazol treated tomato plants (*Lycopersicon esculentum* Mill.). *Plant Growth Regulation* 30: 117–123.

CIMEN I, BASBAG S, TEMIZ M, SAGIR A. 2004. The effect od Paclobutrazol, Growth Retardant, on cotton growth and *Verticillium* Wilt (*Verticillium dahlia* Kleb.). *Plant Pathology Journal* 3: 35-39.

COELHO EV, FONTES PCR, CARDOSO AA. 2003. Qualidade do fruto de melão rendilhado em função de doses de nitrogênio. *Bragantia* 62: 173-178.

COHEN R, YARDEN O, KATAN J. 1987. Paclobutrazol and other plant growth-retarding chemicals increase resistance of melon seedlings to *Fusarium* wilt. *Plant Pathology* 36: 558-564.

FONTES PCR, PUIATTI M. 2009. Cultura do melão. In: FONTES PCR (ed.). *Olericultura: teoria e prática*. Viçosa: Editora UFV. p. 407-428.

GOULSTON GH, SHEARING SJ. 1985. Review of the effects of Paclobutrazol on ornamental pot plants. *Acta Horticulturae* 167:339-348.

JOLY AB. 1991. *Botânica: Introdução à taxonomia vegetal*. Companhia Editora Nacional, São Paulo. 778p.

LENTON JR, APPLEFORD NEJ, TEMPLE-SMITH KE. 1994. Growth retardant activity of Paclobutrazol enantiomers in wheat seedling. *Plant Growth Regulation* 15: 281-291.

NZFSA. New Zealand Food Safety Authority. 2010. Plant Residue Standards (Pesticide Maximum Residue Limits - MRLs). http://www.nzfsa.govt.nz/plant/subject/horticulture/index.htm. Acessed 8 June 2011.

OH JIYOUNG. 2008. *Growth regulator effects on watermelon chilling resistance, flowering, and fruiting.* Raleigh, North Carolina: Graduate Faculty of North Carolina State University. 123p. (Master Thesis).

RESENDE GM, COSTA ND, MELO NF, SOUZA RJ. 1999. Efeitos do Paclobutrazol em diferentes concentrações e períodos de imersão na cultura do alho. *Pesquisa Agropecuária Brasileira* 34: 635-639.

SALISBURY FB, ROSS CW. 1992. *Plant Phisiology*. Belmont: Wadsworth, 682p.

SHIMOTSUMA M, JONES MC. 1972. Effects of ethephon and daylength on sex expression of muskmelon and watermelon. *Hortscience* 7: 72-73.

SILVA CMMS, FAY EF. 2003. Impacto Ambiental do Regulador de Crescimento Vegetal Paclobutrazol. Embrapa Meio Ambiente: Jaguariúma, 106p.

SIQUEIRA DL, SALOMÃO LCC. 2002. Efeitos do Paclobutrazol no crescimento e florescimento dos citros. *Laranja* 23: 355-369.

SINGH VK, BHATTACHERJEE AK. 2005. Genotypic response of mango yield to persistence of Paclobutrazol in soil. *Scientia Horticulturae* 106: 53-59.

TAIZ L, ZAIGER E. 1998. *Plant physiology*. 2 ed. Palo Alto, Readward City: The Benjamin/Cummings, 564p.

TEKALIGN T, HAMMES PS. 2005. Growth and biomass production in potato grown in the hot tropics as influenced by Paclobutrazol. *Plant Growth Regulation* 45: 37–46.

TONGUMPAI P. 1991. *Flower induction of mango*. Thailand: Kasetsart University. 19p.

TONLIN CDS. 1997. *The pesticide manual.* United Kigdom: British Protection Council, 1350p.

VAN ANDEL OM. 1968. Shifts in disease resistance induced by growth regulation. *European Journal of Plant Pathology* 74:113-120.

WITCHARD M. 1997. A Simplified technique for detection of Paclobutrazol in Plant sap extracts, using HPLC. *Plant Growth Regulation* 16:213–214.

YIU J, LIU C, KUO C, TSENG M, LAI Y, LAI W. 2008. Changes in antioxidant properties and their relationship to Paclobutrazol-induced flooding tolerance in Welsh onion. *Science of Food and Agriculture* 88:1222–1230.

ZAMORA TZ. 2004. *Determinación de residues de fungicidas en productos vegetales mediante técnicas cromatográficas avanzadas*. Castellon de la Plana, Espanha: Universitat Jaume I de Castellón. 311p. (Doctoral Thesis)

ZHANG WX, LIN TJ, GU HF, JIN CY. 2006. Research of Paclobutrazol application on melon in autumn cultivation. *China cucurbits and vegetables* 4: 9-11.

I - MORPHOPHYSIOLOGICAL CHANGES IN MELON PLANTS PROMOTED BY PACLOBUTRAZOL

Abstract Paclobutrazol (PBZ) has proven to be effective in the inhibition of gibberellins biosynthesis. Despite the potential as a plant growth regulator, researches about the PBZ use in cucurbits are still scarce. This study aimed to evaluate the effects of PBZ doses on growth and development of melon plants. The treatments consisted of eleven PBZ doses (2.0, 4.0, 8.0, 12.0, 16.0, 20.0, 30.0, 40.0, 50.0, 60.0 and 70.0 mg PBZ plant^{-1}) and the control (untreated plants), distributed in a completely randomized design, with five replications. Plant length; leaves and internodes number; indirect chlorophyll measurement; leaf area; root, stem, leaf and total dry weight; and number of male flowers were evaluated and data were submitted to regression analysis. PBZ demonstrated to be extremely effective in reducing the size of melon plants. However, doses above 2.82 and 3.10 mg plant^{-1} promoted, respectively, plant growth stoppage and flowering inhibition in melon.
Keywords: *Cucumis melo* L., plant growth regulation, gibberellins inhibition, morphophysiological changes

Resumo O Paclobutrazol (PBZ) tem provado ser eficaz na inibição da biossíntese de giberelinas. Embora de seu potencial como regulador de crescimento de plantas, pesquisas sobre o uso do PBZ em cucurbitáceas ainda são escassas. Objetivou-se, por meio desse estudo, avaliar os efeitos de doses de PBZ sobre o crescimento e desenvolvimento de plantas de melão. Os tratamentos consistiram de 11 doses de PBZ (2,0; 4,0; 8,0; 12,0; 16,0; 20,0; 30,0; 40,0; 50,0; 60,0 e 70,0 mg PBZ planta^{-1}) e o controle (plantas não tratadas), distribuídos no delineamento inteiramente casualizado, com cinco repetições. O comprimento de plantas; número de folhas e entrenós; medida indireta de clorofila; área foliar; massa de matéria seca de raiz, caule, folhas e total e número de flores masculinas foram avaliados e os dados submetidos à análise de regressão. O PBZ demonstrou ser extremamente eficaz na redução do crescimento de plantas de melão. Entretanto, concentrações superiores a 2,82 e 3,10 mg PBZ planta^{-1} promoveram, respectivamente, a paralisação do crescimento de plantas e a inibição da floração em meloeiro.
Palavras-chave: *Cucumis melo* L., regulador de crescimento de plantas, inibição da síntese de giberelinas, alterações morfofisiológicas

INTRODUCTION

The Paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol] or PBZ, has proved to be an effective plant growth regulator acting as an inhibitor of gibberellin synthesis. This compound has been used in plants with the main purpose of promoting the reduction of growth due to its action mechanism in blocking the kaurene oxidation in the way to kaurenoic acid synthesis of gibberellin substances, therefore, called anti-gibberellins. Besides reducing internodes length and leaves width, this plant growth regulator promotes a series of physiological changes in plants, such as changes in carbohydrates partitioning; stimulates the production of flowers and fruits; and has fungicidal activity with low toxicity (Salisbury & Ross, 1992).

PBZ effects have been reported in several plant species. However, the ornamental species cultivated in vases and kept into greenhouses have been the most benefited from the advances resulted from the use of this compound due to the significant reductions in stem elongation (Taiz & Zaiger, 2002). Growth retardants have also been used successfully in several fruit species from tropical and temperate regions in order to reduce plant growth and increase crop production (Ferrari & Sergent, 1996). In vegetables, researches about PBZ are few and restricted to a small number of species. Promising results have been found in garlic (Resende et al., 1999), potatoes (Tekalign & Hammes, 2005), tomatoes (Berova & Zlatev, 2000) and onions (Yiu et al., 2008).

Information about the use of plant growth regulators, specifically the use of PBZ in cucurbits, is still scarce. In melon, for example, which has a vigorous and creeping stem, the application of growth regulators that allow reducing the size of the plants in height without reducing productivity and fruit quality, would be of great value, especially when cultivated in greenhouse (Fontes & Puiatti, 2009). Due to the lack of studies about PBZ use in cucurbits, this research aimed to evaluate the morphophysiological changes promoted by the application of PBZ in melon.

MATERIALS AND METHODS

Site description

The experiment was done in glasshouse at the Federal University of Viçosa, Minas Gerais, Brazil, during September-November 2009. During this period the mean of minimum and maximum temperatures were 16°C to 34°C, respectively, and the relative humidity average was 74%. Seeds of melon hybrid Gália (Gália 7681 - Enza Zaden) were sowed in polystyrene trays filled with commercial substrate (Plantmax®, Eucatex, Paulínia, Brazil). Fifteen days after sowing, with the first true leaf expanded, seedlings were transplanted into plastic pots with a capacity of 5 L, placing one plant per pot.

The pots were filled with Red-Yellow Ultisols (Embrapa, 2006), presenting the following chemical characteristics: pH in water (1:2.5) = 6.1; P = 175.4 $mg.dm^{-3}$; Prem = 27.1 $mg.L^{-1}$; K = 163 $mg.dm^{-3}$; Ca^{+2} = 5.7 $cmol_c.dm^{-3}$; Mg^{+2} = 1.2 $cmol_c.dm^{-3}$; Al^{+3} = 0.0 $cmol_c.dm^{-3}$; H + Al = 2.15 $cmol_c.dm^{-3}$; SB = 7.32 $cmol_c.dm^{-3}$; CEC_e = 7.32 $cmol_c.dm^{-3}$; CEC = 9.47 $cmol_c.dm^{-3}$; BSI = 77%; ASI = 0.0% ; SOM = 2.1 $dag.kg^{-1}$. The size analysis showed the following results: thick sand = 18%, thin sand = 11%, silt = 20% and clay = 51%. The fertilization was performed according to soil analysis and recommendations for culture (Fontes & Puiatti, 2009).

Treatments

The experiment consisted of 12 treatments with 11 PBZ doses (2.0, 4.0, 8.0, 12.0, 16.0, 20.0, 30.0, 40.0, 50.0, 60.0 and 70.0 mg PBZ $plant^{-1}$) using the formulation Paclobutrazol® 100 CE (Agro Comercial Wiser Ltda, Diadema, Brazil), plus a witness that did not receive the plant growth regulator. Treatments were arranged in completely randomized design, with five replications. Each PBZ dose (treatment) was divided into five applications, spaced seven days, and the first application performed seven days after transplanting to pots. The product was diluted in 40 ml of distilled water and applied by drench. Soil humidity was maintained near field capacity, taking care to place a dish under the pot to collect and replace any percolated liquid.

Data recorded

At the end of the experiment (42 days after the first application of PBZ) were evaluated: plant length (PL); leaves (LN) and internodes (IN) number; indirect chlorophyll measurement (SPAD); leaf area (LA); roots (RDW), stem (SDW), leaf (LDW) and total (TDW) dry matter weights. The number of male flowers (NMF) was daily measured.

PL was obtained from the plant base to the apical bud. IN and LN were determined by direct counting. The SPAD index was done with a portable chlorophyll meter SPAD-502 (Konica Minolta, Ramsey, USA), and evaluations were taken in the two youngest and fully expanded leaves. On each leaf, three evaluations were made in equidistant regions, using the average of these. LA was measured in leaf area integrator model LI-3100 (Li-Cor, Lincoln, USA). After separate individually, the plant parts (roots, stems and leaves) were dried in a forced-air oven at 60 ºC, until constant mass, and weighed on analytical balance.

Statistical analysis

The data were analyzed by regression analysis and the models have been chosen based on the significance of regression coefficients using the "t" Test, at a level of 5% of probability; the determination coefficient (R^2) and the biological phenomenon in study.

RESULTS AND DISCUSSIONS

Melon was extremely sensitive to PBZ, once that treated plants exhibited significantly smaller than untreated plants (Figure 1). The control had PL of 14.06 cm, while treated plants with 2 $mg.plant^{-1}$ (lower dose of PBZ) had an average of 8.26 cm, corresponding to 41% of reduction in PL (Figures 2).

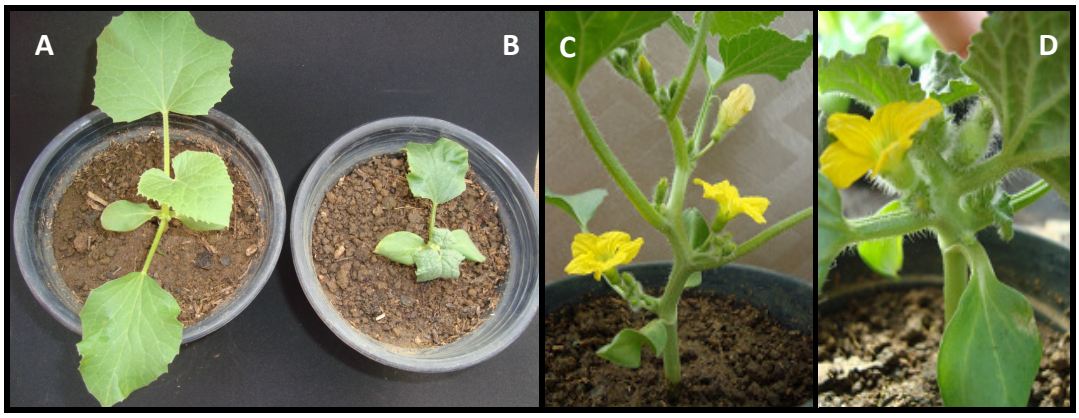

Figure 1. Paclobutrazol effect on size reduction of melon plants. A- Untreated plant with 15 days age after seedling transplantation. B- Melon plant 15 days age treated with 40 mg PBZ plant^{-1}. C- Untreated plant 25 days age after seedling transplantation. D- Melon plant 25 days age treated with 40 mg PBZ plant^{-1}.

According to Linear Response of Plateau in this experiment, PBZ concentrations equal or greater than 2.82 mg.plant^{-1} caused the growth stoppage, maintaining the average growth at 5.85 cm (Figure 2).

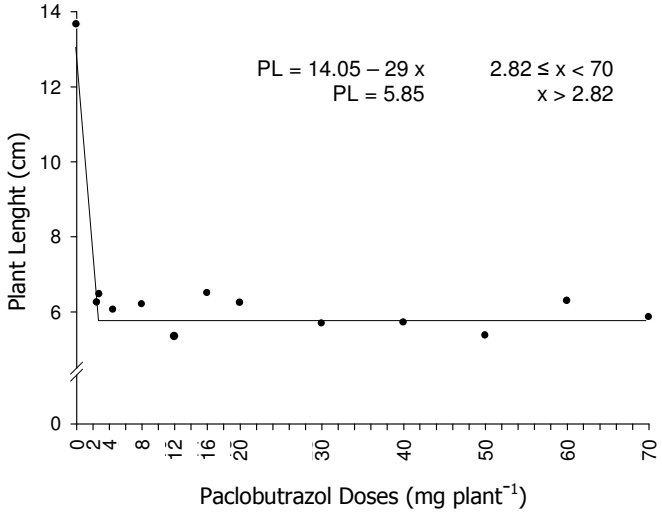

Figure 2. Plant length of melon plants (PL) according to Paclobutrazol (PBZ) doses. Plants with 42 days age after the fist application of PBZ.

Zhang et al. (2006) observed that PBZ concentration ranging from 50 to 150 mg plant^{-1} controlled strongly the internodes length and plumule axis, preventing effectively

the excessive growth of melon seedlings. Similar results were also reported by Huang et al. (1989) in watermelon, which PBZ doses ranging from 200 to 2000 mg.plant^{-1} in foliar application inhibited plant growth strongly and influenced fruit size and quality.

PBZ caused reduction in the IN, having a remarkable decrease until 20 mg.plant^{-1}. The lowest values were observed at 49.44 mg PBZ.plant^{-1}, promoting decrease of 77.6 % (Figure 3A). Gibberellin is a growth promoter hormone that acts on the extensibility of the cell wall, membrane permeability, enzyme activities and mobilization of sugars, in addition to cell elongation (Taiz & Zeiger, 2002). Thus, the IN reduction is due to the action of PBZ in the inhibition of gibberellins biosynthesis. In citrus, the reduction in branches growth was also attributed to the reduction of total available sugars in these organs induced by PBZ action (Mehouachi et al., 1996).

