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Management of Fusarium Wilt of Tomato by Using Different Plant Parts of *Brassica Nigra* (Mustard)

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Abstract

Fusarium wilt of tomato is one of the most destructive diseases worldwide and causes considerable losses to tomatoes both in greenhouse and in field conditions. In the present study, five different parts, (leaves, flowers, stem, seeds and oil) of Brassica nigra (Black mustard) were applied in different doses both under in-vitro and in-vivo conditions to manage Fusarium wilt disease of tomato caused by *Fusarium oxysporum* f. sp. lycopersici (Fol). From each plant part five different doses (4, 8, 12, 16 and 20 g/L or ml/L Potato Dextrose Agar medium) were used to study their inhibitory effect on the mycelial growth of Fol, while in the screen house studies doses of 10, 20, 30, 40 and 50 g/kg soil or ml/kg soil were used to control the Fusarium wilt disease of tomato. Each treatment was replicated five times in a Complete Randomized Design (CRD). Extracted oil was found to be the most effective against Fol under in-vitro conditions by inhibiting the % mycelial growth to 65.96%, followed by seeds (56.37%), flowers (54.50%) and leaves (50.64%). Among doses the highest dose of 20 g/L or ml/L was more effective as compared to their corresponding low doses of 4 g/L or ml/L. Mustard stem was found to be least effective and non-significant result was recorded for all doses. In the in-vivo studies, maximum reduction in AUDPC was achieved by oil followed by seeds, flowers and leaves while growth parameters (root length, shoot length and fresh biomass) were effectively enhanced by oil followed by seeds, flowers and leaves. B. nigra plant parts were observed to be more effective in high dose of 50 g/kg soil or ml/kg soil than lower dose of 10 g/kg soil or ml/kg soil except mustard oil which was phytotoxic to tomato plant resulting in reduction of the growth parameters at 50 ml/kg soil. Mustard stem was found to be the least effective. It is concluded that mustard oil @40 ml/kg soil and the other plant parts @50 g/kg soil can be used for effective control of the disease. However, the cost benefit ratio should be worked out carefully.

Keywords: *Fusarium wilt, Tomato (Solanum lycopersicum), Brassica nigra, Plant extracts, Disease control, Plant-based antifungal activity*

1. INTRODUCTION

Tomato (Lycopersicon esculentum Mill) belongs to family Solanaceae and stands out amongst the most eaten vegetables on the planet. It is consumed fresh or in various processed forms (Isah et al., 2014). Along with its derived products, carotenoids are the major constituents of tomatoes, which provide about 80% of lycopene, ascorbic acid, flavonoids, a-tocopherol and potassium in the western diet (Willcox et al., 2003).

Tomato is a tropical plant and grown in every part of the planet. In 2017, total cultivation of tomato worldwide was 484.83 thousand hectares, and the production was 182.30 million tons, China providing 32% of the aggregate followed by India (11.36 %), Turkey (6.99 %) and the USA (5.99 %) as the major tomato producing countries. Pakistan shares only 0.33 % to the total aggregate (FAO, 2017). In Pakistan during 2018 total area under production was 149.75 thousand acres and total production was 622.1 thousand tons. In 2018, tomatoes were grown in Khyber Pakhtunkhwa on 32.12 thousand acres, and the total tomato production was 124.80 thousand tons (Anonymous, 2018). In Pakistan per acre yield of tomatoes is very low. Field losses are caused by many factors e.g. tomato plants susceptible to many pests and pathogens, on the other hand improper handling also cause many post-harvest losses (Hussain, et al.,2016).

Many plant pathogens such as viruses, bacteria and fungi infect tomato plants. Bacteria and fungi cause stem, fruits, root and foliar diseases. As a result of tomato diseases, tomato growers suffer from huge yield losses. Tomato plants are attacked by many plant pathogens, including *Fusarium oxysporium* f. sp. Lycopersici (Jha et al., 2018).

Tomato wilt disease is caused by fungus *F. oxysporum* f.sp lycopersici. It is one of the profoundly destructive diseases of tomato, causing infection even in those plants which are grown in green houses for experimental purposes (Borrero1et al.,12004). The pathogen first invades the roots of tomato plant and then multiplies and colonizes in the plant vascular tissues prompting blocking of the water supply of the infected plants (Agrios, 2005). Regular symptoms of the disease are yellowing and wilting of leaves and it progressing to the top of the plant from the base of the stem. At first, just one side of a plant is influenced yet after some time these manifestations spread to the remainder of the plant and finally kill the host. Because of prolonged survival as a saprophyte in soil and as resistant structures, the control of *F. oxysporum* f.sp lycopersici is quite difficult (Khan & Khan, 2002; Borrero et al., 2004). Yield loss of tomato due to *F. oxysporum* f.sp lycopersici infection varies from 10 to 90% and it relies upon the phase of the plant development and the natural environmental conditions (Singh,2005).

Three physiological races (1, 2, and 3) of the pathogen are recognized to tomato cultivars by their pathogenicity (Kawabe, 2005) and are distinguished by specific pathogenicity on analyzer plants which carries vertical resistance genes (Cai et al., 2013). In 1886, for the first time race 1 was observed, race 2 was reported for the first time in Ohio in 1945 while in 1978 in Australia, race 3 was initially identified and subsequently observed in various U.S (Amini, 2009). States and Mexico *F. oxysporum* pathogen is the most widely spread and is present all around the globe (Agrios, 2005). No known sexual stage is found yet in *F. oxysporum,* but they produce three types of asexual spores which are chlamydospores, macroconidia, and microconidia. Spores which are produced most abundantly are microconidia. It is a very common pathogen of soil and saprophyte that feeds on dead and rotting natural organic material. The pathogen overwinters as all types of spores and as a mycelium in the soil debris but from the soil the fungus is recovered as chlamydospores most commonly (Snyder and Hansen, 1940).

Like many other diseases which infecting the plants, control of fusarium wilt is also accomplished by systemic fungicides application and utilization of tomato resistant cultivars (Cook 1993; Agrios 2005). However, breeding for host resistance is a time consuming and very laborious and difficult process. Furthermore, the appearance and development of a new pathogen race may break down the resistance easily. On the other hand, fungicides are also too expensive and may pollute the environment. Therefore, an alternate management strategy for the control of Fusarium wilt disease is required, which is environmentally friendly and affordable.

