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Research Article
Use of Biofumigation for Controlling Sesame Root Rot in North Sinai

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Abstract
Background and Objective: Sesame is an oil crop that has been cultivated in Egypt for hundreds of years. Sesame crop suffers from various soil-borne diseases. Charcoal root rot caused by *Macrophomina phaseolina* (Tassi.) Goid, is considered one of the main destructive diseases of this crop. The current research was conducted to evaluate the efficacy of biofumigation using the Brassica crops (rocket, cauliflower and radish) on root rot disease of sesame. **Materials and Methods:** Naturally infected roots of the diseased sesame plants were collected in two successive seasons (2017 and 2018), three biofumigant crops i.e. radish (*Raphanus sativus*), cauliflower (*Brassica oleracea* var. Botrytis) and rocket (*Eruca sativa*) were used as compared with the fungicide Rhizolex-T on mycelial growth of *Macrophomina phaseolina in vitro*. Obtained data were statistically analyzed using one-way analysis of variance MSTAT. **Results:** In the first season the cauliflower treatment was not significantly different from that of the fungicide Rhizolex-T in the field experiment. Obtained data showed that Cauliflower biofumigation and Rhizolex-T treatments have resulted in the lowest disease severity levels (2.000, 2.000) 80 days after transplanting. Moreover, Cauliflower-biofumigation and Radish-biofumigation showed the lowest severity levels 2.000, 2.000 at 80 days after transplanting in the second season respectively. There were no significant differences between all tested treatments 60 days after transplanting in 2017. **Conclusion:** The results of this research demonstrated that biofumigation using the studied brassica crops may provide high efficacy of safe and economical control of charcoal rot of sesame.

Key words: Root rot, sesame, biofumigation, rhizolex-t, soil solarization, cauliflower, rocket, radish

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sesame (*Sesamum indicum* L.), is the most ancient oilseed crop which is grown mainly for its seeds that are used for confectionery purposes and baked products or milled to get high-grade edible oil or paste (tahini)[1,2]. It is typically a crop of small farmers in developing countries. Sesame is considered a drought-tolerant crop due to its extensive root system. Usually, the mature plant height is between 60 and 120 cm. Flowers of sesame are self-pollinated, plant growth is indeterminate i.e. the plants continue to grow and produce leaves, flowers, as well as seed capsules throughout the growing season[3].

In Egypt, sesame is considered a food crop rather than an oilseed crop because most of its seeds are consumed directly. It is grown in many governorates and ranks first among the cultivated oil crops in Ismailia Governorate[4]. The total area under sesame production in Egypt has increased from 28,450 ha in 2005 to 34,000 ha in 2017; and the productivity increased also from 1,250 kg ha^{-1} in 2005 to 2,017 kg ha^{-1} in 2017 [5].

Sesame crops are attacked by several soil-borne diseases. Charcoal root rot caused by *Macrophomina phaseolina* (Tassi.) Goid. is considered one of the main destructive disease in all sesame growing areas[6]. The fungus can infect about 500 plant species in more than 100 families throughout the world. It is of high incidence in Egypt, especially during hot seasons[6-9]. Additionally, *M. phaseolina* causes early maturation, chlorosis and incomplete capsule filling in sesame. It survives as microsclerotia in the soil and infected plant debris. These microsclerotia serve as the primary source of inoculum and have been found to persist in the soil for up to three years[10]. Biofumigation is an eco-friendly method that uses Brassicaceae plants as rotation crops or Greenauer for controlling soil-borne pathogens. The term biofumigation represent suppression of soil-borne pests by compounds released by various Brassica plants[11].

Biofumigation is the practice of using volatile chemicals released from decomposing plant material to suppress soil pathogens, insects and germinating weed seeds. Brassicas have been successfully used for biofumigation[12]. The decomposition of the plant tissues in these plants releases isothiocyanates which are biocidal. Plants have different profiles of isothiocyanates. Biofumigation has been used as an alternative to methyl bromide and other synthetic pesticides in horticulture and agriculture in general.

Different biofumigation crops will have the different potentiality of biofumigation and exhibit different levels of pathogen controlling effects. Under high-value cash crop production farmers often apply some soil disinfestation before planting to reduce the hazard soil-borne pests including fungi[13]. However, this method is eco-friendly and adds organic matter to the soil. There is a need for local research into brassicas that can be used for biofumigation under North Sinai conditions.

