In vitro Response of *Fusarium oxysporum* Isolates to Isothiocyanates Application

Respuesta *in vitro* de Aislados de *Fusarium oxysporum* a la Aplicacion de Isotiocianatos

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Resumen. Algunas cepas de *Fusarium oxysporum* son patogénicas en diferentes especies vegetales durante sus etapas pre y postcosecha y son responsables de serias pérdidas económicas. El manejo de este hongo es generalmente mediante la ayuda de químicos peligrosos al medio ambiente. Otros compuestos biodegradables como los isotiocianatos (ITCs) han demostrado su potencial nematicida, bactericida y fungicida. En esta investigación, el efecto fungitoxico *in vitro* de los ITCs de alilo, bencilo, fenilo, feniletilo y propilo a concentraciones de 0, 0.1, 0.3, 0.5, 1.0, 1.5, y 2.0 µl L^{-1} se evaluaron sobre varias cepas de *F. oxysporum* aisladas de cormos de gladiolo, semillas de jatropha, hojas de mango y frutos de papaya y coahuayote. El crecimiento micelial diario y la germinación conidial de estos cinco aislados se evaluaron en diferentes períodos de incubación. Para verificar la respuesta fungicida o fungistática de los ITCs, estos se resembraron únicamente en medio nutritivo. En general, los conidios de *F. oxysporum* fueron más sensibles que el micelio a los ITCs. Al final del período de incubación, hubo diferencias significativas ($P < 0.05$) en el crecimiento micelial de los hongos tratados con los ITC comparados con los no tratados. Los aislados de *F. oxysporum* más sensibles al ITC de bencilo fueron los que se obtuvieron de hojas de mango y frutos de coahuayote. Los aislados de mango también fueron sensibles a ITC de feniletilo. A concentraciones entre 1.0 a 2.0 µl L^{-1}, el ITC de alilo también suprimió el crecimiento de *F. oxysporum* aislados de frutos de papaya y coahuayote. Con excepción del ITC de fenilo, en el aislamiento del fruto de coahuayote, la germinación conidial de *F. oxysporum* no ocurrió bajo la influencia de los ITCs restantes al mismo tiempo que el tratamiento control alcanzó una germinación del 100%. En general, la concentración aplicada no influyó sobre el crecimiento de los hongos. Una vez que la fuente de los ITCs se quitó de las cajas Petri, el crecimiento y la germinación de los hongos se reinició. En conclusión, los ITCs probados fueron diferentes en su bioactividad mientras que el micelio y los conidios de los diferentes aislados de *F. oxysporum* variaron en susceptibilidad y tolerancia a estos compuestos.

Palabras clave adicionales: Carica mexicana, C. papaya, Gladiolus sp., Jatropha curcas, Mangifera indica.

Abstract. Some strains of *Fusarium oxysporum* are pathogenic to different plant species during their pre- and postharvest stages and are responsible for serious economic losses. Management of this fungus is usually with the aid of environmentally-harmful chemicals. However, other biodegradable compounds such as isothiocyanates (ITCs) have demonstrated their nematicidal, bactericidal and fungicidal potential. In this research, the *in vitro* fungitoxic effect of ITCs of allyl, benzyl, phenyl, phenylethyl and propyl at concentrations of 0, 0.1, 0.3, 0.5, 1.0, 1.5 and 2.0 µl L^{-1} was tested on various isolates of *F. oxysporum* obtained from gladiolus corms, jatropha seeds, mango leaves, papaya and coahuayote fruit. Daily mycelial growth and conidial germination of these five isolates was evaluated at different incubation period. To verify the fungistatic or fungicidal response, the ITCs were removed and the fungi were re-grown only in nutrient media. As average, conidia of *F. oxysporum* were more sensitive to the ITCs than mycelium. At the end of the incubation period, there were significant differences ($P < 0.05$) in mycelia growth of the ITC-treated fungi compared to the untreated ones. The *F. oxysporum* isolates that were the most sensitive to the benzyl

ITC were those obtained from mango leaves and coahuayote fruit. The mango isolate was also very sensitive to phenylethyl ITC. At concentrations ranging from 1.0 to 2.0 µl L^{-1}, the allyl ITC also suppressed the growth of *F. oxysporum* isolated from papaya and coahuayote fruit. Except for the phenyl ITC in the coahuayote fruit, conidial germination of *F. oxysporum* did not occur under the influence of the remaining ITCs, whilst in the control treatment germination reached 100%. As average, the concentration applied did not influence the growth of the fungi. Once the source of the ITCs was removed from the Petri plate re-growth and germination took place in all fungi. In conclusion, the ITCs tested differed in bioactivity and the mycelium and conidia of the different *F. oxysporum* isolates varied in their susceptibility and tolerance to these compounds.

Additional keywords: *Carica mexicana*, *C. papaya*, *Gladiolus* sp., *Jatropha curcas*, *Mangifera indica*.

