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## Effect of Treatment with Plant Lectins on Postharvest Strawberry Fruit-Rot

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### ABSTRACT



Eight lectins, four of which were used in pure forms, *i.e.* lectins of white bean seeds, red bean (Adzuki) seeds, pea seeds and lentil seeds. The other four were used in crude forms for the first time, *i.e.* lectins of white bean seeds, soybean seeds, moringa seeds and orchid tree seeds. All plant lectins tested *in vitro* against the growth of three fungi that cause strawberry fruit-rot, *i.e. Botrytis cinerea, Corynespora cassiicola* and *Alternaria alternata.* The crude lectin exhibited high ability to inhibit radial growth of these fungi compared with the pure lectin, this may be due to the presence of other effective compounds besides lectins in the extract. There was congruence in the general effect of both tested crude and pure lectins on fungal growth, where *Botrytis* growth was the most affected by any used lectin. Moringa crude lectin was high efficient in inhibiting the radial growths with 80.00%, 71.33% and 53.00%, respectively. Postharvest spraying of strawberry fruits with crude lectins succeeded in reducing soft-rot severity compared to control under laboratory conditions outside refrigerators. Fungal treating *in vitro* with crude lectins was consistent with postharvest fruit treating, where moringa crude lectin showed high soft-rot inhibition efficiency (70.40, 66.28 and 30.93%). The results indicated possibility of exploiting crude plant lectins to discourage postharvest fruit-rot and it is tempting to repeat the experiment on plants in the greenhouse and then in the field if the necessary quantities of lectins are available when lectin production gene is transferred to a fast-developing microorganism.

*Keywords:* Crude plant lectins, Postharvest strawberry fruit-rot, *Botrytis cinerea, Alternaria alternata, Corynespora cassiicola.* 

### INTRODUCTION

Strawberry (Fragaria ananassa Duch.) is one of the most important members of the family Rosaceae. It has become one of the most economic vegetable crops in Egypt and considered the main cash crop in Qalyubia, Ismailia, Sharqia and Beheira Governorates moreover, it is one of the most favorite and delicious fruits of which the demand has been increased in Egypt for local consumption and exportation. It is also occupies an important position among the nontraditional vegetable crops due to its multifarious use for local fresh consumption and food processing. Potentially, it is one of the most profitable horticultural Egyptian exports to Europe (El-Shal et al., 2003). Strawberry is the most important grown fresh market vegetable worldwide in America, Turkey, Spain, and Egypt as the four first producers, respectively (FAO, 2017). Under Egyptian conditions, strawberry is liable to be attacked by several diseases which are responsible for considerable quantitative and qualitative losses in the fruit yield. Fungi are the major causative of many diseases such as leaf spots, blotch, scorch and fruit rots.

Elad *et al.* (2016) reported that grey mold fungus, *Botrytis cinerea* attacks about 1,000 known plant species and causes pre-and postharvest losses of cultivated fruits, vegetables, and ornamental flowers. Valda *et al.* (2009) reported that *B. cinerea* was the most fungus detected on the damaged fruits and flowers of strawberry. *B. cinerea* was detected on samples with damages like pale brown fruit, dead flowers and ovaries and dark brown spots on pedicles.

Corynespora cassiicola was recorded as a causal agent to fruit rot on tomato as original host and okra and bell

the most dwide in in industrialized countries and more than 50% in developing

countries (Haggag, Wafaa, 2013). Disease management is based principally on chemical control, but fungicide application may cause problems such as toxic residues on the fruits and selection of resistant isolates of the pathogen (Ma and Michailides, 2005; Myresiotis *et al.*, 2007 and Pande *et al.*, 2010).

pepper as other hosts. (Caetano et al., 2018). Gajbhiye and

Kapadnis (2019) reported that Corynespora cassiicola

able to be present inside stems, leaves, flowers, fruits, and

seeds. It may trigger obvious disease symptoms in the pre-

harvest period or remain quiescent until the post-harvest

period. It is difficult to control because it has a broad host

range, various attack modes, and both asexual and sexual

Many of fungi infected strawberry fruit causing fruit

Fillinger and Elad (2016) reported that B. cinerea is

causing fruit rot on pomegranate fruits in India.

rot Alternaria alternata is sever (Dignand, 2004)

Additionally, fungicide application at flowering may reduce pollen viability and consequently hinder fruit formation (Kovach *et al.*, 2000).

