COATING OF ORANGE FRUIT WITH NANO-SILVER PARTICLES TO MINIMIZING HARMFUL ENVIRONMENTAL POLLUTION BY CHEMICAL FUNGICIDE

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ABSTRACT
This study was aimed to investigate the coating of nanoparticles to minimizing harmful of chemical fungicide in fruit industrial sector. The Effects of coating fruit surface with different concentrations of Nano-silver on the development of mycelium growth of, Penicillium digitatum and Aspergillus niger during storage were estimated. Coated treatments with silver recorded fully reduction of the fungal growth (100%) on surface of fruit with concentration of 50 ppm and 100 ppm as compared with uncoated samples and fungicide. Also, the treatment with 100ppm of silver showed a significant decrease in weight loss compared with uncoated fruit. Application of nanotechnology can be an important way to replacing chemical fungicides in agricultural sector in future with regard controlling fungal post-harvest rot in stored fresh fruit.

Keywords: Nano-silver; Potential Alternative; Synthetic chemical; Orange Fruit.

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INRODUCTION
Nanoparticles a successful and active tool for evolving and working according to the requirements of technological and can increasing advantages of sciences in different sectors (11). Moreover, can be using this material for more uses with some modified and without introducing too much interference depending on purpose of using to simplify their applications in many sector such as agriculture, biological and medicine science (6). Using Nanoparticles materials as an emerging technology and a novel antimicrobials method, with the growing idea of using more effective methods, cheaper and easier and safer for humans and the environment. One of the potential applications of Nanoparticles are to food packaging and management of plant diseases. The unique physical and chemical properties of these materials increases activity of microbial through destroy fungi and bacteria without toxicity effect on the near tissue (19, 27). Using of nano-technology as alternative to chemical pesticides in controlling on pathogen such as fungi and bacteria had become a necessary requirement to replace it. After recorded negative impact by widely using for such as those chemicals on human, animals and environment (4, 12). Nanoparticles such as silver are known to attack a wide types and difference of microbial, microorganisms effect of nano-silver on bioprocesses, functions and structure and membranes of cell and inhibit ATP production (14, 23). Nano-silver have a multiple modes of inhibitory infection against different various plant pathogens in a relatively safer way (13, 22). Until now limited reported has studied and provided activity of coating nano-silver in controlling post-harvest rot under storage condition. The objective of current study to evolution of using silver nanoparticles as the alternative of fungicide to controlling on rot fungi and increase shelf life orange fresh fruit.

MATERIALS AND METHODS
AgNPs solution preparation
AgNPs, WA-CV-WA13B (CV) were used in this experiment. Four concentration of AgNPs of stock solution prepared ranged between 100 to 12.5 by diluted with distilled water. The solutions stored at 4°C until use in the next step.

Fruit collection
Citrus fruit (orange) of medium size and in good physical conditions such as being; damage, insect and disease free, were purchased from local market in Samarra city, Iraq. They were brought and kept under cool condition in laboratory, then had used on the next day for the experiment.

Preparation of fruits
Stored fruits (orange) washed by water, air-dried, after that sterilized using alcohol (ethanol 70%) and left until dried. The fruits were then randomly divided into six groups (Four for nano-treatment + two controls). Fruits were wounded at a (5mm/depth) and (1mm / diameter) using clean and sterilized needle. Then, the wounded fruit inoculated by spraying with the prepared suspension of fungi spores. The treated fruit was left for an hour after spraying to stabilize the spores on the wound. After that, the coated and sprayed fruits were packaged in box carton (40x40x30cm) and incubated under room condition (25ºC±2 and 65-75 % RH) for 21 days. Then the formation of fungal rot and shelf life for fruit were recorded. The experiment repeated with three replicate trials of five fruits per replicate were carried out using the Completely Randomized Design (RBD).

Fungal decay
The Fungal decay of stored fruit was evaluated after 21 days under the storage conditions. The percentage of infection was determined using the scale of 1-5 (25) as explained in table.1.

Table 1. Percentage of infection fungal decay index

<table>
<thead>
<tr>
<th>Percentage infection</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Clean</td>
</tr>
<tr>
<td>1-5</td>
<td>Trace</td>
</tr>
<tr>
<td>5-15</td>
<td>Slight</td>
</tr>
<tr>
<td>15-30</td>
<td>Moderate</td>
</tr>
<tr>
<td>&gt;30</td>
<td>High</td>
</tr>
</tbody>
</table>

Loss Shelf Life Fruit
The percentage of loss shelf life fruit calculated after 21 days from stored employing the following equation (12).

\[ W_L \% = \frac{W_L \text{ before storage} - W_L \text{ after storage}}{W_L \text{ before storage}} \times 100 \]
Data collection and Analysis
Data was collected and analysed using analysis of variance (ANOVA). Duncan’s Multiple range test (P ≤ 0.05) used to determined significant difference between means values. Analysis of variance (ANOVA) which were per-formed using SPSS version 23-2016 (SPSS Inc, Chicago,USA).

