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Original article

The use of previous crops as sustainable and eco-friendly management to fight *Fusarium oxysporum* in sesame plants



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ABSTRACT

Sesame (Sesamum indicum L.), the "Queen of oil seeds" is being infected with pathogens, i.e., fungi, bacteria, virus and nematodes. Fusarium oxysporum sp. sesami (Zap.), is one of the fiercest pathogens causing severe economic losses on sesame. This work aimed to evaluate the impact of the cultivation of some preceding crops and seed inoculation with antagonistic predominant rhizospheric bacteria and actinomycetes on the incidence and development of Fusarium damping-off and wilt disease. Results showed that the lowest pre and/or post-emergence damping-off and wilt of sesame were recorded after onion and garlic, followed by wheat compared to clover in both the 2019 and 2020 seasons. In vitro, soil extracts from plots where onion and garlic have been cultivated slightly decreased the conidia germination and mycelium radial growth of F. oxysporum. The numbers of sesame rhizospheric F. oxysporum and fungi were lower after the cultivation of onion and garlic than those after wheat and clover. However, the numbers of actinomycetes and bacteria were higher in the onion, garlic, and clover rhizosphere than wheat. Among all isolated bacteria and actinomycetes associated with sesame roots cultivated after preceding plants, the Tricoderma viride and Bacillus subtilis (isolate No.3) profoundly reduce F. oxysporum mycelial growth in vitro. When sesame seeds were inoculated with Tricoderma viride, Bacillus subtilis, Streptomyces rochei and Pseudomonas fluorescens, the disease incidence of damping-off and wilt significantly decreased in the greenhouse and field trials conducted in both tested growing seasons, also had highly significant on plant health and growth parameters. Therefore, the current study suggested that using the preceding onion and garlic plants could be used for eco-friendly reduction of damping-off and wilt disease of sesame.

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1. Introduction

Sesame (*Sesamum indium* L.) is one of the world's oldest cultivated oil crops and is considered a productive plant in highyielding desert soil conditions (Bedawy, and Moharam, 2018).

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The cultivated area in Egypt was 34.000 ha, producing 44.000 tonnes (FAOSTAT, 2020). From the seedling stage until maturity, several pathogens can attack and infect sesame plants. The most severe disease is sesame wilt caused by a soil-borne fungus, *Fusarium oxysporum* f.sp. *sesami*, significantly limits sesame production (Ngamba et al., 2020). Many strategies have been applied, including resistant cultivars, crop rotation, and fumigation to mitigate *Fusarium* wilt damage. Although the use of resistant varieties is a practical, sustainable approach, the occurrence and development of new pathogen races that break host resistance is a constant concern (Sales Júnior et al., 2018; Assefa et al., 2020; Zhang et al., 2020). The use of fungicides causes severe harm to the environment as well as high costs. Therefore, investigating crop management and improving plant production through cultural practices

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and biological methods is urgently needed during field trials. This study focuses on using useful preceding plants along with antagonistic microorganisms for the management ofnd antagonistic microorganisms to manage and manage Fusarium wilt disease as alternative eco-friendly options. Several investigators have documented the impact of certain preceding crops on the incidence of certain plant diseases caused by soil-borne fungi, including Fusarium wilt of sesame (Shabana et al., 2014; Islam et al., 2016; Belay, 2018; Assefa et al., 2020). In previous studies, planting sesame after onion and wheat reduced the wilt disease, while broad bean and clover increased the infection (Hosseini et al., 2015; Adhikary et al., 2019). They also observed a reduction in the rhizosphere fungal population and increased bacteria when sesame was grown in the soil previously cultivated with onions. In maize, lentil cultivation before maize highly increased the disease severity of root rot caused by F. solani, while wheat showed the lowest increase in disease incidence (Hulugalle et al., 2020). Likewise, the prevalence of lupin wilt disease caused by F. oxysporum f.sp. lupini was the highest after sorghum in the greenhouse, and cotton resulted in the highest wilt incidence in the field, while maize, sunflower, and groundnut gave the moderate infection (Adhikary et al., 2019). The impact of preceding crops could be attributed to their root exudates affecting stimulation or inhibition of pathogen mycelial growth and germination spores and their effects on stimulating certain microorganisms that suppress the pathogen in the soil. In this regard, the efficacy of root exudates of preceding crops regarding the stimulation or inhibition of mycelial growth and germination of the pathogen spores in vitro was studied. Also, it was reported that root exudates of wheat, sorghum, and maize reduced the growth of F. oxysporum f.sp. cepae, the causal pathogens of tomato damping-off of onion (Hao et al., 2010). Also, root exudates of preceding crops soybean, sorghum, maize, tomato, and cotton reduced the mycelial growth of F. oxysporum f.sp. cumini (F.o.c.), the causal pathogen of cumin wilt (Hao et al., 2010). Whereas root exudates and extracts of garlic Chinese and Giza-20 onion cultivars exhibited a little inhibitory effect against F. oxysporum f.sp. lycopersici, the causal pathogen of tomato wilt disease (Hu et al., 2012). However, the stimulation and activity of numerous microorganisms associated with the soil rhizosphere of useful preceding crops that may suppress the pathogen have been strongly suggested (Gianfreda, 2015; Adhikary et al., 2019).

Application of the biocontrol agents with sesame seed or transplantings by dipping roots before cultivation effectively controlled wilt disease and significantly increased the yield (Elewa et al., 2011; Ziedan et al., 2011; 2012; Hegazy et al., 2019). In this regard, several microorganisms have been isolated from the rhizosphere of sesame plants that exhibit the antagonistic activity to wilt disease pathogen. These antagonists were Gliocladium virens (Jyothi et al., 2011), Bacillus subtilis, Pseudomonas fluorescens MC07 (Amin et al., 2017), Trichoderma harzianum and T. viride, and new strains of Trichoderma spp., (Mahmoud & Abdalla, 2018), where they effectively protected sesame plants from wilt disease. The objective of this study aimed at investigating the effect of some preceding crops on the protection of sesame plants against damping-off and Fusarium wilt, also focus the reduction of wilt disease associated with the cultivation of preceding crops. The antagonistic predominant rhizosphere bacteria and actinomycetes on the incidence and development of damping-off and wilt disease of sesame was studied.