Control of fungal pathogens is based on the use of agronomic practices and pesticides, but the widespread application of chemicals inundates the agro-ecosystems with toxic compounds that affect the balance of the natural food chain. The main problem regarding treatment in the post-harvest period is the presence of toxic residues on fruit, due to the short time elapsing between treatment and

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consumption. Subsequently, there real need to find new methods to control the pathogens.

Lectins are proteins found naturally in all living systems, whether high or low, that are highly specialized in binding to specific carbohydrates, are soluble in water, often withstand high temperatures and extremes of pH without being damaged (Van Damme *et al.*, 1998).

Many authors reported the importance of lectins in medicine such as in the classification of red blood cells (Furukawa *et al.*, 1995), treatment of acute wounds (Bird and Wingham, 1983) and curing of incurable diseases such as cancer (De Mejia and Prisecaru, 2005) AIDS (Mazalovska and Kouokam, 2018), Corona virus (Keyaerts *et al.*, 2007) and C virus through virus restriction and antiviral reverse transcription, and in the field of human immunity in general as an immune receptor (Lepenies and Lang 2019).

While in the agriculture filed lectins were used in Protecting plants which naturally rich in lectins such as legumes from microbial infection, as they act as receptors on the surface of plant cells, recognizing microbes and alerting the immune system to confront them (Gautam *et al.*, 2018).

In Ukraine," Plant Protection Institute" at Kyiv carried out several researches since 2008 (Sergienko *et al.*, 2008), the Institute scientists succeeded in producing a commercial preparation that works as a fertilizer and a natural pesticide called (Azolec) which combines cells of *Azotobacter* bacteria, with some lectin molecules (Kyrychenko, 2021).

There remains the hope of providing large quantities of lectins that are sufficient for spraying on plants, for protecting the environment and saving the cost of pesticides.

### MATERIALS AND METHODS

#### Microorganisms

Three fungal isolates used in the bioassay for plant lectin antifungal activity, *i.e. Botrytis cinerea*, *Corynespora cassiicola* and *Alternaria alternata* isolated from leaves and fruits of strawberry. The identification for these fungi was dependent on their morphological features and the microscopical characteristics according to the descriptions of Gilman (1957), Ames (1961) and Barnett and Hunter (1972) and they verified using molecular characterizations based on 18S rDNA. Fungi used in the study were registered in Gene Bank under accession numbers: OL449703, OL449702 and OL441338 respectively (Soliman H. Y. *et al.*, unpublished data).

### Extraction of crude plant lectins

Four Egyptian cultivar seeds of white bean (*Phaseolus vulgaris*), soybean (*Glycine max*), moringa (*Moringa oleifera*) and orchid tree (*Bauhinia variegata*) were obtained from the Department, Horticultural, Faculty of Agriculture, AL- Azhar University.

The crude plant lectins were prepared according to the method of (Hou *et al.*, 2010). Seed samples (each about 5 g) of white bean, soybean, moringa and orchid tree were grinded with 50 ml of 0.15 M sodium chloride solution (0.85% salt) to maintain the pH required for adhesion activity, filtered in gauze to separate the insoluble materials, and subjected to centrifugation for 20 minutes at 5000 rpm to obtain a supernatant solution containing crude lectin.

### Pure plant lectins

Four pure lectins, *i.e. Phaseolus vulgaris* (white bean seeds) lectin, *Vigna angularis* cv. Adzuki (red bean seeds) lectin, *Pisum sativum* (Pea seeds) lectin and *Lens culinaris* (lentil seeds) lectin were obtained by friendly from

Prof. Dr. Farouk El-Wagih Y. EL-Banoby, Prof. of Plant Pathology at faculty of agriculture, Al-Azhar University and the first Egyptian studied about lectins at Germany in the 1960s. Efficacy of crude plant lectins were comparative with pure plant lectins *in vitro*.

## Effect of plant lectins (pure and crude) on fungal radial growth *in vitro*

Pure cultures 7 days age of fungal isolates *B. cinerea*, *C. cassiicola* and *A. alternata* were used in experiments. The tested lectins were sterilized by syringe filter and added to PDA medium before hardness according to (El-Ghaouth *et al.*, 1992). Each Petri dish contained 20 ml of PDA medium and received 50  $\mu$ l lectin concentration 100mg/1ml. Four replicates were prepared for each test lectin. The free lectin dishes used as control treatments. Each Petri dish was inoculated by one disk (0.5 cm) from pathogenic fungal culture. The plates were incubated at 28  $\pm$  2 °C until the growth of the pathogen covered completely the control plates.

