

# **Effect of** *Brassica nigra* **extract on growth and physiological activities of** *Solanum lycopersicum* **plant infected with** *Fusarium solani* **fungi**

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# **Abstract**

The present work aims to study the growth alterations, productivity, physiological metabolic activities, and some antioxidant enzymes as a result of chemical antagonism of *Brassica nigra* root extract on tomato plants infected with *F solani* fungus. The extract of *B. nigra* root interfering with the fungus had a favorable impact on percentage of germination and some growth parameters of 20- and 60-day-old tomato plants. Also, it was observed that the total carbohydrates, protein, and chlorophyll a and b increased in tomato plants treated with the extract alone or overlapping with *F. solani* in contrast to the control while antioxidant enzymes upregulated in the case of plants infected with the fungus alone. There was a noticeable raise in the N, P, K, Ca, Mg, and Fe contents of the infected plants treated with the *B. nigra* extract at the age of 90 days. The tomato plant productivity and quality of the fruits treated with the *B. nigra* extract improved, either alone or with the fungus. It was found that *B. nigra* root extract had a positive effect by increasing the growth criteria, physiological activities, growth, productivity, and quality of tomato fruits and also by mitigating the harmful effects of *F. solani* fungus on tomato plants.

**Keywords:** tomato, antioxidant, fusarium, extract, growth

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# **Introduction**

It is possible to find a lot of nutritionally beneficial (carbohydrates, lipids, protein, vitamins, and minerals) and phytochemically beneficial (glucosinolates, isothiocyanates, flavonoids, and phenolics) components in *Brassica* plants (Paul et al., 2019). Seeds, stems, roots, flower buds, and sprouts of these plants have all been ingested and used historically (Cartea and Velasco, 2008; Dejanovic et al., 2021; Kapusta-Duch et al., 2012; Paul et al., 2019). *B. nigra* plant was frequently praised for its beneficial effects on health due to

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its high concentrations of glucosinolates (Wang et al., 2016) and phenolic compounds (Duthie et al., 2000), which promote a variety of physiological processes, including antioxidant and antiinflammatory activity, control the production of enzymes, and participate in apoptosis and cell cycle control (Paul et al., 2019). Allelochemicals used to make natural herbicides and manage a variety of weeds are believed to have fewer negative effects on the environment. Allelochemicals released into the environment may additionally have an effect on various types of plants. For example, glucosinolates can be used to prevent weeds from growing (Fenwick et al., 1983). It is usually not the glucosinolates themselves that exert biological effects, but rather

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the byproducts of enzymatic hydrolyses, such as organic cyanides (CN), oxazolidinethiones (OZT), ionic thiocyanate (SCN-), and isothiocyanates, ITC. (Bangarwa et al., 2011).

Thioglucosidases, also known as "myrosinases," come into contact with glucosinolates. The breakdown of the glucose-sulfur connection allows for rearrangement and the generation of biocidal catabolites such as isothiocyanates (Al-Turki and Dick, 2003; Clarke, 2010; Dai and Lim, 2014; Popova and Morra, 2014, 2017). Plants, nematodes, insects, and fungi are all destroyed by glucosinolates released into the rhizosphere (Larkin and Griffin, 2007; Bressan, et al., 2009; Hopkins et al., 2009; Boydston et al., 2011; Avato et al., 2013; Yu and Morishita, 2014).

Allelopathy is a key ecological phenomenon that affects how productive plants are in agricultural practices and vegetational compositions (Tukey, 1969). This is done by influencing plant growth (Rice, 1984). It is recognized as a self-defense tactic when utilized to help plants protect themselves against invasive insects and other surrounding plants (Lovett, 1991). It has a profound impact on community organization, plant variety, and species' capacity for adaptation (Chou and Lee, 1991). The acetate and shikimic acid pathways produce secondary metabolites known as allelochemicals, and they belong to a number of chemical families of phenolic acids, coumarins, flavonoids, terpenoids, alkaloids, and sulfurides, among others (Jiang, 2018, Narwal and Tauro, 1994)

The mechanism was considered to be driven by unique biochemical interactions between plants. However, the propensity of plant species to produce allelopathy may also injure other living organisms such as bacteria and insects (Maqbool et al., 2013). A recent study in this field has discovered the relevant facts.

Siva (2008) observed that leaf extracts from numerous medicinal herbs increased growth metrics (root and shoot lengths, fresh and dry weights of both root and shoot) on *Solanum melogena* L plants treated with *Fusarium oxysorum* vs *Fusarium* alone treated plants. They also discovered that *Adhatoda vasica*, *Jatropha* 

*curcas*, and *Sapindus emarginatus* leaf extracts inhibited mycelial growth of *Fusarium oxysporum*  f. sp. *Melongenae matuo* and *Ishigami* by 100% in water, ethanol, and acetone, and plant extracts of 17 different angiosperms inhibited mycelial growth by 60% to 98%.

According to Rehman et al. (2019) *Brassica* species produce a wide range of allelochemicals that have a significant impact on target plant growth and development. *Brassica* species produce endogenous plant growth regulators such as brassinosteroids, which are a steroidal chemical family required for plant growth and development (Mandava et al., 1981). Glucosinolates are produced by *Brassica* species and accumulate in healthy plants before being released when the plant is injured (Bagger et al*.,* 1999; Bending and Lincoln 1999; Warton et al., 2001).

The majority of *Brassica* species, according to Mayton et al*.* (1996) possess a mechanism for producing and releasing allelochemicals that are poisonous to pests, illnesses, and their vectors. For instance, allyl isothiocyanate produced from the leaf tissues of *Brassica* species effectively reduced potato dry rot (*Fusarium sambucinum*).

Brassinolide was found by Fuiji et al., (1991) to accelerate ripening and increase grain yield in rice. Brassinolide foliar spray has also been confirmed to boost soybean root nodule formation (Terakado et al., 2005). Similarly, a smaller dose of brassinolide enhanced root nodulation, root length, and pod yield in common bean (*Phaseolus vulgaris* L.) (Upreti and Murti, 2004).

