Zagazig J. Agric. Res., Vol. 46 No. (6A) 2019

Plant Protection and Pathology Research

http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

MOLECULAR DETECTION AND CONTROLLING OF SEED-BORNE *Colletotrichum* spp. IN COMMON BEAN AND SOYBEAN

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Received: 18/08/2019 ; Accepted: 08/09/2019

ABSTRACT: Seed-borne fungi cause several diseases of common bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L.). The survey of seed-borne fungi of common bean and soybean was carried out on three Egyptian Governorates (Behera, Dakahlia, and Ismailia) in Egypt. Nineteen fungal species comprising therteen genera were isolated from the collected common bean and soybean seed samples, using standard blotter *i,e*. *Alternaria alternata*, *Alternaria* spp., *Aspergillus niger*, *Aspergillus ochraceous, Aspergillus flavus, Botryodiplodia* sp., *Cladosporium* sp., *Colletotrichum lindemuthianum, Colletotrichum dematium, Fusarium solani, Fusarium moniliforme, Fusarum oxysporum, Macrophomina phaseolina, Myrothecium* sp., *Penicillium* spp, *Rhizoctonia solani, Stemphylium* spp., *Trichoderma* spp. and *Trichothecium* sp. Pathogenicity tests proved that *C. lindemuthianum* and *C. dematium* were pathogenic to common bean and soybean. Polymerase chain reaction (PCR) has many beneficial characteristics that make it highly applicable for detecting *Colletotrichum* spp. of seeds. PCR diagnosis method and DNA extraction considered one of the most important steps and purity of DNA template for successful PCR assay. For the PCR amplification of *C. lindemuthianum* and *C. dematium*, two primers ClF4 and ClF5, stander blotter, agar plate, and deep freezing method were used as seed healthy testing methods. Stander blotter was proved the past and quickly method to detect seed-born of *C. lindemuthianum* and *C. dematium*. *Trichoderma harzianum* reduced linear growth for *C. lindemuthianum* and *C. dematium* followed by *Trichoderma viride* and *Bacillus subtilis* recording (3.3 cm and 3.9 cm, respectively) followed by *Pseudomonas floursence* that display a high linear growth (7.4 cm). On the other hand, fungicide Aetro 30% (Iprodione + Tebuconazole) recorded the least linear growth (0.9 cm) for *C. lindemuthianum* and *C. dematium* compared with negative control recorded (9.0 cm in diameter). Thymus plant extract caused the lowest liner growth (3.7 cm). Plant extract concentrations caused a significant reduction in the growth of *C. lindemuthianum* and *C. dematium.* The highest concentration (1.5 %) gave less fungal growth while the lowest one (0.5%) showed the highest fungal growth compared with negative control.

Key words: Common and soybean, *Colletotrichum lindemuthianum* and *Colletotrichum dematium*, PCR, fungicides, bio-agent and plant extract.

INTRODUCTION

Seeds are the most important for crop production. Pathogen free seed is urgently needed for desired plant populations and good harvest. Several plant pathogens were seed-borne, which cause enormous crop losses **(Dawson and Bateman, 2001; Islam, *et al.* 2009).** Anthracnose has caused serious reductions in the yield of legume crops in many parts of the world, resulting in yield losses as high as 95% (**Chen, *et al.* 2007).**

Colletotrichum lindemuthianum (Sacc. and Magnus) Briosi and Cavara caused bean anthracnose and consider as a serious seed-borne disease of common beans. This pathogen

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is distributed worldwide and causes devastating losses in fields planted with infected seeds. Seed infections can also reduce seed quality and result in the introduction of the disease into new areas or new races into new geographic regions **(Chen *et al.*, 2007)**.

Adegbite and Amusa (2008) reported that, members of the genus *Colletotrichum* have been reported to cause two major diseases in cowpea. These are anthracnose and brown blotch. These diseases are very destructive due to the susceptibility of many cowpea lines to them. **Wrather *et al.* (2003)** isolated *Phythium* sp., *Phytophthora sojaes, Rhizoctonia solani, Fusarium* sp, *Macrophomina phaseolina, Sclerotium rolfsii, Diaporthe sojae* and *Colletotrichum truncatum* from soybean seeds. **(Shovan *et al.*, 2008;Wrather and Koenning, 2009)** collected a total of 33 soybean seed samples from different locations, representing three cultivars and 16 genotypes for detection of the seed borne-fungi of soybean. They detetced ten fungi including nine genera *i.e. Alternaria alternata, Aspergillus flavus, Aspergillus niger, Cheatomium globosum, Colletotrichum dematium, Curvularia luanata, Fusarium oxysporum, M. phaseolina, Penicillium* sp. and *Rhizopus stolonifer*. **Farzana, (2012)** recorded 124 seed-borne fungal infections on four varieties of soybean, six fungi were identified as *F. oxysporum, A. flavus, A. niger, C. truncatum, Rhizopus stolonifer* and *Penicillium* sp.

The blotter and the agar plate methods are two important procedures traditionally applied in routine seed health test for seed-borne fungi detection **(Warham, 1990; Youssef, *et al.* 2018).** Those traditional diagnostic methods seem to have serious disadvantages such as time-consuming and lack of accuracy **(Khiyami, *et al.* 2014).** The failure to adequately identify and detect plant pathogens using conventional morphological techniques has led to the development of nucleic acid-based molecular approaches.

Immune-diagnostic tools can also be successfully employed for differential diagnosis and disease surveillance of seed-borne pathogens of quarantine importance. **Ghoneem, *et al.* (2019**) reported that, soybean seeds were found to have a wide diversity of associated fungi. A collection of thirty-one fungal species comprising nineteen genera were isolated from the collected soybean seed samples, following standard blotter (SB) and agar plate (AP) methods. No differences were observed between the SB (17 genera and 29 species) and AP (18 genera and 28 species) techniques regarding the frequency of the recovered seed-borne fungi.

Polymerase chain reaction (PCR) developed for rapid detection and identification of plant pathogens, but it has not completely replaced traditional cultural and phenotypic tests practiced for the detection of major seed-borne pathogens. In a PCR diagnostic studies, the development of PCR primers is one of the most important steps. Primers are specific to various phytopathogenic fungi. These approaches include using species-specific genes or DNA regions to design PCR primers **(Chen *et al.*, 2007; Mohamed, 2007; Zhonghua and Michailides, 2007; Awad *et al.*, 2019)**. After the evaluation of several methods, DNA extracted one most suitable method described by **Dellaporta *et al.* (1983)** in short and quick detection of different pathogens.

Fungicides have a toxic effect on public health and environment balance and produce fungicides resistant of strain pathogens. Moreover, it causes several problems such as cancer and causes chromosomal abnormalities.

Therefore, biological control of plant pathogens and plant extracts are becoming an important component of plant disease management practices **(Riad *et al.*, 2013).** In this respect, *Trichoderma* spp., *Bacillus subtilis* and *Pseudomonas floursence* represent interesting way in controlling fungal diseases within an environmentally friendly integrated crop protection system through enhancing the resistance of the plant to the pathogen.