2. Materials and methods

2.1. Isolation, purification, and detection of the pathogenic fungi

The fungal causal pathogen was isolated from infected sesame plants showing root-rot and typical wilt symptoms. The infected plants were collected during the growing season 2019 from different localities from the Qena Governorate of Upper Egypt. Each plant sample's root and stem were washed thoroughly with tap water, then cut into small pieces (0.5–1 cm) and surface sterilized by immersing in 1% a sodium hypochlorite (SH) solution for 3 min, then rinsed four times with sterile distilled water (SDW). The disinfected pieces were dried with two layers of sterilized filter papers, plated on Fusarium-selective medium (Maina et al., 2017) supplemented with streptomycin sulphate (0.4 g /1000 ml) selective medium), then incubated at 28 ± 5 °C for 72 h. Single spore and/or hyphal tip isolation was used to purify the growing fungal colonies (Bhimani & LF, 2018). Then, the isolated Fusarium species were then identified according to mycelia's morphological characteristics and spores described by Leslie & Summerell (2006). and confirmed by Assiut University Mycological Centre (AUMC), Pure cultures of all *Fusarium* spp. Isolates were kept at 5°C in a refrigerator for further studies. Stocks were routinely sub-cultured every two weeks on fresh PDA slants.

2.2. Pathogenicity test

Pathogenicity test of all *Fusarium* spp. isolates were performed on sesame plants (Giza-5 cultivar) under greenhouse conditions during 2019-2020. Inoculum of each tested fungi was prepared by infesting fungal growth (three equal disks 5 mm in diameter) taken from new culture into bottles tightly closed with cotton plugs containing autoclaved medium of Barley medium (75 g Barley + 25 pure sand + 2 g sucrose + 0.1 g yeast extract + 100 ml water). Bottles were then incubated at 28 ± 2 °C for three weeks. Four pots (30 cm in diameter) having sandy loam soil (1:1, v/v) were infested with each fungal mixture at the rate of 5% (v/w) of soil weight for each of the isolated fungi, then slightly irrigated every day for a week (El-Gendy et al., 2016). Four pots were inoculated with a sterilized medium at the same rate to serve as a control. Seeds were disinfected by dipping them in a 1 percent SH solution for 3 min, then rinsing them three times in SDW for 5 min before sowing ten seeds per pot. In a randomized design, four pots were used as replicates of each fungal isolate. Pots were checked daily and irrigated when necessary. Pre and/or postemergence damping-off of seedlings percentage were recorded after two and four weeks from sowing, respectively. Then the survival plants were examined weekly for the appearance of wilt symptoms until maturity. Each replication's wilted plants were counted, and the percentage of wilt disease was determined. Also, the individual plants in each replication were rated for the wilt disease severity using a scale of 0–5 according to Ziedan et al. (2011), where the percentage of foliage with yellowness and /or necrosis in an acropetal progression (0 = healthy plants, 1 = 1-20%, 2 = 21-40% ,3 = 41-60% ,4 = 61-80% and 5 = 81-100% wilted plants). The percent of disease severity (DS) of each replicate of each fungal isolate was determined in the following formula:

$$Diseases verity(DS) = \frac{\sum (n.v)}{X.N} \times 100$$
(1)

Whereas: N = total number of all examined plants. n = number of plants at the rate v = the disease rate on the following scale. X = the highest disease severity rate determined from the scale. (Moharam & Negim, 2012). Besides, the main pathogen was reliably re-isolated from infected plants with typical symptoms similar to the original symptoms developed on naturally infected plants.

2.3. Biological control of Fusarium wilt in seasame crop:

2.3.1. Cultivation of preceding crops

These experiments were conducted under field conditions in the Experimental Farm, Faculty of Agric., South Valley University in 2019 and 2020 growing seasons. Seeds of clover, wheat, garlic, and onion were sown in plots (1.8×2.4 m), each contained three apart rows (60 cm) in three replicates were used in a randomized complete block design. For the cultivation of each crop, all cultural practices were followed as is commonplace.

2.3.2. Biological and chemical analysis of cultivated proceeding crops soils

2.3.2.1. Biological analysis. Soil washing and dilution techniques described by Hol et al. (2015) were used for isolating fungal and bacterial microorganisms from the rhizosphere of growing clover. wheat, garlic, and onion. For collecting the soil samples, healthy plants (42-day-old) of each clover, wheat, garlic, and onion crops with roots were carefully drought. The excess soil was gently shaken and discarded, and only that soil adhered strictly to the root system of collected plants was left. Then, 10 g of each raised soil was suspended in 90 ml of tap water in small flasks, shaken for 15 min on the shaker, and serial dilutions from $10^{-1} - 10^{-6}$ were prepared (Desoky et al. 2020a; El-Saadony et al. 2021a; El-Saadony et al. 2021b). Immediately, one ml from each dilution of 10⁻³ and 10⁻⁶ was put in Petri dishes (9.0 cm), each containing 10 ml of the medium used for isolating the fungi and bacteria. Peptone-Rose Bengal agar medium (Dev et al., 2017) supplemented with 40 mg streptomycin sulphate per 100 ml medium was used to isolate fungi. In contrast, a nutrient agar (NA) medium was used for isolating bacteria. The infested dishes were incubated at $28 \pm 2C$. The total counts of the growing colonies of F. oxysporum, bacteria, actinomycetes, and fungi were determined after 2 and 5 days, respectively. Then pure cultures of the isolated bacteria belong to the genus Bacillus spp., pseudomonas spp. A single colony technique A single colony technique was obtained and actinomycetes were obtained and actinomycetes and kept at 5 °C on Nutrient Agar (NA) slant for further studies.

2.3.2.2. Soil texture and chemical analysis. Samples of soil were collected from growing clover plots, wheat, garlic, and onion crops, air-dried, ground, and passed through a two mm screen and then kept until used for soil analysis. Particle size distribution was mechanically carried out by the pipette method, according to Beretta et al. (2014). According to Wang et al. (2015), soil pH was determined in a 1:1 soil-water suspension using a glass electrode. Organic matter content was determined using the method described by Ukalska-Jaruga et al. (2019). NPK content was determined according to the methods described by Oyedeji et al. (2014).