Résumé. Certaines souches de *Fusarium oxysporum* sont pathogènes de différentes espèces végétales avant et après leur récolte, et sont responsables de graves pertes économiques. La gestion de ce champignon passe souvent par l'utilisation de produits chimiques dangereux pour l'environnement. Toutefois, d'autres composés biodégradables tels que les isothiocyanates (ITCs) ont montré leur potentiel nématicide, bactéricide et fongicide. Dans cette recherche, l'effet fongitoxique *in vitro* des ITCs allyle, benzyle, phényle, phényléthyle et propyle à des concentrations de 0, 0,1, 0,3, 0,5, 1,0, 1,5 et 2,0 ul L-1 a été évalué sur plusieurs isolats de *F . oxysporum* obtenus à partir de bulbes de glaïeuls, de graines de jatropha, de feuilles de manguier, de fruits de la papaye et de fruits du coahuayote. La croissance quotidienne du mycélium et la germination des conidies des cinq isolats ont été évalués à différentes périodes d'incubation. Pour vérifier la réponse fongicide ou fongistatique, le champignon a été repiqé dans un milieu nutritif exempt des ITCs. En moyenne, les conidies de *F. oxysporum* étaient plus sensibles que le mycélium à des ITCs. À la fin de la période d'incubation, il y avait des différences significatives ($P < 0,05$) entre la croissance du mycélium de champignons traités avec des ITCs par rapport aux non traités. Les isolats de *F. oxysporum* les plus sensibles au benzyle ITC étaient ceux obtenus à partir des feuilles de manguier et de fruits de coahuayote. Les isolats de manguier étaient également très sensibles au phényléthyle ITC. A des concentrations entre 1,0 et 2,0 ul L-1, l'allyle ITC a également supprimé la croissance de *F. oxysporum* isolé de la papaye et du coahuayote. A part pour l'ITC phényle dans le fruit de coahuayote, la germination des conidies de *F. oxysporum* n'a pas eu lieu sous l'influence d'ITC rémanent alors que le traitement contrôle atteint 100% de germination. En moyenne la concentration appliquée n'affecte pas la croissance des champignons. Une fois que la source d'ITCs a été retirée des boîtes de Pétri, la germination et croissance des champignons ont eu lieu. En conclusion, les ITCs examinés diffèrent dans la bioactivité, et le mycélium et les conidies de différents isolats de *F. oxysporum* varient dans leur sensibilité et tolérance à ces composants.

Mots-clés supplémentaires: *Carica mexicana*, *C. papaya*, *Gladiolus* sp., *Jatropha curcas*, *Mangifera indica.*

Fusarium oxysporum has been implicated in a number of serious diseases in horticultural produce worldwide. *Fusarium* or vascular wilt is the major disease caused by this phytopathogen in several different hosts. This fungus also causes root, stem and corm rots under field or greenhouse conditions and pink or yellow molds of fruits during postharvest storage (Beckman, 1987; Herrera and Ulloa, 1998). Overall, infected plants are usually stunted; their leaves turn pale green to golden yellow and later wilt, wither, die and drop off progressively whilst in fruit and vegetables, *F. oxysporum* is acquired during preharvest life and once ripening progresses this fungus causes internal and external extensive rotting (Leslie and Summerell, 2006; Snowdon, 1990). The use of synthetic fungicides has been the main source of control of *F. oxysporum*. Nevertheless, their intense application and frequently misuse have generated resistance in this fungus, becoming less effective. Due to the importance of this fungus, other methods for controlling are evaluated.

Isothiocyanates (ITCs) are phytochemicals sulphur-containing with the general formula R-N=C=S. Isothiocyanates occur naturally as glucosinolate conjugates in various vegetables (broccoli, cauliflower, turnips etc.) and other botanical families. They are responsible for the typical flavor of these vegetables. Pal Vig *et al.* (2009), reported a wide range of phytopathogenic fungi sensitive to different ITCs. Among them the literature reports that the allyl, benzyl, butenyl, propenyl, 2-phenylethyl, and methyl ITCs have fungitoxic properties. Similarly, for soil-borne fungi, the efficacy of pure isothiocyanates application has been extensively reported. Studies of the suppressing activity of various *Brassica* species on the fungus *Fusarium sambucinum* were strongly associated with ally ITC production of the macerated leaves (Mayton *et al*., 1996).

An array of soil microorganism, including five species of *Fusarium*, was tested against various types of ITCs, resulting in a marked inhibition by 50 and 90% in the presence of 2-phenylethyl ITC (Smith and Kikegaard, 2002). Congruent to these authors the sensitivity to this ITC varied according to genus, being the least tolerant in those belonging to *Sclerotinia* and *Gaeumannomyces*.

In others studies, a significant inhibition of mycelial growth was reported for *Rhizoctonia solani* when nutrient media was amended with allyl ITC at 50 µl L^{-1}(Dhingra *et al*., 2004). In postharvest studies, the ITCs have also proven to be effective in controlling a variety of fungi.

Conidial germination of *Botrytis cinerea*, *Monilinia laxa*, *Mucor piriformis, Penicillium expansum* and *Rhizopus stolonifer* was completely inhibited when treated with some ITCs (Mari *et al*., 1993; 2008). Mycelial growth of *Alternaria alternata* was completely inhibited when exposed to a mixture of various ITCs such as allyl, benzyl, phenyl and phenylethyl (Troncoso *et al*., 2005a). It has

also been reported that response to these natural substance differs according to isolate. In regard to *F. oxysporum*, little is known about their sensitivity to isothiocyanates. Smolinska *et al*. (2003), studied the *in vitro* response of four isolates of *F. oxysporum* which varied across isolate and the ITC tested.

Thus, this paper reports the results of the inhibitory effects of five different ITCs at different concentrations on the mycelial growth and conidial germination of *F. oxysporum* isolated from gladiolus corms, jatropha seeds, mango leaves, papaya and coahuayote fruit.

MATERIALS AND METHODS

Fungal cultures. *Fusarium oxysporum* isolates were obtained from gladiolus (*Gladiolus* spp.) corms (*F. oxysporum* f. sp. *gladioli*), jatropha seeds (*Jathropa curcas*), mango leaves (*Mangifera indica*), papaya (*Carica papaya*) and coahuayote (*Carica mexicana*) fruit showing symptoms provoked by this causal agent. Identification of *F. oxysporum* isolates was according to Nelson *et al*. (1983). The typical reproductive structures of *F. oxysporum* were observed such as micro and macro conidia (5-12 to 2.2-3.5 μL^{-1}), conidiophores and chlamydospores. Once monoconidial cultures were obtained, tests were carried out to verify the pathogenicity of each isolate. Fungal cultures were maintained in PDA for 10 days at 20°C.