Some plant extracts also showed promising results in the control of bean anthracnose of common beans**(Vinale *et al.*, 2008)**. Neem seed extract effectively inhibited both germinations of conidia and mycelial growth of *C. lindemuthianum* **El-Mougy *et al.* (2007)** studied the effects of twenty powdered spicy plants and their extracts against *F. solani* and *R. solani*. They observed high significant inhibitory effect on radial fungal growth for different concentrations of carnation, cinnamon, garlic and thyme. Meanwhile, fennel, marjoram and

chamomile showed a low inhibitory effect on the tested fungi. **Abd El-Kader *et al.* (2012)** stated that, carnation, caraway, thyme, peppermint, and geranium essential oils have been found to have inhibitory effects against the mycelial growth of *F. solani, R. solani, S. rolfsii* and *M. phaseolina* under *in vitro* conditions. Complete inhibition of fungal growth was observed with the use of carnation (4%) and geranium oils. **Hassanein, (2013)** showed that, sclerotial formation of *M. phaseolina* and *R. solani* was significantly inhibited by any of the extracts of clove, cumin, henna, and garlic compared with that of the control and was not affected by the black cumin extract. The clove extract concentrations completely inhibited mycelial growth of all the tested fungi, followed by garlic extract *R. solani, F. semitectum* and *F. oxysporum* were the least affected fungi using the same extract.

The present investigation aimed to detect and identify *C. linemuthinum* and *C. dematum* isolated from common bean and soybean seeds by traditional methods of seed health testing and molecular methods (PCR-based technique using internal transcribed spacer region [ITS region]). In consequently control approaches including plant extracts, biological agents and chemical fungicides were investigated.

MATERIALS AND METHODS

Survey Of Some Legume Seed-Borne Pathogens

Seed samples of common bean and soybean cultivars were collected from different areas at three Governorates *i,e,* Ismailia, Dakahlia, and Behera, Egypt in 2016 and 2017 growing seasons. Samples were then carried out in sterilized paper bags and transferred directly to the Laboratory, of Seed Pathology Research Department, Plant Pathology Research Institute, (ARC), according to the rules of the International Seed Testing Association **(ISTA, 2015).** All samples were kept in a refrigerator at 5 C̊ until using.

Detection And Isolation Of Some Legume Seed-Borne Pathogens In Egypt

The standard blotter method (SBM) technique recommended by (ISTA, 2015), was used for detection of seed-borne fungi. Four hundred seeds of each sample were directly plated on three moistened blotter in Petri dishes (9 cm in diameter) at the rate of 10 seeds per dish. The dishes were incubated for 7 days at 25+2 C̊ for 12hr., under alternating cycles of white fluorescent light and darkness. Frequency percentages of the counted fungi were calculated and tabulated. In this experiment, visual methods using: Stereoscopic microscope (6-50 X magnification) was used to detect seed-borne fungi and study their habit characteristic. The compound microscope was used to confirm the identification.

The developed fungi were carefully transferred onto (PDA) medium. Isolated and purified using the hyphal tip and/or single-spore technique (**Dhingra, and Sinclair, 1973).** The purified cultures were incubated on PDA slant medium for 7days at 28℃ then stored in a refrigerator at 5℃, identification was carried out as mentioned by **Booth (1985), Barnett and Hunter (1998).**

The detected seed-borne fungi were identified according to Common Wealth Mycological Institute Description Sheets, Danish Government Institute of Seed Pathology Publication, and Research work of **Tadja *et al.* (2009).** Identification was kindly confirmed by Taxonomy Dep. Plant Pathology Res. Inst., (ARC), Giza, Egypt.

Pathogenicity Tests

Pathogenicity tests of the isolated fungi was done on common bean (Sonate cv.) and soybean (Clark cv.). Inoculum of *C. lindemuthianum* and *C. dematium* were prepared by growing each fungus individually on autoclaved sorghum: sand: water (2:1:2 *V/V/V*) medium in glass bottles for 21 days at 25±2 C̊ according to **Ghoneem *et. al.* (2019**). Soil infestation was achieved by mixing inoculum of *C. lindemuthianum* and *C. dematium* with the sterilized soil at 5% of soil weight (fungal growth 50 g/kg soil *W/W*), in clay pot (25 cm diam) and watered regularly for five days before planting. Pathogen free autoclaved sorghum medium was added to sterilized soil) in pots to serve as control. Common bean cv. Sonata and soybean cv. clark seeds were surface sterilized by immersing in sodium hypochlorite 1% for three min, then washed with sterilized water to get rid of excess poisonous and sown at the rate

of 10 seeds/pot. Five replicates were used/each treatment. Percentages of pre- post-emergency damping-off and healthy survival plant were calculated at 15, 30 and 45 days post planting, respectively, according to **Abd El-Wahab, (2011)** as follows:-

$$\text{Pre-emergence damping-off (\%)} = \frac{\text{No. of non-emerged seedlings}}{\text{No. of planted seeds}} \times 100$$

$$\text{Post-emergence damping-off (\%)} = \frac{\text{No. of dead seedlings}}{\text{No. of planted seeds}} \times 100$$

$$\text{Healthy survivals (\%)} = \frac{\text{No. of healthy seedlings}}{\text{No. of planted seeds}} \times 100$$

Seed Health Testing Methods

Seed health testing techniques recommended by the **(ISTA, 2015)** namely, standard blotter method (SBM), agar plate method (APM) and deep-freezing method (DFM) were used for the detection of seed-borne fungi. Each of the collected seed samples of common bean and soybean were surface sterilized using 1% aqueous sodium hypochlorite solution (NaOCl) for five minutes then rinsed by sterilized tap water three times and left to dry. Detection and isolation of seed-borne fungi associated with the seeds were then carried out by **(ISTA, 2015).** Random 400 common bean and soybean seeds examined with a blotter, deep freezing and agar plate methods.

Standard blotter method (SBM)

Four hundred seeds from each sample were directly plated on three moistened blotter papers properly soaked in sterilized water in Petri dishes (9 Cm. in diameter) at the rate of five seeds per dish seeds equidistantly under aseptic conditions in 80 replicates each for common bean and at the rate of 10 seeds per dish in 40 replicates for soybean seeds. The dishes were incubated for 7 days at 25+2°C for 12 hr., under alternating cycles of white fluorescent light and darkness according to the rules of **ISTA, (2015).**

Deep freezing method (DFM)

This method was modified from the blotter method **(Neergaard, 1979).** In this method, seeds were placed in petri dishes to be examined as in the (SBM). Dishes were then incubated in a controlled environment room at 25±2°C under the alternating cycle of 12hr., cool light and 12hr darkness for 2 days, then 1day at –20°C (deep freezing) and finally 4 days at 25±2°C of 12/12 hr., light/darkness cycle.

Agar plate method

In the agar plate method, 20 ml of potato dextrose agar (PDA) was poured in a glass Petri plate. After cooling, seeds were placed in petri dishes containing (PDA) media and incubated and examined as mentioned above in the standard blotter method.

Identification by Molecular Method

DNA extraction of *Colletotrichum* spp. by Dellaporta buffer

DNA extraction from cultures

According to **Dellaporta *et. al.* (1983)**, pure cultures of *C. lindemuthianum* and *C. dematium* were used individually and carefully frozen in liquid N2 and ground to a fine powder in a mortar and pestle. The powder was directly transferred to a 1.5ml microfuge tube, and supplement to the end Dellaporta technique. Finally the pellet was air-dried for 1hr., then suspended in 50µl dH_2O. The extracted DNA was then ready for PCR.