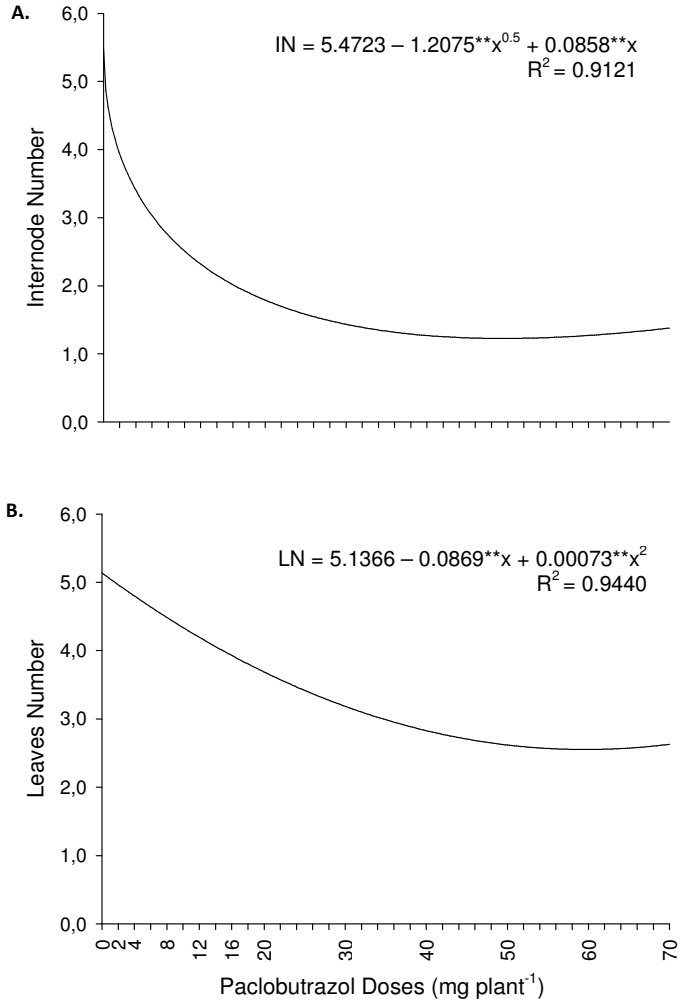

Figure 3. Internodes (IN) (A) and leaves (LN) (B) number of melon plants according to Paclobutrazol (PBZ) doses. Plants with 42 days age after the fist application of PBZ.

Due to leaves arise exogenously and to be positioned on the nodal regions, the internodes number determines the leaves number (Dickison, 2000). Thus, as the PBZ application resulted in reduction of IN, this behavior consequently reduced the LN (Figure 3B). At a PBZ concentration of 59.52 mg.plant^{-1}, was observed a reduction of 49.6 % in LN, compared to control, which had an average of 5.14 leaves. Similar results were observed by Lindon et al. (2001) in two citrus rootstocks (*Citrus macrophylla* and *Citrus aurantium*) whose PBZ application reduced plant growth, producing shorter internodes and smaller leaves.

In relation to LA, a maximum reduction was observed at a PBZ concentration of 51.45 mg.plant^{-1}, reducing 79.3% when compared to the control, that obtained LA equal to 162 dm^2 (Figure 4). Leaves developed after the application of growth regulators tend to be smaller and therefore have less leaf area. According to Khalil & Rahmanb (1995) and Basra (2000) this reduction in the proliferation and growth cells is due to inhibition of gibberellins biosynthesis caused by the PBZ action. In addition, this result can be associated to the fewer LN in the higher doses.

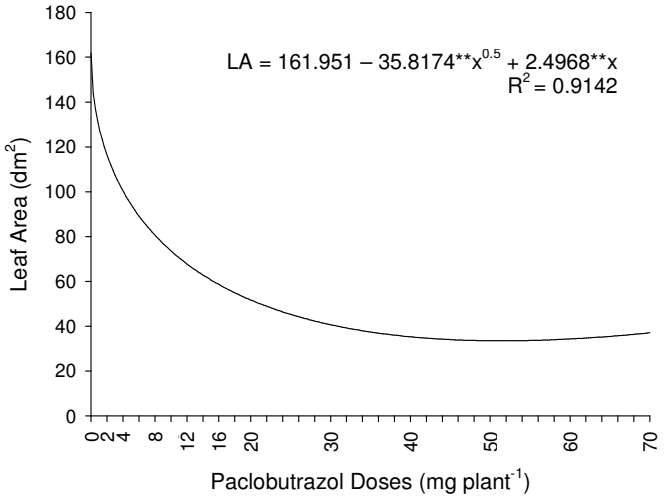

Figure 4. Leaf area (LA) of melon plants according to Paclobutrazol (PBZ) doses. Plants with 42 days age after the fist application of PBZ.

Lower PBZ doses stimulated the production of dry matter of root, leaves and total. However, SDW decreased with increasing PBZ doses (Figure 5).

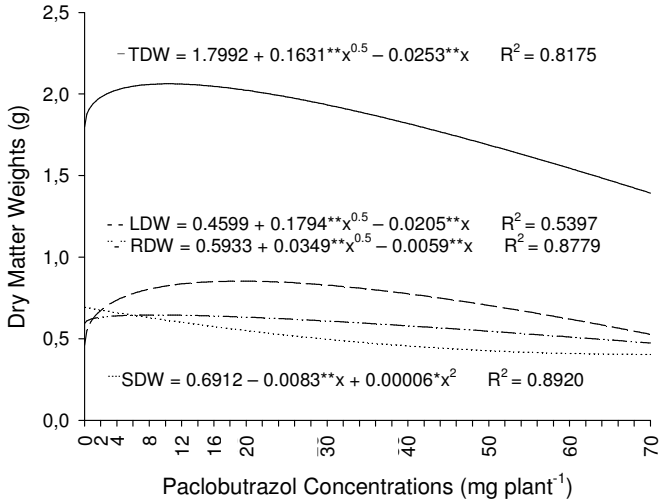

Figure 5. Root (RDW), stem (SDW), leaves (LDW) and total (TDW) dry weight of melon plants according to Paclobutrazol (PBZ) doses. Plants with 42 days age after the fist application of PBZ.

Triazoles have been described as active agents in the physiological process during root formation (Davies & Haissig, 1999). So, PBZ, as a triazole, maintains low levels of gibberellins and thus promotes the roots formation. The maximum value of RDW was obtained by application of 8.75 mg PBZ.plant^{-1}, 8.7% higher than control, which average was 0.59 g. Increasing PBZ doses resulted in lower SDW. At 69.17 mg PBZ.plant^{-1} plants had 41.5% SDW lower when compared to control. It is due to reduction in stem length through the internodes shortening. The reduction of elongation and stem diameters are characteristic effects of triazoles (Davies et al., 1988) and the most known effect caused by PBZ on plant stems (Quilan, 1981). The reduction in SDW can also be related to the decreased number of cells, small cortical cells and decrease in the xylem diameter (Fletcher & Hofstra, 1990).

Although treated plants have showed fewer leaves, smaller size and leaf area, PBZ at lower doses stimulated gains in LDW. In this essay, the maximum LDW was observed at 19.15 mg PBZ.plant^{-1}, 85.3% higher than the control. Leaves became thicker with PBZ application, due to reduction in cell elongation and increased thickness of palisade parenchyma (Tekalign & Hammes, 2005). Increase in leaf thickness in response to PBZ treatment was also confirmed in corn by Sopher et al. (1999). In relation to TDW, PBZ at low doses increased this variable in melon plants, but decrease at doses above 40 mg

PBZ.plant^{-1}. The maximum value (2.06 g) was obtained at 10.39 mg PBZ.plant^{-1}. Low SDW was offset by increasing values of RDW and LDW, promoting higher TDW in melon PBZ-treated plants.

PBZ-treated plants had leaves with more intense green color than the control, which was confirmed by the results of the SPAD index in the evaluation of indirect chlorophyll measurement (Figure 6).

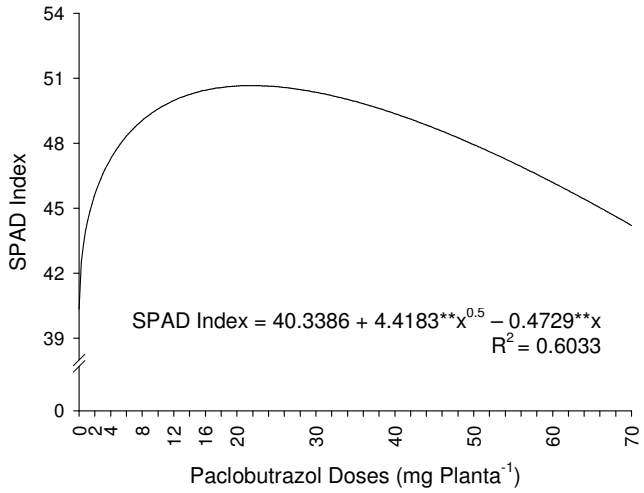

Figure 6. Indirect chlorophyll measurement (SPAD Index) of melon plants according to Paclobutrazol (PBZ) doses. Plants with 42 days age after the fist application of PBZ.

The most intense green color (50.66 SPAD unit) was obtained at 21.82 mg PBZ.plant^{-1}, which corresponds to 25.6% higher than the control (40.34 SPAD units). In most cases, SPAD index increasing is related to increases of chlorophyll content (Davies and Sankhla, 1987). However, this can be simply the result of increase on chloroplasts density caused by the reduction of leaf area (Khalil & Rahmanb, 1995).

During whole experiment the emission of hermaphrodite flowers was not observed in any treatment. However there was a large reduction in the NMF with PBZ application and inhibition of flowering with doses above 3.10 mg.plant^{-1}, according to Linear Response of Plateau (Figure 7).

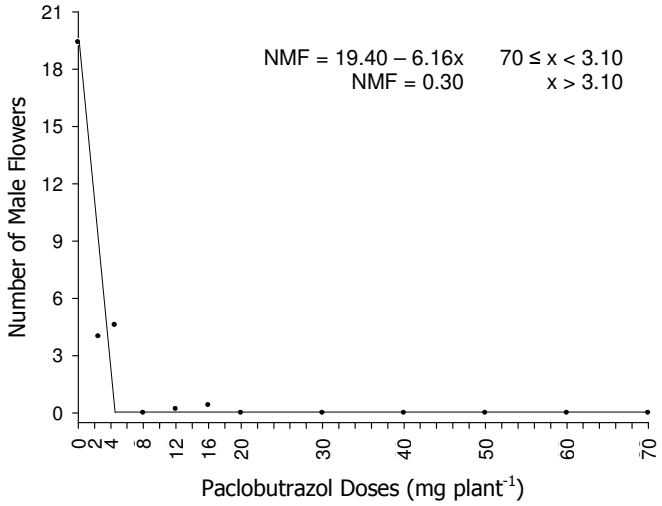

Figure 7. Number of male flowers (NMF) of melon plants according to the Paclobutrazol (PBZ) doses. Plants with 42 days age after the fist application of PBZ.

The relationship between growth regulators and flowering seems to be complex. These influence the production of flowers and can promote or inhibit it, depending on species and time of application. Some reports demonstrate that the flowering inhibition is a result of the use of very high doses of phytoregulators. However, these results can also be attributed to a secondary effect of competition between vegetative and reproductive parts for sugars and nutrients (Basra, 2000; Yuceer et al., 2003). Nevertheless, the specie, form and timing of application, the culture phenological stage, the retention factors of the soil, the time of chemical availability and climatic conditions as well as the plants size may have done a predominant function in the non-inductive flowering process of melon plants. Similar results were observed by Zhang et al. (2006), which stated that melon plants have delayed flowering with the application of PBZ.

Based on present results, this research shows that PBZ is extremely effective in reducing the size of the melon plants, which proved to be very sensitive and responsive specie to this plant growth regulator in relatively low doses. The most pronounced effects were the reduction on PL and the flowering inhibition. Doses above 2.82 and 3.10 mg PBZ.plant^{-1} promoted plant growth stoppage and flowering inhibition in melon, respectively. Therefore, the application of PBZ in commercial melon crop needs adjustments in terms of doses and timing application in order to reduce the plant size without harming the flowering and, consequently, productivity and quality of melon fruit.

ACKNOWLEDGMENTS

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REFERENCES

Basra, A.S. Plant Growth Regulators in Agriculture and Horticulture: Their Role and Commercial Uses. Binghamton: The Haworth Press, 2000. 264p.

Berova, M.; Zlatev, Z. Physiological response and yield of Paclobutrazol treated tomato plants (*Lycopersicon esculentum* Mill.). Plant Growth Regulator, v.30, n.2, p.117-123, 2000.

Davies, T.D.; Sankhla, N. Altered diurnal leaf movements in soybean seedling treated with triazole growth regulators. Plant Cell Physiology, v.28, n.7, p.1345-1349, 1987.

Davis, D.T.; Haissig, B.E. Chemical control of adventitious root formation in cuttings. Bulletin Plant Growth Regulator Society of America, v.18, n.1, p.1-17, 1999.

Davis, T.D.; Steffens, G.L.; Sankhla, N. Triazole plant growth regulators. Horticultural Reviews, v.10, n.1, p.63-105, 1988.

Dickison, W.C. Integrative Plant Anatomy. San Diego: Academic Press, 2000. 533p.

EMBRAPA. Empresa Brasileira de Pesquisa Agropecuária. Sistema Brasileiro de Classificação de Solos. Rio de Janeiro: Embrapa Solos, 2006. 412p.

Ferrari, D.; Sergent, E.A. Promoción de la floración y fructificación em mango (*Mangifera indica* L.) cv. Haden, con Paclobutrazol. Revista de la Faculdad de Agronomía, v.22, n.1, p.9-17, 1996.

Fletcher, R.A.; Hofstra, G. Improvement of uniconazole-induced protection in wheat seedling. Plant Growth Regulation, v.9, n.1-4, p.207-212, 1990.

Fontes, P.C.R.; Puiatti, M. Cultura do melão. In: Fontes PCR (Org.). Olericultura: teoria e prática. Viçosa – MG: Editora UFV, 2009. v. 1, p. 407-428.

Huang, H.; Yina, W.S.; Zhenga, G.F. The effect of Paclobutrazol on watermelon Growth. Scientia Horticulturae, v.39, n.1, p.9-14, 1989.

Khalil, I.A.; Rahmanb, H.U. Effect of Paclobutrazol on growth,chloroplast pigments and sterol biosynthesis of maize (*Zea mays* L.). Plant Science, v.105, n.1, p.15-21, 1995.

Lidón, A.G.; Bernal, I.M.; Martínez, A.C.; Fernández, F.J.B.; Castillo, I.P. Influencia del Paclobutrazol em patrones de cítricos. Investigación agrária, v.16, n.1, p.59-69, 2001.

Mehouachi, J.; Tadeo, F.R.; Zaragoza, S.; Primo Millo, E.; Talon, M. Effects of gibberellic acid and Paclobutrazol on growth and carbohydrate accumulation in shoots and root of citrus rootstock seedling. Journal of Horticultural Science, v.71, n.1, p.747-754, 1996.

Quilan, J.D. New chemical approaches to control of fruit tree form and size. Acta Horticulturae, v.120, n.1, p.95-106, 1981.

Resende, G.M.; Costa, N.D.; Melo, N.F.; Souza, R.J. Efeitos do Paclobutrazol em diferentes concentrações e períodos de imersão na cultura do alho. Pesquisa Agropecuária Brasileira, v. 34, n.4, p.635-639, 1999.

Salisbury, F.B.; Ross, C.W. Plant Phisiology. Belmont: Wadsworth, 1992. 682p.

Sopher, C.R.; Król, M.; Huner, N.P.A.; Moore, A.E.; Fletcher, R. A. Chloroplastic changes associated with Paclobutrazol-induced stress protection in maize seedling. Canadian Journal of Botany, v.77, n.2, p.279-290, 1999.

Taiz, L.; Zeiger, E. Plant Physiology. Sunderland: Sinauer Associates, 2002.672p.

Tekalign, T.; Hammes, P.S. Growth and biomass production in potato grown in the hot tropicsas influenced by Paclobutrazol. Plant Growth Regulation, v.45, n.1, p.37-46, 2005.

Tongumpai, P. Flower induction of mango. Thailand: Kasetsart University, unpaged, 1991.

Yuceer, C.; Kubiske, M.E.; Harkess, R.L.; Land Jr. Effects of induction treatments on flowering in *Populus deltoides*, Tree Physiology, v.23, n.1, p.489-495, 2003.

Yiu, J.; Liu, C.W.; Kuo, C.T.; Tseng, M.J.; Lai, Y.S.; Lai, W.J. Changes in antioxidant properties and their relationship to Paclobutrazol-induced flooding tolerance in Welsh onion. Journal of the Science of Food and Agriculture, v.88, n.7, p.1222-1230, 2008.

Zhang, W.X.; Lin, T.J.; Gu, H.F.; Jin, C.Y. Research of Paclobutrazol application on melon in autumn cultivation. China cucurbits and vegetables v.4, n.1, p.9-11, 2006.

II - PACLOBUTRAZOL EFFECT ON MORPHOPHYSIOLOGICAL FEATURES, PRODUCTIVITY AND FRUIT QUALITY OF MELON.

