



In vitro Investigation on Biocontrol of *Xanthomonas axonopodis* pv. *citri* Cause of Citrus Bacterial Canker by Citrus Antagonistic Bacteria

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Abstract

Citrus Bacterial Canker disease caused by *Xanthomonas axonopodis* pv. *citri* is one of the most important diseases of citrus in south of Iran. In this research, the samples of bacterial flora of citrus leaves were collected from various citrus growing areas of Hormozgan, Jiroft and Mazandaran. The epiphytic bacteria were isolated from those leaves during 2000 and 2001 years. From 453 isolates, 26 strains inhibited growth of *Xanthomonas axonopodis* pv. *citri* under in vitro conditions. The bacterial strains were analyzed based on physiological and biochemical characteristics such as fluorescent pigment production on king B media, oxidase and catalase reaction, gelatin, casein and tween 80 hydrolysis, levan production and utilization of hydrocarbon sources. Based on these characteristics they were grouped on five classes including *Pseudomonas fluorescens* I, *P. fluorescens* V, *P. viridiflava* and *P. syringae*. The last group was aerobic and gram positive bacteria that produced spore and identified as *Bacillus* spp.

Keyword: Citrus bacterial canker, *Xanthomonas axonopodis* pv. *citri*, Biological control, Epiphytic bacteria, Antagonist

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Identification 4 Belonolimids Nematodes from Potato Fields of Khorasan Razavi Province

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Abstract

In order to identify plant parasitic nematodes of potato fields, 55 soil samples and a few tuber samples were collected from Khorasan Razavi province during 2005-2006. Soil samples were washed and nematodes were extracted by centrifugal flotation technique. The extracted nematodes were fixed and transferred to glycerin. Baermann funnel method was used for extracting nematodes from tuber samples. In this survey, 16 species belonging to 12 genera were identified based on morphological and morphometrical characters. Four species were belonged the Belonolimidae such as *Amplimerlinius sikkimensis*, *Tylenchorhynchus shivanandi*, *Geocenamus tartuensis*, *Geocenamus rugosus*. The first three species are new records for nematodes fauna of Iran.

Keywords: Belonolaimid, Potato, Khorasan Razavi

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Detection of (PVY^{NTN}) Strain in Potato Fields of Khorasan Razavi Province

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Abstract

The PVY^{NTN} strain induces potato tuber necrotic ringspot disease PTNRD . PVY^{NTN} strain is sub group of tobacco necrosis veinal PVY^N strain . PVY^{NTN} strain is a recombinant between PVY^N and PVY^O strains. symptoms are often totally symptomless or undetectable by visual inspections at harvest , usually are apparent during post harvest in storage conditions and induce to decrease quality and marketability of potato tubers. Reverse transcription polymerase chain reaction (RT-PCR) is an accurate and reliable tool for differantion and detection of PVY^{NTN} strain and prove to be an important tool in confirmation of symptoms and for The detection of PVY^{NTN} in symptomless tissues .In our survey , during the summer of 2007, 435 tubers from 32 potato fields in Khorasan Razavi province were collected randomly. Tubers passed dormancy period at 4°C and transfered to the greenhouse at 25°C for germination. Sprouts emerging from the tubers were tested by DAS-ELISA with polyclonal antibody. 53 positive samples in DAS-ELISA which had high absorbance values at 405 nm were selected . Total RNA was extracted from 40 infected plants tissue using RNX plus-TM solution. Then two specific primers pairs designed within the coat protein (CP) region, used for cDNA synthesis in Reverse transcription polymerase chain reaction (RT-PCR). Samples infected with PVY produce a band of 569 bp by MOR2 & MOR3 primers. While isolates belonging to PVY^{NTN} strain gave a band of 334 bp by MOR1 & MOR2 primers.

Keywords: PVY^{NTN} , ELISA , RT-PCR

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Study of Different Techniques for Breaking Dormancy and Optimum Temperature for Germination of Russian Knapweed (*Acroptilon repens*)

