



Role of *Ocimum basilicum* var. *thyrsoiflora* (Thai Basil) Aqueous Extract Treated with Yeast Suspension in Enhancing Tomato Plant Resistance to *Fusarium oxysporum*

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Abstract

Tomato (*Solanum lycopersicum* L.) is the most popular vegetable crop in the world. It is grown almost all over the world. The biggest challenge in producing this crop is diseases caused by *Fusarium oxysporum*. In developing eco-friendly antifungal selective substances for controlling *Fusarium* diseases, the role of *Ocimum basilicum* var. *thyrsoiflora* (Thai basil) aqueous extract against stimulated *F. oxysporum* was studied *in vivo*. The presoaked tomato seeds with yeast-treated and untreated *O. basilicum* var. *thyrsoiflora* extracts were cultivated in *F. oxysporum*-infused soil. This part investigated the recovery role of these botanical extracts against the *Fusarium* infection on tomato plants, especially on its growth and biochemical traits. The research showed that the vegetative and floral growth parameters of plant decreased significantly due to *F. oxysporum* infection. The pigment contents, including carotenoids, β -carotene and lycopene in tomato fruits, were also passively affected by fungus infection at variance to phenolic and flavonoid content. Moreover, the *O. basilicum* var. *thyrsoiflora* extract presoaked seeds remarkably enhanced the growth parameters of plant and the fruit pigment content. There was no significant difference in fungus infection recovery between infected plants that got yeast-treated extract and infected plants that got yeast-untreated extract. However, the pathogen inhibition percentage with extract from yeast-treated Thai basil plants *in vitro* increased. This research showed that utilizing *O. basilicum* var. *thyrsoiflora* extract to control *F. oxysporum* infection of tomato plants was possible and available.

Keywords: eco-friendly antifungal; *Fusarium oxysporum*; *Ocimum basilicum* var. *thyrsoiflora* extract; tomato

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the most widely consumed vegetable crop in Iraq due to the abundance and diversity of its daily consumption process. In 2019, tomato production in Iraq reached approximately 400 tons harvested from 20 ha, the total cultivated area in Iraq (FAO, 2021). Tomato production, especially in greenhouses, faces numerous challenges. Fungal infections are one of them. Fungal infections

highly influence tomato production, especially those caused by *Fusarium* spp. (Fayyadh et al., 2017). *Fusarium oxysporum* f.sp. *lycopersici* is a fungus that specifically infects vascular tissues tomato plants and induces severe wilting leaves by blocking xylem transport and impeding water movement. The lack of water movement leads to plant death or a massive crop production reduction (Duniway, 1971). Most management

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strategies for *Fusarium* diseases by chemical fungicides are harmful to the environment or not practical. Thus, alternative strategies like natural products must be applied to prevent or reduce plant disease effects to achieve more sustainable agriculture (Tleuova et al., 2020). This group of natural products including plant extract has the potential effectivity to contribute to sustainable agriculture. As we know, sustainable agriculture consequently enhances the quality and quantity of crop production which is essential for the food security of the growing population (Godlewska et al., 2021).

The utilization of plant extracts for fungus infection prevention has been investigated in numerous studies. Extracts from 12 plants lead to counteraction activities against *Alternaria alternata* and *Fusarium solani* growth *in vitro* (Lira-De León et al., 2014). The application of foliar spray with *Thymus vulgaris* extracts on tomato plants infected by *Alternaria linariae* stimulates the defense system of plants by increasing the defense activity that relates to enzymes peroxidase, polyphenol oxidase and β -1,3-glucanase (Ahmed et al., 2023). Leaves extracts of tansy (*Tanacetum vulgare* L.), sage (*Salvia officinalis* L.), wormwood (*Artemisia absinthium* L.) and yarrow (*Achillea millefolium* L.) lead counteraction activities against the growth of mycelia. The counteraction depends on the type, extract concentration and fungus species (Kursa et al., 2022).

