The African Journal of Plant Science and Biotechnology ©2013 Global Science Books

Evaluation of Preharvest Bioagent Applications for both Production and Biological Control of Onion and Strawberry under Natural *Botrytis* Infections

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ABSTRACT

Botrytis spp. is one of the most important airborne diseases for many plant hosts resulting in poor fruit quality and serious yield loss. This study was performed using different bioagent treatments under natural *Botrytis* infection experiments. Different varieties of strawberry and onion were sensitive to natural *Botrytis* infection more than tolerant varieties. *Botrytis* infection values were significantly different among strawberry organs, being higher in leaves than in stamens. In addition, despite *Botrytis* infection values not differing among different treatments in both strawberry and onion, fungicide was the least efficient treatment under natural conditions. Moreover, treatment with the bioagent yeast, *Pichia anomala*, showed an increase in strawberry flowers and fruits as well as in onion leaves and bulbs in the majority of plants. This study suggests that plant foliar application of microbial antagonists may be not an effective measure for controlling natural *Botrytis* infections but has large impacts on plant production and quality.

Keywords: *Bacillus licheniformis*, biocontrol, *Botrytis* spp., *Pichia anomala*, plant yield
Abbreviations: ATCC, American type culture cell; **CFU**, colony forming units; **EMCC**, Egyptian Microbial Culture Collection; **PDA**, potato dextrose agar; **PDB**, potato dextrose broth; **TSS**, total solid soluble

INTRODUCTION

Botrytis spp. cause serious losses in many crops (Sutton 1990). In strawberry, the fungus, *Botrytis cinerea*, attacks flowers, setting fruits, mature fruits, and leaves. The pathogen may cause latent infection until the fruits ripen. *Botrytis* fruit rot (*B. cinerea*) causing preharvest losses of up to 15% of the fruit on susceptible cultivars (Legard *et al*. 2000). Depending on the cultivar, untreated plants can quickly become infected, damaging both yield and fruit quality. In onion, many species of *Botrytis* have been associated with diseases of *Allium* crops; some of them have been associated with neck rot. Symptoms of neck rot typically develop only after onion bulbs have been harvested and placed in storage, even though infection occurs in the field. The predominantly quiescent nature of field infections (du Toit *et al*. 2004) complicates efforts to manage this disease and to predict losses in storage. Control of fruits and vegetables mainly depends on synthetic fungicides that are the primary means of controlling postharvest diseases. The fact that no cultivar is highly resistant to fruit rot by *Botrytis* spp. make the fungicide application important in order to protect plants from *Botrytis* infection (Legard *et al*. 2000; Li *et al*. 2010). Indeed, fungicide application on infected leaves and flowers could be the major disease management strategy for plant mold. However, alternative control means such as biological control has been used (Boff *et al*. 2002; Hang *et al*. 2005; Haïssam 2011). This is the main reason for the appearance of fungicide-resistant strains of *B. cinerea* (Elad *et al*. 1992; Yourman and Jeffers 1999; Leroux *et al*. 2010; De Miccolis *et al*. 2012). Currently, several antagonistic microorganisms have been identified with potential against a number of postharvest pathogens on a variety of harvested commodities. Food safety requires effective alternatives to fungicides to control pre- and postharvest diseases of fruits and vegetables. Presently, several promising biological approaches including microbial antagonists, naturally-occurring antifungal compounds, and induced resistance have been advanced as potential alternatives to synthetic fungicides for disease control. These treatment types pose no risk to human health or the environment among the proposed alternatives, bacterial and yeast bioagents. One of the bacterial biocontrol agents gaining most attention is the genus *Bacillus* as it produces broad-spectrum antibiotics and maintains viability for a long time as a result of endospore production (Emmert and Handelsman 1999; Tzeng *et al.* 2008). Interestingly, some *Bacillus* species also promote plant growth (Jeon *et al*. 2003; Nautiyal *et al*. 2006; Berg 2009). Previously, *B. licheniformis* was successfully used as an effective biofungicide for controlling gray mold in strawberry plants (Son *et al*. 2002; Lee *et al*. 2006; Kim *et al*. 2007; Essghaier *et al*. 2009). On the other hand, a yeast bioagent *Pichia* spp. is considered to be of widespread biotechnological importance ranging from human therapeutic protein production, food fermentations, biocontrol agents and biofuel production (Walker 2011). *Pichia anomala* is highly competitive and has shown strong biocontrol activity against a variety of fungi (Boysen *et al*. 2000; Druvefors *et al*. 2002; Druvefors and Schnurer 2005; Haïssam 2011). Due to its anti-microbial activity, it is traditionally viewed as a biopreservation agent (Jijakli 2010; Schnurer and Jonsson 2011). *P. anomala* suppresses the growth of several fungi (Masih *et al*. 2000; Laitila *et al*. 2007; Jijakli 2010; Haïssam 2011) as it has antimycotic properties. Consequently, it is being considered a valuable biocontrol agent against fungi of agronomical importance. Previously, *P. anomala* was an antagonist against *Botrytis* infections (Masih *et al*. 2000; Fredlund *et al*. 2004; Mohamed and Saad 2009; Haïssam 2011). Indeed, fungal biological control is an exciting and rapidly developing research area with