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Abstract

Increased knowledge of Russian knapweed biology would facilitate development of an optimum control program. To examine germination characteristic of this weed, several experiments were conducted in Weed Research Department, Plant Pest and Diseases Research Institute of Tehran during 2003. First experiment was a factorial with 3 replications to break seed dormancy of this weed. First factor was water treatments with 2 levels (distilled water and 3% KNO₃). Second factor was duration of storage in concentrated sulfuric acid with 6 periods of scarification (5, 10, 15, 20, 25 and 30 minutes). Second experiment was conducted as a completely randomized design with 26 treatments (constant temperature including 5, 10, 15, 20, 25, 30 and 35°C in both light or darkness condition and fluctuation temperatures including 2/10, 7/15, 12/20, 17/25, 22/30, 27/35 and fluctuation temperatures including 0/10, 5/15, 10/20, 15/25, 20/30 and 25/35. to determine optimum temperature of germination. In order to break of seed dormancy, 20 minutes treatment with concentrated sulfuric acid was required and two water treatments did not have significant effect on seed germination. The Optimum temperature for germination was 30/20°C (16 hours light/8 hours dark) with 42% germination. Seed germination was strongly influenced by temperature. Light did not play a crucial role on seed germination of this weed. Therefore Russian knapweed seeds were not photoblastic and temperature fluctuations increased seed germination. The above characteristics are very important in making Russian knapweed an invasive weed. Having precise information of this traits enables us to a better management and control of this troublesome weed.

Keywords: Russian knapweed (*Acroptilon repens* L.), Seed germination, Seed dormancy, Constant temperature, Fluctuating temperature, Light

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Studying the Effect of Drought and Freezing on Tuberos Root Germination of Lesser Cleandine (*Ranunculus ficaria*)

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Abstract

R. ficaria is an invasive perennial and tuberous roots weed. The most important means of its reproduction and dispersion are tubers. In order to study the effect of droughty and freezing on germination of lesser celandine tubers, two experiments were conducted at weed science laboratory of Faculty of Agriculture Ferdowsi University of Mashhad in 2008. The experiment was a completely randomized design with factorial arrangement of treatments and four replications. In freezing experiment, factors included of time of exposure to freezing (12, 24, 48 and 96 hours) and freezing temperature (0, -5, -10 and -15 °C). To examine the effect of desiccation on ficaria tubers, factors included of , five levels of temperature (5, 10, 15, 20, and 25 °C), four levels of osmotic potential (0, -5, -10 and -15 bar) and two tuber sizes [big (>0.2g) and small (<0.05g)]. In freezing experiment factors and their interactions had significant effect on germination of tubers. Decreasing the temperatures and increasing the storage time in freezing experiment, decreased the germination of tubers. Germination not occurred in tubers which had been stored at -10 °C and -15 °C for beyond 48 hours. Results showed that drought stress had significant effect on germination of tubers. Increasing the osmotic potential and temperature decreased the germination of ficaria tubers. in osmotic potential -15 bar, Germination was zero in all of temperatures. No significant difference was observed among big and small tubers. In all temperature, osmotic potential and tubers size decreasing the tuber weight were significant.

Keyword: *Ranunculus ficaria*, Germination, Tuberos root, Freezing, Drought

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Investigation of the Reaction of Olive Cultivars to Defoliate and Non-Defoliate Isolates of *Verticillium dahliae* Kleb. in Golestan Province

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Abstract

Verticillium wilt of olive caused by *Verticillium dahliae* is one of the main constraints to olive production in Golestan province. In this research, two defoliating and two non-defoliating isolates of the fungus were inoculated in 16-18 months-old olive seedlings. The seedlings consisted of Concervolia, Cronaiki, Kalamon, Zard-roghani, Bladi, Mission, Manzanilla and Sevillana. The seedlings were inoculated using root dip assay. The seedling's root tips were submerged in (4×10^6 spore/ml) spore suspension. The experimental design was conducted as factorial in complete randomized design, with three replicates. All inoculated seedlings were incubated under greenhouse conditions at 25-35°C for 11 weeks. Disease severity was evaluated using 0-5 scale. Mean of disease severity was significantly different ($P \geq 1\%$) in different cultivars by LSD test. Cronaiki and Calamon were less sensitive and the mean disease severities were 1.71 & 1.85, respectively. While, Mission, Conservolia, Zard-roghani and Bladi were severely sensitive and the mean of their disease severities were 3.85, 3.8, 3.75 and 3.33, respectively. Mozanilla and Sevillana were partially sensitive, and the mean of their disease severities were 3 and 2.61, respectively.

