THE EFFECT OF INSECTICIDES ON PEROXIDASE ACTIVITY IN HOT PEPPER PLANTS (*Capsicum annum* L.)

J. L. García-Hernández[1,2¶]; H. Nolasco[1]; E. Troyo-Diéguez[1]; B. Murillo-Amador[1]; A. Flores-Hernández[3]; I. Orona-Castillo[4]; R. D. Valdez-Cepeda[5]

[1]Centro de Investigaciones Biológicas del Noroeste, S. C. Mar Bermejo Núm. 195, Col. Playa Palo Santa Rita, La Paz, Baja California Sur. MÉXICO. Correo-e: luis_garher2000@yahoo.com.mx ([¶]Autor responsable).
[2]Facultad de Agronomía, Universidad Autónoma de San Luis Potosí, MÉXICO.
[3]URUZA. Universidad Autónoma Chapingo, Bermejillo, Durango. C. P. 35230. MÉXICO.
[4]CENID-RASPA, Instituto de Investigaciones Forestales Agrícolas y Pecuarias, Gómez Palacio, Durango. MÉXICO.
[5]CRUCEN, Universidad Autónoma Chapingo, Zacatecas, Zac. MÉXICO.

ABSTRACT

We evaluated the effect of four organophosphoric insecticides on the physiology of hot pepper. Three commercial products (Gusation 35PH®, Paration CE720®, and Tamaron 600 LM®) and an active ingredient with no mixtures (metamidofos) were used. Four rates were utilized for each product: the average rate recommended in the product's label (1.0R), but also 0.5 (0.5R), 1.5 (1.5R), and twice the recommended rate (2.0R). Effects were evaluated through peroxidase activity, which is an enzyme frequently used as a biological marker for oxidative stress. Samples analyzed were taken from photosynthetically active leaves. Results show that the highest insecticide rates caused alterations in the expression of the aforementioned enzyme. Differences were found among insecticide, but all of them increased enzyme activity when applied at rates higher than those recommended, which were used as controls, in the labels of the commercial products studied.

ADDITIONAL KEY WORDS: enzyme activity, organophosphoric, oxidative stress.

EFECTO DE INSECTICIDAS EN LA ACTIVIDAD DE LA PEROXIDASA EN PLANTAS DE CHILE PICANTE (*Capsicum annum* L.)

RESUMEN

Fueron evaluados los efectos de cuatro insecticidas organofosforados sobre la fisiología de chile o ají. Se utilizaron tres productos comerciales (Gusation 35PH®, Paration CE720®, and Tamaron 600 LM®) y un ingrediente activo sin mezclas (metamidofos). Se utilizaron cuatro dosis de cada producto; la dosis media recomendada en la etiqueta del producto (1.0R), así como 0.5 (0.5R), 1.5 (1.5R) y dos veces dicha dosis (2.0R). Los efectos fueron evaluados mediante la actividad de peroxidasa, la cual es una enzima frecuentemente utilizada como marcador biológico de estrés oxidativo. Las muestras bajo análisis fueron tomadas de hojas fotosintéticamente activas. Los resultados mostraron que las dosis más altas de insecticida causaron alteraciones en la expresión de la enzima mencionada. Fueron encontradas diferencias entre los insecticidas, pero todos ellos incrementaron la actividad de la enzima cuando se aplicaron en dosis mayores a las recomendadas en las etiquetas de los productos comerciales que fueron utilizadas como testigo.

PALABRAS CLAVE ADICIONALES: actividad enzimática, organofosforados, estrés oxidativo.

INTRODUCTION

Chili or hot pepper (*Capsicum annum* L.) is one of the most important commercial crops and indispensable condiment as well as vegetable in Mexico and other countries in America, Europe, and Asia. The pepper weevil (*Anthonomus eugenii* Cano), has contributed to excessive use of insecticides on peppers due to difficulties in timely control of adults (Riley, 1997). The apparent lack of non chemicals control tactics and low mortality due to natural enemies exacerbates the problem.