The inhibition of the pathogens growth was taken as an index of antagonistic ability which was calculated according to Zhou and Reeleder (1990) as follows:

### $I = (R_1 - R_2 / R_1) \times 100$

Where: I = Percent of inhibition of fungal growth,  $R_1$  = Fungal growth of the control and  $R_2$  = Fungal growth of the treatment.

### Effect of crude lectins on postharvest strawberry fruit rot

Visual healthy strawberry fruits (Fragaria ananassa vc. Festival) were sterilized by 70% ethanol alcohol for 10 seconds then rinsed in sterilized water several times. Sterilized fruits were dried on sterilized filter paper for 30 moments. Fifty sterilized strawberry fruits were sprayed with 60 ml of the test crude lectin concentration 100mg/1ml for every treatment. Fifty fruits were sprayed with sterilized distilled water alone (not infected) and served as a negative control treatment while another fifty fruits were sprayed with sterilized distilled water and inoculated by pathogenic fungi and served as a positive control treatment. Fruits were separated and placed in disinfested plastic dishes  $10 \times 10$ cm. Treatments (without negative control) were inoculated artificially by one disk 0.5 cm per fruit from pathogenic fungi (B. cinerea, C. cassiicola and A. alternata) and saved under laboratory conditions. The dishes were checked daily to observed soft rot fruits. The percentage of fruits soft rot disease was counted after 7 days.

Assessment of rot degree on the fruits according to Townsend and Heuberger (1943), was depended on the percentage of an average diameter of the infected area from the surface of treated fruit by using the follow of numerical rates: 0 = No rot.

1 = Scattered small rot.

2 =Rots coalescing and including about 25-50 % fruit area. 3 =More than 50% of the fruit area was infected.

To calculated the disease severity, followed the equation suggested by (Townsend and Heuberger, 1943) as follows:

Disease Severity % =	$\sum (n \times r1) + (n \times r2) + (n \times r3)$		
	3N×100		

Where (n) is the number of fruits in each numerical rate; r1, r2 and r3 are ratings and (N) is the total number of inoculated fruits multiplied by the maximum numerical rate 3.

#### Statistical analysis

All the data in the present study, were subjected to analyzed by one-way analysis of variance (ANOVA) by Costat version 3.3 and mean separations were performed by least significant differences (LSD) (Anon, 1986).

### **RESULTS AND DISCUSSION**

### Results

# Effect of plant lectins (pure and crude) on fungal radial growth *in vitro*

In vitro, the mycelial growth inhibition was recorded after five days. Crude lectins had inhibition activity against

the tested fungi. The results showed that, crude lectin of moringa seeds was the best efficient in growth inhibition of *B. cinerea*, *C. cassiicola* and *A. alternata* with a percentages of 80.00%, 71.33% and 53.00% respectively, followed by white kidney bean seeds lectin and orchid tree seed lectin, while the least effect was of soybean crude lectin (Table 1& Fig. 1).

 Table 1. Effect of four sterilized crude lectins of white bean (Phaseolus vulgaris), soybean (Glycine max), moringa (Moringa oleifera) and orchid tree (Bauhinia variegata) on radial growth of B. cinerea, C. cassiicola and A. alternata in vitro.

Europe		Growth inhibitory % by crude lectin of seeds					
Fungus	White bean	Soybean	Moringa	Orchid tree	Control		
B. cinerea	66.66	64.44	80.00	60.00	00		
C. cassiicola	56.66	26.66	71.33	53.33	00		
A. alternata	56.33	16.66	53.00	50.00	00		

LSD at 5% with white bean= 0.37, soybean= 0.08, moringa= 0. 52 and orchid tree= 0.29



Fig. 1. Effect of four sterilized crude lectins of white bean (*Phaseolus vulgaris*), soybean (*Glycine max*), moringa (*Moringa oleifera*) and orchid tree (*Bauhinia variegata*) on radial growth of *B. cinerea*, *C. cassiicola* and *A. alternata in vitro*.

Antifungal activity of pure lectins from seeds of white kidney bean, red bean, pea and lentil was showed that white kidney bean lectin was inhibited *B. cinerea* growth with 71.42%, while the pea lectin was inhibited *A. alternata* 57.50% and red bean lectin was inhibited *C. cassiicola* 50.00%. Lentil lectin was the lowest efficient inhibited 25.00% with *C. cassiicola* (Table 2 & Fig. 2). **Effect of crude lectins on postharvest strawberry fruit rot** 

The results in Table (3) showed that all the tested crude lectins were effective in reducing postharvest strawberry fruit rot severity. Moringa crude lectin showed soft-rot inhibition efficiency (70.40, 66.28 and 30.93%), white bean lectin showed inhibition efficiency (68.53, 61.72 and 53.84%), while orchid tree seed lectin showed inhibition efficiency (39.99, 47.43 and 38.52%) and soybean lectin showed inhibition efficiency (30.93, 14.28 and 15.38 %).