Brassica nigra L. is a significant Rabi season oil-seed crop, which belongs to family Cruciferae (Purendra, 2018). Mustard has been consumed as a green vegetable for centuries, and their products are also used as condiments, and their oils are also used for industrial and edible purposes (Nesi et al., 2008). The seeds of

mustard plant contain high amount of vitamin K and Vitamin A, when consumed uncooked. The flowering shoots of the plant are enormously hot when eaten in fresh form, having bitter taste, but the taste becomes mild after their cooking (Mahmudur et al., 2018).

After collecting seeds, when the plants reach to a certain level, the remaining portion of the plants are used as fodder and the seed residue after extraction of oil, is utilized as the feed for the poultry, dairy and other domesticated animals and aquaculture industries (Mailer et al., 2008)

Mustard oil is utilized for many years to avoid microbial development such as food-spoiling bacteria and due to their antioxidant properties, they are used to increase the shelf life of processed food. Anti-microbial substances include glucosinolates as well as proteins which inhibit the growth of bacteria in foodstuffs (Nielsen and Rios 2000). Antioxidant components such as vitamin E and C, catechin and quercetin in mustard plant have the power to suppress the formation of peroxynitrites, superoxides, hydrogen peroxides and thus decrease the rate of oxidation in food (Parikh et al., 2015). Mustards oil is also used to preserve meat, and meat products such as canned meat as well as sausages, nuggets, salamis burgers (Wendlinger et al., 2014).

The extracts of Mustard plant showed antifungal activity against *Fusarium oxysporum* at various levels. Extract derived from roots showed inhibition of 45% at 10 µg/µL. Extracts derived from stem and leaves showed inhibition (>35% and >30% respectively), in the similar concentration range (Esmail Al-Snafi, 2015).

2. MATERIALS AND METHODS

This study was carried out in the Department of Plant Pathology, The University of Agriculture, Peshawar during 2018-2019.

Collection of mustard plants and preparation of dried powder

Mustard plants (Rai variety of Brassica nigra) in flowering stage were collected from Harichand, District Charsadda, Khyber Pakhtunkhwa, Pakistan in February 2019. After collecting, the samples (stems, leaves and flowers) were washed thoroughly with tap-water and shade dried. Mustard seeds were collected at the end of April 2019 after harvesting the fully matured mustard plant and shade dried at room temperature. Each part of the plant was squashed in electric grinder to obtain fine powder and saved in airtight plastic bags separately. Oil was extracted from mustard seeds through mustard oil machine and saved in airtight bottles, until used.

Preparation of Inoculum

Roots of tomato plants showing symptoms of *Fusarium oxysporum* f. sp. Lycopersici infection were collected from District Charsadda. Infected samples were treated with 1% sodium hypochlorite for 30 seconds for surface sterilization and rinsed with sterilized water to remove the sterilant. Four pieces were plated on Potato Dextrose Agar (PDA) Medium at 25ºC for one week. Subculturing of the isolated pathogen was done to purify the culture. For identification of the pathogen, identification keys were used (Barnett et al., 1998). Suspension of Fungal spores were made by adding 10-ml sterile-distilled water to each-plate and scraping the culture with spatula. The fungal spores were collected in sterile distilled water. Spores' concentration was adjusted to 4×104spores/ml using a Hemocytometer and was used as inoculum for further studies.

Antifungal activity assay of Mustard plant powders

Food poison method (Nasrine et al., 2017) was used to assess the influence of dried powders of different parts of Brassica nigra, against *F. oxysporum* f.sp lycopersici growth under in-vitro condition. Different plant part powders were added at different concentrations 4, 8, 12, 16 and 20g/L or ml/L to PDA medium, at a temperature of 50oC. In aseptic conditions, a plug from 6–7 days old, purified fungus culture was plated on PDA. There were five treatments i.e. stem, flowers, leaves, seeds and oil and five doses in each treatment. Five replications were made for each dose. So, the total experimental units were (5×5+2) ×5= 135. The plates without plant extract served as negative control while the plates with fungicide Searles (Mancozeb plus fungicide contain 54.9% sulfur and 23.5% mancozeb) @2g/L served as positive control (Table 1). The inoculated plates were finally sealed with parafilm and incubated at 25oC for one week. Daily observation was made for fungal development. After one week the antifungal property of extracts

were calculated by measuring the colony diameter with the help of transparent ruler. The percent inhibition of the fungus in treatments was calculated using following formula.
Percent inhibition = C − TC × 100
Where, C is the colony radius in control plate and T is the radial growth of the pathogen in the treated plates (Shivapratap et al., 1996). The statistical design used for the experiment was Completely Randomized Design (CRD) and the data were finally subjected to statistical analysis using ANOVA and LSD tests.

Preparation of Tomato nursery
Tomato transplants for the in-vivo experiments were raised by sowing tomato seeds (Rio Grande variety) in nursery bed, irrigation and fertilization were done as per standard horticultural recommendations for tomato seedlings. One-month old seedlings were transplanted (one seedling/pot) into pots filled with 1kg mixture of sterile sand, clay and farmyard manure.

Inoculation of the potted soil
The effect of different parts of mustard (leaves, stem, flowers, seeds and oil) was tested against fusarium wilt disease under screen-house conditions. Five doses of each treatment were used which were 10, 20, 30, 40 and 50 g/kg or ml/kg soil. Each treatment was replicated five times in a completely randomized design. Total experimental units were (5×5+2) ×5= 135. The dried powders and oil were added to the pots having sterile soil and the soil was thoroughly mixed before transplantation. Potting mixture (soil: sand: FYM = 1:1:1) was pasteurized at 82˚C for 30 minutes. The main roots of each tomato plant were inoculated with 5 ml of prepared pathogen inoculum suspension containing 4×104 conidia/ml in the evening 30 days post transplantation. Soil without plant extract served as negative control while the soil with fungicide Searles (Mancozeb plus) @2g/kg served as positive control.