Thai basil *Ocimum basilicum* var. *thyrsoiflora* from the Lamiaceae family contains lots of Odour-active compounds such as (E)-3-hexen-ol, α -pinene, limonene, γ -terpinene, (E)- β -ocimene, terpinolene, camphor, methyl eugenol, eucalyptol, linalool, thymol, citral and estragole (Łyczko et al., 2020; Sahu et al., 2022). The essential oil of Thai basil lies on all aerial parts of the plant and exhibits antimicrobial and antifungal activities (Avetisyan et al., 2017; Sahu et al., 2024). It makes Thai basil resistant to plant fungal diseases. It also makes the plant resistant to basil downy mildew (BDM). It decreased the susceptibility of plants to BDM infection compared to other *O. basilicum* varieties (Wyenandt et al., 2010). It has been known that biotic and abiotic stress on plants, especially aromatic and medicinal plants, leads to phytoconstituents accumulation alteration. Sahu et al. (2023) find that various agroclimatic regions significantly influence Thai basil essential oil content. Moreover, foliar spray with yeast extract enhances the *O. basilicum* L. volatile oil

percentage. It also increases the percentage of linalool, geranial and neral (Nassar et al., 2015).

In this research, the tomato plant is chosen as a vital model of vegetable crops. *Fusarium oxysporum* is chosen instead of *F. oxysporum* f.sp. *lycopersici*. The plant is observed until it reaches the fruit stage. The extracts of bread yeast treated and untreated *O. basilicum* var. *thyrsoiflora* aqueous are conducted as a nonchemical fungal controller. This research aims to study the controlling efficacy of the yeast-treated and untreated Thai basil extracts on tomato fungus. The study is conducted by observing some of the physiological traits of infected and healthy tomato plants. Some biochemical characteristics in the fruits are also observed to understand better the biochemical changes that occur after the fungus infection and the recovery role of Thai basil extract.

MATERIALS AND METHOD

Preparation of *O. basilicum* var. *thyrsoiflora* aqueous extract

Thai basil plants were cultivated in the Medicinal and Aromatic Unit, Agriculture Collage, University of Basrah greenhouse. The plants were sprayed with 2 g l⁻¹ of bread yeast *Saccharomyces cerevisiae*. Bread yeast-treated and untreated plants were collected at the flowering stage. The plants were washed, dried in the shade at room temperature and ground to fine powder. Then, 50 g of the powder was macerated in 50 ml of distilled water for 48 hours and before being filtered using Whatman no. 1 filter paper. The aqueous extract was stored for further experiments.

Preparation of cultivated soil

Loam soil was sterilized with 5% formalin solution, covered with a polyethylene sheet for seven days and left to dry for two weeks in the open area. Then, 35 cm diameter pots were filled with the sterilized soil and organized into two blocks. Each block had five replicates for different treatments. After that, 3% of the pathogen inoculum was mixed with the potted soil one week before planting.

Tomato plant cultivation and treatments

Tomato seeds (local variety) were obtained from the Agriculture Research Station, University of Basrah. The seeds were divided into three types: 1) 10 hours distilled water-soaked seeds, 2) 10 hours Thai basil extract-soaked seeds and 3) 10 hours bread yeast-treated Thai basil extract-

soaked seeds. Then, all seeds were sown in sterilized soil or *F. oxysporum*-infected soil. Finally, the plants were placed in the nursery, receiving the same service operations.

Pathogen inhibition test *in vitro*

Potato dextrose agar (PDA) medium was prepared by dissolving 39 g of PDA in 1 l of distilled water. Then, 250 mg l⁻¹ of chloramphenicol antibiotic was added to the medium and autoclaved for 20 minutes. Four concentrations of 0%, 50%, 70% and 90% of *O. basilicum* var. *thyrsoflora* extract were added separately to the plates. The *F. oxysporum* was cultured in the plates, triplicates for each extract concentration. The plates were incubated at room temperature for 10 days and the inhibition percentage was recorded by Equation 1.

$$\text{Pathogene inhibition \%} = \frac{(R2-R1)}{R1} \times 100 \quad (1)$$

Where, R2 = Diameter of pathogen growth with extract; R1 = Diameter of pathogen growth with distilled water.

Growth criteria

Plant height, leaf number, length and width of leaf and flower number were measured at the flowering stage of tomato plants. The number of fruits set was measured at the fruiting stage, while fruit biomass was measured after harvesting.

