Seed disinfection of pepper 
(Capsicum annum L.) 
from Alternaria Nees

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SEED DISINFECTION OF PEPPER (CAPSICUM ANNUUM L.) FROM ALTERNARIA NEES

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Abstract

In addition to problems connected with pathogen spread by the seeding, the germination on damp blotting-paper in sterile Petri's dishes is compromised when a mould of early blight envelops the seeds. At Research Institute for Vegetable Crops located in Pontecagnano (SA), different treatments to pepper seeds were compared in order to control serious infections caused by Alternaria alternata (Fr.) Keissl. and A. solani (Ell. et Mart.) Jones et Grout. The compared treatments included: untreated control; heat-treatments using different temperatures and exposure times; UV radiation (253.7 nm) exposure for different days; chemical compounds such as sodium hypochloride at different concentrations of active chlorine; fungicides such as Captan and Enavit-methyl. The observations regarded percentage of germinated seeds, germination average duration (germination index) and percentage of seeds infected by Alternaria at room temperature. With regard to heat-treatments, the best results were obtained with humid-heat at temperature of 50 °C (for 5 and 15 minutes) and 60 °C (for 1 minute). These conditions gave 90-100% of germinated seeds, 15 days of germination average duration and 20% of seeds infected by Alternaria. The seed exposure to UV rays for 4 days exhibited a germination of 90%, 16 days of germination average duration and 7% of infected seeds. Among all the compared chemical and fungicide compounds, the best results were obtained by using both 2.5% sodium hypochloride solution and Captan powder which gave 90% of germinating capability, 19-20 days of average duration of germination and no infected seeds.

1. Introduction

Fungi that mainly colonize the pepper seed coat are numerous. Species of genera Cladosporium Lk. (SATI et al., 1989), Alternaria Nees, Colletotrichum Cda., Curvularia Boed., Fusarium Lk., Phoma Fr. Em. Desm. (MRIDHA and SIDDIQUE, 1989; HASHMI, 1989), Macrophomina (Petr.) Goed. (MALI et al., 1985), Rhizoctonia Khun. (SUREKHA CHITKARA et al., 1986), Aspergillus (Mich.) Lk., Rhizopus Ehrenb. (SETTY et al., 1988) have been isolated from seeds of Capsicum annuum L. It has been demonstrated that some of these fungi can infect the embryo of the seeds and endosperm, particularly at the micropylar end, with microsclerotia or inter- as well as intracellular mycelium. Pathogenicity tests by seed infection have shown their effects by reducing dramati
cally the germination, while Colletotrichum, Cladosporium, Alternaria, Drechslera ito and Curvularia have affected root elongation more adversely than shoot elongation (ADIVER et al., 1987). In many cases, these fungi contaminating seeds can be transmitted to plants acting as primary source of inoculum for field infection (NEERGAARD 1977).

The main reasons that have induced us to carry out this research to obtain fungus-free seeds have been:
- the development of a thick mould of early blight enveloping the seeds of sweet pepper during germination trials;
- the need of improving the percentage of germinated seeds and the germination average duration;
- the observance of the phytosanitary principles regarding the diffusion of pathogens by using vegetative or reproductive materials in agriculture;
- the knowledge of the importance of seed transmission in disease spreading.

2. Materials and methods

Sweet pepper seeds of the cultivar Valdor were used in the therapy experiments. These seeds, under natural infection, showed a thick brown mould during the germination on damp blotting-paper in sterile Petri’s dishes. The identification of seed pathogens was carried out by microscopical analysis of powder material drawn from seed surface; isolation trials on potato-dextrose-agar medium achieved the diagnosis of the seed disease.

With regard to humid-heat, seeds were treated by soaking in sterile hot water. Different water temperatures, ranged from 30 to 70 °C with an interval of 10 °C, were used. The seed exposure times were 1, 5, 15, 30 and 60 minutes. Untreated control consisted of seed soaking in water (at room temperature) at the same times of treated plots.

Concerning dry-heat, seed lots were exposed to different temperatures: 50, 60, and 70 °C, respectively; exposure times of 1, 3 and 24 hours were used. A longer duration dry-heat treatments was chosen because of the best resistance of fungal pathogens to dry-heat compared with humid-heat.