Circumstances like these cause that the use of chemicals for plant protection has been enormously increased

Recibido: 17 de marzo, 2003
Aceptado: 31 de agosto, 2004

(Gliessman, 1998). Pest control supposes to be a judicious combination of several methods including cultural practices, development of resistant varieties and the use of chemicals in an integrated pest approach. It is clear that the use of chemicals to control pests is useful, but the frequent indiscriminate use may have undesirable consequences (Straw *et al*., 1996; Reddy and Rao, 1981). In Baja California Sur and other states of Mexico, some of the farmers apply higher than the recommended concentrations of insecticide to control the resistant pests, and they occasionally report better control, but the yields not increase but even are reduced. This occurs principally in fields having problems with pepper weevil and where hot pepper is grown as a commercial crop on large scale (Calderón-Limón *et al.*, 2002).

Viewing the magnitude that the problem is getting in the northwest of Mexico, this investigation was made in order to give into the details of the effects of widely used organophosphate insecticides, namely Gusation 35 PH®, Paratión CE 720®, Tamaron 600®, and the active ingredient of Tamaron (methamidophos), on the peroxidase activity as physiological stress marker. Other results of this research project have been already reported (García-Hernández *et al.*, 2000; 2001) indicating that higher doses of organophosphate insecticides affect plant growth and yield.

The peroxidase enzime has been used as biochemical indicator because of its activity and composition changes with the physiological status of the organism (Gaspar *et al*., 1991). Peroxidases have been involved in several physiological and biochemical processes, such as cell growth and expansion (Wallace, 1994; Lin and Kao, 1999), differentiation and development (Gaspar *et al*., 1991; Mansouri *et al*., 1999; Lagrimini *et al*., 1997), auxin catabolism (Lagrimini *et al.*, 1997), lignification (Sitbon *et al*., 1999; Otter and Polle, 1997), as well as abiotic and biotic stress responses (Lin and Kao, 1999; Mohan *et al*., 1993; Medina *et al*., 1999). According to the literature, the plant responses to internal or external stimuli increasing peroxidase activity; however, some reports show a decrease in this activity. Many studies indicate a significant correlation between higher levels of peroxidase activity and lower levels of plant growth (Gaspar *et al*.,1991; Sitbon *et al*., 1999; Fang and Kao, 2000).

The peroxidase activity has been used as stress indicator in plants in studies with excess iron, copper and zinc (Fang and Kao, 2000), NaCl (Lin and Kao, 1999), other metals (Ezaki *et al*., 1996) and many other stress factors (Lobarzawsky *et al*., 1991). Almost all of them increase the peroxidase activity in the affected plants, but, in some studies a reduction in peroxidase activity has been reported (Stevens *et al*., 1978).

Phytotoxicity by insecticide excess has been evaluated in some physiological traits in other cultivars of *C. annum*. It has been studied the effects on meiotic system with four organophosphate insecticides by Atale *et al*. (1995). additionally, the effects on the yield and growth were studied using heptachlor-benzene (BHC) and Nuvacron by Reddy and Rao (1981), and similarly, using organo-carbamates by Maheshwari and Singh (1989). Other crops have been studied, i.e: *Picea sitchensis* treated with dimethoate, malathion, primicarb and other combinations (Straw *et al*., 1996), and tomato treated with Abamectin and Cartap (Picanço *et al*., 1998). All these studies have shown that insecticides; applied in higher than recommended doses, cause negative effects on the physiology and yield of plants. In addition, the excessive use of insecticides can motive health problems in human; from minor problems until neurotoxicity or cancer (Abou-Donia *et al*., 1996; Anonymous, 1999). The goal of this work is to study the effect of commonly used organophosphate insecticides on peroxidase activity, and evaluating the role of this enzyme as stress marker in hot pepper leaves.

MATERIALS AND METHODS

The experiments in greenhouse were carried out at The Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR) in La Paz, Baja California Sur, México. This facility is located in the southern portion of this peninsula, at latitude of 26 ° North, and at sea level. Climate at this location is dry most of year with an average of 120 mm annual rainfall, although relative humidity is middle because of moisture from the Sea of Cortéz at the edge of the town. The annual mean temperature oscillates around 18 °C, with maximum of 26 °C and minimum of 12 °C.

