Effect of Postharvest Fruit-Decaying Fungi on Newly Introduced Peach Cultivars to Egypt
2. Enzymatic Activities in Healthy and Diseased Fruits

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ABSTRACT

Enzymatic activities in both healthy fruits and those inoculated with the tested fungi of different peach cultivars (Florda Prince, Desert Red, T. Snow, T. Sweet, Swilling and Meit Ghamr) were studied. *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* significantly differed in their ability to produce pectin methyl esterase (PME), polymethyl galacturonase (PMG), polygalacturonase (PG) and cellulase (Cx) in inoculated fruits of the tested peach cultivars. The highest significant activities of PME, PMG, PG and Cx were observed in fruits of all the tested cultivars infected with *R. stolonifer* and incubated at room temperature (24 – 27 °C). However, at cold storage (27 °C) marked increase in the activities of these enzymes was detected in fruits of all tested cultivars inoculated with *B. cinerea* or *P. expansum*. Generally the activities of pectolytic and cellulytic enzymes in diseases fruits were pronouncly higher than these in healthy ones. At the end of storage period (room temp. or 0°C), fruits of all the tested cultivars inoculated with any of the tested fruit rot fungi exhibited a pronounced increase in enzymatic activities of both polyphenol oxidase (PPO) and peroxidase (PO) except fruits inoculated with *R. stolonifer* and kept at 0°C, compared with those of heathy ones. Meanwhile, fruits of Swilling cultivar inoculated with *B. cinerea* and incubated either at room temperature or at cold storage gave the highest amount of total phenolic compounds in compared to those detected in other cultivars infected with any of the tested fungi. On the other hand, in infected fruits of all tested cultivars phenolic compounds content values showed significant increase compared with those of heathy ones.

INTRODUCTION

Many attempts were done to detect the response of diferent peach varieties towards infection with fruit-decaying fungi. Plant pathogens can produce a series of plant cell wall-degrading enzymes involved in pre- and postharvest diseases (Kaul and Sharma,1992 and Biggs, et al 1997). Pathogens produce cell wall-degrading enzymes in sequence, with pectic enzymes forming first and hemicellulose- and cellulose-degrading enzymes produced later (Bateman and Basham, 1976 and Misaghi,1982). On the other hand, peroxidase and polyphenol oxidase are the enzymes known to be responsible for the oxidation of metabolites of the pathogen as well as those of the host plants including phenolic compounds, enzymes, IAA and toxin (Fric,1976). In general, the higher content of the phenolic compounds plant tissues are the more resistant plant to the attack by pathogen. The phenolic

compounds may be produced prior in response to the attack by plant pathogen (Goodman, et al 1986).

The present work is attempt to investigate the role of pectolytic and cellulytic enzymes in diseased peach fruits of several cultivars after storage under different conditions (at room temperature and at cold storage) and in order to clarify the relation between phenolic compounds and susceptibility of peach cultivars to fruit-decaying fungi. The role of oxidative enzymes (polyphenol oxidase and peroxidase) in disease development, and their correlation to cultivars resistance or susceptibility was also investigated.

MATERISLS AND METHODS

The fungi *Botrytis cinerea, Penicillium expansum* and *Rhizopus stolonifer* were found to be the major fungi isolated from peach fruits and their pathogenicity were confirmed. So, they were tested to estimate their effect on peach cultivars, Florda Prince, Desert Red, T. Snow, T. Sweet and Swilling. These newly imported cultivars have different harvest time. In addition to these newly introduced cultivars, Meit Ghamr cultivar commonly grown in Egypt was also tested for comparison . The fruits were harvested at common commercial harvest stage. Fruits from each cultivar were picked in the early morning, packed in boxes and immediately transported to the laboratory. Fruits free from mechanical injury, scratching and wounding were selected, surface sterilized and inoculated (10^3 sporangiospores per milliliter of *R. stolonifer*, 10^4 conidia per milliliter of *P. expansum* or 10^5 conidia per milliliter of *B. cinerea*) according to procedures described by Hong et al., (1998). Inoculated fruits of each cultivar were then separated into two batches. In the first batch, fruits were incubated at room temperature (24 – 27 °C) for 5 days, while those of the second batch, were incubated in cold storage (0 ± 1°C, with 90-95% RH) for three weeks. Samples were taken at harvest and at the end of storage period for determination the changes in enzymatic activities and total phenolic compounds. The activity of pectin methyl esterase (PME) was measured according to the modified method used by smith (1958), the activities of polymethyl galacturonase (PMG) and cellulase (Cx) was measured according to the method used by Talboys and Busch (1970) and the activity of polygalacturonase (PG) was determined as mentioned by Hancock *et al.*, (1964). For determination of polyphenol oxidase (PPO) activity, the method described by Broesch (1954) was used and the activity of peroxidase (PO) was determined according to the method suggested by Sumner and Somers (1953). Total phenolic compounds was determined according to Swain and Hillis (1959). Tests were carried out through the two successive seasons (2000 & 2001) using three replicates per treatment .