Received: 15 May, 2012. Accepted: 24 June, 2012.

Original Research Paper

implications for plant productivity, animal and human health and food production.

The combined use of two bioagents with a single but different biocontrol mechanism was less effective than that of a single bioagent with both mechanisms (Xu *et al*. 2011). Biological control with multiple mechanisms may be achieved by using one biocontrol agent exhibiting several mechanisms or by applying more than one biocontrol agent in a mixture, provided that each of them has one (or several) distinct mechanism(s). The aim of this study was to determine how the effect of one or a mixture of bioagent(s) on both crop protection and production under natural *Botrytis* infection could be used to reduce chemical treatments that cause damage to humans and the environment.

MATERIALS AND METHODS

Microorganisms and cultivation

Two biocontrol agents, yeast and bacterium, were provided from Cairo Microbiological Resources Centre (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University. The yeast, *Pichia anomala* (EMCC5) was grown on potato dextrose broth (Difco) for 24 to 48 h at 25°C before use. The bacterium, *Bacillus licheniformis* (ATCC11954) was grown on nutrient agar medium (Difco) for 24 h at 30°C. These bioagent cultures were separated from the medium by centrifugation (5.000 rpm for 10 min). Cell concentrations were determined and adjusted to 10^7 CFU/ml.

Pot and field experiments

An experiment utilizing naturally occurring *Botrytis* fungus on both strawberry and onion varieties was carried out during 2010-2011 at the Faculty of Agriculture, Ain Shams University, Egypt. Two varieties of strawberry seedlings used in this study, 'Festival' and 'Florida', were planted in pots (containing growth mixture with 70% peat and 30% vermiculite) and maintained under natural conditions using routine cultivation practices. Two onion varieties, 'Italiani' and 'Giza2', were grown in the field using routine cultivation practices. Each experiment included 10 plants per treatment with three replicates. The four treatments comprised a suspension of *B. licheniformis*, *P. anomala*, a mixture of these two bioagents and the fungicide Captan. Under natural conditions, disease incidence was rated without pathogen inoculation. Natural disease occurrence without any treatment or with only water treatment were the two controls in this experiment. All treatments were applied by a foliar spray weekly immediately after plant establishment and continued until final harvest. Disease occurrence was evaluated by testing samples of each plant organ on modified selective medium.

Preparation of selective medium

The modified selective medium, m1KERS, is the same medium composition previously developed (Abdel Wahab *et al*. 2010) but without adding pentachloronitrobenzene, which was the basis of KERS medium (Kerssies 1990). The composition of this modified medium, m1KERS, was (g/l): glucose, 20; $NaNO_3$, 1; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 0.2; KCl, 0.15; chloramphenicol, 0.05; tannic acid, 5; $CuSO_4$, 2.2; Pyraclostrobin (Cabrio Top fungicide) 0.1; agar, 25. This medium was then autoclaved at 121°C for 20 min. The mycelium growth and the brown halo formation of *Botrytis* spp. were tested against other fungal genera used as negative control, *Aspergillus niger* and *Penicillium expansum*, which were obtained from the collection of the Plant Pathology Department, Faculty of Agriculture, Ain Shams University.

Detection of *Botrytis* spp. on m1KERS medium assay

Strawberry and onion samples were tested for *Botrytis* infection using the modified selective media (m1KERS). To reduce the risk of cross-contamination, samples were dipped separately in sterile water for 5 min, dried on paper towels, then plated onto m1KERS and incubated at 23°C for 3-21 days. The samples were examined daily for development of *Botrytis* isolates which were transferred to potato dextrose agar (PDA; Difco) and preserved in paraffin oil at 4°C.