Growth conditions
The in-vitro studies were carried out in Department of Plant Pathology, The University of Agriculture, Peshawar, to test the antifungal property of Mustard plant against *F. oxysporum* f. sp. Lycopersici while the in-vivo studies were carried out in the screen house at New Developmental Farm, The University of Agriculture, Peshawar, Pakistan under natural conditions. In the screen house temperature ranged 35°C-38°C. Plants were watered up to a saturation level on alternate days.

Data Recording
After seedling transplantation, the experiment was allowed to run for two months. Data was recorded on the following parameters.

Disease severity
Disease severity was recorded weekly using the scale of Sibounnavong et al. (2012): where
1 = no symptoms,
2 = plant showing slight chlorosis and wilting (1 - 20%),
3 = plant showing yellow leaves and wilting (21 - 40%),
4 = plant showing moderate chlorosis and wilting (41 - 60%),
5 = plant showing severe chlorosis and wilting (61 - 80%),
6 = Extreme chlorosis or dead plant (81 - 100%).

Percent Disease Severity (D.S) was calculated according to Tarabulsi et al. (1998) as follows:
% D.S = (∑n)6N × 100
Where ∑n = number of infected leaves per plant and N = total number of leaves per plant and 6 = highest score of severity.

Area under the Disease Progress Curve (AUDPC) was calculated from disease severity data for all the treatments with following formula (Shaner and Finney, 1977):

AUDPC=n=0n−1yi+yi+12×ti+1−ti
Where n = total interval on which disease severity data was recorded, yi = disease severity at the ith (first week) observation, and t = total time at the ith observation. The unit of the AUDPC is Percent development stage unit (% adu).

Fresh biomass
After 30 days of pathogen inoculation each tomato plant was uprooted carefully from pots and weighed in grams with the help of digital balance.

Shoot length
After 30 days of pathogen inoculation each tomato plant was carefully uprooted from pots and measured their shoot length with help of ruler in centimeter.

Root length
After 30 days of pathogen inoculation each tomato plant was carefully uprooted from pots and measured their shoot length with help of ruler in centimeter.

3. DATA ANALYSIS
Statistical analysis of the data was performed using ANOVA-1 protocol in Statistix 8.1 software. LSD0·5 was conducted for mean separation.

TREATMENTS

Table 1: ***List of treatments for the invitro experiment***

S.No	Treatments	Symbols
01	Inoculated and untreated (negative control)	T1
02	2g Searles fungicide/L medium (positive control).	T2
03	4g mustard leaves/L medium	T3
04	8g mustard leaves/L medium	T4
05	12g mustard leaves/L medium	T5
06	16g mustard leaves/L medium	T6
07	20g mustard leaves/L medium	T7
08	4g mustard flowers/L medium	T8
09	8g mustard flowers/L medium	T9
10	12g mustard flowers/L medium	T10
11	16g mustard flowers/L medium	T11
12	20g mustard flowers/L medium	T12
13	4g mustard stem/L medium	T13
14	8g mustard stem/L medium	T14
15	12g mustard stem/L medium	T15
16	16g mustard stem/L medium	T16
17	20g mustard stem/L medium	T17
18	4g mustard seeds/L medium	T18
19	8g mustard seeds/L medium	T19
20	12g mustard seeds/L medium	T20
21	16g mustard seeds/L medium	T21

22	20g mustard seeds/L medium	T22
23	4ml mustard oil/L medium	T23
24	8ml mustard oil/L medium	T24
25	12ml mustard oil/L medium	T25
26	16ml mustard oil/L medium	T26
27	20ml mustard oil/L medium	T27

Each treatment was replicated 5 times so total experimental units were 27×5= 135.

Table 2: ***List of treatments for screen house experiment***

S.no	Treatments	Symbols
01	Inoculated and untreated plant (negative control)	T1
02	2g searles fungicide/L medium (positive control).	T2
03	10g mustard leaves/kg soil	T3
04	20g mustard leaves/kg soil	T4
05	30g mustard leaves/kg soil	T5
06	40g mustard leaves/kg soil	T6
07	50g mustard leaves/kg soil	T7
08	10g of mustard flowers/kg soil	T8
09	20g mustard flowers/kg soil	T9
10	30g mustard flowers/kg soil	T10
11	40g mustard flowers/kg soil	T11
12	50g mustard flowers/kg soil	T12
13	10g mustard stem/kg soil	T13
14	20g mustard stem/kg soil	T14
15	30g mustard stem/kg soil	T15
16	40g mustard stem/kg soil	T16
17	50g mustard stem/kg soil	T17
18	10g mustard seeds/kg soil	T18
19	20g mustard seeds/kg soil	T19
20	30g mustard seeds/kg soil	T20
21	40g mustard seeds/kg soil	T21
22	50g mustard seeds/kg soil	T22

23	10 ml mustard oil/kg soil	T23
24	20 ml mustard oil/kg soil	T24
25	30 ml mustard oil/kg soil	T25
26	40 ml mustard oil/kg soil	T26
27	50 ml mustard oil/kg soil	T27

Each treatment was replicated 5 times so total experimental units were 27×5= 135

Table 3: ***Randomization of treatments***

T1	T15	T25	T20	T6	T1	T9	T22	T3	T18	T2	T17	T3	T10	T4	T23	T4	T13	T8	T14	T22	T17	T21	T8	T18	T24	T15
T11	T1	T13	T23	T7	T22	T16	T9	T26	T12	T26	T23	T3	T7	T27	T20	T24	T9	T25	T15	T5	T16	T12	T5	T19	T14	T25
T27	T1	T24	T11	T17	T1	T18	T6	T19	T21	T6	T20	T14	T23	T16	T7	T4	T10	T19	T12	T24	T18	T20	T5	T21	T23	T19
T2	T27	T10	T13	T6	T14	T12	T15	T7	T10	T2	T26	T3	T20	T3	T22	T27	T13	T15	T21	T5	T8	T26	T17	T25	T13	T9
T24	T2	T6	T19	T18	T26	T8	T11	T17	T25	T2	T16	T10	T11	T12	T27	T4	T8	T7	T21	T4	T22	T11	T5	T9	T14	T16

4.RESULTS

Effect of Mustard plant on the colonial growth of Fusarium oxysporum f.sp. lycopersici under in-vitro conditions