RESULTS AND DISCUSSION

1. Pectolytic enzymes activities.

The present investigation showed that the pectolytic enzyme activities including those of pectin methyl esterase (Fig. 1), polymethyl galacturonase (Fig. 2) and polygalacturonase (Fig. 3), significantly increased in diseased fruits as compared with healthy ones. These findings are in line with those reported by many investigators on peach (Abdel-Malek, 1987; Yash et al., 1989; Kaul and Sharma, 1992 and Biggs *et al.*, 1997), pear (Gaber et al., 1990), banana (Seif El-Nasr et al., 1990), Melon (Shangwu et al., 1998), Muskmelon (Bruton et al., 1998) and apple and grape (Chardonnet et al., 2000).

According to the obtained data, the highest activities of PME, PG and PMG enzymes were noticed in fruits of all tested cultivars inoculated with *R. stolonifer* and kept at room temperature, followed by those inoculated with *B. cinerea,* then *P. expansum*, respectively. Similar results were reported by Bush and Codner (1968), Kamara et al. (1981) and Yash et al. (1989) they found that pathogenic fungi differed in their ability to produce pectolytic enzymes.

At the end of storage period, healthy fruits of any tested cultivars proved to have the lowest activities of PME, PMG and PG enzymes. Zhou et al. (2000) indicated that endo-polygalacturonase, exo-polygalacturonase, pectin esterase and endo-gluconase were present in healthy nectarine fruits after 4 weeks of 0°C storage and after 5 additional days of ripening at 20°C.

2. Cellulases

The obtained data illustrated in Fig. 4 showed that peach fruits inoculated with *R. stolonifer* and kept at room temperature were the highest in their Cx enzyme activity, while the lowest activities of Cx enzymes were recorded in healthy fruits. Fruits inoculated with *P. expansum* and *B. cinerea* were intermediate in this regard. This results was also reported in many plant pathogenic fungi on different host crops (Spalding, 1963; Kamara et al., 1981; Bailey and Pessa, 1990; Gaber et al. 1990 a and Seif El-Nasr et al., 1990). The previously trend was true with all tested cultivars except the case of Swelling cv. fruits inoculated with *P. expansum*, which gave the highest enzyme activity weather the storage was applied at room temperature or at 0°C.

3. Oxidative enzymes activities.

The present data indicated that more oxidative enzymes activities including polyphenol oxidase (Fig. 5) and peroxidase (Fig. 6) were recorded in diseased fruits as compared with healthy ones. Fruits inoculated with *R. stolonifer* and kept at room temperature exhibited the highest levels of polyphenol oxidase and peroxidase activities, followed by those inoculated with *B. cinerea* and *P. expansum.* High levels of these enzymes activities were found to be associated with different pathogens in different host plants (Fric, 1976; Chakraborty and Nadi, 1978; Abdel-Malek, 1987 and Jianzhang et al., 1997). In