Characterization of strawberry and onion

Measurement of strawberry characteristics (leaves number, flowers number, fruits number, fruit weight) and also onion characteristics (leaves number, bulb nick diameter, bulb length, bulb weight) were taken. In addition, the hardness of strawberry fruits was determined using Texture Analyzer (TA-XT2; Stable Micro Systems Ltd., UK) and the maximum force was recorded as fruit's hardness. Total soluble solids (TSS) content of both strawberry fruits and onion bulbs was determined by measuring the refractive index of fruit juice with a hand-hold refractometer (WYT-4; Quanzhou Optical Instrument Co. Ltd., Quanzhou, China).

Statistical analysis

Data from the pot and field experiments, under production conditions, were analyzed using the General Linear Model (GLM) procedure (SAS 1996) in a complete randomized design. Duncan's multiple range test (P = 0.05) was used to compare means in the experiment.

RESULTS

Determination of *Botrytis* infection

Different plant organs were screened to investigate the appearance of brown halo formation around samples after incubation on m1KERS medium during 3-21 days. This brown halo formation resulted from *Botrytis* infection which appeared after 3 days of incubation (Abdel Wahab *et al*. 2010) whereas the negative control did not show any brown halo formation on m1KERS medium. **Table 1A** shows the overall mean values of *Botrytis* infection using different factors (treatment, organ, and variety type). The overall mean value of *Botrytis* infection in strawberry was generally higher in 'Festival' than in 'Florida'. In these two varieties, the highest infection value was determined in leaves, while the lowest one was showed in stamens (**Table 1A**). In addition, the highest overall mean value of *Botrytis* infection was demonstrated using fungicide treatment while the lowest one was showed using *B. licheniformis* treatment. Statistical analysis of *Botrytis* infection values demonstrated significant differences between 'Festival' and 'Florida' (**Table 1B**). Similarly, they were significantly differed among plant organs (**Table 1B**) but the infection values using different treatments were not significantly different. Moreover, **Table 1B** shows that infection incidence values were significantly influenced by the interaction among plant, variety and treatment type. This was due to the highest infection incidence in 'Festival' in comparison with 'Florida' (**Table 1A**). Similarly, The F value of *Botrytis* infection was significantly differed for the interaction effect between plant variety and organ type (**Table 1B**) while it was not significant for the interaction between treatment and organ types (**Table 1B**). In addition, the F value of *Botrytis* infection was not significantly influenced by the interaction among these three factors: variety, treatment and organ types (**Table 1B**).

Table 2A shows that the overall mean values of *Botrytis* infection in onion leaves were generally higher in 'Italiani' than in 'Giza2' variety except when using *P. anomala* treatment. In addition, the highest overall mean value of *Botrytis* infection was observed using fungicide treatment (**Table 2A**). Statistical analysis demonstrated significant differences for *Botrytis* infection between 'Italiani' and 'Giza2' (**Table 2B**) while each treatment type showed no significant effect on infection incidence in both plant varieties (**Table 2B**). Moreover, the interaction effect between plant variety and treatment type demonstrated no significant influence on *Botrytis* infection (**Table 2B**).

Table 1A Effect of biocontrol treatments on *Botrytis* infection in two strawberry varieties.

Var	**Festival**						**Florida**						
Trt	**St**	**C**	**F**	**L**	**O**	**P**	**St**	**C**	**F**	**L**	**O**	**P**	**Overall mean for Trt factor**
C1	0	0.276	0.145	0.866	0.500	0.166	0	0.481	0.433	0.831	-	0.108	0.396
C2	0	0.505	0.333	0.857	0.800	0.400	-	0.436	0.338	0.928	0.400	0.050	0.409
Bl	0	0.395	0.226	0.866	0.500	0.208	0	0.377	0.423	0.952	0.166	0.120	0.339
Pa	0	0.689	0.800	0.452	0.777	0.542	0	0.457	0.461	0.545	0.055	0.291	0.475
Bl+Pa	0	0.218	0	0.759	1	0	0	0.496	0.666	-	0.416	0.230	0.385
Fu	0.225	0.422	1	-	0.750	0.312	0	0.555	0.750	-	0.500	0.244	0.480
Overall mean for Org factor	0.025	0.447	0.454	0.805	0.536	0.202	0.025	0.447	0.454	0.805	0.536	0.202	0.025
Overall mean for Var factor	0.475						0.355						

(-) not detected value. C1 = no treatment, C2 = treatment with only water, Bl = treatment with *B. licheniformis*, Pa = treatment with *P. anomala*, Fu = treatment with fungicide (captan). St = stamen, C = calyx, F = fruit, L = leaf, O = ovary, P = petal. Trt: treatment, Org: organ, Var: variety.