Seeds were germinated in plates. Plants were later transplanted to flowerpots in the greenhouse. A factorial experimental design was arranged completely randomized with three replicates. For the experimental design, the insecticides were assigned as the Factor A: a1) Gusation 35 PH® [active ingredient (a.i).= azinphos methyl]; a2) Paratión CE 720® [a.i. = parathion methyl]; a3) Tamaron 600 CE® [a.i.= methamidophos]; and a4) methamidophos. The doses were assigned as the Factor B: b1) application with manufacturer's recommended dose [1.0R], b2) half recommended dose [0.5R], b3) 1.5 times recommended dose [1.5R], and b4) twice recommended dose [2.0R]. Plants were sprayed weekly for 5 weeks with the treatments during the flowering stage. Application started when 100 % of plants were in flowering stage.

All plants received the same management of fertilization; they were grown in a nutritive solution, applying the essential minerals artificially. Plants were watered daily.

One day after the last spraying, two leaves were taken from each plant. All leaves had a size between 5 to 6 cm long and they were collected from the upper third of the plant. These samples were plenty washed with distilled water and then homogenized in a mortar (in ice water bath) with acetate buffer 50 mM (pH 5.1). The homogenate was centrifuged

at 30000 x *g* for 15 min, and the supernatant was used for assays of peroxidase activity. This activity was assayed by the method of Boehringer (1973) performed with a spectrophotometer SPECTRON 2000 (BAUSCH & LOMB USA). The data were statistically analyzed by means of ANOVA. The protein concentration in the leaf extracts was estimated by the method of Bradford (1976) and carried out with a DU 640 spectrophotometer (BECKMAN, USA).

Native polyacrylamide gel electrophoresis (7.5 %) was carried out at 120 V for 2.5 hrs according to the method reported by Moreno *et al*. (1990) using an 8 x 6 x 0.1 cm gel without SDS. An equal amount of protein (25 g) per sample treatment was applied and the gel was stained for peroxidase activity with a solution of 20 mM of guaiacol for 15 min, and then with 0.03 % of hydrogen peroxide for color development.

RESULTS AND DISCUSSION

Tables 1 and 2 show the statistical analysis for the specific peroxidase activity. These tables explain the statistical differences for the applied treatments. Significant differences between the studied insecticides were observed as follow: the Factor B (doses) showed higher significant values in the variance analysis than the Factor A (insecticides). In the other hand, there were no significant effects in interaction among the two factors in the response of peroxidase activity. The obtained differences confirm the hypothesis that stress by insecticides also influences the antioxidative enzymatic activity.

TABLE 1. Variance analysis of the specific peroxidase activity (protein in U·mg^{-1}) in function of insecticides and doses.

Variation source	Freedom degrees	Sum of Squares	Mean Squares	Calculated F	Significance
Insecticides	4	3.350	0.837	3.480	*
Doses	3	8.767	2.922	12.145	**
Insecticides X Doses	12	3.276	0.273	1.134	NS
Error	40	9.625	0.240		
Total	59	25.020			

NS, *, **; not significant and significant at a $P \leq 0.05$ and 0.01.
Coeficient of variability = 15.91 %.

TABLE 2. Comparison of the specific peroxidase activity in extracts from leaves of hot pepper plants sprayed during 5 weeks with different treatments of insecticides.

Dose[z]	Mean (protein U·mg^{-1})	Insecticide	Mean (protein U·mg^{-1})
0.5	0.0695 c[y]	Control	0.1077 b
1.0	0.1962 bc	Gusation	0.3721 b
1.5	0.5290 b	Tamaron	0.8361 a
2.0	1.0581 a	Paration	0.5373 ab
		Metamidophos	0.4627 ab

[y]Values at the same column with the same letter are equal according to the Tukey's test with a $P \leq 0.05$.
[z]Concentration of recomended dose.