Isothiocyanates assays. The pure ITCs tested in this study were those of allyl, benzyl, phenyl, phenylethyl and propyl (Aldrich Chemical Co., St. Louis and Alfa Aesar, Johnson Matthey Co.) at concentrations of 0.1, 0.3, 0.5, 1.0, 1.5 and 2.0 µl L^{-1}. The application of the ITCs was carried out following the methodology reported by Troncoso *et al*. (2005b). Plugs of 5 mm of diameter were cut from the five actively growing *F. oxysporum* isolates. They were placed in the center of 9 cm-diameter Petri plates containing PDA. The ITCs were pipetted according to the above concentrations into 3 cm-diameter sterile filter paper discs (Whatman # 1). The discs were placed in the cover of the inoculated Petri plates and after sealing them were placed upside down and incubated at 20°C. Control Petri plates consisted of growing each isolate only in PDA.

Variables evaluated. For mycelial development, daily radial measurements of growth were taken until the fungus reached the end of the plate which was eight days for coahuayote isolate, nine for jatropha, 12 days for gladiolus ones and 13 days for mango and papaya isolates. For germination testing, conidia were harvested by scraping off the agar of each of the five *F. oxysporum* 10 day-old cultures. The number of spores mL^{-1} of the filtrate was adjusted to 10^5. Aliquots of 50µl of the above spore suspension were placed onto 20-mm diameter PDA disks. Then, discs with each isothiocyanate were placed in the middle of the Petri plate. After the given 7 h incubation period, germination was stopped by adding lactophenol-safranin. Data were evaluated as percentage germination.

Evaluation of the fungicidal or fungistatic effect on treated mycelia and conidia. To evaluate a possible re-growth of *F. oxysporum* isolates after ITC-treatments, small portions of mycelia taken from the edge of each *F. oxysporum* isolate previously treated with the ITCs were placed again in the center of Petri plates of 5 cm of diameter containing only PDA. Treated spores and nonfixed ones with lactophenol- safranin were also placed upside down in Petri plates with PDA discs; both mycelia and spores were incubated at 25°C.

Statistical analysis. Treatments were arranged in a completely factorial design. For each isolate, five Petri plates and six PDA discs per treatment were considered as an experimental unit for mycelial growth and germination, respectively. Mean and standard deviations were calculated. Data of the final mycelial growth were subjected to ANOVA and means comparison by Tukey test at $P \leq 0.05$. Square root transformation was carried out to fulfil the ANOVA assumptions. Experiments were repeated twice and data were pooled since they were very similar.

RESULTS

Daily mycelial growth. Different responses of *F. oxysporum* were observed according to isolate, type of ITC and concentration. As seen in Figure 1, daily growth of *F. oxysporum* incubated in the presence of ally ITC varied according to the isolate tested. Except for the *F. oxysporum* obtained from gladiolus corms, the best concentrations to delay mycelial growth ranged from 0.1 to 2.0 µl L^{-1}. The most sensitive isolates were those from coahuayote and papaya fruit since no growth was observed at the highest concentration during the eight and 13 day of incubation periods. A delay in the onset of growth from four to six days was observed in the isolates obtained from coahuayote fruit and mango leaves. In regard to benzyl ITC (Figure 2), the most sensitive *F. oxysporum* isolates were those obtained from mango and coahuayote, since growth was completely suppressed during the entire incubation period at all concentrations. The least sensitive *F. oxysporum* isolate was that obtained from gladiolus corms. In Figure 3 it is observed that except for the gladiolus isolate, the application of phenyl ITC delayed the initial growth in the remaining *F. oxysporum* isolates tested. In comparison with the control, most isolates had lower mycelial growth at concentrations up to 0.1 µl L^{-1}. The application of phenylethyl ITC completely inhibited the growth of *F. oxysporum* from mango at all concentrations during the 13-day incubation period (Figure 4), followed by a lower inhibition in the papaya and coahuayote isolates held at concentration up to 0.1 µl L^{-1}. Gladiolus isolate did not respond to the application of this ITC. Figure 5 shows that development of *F. oxysporum* from gladiolus corms was delayed for up to four days and slowed down at the highest concentration of 2.0 µl L^{-1}. Except for the mango isolate, the growth of coahuayote, papaya and jatropha isolates was affected by propyl ITC but they were not completely inhibited.

Final mycelial growth. At the end of the incubation period, mycelial growth of *F. oxysporum* from the isolates tested was significantly different ($P \leq 0.05$) among the treatments compared with the control (Table 1). Overall, there was not a specific pattern associated with mycelial inhibition and ITC concentration. In some instances, such