Table 1B Determination of different factors effect and their interaction on the *F* values of *Botrytis* infection in strawberry.

Main factor effect	**Var**	**Trt**	**Org**	
F value	0.0006 (S)*	0.237 (N.S)**	0.0001 (S)*	
Interaction between factors effect	Var*Trt	Var*Org	Trt*Org	Var*Trt*Org
F value	0.013 (S)*	0.031 (S)*	0.182 (N.S)**	0.121 (N.S)**

(S) *: Significant differences, (N.S) **: Not significant differences. The analysis of variance was applied on infection incidence values, and statistical significance (*) was judged at $P < 0.05$. Trt: treatment, Org: organ, Var: variety

Table 2A Effect of biocontrol treatments on *Botrytis* infection in two onion varieties.

Trt	**Italiani**	**Giza2**	**Overall mean for Trt factor**
Bl	0.234	0.133	0.188
Bl+Pa	0.154	0.148	0.150
Fu	0.542	0.085	0.314
C2	0.341	0.041	0.191
C1	0.142	0.055	0.105
Pa	0.133	0.144	0.139
Overall mean for Var factor	0.262	0.108	

C1 = no treatment, C2 = treatment with only water, Bl = treatment with *B. licheniformis*, Pa = treatment with *P. anomala*, Fu = treatment with fungicide (captan). Trt: treatment, Var: variety.

Table 2B Determination of different factors effect and their interaction on the *F* values of *Botrytis* infection in onion.

Main factor effect	**Var**	**Trt**
F value	0.014 (S)*	0.452 (N.S)**
Interaction between factors effect	Var*Trt	
F value	0.182 (N.S)**	

(S) *: Significant differences, (N.S) **: No significant differences. * statistical significance was judged at $P < 0.05$.

Effect of bioagent treatments on plant characteristics of both strawberry and onion

Table 3A shows the overall mean values of strawberry characteristics (leaves number, flowers number, fruits number, TSS, fruit weight and hardness) after using different bioagent treatments (**Table 3A**). This table demonstrated increasing in all vegetative and fruit characteristics in both 'Festival' and 'Florida' using *P. anomala* treatment except for the number of leaves in 'Florida'. In addition, results showed that *F* values were highly significant in all vegetative and fruit characteristics except neither for the number of flowers in both 'Festival' and 'Florida' nor the number of leaves in 'Florida' (**Table 3A**). Furthermore, the *F* value resulted from the interaction between treatment type and plant variety were influenced significantly on fruit characteristics (fruit number, weight, hardness and TSS) (**Table 3B**).

Table 4A shows the overall mean values of onion characteristics (leaves number, bulb nick diameter, bulb length, bulb weight and TSS) after using different bioagent treatments (**Table 4A**). This table demonstrated that the overall mean values of these characteristics were higher after using *P. anomala* treatment than others in both 'Italiani' and 'Giza2'. In addition, results showed that the *F* values of bulb length and weight were significantly high in both 'Italiani' and 'Giza2'and also in leaves number in 'Giza2' (**Table 4A**). Furthermore, the *F* value of bulb weight was significantly influenced by the interaction between treatments and varieties (**Table 4B**).

DISCUSSION

This research aimed to study the application of a biofungicide at the pre-harvest stage under natural infection of *Botrytis* as bioagents can suppress a pathogen at the source, may reduce harmful microorganisms upon infection, and may protect the environment and human health (Tian *et al.* 2004; Ippolito *et al.* 2005; Mommaerts *et al.* 2011). Infection incidence values were significantly different among plant organs but were highest in strawberry leaves. This is in agreement with previous studies (Bulger *et al.* 1987; Braun and Sutton 1988). Moreover, the infection incidence values were higher in sensitive varieties of both strawberry and onion. In addition, all treatments did not significantly decrease *Botrytis* infection under natural condition in all plants. The highest infection values were shown using fungicide treatment in both strawberry and onion. This is perhaps due to in part to the development of pathogen resistance for chemical fungicide. Since this study was done under natural infection in the field, resistant strains against fungicide may exist. Furthermore, culture supernatant of bioagents could be more effective than bioagent cells (Kim *et al.* 2007; Wang *et al.* 2012). In fact, plant cultivation in an open field is a risk as it exposes the plant surface to the removal of the biofungicide by rainfall (Kim *et al.* 2007). This means that spray schedule of the biofungicide should be applied under plastic house or more than once a week in order to increase the effect of biofungicide application on the control of *Botrytis* spp. In general, yeasts have several important properties making them useful for biocontrol: (i) most are non-pathogens and do not produce mycotoxins or allergenic spores; (ii) most can utilize a broad range of nutrients, and many can grow at low water activity and oxygen levels. Biocontrol yeasts have been commercially available since 1995 as they inhibit the growth of *Penicillium* and *Botrytis* spp. on fruits (El-Neshawy 1997; Sui *et al.* 2012). Moreover, previous studies had shown the effect of the antagonistic yeast *Pichia* on the inhibition of growth of pathogenic fungi in cherry tomato fruit (Zhao *et al.* 2009, 2010). In contrast, another study showed no effect of using different yeasts on decreasing *Botrytis* infection (Robiglio *et al.* 2011). Reports on the efficacy of yeast and bacteria