The aim of the study was to study the effects of toxic volatiles released from tested Brassica crops (radish, Cauliflower, salad rocket) on radial growth of sesame root rot *in vitro* and compare the effects of toxic volatiles released from tested Brassica crops (radish, Cauliflower, salad rocket) with a certain chemical fungicide (Rhizolex-T) in controlling charcoal disease of sesame under field conditions

MATERIALS AND METHODS

Laboratory studies (Isolation, purification and identification of the causal organisms)

Isolation: The collected naturally infected roots of the diseased sesame plants were thoroughly washed with running tap water for several times to remove the adhered soil particles. These roots were then cut into small pieces (0.5-1 cm), excised tissues were surface disinfected by immersing them into sodium hypochlorite solution (0.5%) for 1-1.5 min, passed in sterilized distilled water and dried between two sterilized filter papers. After that, sterilized pieces were separately placed into Petri plates (9 cm) containing Acidified Potato Dextrose Agar (APDA) medium at the rate of 4 or 5 pieces for each dish. Plates were carefully closed with Para-film before incubation at $28\pm2°C$ for two weeks[14].

Purification: During the incubation period, any emerged fungus was purified using the hyphal tip technique[15].

Identification: Plates were examined daily for two weeks and the developed fungi were identified according to their morphological and cultural features[16-18].

The effects of three Brassica on *Macrophomina phaseolina* growth *in vitro*: These studies were carried out at Plant Pathology Laboratory, Plant Production Department, Faculty of Environ. Agricultural Science, Al-Arish between 2017-2018. This experiment was carried out to compare the effects of volatiles released from three crops i.e. radish (*Raphanus sativus*), cauliflower (*Brassica oleracea* var. botrytis) and rocket (*Eruca sativa*) on *Macrophomina phaseolina* growth. Tested crops seeds were planted at the experimental farm of Faculty of Environmental Agriculture Science., El-Arish.

The Agar plugs (6 mm diameter) were taken from the edge of *M. phaseolina* colonies actively growing in APDA medium (27°C, 7 days of incubation in the dark) were cut and transferred to the center of Petri dishes (9 cm diameter) containing fresh APDA medium.

Tissues including root pieces and green above-ground tissues in equal amounts were collected at the 10-leaf stage and washed with tap water and dried out. The tissues were disinfected in ethanol 10% for 10 sec, rinsed in sterile distilled water (SDW) for 5 min, dried on autoclaved filtering paper and macerated using a sterile mortar and pestle.

Two grams of fresh macerated tissues of each of the three Brassica species were placed in the lids of each Petri dish under sterile conditions. All dishes, including plates without any plant tissues (control), were sealed carefully with two layers of parafilm to prevent any possible vapor leak of volatiles from those plates. Plates were incubated upside down at 27°C for two weeks. The diameter of *M. phaseolina* colonies was measured 7 and 14 days after incubation. Each treatment (plant tissue, pathogen combination) had four replicates. This experiment was repeated twice during the 2017 and 2018 seasons.

As for the tested fungicide (Rhizolex-T), (50% WP) it was added to APDA medium before solidification under aseptic conditions. The media was poured in Petri dishes (9 cm) with four replicates. Similar to the Brassica tissue treatments, agar plugs (6 mm) taken from the edge of 7 days old cultures of *M. phaseolina* were transferred to the center of the agar surface of every replicate. Plates were also incubated at 27°C for two weeks. All tested dishes were incubated as mentioned before. linear growth of tested dishes was measured by measuring two perpendicular diameters in cm and the average was recorded and the percent reduction of radial growth in each treatment was measured 7-14 days after inoculation by the formula Abdullah *et al.*[19]:

$$I = \frac{C-T}{C} \times 100$$

where, I is percent growth inhibition, C is colony diameter of the pathogen in control and T is colony diameter of the pathogen in treatment. This experiment was conducted during two seasons with four replicate for each treatment.

Field studies

Preceding crops planting: Cauliflower seeds were sown in the nursery November, 14 and 23 during the 2017 and 2018 seasons, respectively. Radish and Watercress were sown in the field directly. The ideal agricultural practices were performed until seeds and cauliflower seedlings were transplanted into the field on December, 25 and 30 during the 2017 and 2018 seasons, respectively.