Peroxidase activity response was significantly more affected by Tamaron as compared to the control, but the effects were more significant in relation to the doses, and confirmed by statistical differences between the means of treatments (Table 2).

The insecticides applied in lower doses did not origin significant differences as compared to control (Figure 1), but a higher dose significantly increases in peroxidase activity. Similar tendencies have been reported in studies related to physiological injuries by insecticides in hot pepper (García-Hernández *et al*., 2000; Atale *et al*., 1995; Lakshmi *et al*., 1988). Into this context, generally the manufacturer's recommended dose does not originate negative effects on plants, and even some researchers have reported that specific insecticides could act as growth stimulant in low doses. Foster and Brust (1995) found a response as growth promoter in watermelon treated with carbofuran; they experimented with several foliar and soil insecticides and they did not reach significant effects of phytotoxicity, thus, they believed that carbofuran acted as positive stimulant. Nevertheless, it could be discussed if their results were effectively because of the benefices from carbofuran or whether the differences respect to another insecticides were caused by a higher phytotoxicity from these other insecticides. For that reasons it is important to study deeply the phytotoxicity by insecticides, taking into account that most of studies into this topic have pointed out that insecticides do not have physiological benefices in plants (Picanço *et al*, 1998; Atale *et al.*, 1995; Devadas *et al*., 1986, Amer and Ali, 1983).

The peroxidase activity has a positive association with the application doses. The behavior of the polyacrylamide

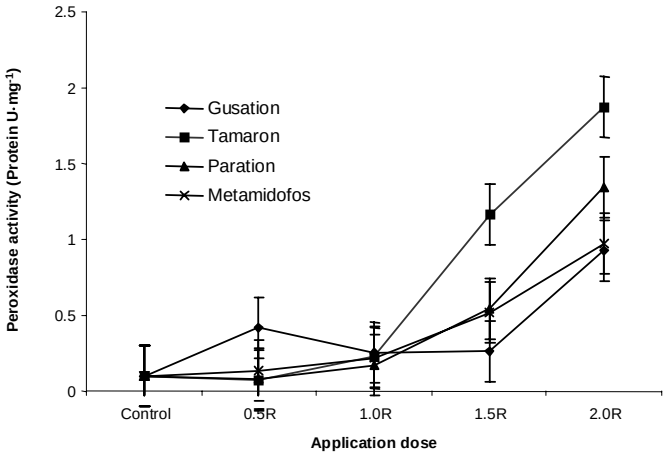

FIGURE 1. Specific peroxidase activity in extracts from leaves of hot pepper plants sprayed during 5 weeks to different treatments with insecticides. Control: plants without insecticide treatment; 0.5R, half of recommended dose; 1.0R, recommended dose; 1.5R, 1.5 times the recommended dose; 2.0R, twice the recommended dose.

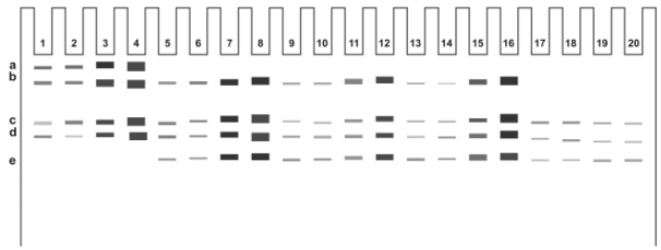

FIGURE 2. Polyacrylamide gel electrophoresis of extracts from leaves of hot pepper plants sprayed during 5 weeks with different treatments of insecticides. 1 to 4: Gusation; 1) 0.5R, 2) 1.0R, 3) 1.5R, 4) 2.0R.; 5 to 8: Tamaron; 5) 0.5R, 6) 1.0R, 7) 1.5R, 8) 2.0R.; 9 to 12: Paration: 9) 0.5R, 10) 1.0R, 11) 1.5R, 12) 2.0R.; 13 to 16: methamidophos; 13) 0.5R, 14) 1.0R, 15) 1.5R, 16) 2.0R.; 17 to 20: Control. R: concentration of recomended dose.

gel electrophoresis coincides with this last (Figure 2) and explain much clearer the observations. The higher intensity in the coloration occurred in the places or columns from 5 to 8 with the samples treated with Tamaron (Figure 2), emphasizing the places 7 and 8 with the highest doses. Coinciding with results from Table 2, the Figures 1 and 2 show that even having the same active ingredient, Tamaron caused an apparent higher affectation than metamidophos, it can be interpreted as an interaction between the active ingredient and the surfactants from the commercial formulation. It was observed that for each insecticide treatment, the highest dose caused the most intense concentration (Figure 2).