Abstract Paclobutrazol (PBZ) is a plant growth regulator that promotes a series of physiologic changes, like decrease of internodes length and leaf expansion. Despite its potential, researches about this phytoregulator in *Cucurbitaceae* family are scarce. This research aimed to evaluate the PBZ effect on morphophysiological features and fruit quality in melon crop. Were used four PBZ doses (0.4, 0.8, 1.2 and 1.6 mg plant^{-1}) and the control. Were realized morphophysiological analysis and verification of the post-harvest quality of melon fruits. All data were submitted to regression analysis. PBZ increased internodes, leaf and tendril numbers; net assimilation rate and indirect chlorophyll measurement. However, PBZ decreased stem diameter, leaf area, leaf area ratio and specific leaf area. Highest values of hermaphrodite flowers and hermaphrodite: male flowers ratio were obtained in 0.25 and 0.23 mg PBZ plant^{-1}, respectively. PBZ application reduced the plant size without affecting productivity and quality attributes of melon fruits, proving to be an effective alternative for management in greenhouse crop. Thus, the best dose was 0.4 mg PBZ plant^{-1} due to positives physiologic responses by melon plants; the higher number of hermaphrodite flowers and the product economy.
Keywords: *Cucumis melo*, PBZ, growth regulation, plant size, yield; post-harvest.

Introduction

The melon is an herbaceous plant of *Cucurbitaceae* family with a vigorous creeping stem (Fontes and Puiatti, 2009). Due to low temperature in fall-winter of the central and southern regions of Brazil, its culture can only be cultivated during the summer. However, in this season, is common the occurrence of intense rainfall, which favors the incidence of diseases in field crop. Thus, the use of greenhouses is common to vegetables that have limited cultivation, like melon crop.

One problem encountered in this kind of cultivation system is the difficulty in management, leading to increased costs of deployment, once that plants should be tutored, with the stem being conducted without ground contact, requiring a support with high altitude. Therefore, the use of techniques that reduce the plant size in height, without reducing productivity and fruit quality would be of great value.

The plant growth retardants act as chemical signals in the regulation of growth and development of plants. Among them, Paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol], known as PBZ, is a gibberellins (GA) biosynthesis inhibitor, which has proven to be an effective plant growth regulator and a plant protector with fungicidal activity of low toxicity, and so, shows a large potential use (Tongumpai, 1991). PBZ promotes a series of physiological changes in plants, including the partitioning of carbohydrates, reduction of internodes length and leaves thickening; however, adverse effects, including inhibition of flowering and fruiting are also reported in the literature (Basra, 2000).

Despite its potential, there are few studies about the PBZ use in vegetables, especially in cucurbits. Thus, this paper aimed to evaluate the effect of PBZ doses on morphophysiological features, crop productivity and fruit quality of melon plants.

Materials and Methods

Site description

This experiment was conducted in greenhouse, at the experimental field of the Federal University of Viçosa/MG-Brazil, during September/2009 to January/2010. The greenhouse had chapel style, with 120 m^2 (12 x 10, length and width, respectively), containing eight plots of 10 m^2 (10m x 1m) each one, covered with plastic film LDPE (150 μm), curtains in front, to make the management of closing and opening when needed. Temperature (°C) and air relative humidity (%) inside the greenhouse were recorded during the entire experimental period, by digital thermal hygrometer (HT-210, Instrutherm, São Paulo, Brazil), being adjusted at the height of the plant canopy. Were recorded an average of 22.6 ºC, 31.6 ºC and 63.4% for minimum and maximum temperature and relative humidity inside the greenhouse, respectively. To assist in pollination, two hives of honey bees (*Apis mellifera*) were placed outside of the greenhouse during the flowering period.

The plots were filled with soil classified as Clay Loam soil (EMBRAPA, 2006) with the following chemical characteristics: pH (H_2O)= 6.5; P= 82.7 mg dm^{-3}; Prem = 31.1 mg L^{-1}; K = 112 mg dm^{-3}; Na = 19 mg dm^{-3}; Ca^{2+} = 11.5 $cmol_c$ dm^{-3}; Mg^{2+}= 3.5 $cmol_c$ dm^{-3}; Al^{3+} = 0.0 $cmol_c$ dm^{-3}; H+Al = 4.62 $cmol_c$ dm^{-3}; SB = 15.37 $cmol_c$ dm^{-3}; CECe = 15.37 $cmol_c$ dm^{-3}; CEC = 19.99 $cmol_c$ dm^{-3}; BSI = 77%; ASI = 0.0%; NaSI =

0.54%; SMO = 6.9 dag kg^{-1}; Zn = 23.4 mg dm^{-3}; Fe = 49.7 mg dm^{-3}; Mn = 52.7 mg dm^{-3}; Cu = 0.4 mg dm^{-3}; B = 0,7 mg dm^{-3}, containing 35% of clay, 29% of silt, 26% thick sand and 10% of thin sand. The soil was previously corrected according to recommendations for the culture (Fontes and Puiatti, 2009). The irrigation was performed daily by located drip system.

Plant culture

Were used the hybrid 'Torreon' (*Cucumis melo* L. group Cantalupensis), which presents reticulated rind and salmon-pulp. The seedlings were prepared into polystyrene trays with 128 cells filled with substratum Plantmax® (Eucatex, Paulínia, Brazil) and the transplant occurred when the second leaf was expanded. The culture was led vertically into single stem, being pruned when reached 2 m in height. One fruit per plant was led in secondary branch, with the pruning realized two leaves after fixation. All other branches and fruits were eliminated. The phytosanitary control with fungicides and pesticides registered for melon culture was performed when necessary.

Treatments

Treatments were consisted of four PBZ doses (0.4; 0.8; 1.2 and 1.6 mg PBZ $plant^{-1}$), using the formulation Paclobutrazol® 100 CE (Agro Comercial Wiser Ltda, Diadema, Brazil) by drench, 10 cm around the base of the melon plants; and the control (untreated plants). The doses were divided in five applications, every seven days, with the first application at 15 days after transplanting. The experiment was conducted using a randomized complete block design with eight blocks. The plot was two rows of 1.80 m, spaced 0.80 m, with 6 plants each, spaced at 0.30 m. To avoid possible interferences between treatments (PBZ doses), the plots were separated with plastic film (LDPE, 150 µm) placed to a depth of 0.30 m transversely to the rows. The useful parcel was consisted of four central plants of each row, resulting in a total of eight plants per plot.

Data recorded

Stem diameter (SD); internodes (IN), tendrils (TN) and leaves (LN) number; leaf area (LA); stem (SDM), leaf (LDM) and total (TDM) dry mass; number of male (NMF)

and hermaphrodite (NHF) flowers; male and hermaphrodite flowers ratio (MHR); and indirect chlorophyll measurement (SPAD) were evaluated.

The SD was measured using a millimeter caliper, 0.30 m from the ground level, and LA was measured using a leaf area integrator, model LI-3100 by Li-Cor (Li-Cor, Lincoln, USA), and expressed in "dm^2". The plant parts (stems and leaves) were dried in a forced-air oven at 60 ºC, until constant mass, and weighed on analytical balance, being expressed in "g". SPAD was measured by a portable chlorophyll meter, model SPAD-502 (Konica Minolta, Ramsey, USA). This analysis was realized in 3 leaves picked from the top, middle and basal canopy area at the same time. The data were taken of equidistant regions of each leaf and then calculated the average with this portable meter. NMF, NHF and MHR (NMF/NHF) were measured daily until the end of the experiment and IN, TN and LN were analyzed each 7 days.

To obtain physiological indices of growth analysis were evaluated: absolute growth rate (AGR), relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA), leaf (LWR) and stem (SWR) weight ratio. The average value of reference growth parameters for different time intervals were calculated according to the following standard formulas of growth analysis:

AGR = $(W_2\text{-}W_1)/(t_2\text{-}t_1)$ expressed as g day^{-1} (Hurt 1990);
RGR = $[(lnW_2\text{-}lnW_1)/(t_2\text{-}t_1)]*(1/W_1)$ expressed as g g^{-1} day^{-1} (Hurt 1990);
NAR = $[(W_2\text{-}W_1)/(t_2\text{-}t_1)]*[(lnL_{A2}\text{-}lnL_{A1})/(L_{A2}\text{-}L_{A1})]*1000$ expressed as g dm^{-2} day^{-1} (Gardner et al. 1985);
LAR = $(L_{A2}/W_1+L_{A1}/W_2)/2$ expressed as dm^2 g^{-1} (Hurt 1990);
SLA = $[(L_{A1}/W_{L1})+(L_{A2}/W_{L2})]/2$ expressed as dm^2 g^{-1} (Gardner et al. 1985);
LWR = $[(W_{L1}/W_1)+(W_{L2}/W_2)]/2$ expressed as g g^{-1} (Hurt 1990);
SWR = $[(W_{S1}/W_1)+(Ws_2/W_2)]/2$ expressed as g g^{-1} (Magalhães 1986).

Where: L_{A1} and L_{A2} are the LA at time 1 (t_1) and time 2 (t_2), respectively; W_1 and W_2 are the TDW at time 1 (t_1) and 2 (t_2), respectively; W_L is the LDW, W_{L1} and W_{L2} at time 1 (t_1) and 2 (t_2), respectively; and W_S is the SDW, Ws_1 and Ws_2, at time 1 (t_1) and 2 (t_2), respectively.

The fruit weight (g) (FW) and yield (kg m^{-2}) (YIE) at the plot were analyzed. To verify the attributes of fruits quality, three representative fruits from each plot were taken randomly within the useful parcel. Were analyzed: fruit weight (g) (FW); longitudinal (LD) and transverse (TD) fruit diameters, pulp thickness (PTN), shell thickness (STN), internal cavity (ICV) and fruit shape index (FSI), expressed in "mm";

firmness (N) (FIR); hydrogen potential (pH); percentage (%) of total soluble solids (TSS), total acidity (TAC) and the maturation index (MAI); vitamin C (AA), expressed as mg of vitamin C per 100 g of pulp; total (TSG), reducing (RSG) and non-reducing (NSG) sugars, expressed in "mg mL^{-1}"; rind reticulation (RRT); pulp (PHA) and rind hue angle (RHA); and pulp (PCH) and rind croma (RCH).

A semi-analytical balance was used to measure FW and YIE. Diameters (LD and TD) and ICV were obtained by a millimeter ruler; and PTN and STN values, with a caliper. The FSI was calculated by the ratio of LD and TD. The FIR was determined by digital penetrometer (PDF-200, Soilcontrol, São Paulo, Brazil) in the pulp of melon fruits longitudinally divided.The values were obtained in pounds (Lbf) and transformed to Newton (N), using the conversion factor of 4.45.

The pH was determined by digital potentiometer DM-22 (Digimed, São Paulo, Brazil) and TSS by digital refractometer (Pal-1, Atago, Tokyo, Japan), being expressed in percentage (%) of glucose. TAC was determined in duplicate, using a rate of 10 mL of juice added to 40 mL of distilled water and 5 drops alcoholic phenolphthalein (1%), titrating up to the turning point with NaOH 0,1 N previously standardized and the results expressed as percentage (%) of citric acid. MAI was given by the ratio TSS/TAC. AA was obtained by direct titration with a Tillman solution according to methodology of Strohecker and Henning (1967). TSG, RSG and NSG were determined by refractometry in a Spectrophotometer UV – 1601 (Shimadzu, Tokyo, Japan) at 620 nm by Antrona method according to Yemn and Willis (1954), using a filtered juice of selected longitudinal slices of melon pulp.

RRT was evaluated according to grading scale by methodology of Rizzo and Braz (2001), using the value '1' to fruits with intense reticulation, '2' to medium reticulation and '3' to weak reticulation on fruits rind. PHA, RHA, PCH and RCH were determined by Colorimeter (Minolta Chroma Meter CR-200B, Konica Minolta, Ramsey, USA), calibrating in white surface under lighting conditions and expressed in a* and b*. The Hue angle and Croma index was obtained using the following equations: Hue angle = $(\tan^{-1} b^*/a^*)$ and Chroma= $\sqrt{(a^{*2}+b^{*2})}$ (McGuire 1992).

Statistical analysis

The data were analyzed by regression analysis and the models have been chosen based on the significance of regression coefficients using the "t" Test, at a level of 5%

of probability; the determination coefficient (R^2) and the biological phenomenon in study.

Results and Discussions

Morphophysiological parameters

The SD decreased and IN increased linearly in increasing PBZ doses, reaching to 6.35% lower and 19.96% higher at the highest PBZ dose (1.6 mg plant^{-1}) respectively, when compared to control, that had a SD of 0.68 cm and 23.02 internodes (Fig. 1). These results indicate a PBZ effect on the plants compaction. The reduction of plant height by internode and stem diameter shortening is a characteristic effect of Triazoles compounds (Davies et al. 1988) and the best-known effect caused by PBZ in plants stems (Quilan 1981). This is due to GA inhibition, exemplified by the reduction of internodes elongation. In addition, smaller SD has been associated with decreased number of cells, small cortical cells and decreases in xylem diameter (Fletcher and Hofstra, 1990). The reduction of branches growth is also attributed to the decrease of the total available sugars, induced by PBZ effect (Mehouachi et al. 1996).

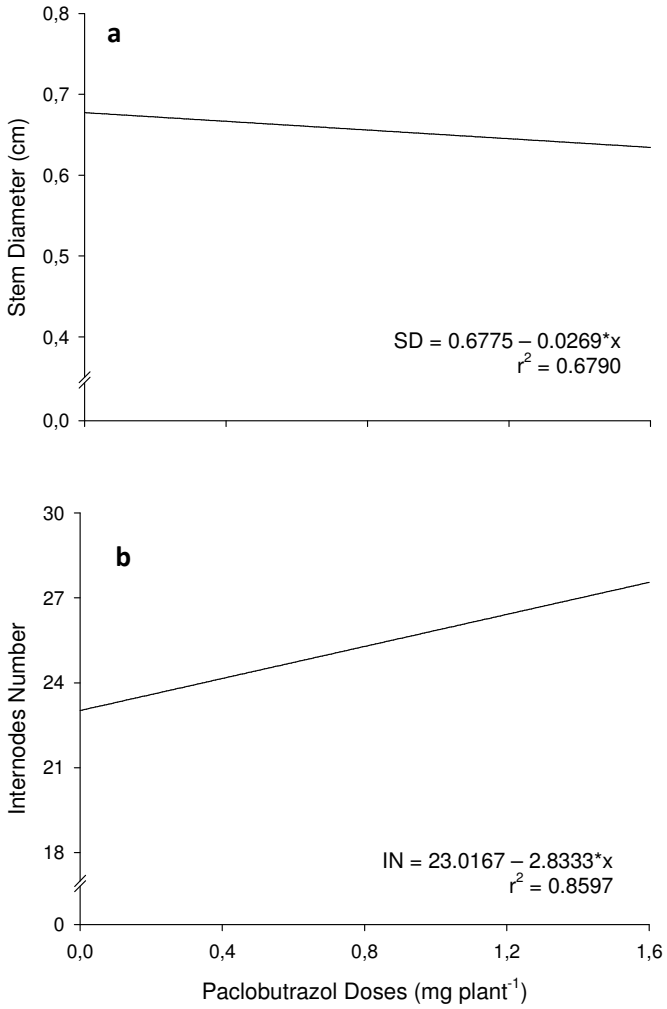

Fig. 1 Stem diameter (ST) (a) and internodes number (IN) (b) of melon plants according to PBZ doses. Plants with 100 days age after planting and 50 days after last PBZ application.

Since PBZ promoted internodes shortening and consequent increases of IN, were observed linearly increases in LN and TN. At highest PBZ dose (1.6 mg plant^{-1}), the LN and TN was 16.76 % and 20.72 % higher than the control, averaging 27.57 internodes and 16.89 tendrils, respectively (Fig. 2). The common feature to all stems are the nodes, internodes, lateral leaves and associated axillary buds, which occur exogenously and are positioned at the nodal regions (Dickison 2000), thereby determining the LN. As the tendrils are modified leaves or branches formed in the leaves axil (Raven et al. 1996), as greater the LN, greater will be the TN in plants.

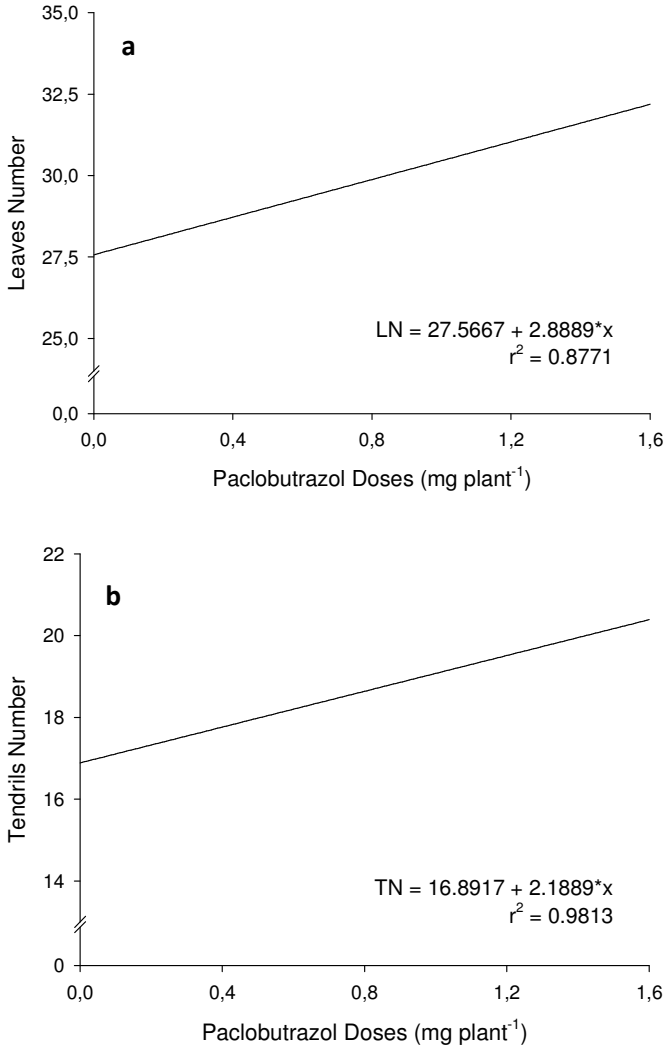

Fig. 2 Leaves (LN) (a) and tendril (TN) (b) number of melon plants according to PBZ doses. Plants with 100 days age after planting and 50 days after last PBZ application.