Biochemical criteria

Tomato fruits were dried in the shade at room temperature, grinded to a fine powder and observed to estimate biochemical criteria.

Pigments content

First, 0.25 g of tomato powder was homogenized with 5 ml of cold acetone and filtered with filter paper no. 1. The filtrate was transferred to a separate funnel with the same volume of petroleum ether and water mixture. The two layers were separated and the lower phase was discarded. The upper ether layer was washed 4 to 5 times with water to eliminate residual acetone. Water was removed using anhydrous sodium sulfate, making petroleum ether extract dry at room temperature (Rodriguez-Amaya, 2001). Next, yellow (C^Y) and red (C^R) isochromic carotenoid concentrations were assessed spectrophotometrically in 2.5 ml of acetone at 472 and 508 nm. Total carotenoid (C^T) was obtained from the absorbencies of both isochromic yellow and red carotenoids using Equation 2 and 3 (Hornero-Méndez and Mínguez-Mosquera, 2001).

$$C^Y = \frac{((A472 \times 1724.3) - (A508 \times 2450.1))}{270.9} \quad (2)$$

$$C^R = \frac{((A508 \times 2144.0) - (A472 \times 403.3))}{270.9} \quad (3)$$

Where, $C^T = C^Y + C^R$.

β -carotene was determined according to a method described by Onwuka (2005). Firstly, 200 μ l of distilled water and 0.25 g of tomato powder were placed in appropriate tubes, and 200 μ l of alcoholic KOH was added to the tubes and vortexed for 10 to 20 seconds. Then, tubes were placed in a water bath at 55 °C for 20 minutes chilled to room temperature. After that, 200 μ l of xylene-kerosene mixture was added and vortexed for 30 seconds. The tubes were centrifuged at 600-1000 xg. The supernatant was taken using a constriction micropipette connected to a rubber tube. Then, it was placed in a spectrophotometer cuvette for reading at 460 nm against the blank. β -carotene content was obtained by Equation 4.

$$\beta\text{-Carotene} = (A460) \times 480 \quad (4)$$

Phenolic content

Initially, the hydrophilic and hydrophobic extract from tomato powder was prepared. To obtain a hydrophilic extract, 0.25 g of tomato was homogenized with 2 ml of 50% methanol incubated for 1 hour at room temperature. Then, the powder was centrifuged at 5000 rpm for 12 minutes and the suspension was collected in separate tubes. Then, 2 ml of 70% acetone was added to the residue, incubated for 1 hour at room temperature, and centrifuged at 5000 rpm for 17 minutes. Then, the residue suspension was collected and added to the first suspension. After that, 5 ml of distilled water was added to the previously collected suspension and placed in separate tubes to reach 9 ml final volume. As much as 2 ml of petroleum ether was added to hydrophilic extract residue to obtain hydrophobic extract. It was incubated for 1 hour, centrifuged at 5000 rpm for 12 minutes and the suspension was collected in separate tubes. After repeating a previous procedure, the suspension volume was made up to 9 ml. According to Waterhouse (2002), phenolic content was concluded in the hydrophilic extract of tomato powder, 0.5 ml of hydrophilic extract was added to tubes with 2.5 ml of 10% Folin-Ciocalteu solution. Two milliliters of 4% sodium carbonate solution were added to the tubes and incubated in the dark for 1 hour. The production of blue color was measured

spectrophotometrically at 750 nm. The phenolic content of the samples was calculated using the equation of the straight line obtained from the established gallic acid standard curve.

Flavonoids content

According to Zhishen et al. (1999), the standard flavonoid content in tomato powder was estimated using Quercetin. First, 250 μ l of tomato hydrophilic extract was mixed with 1.25 ml distilled water and 75 μ l of 5% NaNO₂ solution and incubated for 6 minutes. After that, 150 μ l of 10% AlCl₃ solution was added and incubated for 5 minutes. Then, 0.5 ml of 1 M NaOH was added. The total volume was 2.5 ml with distilled water and the absorbance was measured at 510 nm. The total flavonoid content in tomato samples was calculated using the obtained equation of the Quercetin calibration curve.