Table 3A Effect of bioagent treatments on the vegetative and fruit characteristics of two varieties of strawberry.

Trt	Festival						Florida					
	No. of leaves	No. of flowers	No. of fruits	Fruit weight	T.S.S (%)	Fruit hardness	No. of leaves	No. of flowers	No. of fruits	Fruit weight	T.S.S (%)	Fruit hardness
C1	3	1	1	4.6	3	230	4	1	1	1.866	9	178
C2	4	1	1	2.1	4	158	5	1	1	1.533	9	159
Bl	5	1	2	13.4	6.5	175	5	1	1	8.466	8.7	130
Pa	7	2	4	22.2	7	258	4	2	5	16.733	14	235.33
Bl+Pa	4	1	2	16.2	7	235	4	1	3	9.166	13	224.66
Fu	4	1	1	12.5	6	120	4	1	2	8.133	10	99.66
F value	0.0064*	0.8891	0.0001*	0.0001*	0.0045*	0.0001*	0.8661	0.8571	0.0008*	0.0001*	0.0001*	0.0001*

C1 = no treatment, C2 = treatment with only water, Bl = treatment with *B. licheniformis*, Pa = treatment with *P. anomala*, Fu = treatment with fungicide (captan)
*: Significant differences, statistical significance was judged at $P < 0.05$. Trt: treatment.

Table 3B Demonstration of different factors affecting the vegetative and fruit characteristics of strawberry.

Overall mean values						
Factor type	**No. of leaves**	**No. of flowers**	**No. of fruits**	**Fruit weight**	**TSS (%)**	**Hardness**
Florida	4.50	1.167	2.16	7.65	10.61	171.1
Festival	4.50	1.000	2.00	11.85	5.58	196.2
F value of Trt*Var interaction	0.106	1	0.016*	0.0001*	0.0001*	0.0001*

Trt: treatment, Var: variety, *: Significant differences, statistical significance was judged at $P < 0.05$.

Table 4A Effect of different bioagent treatments on the plant characteristics of two varieties of onion.

Trt	Italiani					Giza2				
	No. of leaves	Bulb neck diameter (cm)	Bulb length (cm)	Bulb weight (g)	TSS (%)	No. of leaves	Bulb neck diameter (cm)	Bulb length (cm)	Bulb weight (g)	TSS (%)
C1	7	2.01	64.00	113	4.83	6	1.3	64	71	5
C2	7	2.03	66.00	116	4.50	6	1.5	65	73	5
Bl	9	2.16	70.00	168	4.43	7	2.0	70	108	6
Pa	11	2.40	75.00	178	5.00	9	2.3	72	114	6.5
Bl+Pa	9	2.20	64.00	158	5.00	7	1.8	62	103	6.4
Fu	8	2.30	70.00	160	5.00	7	1.4	65	75	5
F value	0.0014*	0.1103	0.0009*[1]	0.0001*	0.8495	0.4967	0.1234	0.0185*	0.0001*	0.8606

C1 = no treatment, C2 = treatment with only water, Bl = treatment with *B. licheniformis*, Pa = treatment with *P. anomala*, Fu = treatment with fungicide (captan).
*: Significant differences, statistical significance was judged at $P < 0.05$.

Table 4B Demonstration of different factors effect and their interaction on onion characteristics.