Regarding the flowers number, PBZ application at 0.25 and 0.23 mg plant^{-1} promoted the highest values of NFH (2.74) and RMF (0.18), promoting increases of 34.3% to both variables (Fig. 3). There were no significant statistical changes in the NMF.

GA synthesis has influence in the initiation and flower differentiation. Thus, these results may be a direct consequence of changes in GA concentrations (Basra 2000). In addition, Taiz and Zeiger (1998) state that GA application in cucurbits promotes the formation of staminate flowers and its inhibition promotes the formation of pistillate flower. As the PBZ acts inhibiting the GA synthesis, there was stimulation on hermaphrodite flowers production. However when applied in higher doses, PBZ can

promote the reduction on flowers number (Basra 2000). This was observed in this experiment at doses above 0.8 mg PBZ plant^{-1}.

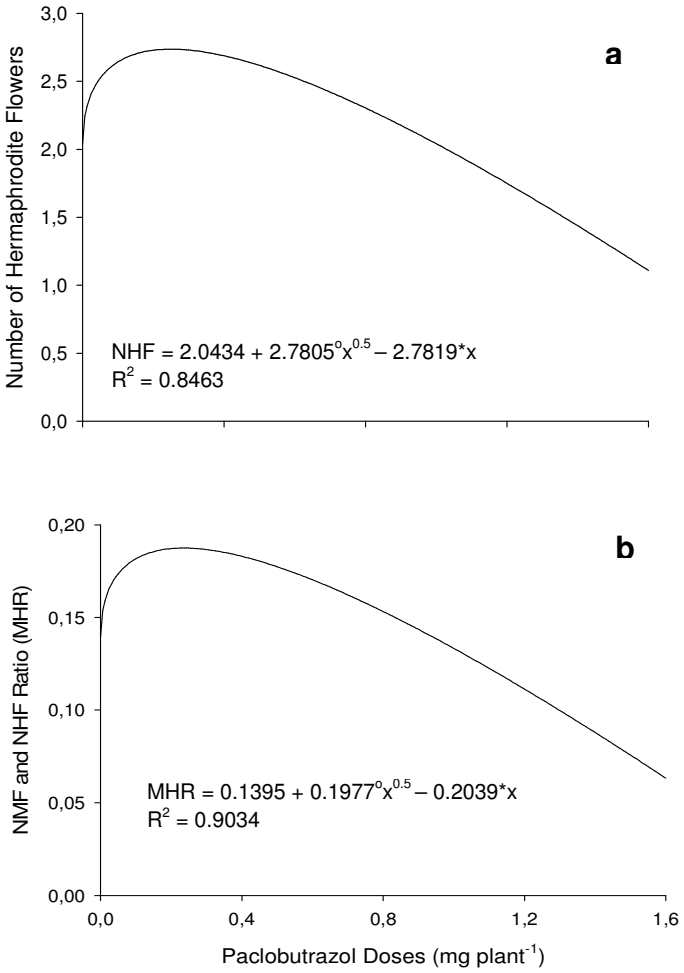

Fig. 3 Number of hermaphrodite flowers (NHF) (a) and number of male (NMF) and hermaphrodite (NHF) flowers ratio (MHR) (b) on melon plants according to PBZ doses. Plants with 100 days age after planting and 50 days after last PBZ application.

PBZ-treated plants had leaves with more intense green color than the control, which was confirmed by the results of the SPAD index (Fig. 4). In most cases, this increase is related to increases in chlorophyll content or simply a concentration effect due to reduced leaf expansion (Davies and Sankhla 1987). The highest SPAD value was 38.61, at 1.37 mg PBZ plant^{-1}. This amount is 4.1% higher than the control which SPAD index was 37.09.

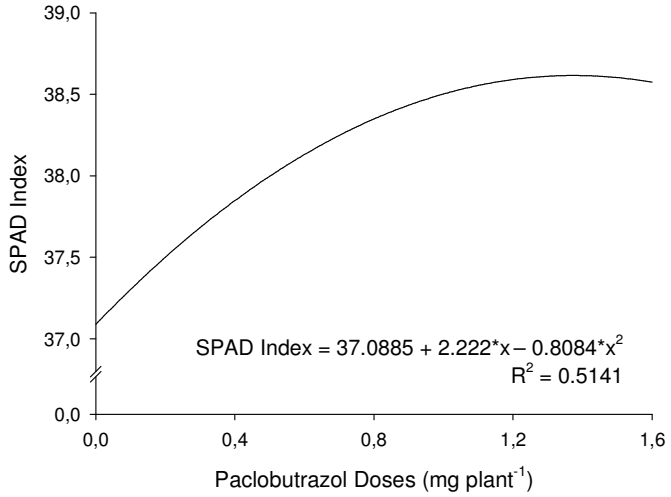

Fig. 4 Indirect chlorophyll measurement (SPAD Index) of melon plants according to PBZ doses. Plants with 100 days age after planting and 50 days after last PBZ application.

There were no significant differences for the variables SDW, LDW and TDW. These variables were possibly offset by the greater number of internodes and leaves, reduced leaf expansion and cuticle thickening (Basra 2000.)

Growth parameters

The lower LA value (4790.81 dm^2) was observed at 0.83 mg PBZ $plant^{-1}$, 26.5 % lower than the control, that showed a LA equal to 6,516.02 dm^2 (Fig. 5A). Leaves that develop after the application of growth regulator tend to be smaller, but thicker due to the production of another mesophyll cells layer (Benton and Cobb 1995; Basra 2000). This response is a result of reduction in proliferation and cell growth by inhibition of GA biosynthesis caused by PBZ (Khalil and Rahmanb 1995). Due to LA reduction, LAR tended to decrease with increasing PBZ doses. The lowest values of LAR (98.69 $dm^2\ g^{-1}$) and SLA (169.65 $dm^2\ g^{-1}$) were observed in PBZ doses of 0.88 mg $plant^{-1}$ and 0.64 mg $plant^{-1}$, 20.35% and 20.56% lower, respectively (Fig. 5B and 5C). On thick leaves, chlorophyll levels, stomatal density and photosynthesis had specific increases per unit area, inferring for this reason the reduction of SLA (Basra 2000). LAR expresses the useful photosynthesis area and the potentially respiratory components of the plant; and SLA is related to density or relative thickness, because express the ratio

between LA and LDW (Hurt 1990). Thus, PBZ-treated plants had reduced photosynthetic rate per mass and higher photosynthetic rate per area.

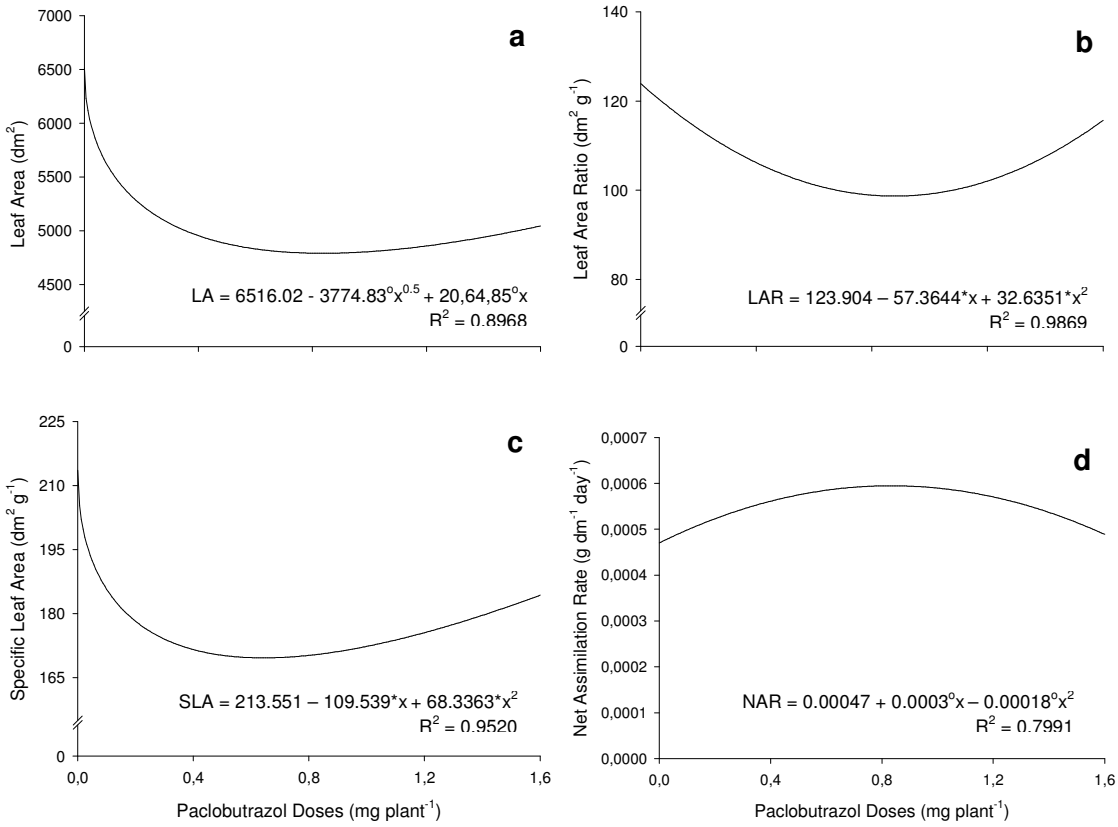

Fig. 5 Leaf area (LA) (a), leaf area ratio (LAR) (b), specific leaf area (SLA) (c) and net assimilation rate (NAR) (d) of melon plants according to PBZ doses. Plants with 100 days age after planting and 50 days after last PBZ application.

PBZ application promoted increase up to 26.8% on NAR at 0.83 mg plant^{-1} averaging 0.0006 g dm^{-2} day^{-1} (Fig 5D). The NAR increase on PBZ-treated plants was caused by the effect of this plant growth regulator on LN. The lower LN provided efficient CO_2 assimilation and dry matter partitioning during the crop cycle, which is useful in assimilates allocation, allowing greater assimilates availability to the fruit (Martin et al. 1987).

There were no significant differences for the variables AGR, RGR, LWR and SWR when subjected to PBZ doses, showing that the tested PBZ doses did not affect the production, accumulation and allocation of biomass in melon plants.

Post-harvest quality of melon fruits

Analyzed fruits satisfied the International Standard FFV-23 concerning the marketing and commercial quality control of melon of the United Nations Economic Commission for Europe - UNECE (UNECE 2010). In addition, there were no significant differences for fruits quality (Table 1). These results indicated that application of PBZ did not affect the internal and external appearance of melon fruits, which had desirable characteristics for marketing.

Table 1 Mean values of variables: longitudinal diameter (LD), transverse diameter (TD), fruit shape index (FSI), pulp thickness (PTN), shell thickness (STN), internal cavity (ICV), rind reticulation (RRT), firmness (FIR), fruit weight (FW), yield (YIE), total soluble solids (STT), hydrogen potential (pH), total acidity (TAC), maturity index (MAI), total sugars (TSG), reducing sugars (RSG), non-reducing sugars (NSG), vitamin C (AA), rind Hue angle (RHA), pulp Hue angle (PHA), rind chroma (RCH) and pulp chroma (PCH), according to the application of PBZ doses.

VARIABLES	MEAN	VARIABLES	MEAN
LD	135.45 mm	pH	6.97
TD	116.15 mm	TAC	0.06 %
FSI	26.29 mm	MAI	162.0
PTN	6.04 mm	TSG	0.12860 mg ml^{-1}
STN	71.42 mm	RSG	0.01072 mg ml^{-1}
ICV	1.12 mm	NSG	0.11788 mg ml^{-1}
RRT	2 (medium reticulation)	AA	23.22 mg 100 g^{-1}
FIR	21.43 N	RHA	91.97º
FW	954.42 g	PHA	70.69º
YIE	5.72 kg m^{-2}	RCH	31.11º
STT	9.42 %	PCH	36.82º

A good yield average (5.72 kg m^{-2}) was obtained, considering that all plants led one fruit per plant. Rizzo and Braz (2001) obtained a yield average ranging from 8.32 to 13.15 kg m^{-2}, analyzing the yield of melon cultivars at greenhouse crop, however, managing three fruits per plant.

The fruits showed intense arome, rounded shape and medium reticulation over the surface (Table 1). Due the crops have been harvested 5 to 10 days after the commercial point of harvest, there was a FIR variation of 19.47 N to 24.38 N, slightly below the recommended for export, which is 30 N (Alves et al. 2000). The STT attended the requirements for international marketing of melon from Cantaloupensis group, which requires a ratio greater than 8 °Brix (UNECE 2010).

Whereas: the Chroma is described as the intensity or color saturation, which 0 (zero) is impure color and 60 is pure color (McGuire 1992); and the Hue angle, shows the color location in a diagram, which angle 0º (zero) represents the pure red, 90º represents the pure yellow, 180º the pure green and 270º the blue (Shewfelt et al. 1988), we can affirm that were obtained fruits showing color and color intensity common to melon fruits hybrid 'Torreon', which has green-yellow rind and salmon-pulp (EMBRAPA 2011).

In conclusion, the PBZ application reduced the plant size without affecting productivity, the appearance of the melon fruit and its quality attributes, proving to be an effective alternative for crop management in greenhouse. Thus, the best dose was 0.4 mg PBZ $plant^{-1}$ due to the positives physiologic responses by the melon plants, the higher number of hermaphrodite flowers and the product economy.

Acknowledgments

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References

Alves RE, Pimentel CR, Maia CE, Castro EB, Viana FM et al (2000) Manual de melão para exportação. Embrapa, Brasília, 51p.

Barreiro AP, Zucareli V, Ono EO, Rodrigues JD (2006) Análise de crescimento de plantas de manjericão tratadas com reguladores vegetais. Bragantia 65:563-567

Basra AS (2000) Plant Growth Regulators in Agriculture and Horticulture: Their Role and Commercial Uses. The Haworth Press, Binghamton, 264p.

Benincasa MMP (2003) Análise de crescimento de plantas: noções básicas. FUNEP, Jaboticabal, 41p.

Benton JM, Cobb AH (1995) The plant growth regulator activity of the fungicide, epoxyconazole, on *Galium aparine* L. Plant Growth Regul 17:149-155

Davies TD, Sankhla N (1987) Altered diurnal leaf movements in soybean seedling treated with triazole growth regulators. Plant Cell Physiol 28:1345-1349

Dickison WC (2000) Integrative Plant Anatomy. Academic Press, California

EMBRAPA (2011) Empresa Brasileira de Pesquisa Agropecuária. Sistema de Produção de Melão: Cultivares. http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Melao/SistemaProducaoMelao/cultivares.html. Acessed 05 June 2011

Fletcher RA, Hofstra G (1990) Improvement of uniconazole-induced protection in wheat seedling. J Plant Growth Regul 9:207-212

Fontes PCR, Puiatti M (2009) Cultura do melão. Olericultura: teoria e prática. Suprema Gráfica e Editora, Visconde do Rio Branco, 486p.

Gardner FP, Pearce RB, Mitchell RL (1985) Physiology of crop plants. Iowa State University, Ames, 327p.

Hurt R (1990) Basic Growth Analysis. Unwin Hyman, London, 114p.

Khalil IA, Rahmanb HU (1995) Effect of Paclobutrazol on growth, chloroplast pigments and sterol biosynthesis of maize (*Zea mays* L.). Plant Sci 105:15-21

Magalhães ACN (1986) Análise quantitativa de crescimento. In: Ferri MG (ed) Fisiologia vegetal. 1st edn. EPU, São Paulo, pp 331 - 350.

Martin GC, Yochikawa F, LaRue JH (1987) Effect of soil applications of Paclobutrazol in vegetative growth, pruning time, flowering, yield and quality of "Flavorclast" peach. J Am Soc Hortic Sci 112:915-921

McGuire RC (1992) Reporting of objective colour measurements. HortSci 27:1254-1255

Mehouachi J, Tadeo FR, Zargaoza S, Primomillo E, Talon M (1996) Effects of gibberellic acid and Paclobutrazol on growth and carbohydrate accumulation in shoots and root of citrus rootstock seedling. J Hortic Sci 71:747-754

Quilan JD (1981) New chemical approaches to control of fruit tree form and size. Acta Hortic 120:95-106.