DNA extraction from common bean and soybean seeds

The artificial infected and uninfected seeds with *C. lindemuthianum* and *C. dematium* were surface-sterilized in 70% ethanol for 30 sce. and washed three times in sterilized distilled water. The seeds were dried on sterilized filter paper, and batches of healthy and diseased seed were separately ground into a fine powder through crushing using a Warring blender and coffee grinder at maximum speed **(Chen *et al.*, 2007).** Samples were frozen in liquid N2 and ground to a fine powder in a mortar and pestle. The powder was transferred to a 1.5ml microfuge tube using a modified Dellaporta extraction method **(Dellaporta *et al.*, 1983)**. also DNA was extracted from cultures of *C. lindemuthianum* and *C. dematium*. **(Awad *et al.*, 2019).**

PCR primers

To develop a tool to identify fungi and classify them according to their phylogenetic

group, the advantage of the sequence diversity of the intragenic spacer regions of fungi was considered. Three PCR primers were shown to amplify three fungi. Each of these primer pairs was specific for each fungus, and they did not produce PCR products of the correct size from any other fungi group. Primers used not produced PCR amplification products in the accurate size of healthy plant DNA. These primers could serve as actual for identifying particular fungi in field samples according to **Drori *et al.* (2013).** For the amplification of *C. lindemuthianum* and *C. dematium*, using the primers combined with the reverse primer ITS4 ($^{5'}$TCCTCCGCTTATTGATATGC$^{3'}$),two forward primers ClF4 ($^{5'}$TCCCCCCTGCCC CGCTCG$^{3'}$) and ClF5 ($^{5'}$CGCCGGAGGAAA ACCCAAC$^{3'}$) **(Sreenivasaprasad *et al.*, 1996; Chen *et al.*, 2007)**.

PCR amplification

Each PCR tube contained the following reaction mixture volumes 25 µl consisted of 2.5 µl 10× PCR buffer, 2.5 µl of Mg+cl2, 0.3 µl of dNTPs, 0.5 µl of each forward and reverse primer, 0.4 µl of Taq DNA polymerase and 3 µl of sample DNA. PCR has performed in a Bio-Rad DNA engine Peltier thermal cycler. The PCR program was optimized and consisted of initial denaturation at 95℃ for 5 min, followed by 30 cycles at 94℃ for 30 sec consisted of denaturation, at 50℃ annealing temperature for 45 sec, 1 min at 72℃ for primer extension, and final extension with 1 cycle of 5 min at 72℃.

Following amplification, the PCR products were separated on a 1% Agarose gel in 1× TBE buffer at 120 V for 1hr., and visualized by staining with ethidium bromide (10 µl/ml) **(Sambrook, *et al.*, 1989)** then, photographed under UV light using Gel-Documentation System (GELDOC 2000, Bio-Rad, USA). The size of the fragment is determined using the 100bp DNA ladder molecular weight markers.

Controlling Assessment

Biological control

Effect of some bioagents on the growth of *Colletotrichum* spp. *in vitro*

Under laboratory conditions four bioagents *i.e., Trichoderma harzianum, Trichoderma viride, Pseudomonas flourscence* and *Bacillus subtilis* isolated previously from seeds, were used to evaluate their antagonistic effect against *C. linemuthinum* and *C. dematum* (**Ghoneem *et al.*, 2019**). PDA was used for *T. harzianum* and *T. viride* and nutrient agar medium was used for *P. flourscence* and *B. subtilis*. To detect the antagonistic effect, discs (5 mm in diameter) were taken from 7 days old culture of different bio-agents and plated inside of PDA on Petri dishes, on the other hand equal discs 5 mm in diameter. were taken from 7 days old culture of *C. linemuthinum* and *C. dematum* and plated in opposite side of PDA Petri dishes. Plates with a fungus alone were served as the control treatment. 1000 ppm from (Iprodione 20% + Tebuconazole 10%) solution was added to flasks PDA medium then flows into Petri dishes and left to solid, then discs (5 mm in diameter) were taken from 7 days old culture of different pathogens and plated inside PDA Petri dishes and served as positive control treatment. Five plates were used for each treatment then incubated untile mycelium growth of control treatment covered the surface of the plate, linear growth of fungi were recorded and percentage of reduction in each treatment was calculated as follows:

$$\text{Reduction (\%)} = \frac{G_1 - G_2}{G_1} \text{ X } 100$$

Whereas:

G1 = Fungal linear growth of the control (mm)

G2=Fungal linear growth of the treatment (mm)

Effect of some plant extracts on the growth of *Colletotrichum* spp. *in vitro*

Three medicinal and aromatic plants were evaluated for their effect on fungal radial growth of the pathogenic fungi *in vitro* according to the method mentioned by **El-Mougy *et al.* (2007).** Plant materials obtained from the Medicinal and Aromatic Plant Pathology Research Department, Plant Pathol. Res. Inst., ARC, Egypt. Plant materials (thymus, clove and garlic) were washed with distilled water and air-dried. The dried plant materials were then finely ground to a fine powder. Fifty grams of each dried plant powder was homogenized by laboratory blender for 10 min in ethanol (96 %) and distilled water

(20: 80, *V/V*), then incubated in a dark bottle of glass for 72 hr., for tissue maceration. The extracts were filtered through thin cheese cloth sheets. The final extracts were collected separately in other dark glass bottles and exposed to 60℃ in a water bath for 15 min for ethanol evaporation and sterilized using Seitz's filter. Then stored in a refrigerator at 5℃ until used according to **Hassanein (2013)**.

Extracts were added to sterilize PDA flasks before solidifying to obtain the proposed concentrations of 0.5%, 1% and 1.5%. The amended medium was poured into 9 cm diameter Petri dishes, and another set of extract free PDA medium was used as the negative control treatment. Discs (5 mm in diameter) were taken from 7 days old culture of *C. lindemuthianum* and *C. dematium* were plated in the center of treated and untreated PDA Petri dishes.1000 ppm from Aetro 30% Fungicide solution was added to flasks PDA medium then flows into Petri dishes and left to solid, then discs (5 mm in diameter) were taken from 7 days old culture of different pathogens and plated in the center of PDA Petri dishes and served as positive control. Five plates were used for each treatment then incubated for when mycelial growth of control treatment covered the surface of the plate.

Measurements of colonies were taken using the control plates as a reference **Zedan *et al.* (2011)** and the percentage of reduction in each treatment was calculated as previously mentioned.

Greenhouse Experiments

Effect of fungicide, plant extracts and bio-agents materials on *Colletotrichum lindemuthianum* and *Colletotrichum dematium*

In greenhouse experiments, pots (25-cm-diam.) filled with soil (1 sand : 2 clay, *W/W*) were used for common and soybean planting. The formaline sterilized pots were infested with a *C. lindemuthianum* and cultivated with common bean (Sonate cv.) and/or infected with *C. dematium* and cultivated with soybean (Clark cv.) each alone. Soil in pots were then continously irrigated for one week to allaw the infection and spread of tested fungs. Seeds of common bean (Sonate cv.) and soybean (Clark cv.) were sterilized as mentioned above then some in infected soil.