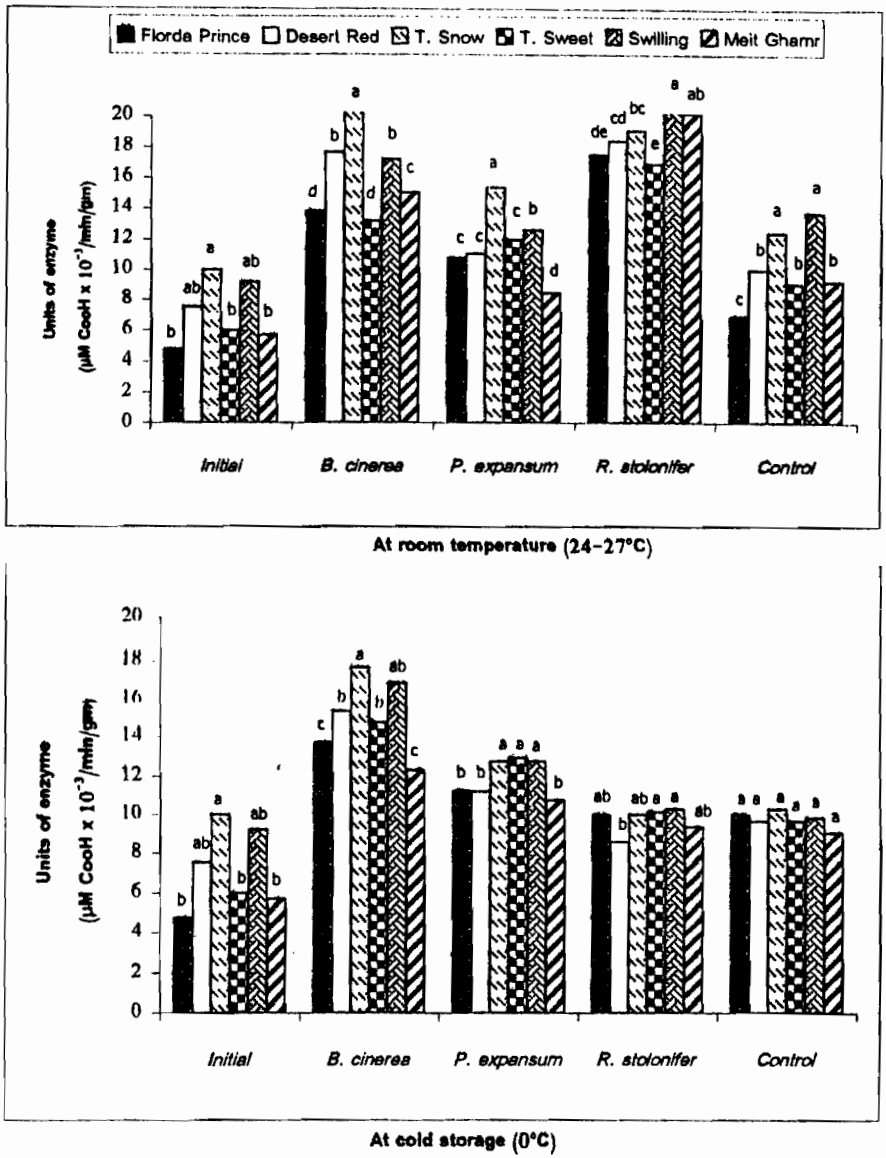
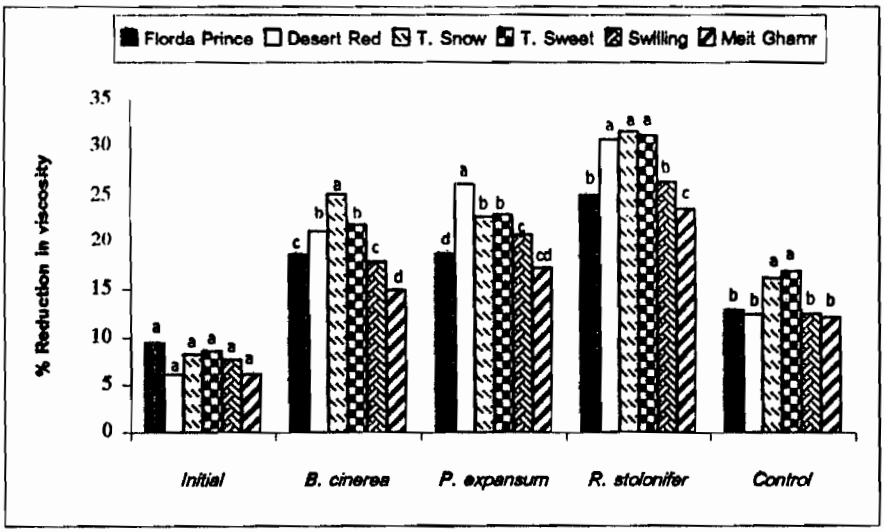


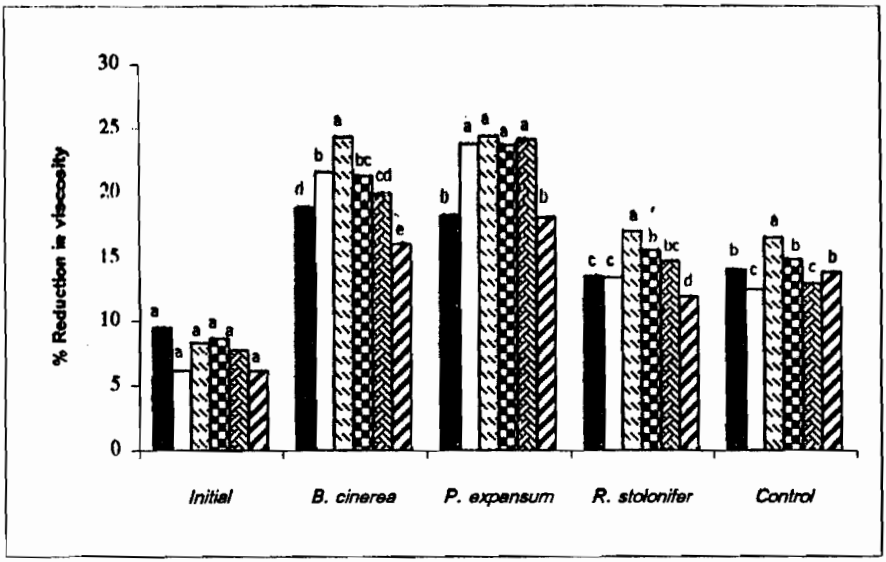
Fig. (1). Pectin Methyl esterase activities (units of enzyme) in fruit of different peach cultivars inoculated with the main fruit-decaying fungi and kept under different storgae conditions.