2.4. In vitro effect of soil extracts on F. Oxysporum mycelial growth and conidia germination

Samples of soil were collected from plots where previous crops had been cultivated. Then soil samples of each previous plant were mixed and well homogenized by grinding. Later, 10 g soil of each previous plant was added to 100 ml of SDW in conical flasks. Then flasks were shaken vigorously on the shaker at room temperature. After 30 min, the soil suspension was centrifuged at 2500 rpm at four °C for 5 min. The stock extracts (10% of each) were then filtered using a Millipore filter (0.45 μ m) in sterile small's bottles and stored at five °C until use. Later, the soil extract (10%) was added to PDA medium before solidification to obtain (1, 3, and 5%) final concentrations. Instead of soil extracts, the control was PDA medium amended with SDW. Each experiment was repeated twice in a factorial design with four replicates for each treatment. Plates were then inoculated with one ml of spore suspension (10^3 conidia ml⁻¹) obtained from the 10-day-old culture of *F. oxysporum* and incubated at 25 °C. After 24 h, the germination was microscopically checked following conidiospores staining with lactophenol blue. A hundred spores per plate were examined, and the percentage of germinated spores was calculated. Other treated plates with soil extracts were inoculated with the fungal disc (0.5 cm) and then incubated at 28 ± 2 °C till control Petri dishes reached 9 cm radial growth. Mycelium in treated Petri dishes was checked, and the percentage of inhibition over control was also calculated using the following formula:

Reduction % =
$$\frac{C-T}{C} \times 100$$
 (2)

Where:

C = Average growth of the pathogenic fungal in the control treatment (cm).

T = Average growth of the pathogenic fungal growth in a targeted treatment (cm).

2.5. Effect of cultivation of preceding crops on the incidence and severity of Fusarium oxysporum in sesame crop

These experiments were carried out in the same soil field following the harvesting of preceding crops in the 2019 and 2020 growing seasons for sesame cultivation. Inoculum of isolate No. 2 of *F. oxysporum*, the highly pathogenic one, was prepared as described before. Disinfected seeds of sesame Giza-5 cv were sown in plots, each with three rows adopted in a completely randomized block design with three replicates, as mentioned previously. Each row contained six hills spaced 20 cm, and each hill was sown with five seeds. Equal inoculum amounts (70 g each) were placed in each hill simultaneously as seed sowing. Disinfected sesame seeds planted in non-inoculated plots with the pathogen served as control. All cultural practices were followed as commonly known for sesame cultivation. Percent of pre-and post-emergence dampingoff of seedlings, as well as wilt disease and severity, were estimated as mentioned before.

2.6. Effect of preceding crops on soil microorganisms of sesame rhizosphere

Soil washing techniques described by Xiao et al. (2019) and soil dilution were used to isolate bacteria and fungi from the sesame rhizosphere, as described by Sangale et al. (2019. For collecting the soil samples, healthy sesame plants (4-6 week-old) with roots were carefully drought, the excess soil gently shaken off, discarded, and the sticky soil with root system was left. Roots were removed, cut to pieces, and placed in flasks containing 100 ml sterile water. Flasks were gently shaken until most of the sticky rhizosphere soil was removed, then roots removed, placed into other flasks containing 100 ml sterile water, and flasks were shaken again. Suspensions from both flasks were mixed, and serial dilutions were prepared (Desoky et al., 2020b; El-Saadony et al., 2021c; El-Saadony et al., 2021d). One ml of each dilution was used to isolate the bacteria and fungi associated with sesame root on PDA and NA media, respectively. The total count of growing colonies of bacteria, actinomycetes, and fungi was determined after 2, 2, and 5 days, respectively.

2.7. Isolation and identification of bioagents associated with the sesame roots

Pure cultures of the isolated bacteria and *Actinomycetes* spp., were isolated from the crucifers plants rhizosphere on King's medium b (KMB) and tentatively identified through microbiological and biochemical tests according to Celandroni et al. (2019). Also, *Trichoderma* spp. was isolated from healthy sesame rhizosphere and identified at Assiut University Mycological Centre (AUMC).

2.8. In vitro antagonistic activity of isolated bioagent against F. oxysporum

Total isolates of Bacillus subtilis. Pseudomonas fluorescens. Trichoderma viride and Streptomyces rochei obtained from the rhizosphere of sesame plants and preceding crops were used in this study were evaluated for their sensitivity against F. oxysporum isolated from sesame plants. At one cm from the plate edge, a vigorously growing culture of the aggressively tested isolate of F. oxysporum was inoculated with a mycelial disc (5 mm in diameter) of a vigorously growing culture. The aggressively tested isolate of F. oxysporum. A disc of freshly Trichoderma viride growth (5 mm in diameter) or a streak line with a loop-full of 2 days-old culture from Bacillus subtilis and Pseudomonas fluorescens was placed a constant distance opposite to the other edge of the Petri plate and incubated at 28 °C for 7 days. Petri plates were inoculated with the pathogen as the same without bioagents and used as control. When fungal growth has completely covered the surface of control plates, the inhibition zones of fungal growth in the treatment were measured. The ability of an antagonist to inhibit Fusarium oxysporum growth was scored as described by Agarry and Osha (2005) using the following formula:

Percent of mycelial growth inhibition =
$$[H-N/H] \times 100$$
 (3)

Where H = diameter of the untreated mycelium growth; N = diameter of the treated mycelium growth.

The inhibition zone between bacteria and the pathogen was measured as described by Yasmeen et al. (2012). All experiments were carried out with five replicates for each treatment. The data obtained were statistically analyzed.

2.9. The control F. Oxysporum by antagonises

2.9.1. Preparation of bacterial and actinomycetes inocula

Strains of *B. subtilis, P. fluorescens* and *S. rochei* were cultured separately in a nutrient broth medium and incubated at 28 °C for 48 hr. The resultant cell suspension of each strain was adjusted to provide 10^9 cfu/ml.