Plant extracts of *Azadirachta indica*, *Atropha belladona*, *Calotropis procera*, *Ocimum basilicum, Eucalyptus amygdalina*, *Ailanthus excelsa*, and *Lantana camera* inhibited *Fusarium* mycelial development (Bansal and Gupta, 2000). *Brassica carinata* and *Brassica calyicina* extracts exhibited high antifungal activity, and the extracts with the strongest inhibitory effect on *Brassica carinata* had a high total phenolic content, acidity, and pH values (Rongai et al., 2015).

According to Fierro et al., (2013), *F. oxysporum* resistance to commercially available fungicides, as well as the frequent use of synthetic fungicide treatments, mandates the development of innovative management strategies.

The *Brassicaceae* family has been identified to have antimicrobial chemicals. All *Brassica rapa* extracts demonstrated dose-dependent antifungal activity at various dosages. Rootderived extract revealed inhibition percentages greater than 45% between 10 and 0.1 g/l. Stemleaf and seed-derived extracts inhibited the enzyme quite well (> 30% and > 35%, respectively) in the same concentration range.

When compared to the untreated control, fourteen plant extracts were efficient in inhibiting the radial growth of *F. oxysporum* f.sp. *lycopersici*, according to Rongai et al., (2015). Extracts from six plant families (*Alliaceae*, *Brassicaceae*, *Lythraceae*, *Lamiaceae*, *Solanaceae*, and *Verbenaceae*) were similarly shown to have excellent antifungal activity against *F. oxysporum* f.sp. *lycopersici*, completely preventing conidial germination.

*Brassica carinata*, according to Avita (2013), contains a high concentration of glucosinolates, which generate cytotoxic compounds with antifungal activity when digested by enzymes. Carbon disulfide, dimethyl disulfide, dimethyl sulfide, and methanethiol, which contain compounds generated during the breakdown of glucosinolates, may play an important role in fungal suppression. *Brunfelsia calycina* is a *Solanaceae* plant that contains alkaloids, flavonoids, saponins, tannins, and glycosides.

Sesquiterpenes substantially inhibit *F. oxysporum* mycelia growth and spore germination, according to Abdelgaleil et al. (2011), and this antifungal effect is based on the permeability of fungus cellular walls. The interactions of sesquiterpenes with the -SH group of amino acids, proteins, and enzymes are also known to be important in their bioactivity. Furthermore, phenolic compounds found in the *Lythraceae*, such as punicalagin and ellagic acid, may be responsible for inhibiting fungal mycelia growth.

The goal of the present study was to use a safe *B. nigra* root extract to reduce the detrimental effects of *F. solani* on the tomato plant.

## **Materials and Methods**

#### **Plant material**

*Brassica nigra*, plants were obtained from the Giza, Egypt, Agricultural Research Center and *Solanum lycopersicum* (Tomato cv. Gawaher Hybrid.) seeds were supplied by Al-Basatin Research Institute, Giza, Egypt. The healthy grains were examined for unity of size and form before being thoroughly cleaned with 2.5% sodium hypochlorite for five minutes and immersed in pure distilled water.

#### **Preparation of aqueous extracts**

*Brassica nigra* root was washed thoroughly, cut into thin slices, and dried in the air for five days until completely dry. It was then ground in an electric grinder into very fine particles, sieved with an No. 80 mesh sieve, and a 7% aqueous extract was prepared (as a result of testing several percentages as a preliminary test) by soaking in distilled water for three days at room temperature using a shaking machine*.* Through a doublelayered filter paper, filtration was conducted (Whatman No.1). In the control treatment, distilled water was used in the experiment for comparison with other treatments.

#### **Fungus**

The fungus strain YMM20 was identified from the Beheira Governorate's agricultural land in Egypt. It was determined to be *F solani* based on its molecular and morphological characteristics (Identifiers of MN960159's GenBank entries). We frequently subcultured on a PDA slant at 28 ℃, pure YMM20 colonies and after that, maintained them at 4 ℃.

#### **Inoculum preparation of YMM20**

*F. solani* YMM20 was grown on PDA Petri plates, and then they were incubated at 28 On PDA Petri plates for five days. Using an isolation loop and sterile deionized water was used in 15 ml the fungal culture was scraped. In order to get the necessary inoculum, supply of  $4.5 \times 10^6$  conidia/ml in suspension, the spores were collected and counted with the aid of a hemocytometer.

Table 1 The employed soil sample's physiochemical criterion



#### **Germination and seedling experiment**

*S. lycopersicum* seedlings were germinated at 23 ± 2  $°C$  in 0.02 m<sup>2</sup> plastic pots containing 780 g of sandy soil disinfected through acid washing. Part of the seeds under study were treated by soaking in *B. nigra* root extract for 24 hours at room temperature and the control seeds were treated by soaking in distilled water for 24 hours.

The seeds treated with the extract were divided into two groups. The first group contained the uninfected seeds (Ext.), and the second group contained the seeds after being infected with *F. solani* (fungus + Ext.), by soaking them in spore suspension for one minute with mechanical vibration. The seeds soaked in distilled water were divided into two sections. The first section was control (C.), and the second section contained the seeds after being infected with *F. solani* (fungus). Each group was grown in a plastic pot including 10 seeds with three replicas irrigated with the same volume of distilled water and kept in the darkness for two days. The seedlings were then exposed to 10:14 hours (light/darkness) photoperiod in the growth chamber. The seeds were collected after 20 days to assay growth parameters and the percentage of germination, radicle and plumule lengths, and fresh and dry weights.

#### **Pot experiment**

In order to achieve realism in the effect within the study (where electrical conductivity of extract of 1:5 soil at 25 °C was 0.71 m mohs  $cm<sup>-1</sup>$  and dirt suspension with a pH of 1:5 was 7.4), sand-clay soil 12 v/v as a clay soil sample was randomly collected from agricultural lands from the surface layer (0- \_35 cm) in the middle of the delta. In addition, the sand was washed with hydrochloric acid and distilled water. The properties of the soil are presented in Table 1.

Five seedlings were sown into the pots after being divided into four groups (Control, Ext., Fungus and

Fungus + Ext). A sample of irrigated soil at a depth of 10 centimeters was weighed, and the weight value was compared to that of completely dry soil from the same mixture as a control, so as to figure out the percentage of the water content of all pots, just prior to the date of irrigation, which was fairly constant. Irrigation was carried out in fixed quantities until the beginning of the saturation level for each pot, with pot places changing daily. The entire growth period was spent without the use of any pesticides, fungicides, or herbicides.