Control = non-inoculated fruits.

Bars within the same pathogen, control or initial with the same letter do not differ significantly according to Duncans multiple range test at 0.05 level of probability



At room temperature (24-27°C)

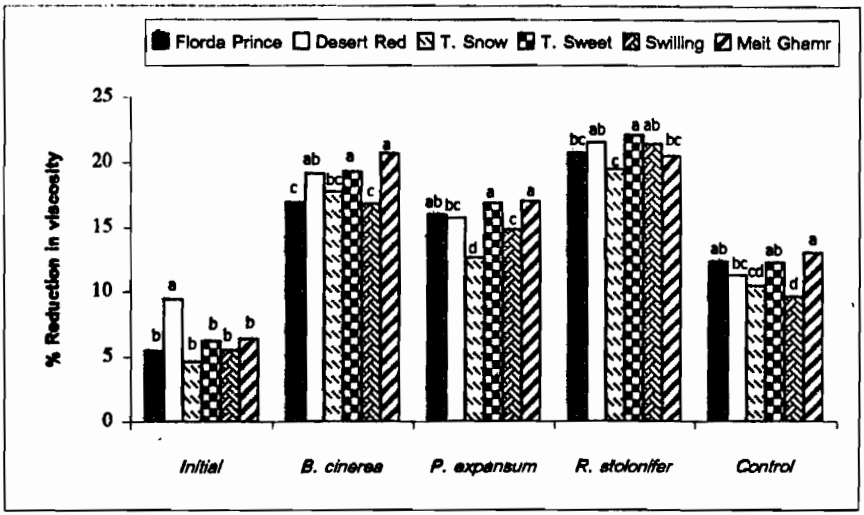


At cold storage (0°C)

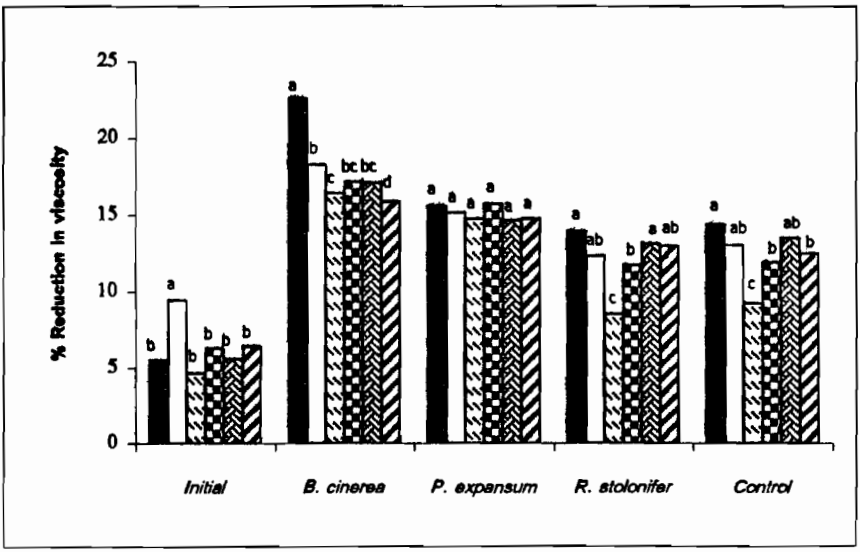
Fig. (2). Polymethyl galacturonase activities (% reduction in viscosity) in peach fruits of different cultivars inoculated with the main fruit-decaying fungi and kept under different storgae conditions.

Control = non-inoculated fruits.

Bars within the same pathogen, control or initial with the same letter do not differ significantly according to Duncans multiple range test at 0.05 level of probability.



At room temperature (24–27°C)



At cold storage (0°C)

Fig. (3). Polygalacturonase activities (% reduction in viscosity) in peach fruits of different cultivars inoculated with the main fruit-decaying fungi and kept under different storgae conditions.

Control = non-inoculated fruits.

Bars within the same pathogen, control or initial with the same letter do not differ significantly according to Duncans multiple range test at 0.05 level of probability.