Raven PH, Evert RF, Eichhorn SE (1996) Biologia Vegetal. Guanabara Koogan, Rio de Janeiro, 856p.

Rizzo AAN, Braz LT (2001) Características de cultivares de melão rendilhado cultivadas em casa de vegetação. Hortic Bras 19:237-240

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Oliveira JB, et al (2006) Sistema Brasileiro de Classificação de Solos. Embrapa, Rio de Janeiro, 306p.

Shewfelt RL, Thai CN, Davis JW (1988) Prediction of changes in color of tomatoes ripening at different constant temperatures. J Food Sci 53:1433-1437

Strohecker R, Henning HM (1967) Analisis de vitaminas: métodos comprobados. Paz Montalvo, Madrid, 428p.

Taiz L, Zaiger E (1998) *Plant physiology*. Readward City, Palo Alto, 792p.

Tongumpai P (1991) Flower induction of mango. Kasetsart University, Thailand, n.19.

UNECE (2010) United Nations Economic Commission For Europe - Unece Standard FFV – 23: Concerning the marketing and commercial quality control of Melons.http://www.unece.org/trade/agr/meetings/ge.01/2010_Nov/WP.7_GE.1_2010_13E_Melons.pdf. Acessed 9 January 2011

Yemn EW, Willis AJ (1954) The estimation of carbohydrate in plant extracts by anthrone. Biochem J 57:505-514

III - DETERMINATION OF PACLOBUTRAZOL IN MELON FRUITS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Abstract Paclobutrazol (PBZ) is a triazole compound and plant growth regulator that when applied in a large amount results in high levels of growth regulator residues in crops. This is why application of plants growth regulators is now restricted. Therefore, this study aimed to detect and quantify PBZ contends in melon pulp using High Performance Liquid Chromatography (HPLC). The experimental design was randomized blocks with five treatments, eight blocks and eight repetitions. The treatments were fruits harvested from PBZ-treated (0.4, 0.8, 1.2 and 1.6 mg PBZ L^{-1}) and untreated plants (witness), 100 days after planting. Eight fruits were harvested of each block for PBZ analysis in melon pulp. PBZ was not present into melon pulp obtained from treated plants, even at the highest dose, being harmless to human health.

Keywords Plant growth regulator, triazole compound, HPLC, chemistry residue, melon pulp, human health

Introduction

The presence of toxic substances in food may be caused by several sources of contamination. A major source of food contamination is due the use of agrochemicals, which are substances used to enhance the quality and quantity of food needed to sustain the population (Vega and Florentino, 2000). For instance, phytoregulators, organic compounds non-toxic to plants, are used to regulate plant growth without changing developmental patterns (Rademacher, 2000).

The largest group of plant growth regulators consists of chemicals antagonistic of gibberellins (Fletcher et al., 2000). Among this group, the Paclobutrazol (PBZ) [(2RS,3RS) - 1 - (4 - chlorophenyl) - 4, 4 - dimethyl - 2 - (1H - 1, 2, 4 - triazol- 1 - yl) pentan - 3 - ol] (Fig.1) is a triazole compound and plant growth regulator that is applied as a foliar spray (El-Khoreiby et al., 1990; Lewis and Ju, 1993), soil drench (Kawabata and DeFrank, 1993; Keever and Cox, 1989) or, trunk injections (Cox, 1990). In many of these methods, a large amount of applied compound results in high levels of growth regulator residues in crops. These residues in roots and shoots of edible crops are known

to be hazardous to human and other animals. This is why application of plant growth regulators is now restricted (Davies et al., 1988).

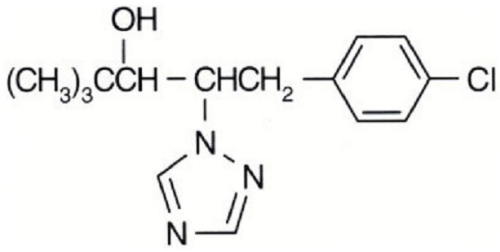

Fig. 1 Molecular structure of Paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol]

To ensure that maximum residue levels are complied, analytical methodologies used to the determination of toxic substances in food should be able to quantify residues of these substances in very low concentrations, as well as identify them unequivocally. Due to the complexity of food matrices involving mixture of water, proteins, lipids, carbohydrates, vitamins and minerals (Lehotay and Hajšlová, 2002), is often required intensive sample preparation, as well as the coupling of analytical techniques to obtain higher selectivity and detectability. A simpler approach is to analyze plant material utilizing HPLC, proving to be advantageous in this sense (Witchard, 1997).

The objective of this study was to detect and quantify PBZ contend in melon pulp using High Performance Liquid Chromatography (HPLC).

Material and Methods

Fruits of the hybrid 'Torreon' (*Cucumis melo* L. group Cantalupensis), presenting reticulated rind and salmon-pulp were used. The seedlings were prepared in polystyrene trays with 128 cells filled with substratum Plantmax® (Eucatex, Paulínia, Brazil) and the transplant occurred when they presented the second expanded leaf. The experimental design was randomized blocks with five treatments and eight repetitions. The treatments were consisted of four PBZ doses (0.4, 0.8, 1.2 and 1.6 mg PBZ L^{-1}), using the formulation Paclobutrazol® 100 CE (Agro Comercial Wiser Ltda, Diadema, Brazil) applied to the soil 10 cm around the base of the melon plants; and the control, which did

not receive the phytoregulator, been treated with distilled water. The applications were divided in 4 times, spaced in 7 days, being the first done 15 days after the transplanting.

Plants were led vertically in single stem, being pruned when they reach 2 m in height. One fruit per plant was led in secondary branch, with pruning realized two leaves after fixation. All other branches and fruits were eliminated. The phytosanitary control with fungicides and pesticides registered for culture was performed when necessary. At harvest, 100 days after planting, the fruits of melon plants were harvested for analysis of residual PBZ into pulp.

Extraction and HPLC Analysis

The method for extraction and HPLC analysis was modified from Lautié et al., (2000), Sharma and Awasthi (2005) and Sancho et al. (2003). In each treatment, pulp samples were taken at equidistant parts of 8 melon fruits, forming a composite sample. Then, 25 g of each sample was triturated in mixer with 10 mL of Absolute Methyl Alcohol (Merc, Darmstadt, Germany) and filtered through qualitative filter paper 80 g. The methanol dissolved any crystallized PBZ contained in the sap. The residue retained on the paper was washed four times with methanol and the volume completed to 50 mL in volumetric flask.

Samples were concentrated for better PBZ detection. A 50 mL amount of each sample was centrifuged twice for 5 min at 1000 ppm, the supernatant was stored. Then, they were putted into flasks in a Rotavapor, model R-200 (BUCHI Labortechnik, Flawil, Switzerland) equipped with a vacuum pump TE-058 (Tecnal, Piracicaba, Brazil) at -600 mmHg and 60±1 ºC, for approximately 13 min to obtain a concentrate containing an average volume of 2.0 mL. The volume of 1.5 mL of the extract was transferred to eppendorf tubes. The tubes were then shaken, centrifuged 2 times at 10000 ppm during 5 min, and supernatants transferred to vials and submitted to analysis by High Performance Liquid Chromatography (HPLC).

The analysis system consisted of an LC10A spectrophotometric HPLC detector (Shimadzu, Tokyo, Japan) with a UV wavelength detector operating at 227 nm (Early and Martin 1988) with a column CLC-ODS (C18) (4,6 mm x 26 cm x 5 µm) (Shimadzu, Tokyo, Japan). An LC10A HPLC pump operated at a flow rate of 0.9 mL min^{-1} and with an injection "loop" of 20 µL. The liquid phase of the system was methanol : deionized water (70/30, v/v) and the retention time was 20 min.

Standard solutions from 40 mg L^{-1} PBZ in absolute methanol were created from solid PBZ. The pure PBZ utilized was Paclobutrazol PESTANAL® (FL-46046, Sigma-Aldrich, Saint Louis, USA). This standard was used to determine relative concentrations of PBZ in the pulp samples of melon fruits.

Results and Discussion

PBZ was identified on the standard solutions by a distinct peak occurring about 12 min 11 s after column injection (Fig. 2). However, PBZ peaks did not occur in any melon sample, demonstrating that used concentrations of PBZ were not enough to let residues on pulp of melon fruits obtained from treated plants, even at the highest concentration (1.6 mg L^{-1}), 65 days after treatment. Taking into consideration the sanitary regulations for PBZ (Kaloyanova 1993), it can be assumed that the fruits of treated plants are harmless to human health from a phytosanitary point of view.

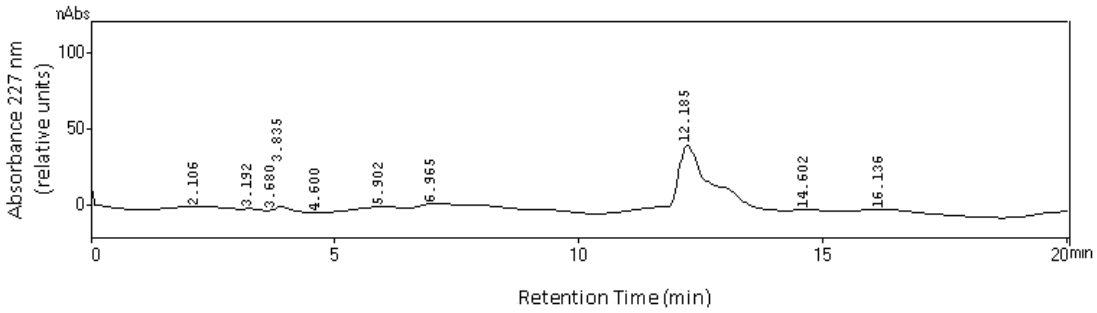

Fig. 2 Detection and quantification of Paclobutrazol (PBZ) on 40 mg L^{-1} PBZ-Standard solution. The PBZ peak occurs at 12 min 11 s.

A large peak encountered nearly at 4 min probably represented a complex of no indentified compounds within the melon pulp (Fig. 3). This peak returned to baseline well before PBZ was detected.

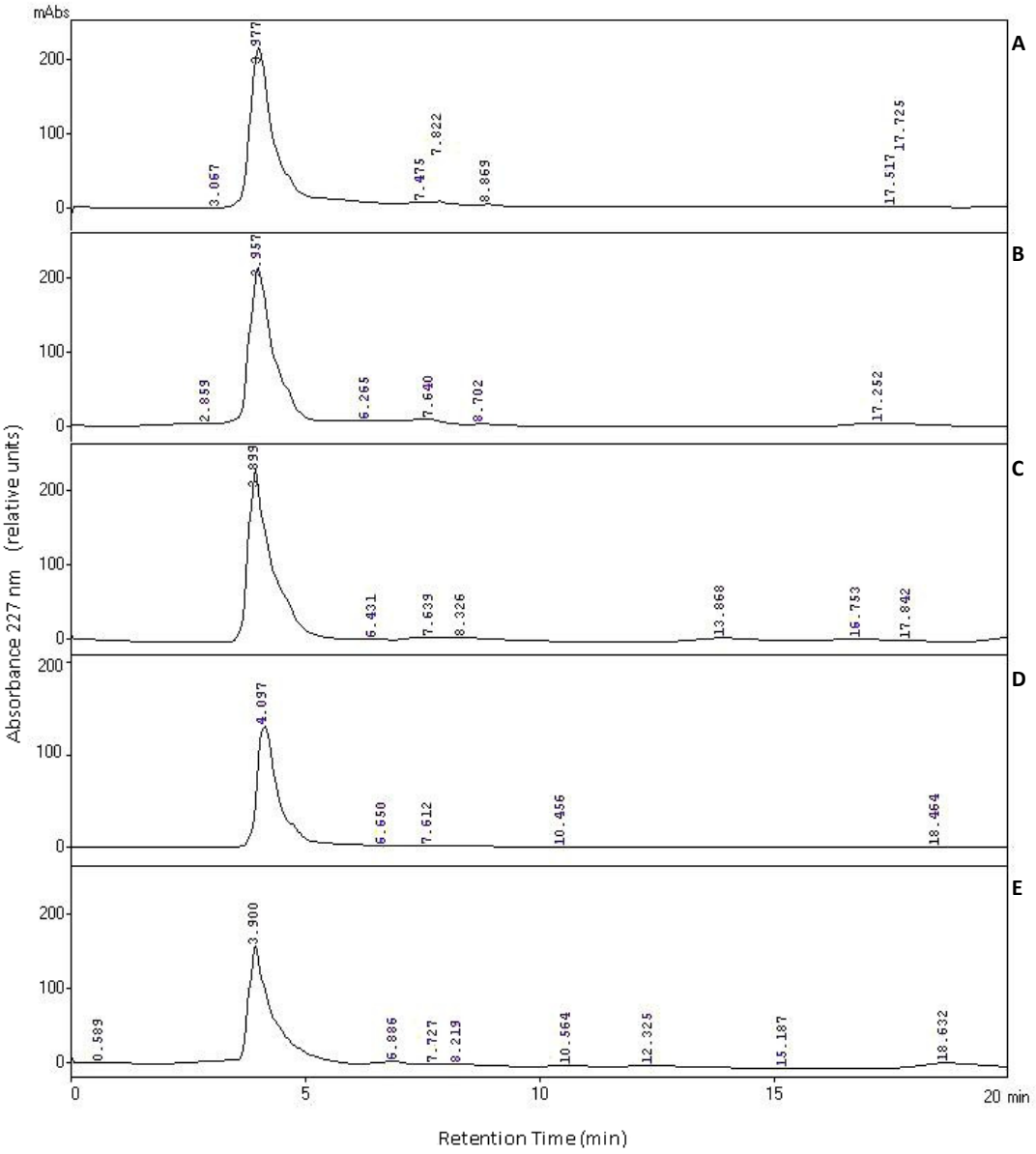

Fig. 3 Detection and quantification of Paclobutrazol (PBZ) on melon pulp samples by HPLC analysis. Pulp samples of untreated plants (A) and PBZ-treatment plants with: 0.4 (B), 0.8 (C), 1.2 (D) and 1.6 (E) mg PBZ L^{-1}.

One of the most common solvents used for extraction of phenolic compounds from foods is methanol (Naczk & Shahidi, 2004). Thus, this conduct can be due to the extraction of these compounds presented within melon pulp. Seeram et al. (2004) reported that extractions with different solvents showed that the methanol yielded the most peaks in the HPLC chromatogram, identifying glycosides, elagic acid based compound, flavanols and hydrolyzable tannins in the first 5 min of retention time in strawberry fruits. In addition, phenolics compounds were extracted on bitter melon fruit

(*Momordica charantia*) (Budrat and Shotipruk 2008) and grapes (Kammerer and Carle 2008, Oszmianski and Lee 1990) by similar methodology.

To confirm the extraction of unknown compound presented in melon fruits, samples of a different melon (Yellow Melon, Hybrid AF-646) were purchased at retail and analyzed by HPLC using methanol as extractor. These samples showed similar peak when compared to samples of melon pulp obtained from untreated and PBZ-treated melon plants (Fig. 4).

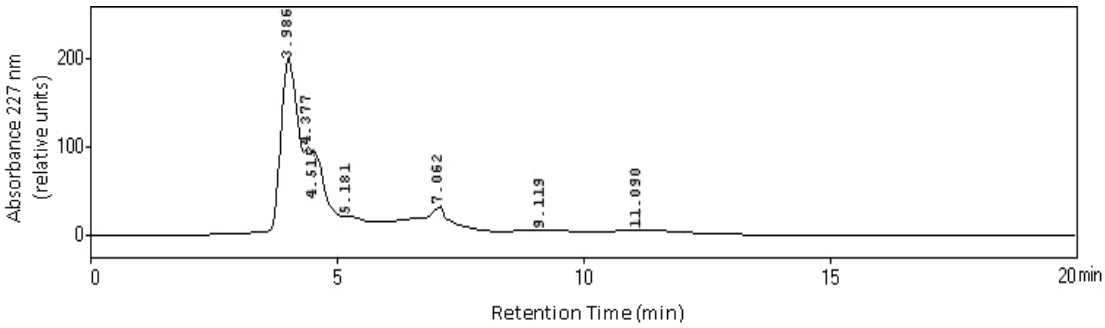

Fig. 4 Detection of unknown compounds on pulp samples of Yellow Melon by HPLC analysis.

The advantages of this presented technique are that it is a relative inexpensive, less labor intensive, relatively rapid and very accurate. Furthermore, this experiment showed that PBZ was not present in pulp sample of melon fruits obtained from treated plants, being harmless to human health.

Acknowledgments

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References

Budrat P, Shotipruk A (2008) Extraction of phenolic compounds from fruits of Bitter Melon (*Momordica charantia*) with subcritical water extraction and oxidant activities of these extracts. Chiang Mai J Sci 35:123-130.

Cox C (1990) Growth retardants and street tress. Aust Parks Recreat 26:18-21.

Davies TD, Steffens GL, Sankhla N (1988) Triazole plant growth regulators. Hortic Rev 10:63-105.

Early JD, Martin GC (1988) Translocation and breakdown of ^{14}C-labeled Paclobutrazol in Nemaguard peach seedlings. HortScience 23:196-200.