A set of five replicates were used for each fungicide and the plant extracts *i.e*. clove, thymus and garlic extracts at 1.5 % according to **Hassanein (2013)**. The fungal spores of *T. harzianum* and *T. virid* were gently scraped from 7 day old cultures grown on PD liquid medium. Bacterial suspension of *P. florescence* and *B. subtilis* were collected from 3 days-old culture grown on nutrient broth medium according to **Sallam *et. al.* (1978) and Kamel (2017).** Spore or cell suspension *T. harzianum* and *T. virid*, adjusted to with sterilized water to be 3×10^4 cfu/ml and cell suspension of concentration *P. florescence* was 1×10^7cells/ml and *B. subtilis*. The tested bio-agents were supplemented in sodium carboxymethyl cellulose (CMC)1% solution were subsequently added individually to one hundred grams of common bean and soybean seeds during coating process by a shaker for 10 min, at 130 rpm according to **Abd El-Wahab (2011) and Youssef *et. al.* (2018).** Subsequently, the seeds were air- dried on filter paper for 1 hour in a laminar flow hood and stored in refrigerator at 5℃ until required. Then they were sown at the rate of 10 seeds /pot, Five replicates used /each treatment. The fungicide Atero 30% at 3 g / kg seeds were used to compare its inhibitory effect with alternative materials. Seeds were soaked in the fungicides or in water only as control for 20 minutes and planted in the infested soil. Data were recorded as survivals plants at 30 days post planting according to **Hassanein (2013)**.

Statistical Analyses

Data were analyzed using analysis of variance (ANOVA), and the means were compared by the least significant differences (LSD) at $P \geq 0.05$ described by **Snedecor and Cochran (1980)** using COSTAS software v 6.3. The significant mean differences between treatment means were separated by Duncan's Multiple Range Test **(Duncan, 1955)**.

RESULTS AND DISSCUSION

Isolation and Identification of Common Bean and Soybean Associated Fungi

Common bean and soybean seeds were found to have a wide diversity of associated fungi. A collection of nineteen fungal species comprising

thirteen genera were isolated from the collected soybean seed samples, using standard blotter (SB) method. The frequency percentage of seed-borne fungi of soybean and common bean seeds were shown in Tables 1 and 2. The isolated fungi were *Alternaria alternata, Alternaria* sp., *Aspergillus niger, Aspergillus ochraceous, Aspergillus flavus, Botryodiplodia, Cladosporium* sp., *Colletotrichum lindemuthianum, colletotrichum dematium, Fusarium solani, Fusarium moniliforme, Fusarum oxysporum, Macrophomina phaseolina, Myrothecum* sp., *Penicillium* spp., *Rhizoctonia solani, Sclerotium batatecola, Stemphylium* spp., *Trichoderma* spp. and *Trichothecum* sp. The highest average of incidence was recorded by *Cladosporium* sp. being (49.7%), where the least was of *Myrothecum* sp. (3.0%). On the other hand, the average of incidence of Behera, Dakahlia and Ismailia Governorates were 24.3, 16.5 and 21.3% in season 2016 and 26.5, 43.9 and 36.4% in 2017 season, respectively. Frequency percentage for *Colletotrichum* spp. (*C. lindemuthianum* and *C. dematium*) was recorded in, Behera, Dakahlia and Ismailia being 2.1, 0.0 and 0.0 in season of 2016 and 2.4, 0.32 and 0.8 in 2017 season.

Results in Table 2 indicate, the average of common bean seed-borne fungi in 2016 – 2017 growing seasons. Were *Alternaria alternata, Alternaria* sp., *Aspergillus niger, Aspergillus ochraceous, Aspergillus flavus, Botrydiplodia* sp., *Cladosporium* sp., *Colletotrichum* spp., *Fusarium solani, Fusarium moniliforme, Fusarum oxysporum, Macrophomina phaseolina, Myrothecum* sp., *Penicillium* spp., *Rhizoctonia solani, Stemphylium* spp., *Trichoderma* spp. and *Trichothecum* sp. The highest incidence average of the isolated fungi was of *Cladosporium* sp. being 49.6% where the least was of *Myrothecum* sp. being 2.8%. However, the incidence average of the different Egyptian Governorates *i,e*, Behera, Dakhlia and Ismailia (38.9, 23.3 and 34.9) in 2016 season and (43.7, 27.5 and 38.9) in 2017 season, respectively. The heighest frequency (%) for *Colletotrichum* spp. (*C. lindemuthianum* and *C. dematium*) was recorded *i,e*, Behera, Dakhlia and Ismailia governorates were (2.5, 0.0 and 0.0) in 2016 season and 2.8, 0.5 and 0.7 in 2017 season, respectively.

Pathogenicity Tests

Pathogenicity tests

Pathogenicity tests proved that, *Colletotrichum lindemuthianum* and *Colletotrichum dematium* species were pathogenic to common bean and soybean with different values (Table 3). The tested fungi significantly caused high percentage of pre-emergence damping-off to common bean and soybean compared with the control. *Colletotrichum lindemuthianum* recorded the highest percentage of pre- emergence damping-off for common bean (26%), while, *C. dematium* recorded the highest % (38%) on soybean and common bean (25.8). *C. lindemuthianum* showed the heighest % of post-emergence on common bean (20%). but *C. dematium* recorded 13.9% and 13.5%, respectively. Similar results were previously reported by **Infantin *et al*. (2006), Mazen *et al*. (2008), Gomaa (2010), Abd El-Wahab (2011) and Kamel (2017).**

Seed Healthy Testing Methods

Several detection methods have been developed over the years for various seed borne pathogens. Results in Table 4 indicate that, the blotter test being the common but not efficient method of detecting seed borne fungal pathogens in seeds. According to rules of ISTA the method involves plating of 400 seeds on some layers of moistened filter paper. The average of incidence of fungi as shown revealed the highest incidence of *Cladosporium* sp. (20.3%) but the least was of *Colletotrichum* spp. (1.9%). On the other hand, the incidence average by methods *i.e*., stander blotter, agar plate and deep freezing method (30.1, 19.9 and 4.7%) in common bean and 45.2, 28.4and 6.6% in soybean seeds, respectively. Incidence (%) for *Colletotrichum* spp. recorded by tested methods *i.e.* stander blotter, agar plate and deep freezing method were 2.3, 1.5 and 1.2% in common bean and 4.2, 2.1 and 2.4% in soybean seeds, respectively. Current results proved that traditional seed health tests could not be enough to depend on their results since they display markedly varied results. These results are in harmony with those obtained by **Mathur and Cunfer (1993**) who pointed out the importance of adequate plant quarantine, correct diagnosis of symptoms and/or methods of detection and isolation of such dangerous pathogen which could be transferred through seeds. Therefore, most countries have to examine seed samples carefully and/or have to treat seeds with fungicides. In contrary **Khiyami *et al.* (2014)** discussed the common disadvantage of the traditional diagonstic methods is that they are time consuming and lack accuracy.