All plant parts significantly (P=0.000) reduced the colony diameter of Fol even when used at the lowest dose of 4g/L medium. The powder of leaves, flowers, seed and oil of B. nigra were found to be more effective than stem in inhibiting mycelial growth of Fol (Table 4). Overall, oil gave maximum inhibition percentage of mycelial growth of Fol which was 65.96% followed by seeds, flowers and leaves with 56.37%, 54.50% and 50.64% growth inhibition respectively. Among doses 20 g/L of ml/L were found to be more effective in reducing the mycelial growth of Fol and oil at 20 ml/L was observed to be even more effective than the fungicide Searles (Fig. 5). Similarly seeds gave maximum inhibition of 76.84% at 20 g/L and minimum of 24.09% at 4 g/L (Fig. 4) while the maximum inhibition of flowers and leaves at 20g/L was 82.22% (Figure 2) and 86.08% (Fig. 1) respectively and minimum at 4g/L which was 18.47% and 15.08% respectively. Stem was least effective than other parts of B. nigra used and resulted in non-significant reduction in the mycelial growth even when used at the highest concentration of 20 g/L as compared with lowest dose of 4 g/L (Fig. 3) the mean percent inhibition of all doses of stem was 37.89%.

Table 4: ***Antifungal effect of Brassica nigra at various concentrations on in-vitro mycelial growth of F. oxysporum f. sp. lycopersici after 7 days incubation***

Treatments	Colony Diameter (cm)	% inhibition
Inoculated and untreated (negative control)	8.55 A	0 %
2g searles fungicide/L medium (positive control).	1.02 M	88.07 %
4g mustard leaves/L medium	7.26 B	15.08 %
8g mustard leaves/L medium	5.31 DEF	37.89 %
12g mustard leaves/L medium	4.01 G	53.09 %
16g mustard leaves/L medium	2.32 I	72.86 %
20g mustard leaves/L medium	1.19 LM	86.08 %
4g mustard flowers/L medium	6.97 B	18.47 %
8g mustard flowers/L medium	5.18 E	39.41 %
12g mustard flowers/L medium	3.23 H	62.22 %
16g mustard flowers/L medium	2.54 I	70.29 %
20g mustard flowers/L medium	1.52 K	82.22 %
4g mustard stem/L medium	5.37 DEF	37.19 %
8g mustard stem/L medium	5.23 F	38.83 %
12g mustard stem/L medium	5.24 EF	38.71%
16g mustard stem/L medium	5.20 F	39.18 %
20g mustard stem/L medium	5.54 D	35.20 %
4g mustard seeds/L medium	6.49 C	24.09 %
8g mustard seeds/L medium	3.97 G	53.56 %
12g mustard seeds/L medium	3.72 G	56.49 %
16g mustard seeds/L medium	2.49 I	70.87 %
20g mustard seeds/L medium	1.98 J	76.84 %
4ml mustard oil/L medium	5.53 DE	35.32 %
8ml mustard oil/L medium	3.98 G	53.45 %
12ml mustard oil/L medium	2.99 H	65.02 %
16ml mustard oil/L medium	1.41 KL	83.50%
20ml mustard oil/L medium	0.66 N	92.28 %
CV	5.94	
$LSD_{(0.05)}$	0.300	

Means followed by different letters are significantly different from one another at 5% level of probability

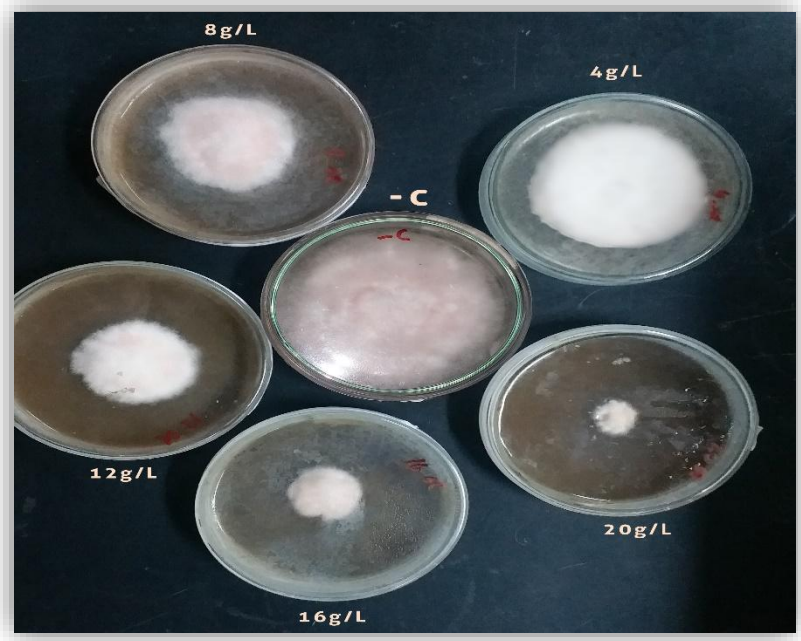

Figure 1: *Effect of different doses of dried powder of Brassica nigra leaves on the mycelial growth of F. oxysporum f. sp. Lycopersici (Fol)*

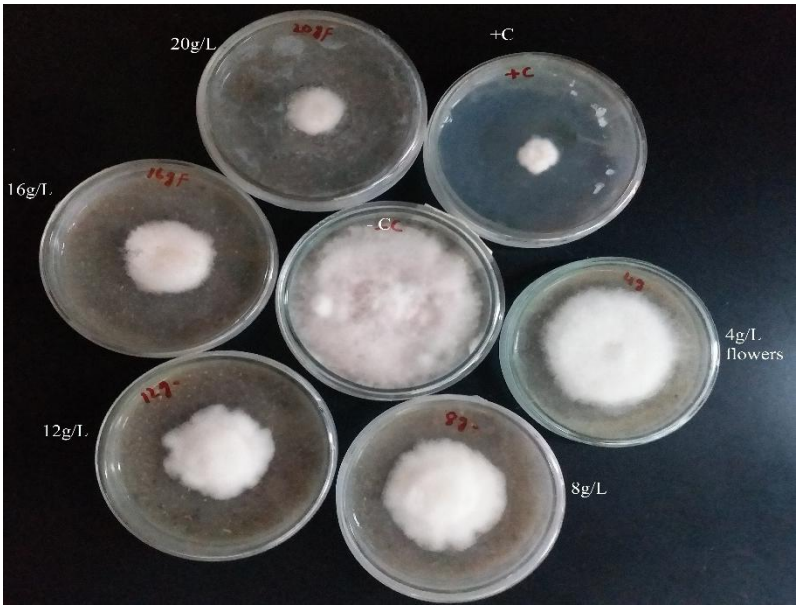

Figure 2: *Inhibation of mycelial growth of Fol by dried powder of B.nigra flowers at different doses*