Keywords: *Verticillium dahliae*, Olive, Cultivars, Resistance, Defoliate, Non-Defoliate

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Investigation of Imidacloprid Residue in 21 Days latent Period and its Decrease with Different Treatments in Greenhouse Cucumber

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Abstract

According to the high application of imidacloprid insecticide in greenhouse cucumber cultivate, this study researched about daily change of imidacloprid residues in 21 days latent period. The samples were collected from a commercial greenhouse at Mahmud Abad (Mazandaran province, Iran) after treatment in 21 days. Also, the effects of different removing treatments such as washing, peeling and refrigeration storage at 4 °C for 48h were investigated in this research. Imidacloprid residue concentrations determined by high performance liquid chromatography (HPLC) with obtained recoveries 104% and limit of detection 0.001 mg l⁻¹. The results showed that maximum concentration of imidacloprid residue detected as in the firstly days after poisoning and this trend decreased till the end of deficiency period. The half-life of imidacloprid that applied on cucumbers was 2.8 days. After 7 days of imidacloprid application, imidacloprid residue concentration was higher than Codex MRL. Additionally, obtained results showed that washing was the most effective way to reduce the imidacloprid residues of the cucumber samples. According to the results of this research, it is recommended that a 7 days waiting period after imidacloprid poisoning and household processing usage are required to lower down the residue levels than Codex acceptable limit to safety consumption of cucumber.

Keywords: Imidacloprid residue, MRL, Greenhouse cucumber, Food safety

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Identification and Sensitivity of *Pythium ultimum* Isolated From Razavi and North Khorasan Provinces to Metalaxyl

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Abstract

Some agricultural fields of Razevi and North khorasan provinces were sampled for identification and resistance to metalaxyl of *Pythium ultimum* species isolates. Isolation was done between years of 2007 to 2009. Van der plaats- niterink key to species of Pythium and specific ITS region of rDNA were used for morphological and molecular identification respectively. Micrometric characteristics of isolates sexual and asexual organs corresponded to van der Plaats-niterink criterion. Fourteen isolates were randomly selected among fourthy identified isolates of *Pythium ultimum* for determination of sensitivity to metalaxyl. Concentration of metalaxyl which causes fifty percents growth inhibitions (EC50) obtained using logistic function; $I=KC (e^{rC})$. That varied between 0.0228-0.044 $\mu\text{g ml}^{-1}$ i.e. the results indicate that all isolates were sensitive to metalaxyl, but there was significantly differences among isolates in response to metalaxyl.

Keywords: *Pythium ultimum*, Metalaxyl sensitivity, Cucumber, Khorasan

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Identification of Plant Parasitic Nematodes of *Rosmarinus officinalis* in Campus Ferdowsi University of Mashhad

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Abstract

In order to identification of plant parasitic nematodes of *Rosmarinus officinalis* rhizospher during 2008-2009, fifty soil and root samples were collected from campus of Ferdowsi University. Soil and root samples were washed and nematodes were extracted by centrifugal flotation technique. According to De Grisse, 1969 the extracted nematodes were fixed and transferred to glycerin. In this survey, nine species belonging to four genera of sub order Tylenchina were identified as follows: *Helicotylenchus pseudorobustus*, *H. indicus*, *H. californicus*, *H. nigeriensis*, *Merlinius microdorus*, *M. indicus*, *Boleodorus thylactus*, *Psilenchus minor* and *P. hilarulus*. Three species *H. indicus*, *H. nigeriensis* and *M. indicus* were reported for the first time from Iran.

Keywords: Plant parasitic nematodes, *Rosmarinus officinalis*, Tylenchina, *Helicotylenchus*, *Merlinius*, Mashhad

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Investigation Biological Control two Strains of Antifungal Actinomycetes Against *Phytophthora parasitica* and *P. citrophthora* in vitro and in vivo Conditions

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Abstract

This research was investigated activity of antagonistic activity of 200 soil Actinomycete isolates assayed against *Phytophthora parasitica* and *P. citrophthora* in Kerman Province. Among all Actinomycetes, strains of 19 and 29 showed high level of activity in Agar disk and Well-diffusion methods. In both isolates, the active substance was water and metanol soluble but insoluble in chloroform. The investigation Greenhouse showed effects of four treatments pathogen, pathogen by antagonist ,antagonist and control on high and dry wet root seedlings and length , wide leaves were significantly different at level 0/05% in completely randomized experimental design. Strin 19 on high and dry wet root seedlings and lenth leaves isolate and Strin 29 on wide leaves were most effective.From the results of our studies it is clear that usage of strain 19 and 29 as a biofungicidal natural product for application as an amendment in greenhouse soil mix inhibits or reduces the pathogen adverse effects. Antagonistic activity of two isolates in vivo confirm activity of biocontrol them in Greenhouse experiments.