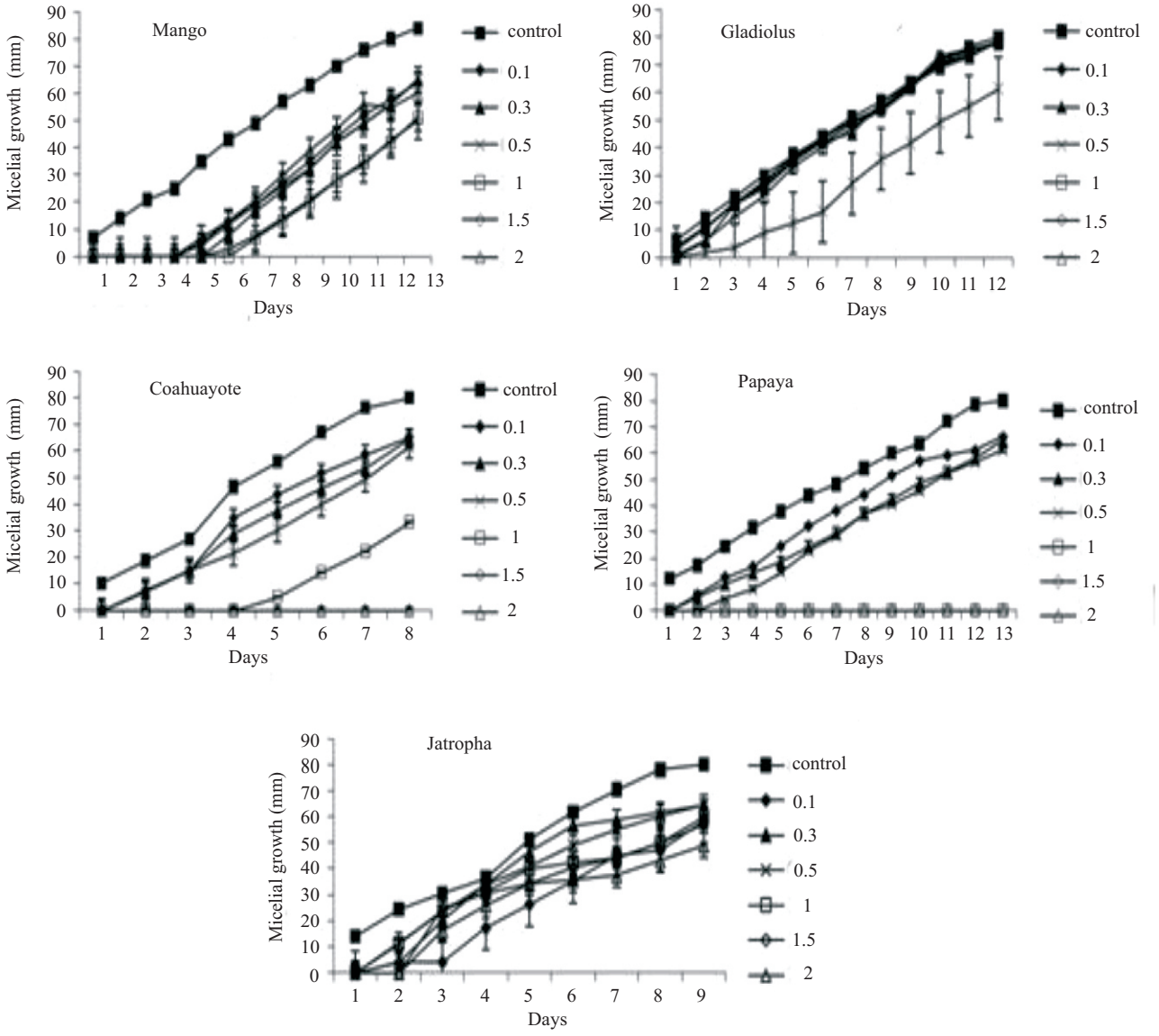

Figure 1. Daily mycelial growth of various *Fusarium oxysporum* isolates subjected to different concentrations of ally ITC for a given incubation time.

as that of the isolate obtained from coahuayote fruit, mycelial inhibition decreased as concentration increased in all ITCs tested. Except for the propyl ITC, this similar pattern was observed with *F. oxysporum* isolated from papaya fruit. Growth suppression up to 50% was rarely registered.

Conidial germination. With respect to the conidial germination of the five isolates tested, a complete inhibition was shown for most ITCs at all concentrations, whilst the untreated isolates had 100% germination (data not shown). Germination took place only in *F. oxysporum* isolated from coahuayote fruit when treated with phenyl ITC. In this treatment, germination decreased as ITC concentration increased. It ranged from 47 % (at 0.1 µl L^{-1} concentration) to 5.3 % (at 2.0 µl L^{-1} concentration).

Evaluation of the fungicidal or fungistatic effect. *F. oxysporum* mycelia and conidia of the five isolates previously subjected to the ITCs treatments followed normal growth and germination once they were placed in PDA Petri plates.

DISCUSSION

The present study showed that effectiveness of the ITCs varied across *F. oxysporum* isolates, fungi

