Journal of Basic & Applied Mycology (Egypt) 9 (2018): 1-7 **p-ISSN 2090-7583, e-ISSN 2357-1047**
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Potentiality of some *Brassica* plants toward the control of tomato-fusarial-wilt

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Received 10/4/2018,
Accepted 17/5/2018

Abstract: In the present study, biofumigation through the use of two *Brassica* plants namely, mustard and turnip was adopted to control tomato-fusarial wilt caused by *Fusarium oxysporum* f.sp. *lycopersici*. Brassicaceous plants were applied, in three forms, as green-manure, seed-meal, and also as pre-plant. The results clearly indicated that all forms applied revealed very reasonable effects on the rate of disease suppression by comparison with the effect induced by the chemical fungicide micronized-sulfur which has-been used as a positive control. By comparison, brassicaceous plants used as pre-plant tended to be more efficient than being used as seed-meal or green-manure. The rate of disease suppression induced by both plants (as pre-plant) came down in the first season from 56 % to an average of 18 %, then getting better in the second season (down to 4 %), and becoming best (0 %) in the third season. If the values of disease suppression, cost-effect and safety were regarded, the efficacy of disease control by brassicaceous plants would be considered more feasible than micronized-sulfur.

Key words: Brassica, mustard, turnip, tomato, wilt, *Fusarium*, plant disease, biofumigation, biocontrol.

Introduction

Fusarial-wilt is one of the most devastating soil-borne diseases. Apart from being able to attack wide range of host plants, it is able to overwinter in the soil for long periods. Furthermore, chemical treatments of the disease remained the most effective strategy of its control up to the present. However, most of used routine fungicides proved to be hazardous and the systemic ones became very expensive. Due to these impacts, a great concern was given, by investigators of plant disease control worldwide, toward alternative safe techniques of less cost effect. After the emergence of chemical fumigation by methyl-bromide and other related volatile compounds in the early 1950s, to control soil-borne pathogens and seed-storage insects, serious problems related to human health and environmental disturbances have been encountered and their negative impacts were badly increased by time. Due to this, it was recommended in The Monterial Protocol (Gullino *et al*. 2003 and Environmental Protection Agency 2005) that the production of these chemicals must-be phased out by January 2005.

In the early 1970s, biofumigation through soil treatment with brassicaceous plants, as an alternative safe technique, came to light and since then the technique was receiving great concern and the experimental results so far obtained by investigators, in different geographic regions, were proving its efficacy as a management strategy toward the control of soil-borne infections (Friberg *et al*. 2009) including those induced by fungi (Mayton *et al*. 1996), bacteria (Ayaz *et al*. 2008), nematodes (Zasada & Ferris 2004) as well as herbaceous plants (Al-Khatib *et al*. 1997).

It would be mentioned that a good number of worldwide studies adopting the technique of biofumigation through soil amendment with brassicaceous plants indicated clearly that the technique could be regarded as very promising though some of these studies referred toward the presence of some inconsistency and selectivity among soil treatment with various parts from different *Brassica* species (Smith & Kirkegaard 2002 and Friberg *et al*. 2009).

In Egypt, the number of previous studies adopting biofumigation using brassicaceous plants as a biocontrol approach was rather limited to make generalizations. Only three trials could be considered as most relevant although all have been using only the form of seed-meal. The first trial, by Fayzalla *et al*. (2004) toward the control of soybean-root infections induced by various pathogens, the second by El-Barougy (2008) toward the control of soybean damping-off induced by *F. oxysporum*, while the third by Shaban *et al*. (2011) toward the control of fusarial-wilt of lupine. The present study was conducted to evaluate the efficacy of two *Brassica* plants, in three different forms, toward the suppression of tomato-fusarial-wilt Micronized-sulfur was used as an effective soil chemical fungicide for comparison.

Materials and Methods

At present, in Egypt there are more than 10 tomato cultivars in use, but the validity for their cultivation vary greatly from one Governorate to another depending on soil suitability and prevailing environmental conditions. In the area of Kafr-Sakr (Sharkiya-Governorate) two cultivars were extensively used by farmers. This adoption was based on several features such as: early flowering, number of flowers, product quantity and quality and validity for planting in various types of soil. These cultivars were namely, **Aliasa** and **GS-12**. Both are Dutch products, the first by Nunhems-Netherlands and the second by Syngenta-Seeds- Netherlands. A preliminary test for the germination rate showed clearly that the percentage in both cultivars was distinctly high but being slightly greater in **Aliasa** (by scoring 96 %) than **GS-12** which showed 91 %.