With regard to UV light use, different lots of seeds were exposed to far-ultraviolet rays of 253.7 nm wavelength (LEACH, 1962) for 1, 4 and 7 days, respectively. The UV radiation was generated from OSRAM HNS 30W ofr lamp. The treatments were tested by comparisons among them.

Concerning chemical treatments, five samples of seeds were treated by soaking in so many solutions of sodium hypochloride for 1 and 5 minutes. The concentrations of the compared solutions were: 0 (untreated control), 2, 2.5, 3.3, and 5% NaClO, equal to 0, 1.1, 1.4, 1.9, and 2.8% of active chlorine, respectively.

Other seed samples were treated with Captan powder (by using g 0.5 of Captan per 100 g of seeds) and Enovit-methyl (spraying the seeds with a suspension of 0.001%) compared with untreated control.
From each seed lot of pepper treated with the different ways above described, 30 seeds were collected and placed on damp blotting-paper in sterile Petri's dishes at room temperature; three replicates of 10 seeds were effected.

The observations regarded: the percentage of germinated seeds; the germination average duration (germination index) by using Pieper's formula; percentage of seeds infected by Alternaria.

Data were transformed into arcsin\[\sqrt{\frac{x}{n}}\] (SOKAL and ROHLF, 1977), before analysis of variance (ANOVA), and compared by using Duncan's test for multiple comparisons among treatments and a control through MSTAT statistical analysis program (MSTAT, 1997).

3. Results

3.1. Pathogen studies

The microscopical observations and the isolation trials of pathogen fungi allowed to attribute the observed conidia to Alternaria alternata (Fr.) Keissl. and A. solani (Ell. et Mart.) Jones et Grout.

3.2. Humid-heat treatments

Concerning the humid-heat, the best results were obtained when the seeds were exposed to 50 °C (for 5 and 15 minutes) and to 60 °C for 1 minute. These conditions significantly gave the highest percentage of germinated seeds (ranging from 90 to 100%), the 20% of seeds infected by Alternaria (Table 1) and the lowest germination average duration (Table 2). Exposure times over 15 minutes at 50 °C, over 1 minute at 60 °C and temperature of 70 °C decreased the affected seed percentage giving the best fungus eradication but considerably decreased the germinated seeds (no germinated seeds). The untreated control gave about 53% of germinated seeds, 100% of infected seeds and about 20 days of germination average duration (Table 1 and 2).

3.3. Dry-heat treatments

Regarding the dry-heat, poor results were obtained for all cases (Table 3). In fact, temperatures ranging from 50 to 70 °C gave a few germinated seeds (from 50 to 60%), many infected seeds (from 50 to 90%) and showed a germination index from 12 to 22 days. The lowest germination duration could be explained with the favorable effects of the high temperature on germination biochemical mechanisms. Temperature over 70 °C gave no germinated seeds even for the lowest exposure times.

3.4. Exposure to far-ultraviolet radiation

UV light for 4 and 7 days of exposure gave good results in the control of seed pathogens. The seed exposure significantly reduced the
infection, since a very few seeds affected by *Alternaria* were observed: the percentage values were of 6.7 and 3.3% for exposure times of 4 and 7 days, respectively (Table 4). Unfortunately, exposure times over 4 days significantly decreased the germinated seed percentage from 86.7 to 76.7%. Untreated control and one day of UV ray exposure exhibited high infected seed percentage (100 and 93.3%, respectively) and low percentage of germinated seeds (56.7 and 60%, respectively).

3.5. Chemical applications

By soaking seeds in different sodium hypochloride solutions, the best results (about the significant reduction of *Alternaria* on the seeds) were obtained by using those with NaClO concentration ranging from 2.5 to 5% (from 1.4 to 2.8% of active chlorine). In all these cases, many seeds resulted free from pathogen fungi; they showed a percentage of germinated seeds about of 90% and exhibited a germination index ranging from 16 to 19 days. Untreated control exhibited 100% of seeds infected by *Alternaria*, low germinating percentage and the longest germination average duration. The lowest concentration gave poor results in connection with the affected seeds and the vitality parameters (Table 5).

As a result of fungicide use (Captan and Enovit-methyl), the best eradication of *Alternaria* was obtained with Captan: sprays with Enovit-methyl, giving 20% of affected seeds, appeared to be less effective in killing the pathogens than powder Captan. Untreated control showed all the seeds infected and the lowest values of seed vitality (Table 6).