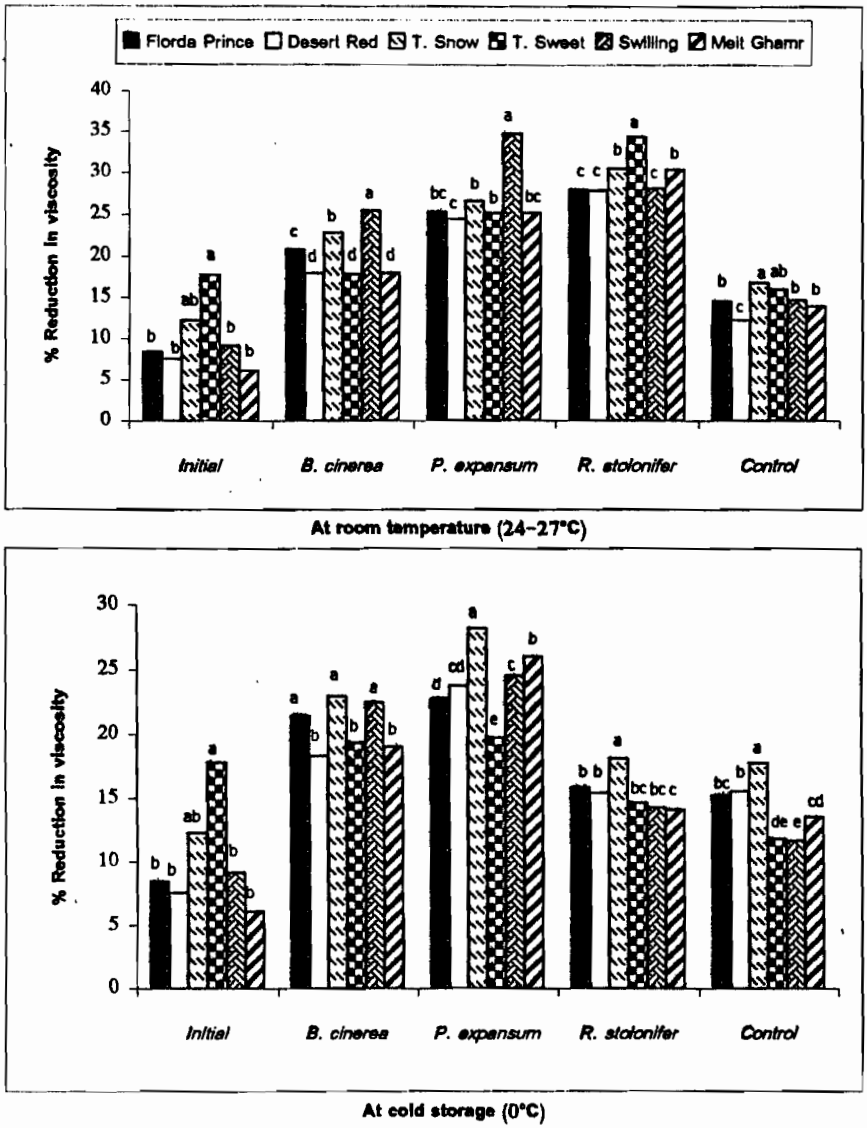


Fig. (4). Cellulase activities (% reduction in viscosity) in peach fruits of different cultivars inoculated with the main fruit-decaying fungi and kept under different storage conditions.

Control = non-inoculated fruits.

Bars within the same pathogen, control or initial with the same letter do not differ significantly according to Duncans multiple range test at 0.05 level of probability.

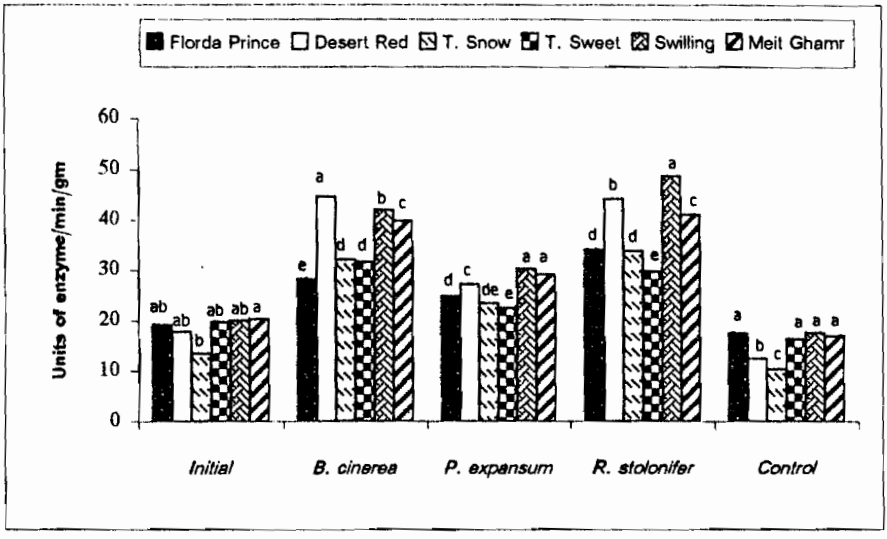
addition, many authors reported that such increase in oxidative enzymes activities was proved to be associated with host resistance reactions (Kaul and Munjal, 1980; Baraka et al., 1987; Gaber et al., 1990 ; Gradziel et al., 1998; Trandafirescu and Indreias, 1999 and Borua and Das, 2000).

