CONTROLLING COMMON BEAN WHITE MOULD CAUSED BY *Sclerotinia sclerotiorum* (Lib.) de Bary

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Received: 09/12/2019 ; Accepted: 05/01/2020

**ABSTRACT:** Bean white mould caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a widespread and destructive disease under protective and open field cultivation in Egypt and worldwide. The vegetative sclerotial fungal structures formed by such pathogen give capability to overwinter between seasons. Different strategies are used efficiently to control the disease and eradicate its causal pathogen. The study was carried out to control the disease under Egyptian conditions. Results revealed that variability in virulence among the tested isolates was correlated with their oxalic acid production. Large white lima cultivar was the most resistant while Cranberry beans was the most susceptible in both detached leaf technique or under field conditions. Among the nine *Trichoderma* spp. used as biological control agent, *T. asperellum* inhibited *S. sclerotiorum* growth in vitro and controlled disease incidence in the field more efficiently. Epidemiological studies also revealed that high soil temperature inhibited sclerotial formation even in presence of high soil moisture while dry soil alleviated the inhibitory effect of high temperature. Sowing the selected resistant cultivars on early September at 50 cm distance with eight days interval period irrigation and organic compost for fertilization gave the most significant control for disease incidence and severity under field conditions.

**Key words:** Bean white mould, *Sclerotinia sclerotiorum*, resistant cultivars, oxalic acid, *Trichoderma asperellum*, epidemiological studies.

**INTRODUCTION**

*Sclerotinia sclerotiorum* (Lib.) de Bary is considered as a widespread and destructive pathogen, thrilling disease on more than 500 plant species causing white mould symptoms with fluffy white mycelium cottony appearance *(Sharma et al., 2015)*. The pathogen severely affecting bean plants from December till early March, causing significant economic losses in bean fields.

After beans harvest, *S. sclerotiorum* survives in soil and crop debris as sclerotia, a dormant and resistant stage, on infected plant tissue typically are incorporated into the soil and can be endured fertile for up to 10 years *(Lopes et al., 2010)*. Important role in disease cycles refers to the somatically structure (sclerotia), as they are the primary structures for their long-term survival and produce inocula for further infection. Sclerotia are germinate either vegetatively for local colonization or carpogenically to initiate the sexual cycle including the production of apothecia from which ascospores are released *(Bolton et al., 2005)* and in crops grown under low aeration and light penetration, white mould is even more aggressive.

Different variations between eight *S. sclerotiorum* isolates previously isolated from upper Egypt were differed in their culture morphology and sclerotial production apothecia *(Abdel-Razik et al., 2012)*.

Oxalic acid plays an important role in pathogenesis of *S. sclerotiorum* infections and the role of fungal secreted oxalic acid in virulence of different isolates of *S. sclerotiorum* have been investigated *(Noyes and Hancock, 1981; Li et al., 2008; Williams et al., 2011)*.
High soil temperatures (40 to 50°C) and low content of soil moisture (–152 to –366 MPa) depressed the durability of sclerotia of Sclerotinia minor and Sclerotinia sclerotiorum (Adams, 1987). Sclerotia of S. minor Jagger and S. sclerotiorum were infertile after three weeks in overflowed soil at mean temperatures ranging from 30 to 33°C (Matheron and Porchas, 2005). Under meteorological conditions favorable to S. sclerotiorum development, such as high humidity and mild temperatures from 15 to 20°C, common bean may afford losses of 30% or more, ascending to 100% in rainy seasons, when protective procedures are not considered (Singh and Schwartz, 2010).

The low content of organic matter in the new cultivated bean areas in Egypt have motivated some farmers to add organic stimulants to decrease disease incidence or carpogenic germination of Sclerotinia sclerotiorum in other crops (Asirifi et al., 1994). Liquid extracts of fermented agricultural debris, enhanced colonization of sclerotia by mycoparasitic fungi Trichoderma spp. (Huang et al., 1997). Agricultural mature composts and their water extract could play a role in decreasing disease (El-HMasry et al., 2002).

Different agricultural practices can cause physical, biological, and chemical variations, such as modifications in soil structure. One or more of these changes may play a role in modifying soil microbial communities and decreasing the survival of soil-borne pathogen propagules (Napoleão et al., 2005).

The most efficient manner for controlling white mould is the physiological resistance of bean cultivars. Virulence of S. sclerotiorum depends on production of toxic compounds (oxalic acid), cell-wall degrading enzymes and growth regulatory factors during infection to the secretion of oxalic acid. Toxins can depress the physiological processes in plants. Moreover, lytic enzymes promote colonization of the plant cell through the function of cell wall degradation enzymes such as cellulases, hemicellulases, endopeptinases, exopeptinases, glycosidases, proteases and xylanases.

Host resistance responses may act in various appearances in plant tissues, depending on the closeness of the region colonized by the pathogen and on the compounds constituted, they may even play as stimulants for production of new enzymes (Ribera and Zuniga, 2012).

This work aims to study several measurements to control white rot disease on common bean and characterize the efficiency of each method under field conditions, including varietal resistance, biological control, environmental factors and agricultural practices.

MATERIALS AND METHODS

Isolation, Purification and Identification of the Causal Pathogen

The causal organism was isolated from stems, branches, and pods exhibiting typical symptoms of white mould collected from different districts. Sclerotia associated with diseased samples of the causal organism were separated and superficially sterilized by agitation in 0.5% solution of NaClO (1:9 dilution of household bleach) for three minutes. Sterilized samples were rinsed several times in sterile distilled water and dried between two sterile filter papers then single sclerotia from each sample was plated onto surface of Petri dish containing potato dextrose agar (PDA) medium and incubated at 20 ±2°C for 5 days to obtain pure cultures. The process was repeated twice for each isolate (Hao et al., 2003). Pure culture of each isolate was obtained using the hyphal tip technique (Brown, 1924). The developed fungi were carefully transferred onto PDA medium slant and kept in refrigerator at 5°C for further studies. The isolated fungi were microscopically identified using the description of Morrall et al. (1972), Price and Colhoun (1975) and Kirk et al. (2001). Identification of the selected isolates was kindly confirmed by the Mycological Research and Diseases Survey Dept., Plant Pathology Research Institute, ARC, Giza, Egypt.