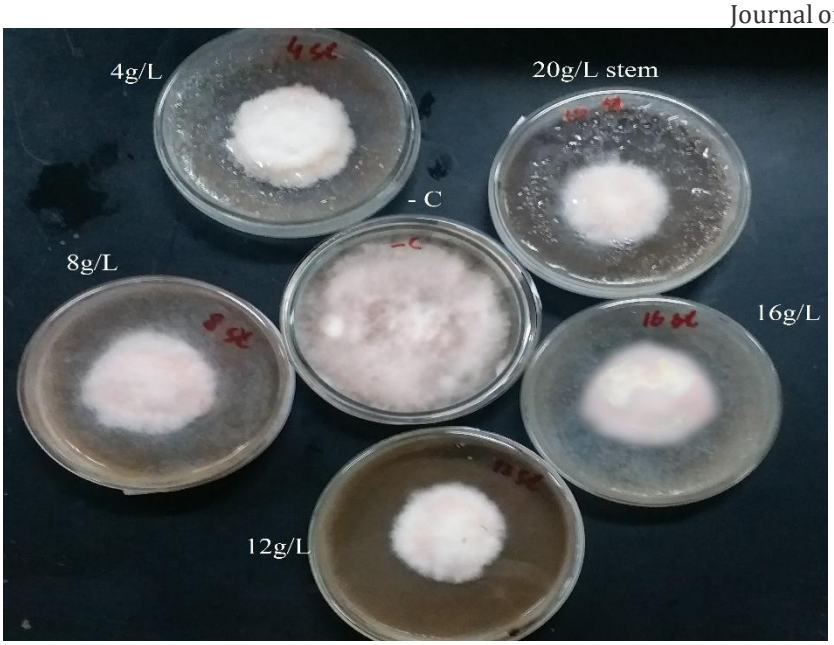

Figure 3: *Non-significant control of Fol growth by dried powder of B. nigra stem at different doses*

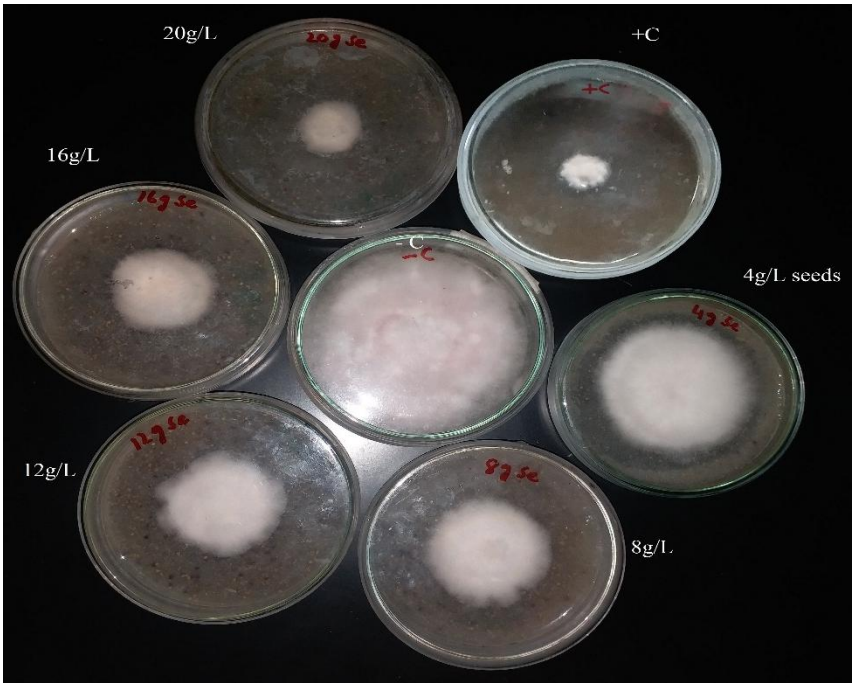

Figure 4: *Inhibitory effect of different doses of dried powder of B. nigra seeds on mycelial growth of Fol*

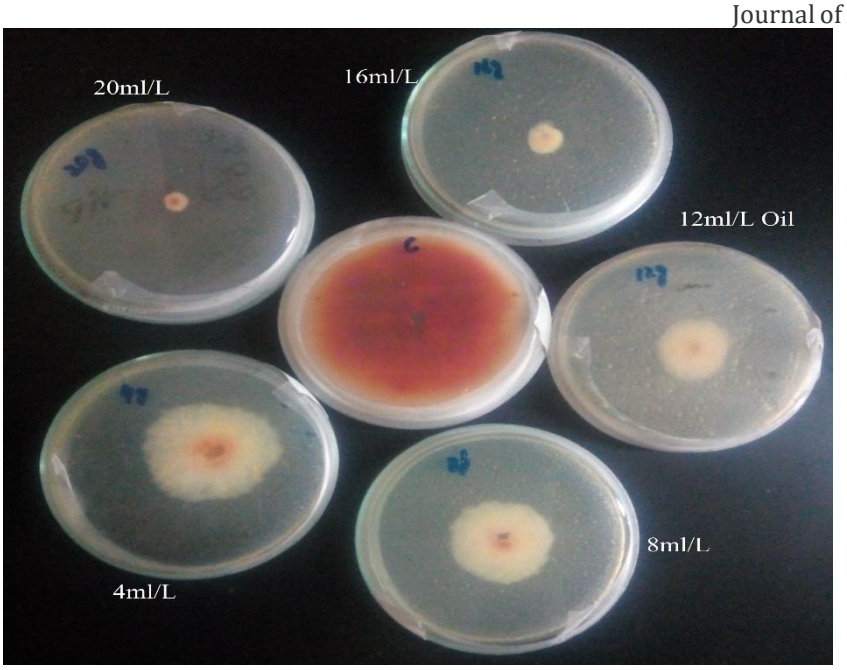

Figure 5: *Inhibitory effect of different doses of dried powder of B. nigra oil on mycelial growth of Fol*