Biofumigation and soil solarization treatments:

- The preceding grown crops were directly incorporated into the soil at maturity stage and after flowering i.e. April, 13 and 21 during 2017 and 2018 seasons, respectively using a tractor for maximum tissue disintegration: plowing was performed to a depth no greater than 15 cm, producing a fine tilth as a mulch to trap the isothiocyanates (ITCs) gasses[20,21]
- The experimental field was watered by drip irrigation[20,22]
- Cover the soil surface tightly with a transparent plastic film for four weeks to retain the influence of the gases produced from the biodegradation of the organic matter. Bare soil was plowed, watered then covered with transparent plastic film and left undisturbed for four weeks (soil solarization treatment). Bare soil was plowed, watered then covered with transparent plastic film and left undisturbed for four weeks (soil solarization treatment)
- The film was removed 4 weeks after May during the 2017 and 2018 seasons, respectively. The soil was slightly disrupted to permit the gases to escape from soil. Sesame seedlings were planted 24 h later[22]

Control treatment: Control plants remained untreated and uncovered but were similarly irrigated to field capacity.

Fungicide treatment: Soil drenching with the fungicide was applied at three intervals, i.e. 15, 30 and 45 days after transplanting. The application rates were 1.5 gm/l water for the chemical fungicide Rhizolex-T 50% WP. The fungicide was applied individually. Each plant received 250 ml of the tested fungicide solution[23].

Sesame planting: Sesame seeds of Chandawel 3 cultivar were sown in soil directly on two different dates. The ideal agricultural practices were performed after previous crops were removed. The experiment consisted of 12 rows 8.5 m long and 6 m width. Each row contained 34 plants at 25 cm distances in-betweens. The experiment contained six treatments with four replicates, for each treatment. Plants were irrigated using a drip irrigation system. Fertilization was done through the drip irrigation system weekly. The ideal agricultural practices were carried out as usual[24].

Measurements

Disease severity index: Disease severity of root rot and any discoloration of tissue were recorded according to Haware and Nene [25] based on 0-4 scale according to percentage of foliage yellowing or necrosis (0 = 0%, 1 = 1-33%, 2 = 34-66%, 3 = 67-l00% and 4 = dead plant)

Statistical analysis: Data were statistically analyzed using MSTAT computer program. The least significant difference (LSD) at 0.05 level was used for comparing the differences between means.

RESULTS AND DISCUSSION

Laboratory studies

Effect of three Brassica species on mycelial growth of *Macrophomina phaseolina, in vitro*: This experiment was carried out to study the effects of volatile compounds released by macerated tissues of three Brassica species i.e. cauliflower, radish and rocket as compared with the fungicide Rhizolex-T on mycelial growth of *Macrophomina phaseolina in vitro*.

All tested Brassica species induced growth reduction of *M. phaseolina* (Table 1) in both experiments (2017, 2018). Cauliflower and radish were significantly more effective than a rocket in *M. phaseolina* growth suppression.

Table 1 showed that the effect of volatile inhibitors produced from fresh macerated tissues against *M. Phaseolina* isolate growth was significantly different from untreated controls. Comparing the Rhizolex-T treatment with the control treatment, it appears that the fungicide significantly reduced *M. phaseolina* growth by 39.5 and 38.79% in both seasons respectively. As for the Cauliflower treatment also resulted in a significant reduction in *M. Phaseolina* radial growth with 26.12 and 20.15% in both seasons, respectively. In addition, the rocket was the least effective treatment in suppressing the mycelial growth of *M. phaseolina* with 9.18 and 10. 40% reduction, respectively.

The suppression of fungal mycelial growth using various Brassica species has been reported by various researchers[26-28].

Table 1: Effects of volatiles released from three species of Brassica on linear growth (cm) of *Macrophomina phaseolina*

	Linear growth (cm) / Days after incubation		
Treatments	7	14	Reduction (%)
First season (2017)			
Rocket	7.525^{b}	8.247^{a}	9.188^{c}
Cauliflower	5.525^{c}	6.698^{bc}	26.12^{ab}
Radish	7.170^{b}	7.935^{ab}	12.63^{bc}
Rhizolex-T	1.033^{d}	5.485^{c}	39.50^{a}
Control	8.658^{a}	9.083^{a}	0^{c}
LSD 0.05	1.057	1.272	13.89
First season (2018)			
Rocket	7.525^{ab}	7.842^{ab}	10.40^{bc}
Cauliflower	5.797^{b}	6.965^{b}	20.15^{b}
Radish	7.122^{ab}	7.412^{ab}	14.81^{bc}
Rhizolex-T	0.9475^{c}	5.360^{c}	38.79^{a}
Control	8.330^{a}	8.770^{a}	0^{C}
LSD 0.05	1.724	1.558	17.72

*Means in column followed by the same alphabetical letter are not significantly different at 5% level according to LSD, *Each figure represents the mean of four replicate

