Antagonistic effects of *Securigera securidaca* extracts, *Bacillus cereus* and *Pseudomonas fluorescens* against *Aspergillus* sp., *Fusarium solani*, and *Rhizoctonia* sp. in vitro

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ABSTRACT
The study was carried out in the Diyala Agriculture Directorate, Plant Diseases Laboratory, during the year 2021 to evaluate the performance of *Securigera securidaca* concentrations (5%, 10%, and 15%) and two bacteria *Bacillus cereus* and *Pseudomonas fluorescens* against *Aspergillus* sp, *Fusarium solani*, and *Rhizoctonia* sp in vitro. The results showed that the extract concentrations 5,10 and 15% of *S. securidaca* inhibited the mycelial growth of *Aspergillus* sp. with inhibition percentage 45.9 %, 55.2 % and 39.4 % respectively. The highest inhibition effect of plant extract on the mycelial growth of *Fusarium solani* was 49.9% at the concentration 5%, while the inhibition percentage was 22.3% and 26.5% at the concentrations 10 and 15% respectively. The concentration 15% was superior in inhibition of mycelial growth of *Rhizoctonia* sp. 58.1%, followed by the concentration 5% and 10%, which reached 52.0% and 41.6% respectively. The bacteria *P. fluorescens* inhibited the mycelial growth of *Aspergillus* sp.,*Fusarium solani* and *Rhizoctonia* sp. with inhibition percentage 15.2 %, 22.4% and 61.1% respectively, whereas the bacteria *B. cereus* inhibited the mycelial growth of previous fungi with inhibition percentage 12.4 %,18.3 % and 58.8 % respectively.

Keywords: *Bacillus cereus*, *Pseudomonas fluorescens* and *Securigera securidaca*

INTRODUCTION

The main problem in the yield reduction of agricultural crops, as well food and feed storage is spoilage and poisoning caused by microorganisms such as fungi and bacteria. Plant diseases cause significant damage for plants during their growth stages, resulting in a decrease in yield and product quality **(21)**. There are many fungi such as *Fusarium* sp, *Rhizoctonia* sp and *Aspergillus* sp causes great economic losses worldwide, besides its produce mycotoxins that cause potential health risks. Fusarium species exists in soil in both tropical and temperate regions as chlamydospores and mycelia, then attacks and colonizes xylem vessels and causing plant wilting, stunting, death and resulting in significant economic losses **(19,20).** *Rhizoctonia* sp attacks a plant at all growth stages and become dangerous in the early stages of plant **(12).** *Aspergillus* sp produces mycotoxins and is one of the most common food-contaminating organisms **(9).** There is an urgent need to reduce the use of chemical pesticides in order to reduce environmental pollution and improve plant growth, it has become necessary to use eco-friendly methods to improve crop health and yield, as a result, botanical extracts and microorganisms are the best alternatives to these dangerous chemicals. The medicinal plants are sources of many compounds such as alkaloids, flavonoids, tannins and phenolic (**16)**.*Securigera securidaca* (goat pea) is a herbaceous plant belong to the Fabaceae family and called goat pea **(8).** *S. securidaca* seeds extract possesses various curative properties, due to it have cardenolides , coumarins, saponins, sterols and flavonoids **(15,11).** In spite of the numerous therapeutic properties to *S. securidaca* seeds extract regarding human health, there is not much scientific data available about its antifungal activity. Bio-control mechanisms occur in the nature against different pathogens

and pests and it is an eco-friendly approach and also can be successfully exploited in the integrated disease and pest management **(19).** *Bacillus* species possess antagonistic ability against pathogens due to their activity in reproduction as well as tolerate severe ecological conditions and their production many antimicrobial compounds such as enzymes, antibiotics and lipopeptides in addition their compete with other pathogens for space and nutrients **(24**). *Bacillus cereus* is facultative anaerobic and forming endospores, which exist everywhere in the soil **(23,10,14,26).** There are different mechanisms to *Pseudomonas fluorescens*, which act to suppress the soil borne pathogens, including antibiosis, rhizosphere colonization, and iron chelation by siderophore production **(13).** Also *Pseudomonas* acts as an antimicrobial and produce several antibiotics against various microorganisms **(27).** The objective of this study was to evaluate the antagonistic activity of *S. securidaca* seeds extract, with *B. cereus* and *P. fluorescens* in vitro against *Aspergillus* sp, *Fusarium solani*, and *Rhizoctonia* sp.

MATERIAL AND METHODS

Samples Collection

The study was carried out in the Diyala Agriculture Directorate, Plant Diseases Laboratory, during the year 2021.