Pathogenicity Test and Aggressiveness of Isolates using the Detached Leaf Assay

Pathogenicity test of the previously identified isolates was carried out under laboratory conditions using detached leaves technique (Kull et al., 2003). Sterilized plastic pots (20 cm in diameter) were filled with 3 kg previously formalin sterilized sand -loam field soil. Healthy surface sterilized four seeds of White kidney
bean cultivar kindly obtained from Hort. Dept., Fac. Agric., Zagazig Univ., Egypt were sown in each pot. Pots were irrigated and fertilized as usual, under greenhouse conditions.

Looking healthy plant middle leaves were gathered at flowering stage. Leaves were surface-sterilized in a solution containing 47.5% ethanol and 2.6% sodium hypochlorite for 5 sec. Sterilized leaves were placed in 15 cm Petri dishes with sterilized moisten filter paper. The lower surface for each leaf was inoculated with fungal growth agar disc 0.5 cm in diameter taken from the edge of 7 days old *S. sclerotiorum* culture. Three replicates (Petri dishes containing bean leaves) were used for each isolate and incubated at 20±2°C for five days. After incubation leaflets were visually rated three days after inoculation and the lesions diameter (percentage of affected necrotic leaflet area) was measured for each isolate to identify white mould incidence and aggressiveness for each isolate (Wegulo et al., 1998). The pathogen severity percentage ranked from absence to very high as follows: 0% (absence), 1-25% (weak), 26-50% (moderate), 51-75% (high), and 76-100% (very high) according to Hall and Phillips (1996).

**Oxalic Acid Production by *S. sclerotiorum* Isolates**

**Detection of oxalic acid by visual degree of color change**

Oxalic acid production was visually investigated using PDA medium amended with bromophenol blue (Bb) in plates according to Steadman et al. (1994). The change of color from purple to yellow refer to a clue of oxalic acid production by the fungus. Oxalic acid secretion by an isolate was rated on a scale from no production (-) to maximum production (+++) depended on the visual degree of color modification observed on PDA+Bb medium plates.

**Oxalic acid quantitation**

Oxalic acid quantity production was determined by transfer one agar growth disc (8 mm diameter) of each isolate to 60 ml Erlenmeyer flasks containing 15 ml of potato dextrose broth (PDB) medium adjusted to pH 6. Four flasks for each tested isolate were incubated for 5 days at 25±2°C. Cultures growth were vacuumed and oxalic acid was determined in the supernatant of each isolate with catalytic kinetic spectrophotometric method, as described elsewhere (Xu and Zhang, 2000). Oxalic acid concentration was determined comparing with a standard curve and was assayed as mg oxalic acid/Liter PDB medium.

**Evaluation of Pathogenesis of *S. sclerotiorum* on Different Cultivars by Detached Leaf Assay**

Seeds of eight common bean cultivars (Giza 6, Bronco, Paulista, Pinto, White kidney bean, Cranberry bean, Black bean and Large white lima bean) kindly obtained from Horticulture Research Institute, Agricultural Research Center, Cairo were grown in the greenhouse for 21 days. Detached leaf assay previously mentioned in pathogenicity test evaluation was used herein. Lesion diameter in cm was calculated after inoculation by *S. sclerotiorum*, of New Nubaria isolate.

**In Vitro Biological Control Experiments**

Six *Trichoderma* species were kindly provided by Dr. Hesham Kamel (Plant Pathology Research Institute, Agricultural Research Center, Cairo) as well as three *Trichoderma* isolates were isolated and identified according to the method of Kirk et al. (2001) from bean rhizosphere and tested for their effect on mycelial growth of *S. sclerotiorum* under laboratory conditions.

For all tests, discs of 7mm diameter collected from the growing edge of the twelve pathogenic fungal isolates and the nine potential biocontrol agents were located on opposite sides of sterile Petri dishes (9 cm diameter) poured previously with potato dextrose agar (PDA) medium. PDA plates inoculated with *S. sclerotiorum* only served as control. Three plates were prepared for each of the pathogen/ potential biocontrol agent combinations. The dishes were incubated in the dark at 20 ±2°C for 5 days. The parameters were measured after 5 days as shown in Fig. 1 s1 (distance between the pathogen discs sowing point and furthest point of the colony) and s2 (distance between the pathogen plug sowing point and the edge of the colony) from where *S. sclerotiorum* and *Trichoderma* mycelia came into contact. Thus, the percentage of inhibition in the direct confrontation assay (ID) was calculated.
Fig. 1. Parameters used to calculate the growth inhibition percentage in direct confrontation ID 
%= [(s1−s2)/s1]x100. (A) *S. sclerotiorum* control plate (B) Growth of *Trichoderma* (right) and 
*S. sclerotiorum* (left) indirect confrontation assays

by the formula: ID% =[(s1−s2)/s1]x 100 (*Mayo et al.*, 2015). Moreover, the number of sclerotia 
on each PDA plate, was counted.

**Mycelial Growth, Sclerotial Formation and Germination as Affected by Epidemiological Factors**

**Relative humidity**

Five levels of relative humidity (32.5, 74, 85, 92.5 and 100% were prepared in Petri dishes 
according to *Solomon* (1951) and used to study their effect on mycelial covering growth and 
production of sclerotia under laboratory conditions as previously mentioned.

**Soil temperature and moisture levels**

Needed sclerotia for experiment was prepared according to *Matheron and Porchas* (2005). 
Sandy-loam soils were collected from dry fields to a depth of 10 cm and air dried then ground 
into fine particles. Two different levels of moisture (dry and wet soil) and five different degrees of 
soil temperature (0, 10, 20, 30 and 40°C) were prepared also according to *Matheron and 
Porchas* (2005). Jars contain soil and sclerotia were placed in growth chamber (150 x 150 x 80 
cm) to control the temperature and moisture. Viability was estimated by the capability of 
sclerotia to germinate on the growth medium and subsequently produce daughter sclerotia. 
Experiment was carried out in split-split plot design.