2.10. Preparation of fungal inocula

F. oxysporum as a pathogen and *T. viride* as an antagonist were grown separately on potato dextrose agar (PDA) medium at 28 °C for seven days. Conidia was harvested in sterilized water sterile brush and filtrated through four layers of sterilized cheesecloth to remove the mycelium. The spore suspension was adjusted to 1×10^6 spores/ml using a hemocytometer (Varshney et al., 2014)

2.10.1. Greenhouse experiments

This experiment was carried out in the tested growing season (2019–2020). Sterilized pots (35 cm in diam.) containing sterilized soil were infested by adding 35 ml of a suspension of *F. oxysporum* 10^6 spore/ml. After seven days, equal amounts of inocula of the antagonistic fungi, bacteria and actinomycetes were separately added to each pot and thoroughly watered. Also were sown in the infected soil. Pots containing inoculum of *F. oxysporum* only were used as control. Four replicates were used for each treatment and eight sterilized sesame seeds Giza 5 cultivar were sown in each pot. Plants were irrigated when necessary and examined periodically and daily examined. Pre and/or post-emergence damping-

off of seedlings percentage, as well as wilt disease and severity, were estimated as previously mentioned.

2.10.2. Field experiments

These experiments were carried out in the 2019 and 2020 growing seasons—the sowing. Seeds of sesame Giza-5 cv. were disinfected, inoculated with antagonistic fungi, bacteria and actinomycetes, and sown in the plots, as mentioned before. Four plots were used for each treatment in a randomized complete block design. Equal inoculum amounts (70 g each) of isolate No. 2 of *F. oxysporum* were placed in each hill of the plots simultaneously with seed sowing. Disinfected sesame seeds sowed in noninoculated plots, with the wilt pathogen served as control. All cultural practices were followed as commonly known for sesame cultivation. Pre-and post-emergence damping-off of seedlings Percentage, as well as wilt disease and severity, were estimated as previously mentioned.

2.10.3. Plant health and growth parameter of sesame

At harvest time, plant samples (ten healthy plants each) were taken at random from each plot to determine Morphological characters, *i.e.*, length of the plant (cm), number of bearing branches/plant, number of pods/plants, seed yield/plant (g), and percentage of oil content according to Ziedan et al. (2010).

2.11. Antioxidant enzymes activity

2.11.1. Enzyme extraction

The extraction was performed using the method described by Vitória et al. (2001). In an ice-box, one gram of leaf fresh, healthy, infected and treated samples were collected and taken to the lab. The leaves were washed in distilled water and dried to remove any moisture on the surface. With a pre-chilled mortar and pestle, a five-tenth gram of leaf was homogenized in ice-cold 0.1 phosphate buffer (pH7.5) containing 0.5 M EDTA (ethylenediaminetetraacetate).

The homogenate was centrifuged at 4 $^{\circ}$ C in Beckman refrigerated centrifuge at 15000 rpm for 15 min. The supernatant was transferred to 30 ml tubes and referred to as enzyme extract.

Peroxidase activity (PO) was determined in sesame leaves according to Pallavi and Vijaykumar's (2019) method. The enzyme was assayed using guaiacol substrate. 3 ml phosphate buffer (0.1, pH 7.0), 30 ml H_2O_2 (20 M), 50 ml enzyme extract, and 50 ml guaiacol (20 M) made up the reaction mixture. At room temperature, the reaction mixture was incubated in a cuvette for 10 min. The enzyme activity was expressed as a number of absorbance units g-1 fresh weight of leaves and the optical density was 436 nm.

2.11.2. Proline content

The rapid colorimetric method suggested by Varamin et al. (2020) was used to calculate proline content (in g/g–1 dry weight of leaf). A proline was extracted by grinding 0.5 g dry weight of leaf tissue in 10 ml of 3 percent (v/v) sulpho salicylic acid. The mixture was then centrifuged for 10 min at 10,000 rpm. In a test tube, 2 ml of the supernatant was applied to 2 ml of freshly prepared acid-ninhydrin solution. The tubes were incubated in a water bath at 90 °C for 30 min before being placed in an ice bath to stop the reaction. After that, 5 ml of toluene was added to each reaction mixture and vortex-mixed for 15 s. The tubes were left in the dark for at least 20 min at room temperature to separate the toluene and aqueous phases. Then, the toluene phase was carefully collected in a test tube and its absorbance was measured at 520 nm. Each sample's concentration was calculated using an analytical grade Pro standard curve and expressed on a 100 g–1 DW basis.

2.12. Statistical analysis

The SAS software was used to analyze the data (SAS ver. 15.1, SAS 2018). The revised LSD was used to compare means for each trait.

3. Results

3.1. Isolation and identification of the causal pathogen

Eight isolates of *Fusarium* species were recovered from wilted sesame plants (*Sesamum indicum* L.). Results are shown in Table 1. The isolates were identified as *F. oxysporum* Schlecht (isolates3), *F. solani* Mart (3 isolates), and *F. clmourum* (2 isolates). Results of pathogenicity tests in Table 1 showed that only isolates of *F. oxysporum* were significantly pathogenic to Giza-5 cultivar of sesame and caused the same ideal wilt symptoms among the eight isolates obtained. Isolate No. 2 of *F. oxysporum* was the highly pathogenic one and caused 88.09% and 61.35% of wilt infection and DS%, respectively. In comparison, isolate *F. oxysporum* (isolate 1) was weak and caused 48.16% and 34.75% of infection and DS, respectively. On the other hand, the other five isolates of *F. solani* and *F. clmourum* did not induce wilt symptoms, but they significantly caused the only pre-and post-emergence damping-off and root rots of sesame seedlings.

3.2. Soil biological and chemical analysis of cultivated preceding crops

3.2.1. Biological analysis

Data in Table 2 show the biological analysis of rhizospheric microflora of preceding plants cultivated clover, wheat, garlic, and onion. In this analysis, the rhizospheric *F. oxysporm* and fungi were lower of cultivated onion and garlic than those of wheat and clover. However, the numbers of actinomycetes and bacteria were higher in the rhizosphere of cultivated onion, garlic, and clover than those of wheat.

3.3. Soil texture and chemical analysis

After the cultivation of the preceding crops, soil texture is loamy clay with particle size distribution ranged from 3.67 to 3.70%, 15.31–15.35%, and 38.92–38.95% of sand, silt, and clay, respectively (Table 3). After the cultivation of the preceding crops, the soil pH showed nearly the same values ranging from 7.88 to 8.16. However, the values slightly decreased after cultivation of wheat, garlic, and onion compared to that after clover. Also, the percent of

Table 1

Pathogenicity test of Fusarium species isolates on Giza-5 sesame cultivar in vivo conditions

organic matter and N, P, and K (%) decreased in the soil after cultivation of garlic, onion, and wheat compared to those after clover.