#### **Estimation of photosynthetic pigments**

Fresh leaf weights from the previous sixty days were immediately homogenized and centrifuged for 15 minutes at 3000 XG in 5 ml of 85% cold acetone, before they were stored in a refrigerator for one day. A suitable volume of the acetone extract was diluted at 663, 644, and 452.5 nm, and its color intensity was measured (Metzner et al., 1965). The following equations were applied for calculating the pigment concentration of the experimental plants:

10.3 E663 - 0.918 E 644= chlorophyll (a)

3.87 E633 - 19.7 E 644= chlorophyll (b)

Carotenoids = 4.2 E 452.5 - (0.0264 chlorophyll a + 0.426 chlorophyll b)

In terms of fresh weight, pigment fractions were represented as  $\mu$ g.

#### **Estimation of total carbohydrates and proteins**

Employing a buffer of borate (28.63 g of boric acid, 29.8 g of potassium chloride, and 3.5 g of sodium hydroxide in one liter of filtered water), aliquots (100 mg) of finely powdered 60-day-old tomato plant dry shoot and root were extracted. Before centrifuging for 15 minutes at 3000 xg, the pH was adjusted to 8.0 and allowed to stand for 24 hours at 4 ℃. For the purpose of estimating the amount of polysaccharides, the residue was washed repeatedly and dried at 80 ℃. In order to assay total carbohydrates and proteins, the supernatant and residue washings were collected. The carbohydrate sugars were extracted in a buffer of borate (pH 8) as 0.1 g dry mass 10  $cm^{-3}$  buffers.

Carbohydrate sugars were quantified using (Nelson, 1944), modified in some ways made by (Naguib, 1963). Modifications included the addition of 10 mg of unwet plant matter to a borate salt buffer after extraction, along with 0.2 ml of 0.1 % (w/v) enzyme and 0.1 milliliter of acetate buffer (six milliliters of 0.2 N acetic acid and four milliliters of 0.2 N Na- acetate), made to three milliliters according to (Classics Lowry et al., 1951).

#### **Antioxidant enzymes assays**

To measure antioxidant enzyme activities, 0.5 g of a sample fresh plant leaves were immediately crushed in liquid nitrogen at 4 ℃, and 50 mM cold phosphate buffer was added, 8 ml of which were homogenized with a pH of 7.0 (Beauchamp and Fridovich, 1971). Centrifuging the homogenates at 4000 rpm for 20 minutes separated the mixtures. The supernatant was incubated at -80 ℃ and further utilized as a raw extract for enzymatic analyses.

## *Peroxidase (POX) activity [EC 1. 11. 1. 7]*

The reaction mixture contained 0.5 ml of diluted enzyme, 1.5 ml of 0.05 M pyrogallol, and 0.5 ml of  $1\%$  H<sub>2</sub>O<sub>2</sub>. Thirty second intervals of the 420 nm absorbance variations were recorded for 3 min. The changes in absorbance in terms of min<sup>-1</sup>g<sup>-1</sup> of leaf tissue served as an indicator of enzyme activity (Hammerschmidt et al., 1982).

## *Catalase (CAT) activity [EC 1. 11. 1. 6]*

Initially disappearing rates of  $H_2O_2$  and tetraguaiacol, respectively, were used to test for catalase (Kato and Shimizu, 1987).  $H_2O_2$ 's coefficient of extinction (40 mM  $cm<sup>-1</sup>$  at 240 nm), which is used to determine activity, was used to assess the change in absorbance brought on by the reduction in  $H_2O_2$  at 420 nm.

### *Polyphenol oxidase (PPO) [EC 1. 10. 3. 1]*

According to the methodology outlined by Mayer et al. (1966), a reaction mixture containing 1.5 ml of 0.1 M sodium phosphate buffer and 200 µL of enzyme extract were prepared (pH 7.0). To begin the reaction, 200 µL of 0.01 M catechol were added. Variations in terms of increases in absorbance at 495 nm were recorded at 10 second intervals over the course of 1 minute. Changes in absorbance in gram per minute of leaf tissue were used to express PPO activity.

## **Determination of mineral contents**

The mineral content of roots and shoots of 90-dayold tomatoes were assessed. In order to determine the minerals, the mixed acid-digestion method was utilized, as stated in (Allen et al., 1974). Half a gram of oven-dried plant samples was mixed using 3 ml of hydrogen peroxide and 5 ml of nitric acid. The mixture was heated gently until the whole mixture turned to a clear solution without charring. After that, distilled water was used to dilute the solution to a constant amount (50 ml). The amount of potassium, magnesium, calcium, and iron minerals present in the extract were determined spectrophotometrically through measuring atomic absorption and flame emission (Model Perkin Elmer 2380 Atomic Absorption Spectrophotometer).

## **Determination of nitrogen**

An estimate of the nitrogen content was made following Tetlow and Wilson (1964). The digested samples were titrated against 0.6 N NaOH using few drops of phenolphthalein as an indicator until a faint pink color appeared. Thereafter, 1 ml of phenol-sodium nitroprusside reagent was added and mixed with 1 ml of sodium hydroxide-sodium hypochlorite reagent. A water bath was used to incubate the combination at 37 ℃ for 15 min, which was then diluted to 10 ml, and by using a spectrophotometer, optical density was determined at 630 nm.



Effect of *Brassica nigra* root extract on percentage of germination, fresh and dry weights, and radicle and plumule lengths of 20 day old *Solanum lycopersicon cv* seedlings infected with –ve and +ve *Fusarium solani* fungus



Means that do not share a letter are significantly different; FW: fresh weight, DW: dry weight, Ext: *Brassica nigra* root extract.

