Postharvest application with propolis for controlling white rot disease of green bean pods

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Abstract: White rot is the most severe disease attacking green bean pods during pre and postharvest stages. Ethanol extracted propolis (EEP) at four concentrations *i.e*. 0, 5%, 10% and 15% was tested to study its effect on growth inhibition zone of the causative fungus *Sclerotinia sclerotiorum*. Results indicated that all tested concentrations of EEP significantly increased the inhibition zone of *S. sclerotiorum* growth. The highest increase was obtained with at 15% where complete reduction in sclerotia germination occurred. At EEP 10%, the sclerotia germination was reduced by 91.6%. When the same concentrations were tested to study their effect on white rot disease of green bean pods during storage, all EEP significantly reduced the percentage of white rot incidence and severity. The highest reduction was obtained with EEP at 15%; reducing the disease incidence and severity by 90% and 91.8% respectively, followed by EEP at 10% which reduced the incidence and severity by 78% and 82% respectively. EEP at 5% showed moderate effect. EEP is suggested as good biosystem within integrated management of pathogens and safe alternative for controlling postharvest diseases of green bean pods.

Keywords: ethanol extracted propolis, green bean pods, pre and postharvest stages, white rot

Citation: Abd-El-Kareem, F., N. M. Saied, and M. M. M. Abd-Elgawad. 2017. Postharvest application with propolis for controlling white rot disease of green bean pods. Agricultural Engineering International: CIGR Journal, Special issue: 310–314.

1 Introduction

Green bean (*Phaseolus vulgaris* L.) is considered one of the most important leguminous crops cultivated in Egypt for both local consumption and export for Europe and Arab countries. Green bean pods decay during their growth in the field, storage, transport, marketing or exportation by a variety of fungi mainly: *Sclerotinia sclerotiorum* (Lib.) de Bary, *Botrytis cinerea* Pers., and *Pythium aphanidermatum* (Edson) Fitzp. (Siviero and Motton, 2000; Naffa and Rabie, 2006; Khalil, 2017). Controlling this disease mainly depends on chemical fungicides which confront environmental issues. Therefore, alternative safe treatments for controlling postharvest diseases are desperately needed (Abd-El-Kareem and Haggag, 2015; Abd-El-Kareem et al., 2015; Abd-El-Kareem and Saied, 2015; Elshahawy, et al., 2015; Abd-El-Kareem, 2016; Saied, 2016).

Propolis is a naturally occurring brownish-green resinous product that honeybees collect from different plant exudates. It possesses favourable biological attributes, including antibacterial, antiviral, and antifungal properties, and has been used for pharmacological applications (Bosio et al., 2000; Şahinler and Kaftanoğlu, 2005). Its antimicrobial activity against human pathogenic fungi, bacteria and viruses has been demonstrated (Burdock, 1998; Kujumgiev et al., 1999). Yet, very few in vitro and in vivo studies have been conducted against plant pathogenic microorganisms (Fahny and Omar, 1989; Abd Al-Fattah et al., 1995; Quiroga et al., 2006). The application of ethanol-extracted propolis (EEP) inhibited *Penicillium digitatum* (Pers.) Sacc. growth in vitro (Soylu et al., 2004, 2008) and limited the growth of *B. cinerea* on strawberry (La Torre et al., 1990).

The application of 5% and 10% concentrations of

Received date: 2017-09-29 **Accepted date:** 2017-12-29

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EPP extended the storage life of Fremont mandarins, as compared to untreated control fruits (Ozdemir et al., 2005). Treatment with EEP was also effective in preventing fungal decay in cherries stored for four weeks, but adversely affected sensory quality and stem color (Çandır et al., 2009). The purpose of the present study is to evaluate the effects of EEP on *Sclerotinia sclerotiorum*. We examined its efficacy on white rot disease of green bean pods during storage.

2 Materials and methods

Sclerotinia sclerotiorum, the causal organism of white rot disease of green bean pods, was obtained from Plant Pathology Dept., National Research Centre, Egypt and maintained on potato dextrose agar (PDA) for further study. Green bean pods cv. Valentino were obtained from the Department of Vegetable Crop Research, Agricultural Research Centre, Giza, Egypt.

2.1 Effect of different concentrations of EEP on growth inhibition of *S. sclerotiorum*

Ethanol extracted propolis at four concentrations *i.e.* 0, 5%, 10% and 15% were tested to study their effect on inhibition zone of *Sclerotinia sclerotiorum* growth. Sterilized filter paper disks were used as carrier material for testing chemicals. Disks (10 mm diameter) were dipped in tested concentrations of EEP, Ethanol or sterilized water then air dried and transferred to Petri plates containing PDA medium inoculated with mycelial suspension (10^6 cfu mL^{-1}) of *S. sclerotiorum*. Inoculated plates were incubated at 20°C for 5 days and diameter of inhibition zone (mm) was measured.