Antioxidant activity

Antioxidant activity was assessed in hydrophilic and hydrophobic tomato extract (Xiao et al., 2020). At first, 100 μ l of tomato extract was placed in separate tubes. Then 3.9 ml of 0.004% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanol solution (w/v) was added and incubated for 30 minutes in the dark. The absorbance was read spectrophotometrically at 515 nm and the percentage of DPPH scavenging was calculated using Equation 5.

$$\text{Scavenging \%} = (\text{AC}-\text{AM}) \times \frac{100}{\text{AC}} \quad (5)$$

Where, AC = Absorbance of control (100 μ l of 50% methanol and 70% acetone solution + 3.9 ml of DPPH solution); AM = Absorbance of sample.

Experiment design and statistical analysis

The experiment was designed as a completely randomized design (RCBD) in two blocks with five replicates. The results were analyzed using the Graph Prism program. The averages were compared at 0.05 by one-way or two-way analysis of variance (ANOVA) at the probability level.

RESULTS AND DISCUSSION

Growth traits

Figure 1 illustrates the effect of *O. basilicum* var. *thyrsiflora* extract on vegetative growth of healthy and *F. oxysporum*-infected tomato plants. Plant height (Figure 1a), leaf number (Figure 1b), and the length and width of the leaf (Figure 1c and 1d) were markedly reduced due to the infection of *F. oxysporum*. However, the vegetative growth parameters of *O. basilicum* var. *thyrsiflora* extract presoaking tomato seeds

escalate. The infection with *F. oxysporum* significantly reduced the number of flowers (Figure 2a), number of fruits set (Figure 2b) and biomass of fruits per plant (Figure 2c) compared with healthy plants. Plants from seeds soaked with *O. basilicum* var. *thyrsiflora* extract enhanced the vegetative and floral growth compared with infected plants with *F. oxysporum* (Figure 1 and 2).

The reduction in all growth parameters of tomato plants is due to the decreasing chloroplast formation caused by *F. oxysporum* toxins. This case leads to the failure or inability of the plants to capture light and carry out the process of photosynthesis (Chand et al., 2020). Moreover, phenolic accumulation related to fungal elicitors and cell wall degradation reduce growth in tomato plants (Akladios et al., 2015). However, seeds treated with *O. basilicum* var. *thyrsiflora* extract significantly enhanced plant growth. The escalation in plant growth could be explained in several ways, including the antibiotic effect of *O. basilicum* var. *thyrsiflora* extract (Avetisyan et al., 2017). It escalates the toxic resistance and diminishes the fungitoxicity (Akladios et al., 2015). The researchers found that the recovery effect of the yeast-untreated Thai basil extracts was quite significant compared to yeast-treated Thai basil extracts in most growth parameters of tomato plants. Due to yeast treatment, the less effectiveness possibly caused by the enhancement of Thai basil volatile compounds such as Linalool, Estragole, Tau-cadinol and Silanol which inhibits tomato plant growth (Sidorova and Plyuta, 2021).

Biochemical traits

Pigments content

Figure 3 showed a negative influence of *F. oxysporum* infection on total carotenoids, β -carotene and Lycopene content in tomato fruits. The content of total carotenoids in tomato plants soaking seeds with *O. basilicum* var. *thyrsiflora* extract was higher than the other seeds (Figure 3a). Moreover, β -carotene content was affected by *F. oxysporum* in the infected plants. Meanwhile, the soaking seeds with yeast-treated *O. basilicum* var. *thyrsiflora* extract enhanced β -carotene content in treated plants (Figure 3b). The maximum Lycopene content was observed in the healthy plants (control). Then, it was compared to healthy or infected plants that grew from soaked seeds with *O. basilicum* var. *thyrsiflora*. Reducing the leave number, length and width could cause diminishing pigment content. Thus a decrease in photosynthesis.