#### **Determination of phosphorus**

Using the molybdenum blue technique, phosphorus was measured (following Allen et al. (1974). One ml of the digested samples extract was titrated against 8 N NaOH solution using phenolphthalein indicator until the faint pink color appeared followed by the addition of 1 ml ammonium molybdate reagent (using 400 ml of distilled water, 25 g of ammonium molybdate was dissolved + 280 ml concentrated  $H_2SO_4$ ). Thereafter, 1 ml stannous chloride reagent (0.5 g stannous chloride dissolved in 250 ml 2% v/v HCl) was added and then diluted to 10 ml and left for 30 min. The absorption was measured spectrophotometrically against blank at 700 nm. Using a calibration curve of standard phosphorus solutions, the phosphorus concentration was calculated as mg/g dm.

#### **Productivity and fruit quality**

At physiological maturity, the crop yield was harvested and the yield characteristics were evaluated via measurements for all the treatments including number of fruits per plant, weight of a typical fruit (g), and number of seeds per gram derived as the yield variables. On the other hand, based on the technique developed by Fish et al. (2002), lycopene co tents of tomato fruits were quantified using a spectrophotometer at a wavelength of 503 nm.

#### **Statistical Analysis**

The level of statistical significances between treatments were assessed, and Fisher's individual error rate was used in a one-way analysis of variance (ANOVA) design. The least significant difference test (LSD) was used to compare the means at p<0.05. Using the Minitab software



Fig. I. Effect of *Brassica nigra* root extract on shoot height and root length of 60-day old *Solanum lycopersicon cv* plants infected with –ve and +ve *Fusarium solani* fungus; different letters (a-c) on the bars indicate significant differences according to LSD test ( $P \le 0.05$ ).

version 16, three replicas of each experiment were run.

#### **Results**

#### **Germinating percentage and growth criteria**

Table 2 reveals that the germination percentage of tomato seedlings treated with *B. nigra* root extract was somewhat higher than the control. The interaction of fungus with the extract significantly increased when compared to tomato seedlings infected with fungus alone. When compared to tomato seedlings infected with fungus alone, the interaction of the fungus and *B. nigra* root extract boosted fresh and dry weights and plumule, and radicle lengths.

#### **Pot experiment and growth criteria**

Combined treatments of fungus and *B. nigra* root extract increased shoot height and root length of



Fig. II. Effect of *Brassica nigra* root extract on shoot fresh and dry weights of 60-day old *Solanum lycopersicon cv* plants infected with –ve and +ve *Fusarium solani* fungus; different letters (a-c) on the bars indicate significant differences according to the least significant difference (LSD) test (P≤0.05).



Fig. III. Effect of *Brassica nigra* root extract on root fresh and dry weights of 60-day old *Solanum lycopersicon cv* plants infected with –ve and +ve *Fusarium solani* fungus; different letters (a-c) on the bars indicate significant differences according to the least significant difference (LSD) test (P≤0.05).

the tomato plants when compared to those infected with the fungus alone (Fig. I). The weights of fresh and dried tomato plant shoots and roots were measured using the same observations (Figs. II and III).

#### **Total carbohydrate contents**

Shoot and root total carbohydrates considerably enhanced in tomato plants treated with *B. nigra* root extract compared to controls, with the



Fig. IV. Effect of *Brassica nigra* root extract on shoot and root total carbohydrates of 60-day old *Solanum lycopersicon cv* plants infected with –ve and +ve *Fusarium solani* fungus; different letters (a-d) on the bars indicate significant differences according to the least significant difference (LSD) test (P≤0.05).



Fig. V. Effect of *Brassica nigra* root extract on shoot and root total proteins of 60-day old *Solanum lycopersicon cv* plants infected with –ve and +ve *Fusarium solani* fungus; different letters (a-d) on the bars indicate significant differences according to the least significant difference (LSD) test (P≤0.05).

combination of the fungus and extract having a more noticeable effect than fungus treatment alone (Fig. IV).

#### **Total protein contents**

Tomato plants infected with fungus had significantly suppressive total shoot and root proteins, whereas the interaction of *B. nigra* root extract recovered the harmful effect of fungus on tomato plants (Fig. V). The results were also highly



Fig. VI. Effect of *Brassica nigra* root extract on pigment contents of the leaves of 60-day old *Solanum lycopersicon cv* plants infected with –ve and +ve *Fusarium solani* fungus; different letters (a-d) on the bars indicate significant differences according to the least significant difference (LSD) test (P≤0.05).

visible in plants treated with *B. nigra* root extract without fungus in comparison to the control.

#### **Pigment contents**

Chlorophyl *a* contents rose to a greater amount in tomato plants treated with *B. nigra* root extract than in controls, and the results were more noticeable in the interaction of fungus with extract than in plants infected simply with fungus (Fig. VI). Chlorophyl *b* content of tomato plants treated with *B. nigra* root extraction decreased somewhat when compared to the control; however, the combination of a fungus and extract resulted in a significant increase in chlorophyl *b* content when compared to fungus-infected plants.

Carotenoids were substantially higher in tomato plants infected with fungus versus those treated with *B. nigra* root extract in combination with fungus. Total pigments accumulated more in infected tomato plants treated with *B. nigra* root extract than in untreated plants; however, there was a minor drop in total pigments in tomato plants treated with *B. nigra* root extract compared to control.

#### **Activities of antioxidant enzymes**

Antioxidant enzyme activities (POX, CAT, and PPO) were higher in tomato plants infected with fungus alone as compared with the tomato plants treated with fungus and *B. nigra* root extraction, with an appreciable increase in enzyme activities in



Fig. VII. Effect of *Brassica nigra* root extract on the activities of (a) peroxidase, (b) catalase, and (c) polyphenol oxidase enzymes of the leaves of 60-day old *Solanum lycopersicon cv* plants infected with –ve and +ve *Fusarium solani* fungus. Different letters (a-d) on the bars indicate significant differences according to the LSD test (P≤0.05).

tomato plants treated with extraction compared to control (Fig. VII. A, b, and c).

#### **Mineral contents**

Fig. (VIII) shows that tomato plants treated with *B. nigra* root extraction had a noticeable increase in shoot and root N, P, K, Ca, Mg, and Fe contents when compared to controls. The interaction of fungus with extraction has also recovered the fungus's inhibitory effect on the shoot and root



Fig. VIII. Effect of *Brassica nigra* root extract on the shoot and root mineral contents of 90-day old *Solanum lycopersicon cv* plants infected with –ve and +ve *Fusarium solani* fungus; different letters (a-d) on the bars indicate significant differences according to the LSD test (P≤0.05).