Table 1. Incidence and frequency percentage of seed-borne fungi associated with soybeans seeds collected from different Governorates in Egypt

Isolated fungi	2016						2017						Mean
	Behera		Dakahlia		Ismailia		Behera		Dakahlia		Ismailia		
	In	F	In	F	In	F	In	F	In	F	In	F	
Alternaria alternata	34.2	7.8	11.1	3.7	30.2	7.9	36.4	7.6	32.1	7.7	13.8	4.0	26.3
***Alternaria* sp.**	8.9	2.0	6.8	2.3	11.9	3.1	11.1	2.3	13.8	3.3	9.5	2.7	10.3
Aspergillus niger	39.1	8.9	9.5	3.2	40.1	10.5	41.3	8.7	42	10.1	12.2	3.5	30.7
Aspergillus ochraceous	20.7	4.7	10	3.4	21.7	5.7	22.9	4.8	23.6	5.7	12.7	3.7	18.6
Aspergillus flavus	27.5	6.3	7.9	2.7	19.9	5.2	29.7	6.2	21.8	5.2	10.6	3.1	19.6
Betryodiplodia	19.1	4.4	19.1	6.4	19.1	5.0	21.3	4.5	21	5.0	21.8	6.3	20.2
***Cladosporium* sp.**	48.6	11.1	48.6	16.4	48.6	12.7	50.8	10.6	50.5	12.1	51.3	14.8	49.7
Colletotrichum lindemathianum	4.3	1.0	0	0	0	0	5.3	1.1	0.52	0.12	1.4	0.43	1.92
Colletotrichum dematium	4.9	1.1	0	0	0	0	6.1	1.3	0.43	0.2	1.3	0.40	2.13
Fusarium solani.	30.2	6.9	30.2	10.2	11.1	2.9	32.4	6.8	13	3.1	32.9	9.5	25.0
Fusarium moniliforme	34.2	7.8	1.1	0.4	34.2	8.9	36.4	7.6	36.1	8.7	3.8	1.1	24.3
Fusarum oxysporum	39.1	8.9	61.1	20.6	37.1	9.7	41.3	8.7	39	9.4	63.8	18.5	46.9
***Penicillium* spp**	14.6	3.3	10.2	3.4	16.2	4.2	16.8	3.5	18.1	4.3	12.9	3.7	14.8
***Myrothecum* sp.**	1.3	0.3	1.3	0.4	3	0.8	3.5	0.7	4.9	1.2	4	1.2	3.0
Rhizoctonia solani	27.6	6.3	17.6	5.9	27.6	7.2	29.8	6.2	29.5	7.1	20.3	5.9	25.4
Sclerotium batatecola	25.3	5.8	11.1	3.7	21.7	5.7	27.5	5.8	23.6	5.7	13.8	4.0	20.5
***Stemphylim* spp.**	12.7	2.9	10.3	3.5	9.3	2.4	14.9	3.1	11.2	2.7	13	3.8	11.9
***Trichoderma* spp.**	27.6	6.3	17.6	5.9	13.6	3.6	29.8	6.2	15.5	3.7	20.3	5.9	20.7
***Trichothecum* sp.**	17.6	4.0	23.6	7.9	17.6	4.6	19.8	4.2	19.5	4.7	26.3	7.6	20.7
Mean	24.3		16.5		21.3		26.5		43.9		36.4		------

F.= Frequency (%) = (No. of infected samples) / (Total No. of tested samples) ×100 In.= incidence
Mean of sample infection = (Σ fungus incidence in all examined samples) / (Total No. of examined samples).

Table 2. Incidence and frequency percentage of seed-borne fungi associated with common bean seeds collected from different Governorates in Egypt

Isolated fungi	2016						2017						Mean
	Behera		Dakahlia		Ismailia		Behera		Dakahlia		Ismailia		
	In	F	In	F	In	F	In	F	In	F	In	F	
Alternaria alternata	34.2	9.3	11.1	5.0	30.2	9.1	36.5	8.8	12.9	4.9	31.9	8.6	26.1
***Alternaria* sp.**	8.9	2.4	6.8	3.1	11.9	3.6	11.2	2.7	8.6	3.3	13.6	3.7	10.2
Aspergillus niger	39.1	10.6	9.5	4.3	40.1	12.1	41.4	10.0	11.3	4.3	41.8	11.3	30.5
Aspergillus ochraceous	20.7	5.6	10	4.5	21.7	6.5	23	5.5	11.8	4.5	23.4	6.3	18.4
Aspergillus flavus	27.5	7.4	7.9	3.6	19.9	6.0	29.8	7.2	9.7	3.7	21.6	5.8	19.4
***Botrydiplodia* sp.**	19.1	5.2	19.1	8.6	19.1	5.8	21.4	5.1	20.9	8.0	20.8	5.6	20.1
***Cladosporium* sp.**	48.6	13.2	48.6	22.0	48.6	14.6	50.9	12.2	50.4	19.3	50.3	13.6	49.6
Colletotrichum lindemathianum	4.5	1.23	0	0	0	0	5.6	1.3	0.50	0.3	1.6	0.4	2.03
Colletotrichum dematium	4.7	1.27	0	0	0	0	5.8	1.5	0.45	0.2	1.1	0.3	2
Fusarium solani.	13.5	3.7	8.6	3.9	8.8	2.7	22.5	5.4	20	7.6	18.8	5.1	15.4
Fusarium moniliforme	5.5	1.5	2.9	1.3	5.9	1.8	6.5	1.6	2.9	1.1	5.9	1.6	4.9
Fusarum oxysporum	29.7	8.0	24.9	11.3	26.8	8.1	31.4	7.6	26.9	10.3	28.8	7.8	28.1
Macrophomina phaseolina	14.6	4.0	10.2	4.6	16.2	4.9	16.9	4.1	12	4.6	17.9	4.8	14.6
***Myrothecum* sp.**	1.3	0.4	1.3	0.6	3	0.9	3.6	0.9	3.1	1.2	4.7	1.3	2.8
***Penicillium* spp.**	27.6	7.5	17.6	8.0	27.6	8.3	29.9	7.2	19.4	7.4	29.3	7.9	25.2
Rhizoctonia solani	25.3	6.8	11.1	5.0	21.7	6.5	27.6	6.6	12.9	4.9	23.4	6.3	20.3
***Stemphylim* spp.**	12.7	3.4	10.3	4.7	9.3	2.8	15	3.6	12.1	4.6	11	3.0	11.7
***Trichoderma* spp.**	27.6	7.5	17.6	8.0	13.6	4.1	29.9	7.2	19.4	7.4	15.3	4.1	20.6
***Trichothecum* sp.**	4.3	1.2	3.7	1.7	7.6	2.3	6.6	1.6	5.5	2.1	9.3	2.5	6.2
Mean	38.9		23.3		34.9		43.7		27.5		38.9		------

F.= Frequency (%) = (No. of infected samples) / (Total No. of tested samples) ×100 In.= incidence
Mean of sample infection = (Σ fungus incidence in all examined samples) / (Total No. of examined samples).

Table 3. Pathogenicity test of *Colletotrichum lindemuthianum* and *Colletotrichum dematium* with common bean and soybean plant

Fungi	Common bean				Soybean			
	Pre-emergence	Post-emergence	Survivals	Root rot	Pre-emergence	Post-emergence	Survivals	Root rot
Colletotrichum lindemuthianum	26 a	20 a	54 a	5.5	22.4a	11.6a	66.0a	4
Colletotrichum dematium	25.8a	13.9b	61.3b	2	38 b	13.5a	48.5b	3
Control	0.0 b	0.0 c	100 c	0.0	0.0 c	0.0 b	100c	0.0
LSD at - 0.05	2.12	1.47	2.06	-----	2.01	3.18	3.92	-----
Coefficient of variation	6.44	5.61	2.06	-----	5.70	13.98	3.90	-----

Identification of *Colletotrichum* spp. Using Internal Transcribed Spacer Region (ITS region)

Fig. 1 shows typical PCR amplification of common bean and soybean seeds infected with the pathogenic fungus *C. lindemuthianum* and *C. dematium*, even a minimum amount of DNA template was amplified by the used primers pair ITS4 and CIF5 that migrated in agarose gel electrophoresis. Lanes 3 and 4 resulted in approximately 593bp two band fragments of *C. dematium* generated with the ITS4 primer and CIF5. However lanes 1 and 2 resulted in approximately 461bp other two bands fragments of *C. lindemuthianum* generated with the ITS4 primer and CIF5 while negative control represents (lane 5).