According to the obtained results the highest levels of the two tested oxidative enzymes activities at harvest time were observed in healthy fruits of Meit Ghamr cv., which proved to be the least susceptible cultivar to infection with any of the tested fruit-decaying fungi. On the other hand, fruits of Swilling and T. Snow cultivars gave the lowest oxidative enzymes activities at harvest, meanwhile, these cultivars were proved to be the most susceptible cultivars to infection with the tested fungi. For this reason, resistance may be correlated with the production of oxidative enzymes in fruit tissues during invasion by pathogens. Oku (1958) reported that phenolic compounds are oxidized to quinone by the host enzymes such as polyphenol oxidase and peroxidase. Quinone, which then polymerize into brown products, are fungitoxic substances and in many cases oxidized phenolic compounds have been reported to be responsible for disease resistance (Hegazi et al. 1993 and Radi et al., 1997).

4.Total phenolic compounds content

Results of the present investigation illustrated in Fig. 7 revealed that inoculation of any of the tested cultivars with the applied fruit-decaying fungi and kept at room temperature resulted in a significant accumulation of total phenols compared with that of healthy fruits. The highest total phenolic content was recorded in inoculated Swilling cv. fruits. Moreover, this cultivar inoculated with *R. stolonifer* gave the highest degree of infection after storage for 5 days at room temperature. These results were in agreement with those found by Hussin (1976), Abdel-Malek (1987), Baraka et al. (1987) and Hegazi et al. (1993). The increase of phenolic compound content in infected fruits could be considered as a response of host tissues to infection with the fungus (Goodman et al., 1986).

Results of the present investigation also indicated that in healthy fruits of all tested cultivars, phenol compounds content mostly decreased at the end of storage period (at room temperature or 0°C) compared with that estimated at harvest. For this reason, peach fruits at over-ripe stage might be more susceptible to infection with postharvest pathogen. Saring et al. (1998) and Tradafirescu and Indreias (1999) declared this point as they mentioned that a direct relationship between accumulation of phenolic compounds in tissues and their resistant to invader fungi. In addition, Sathianathan and Vidhyasekaran (1981) concluded that not the accumulation of phenolic compounds, but the speed with which the phenols accumulate, may be related to the disease resistance. So, these phenolic compounds may have a role in minimizing or preventing infection by fruit rot fungi.



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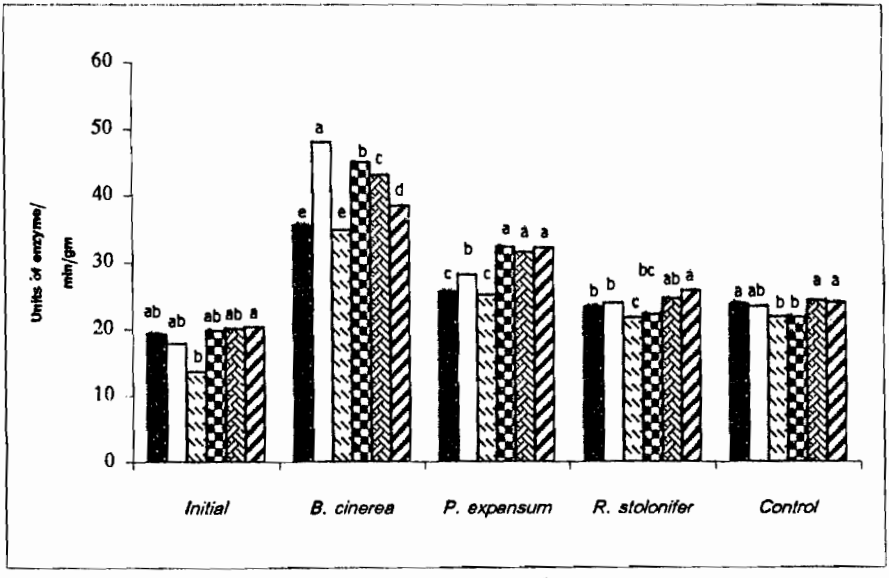
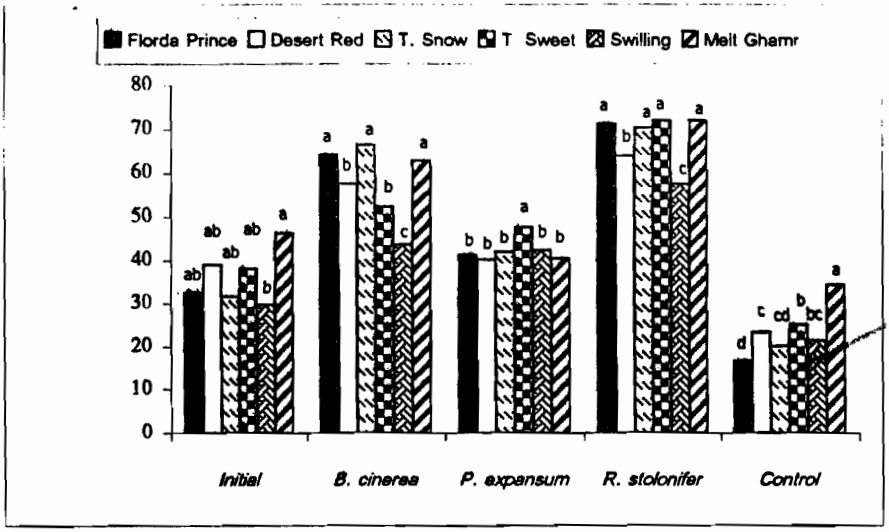