<b>Treatments</b>	Number of fruits/plant ±SD	Average fruit weight $(g)$ ±SD	Number of seeds/g ±SD	Lycopene contents $(mg.100g^{-1} f.w) \pm SD$
Control	$18.67 \pm 0.47$ <sup>A</sup>	46.17 ± 0.39 A	$160 \pm 1.63$ Å	$3.18 \pm 0.05$ <sup>A</sup>
Ext.	$19.33 \pm 0.47$ <sup>A</sup>	47.43 ± 0.33 <sup>B</sup>	$165 \pm 1.70$ <sup>B</sup>	$3.26 \pm 0.01$ B
Fungus	3.33 ± 0.47 $\degree$	$12.10 \pm 0.22$ <sup>D</sup>	$23 \pm 0.82$ <sup>D</sup>	$1.10 \pm 0.01$ <sup>D</sup>
Fungus + Ext.	$11.67 \pm 0.47$ <sup>B</sup>	38.60 ± 0.54 $\textdegree$	112.67 ± 1.25 $C$	$2.51 \pm 0.01$ <sup>C</sup>

Table 3 Effect of *Brassica nigra* root extract on productivity and fruit quality of 90-day old *Solanum lycopersicon cv* plants infected with –ve and +ve *Fusarium solani* fungus.

Means that do not share a letter are significantly different; Ext: *Brassica nigra* root extract.

nitrogen content increasing by 39.4% and root nitrogen content increasing by 19.2%. The shoot phosphorus content grew by 136.2%, whereas the root phosphorus content increased by 34.6%. Shoot potassium content grew by 39.1%, whereas root potassium content increased by 17.7%. The shoot calcium content grew by 140.2%, whereas the root calcium content increased by 25%. The shoot Mg content rose by 75.8%, whereas the root magnesium content increased by 74.9%. The iron content of the shoot grew by 144.4% while the root iron content increased by 200%.

#### **Productivity and fruit quality**

The data in Table 3 revealed a significant increase in average tomato fruit weight, number of seeds per gram, and lycopene content regardless of the number of fruits per plant in tomatoes treated with *B. nigra* root extract compared to the control while the interaction of fungus with *B. nigra* root extraction significantly improved all of the abovementioned productivity and fruit quality without exception.

#### **Discussion**

*Fusarium* wilt is a soil-borne, systemic, and very destructive tomato disease (McGovern 2015; Ramaiah and Garampalli 2015). Using biological management strategies to manage plant diseases is a promising, cost-effective, and ecologically friendlier strategy. Plant growthboosting qualities, as well as direct and indirect antagonism mechanisms are responsible for bio control agents' militancy against plant diseases. According to Mazzola et al. (2012), Narender and Ashok (2014), and Taylor et al. (2014), *Brassicas* are among the plant groups having significant Glucosinolate concentration in their tissues.

Enzymatic hydrolysis of Glucosinolates produces a variety of Sulphur compounds, some of which hav antibacterial properties. If integrated, these agricultural leftovers lower the number of disease propagules in the soil. Some tomato lines exhibit resistance to the wilt pathogen and can thus be used in the integrated use of beneficial organisms and other bio-intensive disease-suppressing practices, which may improve the level and consistency of control by providing multiple mechanisms of action, a more stable rhizosphere community, and efficacy across a wide range of environmental conditions.

Fungi and fungal communities serve critical functions in soil ecology, and the diversity of fungal communities can be inherently hostile to a variety of plant infections (Gao et al., 2012). Previous research revealed that *Brassica* and *Allium* are bioactive plants capable of producing bioactive substances that may be used to reduce soil-borne pests and illnesses (Xiao et al.*,* 2013; Nagla et al., 2015). In contrast, the study found that suppressing *Rhizoctonia solani* with *Brassica napus* seed meal was connected with particular alterations in soil microbial populations and had nothing to do with glucosinolate levels (Cohen et al., 2005)

The interaction of *F solani* with *B. nigra* extraction resulted in significantly higher fresh and dry weights, plumule, and radicle lengths of tomato seedlings when compared to fungus-infected tomato seedlings alone. These findings agreed with many other researchers' findings (El-Marzoky and Abdel-Sattar, 2008; Hassan et al., 2014; Wang et al., 2017c; Jaber and Alananbe, 2018). Lettuce seedlings' root length, competitiveness for sunlight, competitiveness for nutrients in the soil, and moisture content

dramatically enhanced when exposed to low concentrations of goldenrod leaf extracts (Duke et al., 2006, Prithiviraj et al., 2007). The growth performance of the native species that co-occur can therefore be improved by goldenrod with a degree of light invasion. One theory is that the allelochemicals generated by extracts of goldenrod leaves at low concentrations can cause the production of reactive oxygen molecules during plant cell expansion, which in turn has a favorable impact on lettuce seedlings growth and the germination of seeds (Duke et al., 2006, Prithiviraj et al., 2007). *Fusarium* produces significant disruption in growth hormones, resulting in an obvious deficiency in cell biology (Verma et al., 2019; El-Maraghy et al., 2020). Zhao et al. (2022) discovered that grafted seedlings combined with *Allium fistulosum* and *Brassica juncea* had significantly higher dry weights (p<0.05) compared with those planted in untreated replanted soil. Furthermore, plant height, trunk diameter, and branch length were enhanced by 12.72%, 15.42%, 14.67%; 12.06%, 52.88%, and 71.79%, respectively.