Table 2: Biofumigation efficacy of three previous crops on root rot disease of sesame plants during 2017 and 2018 seasons

	Disease severity index / Days after transplanting	
Treatments	60	80
First season (2017)		
Rocket-biofumigation	1.750^{a}	3.500^{a}
Cauliflower-biofumigation	1.750^{a}	2.000^{b}
Radish- biofumigation	1.500^{a}	3.000^{a}
Control	2.500^{a}	3.500^{a}
Rhizolex-T	2.000^{a}	2.000^{b}
Soil solarization	1.750^{a}	3.000^{a}
LSD 0.05	1.238	0.9266
Second season (2018)		
Rocket-biofumigation	1.500^{bcd}	3.250^{a}
Cauliflower-biofumigation	2.000^{b}	2.000^{b}
Radish-biofumigation	1.250^{cd}	2.000^{b}
Control	3.000^{a}	3.250^{a}
Rhizolex-T	1.750^{bc}	2.500^{ab}
Soil solarization	1.000^{d}	2.500^{ab}
LSD 0.05	0.5066	0.8405

*Means in column followed by the same alphabetical letter are not significantly different at 5% level according to LSD, *Each figure represents the mean of four replicates*

Fields studies

Effects of biofumigation on root rot of sesame

Disease severity index (DSI): Data in Table 2 revealed that biofumigation significantly reduced root rot disease of sesame as compared with the control treatment. Cauliflower-biofumigation and Rhizolex-T-T showed the lowest level of disease severity index of (2.000, 2.000) at (80 days after transplanting) in 2017, respectively. However, in 2018, biofumigation with both cauliflower and radish gave similar results after 80 days of transplanting. The disease severity index was 2.0 for both treatments. There were no significant differences between all tested treatments at (60 days after transplanting) in 2017.

The pesticidal effect of biofumigation has been attributed to the chemical breakdown products of glucosinolates (GLS), the characteristic constituents of brassica crops.

Isothiocyanates (ITCs) that have fungicidal properties are released in soil when GLS hydrolysis takes place among other secondary compounds[29-31] and Manici *et al*.[32] showed that many Brassica species produce significant levels of glucosinolates (GLS), which are held in plant cells separately from the enzyme myrosinase and after chopping brassica plants and incorporating them into the soil in presence of water, the enzyme myrosinase is released and hydrolysis of GLS occurs producing the isothiocyanates.

Scientists ITCs toxicity, hence Brown and Morra[29] revealed that ITCs had the same effect of the active ingredient in the commercial fumigants dazomet and metham sodium and were highly toxic to pathogens. Similarly, Sarwar *et al*.[12] also observed that the Brassicaceae family suppresses pests and disease organisms. In general, this effect is attributed to a range of biocidal compounds that are released into the soil when glucosinolates are transformed into various bioactive fungicidal, insecticidal, nematocidal and herbicidal compounds.

In addition to the above mentioned pesticidal activity of glucosinolates, Many researcher have stated that incorporating large amounts of organic matter (Brassicas) into the soil also improved soil structure, increased nutrient availability, increased water holding capacity and stimulation of beneficial pathogen-suppressive microbial communities as reported by Kumar[33]. In addition, Cohen *et al*.[34] confirmed that there is an inverse relationship between the presence of organic matter in the soil and plant root disease.

The differences in the influence of the species of Brassica on the pathogens attributed by Mithen[35]. Kirkegaard and Matthiessen[36] and Sarwar[12] found that Brassicas are the most widely used plant species as biofumigants. The profile, concentration and distribution of different glucosinolates vary within and between Brassica species and in different plant tissues and consequently, the concentration and type of biocidal hydrolysis products evolved also varies[37].

CONCLUSION

The obtained data in this study demonstrated that brassica crops may be successfully applied as a safe and economical control measure for sesame charcoal root rot disease. Such feasibility of biofumigation crops may be improved by integrated disease management with other control measures for controlling charcoal rot of sesame.

SIGNIFICANCE STATEMENT

The current study demonstrated the positive and significant effects of applying biofumigation as a safe control measure that could be added to an integrated management program of charcoal rot disease of sesame crop. The results of this work could help other researchers to study biofumigation as a control measure that may help decrease our dependence on chemical fungicides and thus avoid the probability of developing resistance to those fungicides. The findings of this study may also provide insight into the mechanisms by which biofumigation effect soil-borne diseases. Also verified the potentiality of applying biofumigation as a biological control measure for soil-borne diseases at the field scale.

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