**Organic fertilizers**

Some organic fertilizers (animal manure, poultry manure and compost) were obtained 
from commercial marked and tested for their effect on *S. sclerotiorum* under laboratory 
conditions. Sclerotia of *S. sclerotiorum* was promoted as mentioned before.

Moisture of fertilizers was adjusted to 20% volume / volume. Cylindrical shape plastic 
containers 10 x 7 cm were filled with organic fertilizers. Each twenty sclerotia of new Nubaria 
iso late were put in nylon bag 3x6 cm. Nylon bags with sclerotia were distributed in the 
middle of plastic containers during filling with organic fertilizers. Organic fertilizer free field 
soil was served as control and equiped by the same method. Twelve replicates were used for 
each treatment. All treatments were incubated at 20 ±2°C for 4 weeks. Three replicates of each 
treatment were weekly taken and sclerotia, were removed. Sclerotial viability was estimated by 
the capability of sclerotia to germinate on growth medium plate as mentioned before.

**Field Experiments**

For all field experiments, random complete block design with three replicates were carried 
out under naturally infested at previously recorded white mould infection fields (New Salhia 
district, Sharqia Governorate). White kidney bean cultivar was investigated in this
experiment. Agricultural practices were followed as normal except for the agricultural practice factor under study. Plants were evaluated for disease incidence and severity at flowering stage and complete pods formation. Percentage of white mould infected stems and pods were calculated.

An area of 1.0 m$^2$ in the plots was investigated separately at 90 days after emergence (DAE) for white mould evaluation. Disease incidence was calculated as the percentage of plants with symptoms. As well as, stems, branches, and pods of bean plants were investigated to the rate of their severity on a scale of 0, 1-25%, 26-50%, 51-75% and 76-100% according to Hall and Phillips (1996). Severity scale was transformed in the McKinney (1923) index (MI %) suggested in accordance to this equation:

$$\text{MI} \% = \frac{\sum \text{(class score} \times \text{No. of plants with this score)}}{\text{(total No. of plants} \times \text{greater score)}} \times 100$$

### Reaction of some bean cultivars

The previously mentioned 8 bean cultivars were tested for their susceptibility to white mould caused by naturally infested field with *S. sclerotiorum* in two successive growing seasons 2017 and 2018 in New Salhia, Sharqia Governorate. Cultivars were sown in separated plots (plot area = 10.5 m$^2$) each plot consists of 7 rows. Seeds were sown on rows at hill (distance between hills was 30.0 cm) each hill contains 2-3 seeds.

### Agricultural practices

Organic fertilization, irrigation interval days, sowing dates and sowing distance were tested for their effect on bean white mould disease incidence under naturally infested field conditions in two successive growing seasons 2017 and 2018 in New Salhia, Sharqia Governorate.

#### Sowing dates

Four sowing dates (10$^{th}$ September 20$^{th}$ September, 1$^{st}$ October and 10$^{th}$ October) were tested for their effect on bean white mould disease incidence and severity.

#### Irrigation interval periods

Four irrigation interval periods (5, 6, 7 and 8 days) were studied for their effect on bean white mould disease incidence and severity.

### Planting distances

Seeds were sown in plots (3.5 m x 5 m) included 5 ridges, 60 cm apart. Seeds were hand sown on one side of the ridge in hills. Four planting distances (20, 30, 40 and 50 cm) were evaluated for their effect on bean white mould disease incidence and severity.

### Organic fertilization

Soil in New Salhia, Sharqia Governorate was prepared as normally and organic fertilizers (animal manure, poultry manure and compost) were obtained from Elsalhia Company for Industry and Trading. Organic fertilizers added at a rate of 20 m$^3$/faddan (0.05 m$^3$/plot).

### In vivo biological control experiments

Potential antimicrobial ability of *Trichoderma* spp., as one of the well-known bioagents, was evaluated on beans white mould disease. *In vitro* screening of nine *Trichoderma* isolates inferred that *T. asperellum* was the most efficient biocontrol agent against the white mould pathogen, *S. sclerotiorum*. Thus, further evaluation of the biocontrol capability of *T. asperellum* against white mould disease under greenhouse conditions during two successive seasons, was carried out.

The pathogen, *S. sclerotiorum*, was propagated on potato dextrose agar plates for one week and the freshly emerged sclerotia were harvested and used for inoculation. Inoculum of *T. asperellum* was propagated on gliotoxin fermentation broth (Anitha and Murugesan, 2005). Inoculum concentration in the collected filtrate was adjusted to $10^5$ cfu/ml with the aid of haemocytometer and stored at 4°C till further use. Five white kidney bean seeds were sown in 30-cm pots containing five kg light clay soil. Plants were irrigated and fertilized when needed. Inoculation was carried out on 45-days old plants by stem slashing technique, then three mature sclerotia were placed around the crown area and covered with wet cotton plugs. Following inoculation, plants were moved to greenhouse covered black mesh shade cloth to reduce sunlight intensity and to create conducive environment for white mould disease development and progress (Kraft and Pfleger, 2001). Symptoms development was monitored at two weeks after inoculation as previously
mentioned in pathogenicity test. Biocontrol treatment was done using *T. asperellum* inoculum as spraying application. Bioagent inoculum was applied once every two weeks after symptoms development. Five additional pots were sprayed only with water to serve as control. Data were recorded after each biocontrol application by calculating percentage of disease severity, disease incidence, infected stems and infected pods. Disease severity percentage was calculated based on the scale suggested by Porter *et al.* (2009), using the formula:

\[
\text{Disease severity (\%)} = \frac{\sum (\text{class frequency} \times \text{score of rating class})}{(\text{total number of plants}) \times (\text{maximal disease index})} \times 100
\]

Percentage of disease incidence was calculated according to the following formula:

\[
\text{Disease incidence (\%)} = \frac{\sum \left(\frac{\text{total number of plants within treatment}}{\text{number of infected plants}}\right)}{\text{total number of plants}} \times 100
\]

Percentage of infected stems and infected pods was calculated using the same formulae.

**Statistical Analysis**

The obtained data statistically analyzed by the analysis of variance (ANOVA) using MSTAT-C (1991). The least significant difference (LSD) test and Duncan’s Multiple Range Lest (Duncan, 1955) were used to find out the significiance of the means of various treatment (Gomez and Gomez, 1984). All analyses were performed at \( P = 5\% \) level.