In-vivo antifungal assay

Effect of different doses of plant parts of B. nigra on Area Under Disease Progress Curve (AUDPC) of fusarium wilt of tomato

Disease severity (%) was recorded weekly and AUDPC values were calculated to find out the disease progress over time, significant difference (P=0.000) was noticed among various treatments (Table 5). In inoculated and untreated tomato plants (negative control), highest 1431.2 AUDPC was observed while the lowest 144.87 AUDPC was recorded in mancozeb treated tomato plants @2 g/kg soil. Among different plant parts the oil was more effective than other parts of mustard plant to suppress the fusarium wilt disease in tomato and recorded 390.84 AUDPC followed by seeds (435.10), flowers (496.02) and leaves (584.75). Among doses, 50 g/kg soil or ml/kg soil of all plant parts (except stem) of B. nigra were found to be most effective while 10 g/kg soil or ml/kg soil was found to be least effective. The least AUDPC of 240.42 were recorded in those tomato plants which were treated with oil at the rate of 50 ml/kg soil while 527.67 AUDPC was recorded in tomato plants, treated with oil at the rate of 10 ml/kg soil. Similarly seeds, flowers and leaves at 50 g/kg soil greatly reduced the disease severity of tomato plants by recording 271.34, 333.70 and 383.42 AUDPC respectively while the corresponding AUDPC at 10 g/kg soil was 568.29, 633.87 and 820.07 respectively. Although, the dried powder of stem of B. nigra was effective in reducing the AUDPC as compared with inoculated control, it was the least effective as compared to the other plant parts of B. nigra.

Effect of different doses of dried powders of B. nigra plant parts on shoot length (cm) of tomato plants, inoculated with F. oxysporum f.sp. lycopersici

Shoot length of tomato plants were significantly different (P=0.000) among different treatments (Table 5). Maximum shoot length of 69.48cm was recorded in positive control (treated with mancozeb @2 g/kg soil) while minimum shoot length of 50.45cm were observed in negative control (inoculated and untreated). Among the different plant parts, highest shoot length of 62.78cm was recorded in tomato plants treated with seeds of mustard plant followed by flowers, oil and leaves having shoot length of 60.93cm, 59.06cm and 58.9cm respectively. Among doses, plants treated with 40 ml oil/kg soil, registered maximum shoot length of 73.04cm but the shoot length decreased as the dose decreased. Shoot length of tomato plants treated with 30, 20 and 10 ml oil/kg soil was 58.79cm, 56.52cm, and 53.89cm respectively. On the other hand, the shoot length of tomato plant also decreased significantly to 53.10 when the dose of 50 ml oil/kg soil was used. This show that high doses of oil are phytotoxic to tomato plants. Application of dried powdered seeds at @50 g/kg soil resulted in maximum shoot length of 71.12 cm but the shoot length decreased as the dose decreased. Plants showed minimum shoot length as the soil was treated with 10 g/kg mustard seeds. Similar pattern of response was observed when leaves or flowers were applied as dried powder to the roots of tomato plants with maximum shoot length of 65.02 cm and 68.74 cm respectively. While the lowest dose of 10 g/kg soil resulted in shoot length of 52.67 cm and 54.39 cm respectively.

Among all parts of mustard plant, stem was the least effective. Minimum shoot length of 51.28cm was recorded in tomato plants treated with dried powder of mustard stem. No significant difference was observed among the different doses of stem.

Effect of different doses of dried powders of B. nigra plant parts on root length (cm) of tomato plants, inoculated with F. oxysporum f.sp. lycopersici

Regarding root length, significant differences (P=0.000) were recorded among the different treatments (Table 5). Tomato plants treated with mancozeb @2 g/kg soil, maximum root length of 36.78cm was recorded while in inoculated and untreated control minimum root length of 23.05cm was observed. Among different plant parts of mustard, dried powder of mustard seeds was highly effective against fusarium wilt disease, and the highest dose of 50g/kg soil was found to be far with mancozeb. The root length (mean of all doses) of tomato plants was maximum (30.45cm) when they were treated with seeds followed by oil (29.85cm), flowers (29.56) and leaves (27.45cm). Minimum root length of 24.62cm was recorded when the tomato plants were treated with mustard stem. Among different doses 50 g/kg soil was found to be the most effective than other doses of the same plant part of mustard except oil which resulted in least root length of 23.33cm when they were treated with 50 ml/kg soil but oil at 40 ml/kg soil the tomato plants yielded maximum root length of 36.41cm greater than all doses but decreasing the doses from 40 ml/kg root length of tomato plant was also decreased. For 30, 20 and 10-ml oil/kg soil, the root length recorded was 32.58 cm, 29.66 cm and 27.30cm respectively. For seeds, flowers and leaves, the tomato plants showed maximum root length of 36.09cm, 34.02cm and 31.23cm respectively at 50 g/kg soil doses while minimum root length of 25.20cm, 24.97cm and 24.39cm respectively was observed at 10 g/kg soil dose. Dried powder of mustard stem was the least effective in all doses as the root length of 24.53cm was recorded for 50 g/kg and 24.80cm for 10 g/kg soil.