RESULT AND DISCUSSION

Fungal decay index
The effect of fruit coating with different treatments of Silver (100 to 12.5 ppm) on the development of mycelium growth, *P. digitatum* and *A. niger* (colony diameter/cms) on the orange fruit surface during storage for 21 days at 25°C±2 and 65-75% RH is shows in Table 2. The rate of growth inhibition in coated fruit with different concentration of silver recorded increases percentage as compared with uncoated fruit. The results showed decline of the fungal decay index by *P. digitatum* and *A. niger* represented by 6.6% and 11.1% at a concentration of 12.5ppm respectively, while the uncoated fruits had a percentage of growth of, 81.1% and 91.1% respectively. At concentration of 25 ppm, no growth observed for *P. digitatum* while for *A. niger* we observed a growth of 2.2% only. Nevertheless, no growth observed for both fungi at 50, 75 and 100 ppm of Silver. While coating the fruits with 12.5ppm or fungicide showed approximately same result. The decay index rate of growth for Green mold and Black rot was, 6.66 and 11.10 respectively. Also, there was no statistical difference (P ≤ 0.05) between 25, 50 and 100 ppm treatments. The three dilution exhibited significant decrease in disease severity trace degree to completely inhibition of infections (0%), while with 12.5ppm and fungicide the recorded was, 6.66 and 11.10 decay index for *P. digitatum* and *A. niger* respective results of this study coincide with the previous studies using nanoparticles as antimicrobial components such as titanium dioxide, zinc oxide and magnesium oxide and can be employed a safer method in food packaging. Also cheaper and safer than from chemical fungicide (9, 10, 24). In finding of (21) finding that, used Nano-particle and nanomaterial technology in synthesis chemical and biological pesticides led to higher efficiency compared with conventional pesticides. Probably due to some of the properties of nanostructures such as, small-sized, low toxicity, high mobility, higher surface area and higher solubility. Also, the findings of (2, 18) are in agreement with our result that, Nano silver increased inhibition growth of fungi that isolated from fresh fruit.

Fruits shelf life
The effect of different concentration of coatings and period storage on weight loss presented in Table.3 and Fig.1. Both storage period and type of coating material showed a significant impact on percentage of weight loss for coated fruit with recorded low percentage of weight in coated fruit oranges compared with uncoated fruit. The high percentage of weight loss recorded after four week from storage with mean value ranged between 4.9 to 6% . While short time storage (7 days) had low percentage were there were 2.9 to 5% compared with uncoated fruit that recorded high loss of weight reached to 10.3 and 18.76 for first week and fourth week sequentially. Regarding effect of concentration on weight loss, the mean weight loss at 100ppm, 50ppm, 25ppm and 12.5ppm were 3.8%, 3.9%, 5.42%, and 5.5% respectively ,while uncoated fruits were 13.66% after four weeks of storage. Results from Table.3 using nano-silver with concentration 100ppm shows a significant decrease in weight loss compared with uncoated fruit and other treatment

Table2. Effect of coating fruit with different concentrations of Silver (12.5 to 100 ppm) on the development of mycelium growth

*Penicillium digitatum* and *Aspergillus niger* (colony diameter/cms) on the orange surface during storage for 21 days at 25°C±2 and 65-75% RH

<table>
<thead>
<tr>
<th>Treatments (ppm)</th>
<th><em>Penicillium digitatum</em></th>
<th><em>Aspergillus niger</em></th>
<th><strong>Infection</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>6.66 b</td>
<td>11.10 c</td>
<td>Slight</td>
</tr>
<tr>
<td>25</td>
<td>0 a</td>
<td>2.22 b</td>
<td>Trace</td>
</tr>
<tr>
<td>50</td>
<td>0 a</td>
<td>0 a</td>
<td>Trace</td>
</tr>
<tr>
<td>1000</td>
<td>0 a</td>
<td>0 a</td>
<td>Trace</td>
</tr>
<tr>
<td>Water</td>
<td>86.66 c</td>
<td>91.10 d</td>
<td>High</td>
</tr>
<tr>
<td>Fungicides</td>
<td>6.66 b</td>
<td>11.10 c</td>
<td>Slight</td>
</tr>
</tbody>
</table>
Table.3 Effect of coating fruit using nano-silver solution on weight loss (%) that storage at 25±2°C and 65-75 % RH for four weeks (28S) days

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Time</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
</tr>
</thead>
<tbody>
<tr>
<td>100ppm</td>
<td>2.9 a</td>
<td>3.5 a</td>
<td>3.9 a</td>
<td>4.9 b</td>
<td></td>
</tr>
<tr>
<td>50ppm</td>
<td>3.5 b</td>
<td>3.5 a</td>
<td>4 b</td>
<td>4.63 a</td>
<td></td>
</tr>
<tr>
<td>25ppm</td>
<td>5 c</td>
<td>5.5 b</td>
<td>5.5 c</td>
<td>5.70 e</td>
<td></td>
</tr>
<tr>
<td>12.5ppm</td>
<td>5 c</td>
<td>5 b</td>
<td>6 d</td>
<td>6 d</td>
<td></td>
</tr>
<tr>
<td>Uncoated</td>
<td>10.3 c</td>
<td>11.9 d</td>
<td>13.7 c</td>
<td>18.76 e</td>
<td></td>
</tr>
</tbody>
</table>

* Alphabets different in the same columns shows significant difference at (P≤0.05) between concentrations and average was calculated from three replicates.

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