El-Khoreiby AM, Unrath CR, Lehman LJ (1990) Paclobutrazol spray timing influences apple tree growth. HortScience 25:310-312.

Fletcher R, Gilley A, Sankhla N, Davies T (2000) Triazoles as plant growth regulators and stress protectants. Hortic Rev 24:55-138.

Frenich AG, Vidal JL, López TL, Aguado SC, Salvador IM (2004) Monitoring multi-class pesticide residues in fresh fruits and vegetables by liquid chromatography with tandem mass spectrometry. Chromatogr 1048:199-206.

Kaloyanova F (1993) Pesticids-information sheets for secure application. Sofia: Medicine (Bg). In: Berova M, Zlatev Z (2000) Physiological response and yield of Paclobutrazol treated tomato plants (*Lycopersicon esculentum* Mill.). Plant Growth Regul 30:117-123.

Kammerer DR, Carle R (2008) Process strategies for the recovery and isolation of phenolic compounds from winery by-products. Electr J Environ Agric Food Chem 7:3226-3230.

Kawabata O, DeFrank J (1993) Purple nutsedge suppression with soil-applied Paclobutrazol. HortScience 28:59.

Keever GJ, Cox DA (1989) Growth inhibition in marigold following drench and foliar-applied Paclobutrazol. HortScience 24:390.

Lautié JP, Stankovic V, Sinoquet G (2000) Determination of chlormequat in pears by high-performance thin layer chromatography and high-performance liquid chromatography with conductmetric detection. Analusis 28:155-158.

Lehotay SJ, Hajšlová J (2002) Application of gas chromatography in food analysis. Trends Anal Chem 21:686-697.

Lewis JC, Ju HY (1993) The effect of foliar application of five plant growth regulators on the growth and yield of lowbush blueberry. Can J Sci 73:607-610.

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. J Chromatogr A 1054:95-111.

Nunez O, Moyano E, Galceran MT (2005) LC-MS/MS analysis of organic toxics in food. Trends Anal Chem 24:683.

Oszmianski J, Lee CY (1990) Isolation and HPLC determination of phenolic compounds in red grapes. Am J Enol Vitic 41:3:204-206.

Rademacher W (2000) Growth retardants: Effect of gibberellin biosynthesis and other metabolic pathways. Annu Rev Plant Physiol Plant Mol Biol 51:501-531.

Sancho JV, Pozo OJ, Zamora T, Grimalt S, Hernandéz F (2003) Direct determination of Paclobutrazol residues in pears samples by liquid chromatography-electrospray tandem mass spectrometry. J Agric Food Chem 51:4002-4206.

Seeram NP, Lee R, Heber D (2004) Bioavailability of ellagic acid in human plasma after the consumption of ellagitannins from pomegranate (*Punica granatum* L.) juice. Clin Chim Acta 348:63-68.

Sharma D, Awasthi MD (2005) Uptake of soil applied Paclobutrazol in mango (*Mangifera indica* L.) and its persistence in fruit and soil. Chemosphere 60:164-169

Taylor MJ, Hunter K, Hunter KB, Lindsay D, Bouhellec SL (2002) Multi-residue method for rapid screening and confirmation of pesticides in crude extracts of fruits and vegetables using isocratic liquid chromatography with electrospray tandem mass spectrometry. J Chromatogr 982:225-236.

Vega PV, Florentino BL (2000) Toxicologia de Alimentos. 1 st ed. Centro Nacional de Salud Ambiental, Ciudad de México. 261p.

Witchard M (1997) A simplified technique for detection of Paclobutrazol in plant sap extracts, using HPLC. J Plant Growth Regul 16:213-214.

IV - ALTERAÇÕES MORFOFISIOLÓGICAS E POTENCIAL ELICITOR DO PACLOBUTRAZOL CONTRA *C. lagenarium* EM PLANTAS DE PEPINO

RESUMO

O Paclobutrazol (PBZ) é um fitorregulador derivado do grupo dos triazóis também utilizado na ativação e regulação de processos envolvidos na biossíntese de defesa de plantas. Este trabalho foi dividido em três experimentos distintos e objetivou: verificar a ação do PBZ sobre as alterações morfofisiológicas em plantas de pepino; a sensibilidade *in vitro* de isolados de *C. lagenarium* ao PBZ; e seu potencial elicitor sobre este patógeno. No bioensaio, foram testados dois isolados de *C. lagenarium* (CLKJ11 e CLKJ25) em seis tratamentos: meio BDA puro (testemunha) e acrescidos com concentrações de PBZ (50, 100, 200 e 400 mg L^{-1}), FTT (Tebuconazole + Trifloxystrobin) (100 mg L^{-1}) e FTB (Tebuconazole) (200 mg L^{-1}). Para avaliar as alterações morfofisiológicas e verificar o potencial elicitor sobre o patógeno, plantas de pepino foram tratadas com quatro doses de PBZ (50, 100, 200 e 400 mg planta^{-1}) e suas folhas inoculadas com a suspensão do isolado CLKJ25, na concentração $1x10^{-8}$ conídios mL^{-1}. O PBZ inibiu o crescimento micelial *in vitro* de *C. lagenarium* e demonstrou ser extremamente eficaz na redução do crescimento de plantas de pepino. Além disso, as alterações morfofisiológicas decorrentes da sua aplicação atrasaram a infecção do patógeno nas folhas, proporcionado redução na área foliar lesionada. A melhor concentração de PBZ foi a de 50 mg PBZ L^{-1} ou 50 mg PBZ planta^{-1}. Apesar desses resultados, este fitorregulador não pode ser considerado um agente indutor de resistência de plantas devido ao efeito tóxico sobre o patógeno desafiante.

Palavras chave: *Cucumis sativus*, antracnose, PBZ, regulador de crescimento, potencial elicitor.

IV - MORPHOPHYSIOLOGICAL CHANGES AND ELICITOR POTENTIAL OF PACLOBUTRAZOL AGAINST *C. lagenarium* IN CUCUMBER PLANTS

ABSTRACT

Paclobutrazol (PBZ) is a plant growth regulator derived from triazole group also used in the activation and regulation of processes involved in the plant defense biosynthesis. This research was divided in three distinct experiments and aimed: to verify PBZ action on morphophysiological changes in cucumber plants; the *in vitro* sensitivity of *C. lagenarium* isolates to PBZ; and its elicitor potential on this pathogen. In the bioassay were tested two *C. lagenarium* isolates (CLKJ11 and CLKJ25) in six treatments: pure BDA medium (control) and plus PBZ (50, 100, 200 and 400 mg L^{-1}), FTT (Tebuconazole + Trifloxystrobin) (100 mg L^{-1}) and FTB (Tebuconazole) (200 mg L^{-1}) concentrations. To evaluate morphophysiological changes and verify the elicitor potential on the pathogen, cucumber plants were treated with four doses of PBZ (50, 100, 200 and 400 mg $plant^{-1}$) and leaves inoculated with suspension of the isolate CLKJ25 at concentration of $1x10^{-8}$ conidia mL^{-1}. PBZ inhibited the *in vitro* micelial growth of *C. lagenarium* and showed to be extremely efficient in reducing the growth of cucumber plants. Moreover, morphophysiological changes caused by its application delayed the pathogen infection in leaves, promoting reduction of lesioned leaf area. The best PBZ concentration was 50 mg PBZ L^{-1} or 50 mg PBZ $plant^{-1}$. Despite these results this phytoregulator can not be considered a plant resistance inducer due to its toxic effect on the tested pathogen.

Keywords: *Cucumis sativus*, PBZ, anthracnose, plant growth regulator, elicitor potential.

INTRODUÇÃO

Antracnose em pepino (*Cucumis sativus* L.) causado pelo fungo *Colletotrichum lagenarium* (Pass.) Ellis & Halsted sin. *C. orbiculare* (Berk & Mont.) Arx e *C. gloeosporioides* f. sp. Cucurbitae), é uma das doenças mais destrutivas em cucurbitáceas, ocorrendo em todos os lugares onde o pepino é cultivado (Tian *et al*., 2008). Os sintomas iniciam-se em folhas mais velhas com lesões encharcadas, seguida

de necrose e manchas circulares circundadas por um halo de tecido amarelado. As lesões crescem rapidamente, tornando-se marrons com centro mais claros (Rego & Carrijo, 2000). O surto e a desfolha causada pela infecção no caule e nas folhas infectadas podem resultar em reduções no rendimento das culturas ou até mesmo perda total em alguns campos de produção (Amin & Ullasa, 1981; Latin, 1993). Atualmente, o controle de antracnose é realizado principalmente através da aplicação de fungicidas protetores. Consequentemente, pesquisas tem objetivado a criação de novas técnicas para o controle da doença, oportunando o estudo da ativação da resistência induzida em plantas (Tian *et al*., 2008).

A Resistência Sistêmica Adquirida (SAR) envolve a ativação de mecanismos de defesa latentes existente nas plantas em resposta ao tratamento com agentes bióticos ou abióticos chamados de elicitores (Cohen *et al*., 1993; Smith, 1996). Estes compostos aumentam o nível de resistência da planta, podendo atuar por meio da indução de alterações estruturais como o espessamento de cutícula, lignificação, papilas, halos, densidade de tricomas, densidade e conformação de estômatos (Agrios, 2005).

A diversidade de substâncias com natureza química variada demonstra a não existência de característica estrutural única na determinação da atividade elicitora. Por exemplo, algumas substâncias químicas como o ácido jasmônico (Shoresh *et al.,* 2005), o acibenzolar-S-methyl (ASM) (Bubici *et al.*, 2006), o tidiazol (Fan *et al*., 2009), o probenazole (Oostendorp *et al*., 2001), o tetrazonazol (Ronchia *et al*., 1997) e o trifluralin (Starratt & Lazarovits, 1999) apresentam essas características. Alguns reguladores de crescimento, como o acylcyclohexanediones e os trinexapac-ethyl, também têm apresentado atividade elicitora. Estes compostos reduzem a suscetibilidade de plantas a bactérias e fungos, atuando como retardador de crescimento e afetando parâmetros morfológicos e histológicos em folhas e brotos (Spinelli *et al*., 2005).

O Paclobutrazol (PBZ) é um regulador de crescimento derivado do grupo dos triazóis (Salisbury & Ross, 1992). Este composto tem sido utilizado na ativação e regulação de processos envolvidos na biossíntese de defesa da planta apresentando, dessa forma, potencial elicitor. Apesar da potencialidade do PBZ como regulador de crescimento e agente elicitor pouco se conhece sobre o real efeito deste composto sobre a cultura do pepino e sobre a indução de resistência ao patógeno *C. lagenarium*. Desta forma, o presente trabalho teve como objetivo verificar a ação do PBZ sobre as alterações morfofisiológicas em plantas de pepino; a sensibilidade *in vitro* de isolados de *C. lagenarium* ao PBZ; e seu potencial elicitor sobre este patógeno.

MATERIAL E MÉTODOS

Este trabalho consistiu em três experimentos distintos. No primeiro foi realizado um bioensaio para avaliar a sensibilidade micelial de isolados de *C. lagenarium* ao PBZ. No segundo, plantas de pepino foram tratadas com PBZ, para verificação das alterações morfofisiológicas; e finalmente, as folhas dessas plantas foram inoculadas com uma suspensão de conídios do isolado mais virulento de *C. lagenarium* obtido no primeiro ensaio, objetivando verificar o potencial elicitor do PBZ sobre a antracnose.

Sensibilidade in vitro de isolados de C. lagenarium ao PBZ

O bioensaio foi realizado no Laboratório de Proteção de Plantas do Departamento de Fitopatologia/UFV, Viçosa/MG. Foram utilizados dois isolados de *C. lagenarium* (CLKJ11 e CLKJ25), obtidos a partir de lesões encontradas em folhas de pepino. Para a obtenção dos isolados, fragmentos do tecido das folhas de pepino foram desinfestadas superficialmente com hipoclorito sódico (1:3, v:v) durante 1 minuto e lavadas mais duas vezes em água destilada autoclavada para a eliminação do excesso de hipoclorito. Em seguida, foram transferidos para placas de Petri, contendo meio de cultura batata-dextrose-ágar (BDA) acrescido de 500 mg L^{-1} de estreptomicina, sendo incubados por 7 dias à temperatura de 25+2ºC, sob luminosidade contínua. As espécies fúngicas crescidas em meio BDA foram repicadas, para a constituição de uma população pura, sendo posteriormente identificada em microscópio ótico. Discos de micélio dos isolados, medindo 5 mm de diâmetro, foram transferidos para placas de Petri contendo BDA com os compostos químicos testados e então incubadas por 10 dias em BOD, a 25+2ºC com luminosidade constante.

O bioensaio foi composto por sete tratamentos distribuídos em delineamento experimental inteiramente casualizado, dois isolados do patógeno e dez repetições. A unidade experimental foi constituída de uma placa de Petri contendo 1 disco do fungo. Os tratamentos consistiram na utilização de meio BDA puro (testemunha) e acrescido com concentrações dos compostos PBZ (50, 100, 200 e 400 mg L^{-1}), FTT (100 mg L^{-1}) e FTB (200 mg L^{-1}) (Tabela 1). O PBZ foi obtido a partir da formulação de Paclobutrazol® 100 CE (Agro Comercial Wiser Ltda, Diadema, Brasil). Os compostos denominados FTT e FTB, também conhecidos pelo nome comercial de Nativo® (Bayer Crop Science, Leverkusen, Alemanha) e Triade® (Bayer Crop Science, Leverkusen,

Alemanha) respectivamente, são fungicidas recomendado para o controle de *Colletrotrichum* sp. em diversas culturas. Estes também são pertencentes ao grupo dos triazóis e apresentam mecanismo de ação semelhante ao PBZ (inibidores da biossíntese de ergosterol) (Richardson & Warnock, 1993).

Tabela 1. Composição de meios para a avaliação da sensibilidade *in vitro* de *C. lagenarium* aos compostos triazólicos.

Tratamento	Composição
BDA	Batata (100 g L^{-1}) + Dextrose (5 g L^{-1}) + Agar (17 g L^{-1})
PBZ 50	BDA + Paclobutrazol (50,0 mg L^{-1})
PBZ 100	BDA + Paclobutrazol (100 mg L^{-1})
PBZ 200	BDA + Paclobutrazol (200 mg L^{-1})
PBZ 400	BDA + Paclobutrazol (400 mg L^{-1})
FTT	BDA + Tebuconazole (200 g L^{-1}) + Trifloxystrobin (100 g L^{-1})
FTB	BDA + Tebuconazole (400 g L^{-1})

Para determinar a sensibilidade *in vitro* do *C. lagenarium* às concentrações de PBZ, FTT e FTB avaliou-se o crescimento micelial (CM) e a porcentagem de inibição do crescimento micelial (PIN) em relação à testemunha. As avaliações do CM foram realizadas durante oito dias, a cada 48 horas, por meio de medição do crescimento radial do micélio na superfície do meio de cultura. As médias para cada uma das repetições foram estabelecidas por meio de duas medidas feitas em direções ortogonais nas placas, com o auxílio de uma régua milimetrada. Os dados obtidos foram submetidos à análise de variância e as médias comparadas pelo teste de Tukey a 5% de probabilidade.

Alterações morfofisiológicas em plantas de pepino causadas pela aplicação de PBZ

Foram utilizadas sementes de pepino híbrido 'Safira' (Sakata, Kanagawa, Japão). As mudas foram preparadas em bandejas de poliestireno de 128 células preenchidas com substrato comercial (Plantmax®, Eucatex, Paulínia, Brasil). O transplante, para vasos com 3 L de capacidade, ocorreu quando as plantas apresentaram a segunda folha completamente expandida. O solo foi classificado como ARGISSOLO VERMELHO-AMARELO (EMBRAPA, 2006), apresentando as seguintes características: pH em água (1:2.5) = 6.1; P = 175,4 mg dm^{-3}; P-rem = 27,1 mg L^{-1}; K = 163,0 mg dm^{-3}; Ca^{+2} = 5,7 $cmol_c$ dm^{-3}; Mg^{+2} = 1,2 $cmol_c$ dm^{-3}; Al^{+3} = 0,0 $cmol_c$ dm^{-3}; H + Al = 2,15 $cmol_c$ dm^{-3}; SB = 7,32 $cmol_c$ dm^{-3}; $CTC_{(t)}$ = 7,32 $cmol_c$ dm^{-3}; $CTC_{(T)}$ = 9,47 $cmol_c$ dm^{-3};

V = 77,0 %; m = 0,0 %; MO = 2,1 dag kg^{-1}. As temperaturas mínimas e máximas obtidas dentro da casa de vegetação foram 18,7 ºC e 29,7 ºC, respectivamente.

Os tratamentos consistiram de 4 doses de PBZ (50, 100, 200 e 400 mg $planta^{-1}$) usando a formulação Paclobutrazol® 100 CE (Agro Comercial Wiser Ltda, Diadema, Brasil) aplicados diretamente no solo, 10 cm ao redor da base das plantas; e a testemunha, que recebeu água destilada, e 5 repetições (cada unidade experimental foi constituída de uma planta por vaso). Foi realizada uma aplicação única 15 dias após o transplante das mudas e 25 dias após este tratamento foram avaliados: comprimento de plantas (CP); diâmetro de caule (DC); número de entrenós (NE), número de folhas (NF); área foliar (AF); material seca de raiz (MSR), caule (MSC), folha (MSF) e total (MST); número de flores masculinas (NFM) e femininas (NFF); e a medida indireta de clorofila (SPAD).