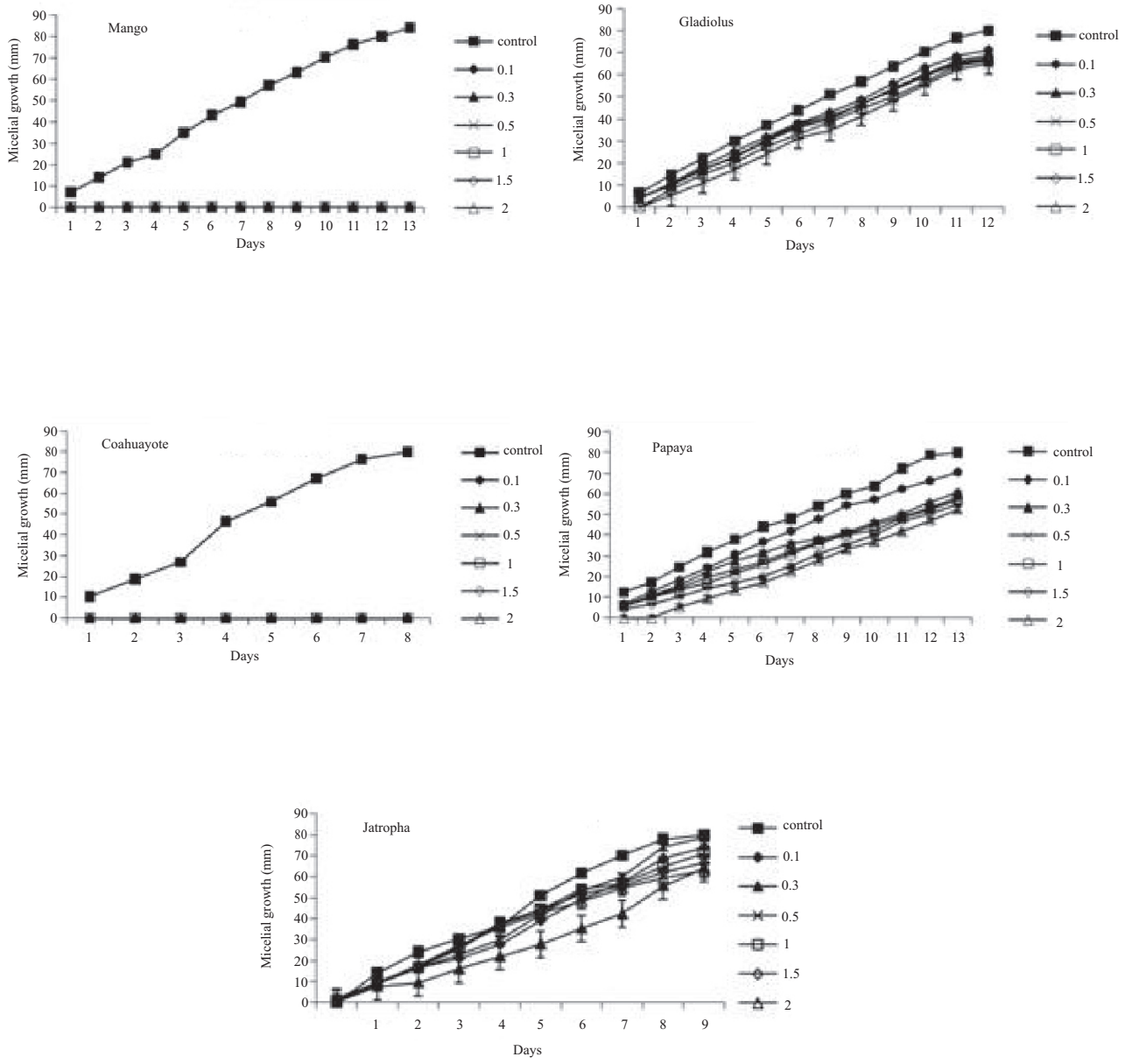

Figure 2: Daily mycelial growth of various *F. oxysporum* isolates subjected to different concentrations of benzyl ITC for a given incubation time.

development stage and ITC type tested. Overall, the most sensitive isolates to the ITCs were those obtained from mango and coahuayote and the highest toxicity was present in the benzyl and phenylethyl ITCs, followed by the allyl ITC on papaya and coahuayote isolates. We observed that while fungi were in contact with the benzyl and phenylethyl ITCs, there was a complete inhibition of growth. In the case of the allyl ITC, only the highest concentrations stopped growth. Coinciding with our results, Sarwar *et al*. (1998), reported that the aromatic benzyl and 2-phenylethyl ITCs suppressed growth by 50% on various fungi such as *Rhizoctonia*, *Fusarium*, *Bipolaris* and *Gaeumannomyces*. In other studies, higher inhibition of the germ tube elongation was recorded when two species of *Alternaria* were treated with benzyl compared to the inhibition reported with the allyl ITC (Sellam *et al*., 2007). The toxicity of these ITCs may be associated to their chemical structure. Previous studies revealed that aromatic forms are more biologically

Table 1. Summary of the mycelial growth of *Fusarium oxysporum* i solated from various hosts and treated with various ITCs.

Isothiocyanate and concentration ($\mu 1L^{-1}$)	Host				
	Gladiolus corms	Jatropha seeds	Mango leaves	Papaya fruit	Coahuayote fruit
Control	80[a]	80[a]	80[a]	80[a]	80[a]
Allyl					
0.1	78.6[b]	58.1[c]	64.3[bc]	66.3[b]	65.1[b]
0.3	78.3[b]	64.5[b]	65.0[b]	64.9[bc]	64.4[b]
0.5	71.3[c]	64.4[b]	65.0[b]	61.1[c]	61.6[bc]
1.0	78.5[b]	59.6[bc]	51.1[e]	0.0[d]	33.0[d]
1.5	77.7[bc]	56.9[d]	50.0[e]	0.0[d]	0.0[e]
2.0	78.0[b]	48.9[c]	59.5[d]	0.0[d]	0.0[e]
Bencyl					
0.1	71.2[b]	74.8[c]	0.0[b]	70.4[b]	0.0[b]
0.3	67.7[c]	64.2[f]	0.0[b]	60.8[c]	0.0[b]
0.5	66.9[cd]	78.8[b]	0.0[b]	58.4[cd]	0.0[b]
1.0	66.3[cd]	71.2[d]	0.0[b]	57.2[d]	0.0[b]
1.5	67.3[cd]	66.5[e]	0.0[b]	54.7[e]	0.0[b]
2.0	68.9[c]	63.1[fg]	0.0[b]	52.5[ef]	0.0[b]
Phenyl					
0.1	53.0[c]	58.4[bc]	34.0[b]	69.5[b]	60.2[b]
0.3	51.3[c]	53.4[d]	30.0[bc]	68.2[b]	59.9[b]
0.5	39.3[d]	58.6[bc]	26.0[d]	67.3[c]	56.6[bc]
1.0	58.9[b]	60.3[b]	19.0[e]	65.4[d]	55.5[cd]
1.5	42.8[d]	56.6[ef]	18.0[e]	50.8[e]	55.5[cd]
2.0	36.6[de]	53.0[de]	14.0[f]	45.3[f]	36.6[e]
Phenylethyl					
0.1	76.1[b]	64.6[f]	0.0[b]	50.3[b]	66.9[b]
0.3	76.7[b]	70.0[d]	0.0[b]	49.3[bc]	63.8[bc]
0.5	76.7[b]	80.0[b]	0.0[b]	48.6[c]	62.8[bc]
1.0	75.7[bc]	80.0[b]	0.0[b]	47.4[cd]	56.9[d]
1.5	75.2[bc]	69.2[e]	0.0[b]	45.4[e]	56.6[d]
2.0	75.2[bc]	76.2[c]	0.0[b]	44.4[e]	55.9[d]
Propyl					
0.1	78.3[bc]	77.1[b]	78.0[c]	57.6[g]	79.5[b]
0.3	77.1[d]	77.3[b]	78.0[c]	60.4[f]	78.9[b]
0.5	79.3[b]	77.3[b]	81.0[b]	62.1[e]	45.2[bc]
1.0	78.0[bc]	67.1[c]	81.0[b]	64.1[d]	70.9[d]
1.5	79.1[b]	63.8[d]	79.0[bc]	66.2[bc]	68.2[de]
2.0	41.6[c]	62.5[de]	89.0[b]	67.6[b]	66.0[f]