The tomato wilt pathogen has a considerable influence on physiological indices of tomato plants (Jamil, 2021). These characteristics were greatly enhanced by *Trichoderma spp.* According to Attia et al. (2022), the first sign of systemic resistance in plants is the use of plant growth-promoting fungi, which reduce the diversity severity percentage while also providing protection against *F. oxysporum*. It can be claimed that an invader with a low degree of invasion can place the native species it coexists with under minimal stress, which will then support the emergence of seedlings and the growth of native species (Ye et al., 2014; Hossain et al., 2016; Wang et al., 2017a; Wang et al., 2017b; Wang et al., 2018). This may be partially linked to hormone influences, which are typically regarded as ecological methods for plant species' responses to outside demand as their main driving force (Takao et al., 2011). According to Attia et al. (2022), tomato plants infected with *F. oxysporum* had a considerable decrease in shoot length (40.4%), root length (85%), and leaf number (50.6%) when compared to healthy control plants. The results showed that extracts of all plant growth-promoting fungus

considerably enhanced different growth indices (shoot length, root length, and the number of leaves). Infected plants, on the other hand, exhibited promising recovery when treated with tested fungal isolates (*A.niger*, *A.flavus*, *M.circinelloides*, and *P.oxalicum*). According to Hung and Lee Rutgers (2016), *Aspergillus spp*. stimulates plant development via producing active chemicals. Another study found that *Aspergillus* isolated from the wheat rhizosphere generated numerous plant growth inducers such as IAA, GA, and siderophores, which resulted in phosphate solubilization and increased seed germination percentage and plant height (Pandya et al., 2018). According to Jovii-Petrovi and Jeremi (2016), *Aspergillus* has a 33% inhibitory rate against *F. oxysporum*.

By treating tomato plants with *B. nigra* root extract, shoot height, root length, and fresh and dry weights increased. The outcomes of this study concurred with those of (Guo et al., 2020) who found that the length of the fava bean roots and its dry weight and plant height considerably increased by the exogenous addition of 0.01 g.mL<sup>-</sup>  $1$  wheat stem and leaf extracts in comparison to the control. These parameters were modestly elevated by the exogenously supplied  $0.05$  g.mL $^{-1}$ wheat stem and leaf extract. Nevertheless, all of the fava bean's growth indicators significantly reduced when  $0.1$  g.mL $^{-1}$  of wheat stem and leaf extract was applied. The potential of these fungal isolates to generate secondary metabolites that boost plant development and stimulate molecules important for the defense was documented by Chand et al. (2020), Ismail et al. (2020), and Hussain et al. (2021).

In this regard, Guo et al. (2020) reported that fava bean dry weight, root dry weight, main root length, and stem dry weight were all significantly boosted when wheat root extract 0.05 g/mL was applied. The plant height, main root length, stem weight, and root length of the fava bean were significantly reduced when wheat root extract concentration was raised to 0.1  $g.mL^{-1}$ , but the other indicators were not significantly affected. *Fusarium oxisporum's* spore germination and mycelial growth were suppressed by the extracts

of fava bean stems, leaves, and roots when used in small doses (Guo et al., 2020)

Comparing the treated group with the untreated control showed that 14 plant extracts were able to inhibit the radial growth of *F. oxysporum* f.sp. *lycopersici*. Alkaloids, flavonoids, and resin in *Rivina humulis*, the known bioactive chemicals that are effective in controlling bacteria and fungi (Joseph and Elvita, 2013).

According to Abdelgaleil et al. (2011), sesquiterpenes considerably reduce the growth of *F. oxysporum's* mycelium and the germination of its spores. This antifungal activity is based on the permeability of fungi's cellular walls.

In the current study, tomato plants with *F. oxysporum* infections displayed a decrease in every growth indicator. This decrease could be the result of the fungus's toxins, which interfere with stomatal function and cause excessive water loss and uncontrolled transpiration, which causes wilted plants. Additionally, a rise in respiration rate and de-compartmentalization brought on by membrane breakdown may be linked to the decline in shoot dry weight (Ahmed et al., 2009). These findings are consistent with those of Hassanein et al. (2008), who discovered that the restriction of growth might also be connected to the buildup and activity of phenolics formed by the breakdown of cell wall lignin, mostly via depolymerization brought on by fungal elicitors.

*B. nigra* root extract enhanced total shoot and root carbohydrates. These results are consistent with those obtained by Couée et al. (2006), who reported that soluble sugars play a vital role in how the body reacts to various stresses by acting as signaling molecules for nutrients and metabolites that activate particular or hormonal transduction pathways and significantly alter gene expression. When compared to non-infected controls, total soluble sugars in the leaves and roots of infected tomato plants treated or untreated with biological agents and/or hormonal elicitors decreased 14 days after pathogen inoculation, and then significantly increased to reach their peak value 42 days after infection (El-Khallal, 2007). Treatment with *Arbuscular mycorrhiza* fungi plus jasmonic acid resulted in the highest levels of root soluble carbohydrates; this may be related to higher levels of jasmonic acid in mycorrhiza roots, which may improve the sinkstrength of mycorrhizal roots and subsequently increase the production of carbohydrates and their movement into the roots from the shoots (Hause et al., 2002). High chlorophyll content and subsequent higher photosynthetic rate of plants treated with *Arbuscular mycorrhiza* and salicylic acid may be related to the elevated levels of soluble sugars in their leaves. These findings show that the buildup of soluble sugars in tomato plants infected with the disease, particularly those that had been exposed to bioagents and hormone elicitors suggest the relation between activation of systemic resistance and sugar regulation. Plant growth-promoting fungi increase carbohydrates, which play a significant role in physiological protection against numerous pathogens by stimulating hormones and other defense mechanisms, resulting in gene expression alterations (Hosain et al., 2017).

Interaction of *B. nigra* root extraction reversed the damaging effect of *F. solani* on the total shoot and root proteins of tomato plants. In this connection El-Khallal (2007) discussed that *F. oxysporum* infection resulted in a considerable decrease in the quantities of total soluble protein and free amino acids in tomato plant leaves and roots. This decrease grew as the pathogen infection period grew longer. When plants are going through natural senescence during *F. oxysporum* attacks, this may speed up the loss of soluble protein. A decrease in energy requirement may be connected to a delay in the synthesis of the primary nitrogenous chemicals in leaves. As a result, it is challenging to predict how the presence of *F. oxysporum* and/or its metabolites would affect the ability of tissues to conserve energy (Nafie, 2003). Total soluble proteins and total carbohydrates in tomatoes decreased dramatically in response to *F. oxysporum* infection, although total phenols and free proline rose significantly (Attia et al., 2022). When compared to control plants, treatment with *Aspergillus niger*, *Aspergillus flavus*, *Mucor circinelloides*, and *Pencillium oxalicum* elicitors increased total soluble proteins and carbohydrates.