4. Discussion and conclusions

Fungi of genus *Alternaria* can colonize not only the external part of the sweet pepper seeds but can also be localized more deeply. They mainly contaminate the surface or the parenchyma of seed coat; rarely they can interest the cotyledons and the embryo.

The research carried out proved that reduction of seed infection or contamination by *A. alternata* and *A. solani* is possible by using heat treatments. The incidence of the heat is different according to this application way. With dry-heat at 70 °C for 3 h up to 24 h the infected seed percentage is reduced but not below an acceptable threshold (about 20%). Better results were obtained by soaking the infected seeds in hot water either at 50 °C for 1 minute up to 15 minutes or at 60 °C for 1 minute. A fall in infected seed number is very important in relation to the disease transmission to the plants during their growth period in open field or in protected cultivation.

This study also demonstrated the importance of UV light in reducing the seed contamination by *Alternaria*. The exposure of seeds to the UV rays showed a great susceptibility of both *Alternaria* conidia and mycelium while the seed vitality was not affected by the quantity and
quality of UV light used for 4 days; longer exposures decreased the
germinated seed number more than the increase of not infected seeds.

The present research proved that a very good reduction of seed
infection by Alternaria is possible by using chemical treatments. By
soaking the seeds in solutions with 2.5% of sodium hypochloride (1.4% of
active chlorine) the pathogen eradication was obtained. Seed
treatments by active chlorine can increase the germinated seeds and the
germination index as a consequence of seed coat softening.

The fungus eradication from C. annuum coat seeds, very highly infec-
ted, is possible to obtain by fungicide treatments. Applications of
Captan powder and Enovit-methyl spray significantly decreased the
infected seed percentage as well as the germination index. However, the
fungicide use could be restricted by phytosanitary legislation.

Better results could be obtained by combining the physical
treatments (heat and UV light) with chemicals (sodium hypochloride and
fungicides). Their combination could be also effected by using less
drastic temperatures, exposure times and chemical doses.

Generally, the seed treatments represent an easy and preventive way
that can help horticulturists in utilizing healthy seeds to control the
spreading of diseases, especially regarding the introduction of new
pathogenic races into our country.

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Table 1 - Humid-heat treatments. Effects of different exposure times on the germinated seed percentage and infected seeds

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1 minute germinated seeds</th>
<th>1 minute infected seeds</th>
<th>5 minutes germinated seeds</th>
<th>5 minutes infected seeds</th>
<th>15 minutes germinated seeds</th>
<th>15 minutes infected seeds</th>
<th>30 minutes germinated seeds</th>
<th>30 minutes infected seeds</th>
<th>60 minutes germinated seeds</th>
<th>60 minutes infected seeds</th>
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<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
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<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Control</td>
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<td>100.0 A</td>
<td>56.7 C</td>
<td>100.0 A</td>
<td>53.3 BC</td>
<td>100.0 A</td>
<td>50.0 B</td>
<td>96.7 A</td>
<td>53.3 C</td>
<td>93.3 A</td>
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<td>100.0 A</td>
<td>63.3 C</td>
<td>100.0 A</td>
<td>50.0 CD</td>
<td>93.3 A</td>
<td>60.0 B</td>
<td>80.0 B</td>
<td>53.3 C</td>
<td>80.0 A</td>
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<td>80.0 B</td>
<td>100.0 A</td>
<td>80.0 B</td>
<td>100.0 A</td>
<td>73.3 B</td>
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<td>60.0 B</td>
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<td>83.3 A</td>
<td>73.3 B</td>
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<tr>
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<td>30.0 B</td>
<td>100.0 A</td>
<td>20.0 B</td>
<td>100.0 A</td>
<td>20.0 B</td>
<td>86.7 A</td>
<td>16.7 C</td>
<td>66.7 B</td>
<td>13.3 C</td>
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<tr>
<td>60</td>
<td>90.0 A</td>
<td>20.0 C</td>
<td>56.7 C</td>
<td>10.0 C</td>
<td>30.0 D</td>
<td>6.7 C</td>
<td>0.0 C</td>
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Means separation in columns by Duncan's multiple range test (P<0.01)

Table 2 - Humid-heat treatments. Effects of different exposure times on germination index