**RESULTS AND DISCUSSION**

**Isolation, Purification and Identification of the Causal Organism**

The pure twelve isolates were identified based on their morphological characters as *Sclerotinia sclerotiorum*. Fungal isolates exhibited typical characters of *S. sclerotiorum* such as the white colony color and the black sclerotia were selected and coded (Morrall *et al.*, 1972; Price and Colhoun, 1975; Kirk *et al.*, 2001).

**Pathogenicity Test and Aggressiveness of Isolates using the Detached Leaf Technique**

All the tested twelve isolates were pathogenic to common bean at different degrees as observed *in vitro* (detached leaves technique). Moreover, virulence assays indicated that the tested isolates have variable degrees of virulence on beans (Table 1). The isolates gave three main categories of responses on the host plant, high, moderate and weak pathogenic. The isolates named NEWN, HOSH, ELKA, and NEWS were highly pathogenic according to their necrotic lesion diameter, BELB and ELKO were weakly pathogenic, while the rest of isolates were moderately pathogenic. Variable virulence degrees of the tested isolates are correlated with their growth rate, sclerotial abundance and oxalic acid ranked and secretion as it will be discussed in the following experiments. Viteri *et al.* (2015) found that isolates from distant geographic regions exhibit variable levels of virulence on common beans. Robison *et al.* (2018) found that resistant bean cultivars have unique physiological responses that decline photosynthesis keeping high leaf surface pH during infection. Also, leaves of resistant cultivars included amino acids, organic acids, phytoalexins, and ureides.

**Oxalic Acid Production by *S. sclerotiorum* Isolates**

Further discrimination between the collected isolates was done based on their extent of oxalic acid (OA) production and degree of virulence on detached bean leaves. Qualitative analysis of oxalic acid production on bromophenol blue medium sorted out the isolates into three main categories where +++ showed the highest level of OA production (Table 1). The isolates named NEWS, ELKA, HOSH and NEWN display the highest potential of OA production while BELB and ELKO were the lowest, and the remaining isolates were moderate. Quantitative analysis of OA by the same isolates gave parallel results. Isolates located in the highest category “+++” produced the highest amount of oxalic acid in culture medium which ranged between 6.17 to 8.8 mg/L. It is worthy to mention that there is a correlation between the highest production of oxalic acid and pathogenic capabilities of tested isolates. Isolate produce high concentration of oxalic acid ranked highly pathogenic one. These findings were in agreement with Li *et al.* (2008).

This result demonstrates an intimate correlation between OA production and fungal virulence. Thus, it is plausible that such pathogen relies on
Table 1. Differences between twelve Sclerotinia sclerotiorum isolates in relation to degree of pathogenic capabilities by detached leaf technique

| Isolate | Pathogenicity | Necrotic lesions diameter (cm) | Oxalic acid rank PDA medium + Bb* | Oxalic acid (mg/L) PDB medium |
|---------|---------------|--------------------------------|---------------------------------|------------------------------|
| NEWN    | High          | 6.70 a                         | +++                             | 8.80 a                       |
| HOSH    | High          | 5.30 b                         | +++                             | 7.30 b                       |
| ELKA    | High          | 4.70 c                         | +++                             | 6.17 c                       |
| NEWS    | High          | 4.50 c                         | +++                             | 6.47 c                       |
| ABOS    | Moderate      | 3.70 d                         | +                               | 5.13 d                       |
| BADR    | Moderate      | 3.50 de                        | +                               | 4.37 e                       |
| ABOK    | Moderate      | 3.40 de                        | +                               | 4.67 e                       |
| KOMH    | Moderate      | 3.20 de                        | +                               | 4.60 e                       |
| HOSI    | Moderate      | 3.00 e                         | +                               | 3.53 f                       |
| SARA    | Moderate      | 3.00 e                         | +                               | 3.37 f                       |
| BELB    | Weak          | 2.20 f                         | +                               | 2.53 g                       |
| ELKO    | Weak          | 2.00 f                         | +                               | 2.13 h                       |

Values with the same letters are not significant at probability = 0.05

*Oxalic acid secretion rate on a scale from no production (-) to maximum production (+++)

OA for invasiveness and colonization of its host tissue. Therefore, the study concluded that the highly virulent isolates of *S. sclerotiorum* are able to grow faster and produce higher amounts of oxalic acid and sclerotia on growth medium. The production of oxalic acid was considered as a pathogenicity determinant in *S. sclerotiorum* as previously mentioned by research workers (Li *et al.*, 2008; Williams *et al.*, 2011). The importance of oxalic acid secretion by this pathogen proposed the following mechanisms to clarify its role in pathogenesis: (1) decreasing the pH of infected bean tissues which boosts the activity of enzymes secreted by the pathogen (Bateman and Beer, 1965), (2) chelating cell wall calcium by the anions of oxalate, which modifies the plant cell walls softness and arrangement the function of Ca-dependent defense responses (Bateman and Beer, 1965), (3) its toxicity on host tissues, which macerates the plant defenses and facilitates pathogen’s colonization (Noyes and Hancock, 1981), (4) inhibiting the plant oxidative burst (Cessana *et al.*, 2000). Oxalic acid also inhibits the plant’s polyphenol oxidase defense enzymes (Xiong *et al.*, 1998).

Evaluation of *S. sclerotiorum* Pathogenesis on Different Cultivars by Detached Leaf Technique

Bean cultivars react differently to the invading pathogens based on the amount of resistance genes they have. The most virulent isolate of the pathogen (NEWN) was selected and inoculated on detached leaves of eight different bean cultivars. Data were recorded as lesion diameter for the detached leave technique. Cranberry Bean was found to be the most susceptible cultivar to the disease followed by Giza 6, Pinto Bean and Paulista while Large white lima and Bronco were the most resistant ones as they exhibited the smallest lesions (Fig. 2). Similar results were obtained by Balasubramanian *et al.* (2014). Previous studies under Egyptian conditions by Hatamleh *et al.* (2013) referred that cultivar Paulista was the lowest cultivar for susceptibility to *S. sclerotiorum* that giving 80% survived plants. This was followed by both saheland amy which revealed the same result with 72% survival plants. While, Giza-4 cultivar was the most susceptible to infect by the fungus, which
employed 32% survived plants. Those findings gave support to the present results as they showed high similarity.