Keywords: Citrus gumosis, *Phytophthora parasitica*, *P. citrophthora* Actinomycetes, Biological control

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Study of Antagonistic Mechanisms of *Bacillus* spp. in Biocontrol of Cucumber Root and Foot rot Caused by *Pythium ultimum* and *P. aphanidermatum*

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Abstract

A total of 270 *Bacillus* strains were isolated from bulk soil and rhizospheric of cucumber in the Tonekabon fields (Mazandaran province). Antifungal activity of *Bacillus* strains was evaluated against *Pythium aphanidermatum* and *P. ultimum* using dual culture method. 13 strains with more than 10 mm inhibition zone were selected for further study. Morphological, physiological, biochemical and protein pattern study of the strains showed that they are belong to *Bacillus subtilis* (B2, B3, B4, B5, B6, B7), *B. marinus* (B1, B13) *B. licheniformis* (B9, B8), *B. circulans* (B10, B12), and *Bacillus* sp.(B11). Antifungal activity of the liquid and volatile metabolites of the strains of *Bacillus* spp. on *Pythium* spp. as well as, their potential to produce antibiotic surfactin and enzymes protease and cellulose were studied as antagonistic mechanisms of the bacterial strains. Results indicated very different comporment of strains against two *Pythium* species. More important variation was noted for liquid and volatile metabolites of strains on the mycelial growth of *Pythium* strains B12, B11, B7, B3 and B9, B8, B6, B3 inhibited 100% mycellial growth of *P. ultimum* respectively. While B14 (liquid metabolites) and B11 (volatile metabolites). Showed very weak inhibitory effect (13.7, 4.44. %). In greenhouse experiment all strains were able to control of the diseases intensity of seedlings in different rates. Strains B7, B8, B13, and B14 were more efficient, they reduced the rate of rotted roots caused by both *Pythium* species between 75 to 100%. Strains B7 and B13 demonstrated also important growth promoting effect on the cucumber seedlings. They increased the seedling wet weight of the varieties superdominos and French Net- wet at the rate of 85.6 and 44.4% related to control respectively. The correlation between antagonistic mechanism index, evaluated in vitro, and biocontrol potential of strains in greenhouse indicated, that there is a good correlation between inhibitory effect of liquid metabolite on mycelia growth of *Pythium* species and their biocontrol potential index. The correlation index were -0.67 and -0.45 for *P. aphanidermatum* and *P. ultimum* respectively. There was a high and significant correlation between the effect of strains on seedling growth and the root rot index in greenhouse. The correlation index was -0.93 and -0.79 for *P. aphanidermatum* and *P. ultimum* respectively.

Keywords: Root and foot rot of cucumber, *Pythium aphanidermatum*, *P. ultimum*. *Bacillus*., Antagonists

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Critical Period of Weed Control in Corn (*Zea mays* L.)

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Abstract

The critical period of weed control (CPWC) is a period in the crop growth cycle during which weeds must be controlled to prevent yield losses. In order to determine the critical period of weed control (CPWC) of corn in Hamedan, an experiment was conducted in 2007 at Agricultural Research Station of Bu-Ali Sina University of Hamedan. The experiment was carried out as a randomized complete block design with three replications. The trial included twelve treatments consisted of six initial weed-free periods in which plots were kept free of weeds for 0, 15, 25, 35, 45 and 55 days after crop emergence (DAE), and then weeds were allowed to grow until harvest and six initial weed-infested periods in which, weeds were allowed to grow for 0, 15, 25, 35, 45 and 55 DAE, after which the plots were kept free of weeds until harvest. Each plot consisted of four rows (six meter per row) with a row spacing of 75 cm. Weed sampling carried out in first series of treatments at the end of growth season and in second series of treatments at the end of infested periods. The results showed that beginning of CPWC ranged from 124 to 204 GDD, at 5% and 10% AYL, which equates to 8 and 16 days after crop emergence (3 and 5 leaf), respectively. The end of the CPWC varied from 395 to 289 GDD, at 5% and 10% AYL, which equates to 31 and 24 days after crop emergence (10 and 7 leaf), respectively. Total dry weight of weeds increased as the duration of weed infested period increased and it was decreased with increasing duration of the weed-free period. Grain yield of corn increased with increasing duration of the weed-free period and decreased as the duration of weed infested period increased.