Effect of B. nigra plant parts with different doses on fresh biomass (g) of tomato plants inoculated with F. oxysporum f.sp. lycopersici

Fresh biomass of tomato plants was found to be significantly different (P=0.000) from each other's among different treatments (Table 5). Tomato plants treated with mancozeb @2 g/kg soil (positive control) registered the maximum fresh biomass of 60.53g while minimum fresh biomass of 30.60g was recorded in inoculated and untreated tomato plants (negative control). Among different dried powders of mustard plant parts, seeds were the most effective against fusarium wilt disease of tomato. Fresh biomass of tomato plant was recorded 45.25g (mean of all doses) when treated with seeds followed by oil (44.68g), flowers (42.27g) and leaves (39.24g). Stem was found to be least effective and resulted in minimum biomass of tomato plant which was 33.51g. Among different doses, tomato plants treated with oil at the rate of 40 ml/kg soil gave maximum fresh biomass of 55.46g which was the highest fresh biomass after positive control but increasing the dose was phytotoxic to tomato plants and the fresh biomass of tomato plants were decreased to 32.85g when the soil was treated 50 ml/kg oil also decreasing the doses from 40 ml/kg soil the fresh biomass of tomato plants decreased. In soil treated with 30, 20 and 10 ml oil/kg soil, the fresh biomass of tomato plant was recorded as 50.31g, 45.33g and 39.46g respectively. Maximum fresh biomass of 53.42g, 49.35g and 47.58g of tomato plants was recorded when soil was treated with 50 g/kg seeds, flowers and leaves respectively but their effect decreased when the dose was decreased. Seeds, flowers and leaves at 10 g/kg soil, registered the fresh biomass of 37.42g, 36.41g and 33.85g respectively. Tomato plants treated with dried powder of mustard stem resulted in non-significant fresh biomass at all doses. The fresh biomass of 33.55g was recorded for 50 g/kg and 33.28g for 10 g/kg soil.

Table 5: ***Effect of dried powder of different parts of Brassica nigra against Fusarium wilt disease of tomato in screen house studies***

Treatments	AUDPC	Shoot Length (cm)	Root Length (cm)	Fresh Biomass (g)
Inoculated and untreated plant (negative control)	1431.2 A	50.452 N	23.05 R	30.60 U
2g searles fungicide/L medium (positive control).	144.87 V	69.488 C	36.78 A	60.53 A
10g mustard leaves/kg soil	820.07 D	52.67 LM	24.39 Q	33.85 R
20g mustard leaves/kg soil	658.21 E	54.40 JK	25.19 M	34.70 Q

30g mustard leaves/kg soil	557.02 H	59.84 GH	27.19 KL	37.78 N
40g mustard leaves/kg soil	505.05 K	62.62 F	29.29 J	42.31 I
50g mustard leaves/kg soil	383.42 P	65.02 E	31.23 G	47.58 F
10g mustard flowers/kg soil	633.87 F	54.39 JK	24.97 MN	36.41 P
20g mustard flowers/kg soil	563.23 G	55.31 IJ	27.17 L	38.48 M
30g mustard flowers/kg soil	514.26 J	61.64 F	29.54 IJ	41.35 J
40g mustard flowers/kg soil	435.05 N	64.57 E	32.13 F	45.78 G
50g mustard flowers/kg soil	333.70 R	68.74 C	34.02 C	49.35 E
10g mustard stem/kg soil	1134.5 C	51.11 N	24.80 NO	33.28 S
20g mustard stem/kg soil	1133.5 C	50.99 N	24.46 PQ	33.38 S
30g mustard stem/kg soil	1140.6 B	51.32 N	24.56 OPQ	33.74 R
40g mustard stem/kg soil	1141.4 B	51.44 MN	24.78 NOP	33.62 RS
50g mustard stem/kg soil	1139.4 BC	51.54 MN	24.53 OPQ	33.55 RS
10g mustard seeds/kg soil	568.29 G	53.86 KL	25.20 M	37.42 O
20g mustard seeds/kg soil	506.92 K	60.07 G	27.51 K	40.40 K
30g mustard seeds/kg soil	472.36 L	62.45 F	29.99 H	45.57 GH
40g mustard seeds/kg soil	356.61 Q	66.41 D	33.47 D	49.48 E
50g mustard seeds/kg soil	271.34 T	71.12 B	23.33 R	53.42 C
10 ml mustard oil/kg soil	527.67 I	53.89 KL	27.30 KL	39.46 L
20 ml mustard oil/kg soil	462.06 M	56.52 I	29.66 I	45.33 H
30 ml mustard oil/kg soil	397.77 O	58.79 H	32.58 E	50.31 D
40 ml mustard oil/kg soil	326.29 S	73.04 A	36.41 B	55.46 B
50 ml mustard oil/kg soil	240.42 U	53.10 L	23.33 R	32.85 T
CV	0.77	1.68	0.92	0.66
$LSD_{(0.05)}$	5.977	1.239	0.329	0.342

Means followed by different letters are significantly different from one another at 5% level of probability

4. DISCUSSION

Tomato (Lycopersicon esculentum Mill.) is one of the most widely cultivated vegetable crops throughout the world and is consumed in fresh fruit form or in various processed forms (Hariprasad et al., 2009). Tomato plants are susceptible to various diseases caused by fungi, viruses, nematodes and bacteria (Giovanni., 2004). One of the most destructive diseases in tomato is fusarium wilt disease caused by *Fusarium oxysporum* f.sp. lycopersici (Sudhamoy et al., 2009). The major symptoms of the fusarium wilt disease are chlorosis, wilting, stunting and premature death of tomato plants with minimal or zero crop yield. Yield losses due to this disease are 30 to 40% but may increase up to 80% when environmental conditions are favorable (Pandey and Gupta, 2013).

Methyl bromide is used under large scale production of tomatoes to manage the Fusarium wilt disease which has severe adverse effect on the environment and beneficial microorganisms (Santos et al., 2006). Also, continuous application of chemicals may result in developing multi-resistant strains (Njue et al., 2012). This requires searching for alternative ecofriendly ways to manage this disease that are affordable and much more effective.

One promising and best management practice is Biofumigation, which is a process used to suppress the soilborne micro-organisms by releasing biocidal compounds in soil. Glucosinolates, thioglucoside are compounds present in brassica plants, which can easily be hydrolyzed to produce isothiocyantes which has biocidal effect (Kirkegaard and Sarwar, 1998). Smolinska in 2000, reported that Fusarium spp. is sensitive to breakdown products of glucosinolate.