Fig. 2 shows typical PCR amplification of common bean and soybean seeds infected with the pathogenic fungus *Colletotrichum* sp. even a minimum amount of DNA template was amplified by the used primer pairs ITS4 and CIF4 and migrated in agarose gel electrophoresis, Lanes 1 and 2 resulted in approximately 461bp two band fragment of *Colletotrichum lindemuthianum* generated with the ITS4 primer and CIF4. However lanes 3 and 4 resulted no *Colletotrichum dematium* bands generated with the ITS4 primer and CIF4 while negative control represents (lane 5).

Chen *et al.* (2007) mentioned that, the insufficient detection of plant pathogens using conventional culture-based morphological methods has led to the development of nucleic acid-based molecular approaches. Modification of DNA extraction methods and PCR amplification may enhance sensitivity and specificity of PCR product plus increasing the usage of DNA in variable modern techniques. Species-specific PCR, using ITS region of rDNA, has been widely advocated for rapid identification of *C. lindemuthianum* and *C. dematium* and for differentiating closely related fungal species (**Freeman *et al.*, 2000; Schiller *et al.*, 2006)**. According to **Serra *et al.* (2011)**, study in the analysis of the ITS sequence of ribosomal DNA for *C. lindemuthianum* and *C. dematium*, all isolates amplified with the ITS4, The amplicon sizes of ITS region in this study were in line with the results of other authors who worked on different species of *Colletotrichum*. For instance, **Lima *et al.* (2013)** recorded the sequences of the ITS region of *Colletotrichum* isolates ranged from 484 to 598 bp. On the other hand, **Photita *et al.* (2005)** reported that the ITS region of *Colletotrichum* spp. which they studied varied from 581 to 620 bp.

Table 4. Incidence percentage of isolated fungi using different methods of common bean and soybean seeds

Isolated fungi	Common bean				Soybean				
	SBM	AP	DFM	Mean	SBM	AP	DFM	Mean	Mean*
Alternaria alternata	18.5	7.5	4.5	10.2	26.3	11.4	4.6	10.7	10.4
***Alternaria* spp.**	12.2	4.1	2.3	6.2	10.3	4.6	2.6	4.4	5.3
Aspergillus niger	19.0	10.1	3.3	10.8	30.7	19.2	4.2	13.8	12.3
Aspergillus ochraceous	13.4	8.1	2.8	8.1	18.6	11.7	4.8	8.8	8.5
Aspergillus flavus	13.8	10.0	1.4	8.4	19.6	13.5	2.1	9.0	8.7
***Botryodiplodia* sp.**	20.2	17.8	2.4	13.4	20.2	19.5	1.8	10.8	12.1
***Cladosporium* sp.**	44.6	18.9	3.2	22.2	49.7	19.7	2.1	18.3	20.3
***Colletotrichum* sp.**	2.3	1.5	1.2	1.7	4.2	2.1	2.4	2.1	1.9
Fusarium solani.	14.8	8.0	1.7	8.1	25.0	16.7	2.1	11.3	9.7
Fusarium moniliforme	4.5	3.8	0.8	3.0	24.3	9.5	1.5	9.0	6.0
Fusarium oxysporum	25.4	14.1	2.5	14.0	46.9	28.7	5.4	20.8	17.4
Macrophomina phaseolina	15.9	18.1	3.4	12.5	27.0	15.0	3.6	11.6	12.0
***Myrothecium* sp.**	4.3	3.2	0.7	2.7	25.4	19.7	6.5	13.1	7.9
***Penicillium* spp.**	21.7	15.2	5.7	14.2	20.5	24.3	4.5	12.8	13.5
Rhizoctonia solani	16.3	22.0	3.2	13.8	27.0	15.0	3.6	11.6	12.7
***Stemphylium* spp.**	16.0	10.2	2.0	9.4	11.9	3.8	1.1	4.2	6.8
***Trichoderma* spp.**	15.1	12.5	3.0	10.2	20.7	18.1	5.5	11.3	10.7
***Trichothecium* sp.**	8.1	4.2	0.5	4.3	20.7	17.7	3.9	10.9	7.6
Mean	30.1	19.9	4.7	------	45.2	28.4	6.6	------	------

Incidence (%) = (No. of infected samples in 2016+2017).

Mean of sample infection = (Σ fungus incidence in all examined samples) / (Total No. of examined samples). SBM = Standard blotter method. AP = Agar plate method. DFM = Deep freezing method.

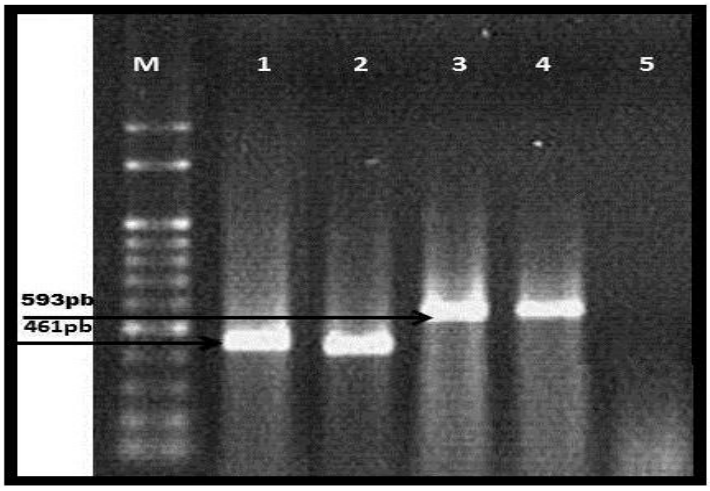

Fig. 1. Agarose gel (1%) electrophoresis pattern of amplified ITS-PCR for common bean seed pathogenic fungi *Colletotrichum lindemuthianum*. Lane M= Genomic DNA marker VC100pb fractionated (100, 200, 300, 400, 500, 600,700, 800, 900, 1000, 1100 and 1200 bp); lane 1 – *Colletotrichum lindemuthianum* DNA template extracted from pure culture; lane 2 – Infected common bean seeds with *Colletotrichum lindemuthianum* DNA template extracted; lane 3 – *Colletotrichum dematium* DNA template extracted from pure culture; lane 4 – infected soybean seeds with *Colletotrichum dematium* DNA template extracted, Lane 5-PCR negative control (sterile distilled water).