*Average of five replicates; means separation within columns by Tukey's multiple test ($P<0.05$) after square root transformation.

active than aliphatic forms due to their higher penetrability, and increasing volatility (Pal Vig *et al*., 2009). Contrary to other studies (Smolinska and Horbowicz, 1999), the inhibitory effect of the ITCs on the *F. oxysporum* isolates tested in our investigation was not usually depenant on the concentrarion applied. Moreover, despite the promising inhibition of the ITCs on mycelia and conidia found in this research, we observed that the fungitoxic activity of the ITCs tested was transitory since re-growth of all *F. oxysporum* isolates was observed once the fungi were removed from the source of inhibition. Similar results were reported by Smolinska *et al*. (2003), in which the toxic effects of various ITCs were also only for a limited time over various strains of *F. oxysporum*. However, in further

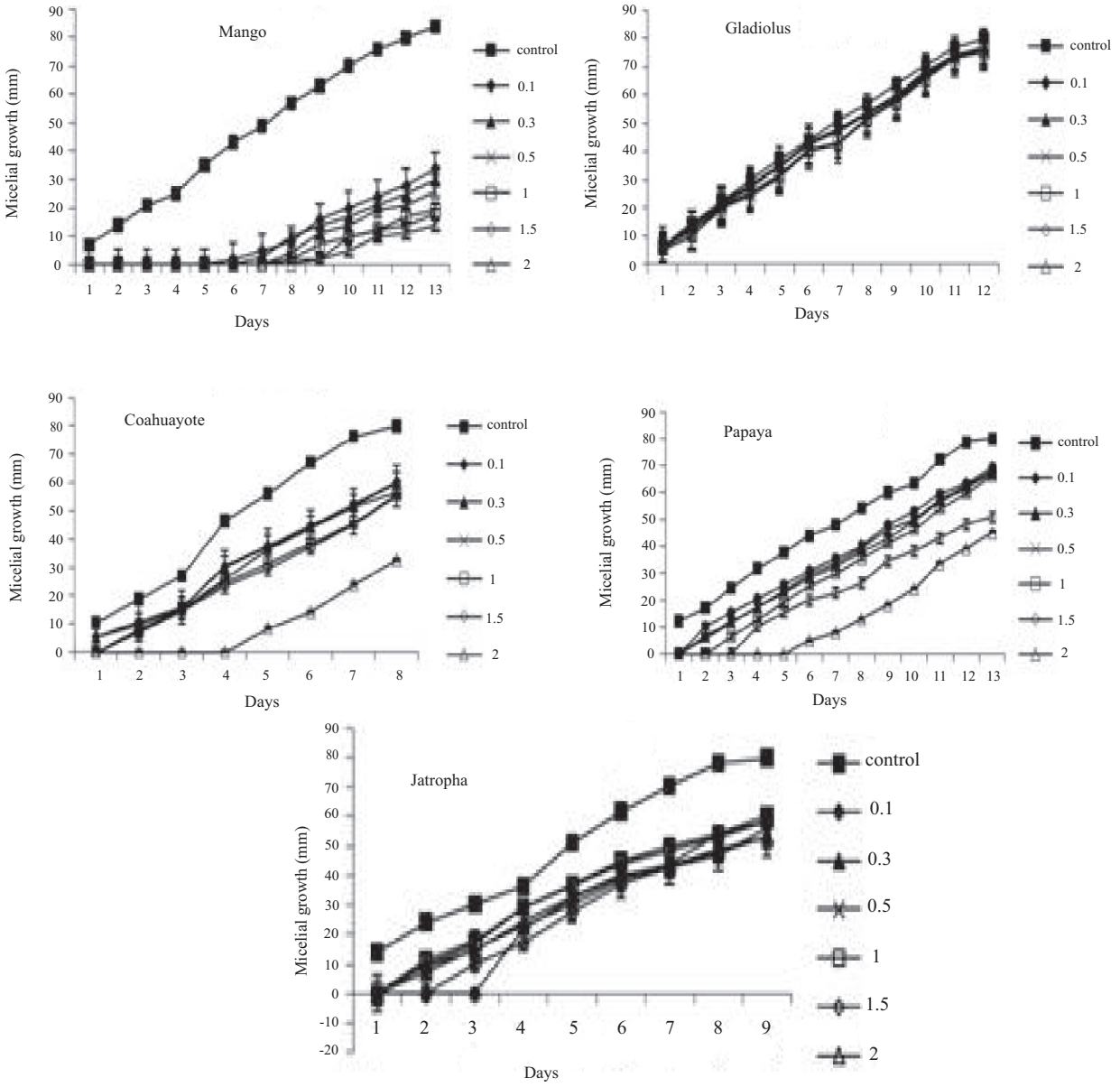

Figure 3. Daily mycelial growth of various *Fusarium oxysporum* isolates subjected to different concentrations of phenyl ITC for a given incubation time.

studies carried out in our laboratory we were able to obtain a permanent toxic effect in higher concentrations or in combination with chitosan (data not shown).

The different reaction of *F. oxysporum* mycelia and conidia to ITC exposure are, to some extent, probably due to the morphology involved in mycelia and conidia. The hyphae wall is perhaps thicker than the conidia wall; hence, the volatile penetration in these structures was easier. Other mechanisms involved maybe associated with the sensibility of conidia and hyphae to exogenous materials. Similar results were reported with conidia of the fungus *Metarhizium anisopliae* (Inyang *et al.,* 1999). In that study, the exposure of 1 µl of 100% v/v of the ITCs of phenylethyl, 2-chlorophenyl and phenyl consistently inhibited conidia germination during the incubation period of this fungus while mycelium growth always took place. In other studies, Mari *et al*. (2008), reported that the effect of various ITCs tested was evident in a shorter period on conidial than mycelial development in *Monilinia laxa* causal agent of brown rot disease in stonefruit. In our observations, we also