**In Vitro Biological Control Experiments**

Results revealed that the highest percentage of mycelial growth reduction was occurred by *T. asperellum* followed by *T. album*, *T. hamatum* while *T. harzianum*, *T. koningii* and *T. viride* display moderate inhibition (Table 2). Three uncharacterized species of *Trichoderma* termed Y, A, and B also gave significant reduction of pathogen’s mycelial growth in vitro. These findings were in accordance with those reported by Sumida *et al.* (2018) who showed that *T. asperellum*, has valuable antagonism in dual-plate confrontation assessments against nine different isolates of *S. sclerotiorum*.

Species of *Trichoderma* are efficient bioagents due to their direct antagonism via secretion of lytic enzymes such as chitinases, β-1,3-glucanases, proteases and by the secondary metabolites (Geraldine *et al.*, 2013). Also, one of the main mechanisms of *Trichoderma* species is to affect fungal parasitism by production of cell wall-degraded enzymes. Degradation enzymes are *i.e.* chitinase, protease and β-1,3-glucanases that destroy the cell wall of the fungus and allow biological control agents to penetrate the pathogen cell wall (Geraldine *et al.*, 2013). Abdullah *et al.* (2008) showed that *T. harzianum* are able to form a “hook-like” and “appressorial” structures around the hyphae of *S. sclerotiorum*, which then kill the pathogen’s mycelium through penetrating and colonization of the hyphae (Elad *et al.*, 1982; Viterbo *et al.*, 2002). Consequently, *T. harzianum* can be considered as an antagonist and a mycoparasite of *S. sclerotiorum*, as it can inhibit the myceliogenic and carpogenic germination of sclerotia (Abdullah *et al.*, 2008).

**Epidemiological Studies**

It was mainly noticed that the higher relative humidity the faster fungal growth and sclerotial formation (Table 3). Therefore, it seems that the tested fungus prefers highly humid conditions for optimal growth and reproduction. No significant difference was observed among the tested isolates when exposed to different levels of relative humidity, which indicates that fungal preferable to ambient humidity is not essential for discrimination. Air relative humidity detrimental
Table 2. *In vitro* effect of *Trichoderma* species on mycelial growth reduction percentage and sclerotial production of *Sclerotinia sclerotiorum*

| Bioagent          | Mycelial growth reduction | Sclerotial number |
|-------------------|---------------------------|-------------------|
| *Trichoderma album* | 73.70 c                   | 3.33 f            |
| *Trichoderma asperellum* | 85.18 a                   | 0.00 g            |
| *Trichoderma hamatum* | 66.30 e                   | 5.67 e            |
| *Trichoderma harzianum* | 60.37 f                   | 10.00 c           |
| *Trichoderma koningii* | 53.33 g                   | 14.00 b           |
| *Trichoderma viride* | 62.22 f                   | 8.00 d            |
| *Trichoderma Y*    | 71.48 cd                  | 4.33 ef           |
| *Trichoderma A*    | 70.37 d                   | 5.00 ef           |
| *Trichoderma B*    | 77.41 b                   | 0.67 g            |
| Control            | 0.00 h                    | 30.67 a           |
| LSD 0.05           | 2.64                      | 1.91              |

Values with the same letters are not significant at probability = 0.05

Table 3. *In vitro* effect of different levels of relative humidity on mycelial growth (cm) and sclerotial formation of *Sclerotinia sclerotiorum*

| Isolate | Relative humidity (%) | 100 | 92.5 | 85 | 74 | 32.5 |
|---------|-----------------------|-----|------|----|----|------|
|         |                       | Mgd*| Sclf**| Mgd| Sclf| Mgd| Sclf | Mgd| Sclf | Mgd| Sclf |
| HOSI    | 9.00                  | 15.00 | 7.23 | 11.33 | 4.83 | 5.33 | 3.50 | 0.00 | 0.00 | 0.00 |
| NEWS    | 9.00                  | 14.33 | 7.63 | 9.67 | 5.23 | 5.33 | 3.27 | 0.00 | 0.00 | 0.00 |
| BELB    | 9.00                  | 17.33 | 7.03 | 10.33 | 5.13 | 5.00 | 3.00 | 0.00 | 0.00 | 0.00 |
| ELKO    | 9.00                  | 19.33 | 6.90 | 11.67 | 4.57 | 5.67 | 2.87 | 0.00 | 0.00 | 0.00 |
| ELKA    | 9.00                  | 15.33 | 7.50 | 10.67 | 5.23 | 4.67 | 3.37 | 0.00 | 0.00 | 0.00 |
| ABOK    | 9.00                  | 13.33 | 7.20 | 8.67 | 4.87 | 4.33 | 3.03 | 0.00 | 0.00 | 0.00 |
| ABOS    | 9.00                  | 12.33 | 7.77 | 7.33 | 5.17 | 3.33 | 3.37 | 0.00 | 0.00 | 0.00 |
| SARA    | 9.00                  | 18.67 | 7.10 | 10.67 | 4.70 | 5.00 | 3.00 | 0.00 | 0.00 | 0.00 |
| HOSH    | 9.00                  | 17.33 | 7.87 | 11.33 | 5.20 | 4.33 | 3.47 | 0.00 | 0.00 | 0.00 |
| KOMH    | 9.00                  | 18.33 | 7.27 | 11.00 | 4.57 | 4.33 | 3.27 | 0.00 | 0.00 | 0.00 |
| NEWN    | 9.00                  | 15.67 | 8.07 | 10.67 | 5.43 | 4.67 | 3.60 | 0.00 | 0.00 | 0.00 |
| BADR    | 9.00                  | 14.33 | 7.20 | 9.33 | 4.63 | 5.00 | 3.30 | 0.00 | 0.00 | 0.00 |