3.4. Effect of cultivation of preceding crops on the incidence and development Fusarium oxysporum in sesame crop

Data in Table 4 show that among the preceding crops cultivated, the lowest pre-and/or post-emergence damping-off and wilt of sesame were recorded after onion and garlic followed by wheat compared to those of clover in both tested growing seasons. The lowest means of pre- and post- damping-off and wilt over the two seasons were 14.21 and 4.815%, and 17.96%, respectively, after the onion crop, followed by 13.33 and 4.81%, and 19.95%, respectively, after the garlic crop. While the highest mean of pre-and post- damping-off and wilt were 20.92 and 10.74%, and 45.30%, respectively, after the clover crop. However, the cultivation of sesame after wheat resulted in 15.47 and 6.49%, and 23.32% of pre-and post- damping-off and wilt incidence.

3.5. Effects of soil extracts on conidia germination and mycelial growth of F. Oxysporum in vitro

The data present in Table 5 show that among the soil extracts from plots where preceding crops have been cultivated, the conidia germination of *F. oxysporum* slightly decreased by soil extracts from onion and garlic crops at all tested concentrations of 1, 3, and 5%. However, these extracts do not affect mycelium radial growth. On the other hand, soil extracts from plots where clover and wheat have been cultivated do not affect conidia germination and mycelium radial growth.

3.6. Characteristics of Bacillus subtilis and Pseudomonas fluorescens

Tables 6 and 7 illustrate the examined characteristics of *Bacillus* and *Pseudomonas* isolates. The results of the tested parameters

3.7. Antagonistic activity of isolated bioagent against F. Oxysporum in vitro.

Based on the results obtained, all isolates significantly inhibited the mycelial growth of *F. oxysporum*. Data in Table 8 shows that the bioagent strains decreased the radial growth of *F. oxysporum*, *Trichoderma viride* was more active than *Pseudomonas fluorescens*, *Streptomyces rochei* and *Bacillus subtilis*, in such effect being 66.84, 29.52,25.80 and 18.45%, respectively compared to control while with inhibition percent ranged from 11.93 to 51.10% (Table 8 & Fig. 1). However, isolate of *Streptomyces rochei* exhibited the

Fungal isolates	gal isolates Damping-off (%) Survival plants (%		Survival plants (%)	Wilt disease		Root rot disease		
	Pre-	Post-		Incedance (%)	Severity (%)	Incedance (%)	Severity (%)	
F. oxysporum								
1	7.50	17.50	75.00	48.16	34.75	8.00	3.7	
2	10.00	25.00	65.00	88.09	61.35	12.00	5.9	
3	5.00	17.50	77.50	58.48	38.75	9.50	3.3	
F. solani								
1	32.50	15.00	52.50	10	5.1	27.5	10	
2	30.50	14.00	55.50	7.5	3.9	15	17.3	
3	31.50	13.00	55.50	19.1	4.3	22	15.8	
F. clmourum								
1	30.00	37.50	32.50	0.00	0.00	0.00	0.00	
2	28.00	35.00	37.00	0.00	0.00	0.00	0.00	
Control*	2.50	0.00	97.50	0.00	0.00	0.00	0.00	
L.S.D. at 5%	7.90	7.90	12.41	13.51	5.37	12.46	4.25	

*Non-infested plants, healthy plants. The significant differences between means compared by LSD at $p \leq 0.05$, NS, not significant

Table 2

Microbial composition of rhizospheric zones of cultivated crops preceding sesame plants in the 2019 and 2020 seasons.

Preceding crop	Mean of the total count rhizosphere soil (CFU g ⁻¹ soil)								
	Fusarium spp. (×10 ⁴)	Bacteria (×10 ⁶)	Actinomycetes (×10 ⁶)	Other Fungi ($\times 10^4$)					
Clover	29.5 ab	97.5 a	8.0 a	28.0b					
Wheat	34.0 a	30.5c	4.5c	42.5 a					
Garlic	15.5b	83.0b	12.0b	12.5c					
Onion	14.0b	85.0b	16.0b	13.5c					

Values within columns followed by different letters are significantly different (LSD; p < 0.05).

Table 3

Soil texture and chemical analysis of cultivated crops preceding sesame plants in the 2019 and 2020 seasons.

Preceding crops	Particle size distribution			pH	OrganicMatter	Ν	Р	K	
	Texture	Sand %	Silt %	Clay %					
Clover Wheat Garlic Onion	Loamy Clay	3.67–3.70	15.31–15.35	38.92–38.95	8.16a 7.88b 8.01ab 8.03ab	0.98a 0.38b 0.14c 0.16c	0.051a 0.024b 0.012c 0.014c	0.0021a 0.0011b 0.0013b 0.0015b	0.0047a 0.0021c 0.0032b 0.0033b

Organic matter (%); N (%); P (%), K (%); Means with different lowercases indicate significant differences at $p \le 0.05$

Table 4

Effect of preceding crops on on the incidence and development Fusarium oxysporum in sesame crop under field conditions in 2019 and 2020 growing seasons.

Preceding 2019			2020				Mean					
crops	Damping %	-off	Survival plants %	Wilt %	Damping %	-off	Survival plants %	Wilt %	Damping %	-off	Survival plants %	Wilt %
	Pre-	Post-			Pre-	Post-			Pre-	Post-		
Clover White Garlic Onion	19.26a 14.45b 13.71bc 12.96c	9.26a 5.57b 4.44bc	71.48a 79.98b 81.85bc 82.97c	41.66a 21.35b 18.24bc 16.15c	22.57a 16.49b 12.95bc 15.45c	12.21a 7.40b 5.18bc	65.22a 76.11b 81.57bc 78.91c	48.94a 25.29b 21.66bc	20.92a 15.47b 13.33bc	10.74a 6.49b 4.81bc	68.35a 78.05b 81.71bc 80.94c	45.30a 23.32b 19.95bc 17.96c

Values within columns followed by different lowercase letters are significantly different (LSD; p < 0.05).

Table 5

Mycelial growth and spore germination of Fusarium oxysporum as effected by soil extracts in vitro.