El-Khallal (2007) reported that treatment with the jasmonic acid elicitor alone or in combination with the bio agent *Arbuscular mycorrhiza* fungi had been more effective than all treatments in increasing the amount of free amino acids and total soluble proteins in infected tomato plants. Meuriot et al. (2004) suggested that jasmonate may have an effect on N storages such as soluble proteins and specific VSPs, either directly (transcriptional regulation) or indirectly (through changes in N partitioning). In their study on lupine seedlings, El-Bahay and Moursy (2003) found that salicylic acid exerts its effects on the machinery that produces DNA and RNA at transcriptional and/or translocational levels with magnitudes based on how closely nucleic acid and total soluble protein levels are related in response to the compound. According to El-Khallal (2007), *F. oxysporum* infection predominantly affects the growth of tomato plants, which also experience a reduction in N acquisition as a result of mechanisms for controlling negative feedback. Increasing the rate at which additional amino acids are converted reportedly happens at the expense of soluble proteins, which may be the cause of alterations in tomato plants' total soluble protein concentrations harboring pathogens. Finally, the interaction between the biological agent (*Arbuscular mycorrhiza* Fungi) and the hormonestimulating agents (Jasmonic Acid and Salicylic Acid) had been successful in treating the symptoms of wilt disease, which were brought on by the infection's metabolic changes. The stimulation of plant defense systems through protein synthesis is one of the indirect impacts of plant growth-promoting fungi in disease eradication (Dufossé, 2006). According to phytochemistry and cell biology findings, physiological immunity is the outcome of a variety of biological processes, including changes in the cell wall and the production of defense-related chemicals such as phytoalexins and proteins involved in pathogenesis (Elsharkawy et al., 2015).

Chlorophyll b levels in tomato plants treated with *B. nigra* root extract were somewhat lower; however, carotenoid levels were much higher in the plants infected with *F. solani* alone compared to the plants treated with a combination of *B. nigra* root extract and fungus. In this connection,

Akladious et al. (2015) found that when compared to the healthy control, *F. oxysporum* contaminated tomato plants had significantly reduced levels of total photosynthetic pigments and chlorophyll a and b at the vegetative and blooming stages in their leaves. Total photosynthetic pigments and carotenoid contents in the leaves of both diseased and healthy plants increased after the application of *Ocimum basilicum* extract. The maximum rise in total pigment content was seen during the flowering stage of healthy plants treated with *O. basilicum* extract. This could be due to a factor that has been identified as the current rise in leaf area and number. According to Attia et al. (2022), chlorophyll a and b levels reduced dramatically, whereas carotenoids increased in *Fusarium*infected plants. On the other hand, infected tomato plants treated with the plant growthpromoting fungus showed a significant increase in the concentration of photosynthetic pigments. Treatment with *Mucor circinelloides* strain had the greatest effect on chlorophyll concentration (Attia et al., 2022). The loss in chlorophyll was effectively characterized by Kyseláková and Prokopová (2011), who observed that infection may be caused by oxidative stress, which damages chlorophyll a; this implies that the plant fails to capture sunlight, and therefore photosynthesis is limited or blocked. When seed treatment with *Trichoderma viride* was utilized, Jamil (2021) discovered that the maximum significant increase in chlorophyll content was 42.56% (3.36 mg/g fresh leaves) in the first year and 45.70% (3.02 mg/g fresh weight) in the following year. Choudhury and Panda (2005), Jahan et al. (2020), and Singh et al. (2021) explained the decrease in chlorophyll pigments as a result of oxidative stress after injury caused by the release of free radicals, causing damage or distortion in the formation of chloroplasts, and this means the plant's failure or inability to capture light and carry out the process of photosynthesis.

The quantity of all pigments used in photosynthetic processes produced by the plants increased when *B. nigra* extract was added to seeds. The extract's function as a catalyst for the enzymes that regulate photosynthetic carbon reduction and protect chloroplast from oxidative

damage may be the cause of this. β-carotene, flavone, phenol, and vitamins, all recognized components of *B. nigra* extract, play crucial photo protective roles in all photosynthetic species by scavenging ROS. As free radical scavengers, carotenoids serve as energy carriers and photooxidation defenders, preventing the oxidative destruction of chlorophylls and reducing chlorophyll degradation (Abbas and Akladious, 2013). Increased chlorophyll content caused an increase in net photosynthetic rate, which has a direct influence on plant photosynthetic activity. This is consistent with the findings of Harish et al*.* (2008) who discovered that *Trichoderma* promoted plant growth by increasing leaf chlorophyll levels, resulting in greater photosynthetic activity.

The antioxidant enzyme activities (peroxidase, catalase, and polyphenol oxidase) of tomato plants infected with fungus were higher than those of tomato plants treated with *B. nigra* extract. These outcomes align with those that were attained by (Akladious et al., 2015) who found that the activities of the enzymes catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase (GR) were dramatically enhanced by *F. oxysporum* infection during the vegetative and flowering stages. In comparison to CAT and POX activities, the APX and SOD activities were improved more at all treatments. Additionally, soaking the seeds in *O. basilicum* extract prior to sowing significantly boosted the CAT, POX, APX, SOD, and GR activities in both infected and non-infected plants as compared to the control. Infected plants that had received *O. basilicum* extract showed the highest values in these activities. Infected plants had considerably higher levels of oxidative enzymes (POD and PPO) in the study by Attia et al. (2022). Additionally, applying *M. circinelloides* to the infected plants resulted in the greatest rise in POD and PPO activity, followed by *Aspergillus flavus*, *Pencillium oxalicum*, and *Aspergillus niger*. Induced host resistance is shown by increases in the levels of these biochemical and antioxidants (Woo et al., 2014).

When stressed sweet marjoram plants were grown in vegetative rather than flowering stages, Baâtour et al. (2012) detected an improvement in terms of high phenolic contents and antioxidant activities, which indicate a rise in secondary metabolism.