*Mgd: Mycelial growth (diameter in cm) LSD at (5%) = 0.2088*

**Sclf: Sclerotia formation No. LSD at (5%) = 1.214**
### Table 4. *In vitro* effect of weekly temperature degrees and moisture levels on sclerotial germination of *Sclerotinia sclerotiorum*

| Soil moist type/ Temperature (°C) | 1 Week | 2 Weeks | 3 Weeks | 4 Weeks |
|----------------------------------|--------|---------|---------|---------|
| Wet soil                         |        |         |         |         |
| 0                                | 14.33  | 10.33   | 5.67    | 3.00    |
| 10                               | 12.00  | 8.33    | 5.67    | 2.00    |
| 20                               | 8.33   | 4.67    | 4.00    | 1.67    |
| 30                               | 3.33   | 1.33    | 0.00    | 0.00    |
| 40                               | 0.67   | 0.00    | 0.00    | 0.00    |
| Dry soil                         |        |         |         |         |
| 0                                | 15.00  | 14.33   | 12.33   | 11.33   |
| 10                               | 15.00  | 14.00   | 11.67   | 10.67   |
| 20                               | 15.00  | 12.67   | 11.00   | 10.67   |
| 30                               | 12.00  | 10.00   | 7.67    | 7.33    |
| 40                               | 8.33   | 7.00    | 5.33    | 4.67    |

Values with the same letters are not significant at probability = 0.05.

**Fig. 3.** Effect of organic fertilizers on sclerotial germination percentage of *S. sclerotiorum*, under laboratory conditions
factor for white mould disease caused by *S. sclerotiorum* as the relative humidity be higher than 80% in the environment is one of the determining factors for *S. sclerotiorum* development to cause disease in soybean plants and also other crops (Hannusch and Boland, 1996).

Soil moisture and temperature, on the other hand, had more significant impact on fungal sclerotial germination (Table 4). High soil temperature was almost inhibitory for sclerotial germination even in presence of soil moisture. However, dry soil treatment alleviated the inhibitory effect of high temperature (40°C) and let the examined sclerotia grow normally as compared to the other control treatments. Longer incubation periods were determined to the fungal germination even in wet soil. However, fungal germination was completely diminished in wet soil. This observation was similar to the results obtained by Matheron and Porchas (2005) who found that the sclerotial proportion of *S. sclerotiorum* that germinated in wet soil tended to decline as soil temperature increased from 15 to 40°C with no germination of sclerotia observed after 1 and 2 weeks, respectively, at 40°C.

Plant fertilizers derived from organic matter including animal, chicken manure and compost are useful for plant growth and health as well. Biofertilizers application enhances plant growth parameters and suppress invading plant pathogens. Such effect could occur through variable mechanisms including biocontrol agents and inhibitory metabolites secreted from soil PGPRs (Bonanomi et al., 2007). In the current research trial, all organic fertilizers tested showed inhibitory effect against *S. sclerotiorum* to variable extent (Fig. 3). Compost, in particular, was the most suppressive for sclerotial germination *in vitro*, as germination percentage was dropped to 36.7%, while animal and chicken manure exhibit an average percentage of 45% for each at four weeks post inoculation comparing to 53.3% for the untreated controls. Germination percentage of sclerotia was randomly diminished with the time. Lahoz et al. (2009) studied the inhibitory effect of sterile water extract from manure compost and confirmed its inhibitory action on growth of *S. sclerotiorum*. They found that fungistasis could be one of the possible mechanisms of action and the extract was not phytotoxic. The suppressive action of organic fertilizers was supported by the findings of El-Masry et al. (2002) and Mello et al. (2005).

**Field Experiments**

Reaction of some bean cultivars to white mould under field conditions

Varetial reactions to the tested pathogen in field experiment were parallel to those obtained from detached leaf assay (Table 5). Cranberry Bean, Giza 6, Pinto Bean and Paulista were the most susceptible cultivars as they recorded the highest disease severity percentages which ranged between 46.67 and 73.33%. Susceptibility of bean cultivars to *S. sclerotiorum* infections was similar in the two successive seasons of 2017 and 2018 except that infection percentage was declined in the second season. Most cultivars were completely resistant to seed infections except Giza 6 and Cranberry Bean. As mentioned above, studies by Hatamleh et al. (2013) carried out under Egyptian conditions, gave similar findings as shown in this experiment.

Agricultural practices

Results in Table 6 indicate that the earlier sowing date the better disease escape. Plants sown on 10th September exhibited no infections while those sown month later showed higher levels of disease severity and free seed yield. Therefore, selection of the suitable sowing date is considered among the agricultural practices to manage the disease incidence. Workneh and Yang (2000) announced that annual modifications in air temperature were more valuable to the incidence of white mould on soybean crop as opposed to the patterns of atmospheric humidity and precipitation. It is convenient to mention that inhibiting factors to the incidence of white mould on soybean crop differ from site to site and year to year as a result of the modifications observed in the air temperature and relative humidity regime at a locate site. This assumption supports our finding that early sowing dates provide unfavorable air temperature to the pathogen growth and prevent disease initiation.
Table 5. Reaction of some bean cultivars to bean white mould disease under laboratory conditions by detached leaf and during 2017 and 2018 growing seasons under naturally infested field conditions