The best crude lectins in reducing the severity of fungal infection were moringa lectin then white kidney bean lectin against *B. cinerea*, while the least effective crude lectins were soybean lectin against *C. cassiicola.* (Fig. 3).

Fable	2. Effect of four sterilized pure lectins of white
	bean seeds (Phaseolus vulgaris), red bean seeds
	(Vigna angularis cv. Adzuki), Pea seeds (Pisum
	sativum) and lentil seeds (Lens culinaris) on
	radial growth of B. cinerea, C. cassiicola and A.
	alternata in vitro.

Energy and	Growth inhibitory % by pure lectin of seeds					
Fungus	White bean	Red bean	Pea	Lentil	Control	
B.cinerea	71.42	75.14	65.71	62.85	00	
C. cassiicola	37.50	50.00	27.50	25.00	00	
A. alternata	50.00	40.00	57.50	52.50	00	
LSD at 5% v	vith white be	an=0.25, red	bean=	0.12, Pea	a=6.01 and	

LSD at 5% with white bean=0.25, red bean= 0.12, Pea=6.01 and Lentil=1.72



Fig. 2. Effect of four sterilized pure lectins of white bean seeds (*Phaseolus vulgaris*), red bean seeds (*Vigna angularis* cv. Adzuki), Pea seeds (*Pisum sativum*) and lentil seeds (*Lens culinaris*) on radial growth of *B. cinerea*, *C. cassiicola* and *A. alternata in vitro*.

 Table 3. Effect of crude lectins, white bean (*Phaseolus vulgaris*), soybean (*Glycine max*), moringa (*Moringa oleifera*) and orchid tree (*Bauhinia variegata*) on postharvest strawberry fruit rots severity.

Seed crude	B. cinerea		C. cassiicola		A. alternata	
lectin	Fruit rot severity%	Efficacy%	Fruit rot severity%	Efficacy%	Fruit rot severity%	Efficacy%
P. vulgaries	26.22	68.53	29.77	61.72	33.33	53.84
G.max	57.55	30.93	66.66	14.28	61.11	15.38
M. oleifera	24.11	70.40	26.22	66.28	57.55	30.93
B. variegata	50.00	39.99	40.88	47.43	44.40	38.52
Negative control	0.00	0.00	0.00	0.00	0.00	0.00
Positive control	83.33	0.00	77.77	0.00	72.22	0.00

LSD at 5%: Fungi = N.S; Treatment = N.S; Fungi \* Treatment = N.S.



Fig.3. Effect of crude lectins of white bean (*Phaseolus vulgaris*), soybean (*Glycine max*), moringa (*Moringa oleifera*) and orchid tree (*Bauhinia variegata*) on postharvest strawberry fruit rot severity.

### Discussion

All available experiments of lectin effects on fungal growth are laboratory experiments onto Petri dishes using pure lectins that are difficult to apply under field conditions. Lectins varies according to their type (Merolectin, Hololectin and Chimerolectin). Most fungicidal lectins are small monomeric lectins (Merolectins) (Van Damme *et al.*, 1998).

Physiologically, lectins impair the synthesis and /or deposition of chitin in fungal cell wall and thus inhibit its growth (Selitrennikoff, 2001), as well as affect nutrient uptake and inhibit the germination of fungal spores (Lis and Sharon, 1981& Hamid *et al.*, 2013). This was confirmed by Kumar *et al.* (2012) who indicated that the addition of wheat germ lectin led to arrest of fungal chitin synthesis and growth inhibition and spore germination inhibition of *Trichoderma viride*.

The lectins also cause several morphological changes that render the fungi susceptible to different stress conditions (Ciopraga *et al.*, 1999) such as swelling of the hyphae, unloading of cell content and lysis of the rhyming cell wall upon interaction, thus enhancing sensitivity to osmotic shock (Lis and Sharon, 1981). Moreover, some small lectins also directly penetrate the fungal cell wall and reach the cell membrane where they inhibit enzyme activity by binding to the active sites and thus affect cell wall morphogenesis.