A cada sete dias, o CP foi obtida medindo-se do colo da planta até gema apical com o auxílio de régua e o DC foi mensurado a 3 cm do solo utilizando-se um paquímetro, ambos graduados em milímetros. Para a medição do índice SPAD foi utilizando o medidor portátil de clorofila SPAD-502 (Konica Minolta, Ramsey, USA), sendo as leituras realizadas em 3 regiões eqüidistantes nas 3 folhas mais novas, calculando-se posteriormente a média, com o próprio medidor. Ao final do experimento, 45 dias após o transplante, determinou-se a área foliar, em integrador de área foliar, modelo LI-3100 (Li-Cor, Lincoln, USA); e as partes da planta (raízes, caule e folhas) foram secas em estufa de circulação forçada de ar a 60 ºC, até a obtenção de massa constante, sendo pesadas em balança analítica. O NFM e NFF foram mensuradas diariamente.

Os dados foram submetidos à análise de variância e regressão Os dados foram submetidos à análise de variância e regressão. Os modelos foram escolhidos baseados na significância dos coeficientes de regressão, utilizado-se o teste "t" adotando o nível de significância de 5% de probabilidade, no coeficiente de determinação (R^2) e no fenômeno biológico.

Atividade elicitora do PBZ e proteção de pepino contra a antracnose

Vinte dias após o tratamento com o fitorregulador, as plantas de pepino foram inoculadas com o isolado CLKJ25 do fitopatógeno *C. lagenarium*, por ter demonstrado ser menos sensível ao PBZ no ensaio *in vitro.* A suspensão de conídios do isolado CLKJ25 foi preparada pela adição de água destilada esterilizada à superfície das

culturas crescidas em meio BDA por 7 dias. Em seguida, as suspensões foram filtradas em camada dupla de gaze e ajustadas em hemacitômetro para $1x10^{-8}$ conídios mL^{-1}. A suspensão testemunha consistiu de água destilada esterilizada. Todas as suspensões foram suplementadas com Tween 20 (Sigma, Saint Louis, EUA) a 0,05%. A inoculação foi realizada em todas as folhas da planta até o ponto de escorrimento com o auxílio de um pulverizador manual, e em seguida foram envoltas por uma cobertura plástica, permaneceram em câmara úmida por 48 horas.

Dez dias após a inoculação das plantas, removeram-se as oito folhas mais velhas de cada planta para a avaliação. Cada folha foi fotocopiada em scanner a 300 dpi e a leitura da severidade de doença foi realizada com o auxílio do software QUANT (Vale *et al*., 2003), da Universidade Federal de Viçosa, determinando-se a porcentagem da área foliar lesionada em relação à área foliar total das plantas inoculadas, através da redução de todas as cores presentes em cada folha para apenas 3: amarelo (tecido lesionado), verde (tecido sadio) e branco (fundo) (Figura 1). Foi avaliada a percentagem de área foliar lesionada (AFL). Os dados foram submetidos à análise de variância e regressão. O modelo foi escolhido baseado na significância do coeficiente de regressão, utilizado-se o teste "t" adotando o nível de significância de 5% de probabilidade, no coeficiente de determinação (R^2) e no fenômeno biológico.

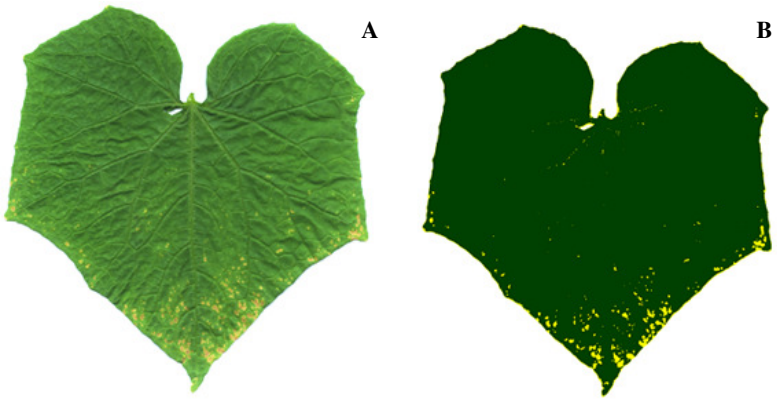

Figura 1. Agrupamento e diferenciação de cores, realizada pelo software QUANT, para a quantificação precisa da área lesionada de folhas de pepino inoculadas com *C. lagenarium*. (A) Folha de pepino scaneada (B) imagem obtida pelo software QUANT após agrupamento e diferenciação de cores.

RESULTADOS E DISCUSSÃO

Efetividade in vitro dos compostos químicos

Os compostos PBZ, FTT e FTB inibiram o crescimento de *C. lagenarium* independente das concentrações testadas (Figura 2). Esta eficiência no controle *in vitro* está relacionada ao mecanismo de ação relativa ao grupo químico a que pertencem (Grupo dos Triazóis), que atua sobre a biossíntese do ergosterol, componente essencial da membrana de fungos. Estes compostos agem diretamente sobre a 14α-demetilase do citocromo P450, responsável pela demetilação do carbono 14α e impede que a enzima continue o processo de demetilação do lanosterol, um precursor do ergosterol prejudicando a obtenção de grande quantidade de energia requerida pelo fungo para o crescimento micelial e formação dos conídios (Edginton *et al.*, 1971).

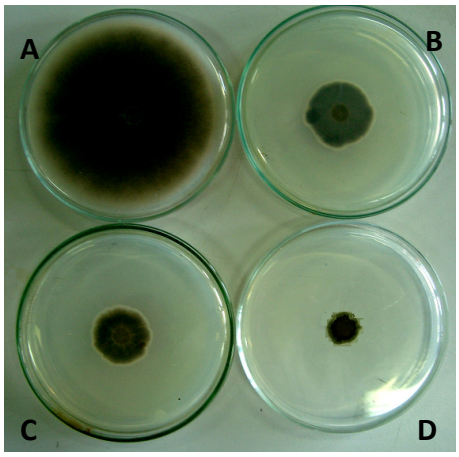

Figura 2. Crescimento micelial *in vitro* de *C. lagenarium* (CLKJ25) em meio de cultura contendo BDA (A); BDA + 100 mg PBZ L^{-1} (B); BDA + 100 mg FTT L^{-1} (C); e BDA + 200 mg FTB L^{-1} (D).

O PBZ apresentou potencial de inibição crescente em função do aumento da concentração do ingrediente ativo, tendo desta forma apresentado o menor CM e, conseguentemente a maior PIN na concentração de 400 mg L^{-1} para os dois isolados testados (CLKJ11 e CLKJ25). Foi observada diferença significativa entre os isolados, sendo o isolado CLKJ11 mais sensível a todos os compostos avaliados (Tabela 2).

Tabela 2. Efeito dos compostos PBZ, FFT e FTB sobre o crescimento micelial (CM) e a porcentagem de inibição (PIN) de dois isolados de *C. lagenarium.*

Tratamentos	Isolado 1 (CLKJ11)			Isolado 2 (CLKJ25)		
	CM (cm)		PIN (%)	CM (cm)		PIN (%)
BDA	8,40	A a	-	8,40	Ab	-
PBZ50	1,34	BC b	84,16	3,54	B a	57,86
PBZ100	1,04	CD b	87,62	3,02	B a	64,05
PBZ200	0,89	CD b	89,40	1,85	C a	77,98
PBZ400	0,00	D b	100,00	0,57	D a	93,21
FTT	1,65	B b	80,36	2,01	C a	76,07
FTB	0,71	Db	91,55	0,82	Da	90,24

Os valores médios seguidos de mesma letra minúscula na linha e letra maiúscula na coluna não diferem significativamente ao nível de 5% de probabilidade pelo teste de Tukey. CV (%)=12,58.

Fungos, principalmente do gênero *Colletotrichum,* têm demonstrado alta sensibilidade aos fungicidas do grupo dos triazóis. A eficiência de fungicidas deste grupo químico na inibição do crescimento micelial *in vitro* já foi comprovada em outros trabalhos (Freeman *et al*., 1997). Não foram encontrados na literatura trabalhos relatando a bioatividade do PBZ em *C. lagenarium.* Entretanto, fungicidas como imazalil, prochloraz, propiconazole e tebuconazole demonstram-se altamente eficientes no controle de *C. gloeosporioides*, sendo 1 mg L^{-1} capaz de reduzir, em média, 83,3% do crescimento micelial dos mesmos (Tavares, 2004; Tavares & Souza, 2005). Além disso, estudos preliminares sobre a utilização de PBZ no controle de fitopatógenos revelaram que este fitorregulador foi eficaz na inibição no crescimento micelial *in vitro* de *Fusaium oxysporum* f. sp. melonis (Cohen *et al.*, 1987) e *Verticillium dahliae* (Cimen *et al.*, 2004).

No isolado 1 (CLKJ11), o composto PBZ proporcionou diferença significativa no CM apenas entre sua maior e menor concentração. Esta última também foi estatisticamente semelhante ao FTT. Em relação ao FTB, houve semelhança deste composto com as três maiores concentrações de PBZ. No isolado 2 (CLKJ25) a maior (93,21%) e a menor (57,86%) PIN foram observado no tratamento PBZ400 e PBZ50, sendo estatisticamente semelhante apenas ao FTB e ao PBZ100, respectivamente. O FTT foi estatisticamente semelhante apenas ao PBZ200 (Tabela 2).

As semelhanças encontradas entre os compostos PBZ50 e FTT e entre PBZ100 e FTB no isolado 1, e entre PBZ100 e FTT e PBZ400 e FTB no isolado 2, indicam que o

PBZ mesmo em menor concentração é mais fungitóxico, tornando-o assim mais eficiente no controle *in vitro* do patógeno. Além disso, apesar de, segundo a classificação toxicológica, o PBZ, o FTT e o FTB serem semelhantes (medianamente tóxico); quando classificados quanto ao potencial de periculosidade ambiental, o FTT e o FTB são produtos muito perigosos ao meio ambiente (Classe II) e o PBZ é um produto medianamente perigoso ao meio ambiente (Classe III) (ANVISA, 2011; SEAB, 2011). Desta forma, podemos afirmar que o PBZ apresentou alta eficiência na inibição do crescimento micelial do patógeno, sendo o mais indicado, considerando a economia do produto, a fungitoxidade e a segurança ambiental.

Alterações morfofisiológicas

Plantas de pepino foram sensíveis à aplicação de PBZ, tendo as características NE, CP, NF, AF, MSC, NFM e NFF decrescido com a aplicação das doses do fitorregulador. O menor NE (7,98) foi observado na concentração 271,20 mg PBZ planta^{-1}, sendo 31,7% menor em relação à testemunha (Figura 3A). A diminuição do NE, promoveu a redução drástica do CP, tendo o menor CP sido observado na dose 37,83 mg PBZ planta^{-1}, o que representa um decréscimo de 60,42% em relação a testemunha (Figura 3B). Não foi observado efeito significativo para o DC (DC médio de 5,2 mm).

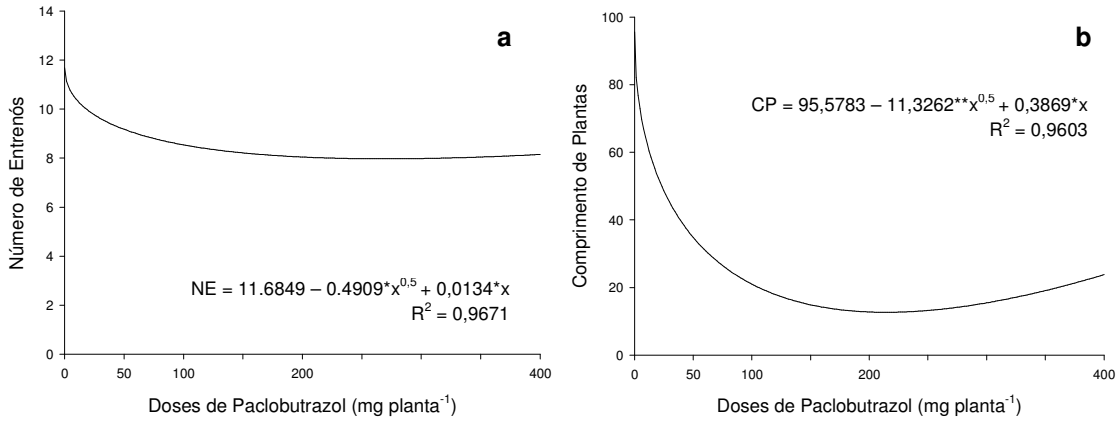

Figura 3. Número de entrenós (NE) (a) e comprimento (CP) (b) de plantas de pepino em função das doses de Paclobutrazol.

Devido às folhas surgirem de forma exógena e posicionada na região nodal, o número de entrenós determina o número de folhas (Dickison 2000). Além disso, folhas que se desenvolvem após a aplicação de PBZ são menores (Basra, 2000). Portanto, a aplicação do fitorregulador provocou a redução do NF e do tamanho das folhas, resultando na redução da AF. O menor NF (7,53) e a menor AF (447,46 dm^2) foram observados nas concentrações 372,19 mg PBZ $planta^{-1}$ e 355,18 mg PBZ $planta^{-1}$, respectivamente (Figura 4).

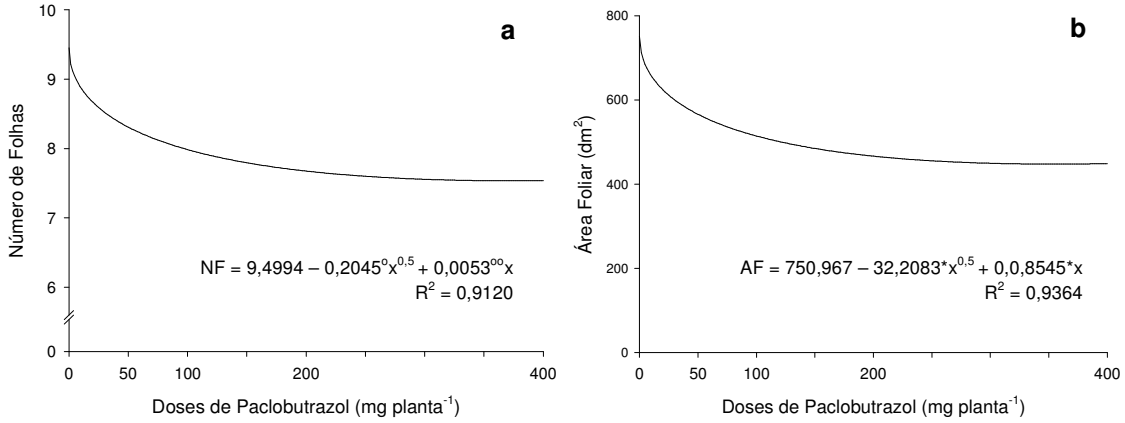

Figura 4. Número de folhas (NF) (a) e área foliar (AF) (b) de plantas de pepino em função das doses de Paclobutrazol.

A MSF aumentou com a aplicação do PBZ. Entretanto, doses superiores a 100 mg $planta^{-1}$ promovem redução desta variável. Sua média máxima (2,19 g) foi obtida na dose 53,44 mg PBZ $planta^{-1}$, correspondendo a um acréscimo de 7,63%. A MSR aumentou linearmente com a aplicação do PBZ. Desta forma, a maior média (0,28 g) foi obtida na maior concentração (400 mg PBZ $planta^{-1}$) e correspondeu a um aumento de 94,64% quando comparado à testemunha. Houve um decréscimo acentuado na variável MSC com a aplicação do PBZ. A dose que proporcionou o valor mínimo de 0,20 g (89,39% inferior à testemunha) foi de 244,53 mg $planta^{-1}$ (Figura 5).

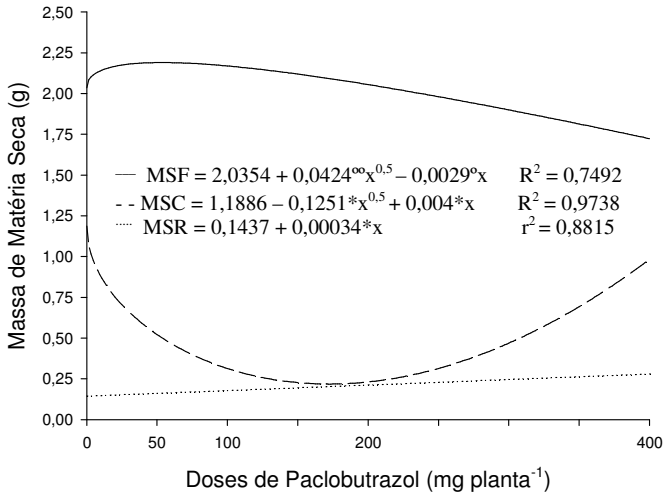

Figura 5. Peso de matéria seca dos órgãos vegetais: raiz (MSR), caule (MSC) e folha (MSF) de plantas de pepino em função das doses de Paclobutrazol.