| Cultivar        | Disease percentage 2017 | Disease severity 2017 | Infected stems percentage 2017 | Infected pods percentage 2017 | Disease percentage 2018 | Disease severity 2018 | Infected stems percentage 2018 | Infected pods percentage 2018 |
|-----------------|------------------------|-----------------------|--------------------------------|-------------------------------|------------------------|-----------------------|--------------------------------|-------------------------------|
| Giza 6          | 74.44 ± 14.44          | 45.56 ± 12.56         | 49.63 ± 12.56                  | 33.33 ± 12.56                 | 40.37 ± 12.56         | 40.37 ± 12.56         | 30.00 ± 12.56                  | 14.44 ± 12.56                  |
| Bronco          | 61.11 ± 17.11          | 42.22 ± 15.56         | 37.04 ± 15.56                  | 14.07 ± 15.56                 | 30.00 ± 12.56         | 17.78 ± 12.56         | 15.56 ± 12.56                  | 0.00 ± 12.56                   |
| Paulista        | 70.00 ± 14.00          | 53.33 ± 12.33         | 46.67 ± 12.33                  | 35.6 ± 12.33                  | 27.78 ± 12.33         | 25.56 ± 12.33         | 0.00 ± 12.33                  | 0.00 ± 12.33                  |
| Pinto Bean      | 73.33 ± 17.33          | 61.11 ± 15.11         | 56.67 ± 15.11                  | 48.89 ± 15.11                 | 48.89 ± 15.11         | 37.78 ± 15.11         | 23.33 ± 15.11                  | 0.00 ± 15.11                  |
| White kidney    | 60.00 ± 16.00          | 53.33 ± 12.33         | 48.89 ± 12.33                  | 21.48 ± 12.33                 | 18.89 ± 12.33         | 18.89 ± 12.33         | 0.00 ± 12.33                  | 0.00 ± 12.33                  |
| Cranberry Bean  | 73.33 ± 15.33          | 67.78 ± 14.78         | 52.22 ± 14.78                  | 37.78 ± 14.78                 | 35.6 ± 12.67          | 21.11 ± 12.67         | 15.56 ± 12.67                  | 0.00 ± 12.67                  |
| Black Bean      | 56.67 ± 17.67          | 38.89 ± 15.56         | 37.78 ± 15.56                  | 17.78 ± 15.56                 | 17.78 ± 15.56         | 17.78 ± 15.56         | 0.00 ± 15.56                  | 0.00 ± 15.56                  |
| Large white lima| 44.44 ± 17.44          | 28.89 ± 15.34         | 14.81 ± 15.34                  | 12.22 ± 15.34                 | 14.44 ± 15.34         | 14.44 ± 15.34         | 0.00 ± 15.34                  | 0.00 ± 15.34                  |

Values with the same letters are not significant at probability = 0.05

Table 6. Effect of different agricultural practices on bean white mould disease incidence and severity during 2017 and 2018 growing seasons, under naturally infested field conditions

| Treatment                     | Disease severity 2017 | Disease severity 2018 | Infected stems percentage 2017 | Infected stems percentage 2018 | Infected pods percentage 2017 | Infected pods percentage 2018 |
|-------------------------------|-----------------------|-----------------------|--------------------------------|--------------------------------|-------------------------------|-------------------------------|
| Sowing date                   | 10<sup>th</sup> July  | 0.00 ± 0.00           | 0.00 ± 0.00                    | 0.00 ± 0.00                    | 0.00 ± 0.00                    | 0.00 ± 0.00                    |
| 20<sup>th</sup> September     | 12.59 ± 2.59          | 3.85 ± 1.92           | 18.89 ± 1.92                    | 7.78 ± 1.92                    | 5.56 ± 1.92                    | 2.22 ± 1.92                    |
| 1<sup>st</sup> October        | 29.63 ± 5.93          | 25.19 ± 4.93          | 44.44 ± 4.93                    | 37.78 ± 4.93                    | 18.89 ± 4.93                    | 6.67 ± 4.93                    |
| 10<sup>th</sup> October       | 39.26 ± 7.89          | 30.37 ± 6.89          | 58.89 ± 6.89                    | 45.56 ± 6.89                    | 26.67 ± 6.89                    | 21.11 ± 6.89                    |
| 5                             | 40.74 ± 6.07          | 30.37 ± 5.07          | 61.11 ± 5.07                    | 45.56 ± 5.07                    | 25.56 ± 5.07                    | 23.33 ± 5.07                    |
| 6                             | 25.93 ± 4.04          | 22.22 ± 3.04          | 38.89 ± 3.04                    | 33.33 ± 3.04                    | 21.11 ± 3.04                    | 18.89 ± 3.04                    |
| 7                             | 20.00 ± 3.00          | 17.04 ± 2.00          | 30.00 ± 2.00                    | 25.56 ± 2.00                    | 16.67 ± 2.00                    | 13.33 ± 2.00                    |
| 8                             | 14.07 ± 2.00          | 10.37 ± 1.00          | 21.11 ± 1.00                    | 15.56 ± 1.00                    | 7.78 ± 1.00                     | 6.67 ± 1.00                     |
| 20                            | 33.33 ± 5.00          | 25.93 ± 4.00          | 50.00 ± 4.00                    | 38.89 ± 4.00                    | 15.56 ± 4.00                    | 10.00 ± 4.00                    |
| 30                            | 28.15 ± 4.04          | 20.74 ± 3.04          | 42.22 ± 3.04                    | 31.11 ± 3.04                    | 11.11 ± 3.04                    | 8.89 ± 3.04                     |
| 40                            | 17.78 ± 3.04          | 13.33 ± 2.04          | 26.67 ± 2.04                    | 20.00 ± 2.04                    | 7.78 ± 2.04                     | 5.56 ± 2.04                     |
| 50                            | 13.33 ± 2.00          | 10.37 ± 1.00          | 20.00 ± 1.00                    | 15.56 ± 1.00                    | 3.33 ± 1.00                     | 2.22 ± 1.00                     |
| Animal                       | 51.11 ± 10.11         | 41.11 ± 9.11          | 34.07 ± 9.11                    | 27.41 ± 9.11                    | 24.44 ± 9.11                    | 20.00 ± 9.11                    |
| Poultry                      | 48.89 ± 9.89          | 38.89 ± 8.89          | 32.59 ± 8.89                    | 25.93 ± 8.89                    | 21.11 ± 8.89                    | 15.56 ± 8.89                    |
| Compost                      | 27.78 ± 8.78          | 21.11 ± 7.78          | 18.52 ± 7.78                    | 14.08 ± 7.78                    | 15.56 ± 7.78                    | 13.33 ± 7.78                    |
| Organic fertilizer           | 56.67 ± 10.67         | 54.45 ± 9.67          | 37.78 ± 9.67                    | 36.30 ± 9.67                    | 30.00 ± 9.67                    | 26.67 ± 9.67                    |

Values with the same letters are not significant at probability = 0.05
Irrigation frequency have also impacted disease levels. Eight days interval period significantly decreased the disease severity comparing to the other shorter irrigation periods. Low water levels dropped the disease severity down to 14% whereas five days interval showed higher disease severity (40.74%). Higher disease severity in case of short irrigation period (5 days) might be due to the increase of soil moisture content necessary for sclerotial germination and hence increased disease incidence and severity. Previous researches showed a great impact of irrigation frequency on severity of white mould disease. Thus, the irrigation strategy that achieves field capacity is recommended by research workers (Paula Júnior et al., 2006), especially when a cultivar with an intensive plant canopy is used (Napoleão et al., 2005).