Soil extra preceding	act of g crop	Conidia germination (%)	Reduction (%)	Mycelium radial growth (cm)	Mycelium reduction (%)
Conc.	(%)				
Clover	0	98.50	1.5b	9.0	0
	1	98.00	2a	9.0	0
	3	98.50	1.5b	9.0	0
	5	98.50	1.5b	9.0	0
Mea	n	98.37	1.63	9.0	0
Wheat	0	98.50	1.5b	9.0	0
	1	98.50	1.5b	9.0	0
	3	98.00	2a	9.0	0
	5	98.00	2a	9.0	0
Mea	n	98.25	1.75	9.0	0
Garlic	0	98.50a	1.5d	9.0a	0
	1	96.50ab	4c	8.5ab	0.5b
	3	94.00b	6b	8.2ab	0.8ab
	5	91.50c	9a	7.8b	1.2a
Mea	n	95.13	4.87	8.38	0.62
Onion	0	98.50a	1.5c	9.0a	0
	1	96.00ab	4b	8.4ab	0.6b
	3	92.50b	8ab	8.0b	2a
	5	90.00c	10a	7.3c	1.7ab
Mea	n	94.25	5.75	8.18	0.82
General	Mean	96.49	3.51	8.64	0.36

Conidia germination Mycelium radial growth L.S.D. 0.05 Soil extract (SE) = 0.86 = Not significant (NS) Conc. (C) = 0.56 = 0.17 SE \times C = 1.12 = NS

Table 6

Biochemical and morphological characteristics of Bacillus subtilis.

Biochemical and morphological paramters	Result
Gram stain	Gram positive
Spores shape	Oval
Spores position	Central
Spores bulging	negative
Casein hydrolysis	positive
Hippurate hydrolysis	positive
Starch hydrolysis	positive
Urease	positive
Chloramphenicol resistance	S
Nalidixic acid	S
Polymyxin B	R
Streptomycin	S
Acid from Fructose	positive
Acid from Galactose	negative
Acid from Lactose	negative
Acid from Xylose	Positive
Citrate utilization	Positive
Growth at 50 0C	Positive
Growth in 10% NaCl	Positive
Anaerobic growth	negative
Nitrate reduction	positive
Oxidase reaction	positive
Voges-Proskauer test	positive

S, Sensitive; R, Resistant

Table 7

Biochemical and morphological characteristics of *Pseudomonas fluorescens* isolates.

Biochemical and morphological properties	Result
Temperature range for growth	15-35 °C
Optimum temperature	30–35 °C
pH range for growth	5-8
Optimum pH	7-8
Phosphate solubilization	positive
Denitrification	positive
Nitrification	positive
Ammonification	positive
Assimilation of carbon source:	
a. Histidine	very good.
b. Glucose	good enough
c.B-alanine	very good.
d. Sorbitol	good

Table 8

Antagonistic activity of isolated *Bacillus* spp., and *Actinomycetes* spp., against *F. oxysporum in vitro*.

Microorganism	Isolate No.	Fusarium growth (mm)	Reduction (%)
Bacillus subtilis	1	7.807a	11.93d
	2	6.889b	21.11b
	3	6.445b	25.55a
	4	7.361ab	16.39c
	5	7.028ab	19.72b
	Mean	7.106	18.94
Streptomyces rochei	1	6.838ab	21.62b
	2	7.415a	15.85c
	3	3.89b	51.10a
	Mean	6.048	29.52
Pseudomonas fluorescens		4.4	45.8
Trichoderma viride		2.3	66.84
Control		9	0.00
L.S.D. 0.05		4.49	

The significant differences between means compared by LSD at $p \leq$ 0.05, NS, not significant

highest antagonistic activity, followed by isolate No.3 of *Bacillus subtilis*, where they caused 51.105 and 25.55% of mycelial growth inhibition, respectively.

3.8. Effect of seed inoculation with antagonistic bacteria and actinomycetes on the incidence and development of disease under greenhouse and field conditions

In the greenhouse experiment conducted in 2020, seed inoculation with *S. rochei* and *B.subtilis* significantly decreased the incidence of damping-off and wilt disease of sesame caused by *F. oxysporum* non-treated control (Table 9). No significant differences between seed treatment with *P. fluorescens*, *S. rochei*, *T. viride* and *B. subtilis* were found in the percents of pre and/or post-emergence damping-off and wilt disease. However, *Trichoderma viride* was better than *Bacillus subtilis* and *P. fluorescens*, where it reduced the pre-and post- damping-off and wilt to 4.00 and 3.00%, and 14.00%, respectively.

In the field trials conducted in both the 2019 and 2020 growing seasons, seed inoculation with *Trichoderma viride* and *Bacillus subtilis* significantly decreased damping-off and wilt disease incidence compared with the non-treated control (Table 10). There was no significant difference between seed treatment with *S. rochei* and *B. subtilis*, *P. fluorescens*, and *T. viride* found in both seasons and the mean. However, seed treatment with *S. rochei* was better than *B. subtilis*, where it reduced the means of pre-and post- dampingoff and wilt to 3.62 and 2.64%, and 14.27%, respectively, compared with the non-treated control.

3.9. Plant health and plant growth parameter of sesame as affected by biological control agents

Data in Table 11 revealed that bioagents' application is a decisive factor in determining the efficacy of each treatment on plant health and growth promoter, *i.e.*, plant height, number of fruiting branches per plant, plant seed yield and percentage of oil content, in the two tested seasons. Values of parameters of sesame plants were significantly increased with the dual inoculation of *T. viride*, *B. subtilis*, *P. fluorescens* and *S. rochei. Tricoderma viride* achieved the highest rates of sesame plant health and growth parameters during two tested seasons, followed by *Bacillus subtilis*. Then, *P. fluorescens* and *S. rochei* recorded the lowest results on all the different measurements.

3.10. Effect of biocontrol agents on physiological parameters of sesame plants inoculated with Fusarium oxysporum

Concerning changes in the activities of oxidative enzymes, peroxidase and proline contents in infecting sesame plants were significantly increased than that of non-infected (control). Data in Table 12 shows that the *T. viride* reveal an increase in all enzymes activity in all tested plants when inoculated with pathogenic fungi followed by *B. subtilis*. The highest peroxidase and proline contents were detected when sesame was inoculated, with *Fusarium oxysporum* being 0.69 (1.20) mg/g leaves. In case of *T. viride*, *B. subtilis*, *P. fluorescens* treatment, also *S. rochei* revealed 0.65(1.17), 0.56 (1.10) ,0.61 (1.01) and 0.52 (0.87) mg/g leaves, respectively.