A redução do comprimento e diâmetro do caule é um efeito característico do tratamento de plantas com compostos triazólicos e mais especificamente do PBZ (Davis *et al.*, 1988; Quilan, 1981). Além disso, esses compostos são descritos como agentes ativos no processo fisiológico de formação das raízes, na redução do alongamento celular e aumento da espessura do parênquima paliçádico (Davis & Haissig, 1990; Tekalign & Hammes, 2005). O MST médio foi de 3,43 g, não havendo diferenças estatísticas para esta variável. Esse resultado é elucidado devido ao fato dos baixos valores obtidos na MSC terem sido compensados pelos altos valores observados nas variáveis MSR e MSF.

Em relação ao número de flores, observou-se um decréscimo linear tanto para o NFM quanto para o NFF, com o aumento das doses de PBZ. Os menores valores (0,14 e 1,17) foram obtidos na maior dose do composto (400 mg planta^{-1}), correspondendo a uma redução de 96% e 66%, para NFM e NFF, respectivamente (Figura 6). A inibição de flores é resultado do uso de altas doses de PBZ. Entretanto, esses resultados também podem ser atribuídos a um efeito secundário da competição entre partes vegetativas e reprodutivas por açúcar e nutrientes (Basra, 2000).

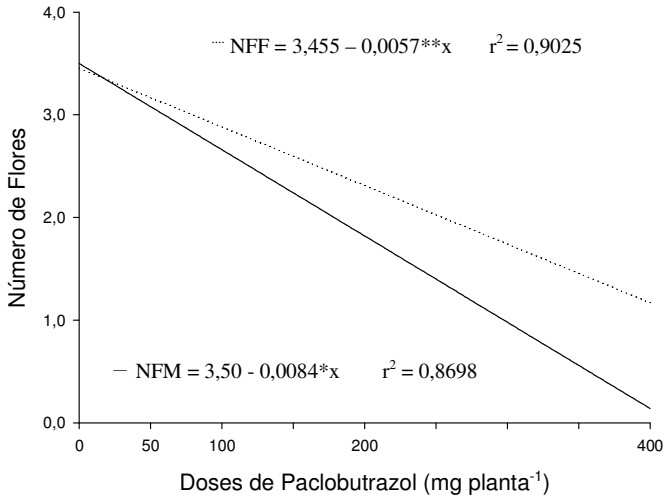

Figura 6. Número de flores femininas (NFF) de masculinas (NFM) de plantas de pepino em função das doses de Paclobutrazol.

Plantas tratadas com PBZ apresentaram folhas com coloração verde mais intensa que o controle, o que foi comprovado pelos resultados obtidos pela medida indireta de clorofila (índice SPAD). O maior índice SPAD (46,57) foi observado com a aplicação de 336,67 mg planta^{-1}, apresentando um acréscimo de 49,65% em relação à testemunha (Figura 7). Na maioria dos casos, este aumento está relacionado ao maior teor de clorofila ou simplesmente ao efeito concentrador, devido à reduzida expansão foliar (Davies & Sankhla, 1987).

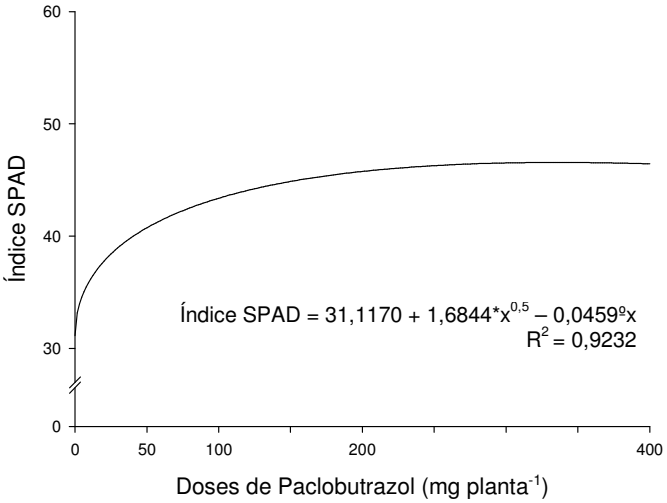

Figura 7. Medida indireta de clorofila (índice SPAD) de folhas de plantas de pepino em função das doses de Paclobutrazol.

Diante dos resultados obtidos, a dose que apresentou melhor resposta para essas características foi a de 50 mg planta^{-1}, levando-se em consideração o menor prejuízo às características morfofisiológicas das plantas e a economia do produto.

Proteção das plantas de pepino contra a antracnose

A partir dos dados obtidos pode-se afirmar que a aplicação do PBZ proporcionou redução da AFL, sendo observado decréscimo acentuado (cerca de 90%) com a aplicação da menor dose do composto (50 mg PBZ planta^{-1}) quando comparado a testemunha, que obteve AFL média de 4,39%. Entretanto, segundo a Resposta Linear de Plateau, doses superiores a esta não exerceram efeito sobre a AFL, mantendo a AFL igual a 3,8% independente das doses utilizadas. Desta forma, a concentração 50,76 mg PBZ planta^{-1} é a mais indicada para o controle da doença (Figura 8).

Figura 8. Imagens de folha de planta de pepino inoculadas com *C. lagenarium* obtidas através do software QUANTI. Planta não tratada (A) e planta tratadas com 50 mg PBZ planta^{-1} (B) apresentando área foliar lesionada (AFL) de 4,39% e 3,8%, respectivamente.

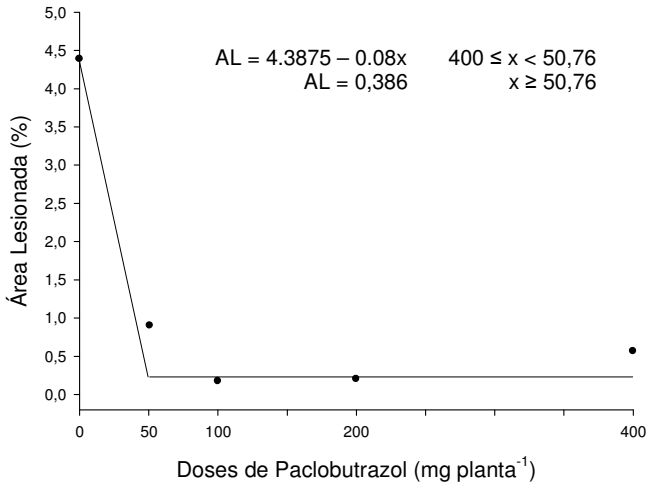

Figura 9. Percentagem de área lesionada (AL) de folhas de plantas de pepino tratadas com PBZ e inoculadas com *C. lagenarium.*

Essas respostas evidenciam a relação das alterações morfofisiológicas promovidas pelo PBZ e a proteção desenvolvida contra a infecção do *C. lagenarium.* Para invadir o tecido hospedeiro, as espécies de *Colletotrichum* utilizam estratégias que variam de hemibiotróficos intracelular a necrotróficos subcuticular, desenvolvendo estruturas especializadas, como por exemplo, tubos germinativos, apressórios e haustórios (Sutton, 1980). A aplicação de PBZ promoveu a redução do alongamento celular e o aumento da espessura do parênquima paliçádico das folhas, indicado pela menor área foliar e maior massa de matéria seca das folhas, que se constituem barreiras mecânicas contra o patógeno (Tekalign & Hammes, 2005; Gao *et al.*, 1998). Desta forma, embora a aplicação do PBZ não tenha afetado a germinação de esporos e a formação de apressórios de *C. lagenarium*, a penetração é reduzida drasticamente nos tecidos das folhas.

Resultados semelhantes forma observados por Cohen *et al*. (1987). Estes autores também sugeriram que a redução da incidência da murcha de Fusário em plântulas de melão foi decorrente do efeito do PBZ sobre os processos metabólicos das plantas e não ao efeito fungitóxico deste composto. Além disso, DeStefano *et al*. (2007) verificaram que a alterações fisiológicas provocadas pela aplicação de PBZ afetaram o desenvolvimento da bactéria *Xillela fastidiosa* em algumas árvores de sombra hospedeiras deste patógeno; e Cimen *et al*. (2004) atestaram que o declínio da Murcha de *Verticillium* em algodão foi uma resposta à diminuição do porte das plantas tratadas com o fitorregulador.

CONCLUSÕES

Diante dos resultados obtidos, pode-se concluir que o PBZ revelou-se eficaz na inibição do crescimento micelial de *C. lagenarium* e que plantas de pepinos foram sensíveis à aplicação do fitorregulador, promovendo alterações morfofisiológicas responsáveis por prejudicar a infecção do patógeno nas folhas. A melhor dose de PBZ foi de 50 mg PBZ L^{-1} ou 50 mg PBZ $planta^{-1}$. Embora a aplicação do PBZ tenha proporcionado redução da área foliar lesionada, este fitorregulador não pode ser considerado um agente ativador da SAR uma vez que exibe efeito tóxico sobre o patógeno desafiante, contrariando uma das regras básicas para a determinação da resistência adquirida de plantas segundo Kessmann *et al.* (1994)

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REFERÊNCIAS

Agrios GN (2005) Plant Pathology. 5ª ed. San Diego, Elsevier Academic Press. 922p.

Amin KS & Ullasa A (1981) Effect of thiophanate on epidemic development of anthracnose and yield of watermelon. Phytopathology, 71:20-22.

ANVISA. Agência Nacional de Vigilância Sanitária (2011). Disponível em: <http://portal.anvisa.gov.br/wps/wcm/connect/55e2fc80409be7e4bf99bf72ea9f08f9/p45.pdf?MOD=AJPERES>. Acessado em: 16 de junho de 2011.

Basra AS (2000) Plant Growth Regulators in Agriculture and Horticulture: Their Role and Commercial Uses. 1ª ed. Binghamton, The Haworth Press. 264p.

Bubici G, Amenduni M, Colella C, D'amico M & Cirulli M (2006) Efficacy of acibenzolar-S-methyl and two strobilurins, azoxystrobin and trifloxystrobin, for the control of corky root of tomato and *Verticillium* wilt of eggplant. Crop protection, 25:814-820.

Cimen I, Basbag S, Temiz M & Sagir A (2004) The effect of Paclobutrazol, growth retardant, on cotton growth and *Verticillium* Wilt (*Vericillium dahliae* Kleb.) Plant Pathology Journal, 3:35-39.

Cohen R, Yarden O & Katan J (1987) Paclobutrazol and other plant growth-retarding chemicals increase resistance of melon seedlings to *Fusarium* Wilt. Plant Pathology, 36:558-564.

Cohen Y, Gisi U & Niederman T (1993) Local and systemic protection against hytophthora infestans induced in potato and tomato plants by jasmonic acid and jasmonic methyl-ester. Phytopathology, 83:1054-1062.

Davies TD & Sankhla N (1987) Altered diurnal leaf movements in soybean seedling treated with triazole growth regulators. Plant Cell Physiology, 28:1345-1349.

Davis DT & Haissig BE (1990) Chemical control of adventitious root formation in cuttings. Bulletin Plant Growth Regulator Society of America, 18:1-17.

Davis TD, Steffens GL, Sankhla N (1988) Triazole plant growth regulators. Horticultural Reviews, 10:63-105.

DeStefano DA, Grybauskas AP, Sherald JL, Momen B, Huang Q & Sullivan JH (2007) Effect of the Growth Regulator Paclobutrazol on growth of the bacterial pathogen *Xyllela fastidiosa*. Arboriculture & Urban Forestry, 33:246-252.

Dickison WC (2000) Integrative Plant Anatomy. 1ª ed. San Diego, Academic Press. 532p.

Edginton LV, Knew KL & Barron GL (1971) Fungitoxic spectrum of benzimidazole compounds. Phytopathology, 62:42-44.

EMBRAPA (2006) Empresa Brasileira de Pesquisa Agropecuária. Sistema Brasileiro de Classificação de Solos. 2ª ed. Rio de Janeiro, Embrapa Solos. 412p.

Fan Z, Shi Z, Zhang H, Liu X, Bao L, Ma L, Zuo X, Zheng Q & Mi N (2009) Synthesis and biological activity evaluation of 1,2,3-thiadiazole derivatives as potential elicitors with highly systemic acquired resistance. Journal of Agricultural and Food Chemistry, 57:4279-4286.

Freeman S, Nizani Y, Dotan S, Even S & Sando T (1997) Control of *Colletotrichum acutatum* in strawberry under laboratory, greenhouse, and field conditions. Plant Disease 81:749-752.

Gao JG, Hofstra G & Fletcher RA (1988) Anatomical changes induced by triazoles in wheat seedlings. Canadian Journal of Botany, 66:1178-1185.

Kessmann H, Staub T, Ligon J, Oostendorp M & Ryals J (1994) Activation of systemic acquired disease resistance in plants. European Journal of Plant Pathology, 100: 359-369.

Latin RX (1993) Diseases and pests of muskmelons and watermelons. 1ª ed. West Lafayette, Purdue University Cooperative Extension Service. 85p.

Oostendorp M, Kunz W, Dietrich B & Staub T (2001) Induced disease resistance in plants by chemicals. European Journal of Plant Pathology, 107:19-28.

Quilan JD (1981) New chemical approaches to control of fruit tree form and size. Acta Horticulturae, 120:95-106.

Rego AM & Carrijo IV (2000) Doenças das cucurbitáceas. In: Vale FXR, Zambolim L & Costa H (Eds.) Controle de doenças de plantas: hortaliças. Viçosa, UFV. p. 535-598.

Richardson MD & Warnock DW (1993) Antifungal drugs. In: Richardson MD & Warnock, DW (Eds.) Fungal infection – Diagnosis and management. London, Blackwell. p. 17-43.

Ronchia A, Farinab G, Gozzoc F & Tonelli C (1997) Effects of a triazolic fungicide on maize plant metabolism: modifications of transcript abundance in resistance-related pathways. Plant Science, 130:51-62.

Salisbury FB & Ross CW (1992) Plant Phisiology. 4ª ed. Belmont, Wadsworth. 682p.

SEAB. Secretaria de Estado da Agricultura e do Abastecimento. Disponível em: <http://www.seab.pr.gov.br/modules/conteudo/conteudo.php?conteudo=120>. Acessado em: 16 de junho de 2011.

Shoresh M, Yedidia I & Chet I (2005) Involvement of Jasmonic Acid/Ethylene Signaling Pathway in the Systemic Resistance Induced in Cucumber by *Trichoderma asperellum* T203. Phytopathology, 95:76-84.

Smith CJ (1996) Accumulation of phythoalexins: defense mechanisms and stimulus response system. New Phytholgist, 132:1-45.

Spinelli F, Speakman JB, Rademacher W, Halbwirth H, Stich K & Costa G (2005) Luteoforol, a flavan 4-ol, is induced in pome fruits by prohexadione-calcium and shows phytoalexin-like properties against *Erwinia amylovora* and other plant pathogens. European Journal of Plant Pathology, 112:133-142.

Starratt AN & Lazarovits G (1999) Herbicide-induced disease resistance and associated increases in free amino acid levels in melon plants. Canadian Journal of Plant Pathology, 21:33-36.

Sutton BC (1980) The Coelomycetes. 1ª ed. Surrey, CMI Kew. 696p.

Tavares GM (2004) Controle químico e hidrotérmico da antracnose em frutos de mamoeiro (*Carica papaya* L.) na pós-colheita. Dissertação de Mestrado. Universidade Federal de Lavras, Lavras, 55p.

Tavares GM & Souza PE (2005) Efeito de fungicidas no controle *in vitro* de *Colletotrichum gloeosporioides*, agente etiológico da Antracnose do Mamoeiro (*Carica papaya* L.). Ciência e Agrotecnologia, 29:52-59.

Tekalign T & Hammes PS (2005) Growth and biomass production in potato grown in the hot tropicsas influenced by Paclobutrazol. Plant Growth Regulation, 45:37-46.

Tian F, Zhu J, Sun M, Jiang J, Wang S & Zhang W (2008) Induction and mechanism of cucumber resistance to anthracnose induced by *Pieris rapae* extract. Frontiers of Agriculture in China, 2:137-140.

Vale FXR, Fernandes-Filho EI & Liberato JR (2003) QUANT: A software for plant disease severity assessment. In: Proceedings 8th International Congress of Plant Pathology, Christchurch. Anais, ICPP. p. 105.

Van Andel OM (1968) Shifts in disease resistance induced by growth regulation. European Journal of Plant Pathology, 74:113-120.

GENERAL CONCLUSION

Despite the PBZ has been effective regarding to the reduction of plant growth and on the mechanisms induction of plant resistance to diseases, its use in melon and cucumber commercial crops needs adjustment in terms of dose, timing and mode of application, once that these cultures were extremely sensitive to this compound and excessive application might leave residue on the cultivated substrate.

Although PBZ should not be considered a plant resistance inducer, the morphophysical changes caused by its application were an important strategy against the disease severity, once that these changes, provided by so low concentrations, served as mechanical barriers, hindering the pathogen infection.

Thus, is necessary to perform further studies that specify the PBZ use in the control of plant size without harm the flowering, yield and fruit quality. Moreover, in face of the potentially as elicitor, more tests have to be done to identify other components involved in plant defense to pathogens, activated by this phytoregulator.