At cold storage (0°C)

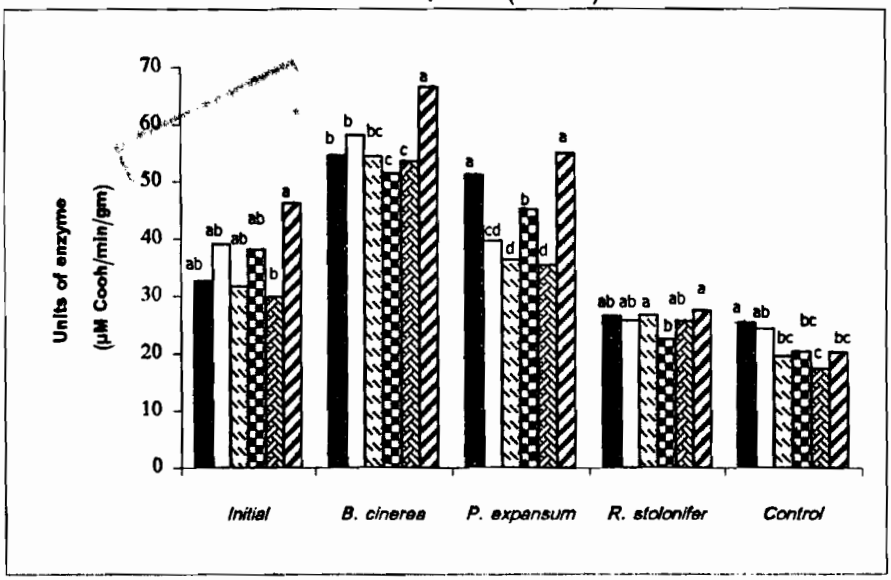
Fig. (5). Polyphenol oxidase activities (units of enzyme) in peach fruits of different cultivars inoculated with the main fruit-decaying fungi and kept under different storgae conditions.

Control = non-inoculated fruits.

Bars within the same pathogen, control or initial with the same letter do not differ significantly according to Duncans multiple range test at 0.05 level of probability.



At room temperature (24-27°C)

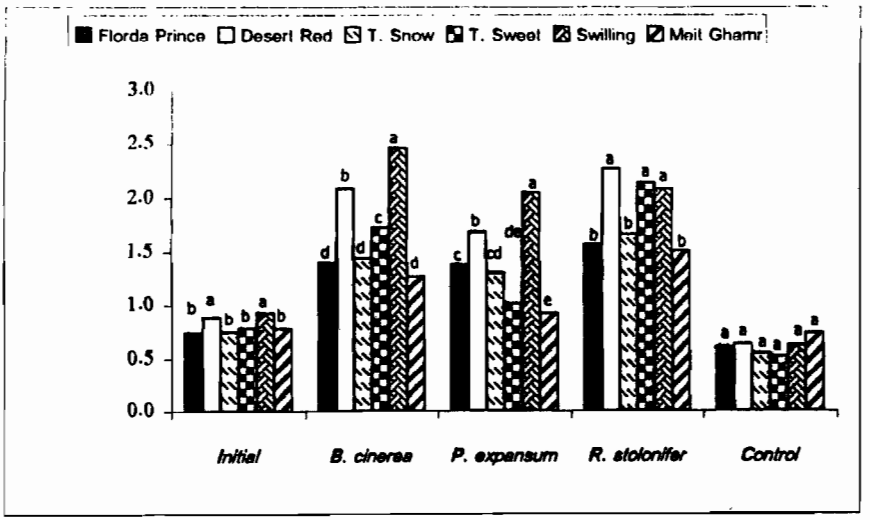


At cold storage (0°C)