Cultivar selection

Healthy seedlings of the two tomato cultivars **Aliasa** and **GS-12** were grown separately in pots containing infested soil at the rate of 5 seedlings per pot. After a growth period of one week, both cultivars started showing wilt morphological symptoms. However, after **70** days it was interesting to notice that some diseased seedlings of the cultivar **Aliasa** revealed a prominent recovery reaching 51 % while seedlings of the cultivar **GS-12** showed permanent wilting without showing any signs of recovery. Therefore, **GS-12** was selected as a test cultivar in this study.

Pathogen isolation and identification

Infected tomato plants showing symptoms of wilt were collected from several fields in the area of Kafr-Sakr. After gentle washing with very diluted soap, infected roots were rinsed with distilled water several times and finally by 70% Ethyl alcohol. Thereafter, infected roots were dried, cutted into 1 cm segments and transferred to sterile petriplates having potato dextrose agar (PDA). Plates were then incubated at 28^{o}C for a period of one week during which mycelial tips of emerging hyphae were picked up and transferred to new sterile plates containing fresh PDA and allowed to grow for two weeks in order to give the colonies enough time to develop all growth characteristics required to allow precise identification. Mature colonies were then examined morphologically and microscopically. In view of the colony colour, diffused secretions in the agar and conidial structures, the pathogen was identified as *Fusarium oxysporum* according to Booth (1971) and Leslie & Summerell (2006). A host specificity test showed clearly that the pathogen under test would be assigned to *F. oxysporum* f.sp. *lycopersici*.

Inoculum preparation and soil artificial infestation

It has to be mentioned that the soil of the experimental-field-site was arid, not cultivated before. A survey of the mycoflora showed poor content and absence of pathogenic fusaria. Therefore, to conduct our experimentation an artificial infestation became a must before launching experiments.

To prepare enough amount of the pathogen to be used as inoculum for artificial infestation, 600 g of sterile crushed-corn were inoculated with a spore-suspension of the identified pathogen and incubated at 28 ^{o}C for two weeks to allow good colonization of crushed-corn. Colonized corn was then mixed well with 5 kg of a sterile natural sandy-loam soil and to this mixture 750 ml of sterile water were added. The whole mixture was incubated at 28 ^{o}C for two weeks to allow good soil infestation. Infested soil was then dispensed in 1 kg capacity pots at the rate of 0.5 kg / pot. The concentration of this inoculum was considered to be 12% according to the following equation.

$$\text{Inoculum concentration (\%)} = \frac{\text{Amount of sterile inoculated corn} \times 100}{\text{Total amount of soil}}$$

$$\text{Total amount of soil} = \frac{600 \times 100}{5000} = 12\ \%$$

Experimental design

To carry out field experimentation, a land site of 24×6 meters within The Botanical Garden of The Department of Botany, Faculty of Science, at Ismailia was selected. The soil of this site was sandy-loam with low mineral content and slightly alkaline by showing a pH of 8.6. The land site was divided into 18 equal plots, each was assigned to accommodate certain soil treatment. Each plot was prepared to include 5 lines each of 280 cm long and 40 cm apart. Each line was planted with 5 seedlings at equal distances. Plots designated with the letter **A** contained treatments using infested soil, while plots designated with the letter **B** contained treatments using uninfested soil. For the general layout of the experimental field and specific treatments see Fig. 1 and for a detailed plot dimensions see Fig. 2.

For soil artificial infestation, each hill in all plots designated with the letter **A** received an amount of 25 g of the previously prepared pathogen inoculum followed by gentile irrigation and left for two weeks to allow soil colonization by the pathogen before planting with healthy tomato seedlings. In the present study, all experiments were repeated three times during three consecutive but climatologically different seasons (winter of 2015, summer of 2015, winter of 2016). Therefore, it became necessary that soil-infestation would be repeated and accordingly the disease

incidence (as percentage) was evaluated every season separately.

Biofumigation

The two candidate *Brassica* plants adopted to be used as a source of volatiles were namely, yellow-mustard (*B. nigra*) and turnip (*B. rapa*). For comparison of efficacy, each of these species was used in three forms: as green- manure, as seed-meal and as pre-plant.

a- As green – manure; fresh-turnip-roots and fresh-mustard plants were crushed and 200 g from each were dispensed in hills of each line at the rate of 40 g/hill where plots 2A and 2B were assigned for turnip and plots 5A and 5B for mustard.

b- As seed- meal; turnip-seed-meal and mustard-seed-meal were dispensed in hills of each line at the rate of 40 g/hill where plots 3A and 3B were assigned for turnip and plots 6A and 6B for mustard.

c- As pre-plant; seedlings of turnip and mustard were grown for a period of 2 months thereafter removed and replaced by healthy tomato seedlings. The plots 4A and 4B were assigned for turnip while plots 7A and 7B for mustard.