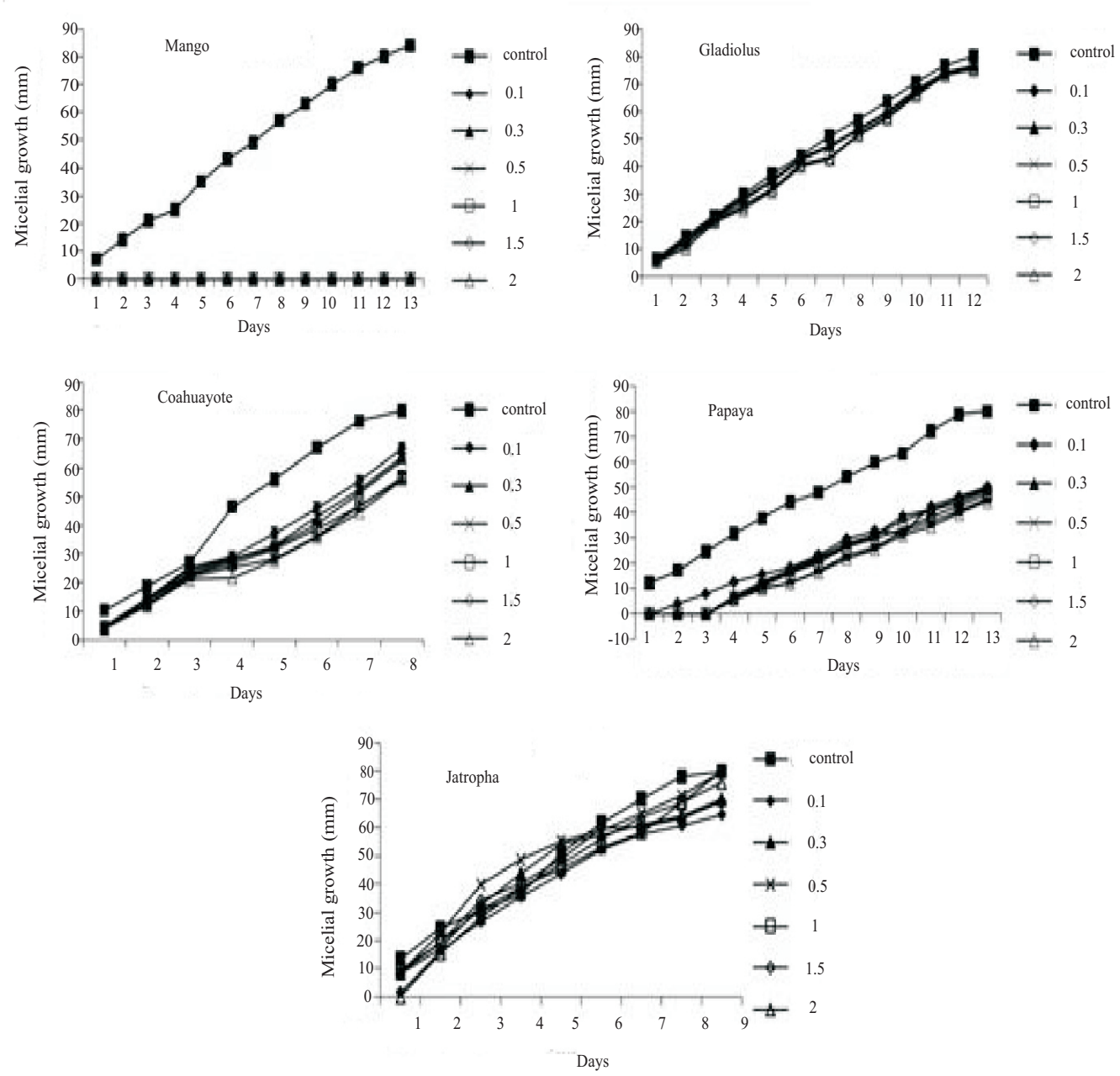

Figure 4. Daily mycelial growth of various *Fusarium oxysporum* isolates subjected to different concentrations of phenylethyl ITC for a given incubation time.

confirm that the ITCs differ in bioactivity, and that the mycelium and conidia of the different *F. oxysporum* isolates also varied in their susceptibility and tolerance to these compounds.

LITERATURA CITADA

Beckman CH. 1987. The nature of wilt diseases of plants. St. Paul MN. American Phytopathology Society.

Dhingra OD, Costa MLN and Silva GJ Jr. 2004. Potential of allyl isothiocyanate to control *Rhizoctonia solani* seedling damping off and seedling blight in transplant protection. Journal Phytopathology 152:352-357.

Herrera T and Ulloa M. 1998. El Reino de los Hongos. Micología Básica y Aplicada. Fondo de Cultura Económica-UNAM. México, D.F. 552p.