The seeds of *S. securidaca* were obtained from the College of Pharmacy, University of Isfahan, Iran and were grown in the fields of Agriculture College , Diyala University by Dr. Ahmed Y. Hassan to increase its quantity. The plates of *Pseudomonas fluorescens, Bacillus cereus* and the plant pathogenic fungi *Aspergillus* sp, *Fusarium solani* and *Rhizoctonia* sp were obtained from the Diyala Agriculture Directorate, plant pathology Lab.

Preparation of aqueous extract

The *S. securidaca* seeds were grounded to get the powder, and 50 g of material was soaked in 500 ml distilled water for 24 hours at room temperature, then added the mixture in a blender for homogeneity and mixing and filtered through a double-layered tissue of muslin, then the extract was stored until use **(22).**

Poisoned food test

The technique of poisoned food was used to assess the performance of *S. securidaca* extract against the tested fungi, three concentrations of 5, 10 and 15 % were prepared with PDA medium (Potato Dextrose Agar), through added 5 , 10 and 15 ml of *S. securidaca* extract separately for 95, 90 and 85 ml of PDA to get concentrations 5, 10 and 15% respectively, then the media was poured separately into petri plates (9 cm) and the control treatment included only PDA medium. After that the agar discs (6 mm) of *Aspergillus* sp, *Fusarium solani*, and *Rhizoctonia* sp from the seven-day-old culture were transferred to the centers of petri plates and incubated at 25±2 ^{o}C for seven days. The treatments were performed in three replications and the mean diameters of fungi growth were calculated. The inhibition ratio was calculated according to following formula:

% Inhibition = (1- control colony/ treatment colony) ×100

Antibacterial ability test against fungi

Agar well Diffusion Method technology was used through making holes 6 mm by a cork borer in the PDA medium in Petri dishes 9 cm at a rate of 4 holes for each Petri dish with three replications for each treatment. A part of each growing bacteria in the nutrient agar medium were transferred by the loop to the nutrient broth medium separately and after growing bacteria *P. fluorescens* and *B. cereus* on nutrient broth medium at 37±2 ^{o}C for two days, each hole was filled with bacterial inoculum separately and the center of each petri dish was inoculated with the pathogenic fungi *Aspergillus* sp, *Fusarium solani*, and *Rhizoctonia* sp separately. Three petri dishes were used as control without bacteria, Then the petri dishes were incubated under room temperature for four days **(18).** The diameters mean of fungi growth was measured and the inhibition ratio was calculated according to following formula.

% Inhibition = (1- control colony/ treatment colony) ×100

Statistical analysis: The results were analyzed with complete randomized design

(CRD) by using the (SAS) program and the averages were compared with Duncan's polynomial test at a probability level of 0.05 **(4).**

RESULTS AND DISCUSSION

The results in Table (1) and Fig (1) showed that *S. securidaca* concentrations 5,10 and 15% led to a significant increase in the inhibition of mycelial growth. The percentages of inhibition for *Aspergillus* sp. were 45.9 %,55.2 % and 39.4 % in the plant extract concentrations 5,10 and 15% respectively compared with control treatment 0%. The highest extract concentration was 5% which significantly inhibited the mycelial growth of *Fusarium solani*, 49.9 % compared with the concentrations 10% and 15% that were 22.3% and 26.5 % without any significant differences. The concentration 15% was superior in inhibition of mycelial growth of *Rhizoctonia* sp. 58.1%, followed by the concentration 5% and 10%, which reached 52.0% and 41.6% respectively. Compared to controls, both *P. fluorescens* and *B. cereus* had a significant inhibition effect on the mycelial growth of the tested fungi without statical differences between treatments (Table (2) and Fig (2)). However, *P. fluorescens* and *B. cereus* showed the percentages of inhibition were 15.2% and 12.4% against Aspergillus sp., 22.4% and18.3% against *Fusarium solani*, and 61.1% and 58.8% against *Rhizoctonia* sp. *B .cereus* and *P. fluorescens* can be used as biocontrol agents against the tested fungi, this result is consistent with **(3)** which found all the *Bacillus* spp led to suppress growth of *Fusarium solani* in change degrees, where *Bacillus cereus* gave the highest inhibition reached 55.70%. *Bacillus cereus* showed suppress growth of *Rhizoctonia solani* on potato dextrose agar (PDA) medium (**1).** *Bacillus cereus* exhibited antagonism against *Rhizoctonia solani* under in-vitro conditions **(7). (2)** reported that *P. fluorescens* possess potential to produce proteases that causes antagonistic activity against many pathogenic fungi by degrading cellulolytic enzymes produced by that fungi. *P. fluorescens* is a biocontrol agent against *Aspergillus niger* **(25).** *P. fluorescens* acts as a bio-agent for inhibiting *Fusarium oxysporum* f. sp. *Cumini* **(17).** *P. fluorescens* inhibited the growth of *R. solani* with the most antagonism and formed higher inhibition zones **(5).** *S. securidaca* extract revealed perfect antifungal effects, due to it contains phytochemicals, where it had an effect on Candida strains **(28).** All concentrations of *S. securidaca* extract possess inhibitory effects on Escherichia coli due to their antimicrobial properties **(6).**

CONCLUSION

The bacteria *P. fluorescens* and *B.cereus* and extract of *S. securidaca* showed antagonistic activity against *Aspergillus* sp., *Fusarium solani* and *Rhizoctonia* sp. in vitro and formed an inhibition zones.