4. Discussion

This study answers the hypothesis management of endemic soil-borne diseases in an efficient, eco-friendly and inexpensive manner. The pathogen was isolated from Qena Governorate, one of the governorates most famous for producing sesame plants and the most influential sesame wilt disease. Isolation results revealed in eight isolates from three *Fusarium* species that were identified as *F. oxysporum* (3 isolates), *F. solani* Mart (3 isolates), and *F. clmourum* (2 isolates). Following the performing of these isolates' pathogenicity test on sesame Giza-5 cv., only the isolates of *F.*



Fig. 1. Antagonistic activity of bioagents against F. oxysporum, where (a) Streptomyces rochei isolate No.3, (b) Bacillus sp. isolate No.3, (c) Pseudomonas fluorescens, (d) Trichoderma viride and (e) Fusarium oxysporum.

Table 9

Effect of seed inoculation with biocontrol agents on the incidence of sesame wilt disease in the greenhouse.

Biocontrol agents	Damping-off %		Survival Plants (%)	Wilt%
	Pre-	Post-		
Bacillus subtilis	5.00	6.00	89.00b	16.00b
Pseudomonas fluorescens	7.00	7.00	89.00b	17.00b
Trichoderma viride	4.00	3.00	93.00a	14.00c
Streptomyces rochei	10.00	11.00	93.00a	14.00c
Control	12.00	27.00	61.00c	74.00a
L.S.D. at 5%	2.81	3.14	6.57	4.88

The significant differences between means compared by LSD at $p \leq$ 0.05, NS, not significant

Table 10

Effect of seed inoculation with biocontrol agents on the incidence of sesame wilt disease under field conditions during the 2019 and 2020 growing seasons.

Biocontrol agents	2019	2019				2020				Mean			
	Dampin (%)	g-off	SurvivalPlants	Wilt %	Dampin	g-off(%)	Survival Plants	Wilt %	Dampin (%)	g-off	Survival Plants	Wilt %	
	Pre-	Post-			Pre-	Post-			Pre-	Post-			
B. subtilis	4.16	3.32	93.82	18.09	5.27	4.15	91.88	18.65	4.72	3.74	94.85a	18.37c	
P. fluorescens	4.72	4.17	91.11	25.32	6.39	3.89	89.72	23.80	5.56	4.03	90.42b	24.56b	
T. viride	3.06	2.22	94.72	16.99	4.17	3.05	92.78	17.55	3.62	2.64	93.75ab	17.27c	
Control	18.61	26.39	55.00	57.96	22.22	29.17	48.61	67.00	20.42	27.78	51.81c	62.48a	
L.S.D. at 5%	2.92	3.42	6.25	4.14	2.75	4.13	5.18	6.50	2.84	3.78	5.72d	5.32	

Means with different lowercases indicate significant differences at $p \leq 0.05$

oxysporum caused the same ideal damping-off and wilt symptoms. The other isolates of *F. solani* and *F. clmourum* did not induce wilt symptoms, but they significantly caused the seedling damping-off. These results agree with those reported by Bashir et al. (2017). Previous studies reported that the cultivation of certain preceding plants effectively controlled various plant diseases caused by soil-borne fungi, including *Fusarium* wilt of sesame (Bashir et al. 2017; El Kichaoui et al., 2017; Assefa et al., 2020). The lowest pre and/or post damping-off and wilt of sesame caused by F. oxysporum were recorded in plots where onion and garlic were cultivated, followed by wheat compared to clover in both the clover 2019 and 2020 seasons. Similarly, Baskar et al. (2017)

found that planting sesame planting after onion and wheat reduced the wilt disease while broad bean and clover increased the infection.

In an attempt to investigate the mechanism of damping-off and wilt disease reduction after useful preceding crops, the effects of the cultivation of preceding plants on soil microorganisms were studied. In this regard, the biological analysis of rhizospheric microflora, including *F. oxysporum* of preceding plants in plots where clover, wheat, garlic, and onion have been cultivated sesame rhizosphere in the same plots after cultivation of preceding plants was performed. Based on the results obtained, the numbers of rhizospheric *F. oxysporm* and fungi were lower in plots where onion

Table	11
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Effect of sesame seed treatments with the test	d antagonists on growth, yield and s	seed oil percentage during 2019, 2020 growing seasons.
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Treatment	Height of plant (cm)		Fruit number/plant		Number of capsules / plant		Seed yield / plant (g)		Oil content (%)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
T. viride	192.7a	189.78	9.15	8.2	279.88	275.4	39.2	38.79	57.99	58.85
Bacillus subtilis	187.6	183.48	7.15	7.05	231.98	230.78	36.42	33.49	54.3	54.66
P. fluorescens	181.4	179.15	7.73	7.7	225.84	217.65	34.51	33.49	52.34	52.96
Streptomyces rochei	179.15	173.93	6.35	6.33	194.48	188.2	30.19	29.19	51.5	50.7
Control	173.93	171.2	6.15	6.28	175.13	175.7	24.19	22.78	45.7	46.95
LSD at 5%	2	NS	0.95	0.74	25.51	19.16	3.15	3.12	6.43	6.96

The significant differences between means compared by LSD at $p \leq$ 0.05, NS, not significant

Table 12

Effect of biocontrol agents on physiological parameters of sesame plants inoculated with *Fusarium oxysporum*.