Keywords: Weed dry mater, Weed infestation, Yield, Yield components

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Hyphomycetous Fungi Isolated from Insects and Their Pathogenic Effect on Colorado Beetle in Hamedan Province

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Abstract

Insect biocontrol by entomopathogenic fungi can be a suitable substituting method for chemical insecticide or at least can be used as complementary the chemical insecticides. In this study 42 fungal isolates belonging to 12 species were isolated and identified from different insects. Among obtained fungal isolates, seven isolates were used in pathogenicity trials on Colorado beetle larva in Hamedan province. The study was achieved under factorial test and completely randomly design. Results showed that all fungal isolates were pathogenic on Colorado beetle larva and mortality percent of larva were increased with increasing concentration of fungal spore suspension. Application of spore suspension in concentration of 10^7 spore per milliliter of fungal isolates comprising *Beauveria bassiana* (two isolates), *Paecilomyces* sp. (two isolates), *Isaria farinosa*, *Trichoderma koningiopsis* and *Fusarium verticillioides* caused 100, 100, 42.2, 42.2, 37.5, 28.37 and 17.7 percent larva mortality respectively. It was conducted that *B. bassiana* isolates were the most pathogenic agent on Colorado beetle larva. In addition effect of insecticides Danito and Desis on spore germination and mycelia growth of *B. bassiana* was investigated. Results showed that these insecticides have a little effect s on *B. bassiana*. Therefore integrated control of Clorado beetle can be achieved by application of *B. bassiana* and these insecticides in concentration of under lethal dose.

Keywords: Biological control, Potato, *Beauveria bassiana*, *Leptinotarsa decemlineata*

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Prediction of Spatial Distribution Pattern of *Hordeum glaucum* steud. Population Using Neural Network Model

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Abstract

Recent interest in describing the spatial distribution patterns of weeds through using interpolation methods has increased to estimate weed seedling density from spatially referenced data and evaluation of applicable to site-specific weed management. In this research, a multi layer perceptron neural network (MLPNN) model was developed to predict the spatial distribution of *H. glaucum* density, with respect to its ability to interpolate and map weed seedling densities. This method was evaluated on data of *H. glaucum* density in a saffron field in Southern Khorasan. Some statistical tests, such as comparisons of the means, variance, statistical distribution as well as coefficient of determination in linear regression were used between the observed point sample data and the estimated weed seedling density surfaces to evaluate the performance of the interpolation method. Results showed that in training MLPNN, test and total phase P- value was greater than 0.49, 0.18 and 0.27 percent respectively, indicating that there was no significant ($p < 0.05$) difference between statistical parameters such as average, variance, statistical distribution and also coefficient of determination in the observed and the estimated weed seedling density. This results suggest that MLP neural network can learn weed density model very well. In addition results indicated that trained MLP neural network has a high capability in predicting weed density at unsampled points. The technique showed that the MLPNN could interpolate and map spatial *H. glaucum* density variability. Patchy weed distribution offers large potential for using site-specific weed control on this field.

Keywords: *Hordeum galucum*, Interpolation, Neural network, Spatial distribution, Weed

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Antixenosis Resistance of Different Poplar Clones to Three Important Pest in East Azarbaijan, Iran

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Abstract

East Azarbaijan province is one of the most important areas for poplar production and has the highest suitability of poplar plantation in Iran. Three species of poplar trees included *Populus alba*, *P. nigra* and *P. x. euramericana* are planted and usually attacked by different pests in this province. *Melasma populi* L., *Archips rosana* (L.) and *Pemphigus filaginis* are some of important pests that cause direct and indirect damage on poplar trees. In this study were investigated antixenosis resistance of different poplar clones based on evaluation of pests accumulation on clones in natural condition. Based on the study results, clones included *P. nigra* Shabestar, *P. alba* Marand and *P. nigra* 62/154 respectively had more susceptibility and *P. nigra* Maragheh, *P. alba* Maragheh, *P. alba* Bostanabad and *P. x. euramericana* Bostanabad clones were the most resistant against *M. populi*. *P. alba* Mianeh clone was the most susceptible to *A. rosana* while, *P. nigra* Miandoab, *P. nigra* Maragheh and *P. alba* Maragheh had the highest resistance. About *P. filaginis* only *P. alba* Miandoab, *P. nigra* Marand and *P. nigra* Mianeh clones were infested to the pest and none of the other clones infested to the aphid.

Keywords: Resistance, Susceptibility, Clone, Pests, Poplar, East Azarbaijan

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Brief Report

Isolation and Identification of the Fungal Agents of Citrus Brown rot in the North of Iran

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Abstract

Brown rot is one of the fungal disease in the north of Iran. Symptoms on infected fruit in the initiation of infection appear as small dusky spots resembling peteca. Then infected tissues were removed and cultured on the PARPH selective medium. Based on morphological characters, growth rates, growth patterns and cardinal temperatures for growth, *P. parasitica* as a causal agent for mandarin varieties and *P. citrophthora* for sweet oranges were identified. In pathogenicity test *Phytophthora* species (*P. parasitica* and *P. citrophthora*) respectively on fruit of mandarin varieties and sweet orange were found to be pathogenic.

Keywords: Citrus, *Phytophthora* fangi, Brown rot

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