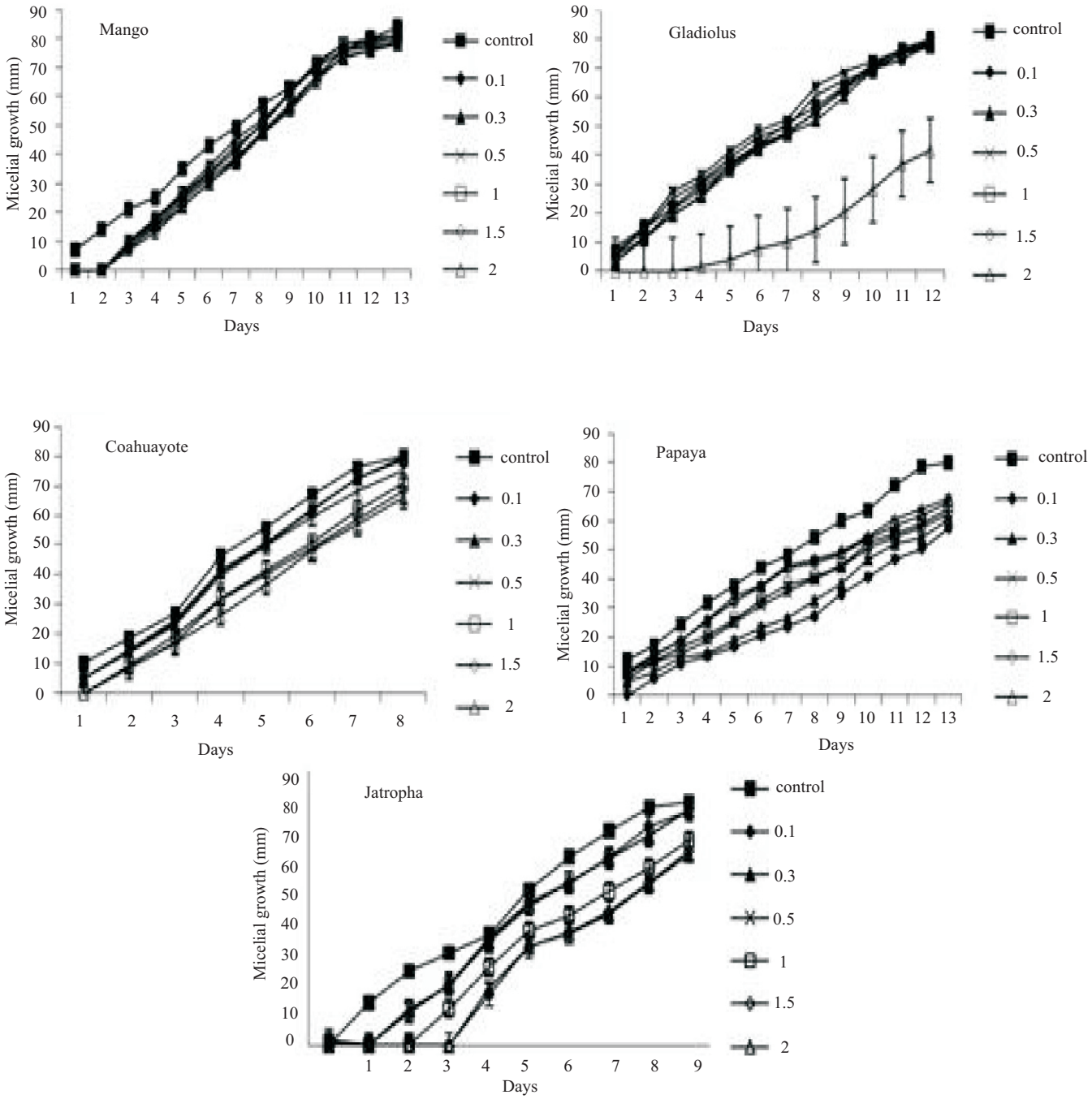

Figure 5. Daily mycelial growth of various *Fusarium oxysporum* isolates subjected to different concentrations of propyl ITC for a given incubation time.

Inyang EN, Butt TM, Doughty KJ, Todd AD and Archer S. 1999. The effects of isothiocyanates on the growth of the entomopathogenic fungus *Metarhizium anisopliae* and its infection of the mustard beetle. Mycology Research 103:974-980.

Leslie JF and Summerell BA. 2006. The Fusarium Laboratory Manual. Blackwell Publishing IOWA USA. 388p.

Mari M, Iori R, Leoni O and Marchi A. 1993. *In vitro* activity of glucosinolate-derived isothiocyanates against postharvest fruit pathogens. Annal Applied Biology 123:155-164.

Mari M, Leoni O, Bernardi R, Neri F and Palmieri S. 2008. Control of brown rot stonefruit by synthetic and

glucosinolate derived isothiocyanates. Postharver Biology and Technology 47:61-67.

Mayton HS, Olivier C, Vaughn SF and Loria R. 1996. Correlation of fungicidal activity of Brassica species with allyl isothiocyanate production in macerated leaf tissue. Phytopathology 86:267-271.

Nelson PE, Toussoun TA and Marasas WFO. 1983. *Fusarium* species: An illustrated manual for identification. The Pennsylvania State University Press, University Park. Pennsylvania, USA. 193p.

Pal Vig A, Rampal G, Singh T and Arora F. 2009. Bio protective effects of glucosinolates – A review. LWT-Food Science Technology 42:1561-1572.

Sarwar M, Kikegaard JA, Wong PTW and Desmarchelier JM. 1998. Biofumigation potential of brassicas III. *In vitro* toxicity of isothyocianate to soil-borne fungal pathogens. Plant and Soil 201:103-112.

Sellam A, Iacomi Vasilescu B, Hudhomme P and Simoneau P. 2007. *In vitro* antifungal activity of brassinin, camalexin, and two isothyocianates against the crucifer pathogens *Alternaria brassicola* and *Alternaria brassicaceae*. Plant Pathology 56:296-301.

Smith B and Kikegaard JA. 2002. *In vitro* inhibition of soil microorganisms by 2-phenyethyl isothiocyanate. Plant Pathology 51:585-593.

Smolinska U and Horbowicz M. 1999. Fungicidal activity of volatiles from selected cruciferous plants against resting propagules of soil-borne fungal pathogens. Journal of Phytopathology 147:119-124.

Smolinska U, Morra MJ, Knudsen GR and James RL. 2003. Isothiocyantes produced by Brassicaceae species asinhibitors of *Fusarium oxisporum*. Plant Disease 87:407-412.

Snowdon AL. 1990. A Colour Atlas of Post-Harvest Diseases and Disorders of Fruits and Vegetables. Vol. 1 General Introduction and Fruits. CRC Press London UK. 302p

Troncoso R, Sánchez Estrada A, Ruelas C, García HS and Tiznado Hernández ME. 2005a. Effect of benzyl isothiocyanate on tomato fruit infection development by *Alternaria alternata.* Journal Sciense Food Agricultural 85:1427-1434.

Troncoso R, Espinoza C, Sánchez Estrada A, Tiznado ME and García HS. 2005b. Analysis of the isothiocyanates present in cabbage leaves extract and their potential application to control *Alternaria* rot in bell peppers. Food Research Technology 38:701-708