According to the different derived bands in the gel, apparently the different insecticides influenced the appareance of different isoforms of the peroxidase enzyme. As it is observed, the places treated with Gusation (1 to 4) showed just two bands that were observed in the control (17 to 20); in the *c* and *d* positions, but Gusation treatments showed another different bands in the position *a* and *b*.

All the other treatments (Tamaron, Paration and metamidophos) showed the same bands than the control in the *c, d* and *e* positions. The differences among these treatments is explained because they show other different band in the position *b*. According to the literature, peroxidase is one the enzymes with more number of isoforms (Lobarzawsky *et al*., 1991), and the apparition of each isoform depends on the physiological status and the type of developing conditions in a plant (Lobarzawsky *et al*., 1991; Moreno *et al*., 1990).

The differences in the peroxidase activity can be reflected in the growth and yield of plants. As some authors claim, peroxidases have been directly or indirectly associated to some physiological processes like abscission, dormancy, apical dominance, resistance to parasites (Lobarzawsky *et al*., 1991), and in some important phases of the metabolism like the auxins catabolism, and lignin formation (Sitbon *et al*., 1999; Fang and Kao, 2000). In this context, Reddy and Rao (1981), made a study with BHC and monochrotophos in *Capsicum annuum*. They observed that both germination and survival rate were affected in all the treatments with BHC, and the injurious effect of Nuvacron on germination and survival of *C. annuum* seeds was greater than with BHC. Both insecticides produced abnormalities in the dividing meristematic root cells. The frequency of these abnormalities was higher in Nuvacron than BHC. Devadas *et al*. (1986) made a study to determine the effects of four organophosphates insecticides, protiophos, diclorvos, phosphamidon, and monochrotophos on germination, survival, and meiotic behaviour in the same species. They found alterations in all the characteristics studied, at different levels.

Lakshmi *et al*. (1988) also evaluated the effects of two organophosphorus pesticides, Ekalux EC 25 and Metasixtos, which were studied using other cultivar of hot pepper in India. Seed germination and survival rate steadily decreased with increased doses of chemicals. There was a reduction of mean chiasmata per cell and there were induced clastogenic changes such as stickiness of chromosomes, formation of univalent, laggards, bridges, and micronuclei in different stages of meiosis. The very apparent association that exists between the increase of peroxidase activity and the decrease in grown and yield in hot pepper indicates that peroxidase activity is an important tool to evaluate the physiological stress by insecticides and to prevent losses in yield and quality of this crop.

CONCLUSIONS

The peroxidase activity was significantly increased by the 1.5R and 2.0R doses of the evaluated insecticides. No significant interactions were observed between insecticide and doses. The most effecting insecticides were Tamaron and methamidophos, but, even being the same active ingredient, Tamaron caused a highest peroxidades activity observed as intensity in coloration in electrophoresis gel and in concentration of peroxidase. Specific peroxidase activity resulted a very recommendable tool to evaluate organophosphate-insecticide physiological-stress in hot pepper plants. The utilization of different approaches to measure the peroxidase; as electrophoresis and spectrophotometer, allowed to evaluate different aspects of the enzymatic activity. The achieved results confirm that indiscriminate use of chemicals affects normal plant growth, which could result in reduction of yield or quality. These results, also confirm that the peroxidase enzyme has a great number of isoforms and that each isoform is activated depending on the class of insecticide. The activation of specific isoforms should be studied in future investigations.