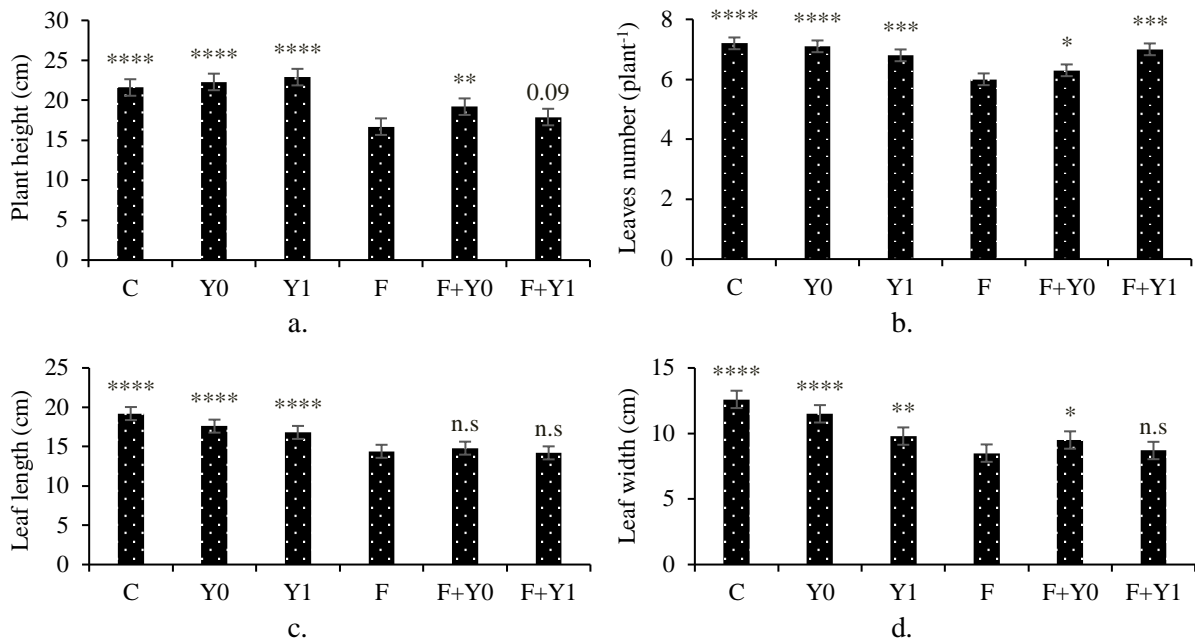
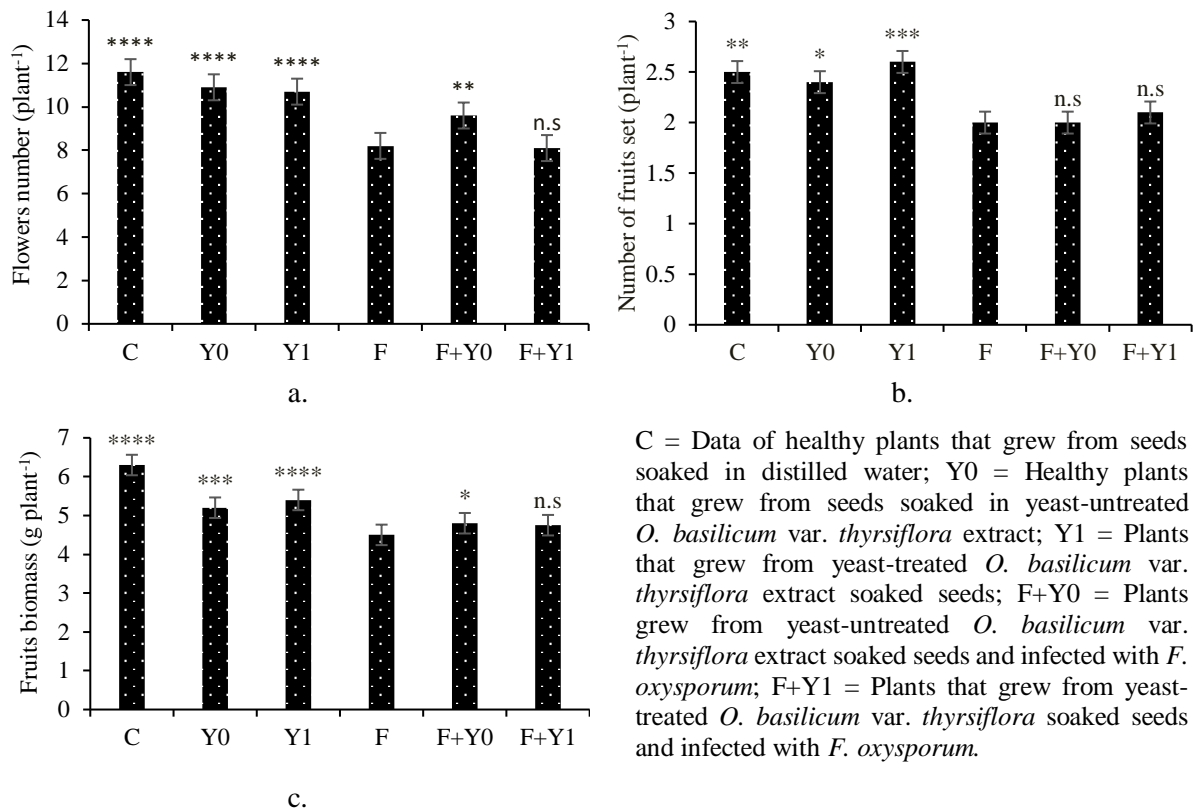