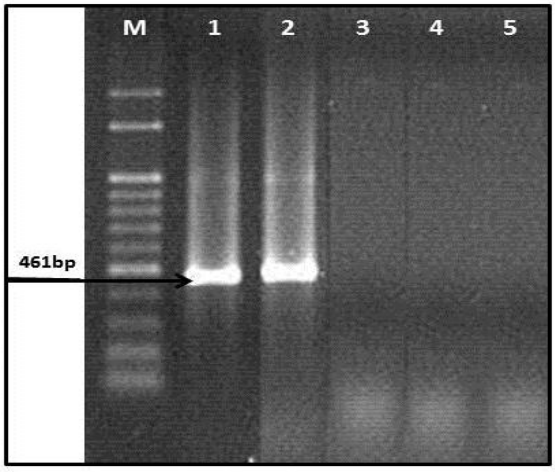

Fig. 2. Agarose gel (1%) electrophoresis pattern of amplified ITS-PCR for common bean seed pathogenic fungi *Colletotrichum lindemuthianum*. Lan M= Genomic DNA marker VC100pb fractionated (100, 200, 300, 400, 500, 600,700, 800, 900, 1000, 1100 and 1200 bp); lane 1-*Colletotrichum lindemuthianum* DNA template extracted from a pure culture; lane 2-Infected common bean seeds with *Colletotrichum lindemuthianum* DNA template extracted; lane 3-*Colletotrichum dematium* DNA template extracted from a pure culture; Lane 4-Infected soybean seeds with *Colletotrichum dematium* DNA template extracted; Lane 5-PCR negative control (sterile distilled water) finely *Colletotrichum lindemuthianum* from common bean seed and *Colletotrichum dematium* from soybean seed

Control Studies

Effect of bioagents on the growth of *Colletotrichum* spp. *in vitro*

Isolates of *Trichoderma viride, Trichoderma harzianum, Bacillus subtilis* and *Pseudomonas floursence* were used as biocontrol against for *C. lindemuthianum* and *C. dematium*. Results in Table 5 showed that, all examined bioagents exhibit different degrees of antagonism to the growth of tested fungi. Results also indicated that *T. harzianum* revealed the least linear growth (2.9 cm) for the tested fungi followed by *T. viride* and *B. subtilis* being 3.3, 3.9 cm, respectively. While *P. floursence* display the lowest reduction percent of linear growth reduction percent (7.4 cm). On the other hand, fungicide Aetro 30% (Iprodione 20% + Tebuconazole 10%) recorded the least reduction liner growth (0.9 cm) for both pathogens tested compared with negative control which recorded 9.0 cm in diameter. Some investigators explained the mode of action of *Trichoderma* spp. against many pathogenic fungi as due to metabolites produced in the medium, which has fungi-static effect on other fungi, who revealed that *Trichoderma* spp. activity against other pathogenic fungi was due to the production of certain antimicrobial such as tricholin, which inhibit the mycelial growth when spread in the medium. There were three modes of the action expressed by the bio-control agent *Trichoderma* spp. was recognized to produces number of antibiotics, *i.e.* trichodermin, trichodermol A and harzianolide. These compounds are responsible for the inhibition of most fungal phytopathogens **(El-Abbasi *et al.*, 2003), (Nawar 2007)** and **(Abd El-Wahab, 2011).**

Effect of plant extracts on the growth of some selected seed-borne fungi of dry bean and soybean *in vitro*

All tested plant extracts such as clove, thymus, and garlic had a significant reduction in the growth of *C. lind* and *C. dematum* (Table 6). Thymus extract caused the lowest liner growth (3.7 cm) followed by clove and garlic extracts (4.2 and 5.8 cm, respectively). Plant extract concentrations caused a significant reduction in the growth of tested fungi. The high concentration (1.5%) gave less fungal growth (3.3 cm). While the lowest one (0.5%) gave the

Table 5. Effect of biocontrol agent on linear growth and efficacy of *Colletotrichum linemuthianum* and *Colletotrichum dematium*

Bioagents (A)	Fungi (B)				Mean (A)
	C. linemuthianum		*C. dematium*		
	L.G	E	L.G	E	
Trichoderma harzianum	3.1	65.6	2.7	70.0	2.9
Trichoderma viride	3.9	56.7	2.8	68.9	3.3
Bacillus subtilis	4.0	55.6	3.9	56.7	3.9
Pseudomonas floursence	7.4	17.8	7.4	17.8	7.4
Iprodione 20% + Tebuconazole 10%	1.2	86.7	0.7	92.2	0.9
Control	9.0	0.0	9.0	0.0	9.0
Mean (B)	4.7		4.4		-------

Coefficient of Variation = 5.914

Critical Difference Values

-	LSD 5%	LSD 1%
Factor A	0.326	0.444
Factor B	0.188	0.256
Treatments/(AxB)	0.462	0.627

Table 6. Effect of plant extracts on linear growth and efficacy (cm) of *Colletotrichum linemuthianum* and *Colletotrichum dematium*

Plant extract (A)	Concentration ppm (B)	Fungi (C)				Mean (AB)	Mean (A)
		C. linemuthianum		*C. dematium*			
		LG	E	LG	E		
	0.5%	8.2	8.9	5.7	36.7	6.9	
Garlic	1%	7.5	16.7	5.1	43.3	6.3	
	1.5%	7.3	18.9	4.6	48.9	5.9	5.8
Iprodione 20% + Tebuconazole 10%		1.2	86.7	0.7	92.2	0.9	
Control		9.0	0.0	9.0	0.0	9.0	
Mean (AC)		6.6		5.0		-------	
	0.5%	5.3	41.1	3.4	62.2	4.4	
Clove	1%	4.6	48.9	2.6	71.1	3.6	
	1.5%	3.9	56.7	2.3	74.4	3.1	4.2
Iprodione 20% + Tebuconazole 10%		1.2	86.7	0.7	92.2	0.9	
Control		9.0	0.0	9.0	0.0	9.0	
Mean (AC)		4.8		3.6		-------	
	0.5%	5.2	42.2	3.8	57.8	4.5	
Thymus	1%	3.9	56.7	3.0	66.7	3.4	
	1.5%	0.8	91.1	0.8	91.1	0.8	3.7
Iprodione 20% + Tebuconazole 10%		1.2	86.7	0.7	92.2	0.9	
Control		9.0	0.0	9.0	0.0	9.0	
Mean (AC)		4.0		3.4		Mean (B)	
	0.5%	6.2		4.3		5.3	
Over all means (BC)	1%	5.3		3.6		4.4	
	1.5%	4.0		2.6		3.3	
Iprodione 20% + Tebuconazole 10%		1.2		0.7		0.9	
Control		9.0		9.0		9.0	
Mean (C)		5.1		4.0		----------	

Control (+) =Effect of Aetro 30% (Iprodione 20% + Tebuconazole 10%) at (1000 ppm) on linear growth (cm). Control (-) =Linear growth (cm) of pathogens.

Coefficient of Variation = 4.8

Critical Difference Values -	LSD 5%	LSD 1%
Factor – A	0.115	0.153
Factor – B	0.149	0.198
Factor – C	0.094	0.125
A x B	0.257	0.342
A x C	0.163	0.217
B x C	0.210	0.280
A x B x C	0.364	0.484

highest fungal growth (5.3 cm). On the other hand, fungicide Aetro 30% (Iprodione 20% + Tebuconazole 10%) recorded the lowest liner growth (0.9 cm) for the pathogens compared with control which recorded 9.0 cm in diameter. Generally, clove, thymus, and garlic extracts have an inhibition effect on *C. lindemuthianum* and *C, dematium.* The application of the crude extracts would probably show better antifungal activities.