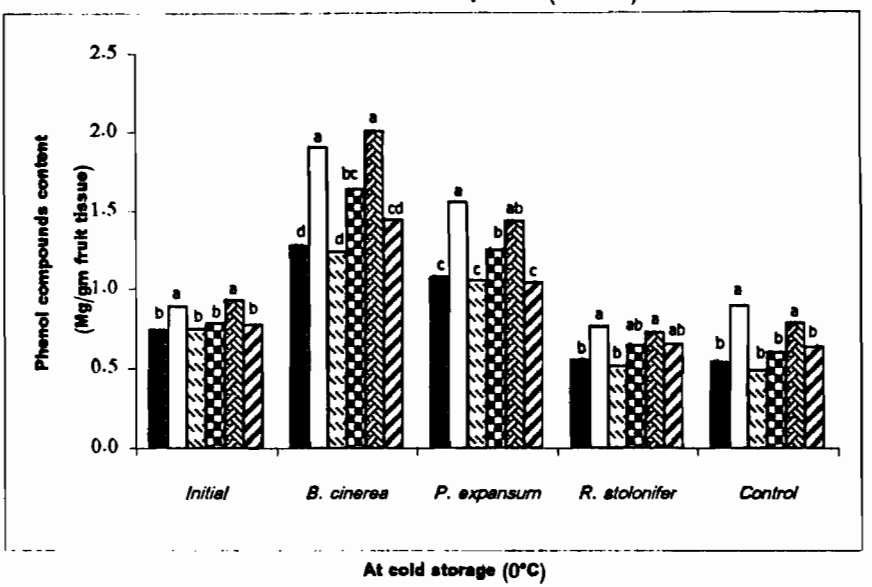
Fig. (6). Peroxidase activities (units of enzyme) in peach fruits of different cultivars inoculated with the main fruit-decaying fungi and kept under different storgae conditions.

Control = non-inoculated fruits.

Bars within the same pathogen, control or initial with the same letter do not differ significantly according to Duncans multiple range test at 0.05 level of probability.



At room temperature (24-27°C)



At cold storage (0°C)

Fig. (7). Phenol compounds content (mg/gm fruit tissue) in peach fruits of different cultivars inoculated with the main fruit-decaying fungi and kept under different storage conditions.

Control = non-inoculated fruits.

Bars within the same pathogen, control or initial with the same letter do not differ significantly according to Duncans multiple range test at 0.05 level of probability

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الملخص العربى

تأثير فطريات عفن الثمار بعد الحصاد على أصناف الخوخ المدخلة حديثا الى مصر.

٢-النشاط الانزيمى فى الثمار السليمة والمصابة.

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تم دراسة النشاط الانزيمى فى ثمار سليمة واخرى محقونة بالفطريات الممرضة وذلك لعدة اصناف من الخوخ (فلوردا برنس ، ديزرت ريد ، تي سلو ، تي سويت ، سويليبنج وميت غمر) . واظهرت النتائج اختلاف الفطـريات بوترايـتس سـينااريا ، بنسليوم اكسبنسيوم ورايزوبس ستولونيفير في قدرتها على إنتاج الإنزيمات البكتيوليتـية والسـليوليتية مـثل إنـزيم بكتين ميثيل استيريز ، إنزيم بولي ميثيل جلاكتيوروناز ، إنزيم بولي جلاكتـيوروناز وإنـزيم السـليوليز في ثمار الأصناف المحقونة بها وثبت أعلى ارتفاع معنوي في نشاط هذه الإنزيمات في الثمار المصابة بالفطر رايزوبس ستولونيفير وذلك في كل الأصناف المختبرة على درجة حرارة الغرفة (٢٤-٢٧°م) ، بينما تحت ظروف التخزين البارد (٠°م) فقد أظهرت الثمار المحقونة بالفطر بوترايتس سينااريا والفطر بنسيليوم اكسبنسيوم زيادة ملحوظة في نشاط هذه الإنزيمات . عموما أوضحت النتائج أن نشاط الإنزيمات البكتينية والسليلوزية كان أكثر وضوحا في الثمار المصابة منها في الثمار السليمة. أدت الإصابة بكل الفطـريات المختـبرة إلى حدوث زيادة واضحة في نشاط كل من إنزيم البولي فينول أوكسيديز والبيروكسيديز مقارنة بالثمار السليمة وذلك في جميع الأصناف تحت الدراسة بعد انتهاء فترة التخزين علي درجة حرارة الغرفة أو تحـت ظـروف التخزين البارد . واتضح أيضا أن ثمار الصنف سويلينج المصابة بالفطر بوترايتس سينااريا سـواء علـى درجة حرارة الغرفة أو تحت ظروف التخزين المبرد تحتوي مواد فينولية أكثر مما في الأصناف الأخرى بعد إصابتها بأي من الفطريات المختبرة . وعموماً فإن إصابة ثمار جميع الأصناف المختبرة تؤدي إلى زيادة محتواها من المواد الفينولية مقارنةً بالثمار السليمة .