The most prevalent, potent, and water-soluble antioxidant that is not an enzyme, ascorbic acid (vitamin C), works to stop or lessen the harm that ROS causes. In addition to using the APX process to convert  $H_2O_2$  to water, it has the capacity to directly scavenge singlet oxygen, superoxide, and hydroxyl radicals (Abbas and Akladious, 2013). During infection, it is possible to facilitate the species' reactive oxygen metabolism via a number of antioxidant enzymes, including POX and CAT. By facilitating its breakdown into  $O_2$  and H<sub>2</sub>O, catalase can shield the cell from the harmful effects of  $H_2O_2$ . Increased  $H_2O_2$ -scavenging ability and protection from lipid peroxidation are benefits of increased APX activity (Sato et al., 2011). More phenol oxidase enzyme is secreted by the host plant as a defense mechanism when tomato plants are exposed to the pathogen and the antagonist. This could explain why infected plants treated with *O. basilicum* extract showed high levels of enzymatic antioxidant activity (Ojha and Chatterjee, 2012). In addition to direct antagonistic effects, pathogen infection causes the bio control agents to upregulate the activity of a number of defense-related enzymes and molecules against the fungi that cause the damping-off disease. Nahak et al. (2011) found *O. basilicum* essential oil effective, and the stimulatory impact of *O. basilicum* extract on boosting antioxidant enzyme activity in infected tomato plants strongly imply that *F. oxysporum* wilt disease prevention by *O. basilicum* extract involves activating antioxidant defense enzymes (Hanaa et al., 2011). According to Hassan et al. (2014), the use of plant growth-promoting fungus promoted the induction of POD and PPO in *Fusarium*-infected plants. These enzymes generate chemicals that demonstrate the early stages of resistance induction as well as phenolic molecules (Sharma et al., 2019).

In comparison to the control, tomato plants treated with *B. nigra* root extract showed a significant rise in shoot and root N, P, K, Ca, Mg, and Fe contents. Regarding the influence of *B. nigra* extract on mineral contents, El-Khallal (2007) observed that in tomato plants infected with *F. oxysporum* 42 days after introducing the pathogen, the N and P contents were much lower than those of the non-infected control. If root tissues are already being attacked by pathogens, it may impact the tomato tissues' ability to absorb nutrients and water from the soil, alternatively, they could be extracted from macerated tissues. There may be a connection between this and the decreased uptake of N and P in tomato tissues (Nafie, 2003). Last but not least, increased biomass and photosynthetic rate may be associated to high N and P contents, which may increase tomato plants' susceptibility to wilt disease brought on by *F. Oxysporum*.

While *B. nigra* extract and/or *F. solani* fungi may have raised the amount of  $K^+$  in infected plants, this may slow the spread of *F. solani* and its toxins by blocking the transfer of nutrients from the host to the infection (Nafie, 2003). The integrity of cell membranes may be compromised, increasing permeability, and causing leakage from tissues affected by *F. oxysporum*. This might be a result of all infected plants having less  $Ca<sup>2+</sup>$  in them than non-infected plants, which could be the cause (healthy control). According to Orcutt and Nilsen (2000), calcium may obstruct the uptake of toxic ions from the soil when *F. oxysporum* pathogenesis is present. Hollister et al. (2013) discovered that *Brassica juncea* seed meal treatment significantly impacted soil fungus populations and that its effects might last longer than those conferred on the bacterial community.

The average fruit weight, the number of seeds per gram, and the lycopene content of tomatoes treated with *B. nigra* root extract increased significantly when compared to the control. In this regard, *Brassica* residues at a rate of 25 kg per plot, with or without solarization, were found to dramatically lower the incidence of illness and the cucumber root galling index when compared to the control (Al-Abed et al., 2022). The yield for all treatments (cultivated crops) significantly increased in comparison to the control. However, compared to solarization alone or simply bio fumigation, the results showed that under combined treatment of soil solarization and bio fumigation, there was less root galling frequency and disease. Additionally, compared to the other treatments, utilizing bio fumigation in conjunction with solarization significantly increased the production of cucumbers (Al-Abed et al., 2022).

Al-Abed et al. (2022) demonstrated that broccoli, radish, cabbage, and mustard all showed stronger antifungal activity against *F. oxysporum* than cauliflower, which only inhibited the fungus by 57%. Additionally, they demonstrated that the cultivated *Brassica* had better antifungal properties than the wild *Brassica* did. They showed that employing *Brassica* residues brought about a substantial drop in the severity of the disease and a reduction in root galling when compared to the control (10 % application rate increase). On the other hand, the effects of several plant species on the *Meloidogyne incognit* did not differ significantly from one another. Molla et al. (2012) and Hasan et al. (2020) discovered higher lycopene levels in *Fusarium oxysporum* f. sp. *lycopersic*i-infected tomato plants treated with *T. harzianum*.

The effects of soil solarization and bio fumigation with organic amendments applied as sheep manure and cabbage residues had a positive influence on controlling corky root (*Pyrenochaeta lycopersici*) and nematodes infection, reducing the damage on tomato roots (Moura et al., 2010)**.** A better tomato production resulted from the most efficient management of corky root and nematode. More study is required to develop a unique method for bio fumigating plants that contain GLS using dried pellets of plant material (Lazzeri et al., 2004). According to Jamil (2021), the findings on the efficiency of *Trichoderma spp.* in disease suppression and plant growth augmentation against *Fusarium* wilt of tomato are promising. Compared to the untreated control, using *Trichoderma spp.* in either form significantly (p<0.05) improved plant growth and yield indices.

# **Conclusion**

According to the findings of the current study, *B. nigra* contains a strong capability for antioxidants and is applicable to creating innovative use of natural antioxidants to the devastation caused by tomato plant *Fusarium* wilt. Results revealed the positive effects of *B. nigra* root extract on the

harmful effects of *F. oxysporum* on tomato plants by improving some growth parameters, total carbohydrates, and proteins. The content of chlorophyll pigments was increased by using *B. nigra* root extract. It was also found that *B. nigra* root extract can increase the enzymatic antioxidant contents such as peroxidase, catalase, and polyphenol oxidase compared to the control. Furthermore, an increase occurred in mineral content under the *B. nigra* root extract treated or not treated with fungus. The productivity and fruit

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quality of tomato plants were significantly improved.

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