Chemical fungicide; for evaluating the efficacy of disease suppressivness by *Brassica* plants, micronized-sulfur as one of the most effective fungicide was adopted, as a positive control (8A and 8B) for comparison at the rate of 40 g / hill.

Normal and negative control; healthy tomato seedlings growing in infested, non-treated soil were used as **negative control** (plot 1A) while healthy tomato seedlings growing in non-infested, non-treated soil were used as **normal control** (plot 1B).

Results and Discussion

Effect of seasonal conditions on disease incidence

The data of Table 1 clearly indicate that the disease incidence is greatly affected by the prevailing environmental conditions especially temperature where the recorded values of the repeated evaluation of the disease incidence over the three consecutive but climatologically different seasons were significantly different.

The obtained data tended to be distinctly greater in summer than in winter seasons. However, throughout the three seasons, the rate of disease incidence followed a similar pattern of change by showing gradual increase upon increasing of the host growth period up to the age of 50 days after which the disease incidence attained constant value. A very related observation was also noticed by several investigators like Stapleton & Duncan (1998) who found that soil amendment with brassicaceous residues when combined with a soil-heating- regime induced greater reduction in the disease incidence. Similarly, Nunez-Zofio *et al*. (2011) reported that soil organic amendment with residues of different *Brassica* spp. followed by soil-plastic-mulching showed significantly lower values of disease incidence. In view of our observation along with those of the preceding investigators, it seems possible that high temperature might increase the rate of volatilization and accordingly the suppressive ability of brassicaceous residues.

Suppressiveness potential of turnip and mustard

The obtained data related to the ability of disease suppression through the application of soil treatment with turnip and mustard, applied in three different forms, is given in Table 2 where the data clearly showed that all forms of the two candidate plants were effective by reducing the disease incidence in variable rates during the three consecutive growing seasons. In view of the recorded values of disease incidence, the effect of the different forms showed a general trend indicating that brassicaceous residues used as preplant revealed the best results, followed by seed-meal and next by green-manure.

In the first season, all forms (except turnip as preplant) showed moderate effect by bringing down the disease incidence from 56 % to 40 % in turnip (as seed-meal) and to 24 % in mustard (as pre-plant).

In the second season, all forms revealed significant reduction of disease incidence ranged between 16 % in turnip (as green-manure) and 0 % in both of turnip and mustard (as seed-meal).

In the third season, the disease incidence came down to 0 % in all forms applied. This inconsistency in the potentiality of disease suppression revealed by the different forms of the two candidate *Brassica* plants during the present study was also reported by many other investigators (Motisi *et al*. 2009, Hossain *et al*. 2012, Klein *et al*. 2016, Mazzola *et al*. 2016). Such inconsistency would be accepted because the chemical profile of glucosinolate-derivatives produced upon the hydrolysis of brassicaceous residues vary greatly not only from one *Brassica* plant to another (Sang *et al*. 1984) but also from a vegetative part to another within the same species (Hill *et al*. 1987). Therefore, we recommend a conduct of pilot-scale experiments to determine what plant species to be selected and in what form to be applied before launching cultivation of crops on a large scale adopting the strategy of fumigation by brassicaceous plants.