Treatments	Peroxidase mg/g leaves	Proline mg/g leaves
Control treatments	0.62	0.59
Fusarium oxysporum	0.69	1.20
Trichoderma viride	0.65	1.17
Bacillus subtilis	0.61	1.10
Pseudomonas fluorescens	0.56	1.01
Streptomyces rochei	0.52	0.87

Averages marked with the same letter in each column are not significant different (P < 0.01) by Duncan's test.

and garlic have been cultivated compared to those in plots where wheat and clover have been cultivated. However, the numbers of actinomycetes and bacteria were higher in the onion, garlic, and clover rhizosphere than wheat. These findings were also similar to those of the sesame rhizosphere in plots after preceding plants' cultivation. These results agree with those reported by El- Xiao et al. (2019). They observed a high reduction of the fungal population and increased bacteria in the sesame rhizosphere when sesame was grown in the soil previously cultivated with onions. These results suggest that the bacteria and actinomycetes might be associated with damping-off inhibition and wilt in sesame. However, the correlation between the disease incidence of succeeding sesame plants and the number of bacteria and actinomycetes after the cultivation of the preceding onion and garlic plants was remarked. The relationship between bacteria and actinomycetes in the soil and reducing and reducing the damping-off and wilt disease still required further studies. Also, the combination of biotic and abiotic soil factors is responsible for preventing damping-off and wilt disease caused by F. oxysporum. Therefore, abiotic should be considered in this study. In this regard, differences in soil pH values, which were among the essential factors related to disease incidence of damping-off and wilt, were not recognized among the plots after the cultivation of preceding plants. However, the percent of organic matter and N, P(%), and K(%) were higher in the soil after cultivation of garlic and onion compared to those after wheat, and not after clover.

On the other hand, the soil extract of onion and garlic slightly decreased the conidia germination and mycelium radial growth of *F. oxysporum* at concentrations of 1, 3, and 5% tested *in vitro*. The soil extracts of these plants have contained their root exudates with several compounds (not studied) that may be affecting inhibition of pathogen mycelial growth and germination spores (Lecomte et al., 2016). Also, the reduction in populations of fungi and *F. oxysporum*, and stimulation of total bacteria and actinomycetes in sesame rhizosphere after the cultivation of onion and garlic that may suppress the pathogen could explain the efficiency of these plants for reducing damping-off and wilt disease of sesame compared to that after clover and wheat plants. The stimulation and activity of numerous bacteria and actinomycetes asso-

ciated with the soil rhizosphere of several preceding crops that may suppress the pathogen and confirming the current results have been previously reported (Nga et al., 2016; Wang et al., 2018).

In this study, among all isolates of bacteria and actinomycetes associated with sesame roots cultivated after preceding plants, only the Streptomyces rochei and Bacillus subtilis, (isolate No.3) profoundly inhibited the mycelial growth of F. oxysporum in vitro. Several isolates of Streptomyces rochei and Bacillus subtilis, were reported to have antagonistic activity against F. oxysporm (Haggag et al., 2014; Anusha et al., 2019). Therefore, they were selected for seed treatment to control damping-off and wilt disease of sesame caused by F. oxysporum under greenhouse and field conditions. Based on results obtained, seed inoculation with *T. viride*. Bacillus subtilis and P. fluorescens significantly decreased the incidence of damping-off and wilt disease in the greenhouse during the 2019 growing season and under field conditions in both tested seasons. The potential use of actinomycetes and bacteria to manage various diseases on many crops has been well demonstrated. In this regard, several bioagents isolates of T. viride, Bacillus subtilis and P. fluorescens, that able to control root diseases of many crops, including sesame in the greenhouse and field, were previously reported (Lecomte et al., 2016; Haggag et al., 2014; Anusha et al., 2019). It is known that the mechanism by which the *Streptomyces* rochei and Bacillus subtilis inhibited F. oxsporum could be attributed to their siderophore, IAA, lipase, protease, b-1,3-glucanase, HCN producing capabilities, and/or to their ability to survive under harsh environments (Anusha et al., 2019). Thus, this study identifies the potential of the preceding onion and garlic plants, Streptomyces rochei and Bacillus subtilis, for eco-friendly reducing of damping-off and wilt disease of sesame.

Regarding the effect of biological control and its relationship to plant health and growth promoters, the study indicated the increase of sesame resistance obtained in this study could be related to *T. viride* as plant growth promoters. Mahmoud and Abdalla (2018) reported that *T. viride* treatments suppressed the disease and enhanced the growth parameters of sesame plants compared to the infected control. The reduction in disease incidence and increasing the yield after treatment by formulations of *T. viride* has been reported in several crops (Babychan and Simon 2017).

According to Dehghan et al. (2018), the application of *B. subtilis* alters the phytohormone balance in the plant, causing more reserve substances to be introduced into storage organs. Hence the plant root length, stem length and dry weight are expected to be improved by applying biocontrol agents. Though added *P. fluorescens*, this may be due to differences in siderophore compounds formed by them and compounds formed by them and their tolerance to field conditions. The high inhibition in growth characters in all bioagent treatments may be related to the toxic compounds produced from *F. oxysporum* to inhibit seed embryo activity. This agrees with Vinothini et al. (2020) found that the ability of *Pseudomonas fluorescens* to produce promoter compounds, *i.e.*, branch No./plant, the height of plant (cm), leaf area/plant (cm2) weight

of 1000 grains (g), Total yield of grains per plot (g) and percentage of oil in grains of sesame crop planted in soil contaminated with *Fusarium* fungi under normal conditions. Also, Li et al. (2015) found that Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) treatments affect plant growth and this effect was more pronounced in both the shoot height and the size of the secondary leaves, which were smaller than those of controls.

By preventing or minimizing oxidative damage caused by reactive oxygen species, bioagents increased peroxidase levels and proline in plant membranes (ROS). Plants have endogenous defense mechanisms that can be activated in response to *F. oxysporum* infection. Protection genes are considered inducible, meaning that specific stimuli must activate the specific stimuli or signals must activate them. A novel plant safety technique is thought to be inducing the plant's defensive mechanisms by using a biological inducer beforehand (Rashid and Chung 2017). Proline, an amino acid, can function as a potent scavenger of reactive oxygen species, preventing the induction of programmed cell death by reactive oxygen species (Liu et al., 2019). The responses differed depending on the type of application.

From a comprehensive perspective, planting previous crops to sesame plays a positive and effective role in changing the rhizosphere system; furthermore, the application of biological control as one of the eco-friendly applications in sustainable management has a profound effect on economic crops.

5. Conclusions

This paper presented to study the eco-friendly role in managing *Fusarium oxysporum* disease on sesame by cultivar previous crops before sesame plants, which had an effective role in reducing disease incidence and its severity to isolating bioagents from the soil in which the previous crops were grown. Before sesame and studying the effect of these bioagents on the growth and spore germination of the pathogen, as well as when application these bioagents to the soil, it had an effective and significant role not only in inhibiting disease but also on plant health under greenhouse and open field conditions, as confirmed by physiological analyses that measure plant resistance to disease.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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