Results of the effectiveness of the present extracts on the inhibition of mycelial growth are, to somewhat, similar to those reported by **Halawa (2004), Hassanin *et al.* (2007) and El-Mougy *et al.* (2007).** Also, the obtained results concerning the increase in the inhibitory effect of each extract by increasing its concentration coincide with **El-Habaa *et al.* (2002) and Shafie (2004).** On the other hand, these extracts might contain fungicidal or fungistatic substances, causing inhibition to the formation of fungal spores and the sclerotial formation and their germination as well as preventing the formation of reproductive of the fungal organs. These results are, to somewhat, similar to those reported by **Ahmed and Sultana (1984)** who stated that garlic and cloves extract inhibited spore germination and mycelial growth of some important fungal pathogens of jute such as *M. phaseolina* and *C. corchari*. The mode of action of the active substances in extracts of medicinal and aromatic plants was interpreted by many scientists. **Zambonelli *et al.* (1996) and Wilson *et al.* (1997)** mentioned that these antifungal substances have high capabilities to damage the structure and function of the enzymatic bioactivity **(Hassanin, 2013).**

Greenhouse Exeperements

Effect of fungicide, plant extracts and bio-agents materials on disease incidence (%), after planting in soil infested with *Colletotrichum lindemuthianum* and *Colletotrichum dematium in vivo*

The effect of plant extracts, bio-agents and fungicides were applied as seed treatments, on disease incidence 30 days post planting in soil artificially infested with *C. lindemuthianum and C. dematium*. Results in Table 7 indicate that, percentages of disease incidence decreased, with all tested treatment. Atero 30% was the most effective followed by *T. harzianum, B. subtilis, T. viride,* thymus extract, clove extract, *P. floursence* and garlic extract (1.5%), respectively. On the other hand,

Table 7. Effect of different seed treatments on disease incidence (%), 30days post planting in soil infested with *Colletotrichum lindemuthianum* on common bean and *Colletotrichum dematium* on soybean under greenhouse conditions

Treatment	*Colletotrichum lindemuthianum*		*Colletotrichum dematium*		Mean	
	Survivals (%)	Disease incidence (%)	Survivals (%)	Disease incidence (%)	Survival (%)	Disease incidence (%)
Trichoderma harzianum	90.1	9.9	93.3	6.7	91.7	8.3
Trichoderma viride	85.0	15.0	86.0	14.0	85.5	14.5
Bacillus subtilis	89.0	11.0	87.5	12.5	88.3	11.7
Pseudomonas floursence	78.5	21.5	76.5	23.5	77.5	22.5
Garlic	73.3	26.7	71.7	28.3	72.5	27.5
Clove	78.3	21.7	83.5	16.5	80.9	19.1
Thymus	83.0	17.0	83.0	16.0	83.5	16.5
Atero 30%	93.7	6.3	94.9	5.1	94.3	5.7
Control (negative)	100	0.0	100	0.0	100	0
Control (posative)	54.0	46.0	48.0	52.0	51	49.0
Mean (B)	84.5	15.5	84.9	15.1	84.7	15.3
LSD 5%	6.724		7.351		----------	

Atero 30%, *T. harzianum, B. subtilis*, *T. viride*, and thymus extract were effect disease incidence on all tested fungi. Atero 30% was the most effective treatment compared with all treatment which gave highly effect on disease incidence of *C. lindemuthianum* and *C. dematium* (6.3 and 5.7%, respectively) followed by *T. harzianum* (9.9 and 6.7%, respectively), *B. subtilis*, *T. viride* and thymus extract. Moreover, garlic extract gave the lowest effective one on *C. lindemuthianum* and *C. dematium* (26.7% and 28.3%), respectively. Compared with negative and positive control. Results of the effectiveness treatments of the present extracts and fungicide on the inhibition of mycelial growth are, to somewhat, similar to those reported by **Zeilinger and Omann (2007)**, **Shovan *et al.* (2008), Abd El-Wahab (2011), Gveroska and Ziberoski (2012), Hassanin (2013), Mohamed *et al.*** (**2013-b**)**, Yousef *et al.* (2016) and Ghoneem *et al.* (2019**).

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الكشف الجزيئى و مكافحة أنواع الفطر *Colletotrichum* spp. المحمول ببذور الفاصوليا وفول الصويا

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أستخدمت معاملات مختلفة للبذور كبدائل للمبيدات الكيماوية لمقاومة الأمراض الفطرية المحمولة ببذور بعض التقاوى البقولية (الفاصوليا وفول الصويا) حيث تم حصر الفطريات المحمولة على بذورها فى ثلاث محافظات مختلفة فى مصروهى البحيرة، الدقهلية، الإسماعيلية. تم عزل ١٩ نوعا فطريا تنتمى الى ١٣ جنس باستخدام طريقة أوراق الترشيح المبللة (Standard blotter) وكانت الفطريات المعزولة كالتالى: *Alternaria alternata, Alternaria* spp., *Aspergillus niger, Aspergillus ochraceous, Aspergillus flavus, Botryodiplodia* sp., *Cladosporium* sp., *Colletotrichum* spp., *Fusarium solani, Fusarium moniliforme, Fusarum oxysporum, Macrophomina phaseolina, Myrothecium* sp., *Penicillium* spp, *Rhizoctonia solani, Stemphylium* spp., *Trichoderma* spp. and *Trichothecium* sp.، وباجراء إختبار القدرة المرضية وجد ان عزلات الفطر(*Colletotrichum* spp.) كانت ممرضة للفاصوليا وفول الصويا . ووجد أن إختبارتفاعل البلمرة المتسلسل (PCR) المستخدم فى الكشف والتشخيص لفطر*C. lindemuthianum* and *C. dematium* المحمول ببذور الفاصوليا وفول الصويا على التوالى أحد الطرق الهامة والسريعة والأكثر دقة فى التعريف والتشخيص المستخدمة بالبحث عن طريق التشخيص الجزيئيى للفطر ولإجراء تفاعل البلمرة يلزم استخدام ثلاث بادئات وهما ITS4, ClF4 and ClF5 مع استخلاص للحمض النووى DNA بصورة نقية، وأجريت اختبارات الطرق القياسية لصحة وسلامة البذور المستخدمة وهى (طريقة اوراق الترشيح المبللة، طريقة أطباق الأجار وطريقة التجميد) وجد أن طريقة أوراق الترشيح المبللة هى الأفضل والأسرع بينهم فى الكشف عن وجود الفطريات المحمولة على البذور، وثبط فطر*Trichoderma harzianum* النمو الطولى لعزلات فطر *C. lindemuthianum* and *C. dematium* سجل ٢,٩ سم لكلتا العزلتين ويليه فطر*Trichoderma viride* ثم بكتيريا *Bacillus subtilis* حيث سجل كلا منهم (٣,٣سم و٣,٩ سم)على التوالى بينما كانت بكتيريا *Pseudomonas floursence* الأقل فى التأثير على النمو الطولى (٧,٤ سم) بالمقارنة بالمبيد الفطرى أيترو٣٠% حيث سجل أقل نمو طولى٠,٩ سم للفطريات *C. lindemuthianum* and *C. dematium* وكذلك سجل المستخلص النباتى لنبات الزعتر أقل نمو طولى ٣,٧ سم وسجل مستخلصى القرنفل والثوم ٤,٢ سم و٥,٨ سم على التوالى ،وأوضحت التركيزات المختلفة للمستخلصات النباتية اختزال فى النمو الطولى لعزلات فطر (*C. lindemuthianum* and *C. dematium*) حيث سجل التركيز ١,٥% أقل نمو طولى ٣.٣سم بينما التركيزين ١%، ٠,٥% سجلا نمو طولى ٤,٤سم ، ٥,٣ سم على التوالى وعلى الجانب الآخر أعطى المبيد الفطرى أيترو ٣٠% الذى يحتوى على المواد الفعالة وهى (Iprodione + Tebuconazole) أقل نمو طولى ٠.٩ سم للفطريات (*C. lindemuthianum* and *C. dematium*) مقارنة بالكنترول ٩سم.

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