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1 min</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
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<tr>
<td>°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
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<td>23.00 A</td>
<td>18.37 A</td>
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<td>30</td>
<td>20.50 A</td>
<td>23.00 A</td>
<td>18.67 B</td>
<td>19.67 A</td>
<td>20.70 A</td>
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<td>40</td>
<td>20.00 A</td>
<td>23.63 A</td>
<td>18.20 B</td>
<td>19.38 A</td>
<td>19.50 A</td>
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<tr>
<td>50</td>
<td>15.00 B</td>
<td>14.30 B</td>
<td>15.80 C</td>
<td>15.56 B</td>
<td>17.80 B</td>
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<td>60</td>
<td>14.33 B</td>
<td>17.33 B</td>
<td>18.14 B</td>
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Means separation in columns by Duncan's multiple range test (P<0.01)
Table 3 - Dry-heat treatments. Effects of different exposure times on the germinated seed percentage, germination index, and infected seed percentage

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>1 hour</th>
<th>3 hours</th>
<th>24 hours</th>
</tr>
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<tr>
<td></td>
<td>germinated seeds</td>
<td>germination index</td>
<td>infected seeds</td>
</tr>
<tr>
<td>50</td>
<td>60.0 A</td>
<td>15.40 A</td>
<td>80.0 A</td>
</tr>
<tr>
<td>60</td>
<td>60.0 A</td>
<td>15.50 A</td>
<td>80.0 A</td>
</tr>
<tr>
<td>70</td>
<td>56.6 A</td>
<td>12.43 B</td>
<td>70.0 A</td>
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Means separation in columns by Duncan's multiple range test (P<0.01)

Table 4 - Seed exposure to far-ultraviolet. Effects of diverse exposure times on the germinated seed percentage, germination index, and infected seed percentage

<table>
<thead>
<tr>
<th>Exposure to UV days</th>
<th>germinated seeds %</th>
<th>germination index days</th>
<th>infected seeds %</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>56.7 C</td>
<td>23.0 A</td>
<td>100.0 A</td>
</tr>
<tr>
<td>1</td>
<td>60.0 C</td>
<td>19.3 B</td>
<td>93.3 A</td>
</tr>
<tr>
<td>4</td>
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<td>16.0 C</td>
<td>6.7 B</td>
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<td>7</td>
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<td>19.0 B</td>
<td>3.3 B</td>
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Means separation in columns by Duncan's multiple range test (P<0.01)
Table 5 - Seed treatments by soaking in different concentrations of sodium hypochloride solutions for 1 and 5 minutes. Effects on germinated seeds, germination index, and seed contamination

<table>
<thead>
<tr>
<th>Concentration in NaClO</th>
<th>Cl₂ concentration</th>
<th>1 minute germinated seeds %</th>
<th>germination index days</th>
<th>infected seeds %</th>
<th>5 minutes germinated seeds %</th>
<th>germination index days</th>
<th>infected seeds %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>53.3 C</td>
<td>23.5 A</td>
<td>100.0 A</td>
<td>56.7 C</td>
<td>22.0 A</td>
<td>100.0 A</td>
</tr>
<tr>
<td>2.0</td>
<td>1.1</td>
<td>63.3 BC</td>
<td>18.0 BC</td>
<td>70.0 B</td>
<td>80.0 B</td>
<td>16.6 C</td>
<td>100.0 B</td>
</tr>
<tr>
<td>2.5</td>
<td>1.4</td>
<td>90.0 A</td>
<td>19.1 B</td>
<td>0.0 C</td>
<td>86.7 A</td>
<td>15.7 C</td>
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<td>16.3 C</td>
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Means separation in columns by Duncan's multiple range test (P<0.01)

Table 6 - Fungicide treatments. Effects on the germinated seed percentage, germination index, and percentage of seeds infected by Alternaria

<table>
<thead>
<tr>
<th>Treatments</th>
<th>germinated seeds %</th>
<th>germination index days</th>
<th>infected seeds %</th>
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</thead>
<tbody>
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<td>Untreated control</td>
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<td>23.0 A</td>
<td>100.0 A</td>
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<tr>
<td>Powdered with Capitan</td>
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<td>19.7 B</td>
<td>0.0 C</td>
</tr>
<tr>
<td>Spray with Enovit-methyl</td>
<td>83.3 A</td>
<td>17.8 B</td>
<td>20.0 B</td>
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Means separation in columns by Duncan's multiple range test (P<0.01)