When testing sowing distance, results revealed that plants sowing at 50 cm apart were safer and healthier as the disease severity was 13.33% while other plants cultured on close distance exhibited higher disease severity percentage (33.33%). Vieira et al. (2005) showed that there was an inhibition of both incidence and severity of white mould when six plants on the contrary of 12 plants per meter were sown. High plant density (12 plants/m) decreases air circulation and increase, the moisture in the canopy, contributing to increase the disease incidence and more severe white mould than low plant density (Tu, 1997). Therefore, no yield shortage using lower plant population in field highly infested with S. sclerotiorum is an important result and implies a lower investment with seeds as well.

The investigated compost exhibited the effective results in terms of disease inhibition. Plants fertilized with compost had 27.78% disease severity percent while animal and chicken manure were conducive for the disease progression which resulted in higher disease severity levels. The importance of inhibitory effect of organic fertilizers on white mould disease of beans was previously discussed and confirmed by El-Masry et al. (2002), Mello et al. (2005) and Lahoz et al. (2009).

**In vivo biological control experiments**

*T. asperellum* gave efficient level of disease reduction after the third spray which significantly decreased the disease severity below 3.70% and completely protected the seeds from infection (Table 7). Repeated application with the bioagent’s inoculum significantly suppressed disease severity in field conditions. Recent results suggest that the performance of Trichoderma spp. can be influenced much more by the time it remains in contact with the pathogen than by the pressure of the disease itself (McLean et al., 2012).

Pinto da Silva et al. (2019) assayed the biocontrol potential of 12 Trichoderma spp. and found that all Trichoderma spp. were able to reduce the severity of white mould disease. According to Zhang et al. (2016) explanation, the white mould biocontrol is facilitated by the synthesis and exudation of Trichoderma spp. compounds which provides growth promoting features and enhance plant growth and increase root mass. Yedidia et al. (2001) suggest that the effect on growth promotion caused by T. harzianum present in soil rhizosphere is due to an increase in root area provided, allowing the plant to exploit a larger area of soil and consequently more nutrient sources.

### Table 7. Effect of spraying with *Trichoderma asperellum* on bean white mould disease incidence during 2017 and 2018 growing seasons, under field conditions

| Spray No. | Disease severity | Disease percentage | Infected stems percentage | Infected pods percentage |
|-----------|------------------|--------------------|---------------------------|-------------------------|
|           | 2017  | 2018  | 2017  | 2018  | 2017  | 2018  | 2017  | 2018  |
| 1         | 23.70 b | 18.52 b | 35.55 b | 27.78 b | 12.22 b | 7.78 b | 2.22 b | 0.00 b |
| 2         | 14.82 c | 10.37 c | 22.22 c | 15.56 c | 5.57 c | 4.44 bc | 0.00 b | 0.00 b |
| 3         | 3.70 d | 1.48 d | 5.56 d | 2.22 d | 2.22 cd | 1.11 cd | 0.00 b | 0.00 b |
| Unsprayed | 36.30 a | 25.18 a | 54.45 a | 37.78 a | 18.89 a | 13.33 a | 13.33 a | 10.00 a |
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مكافحة مرض العفن الأبيض في الفاصوليا الذي يسببه الفطر سكليروتني سكليروشيوريوم

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يعتبر العفن الأبيض في الفاصوليا والذي يسببه عن الفطر سكليروتني سكليروشيوريوم من الأمراض المدمرة واسعة

الانتشار تحت كل من النظام الزراعي المفتوح والمحمية في مصر وفي جميع أنحاء العالم، ويتكون الفطر تراكيب خضرية

تعرف بالأسماك الحربية توغلها للتواجد بين المواسم الزراعية وقضاء فترة التشتيت، ت تعد أساتذة تطبقيات وطرق مكافحة

المرض للتحكم والسيطرة عليه، وتنفيذ هذا الدراسة إلى مكافحة المرض بعد من الطريق، والتي تشمل مقاومة الأصناف

للمرض والمقاومة البيولوجية والعملات الزراعية تحت الظروف العملية والتحليقة، وقد أوضحت النتائج أن من بين أثني

عشرة عزلة تم عزلها من أثنا عشر منطقة من ثلاث محافظات مصرية أن عزلة منطقة الوبوية العربية كانت أكثر

العزلات ضرارة وأظهرت نسبة عالية من نسبة وشده المرض بل وأيضا من المساحة النباتية المبتلة بالأوراق من خلال

تتكيف عدد الأوراق المفصولة، وأظهرت النتائج التباني الواضح بين عزلات السبب المرضي من خلال معدل نموها.

معادل تكوينها للأسماك الحربية ومعادل إفرازها لحمض الأوكسليك، وأوضحت نتائج اختبار الأصناف أن الصنف لارج

وايت ليما من أكثر الأصناف مقاومة وعلى النقيض من ذلك الصنف كرايبري كان من أكثر الأصناف قابلية للأصابة

استخدام نفس التكثيف ومن خلال الغذاء الحقلي، كما أظهرت عزلة الترستوندا سبيسيل يرا مثبطاً عاملاً لنمو الفطر

المرض في العمل ويحكاها في ظهر المرض بالقلق، وكشفت الدراسات البيئية أن الحالة المرتفعة لحرية النبات في ظل

الظروف المرتفعة حدد من تكون الفطر للاكتر من الأسمك الحربية بينما خففت الحرارة الجافة من تأثير الحالة المرتفعة

على معدل تكوين الأسماك الحنارية، أدت زراعة الأصناف التي أظهرت نسب عالية من مقاومة في أول شهر سبتمبر مع

فترة ري كل ثمانية أيام بالإضافة للتمديد الحيوي بالكوبيوست لتحسين صحة وجودة النباتات والتحكم بالعدالة

المرضية تحت ظروف الجدل.

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