Crude lectins isolated from plants were used for the first time in this study *in vitro* and to protect strawberry fruits against postharvest soft-rot. Fungal treating *in vitro* with crude lectins was consistent with postharvest fruit treating. *Moringa* seed lectin was the best efficient in growth inhibition of *B. cinerea*, *C. cassiicola* and *A. alternata*. This result is in agreement with the results of many researches. *Botrytis cinerea* was inhibited *in vitro* by many monomeric lectins, *i.e. Urtica dioica* lectin (Broekaert *et al.*, 1989) and *Hevea brasiliensis* lectin (Van Parijs *et al.*, 1991), *Solanum tuberosum* lectin (Gozia *et al.*, 1993) *Astragalus mongholicus* lectin (Yan *et al.*, 2005), *Amaranthus viridis* lectin (Kaur *et al.*, 2006) and *Gontanthus pumilus* lectin (Dhuna *et al.*, 2007).

Alternaria alternata was inhibited in vitro by red kidney bean lectin (Alizadeh et al., 2011), while as for *Corynespora cassiicola*, this is the first record for the lectin effect on this fungus.

Available references indicated that *Phaseolus vulgaris* lectin is effective against some pathogenic bacteria and fungi such as *Staphylococcus aureus*, *Streptococcus mutants*, *Pseudomonas aeruginosa* and *Candida albicans* (Hamed, Einas *et al.*, 2017). Also, *Glycine max* lectin is effective against some pathogenic fungi such as *Fusarium oxysporum* and *Rhizoctonia solani* (Mohsen, Soad *et al.*, 2018). While *Moringa oleifera* lectin has antifungal effect against some fungi such as *Aspergillus niger*, *A. flavus*, *Candida albicans* (Ayirezang *et al.*, 2020 and Santos *et al.*, 2021). Moreover, *Bauhinia* seeds have effective lectin against many pathogenic fungi such as *Penicillium crysogenum*, *Aspergillus niger*, *Fusarium solani*, *F. moniliforme* and *Colletotrichum lindemuthianum* (Oconnell, 1991; Souza *et al.*, 2011; Moreira *et al.*, 2013 and Silva *et al.*, 2014).

Much research indicates that lectins are a promising tool for inhibiting the growth of pathogenic fungi, for example (Gozia *et al.*, 1993) reported that potato lectin affects against early developmental stages of *Fusarium oxysporum*, this lectin does not inhibit mycelial growth but irreversibly inhibits conidia germination and alters the germ tubes. Also, (Ye *et al.*, 2001) reported that red bean lectin exerted a suppressive effect on growth of fungal species, Fusarium oxysporum, Coprinus comatus and Rhizoctonia solani, the lectin had low ribonuclease and negligible translation-inhibitory activities. Similarly, (Graças et al., 2002) stated that Talisia esculenta seed lectin showed an antifungal effect on Fusarium oxysporum, Colectrotrichum lindemuthianum and Saccharomyces cerevisiae. Also, (Tian et al., 2008) reported that Ophiopogon japonicus root lectin exhibited antifungal activity against Gibberella saubinetii and Rhizoctonia solani. Petnual et al. (2010) stated that Curcuma longa rhizome lectin inhibited fungal growth of Colectrotrichum cassiicola, also Biswas and Chattopadhyaya (2015) stated that the same lectin of Curcuma longa inhibit fungal growth of Exserohilum turicicum and Fusarium oxysporum. Similarly, (Karnchanatat, 2012) stated that Artocarpus gradifolia seed lectin showed growth inhibition of Fusarium moniliforme and Saccharomyces cerevisae.

Lectins of legumes such as Astragalus mongholicus, Phaseolus coccineus, Archidendron jiringa, Bauhinia variegata, Glycine max and Indigofera heterantha were more efficient than others in inhibiting fungal growth such as Colletotrichum spp., Drechslera turcia, Exserohilum turcicum and the human pathogenic fungi, Candida albicans and Penicillium italicum and Aspergillus spp. (Rao et al., 1998; Sharon and Lis, 2001; Qadir et al., 2013; Boleti et al., 2007; Chen et al., 2009 and Yan et al., 2009).

Due to the importance of the activity of lectins, several studies have sought to genetically modify plants to protect them from pathogens and insects by introducing the lectin gene to them, such as introducing the *Urtica dioica* lectin gene into tobacco plants to reduce the spore germination of *Botrytis cinerea*, *Colletotrichum lindemuthianum* and *Trichoderma viride* (Does *et al.*, 1999).

There remains the hope of providing large quantities of lectins that are sufficient for spraying on plants by transferring the appropriate lectin gene to one of the easy-todevelop organisms such as bacteria *E. coli* or yeast *Pichia* and then using the genetically modified isolates when needed to produce lectins, and this is not difficult in return for protecting the environment and saving the cost of pesticides.

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