Overall mean of variety					
Factor type	**No. of leaves**	**Bulb neck diameter (cm)**	**Bulb length (cm)**	**Bulb weight (g)**	**TSS (%)**
Italiani	8.50A	2.18A	68.3A	148.83A	4.79B
Giza 2	6.83B	1.71B	66.3B	90.66B	5.65A
F value for Trt*Var interaction	0.594	0.323	0.681	0.001*	0.374

Trt: treatment, Var: variety, *: Significant differences, statistical significance was judged at $P < 0.05$.

vary, as in some studies they resulted in low to moderate disease suppression, whereas their efficacy in other studies was high (Guetsky *et al*. 2001). The combination of complementary biological techniques for additional and/or synergistic effects could improve the efficacy of biological control. It is important that evaluation of these microorganisms be carried out in a product formulation because the formulation may improve or diminish antagonistic efficacy depending on the concentration of chemical product and the duration of exposure to the treatment (Marie *et al*. 2003). The degree of control obtained by these microorganisms alone is often not satisfactory, so the addition of chemical fungicides at low rates can enhance biocontrol. In addition, a combination of chemical treatment with antagonistic yeast (Buck 2004) or heat treatments (Schirra *et al*. 2011) reduced disease severity caused by a fungal pathogen. Fungicide treatment can reduce decay but effectiveness decreases with the appearance of resistant strains. It is equally important to determine how the interaction between antagonist–pathogen–host and environment can be manipulated to favour antagonistic activity of the agents introduced (Marie *et al*. 2003). In fact, the insufficient efficacy of bioagents against preharvest diseases in practical conditions is still an important factor limiting the implementation of biocontrol methods as the variation of the weather conditions explains the lack of stable and reproducible effectiveness of biological control methods in the field (Jijakli 2011). The lack of effectiveness can be due to the inadequacy and/or the variations of the environmental conditions limiting the effectiveness of the agents of biological control, but also to the difficulty in maintaining the effectiveness of the antagonist for one sufficiently long period (Jijakli 2011).

On the other hand, there was a positive effect of the yeast treatment, *P. anomala*, on flower number, fruit number, fruit weight, TSS and hardness in all treated strawberry plants and also on onion characteristics (leaf number, bulb length and weight and TSS). In fact, there is little information about the efficacy of pre-harvest spraying with *P. anomala* on postharvest decay especially in fruit quality (Zhao *et al*. 2011). In general, few studies investigated the effect of the treatment of biological control agents on both plant prevention of disease occurrence and increasing yield (Utkhede and Mathur 2002; Huang and Erickson 2002; Mommaerts *et al*. 2011) or plant growth promotion (Jeon *et al*. 2003; Nautiyal *et al*. 2006). Previously, quality parameters of treated apples by *P. anomala* had been studied (Jijakli 2011). Other postharvest yeast treatments showed a positive effect of controlling natural *Botrytis* infection without impairing quality parameters of pear fruits (Zhang *et al*. 2008). In contrast, no studies had shown the effect of preharvest spraying with either *P. anomala* or *B. licheniformis* on the quality of strawberry fruit and onion bulb. These encouraged results conducted at least to use *P. anomala* for increasing plant yield and quality. In addition, it may be useful for using biocontrol treatment of natural *Botrytis* infections under specific environmental conditions, because of sensitivity of the biocontrol agents to environmental influences (Elad 2003), or application timing as postharvest applications are more effective than preharvest applications assuming that each one of them has different ecological requirements (Sharma *et al*. 2009).

CONCLUSION

Under natural *Botrytis* infection, results showed significant differences for infection incidence depending on plant variety and organ in both strawberry and onion. All treatments were not differed significantly for decreasing *Botrytis* infection. Contrarily, Treatment with the antagonist yeast *P. anomala* showed significant increasing in flower and fruit characteristics of strawberry. Similarly, onion plants treated with *P. anomala* had showed positive effect on the majority of plant characteristics (leaves and bulbs). Indeed, the preharvest treatment with the yeast, *P. anomala*, increase plant production and quality for both strawberry and onion, whereas it does not affect *Botrytis* infection under natural condition.

ACKNOWLEDGEMENTS

This project was supported financially by the Science and Technology Development Fund (STDF), Egypt, Grant No 2131. Thanks to Prof. Dr. Ali Abdelazeez, Vice president of Ain Shams University and previously Deputy Director of MIRCEN for his permission to use some machines in his lab. Thanks to Prof. Dr. Mohamed Emam, horticulture department, for offering strawberry seedlings during this study. Finally, the authors thank Dr. Jaime A. Teixeira da Silva for significant improvements to English.

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