Table 1. Antifungal effect of *S. securidaca* concentrations on the fungal growth

S. securidaca concentrations (%)	Rate of inhibition (%)		
	Fungal isolates		
	Aspergillus sp	*F. solani*	*Rhizoctonia* sp
5	45.9 A	49.9 A	52.0 B
10	55.2 A	22.3 B	41.6 C
15	39.4 A	26.5 B	58.1 A
0 Control	0.0 B	0.0 C	0.0 D

The letters mean the significant differences between the inhibition percentages

Table 2. Effect of *P. fluorescens* and *B .cereus* on the fungal growth

Bacterial strains	Rate of inhibition (%)		
	Fungal isolates		
	Aspergillus sp	*F. solani*	*Rhizoctonia* sp
P. fluorescens	15.2 A	22.4 A	61.1 A
B .cereus	12.4 A	18.3 A	58.8 A
0 Control	0.0 A	0.0 B	0.0 B

The letters mean the significant differences between the inhibition percentages

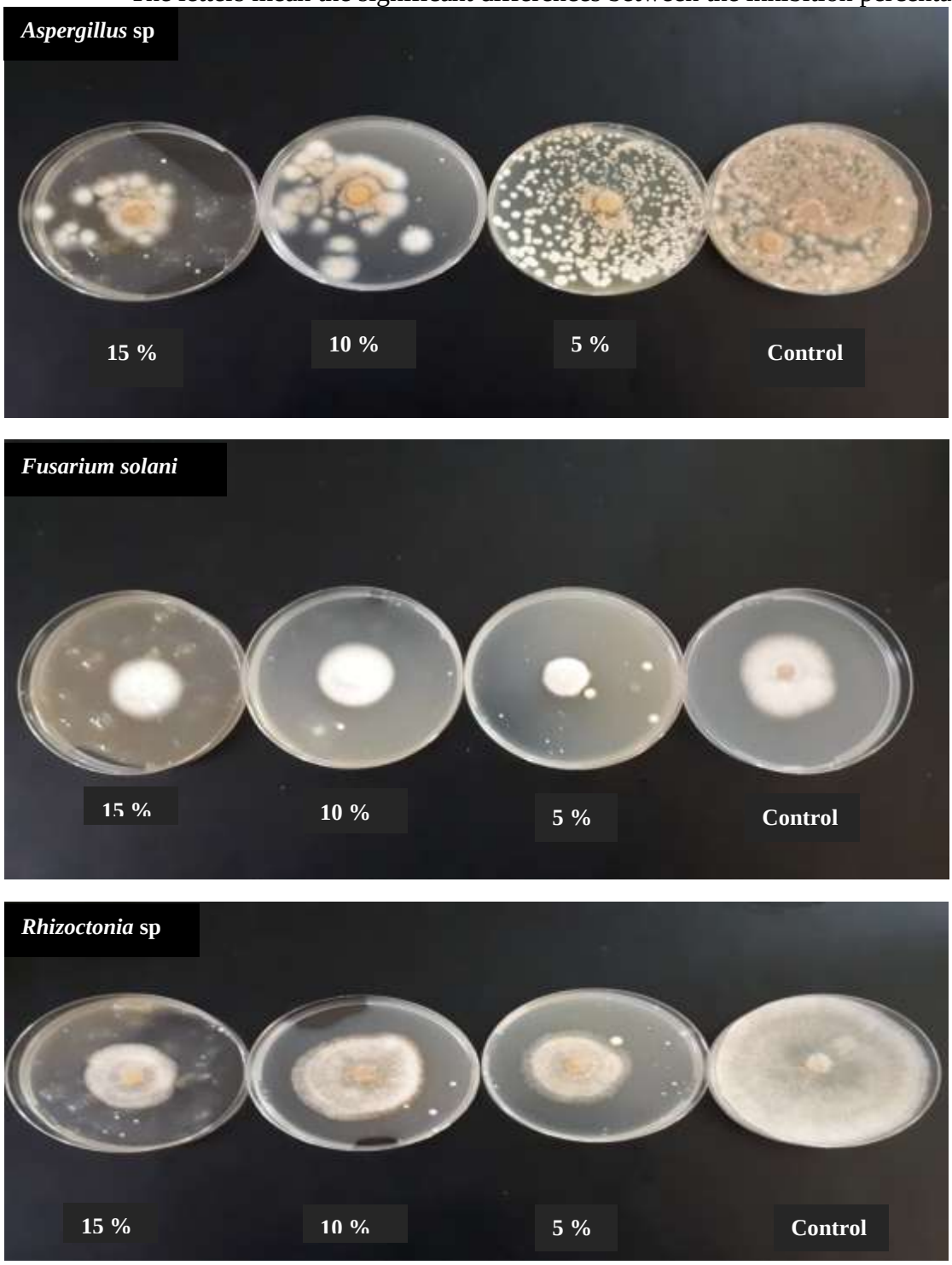

Fig 1. Effect of concentrations of *S. securidaca* extracts on mycelial growth of investigated fungi

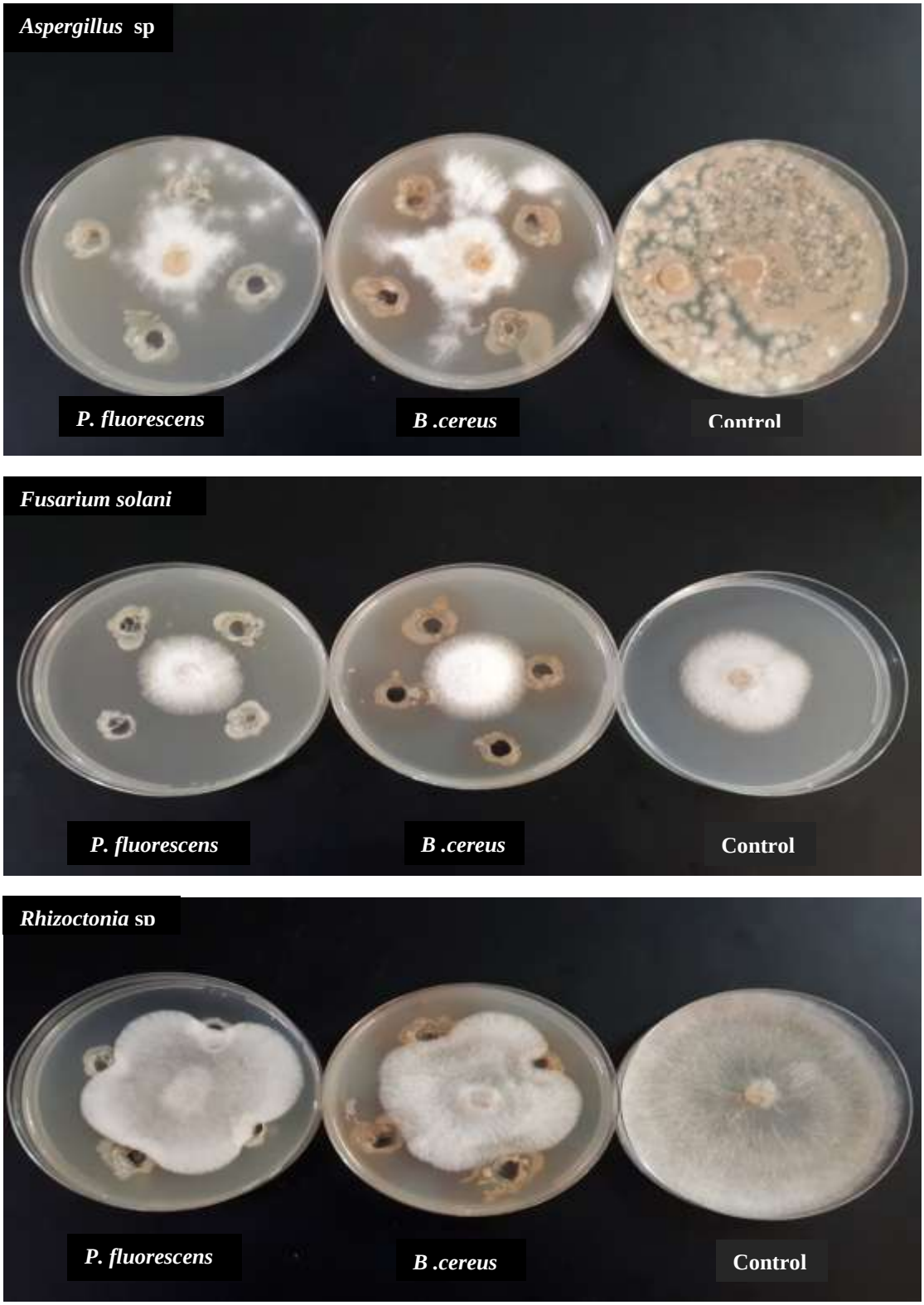

Fig 2. Effect of *P. fluorescens* and *B. cereus* on mycelial growth of investigated fungi

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