2.2 Effect of different concentrations of EEP on sclerotia germination of *S. sclerotiorum* and white rot incidence of green bean pods during storage

The same concentrations of EEP were tested to study their effect on sclerotia germination of *S. sclerotiorum*. The fungal isolate was grown on PDA medium and incubated at 20°C for 14 days. Sclerotia were harvested and sterilized using ethanol 70% for one minute. Sclerotia were then dipped in the tested concentrations of EEP, Ethanol or sterilized water for one minute, air dried and transferred to Petri plates containing PDA medium. Inoculated plates were incubated at 20°C for 7 days and percentage of sclerotia germination was calculated. Five sclerotia per plate and ten plates for each treatment were used. Similarly, the effect of EEP on white rot disease of green bean pods during storage was examined.

2.3 Inoculation of the pods

Inoculum of *S. sclerotiorum* was prepared as a mycelial suspension. The fungus was grown on PDB medium at 20˚C for 10 days, and then mass of growth was blended with sterilized distilled water to get mycelial fragment (Soltan, 1993). Inoculum concentration of *S. sclerotiorum* was prepared as mycelial suspension and adjusted to about 10^6 cfu mL^{-1}. Apparently healthy green bean pods cv. Valentino, obtained from EL-Behera Governorate, were surface sterilized by dipping in 70% ethyl alcohol for one minute and washed several times with sterilized distilled water then dried at room temperature. Sterilized bean pods were treated with EEP at the four concentrations by dipping in the tested suspension of EEP then air dried. Dipped sterilized green bean pods or ethanol served as controls. Treated green bean pods were inoculated with prepared inoculum using an atomizer at the rate of 100 mL suspension (10^6 cfu mL^{-1}) per fifty gram green bean pods. All treatments were kept in foam tray (23×12×4 cm). Four replicates were used for each treatment and stored for 7 days at 20±1°C. After seven days, green bean pods of all treatments were examined. Disease incidence was measured as the percentage of number of infected pods to the total number of pods in the treatment. Disease severity was estimated by determining the weight percentage (g) of infected pods compared to the total weight of the treatment according to Spalding and Reeder (1974) as follows:

$$\text{Severity (\%)} = \frac{\text{Weight of infected parts of diseased pods}}{\text{Total weight of the treatment}} \times 100$$

2.4 Statistical analysis

Tukey test for multiple comparison among means was utilized (Neler et al., 1985).

3 Results

3.1 Effect of EEP concentrations on inhibition zone of *S. sclerotiorum* growth

EEP at four concentrations *i.e*. 0, 5%, 10% and 15%

showed different inhibition zones for *S. sclerotiorum* growth. Results in Table 1 indicate that all tested concentrations of EEP significantly ($P \leq 0.05$) increased the inhibition zone of *S. sclerotiorum* growth. The highest increase was obtained with EEP at 15% which recorded 2.8 mm as inhibition zone of *S. sclerotiorum* growth followed by EPP at 10% and then 5%.

Table 1 Effect of different concentrations of Ethanol-extracted propolis (EEP) on inhibition zone (mm) of *Sclerotinia sclerotiorum* growth

EEP, %	Zone of inhibition growth, mm
5.0	2.0 c
10.0	2.4 b
15.0	2.8 a
Ethanol	0.0 d
Water	0.0 d

Note: Figures with the same letter are not significantly ($P \leq 0.05$) different.

3.2 Effect of EEP on sclerotia germination and rot incidence of green bean pods

EEP at the four concentrations was used to study its effect on sclerotia germination of *S. sclerotiorum*. Results in Table 2 indicate that all tested concentrations of EEP significantly ($P \leq 0.05$) reduced the percentage of sclerotia germination of *S. sclerotiorum*. Complete reduction in sclerotia germination was obtained with EEP at 15%. The highest reduction was obtained with EEP at 10% which reduced the sclerotia germination by 91.6%. EEP at 5% showed moderate effect. Testing EEP on white rot disease of green bean pods indicated that all the tested concentrations significantly reduced the percentage of white rot incidence and severity during storage (Tables 3 and 4). The highest reduction was obtained with EEP at 15% which reduced the disease incidence and severity by 90% and 91.8% respectively, followed by EEP at 10% which reduced the disease incidence and severity by 78% and 82% respectively. EEP at 5.0% showed moderate effect.

Table 2 Percent of sclerotia germination of *S. sclerotiorum* as affected with different concentrations of Ethanol-extracted propolis (EEP).