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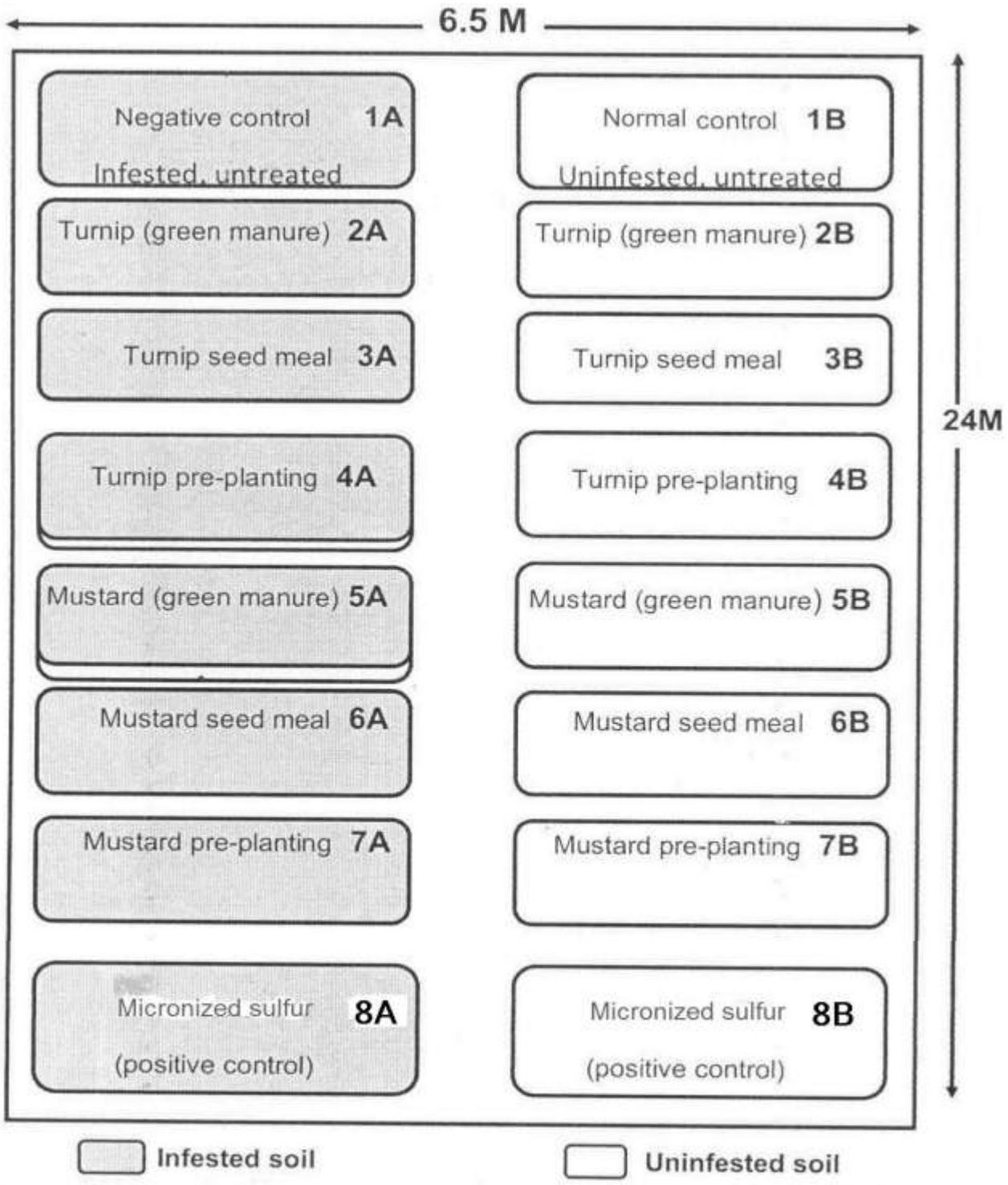