The in-vitro results in the present studies showed that application of Brassica nigra plant shows strong antifungal effect against *F. oxysporum* f.sp. lycopersici. Incorporation of mustard oil at the rate of 20 ml/L to Potato dextrose agar medium was more promising and inhibited the mycelial growth of *F. oxysporum* up to 92.28% followed by 16, 12, 8 and 4 ml/L concentration giving percent mycelial growth inhibition of 83.50%, 65.02%, 53.45% and 35.32% respectively. Similarly, the application of mustard seeds, flowers and leaves showed strong antifungal effect at 20 g/L medium with maximum percent inhibition of 76.84%, 82.22% and 86.08% respectively and minimum percent inhibition of 24.09%, 18.47% and 15.08% respectively at 4 g/L. Stem powder application was found to be least effective and resulted in 37.89% (mean of five doses) percent inhibition. According to Toosi et al., (2013) the antifungal effect of different parts of mustard plant is different because of glucosinolate contents variation. They reported that seeds, flowers, leaf pod, stem and root of Brassica juncea contain 34.8, 30.9, 17.0, 30.1, 2.1 and 4.5 µmol/g Glucosinolate respectively. The above investigation correlated with the previous work of Kedia et al., 2015. He used Brassica nigra oil against Aspergillus niger, Aspergillus ochraceous and Penicillium citrinum and reported that direct contact of mustard oil at 4 µl/ml concentration resulted in 100% fungal growth inhibition. Nielsen and Rios (2000) reported that Brassica juncea resulted considerable percent inhibition (45-68%) against Fusarium monoliforme, Aspergillus niger, Penicillium viridicatum at all doses. Similar results were reported by Nancé et al., 2012 by using all green parts of B. juncea against Rhizoctonia solani, Botrytis cinerea and Fusarium solani which inhibited 100% mycelial growth of all the test fungi at 30 ml/L potato dextrose agar medium.

The screen house experiment showed that soil amended with dried powder of different parts of B. nigra and oil effectively reduced the disease severity and enhance other growth parameters of tomato plants. These results are in line with Gimsing and Kirkegaard (2006), who used different vegetative parts, both fried and fresh, and the seed meal of Brassica plant for pathogen control. Other researchers have reported that mustard plant effectively suppressed many common fungal plant pathogens which included Fusarium spp., Macrophomina phaseolina, Sclertotium rolfsii, Pythium spp., Rhizoctonia solani, Gaeumannomyces graminis and Bipolaris sorokiniana (Chung et al. 2002.; Chung et al. 2003.; Mazzola and Brown 2010).

The suppression of microbial growth is due to the presence of Glucosinolates which are sulfur compounds consisting of sulphonated oxime, a side chain carbon (R-group), and Thio glucose
group (Mayton et al., 1996). Myrosinase enzyme in mustard plant tissues (present endogenously) hydrolyzed glucosinolates and release a wide range of products including nitriles, thiocyanates, oxazolidinethiones and various forms of volatile isothiocyanates which had antimicrobial effect (Gardiner et al., 1999).

Results of screen house experiment in this study showed that the tomato plants treated with dried powders of leaves, flowers, seeds of mustard and oil decreased the disease severity and the recorded AUDPC was 584.75, 496.02, 435.10 and 390.84 respectively at 50 g/kg soil or ml/kg soil and the AUDPC increased with corresponding decreased in the dose. Growth parameters also depended on the plant parts of B. nigra and their applied doses. Seeds, leaves and flowers registered maximum shoot length, root length and fresh biomass at 50 g/kg soil and minimum growth parameters at 10 g/kg soil. Unlike others tomato plants treated with oil give maximum growth parameters at 40 ml/kg soil while above 40 ml/kg doses were phytotoxic to tomato plants and hence the growth parameters were decreased as the soil treated with 50 ml oil/kg soil. Growth parameters were also decreased by decreasing the dose from 40 ml oil/kg soil. Similar findings were reported by Smolinska et al., (1997) that glucosinolate and their hydrolyzed product in B. napus seed meal were highly toxic to fungus *Aphanomyces euteiches* f. sp. pisi. Manici et al., (2000) also reported that damping-off disease caused by Rhizoctonia solani and Pythium irregulare were suppressed in sterile soil when treated with hydrolyzed glucosinolate product in mustard plant. Smolinska and Horbowicz, (1999) showed that chlamydospores of *F. oxysporum* completely lost their viability when exposed to volatile substances from B. juncea. Potter et al., (1998) tested the leaves of six species of Brassica plant against Pratylenchus neglectus for their nematocidal properties. They incorporated the soil

with brassica leaves and found that B. nigra resulted in 56.2% control and B. oxyrrhina result in 95.2% control. Weerakoon and Somaratne (2012) studied the effects of seed meal of B. juncea on the microbial community in the rhizosphere and found that the growth of different organisms was enhanced. They observed that the growth of Mortierella alpine and Trichoderma spp. increased while the growth of Verticillium spp., Mortierella elongata, Cenoccocum geophilum, Cylindrocarpon spp. and Rhinocladiella spp. was increased and hence completely changed the structure of soil microbial community. These few reports showed evidence which supports the anti-microbial capacity of mustard plants.

It is evident from the current study that B. nigra and other Brassica spp. offer potential management tools for ecofriendly control of fusarium wilt and other soil borne diseases of tomato and other related crops. However, the phytotoxic effects of different plant parts on the beneficial microflora such as Trichoderma spp. naturally present in the soil need to be assessed. Moreover, integration of Brassica plant parts with chemical and non-chemical management strategies should also be explored.

5. CONCLUSION

Mustard oil was found to be the most effective in controlling Fusarium wilt disease of tomato when applied @ 40 ml/kg potted soil under screen house conditions. Although more effective in reducing disease severity, the highest dose of 50 ml/kg soil of mustard oil was found to be phytotoxic resulting in suppression of growth parameters including shoot length, root length and fresh biomass. Other plant parts of mustard i.e. seed; leaves and flowers were found to be effective in high doses and effectively control the fusarium wilt disease of tomato and enhanced the tomato growth parameters. Mustard stem powder was observed to be the least effective in reducing disease severity and enhancing tomato growth parameters.

Author Contributions

SU is researcher and author of manuscript. MA supervised study conducted. AZ, DM guided to conduct research in laboratory. HUK, SS, YA helped in formal analysis. MT, TA, F and MUK helped in manuscript writing review and editing.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research

Data Availability

The original data of study is available.

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