EEP, %	Sclerotia germination, %	Reduction, %
5.0	42.0 b	55.8
10.0	8.0 c	91.6
15.0	0.0 d	0.0
Ethanol	95.0 a	-
Water	95.0 a	-

Note: Figures with the same letter are not significantly ($P \leq 0.05$) different.

Table 3 White rot incidence of green bean pods as affected with different concentrations of Ethanol-extracted propolis (EEP)

EEP, %	White rot incidence, %			Reduction, %
	Days after storage			
	3	6	9	
5.0	18.0 b	38.0 b	48.7 b	51.3
10.0	4.0 c	11.0 c	22.0 c	78.0
15.0	0.0 d	4.0 d	10.0 d	90.0
Ethanol	31.0 a	72.0 a	100.0 a	0.0
Control	32.0 a	72.0 a	100.0 a	0.0

Note: Figures with the same letter are not significantly ($P \leq 0.05$) different.

Table 4 White rot severity of green bean pods as affected with different concentrations of Ethanol-extracted propolis (EEP)

EEP, %	White rot severity, %			Reduction, %
	Days after storage			
	3	6	9	
5.0	12.0 b	31.0 b	42.0 b	58.0
10.0	3.2.0 c	9.0 c	18.0 c	82.0
15.0	0.0 d	3.0 d	8.2 d	91.8
Ethanol	27.0 a	62.0 a	100.0 a	0.0
Control	29.0 a	65.0 a	100.0 a	0.0

Note: Figures with the same letter are not significantly ($P \leq 0.05$) different.

4 Discussion

Green bean pods are subjected for decay caused by a variety of fungi mainly, *S. sclerotiorum, B. cinerea,* and *P. aphanidermatum* for extended periods. Such periods start with their growth in the field; expand through storage, transportation and marketing till even before direct consumption. In this respect, Fahiem (2010) isolated seven genera of the common mold fungi from naturally infected green bean pods collected from different Egyptian Governorates. These isolated genera were identified depending on their morphological characters. The highest number and frequency of recorded isolates were related to *S. sclerotiorum.* On the other hand, the present study examined propolis against such pathogens with a clear aim at avoiding environmental pollution and health hazards resulting from chemical fungicides. It possesses many of the above-mentioned biological properties, and has been used for pharmacological applications. Our results indicated that all tested concentrations of EEP significantly increased the inhibition zone of *S. sclerotiorum* growth. The highest increase was obtained with EEP at 15%. Also, results

revealed that all tested concentrations of EEP significantly reduced the percentage of sclerotia germination of the fungus. Complete reduction in sclerotia germination was obtained with EEP at 15% but EEP at 10% reduced the sclerotia germination by 91.6%. In this regard, EEP treatment inhibited *P. digitatum* growth in vitro (Soylu et al., 2004, 2008) and limited the growth of *B .cinerea* on strawberry (La Torre et al., 1990). Also, complete inhibition of naturally occurring green mold disease on wounded and uninoculated grapefruits was reported (Soylu et al., 2004, 2008).

Our results revealed also that all tested concentrations of EEP significantly reduced the percentage of white rot incidence and severity during storage. The highest reduction was obtained at 15% EEP which reduced the disease incidence and severity by 90% and 91.8% respectively. Ozdemir et al. (2005) found that EEP treatment resulted in a slightly lower incidence of fungal decay in Fremont mandarins than in control fruits during the storage period. Also, Çandır et al. (2009) indicated that EEP could provide inhibition of fungal decay in cherries for four weeks of storage, which is a sufficient time for marketing. Moreover, propolis lessened water loss which is the most important factor in the storage of fruits and vegetables; usually accounts for a large portion of the total weight loss observed. That is probably because EEP treatment covered the surface of the fruits and pods. Similar results were obtained in other studies that tested variability in weight loss when treatments and products that covered the surface of fruits were applied (Hagenmaier and Baker, 1995; Ozdemir and Dündar, 2001). Similarly, Ozdemir et al. (2005) and Çandır et al. (2009) reported that weight loss was lower in propolis-treated cherries during storage. Propolis treatment did not cause physiological disorders in cherries during 4 weeks of storage (Çandır et al., 2009), but Ozdemir et al. (2005) reported that propolis treatment reduced physiological disorders in mandarins during 4 months of storage. Çandır et al. (2009) reported that propolis treatment had little or no harmful effect on skin lightness in cherries during storage.

In conclusion, further studies are guaranteed on EEP treatment against white rot attacking green bean pods before it can be commercially used within integrated management of pathogens to reduce fungal decay of the pods. The deployment of such safe chemical fungicide alternatives against several plant pathogenic fungi before their development into registered, ready-for-sale plant protection products are desperately needed.

Acknowledgement

This study was supported in part by NRC In-house project entitled ‘Pesticide alternatives against soilborne pathogens attacking legume cultivation in Egypt.

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