Fig.(1): General layout of the experimental area

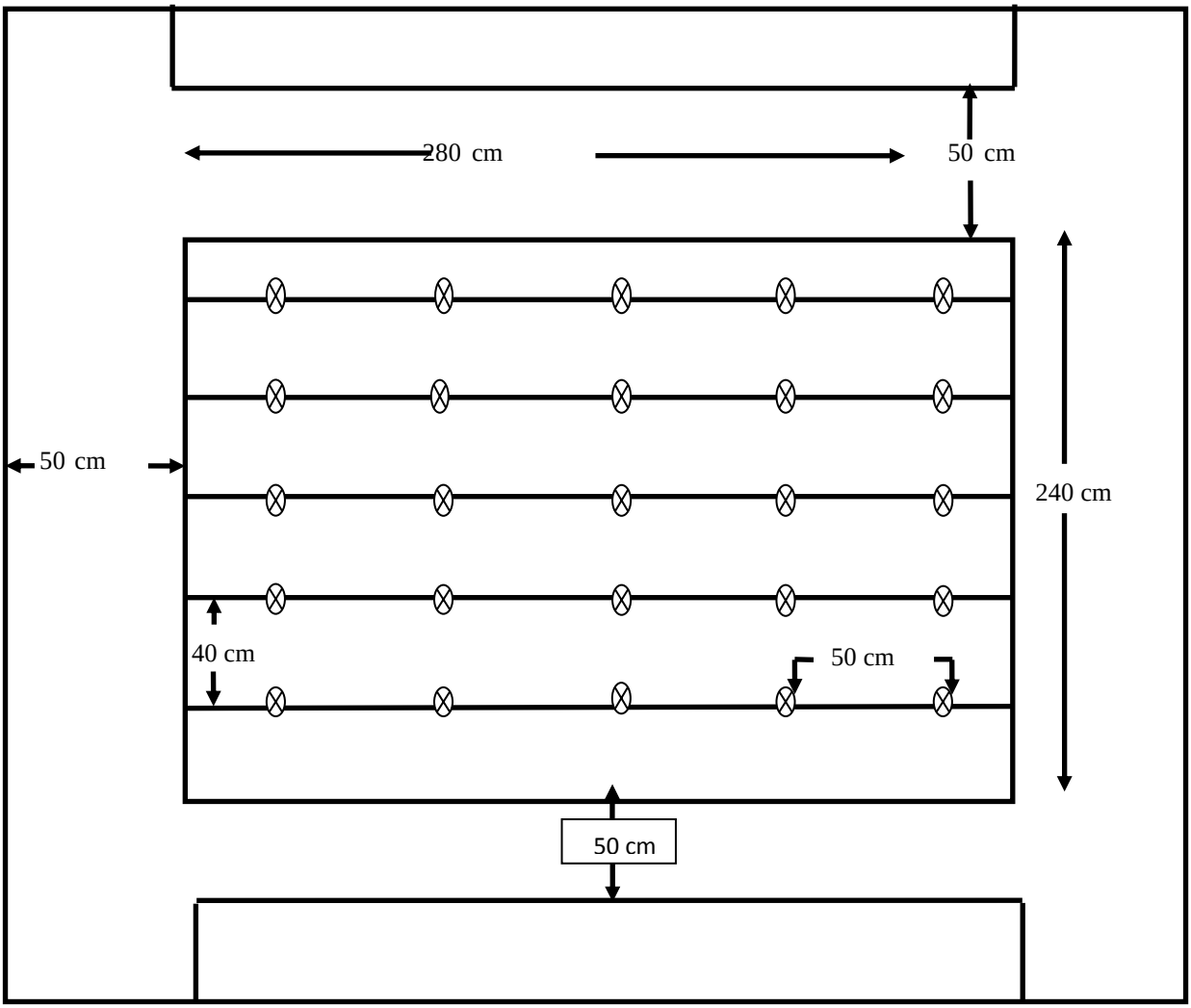

Fig. 2: Detailed dimensions of a single.

Table 1: Mean values of disease incidence (as percentage) in the 9 infested plots during the three growing seasons after variable growth periods.

Disease incidence / Season	34 days after planting	38 days after planting	42 days after planting	46 days after planting	50 days After planting ⊗	54 days After planting ⊗	58 days After planting ⊗
1st season (winter 2015)	32 **±2.31**	70.22 **±2.76**	78.67 **±2.83**	82.67 **±3.13**	83.56 **±3.43**	83.56 **±3.43**	83.56 **±3.43**
2nd season (summer 2015)	31.56 **±2.53**	61.78 **±2.59**	80.89 **±2.81**	91.11 **±2.38**	91.11 **±2.38**	91.11 **±2.38**	91.11 **±2.38**
3rd season (winter 2016)	24.89 **±2.38**	43.11 **±2.89**	56.89 **±1.30**	64.44 **±2.15**	73.78 **±4.01**	73.78 **±4.01**	73.78 **±4.01**

⊗ **Stable values ± Standard error**

Table 2: Mean values of disease incidence (as percentage) in 9 infested plots during the three growing seasons after using *Brassica* plants, in different forms.

Season / Type of treatment	**First season** (winter of 2015)	**Second season** (summer of 2015)	**Third season** (winter of 2016)
Negative control (infested-soil)	56 %	60 %	52 %
Positive control (micronized-sulfur)	4 %	4 %	4 %
as green-manure			
Crushed-turnip in infested soil	36 %	16 %	0 %
Crushed-mustard in infested soil	32 %	8 %	0 %
Mean value of disease incidence	34 %	12 %	0 %
as seed-meal			
Turnip seed-meal in infested soil	40 %	12%	0 %
Mustard seed-meal in infested soil	28 %	8%	0 %
Mean value of disease incidence	34 %	10 %	0 %
as pre-plant			
Turnip-plants in infested soil	12 %	8%	0 %
Mustard-plants in infested soil	24 %	0 %	0 %
Mean value of disease incidence	0 18 %	4 %	0 %