LITERATURE CITED

ABOU-DONIA, M. B.; WIMARTH, K. R.; ABDEL, A. A.; JENSEN, K. F.; KURT, T. L. 1996. Increased neurotoxicity following concurrent exposure to pyridostignine bromide, DEET, and

chlorpirifos, pp. 201-222. *In*: Fundamentals of Applied Toxicology. Academic Press, Orlando, Fla., USA.

AMER, S. M.; ALI, E. M. 1983. Cytological effects of pesticides XIV. Effect of the insecticide dipterex 'trichlorphon' on *Vicia faba* plant. Cytologia 48: 761-563.

ANONYMOUS. 1999. Reconocimiento y Manejo de los Envenenamientos por Pesticidas. Quinta edición. Environmental Protection Agency (EPA). Washington, D.C., USA. 252 p.

ATALE, A. S.; NARKHEDE, M. N.; ATALE, S. B. 1995. Effects of some agrochemicals on meiotic cell division in chilli. J. Maharashtra Agric. Universities 20: 195-197.

BRADFORD, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

BOEHRINGER, M. 1973. Methods of Enzymatic Analysis, 2nd enlarged edition. Verlag Chemie. Weinheim, Germany. 434 p.

CALDERÓN-LIMÓN, B. A.; GARCÍA-HERNÁNDEZ, J. L.; TROYO-DIÉGUEZ, E. 2002. Technique for oviposition of the pepper weevil (Coleoptera: Curculionidae) to obtain massive colonies in the laboratory. Folia Entomol. Mex. 41(2): 249-251.

DEVADAS, N.; RAJAM, M. V.; SUBHASH, K. 1986. Comparative mutagenicity of four organophosphorus insecticides in meiotic system of red pepper. Cytologia 51(4): 645-653.

EZAKI, B.; TSUGITA, S.; MATSUMOTO, H. 1996. Expression of a moderately anionic preoxidase is induced by aluminum treatment in tobacco callus: Possible involvement of peroxidase enzymes in aluminum ion stress. Physiol. Plant. 96: 21-28.

FANG, W. CH.; KAO, CH. H. 2000. Enhanced peroxidase activity in rice leaves in response to excess iron, copper and zinc. Plant Sci. 158: 71-76.

FOSTER, R. E.; BRUST, G. E. 1995. Effects of insecticides applied to control cucumber beetles (Coleoptera: Chrysomelidae) on watermelon yields. Crop Prot. 14: 619-624.

GARCÍA-HERNÁNDEZ, J. L.; TROYO-DIÉGUEZ, E.; MURILLO-AMADOR, B.; FLORES-HERNÁNDEZ, A.; GONZÁLEZ-MICHEL, A. 2001. Efecto de algunos insecticidas y un promotor de crecimiento sobre variables fisiológicas y el rendimiento de tomate *Lycopersicon esculentum* L cv. Río Grande. Agrochimica 45: 189-198.

GARCÍA-HERNÁNDEZ, J. L.; TROYO-DIÉGUEZ, E.; JONES, H.; NOLASCO, H.; ORTEGA-RUBIO, A. 2000. Efectos de la aplicación de insecticidas organofosforados sobre el rendimiento (y sus parámetros) en ají (*Capsicum annuum* L. cv. Ancho San Luis). Phyton 67: 113-120.

GASPAR, T.; PENEL, C.; HAGAGE, D.; GREPPIN, H. 1991. Peroxidases in plant growth, differentiation and development processes, pp. 249-280. *In*: Biochemical, Molecular, and Physiological Aspects of Plan Peroxidases. Univeristy of Geneve, Geneve, Italy.

GLIESSMAN, S. R. 1998. Agroecology: Ecological Processes in Sustainable Agriculture. Sleeping Bear Press. Chelsea, MI, USA. Chelsea, MI, USA. 351 p.

LAGRIMINI, L. M.; GINGAS, V.; FINGER, F.; ROTHSTEIN, S.; LIU, T. T. Y. 1997. Characterizationof antisense transformed plant deficient in the tobacco anionic peroxidase. Plant Physiol. 97: 1187-1196.

LAKSHMI, N.; PRAKASH, N. S.; HARINI, I. 1988. Cytological effects of agricultural chemicals. I Effects of insecticides "Ekalux and metasixtos" on chili (*Capsicum annuum*). Cytología 53: 703-708.

LIN, C. C.; KAO, C. H. 1999. NaCl induced changes in ionically bound peroxidase activity in roots of rice seedlings. Plant Soil 216: 147-153.

LOBARZAWSKY, J. H.; GREPPIN, H.; PENEL, C.; GASPAR, T. 1991. Biochemical, Molecular, and Physiological Aspects of Plant Peroxidases. Univeristy of Geneve, Geneve, Italy. 207 p.

MANSOURI, I. E.; MERCADO, J. A.; SANTIAGO-DOMENECH, N.; PLIEGO-ALFARO, F.; ALPUESTA, V.; QUESADA, M. A. 1999. Biochemical and phenotypical characterization of transgenic tomato plants overexpresing a basic preoxidase. Physiol. Plant. 106: 355-362.

MAHESHWARI, D. K.; SINGH, S. P. 1989. Inhibitory Effects of Two Organocarbamates Nematicides on Growth and Yield of *Capsicum annuum*. Biochemical Physiology. Gustav Fisher. London. U.K. 184 p.

MEDINA, M. I.; QUESADA, M. A.; PLIEGO, F.; BOTELLA, M. A.; VALPUESTA, V. 1999. Expresión of the tomato preoxidase gene *TPX 1* in NaCl-adapted and unadapted suspensión cells. Plant Cell Rep. 18: 680-683.

MOHAN, R.; BAJAR, A. M.; KOLETTUKUDY, P. E. 1993. Introduction of a tomato anionic peroxidase gen (*tap* 1) by wounding in transgenic tobacco and cultivation of *tap 1*/GUS and *tap 2*/GUS chimeric gene fusions in transgenic tobacco by wounding and pathogen attack. Plant Mol. Biol. 21: 341-354.

MORENO, O. A.; VAZQUEZ-DUHALT, R.; NOLASCO, H. 1990. Extracellular accumulation of high specific-activity peroxidase by cell suspension cultures of cowpea. Plant Cell Rep. 9: 147-150.

OTTER, T.; POLLE, A. 1997. Characterization of acidic and basic apoplastic peroxidases from needles of Norway spruce (*Picea abies*, L., Karsten) with respect to lignifying substrates. Plant Cell Physiol. 38: 595-602.

PICANÇO, M.; LEITE, G. L.; GUEDES, R. N.; SILVA, E. A. 1998. Yield loss in trellised tomato affected by insecticidal sprays and plant spacing. Crop Prot. 17: 447-452.

REDDY, S. S.; RAO, G. M. 1981. Cytogenetic effects of Agricultural chemicals. I. Effects of insecticides "BHC and Nuvacron" on chromosomal mechanism in to yield and yield components in chili. Cytologia 46: 699-707.

RILEY, G. D. 1997. The Pepper Weevil and its Management. Texas Agricultural Extension Service, The Texas A&M University. College Station, TX, USA.

SITBON, F.; HENNION, S.; LITTLE, C. H.; SUNDBERG, B. 1999. Enhanced ethylene production and peroxidase activity in IAA-overproducing transgenic tobacco plants is associated with increased lignin content and altered lignin composition. Plant Sci. 141: 165-171.

STEVENS, H.; CALVAN, M.; LEE, K.; SIEGEL, B. 1978. Peroxidase activity as a screening parameter for salt stress in Brasica species. Photochemistry 17: 1521-1522.

STRAW, N. A.; FIELDING, N. J.; WATERS, A. 1996. Phytotoxicity of insecticides used to control aphids on Sitka spruce, *Picea sitchensis* (Bong.) Carr. Crop Prot. 15: 451-459.

WALLACE, G.; FRY, S. C. 1994. Phenolic components of the cell wall: Dynamic aspects. Int. Rev. Cytol. 151: 229-267.