Figure 1. The effect of *O. basilicum* var. *thyrsoiflora* extract on vegetative growth of healthy tomato plants and *F. oxysporum*-infected tomato plants; a) Plant height, b) Number of leaves, c) Leaf length and d) leaf width



C = Data of healthy plants that grew from seeds soaked in distilled water; Y0 = Healthy plants that grew from seeds soaked in yeast-untreated *O. basilicum* var. *thyrsoiflora* extract; Y1 = Plants that grew from yeast-treated *O. basilicum* var. *thyrsoiflora* extract soaked seeds; F+Y0 = Plants grew from yeast-untreated *O. basilicum* var. *thyrsoiflora* extract soaked seeds and infected with *F. oxysporum*; F+Y1 = Plants that grew from yeast-treated *O. basilicum* var. *thyrsoiflora* soaked seeds and infected with *F. oxysporum*.

Figure 2. The effect of *O. basilicum* var. *thyrsoiflora* extract on floral growth of healthy tomato plants and *F. oxysporum*-infected tomato plants; a) Flowers number, b) Number of fruits set, and c) Fruits biomass

Note: A multiple ANOVA is conducted using ordinary one-way ANOVA multiple comparisons in order to compare each treatment with *F. oxysporum* infection treatment. The significance is designated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

The related biosynthetic pathway influenced carotenoid and its isomer formation (Riggi et al., 2008).

Furthermore, resealed transported toxins by fungi relate to the liberation of reactive oxygen species (ROS). It leads to lipid oxidative damage in pigment molecules (Pandhair and Sekhon, 2006). The enhancement of carotenoid content, special β -carotene in tomato fruits that were produced from soaked seeds with yeast-treated *O. basilicum* var. *thyriflora* extract could be concluded as the protective role of the extract in the reduction of fungus toxin, enzymes activation that regulate photosynthetic carbon reduction and protect pigments from oxidative damage (Akladios et al., 2015).

Phenolic and flavonoid content

The phenolic and flavonoid content in tomato fruits of *F. oxysporum* infected sample was higher than the control and the plants produced from seed soaked with *O. basilicum* var. *thyriflora* extract (Figure 4a and 4b). However, the fruits of presoaked seeds with *O. basilicum* var. *thyriflora* extract plants indicated the phenolic content

degradation (Figure 3a). The remarkable increase of the phenolic and flavonoid content to fungus infection is a natural reaction of the plant to the biotic stress. Under biotic and abiotic stress, the plant releases antioxidant compounds to act as ROS suppressers or ROS scavengers, which consequently protect its cell membranes from their oxidative properties (Mona et al., 2017). Phenolic compounds are potential molecular mechanisms that protect plants from oxidative damage (Wallis and Galarneau, 2020).

Antioxidant activity

Hydrophilic extract of tomato fruits from *F. oxysporum* infected plants stimulates higher DPPH scavenging activity than the rest (Figure 3c). The increasing scavenging proportion was caused by the increasing of phenols and flavonoids content in the infected plant fruits. This phenomenon showed the efficacious DPPH scavenging activity against free radicals (Guo et al., 2018). Meanwhile, the hydrophobic extract of tomato fruits of healthy and treated plants showed no significant differences (data not shown).

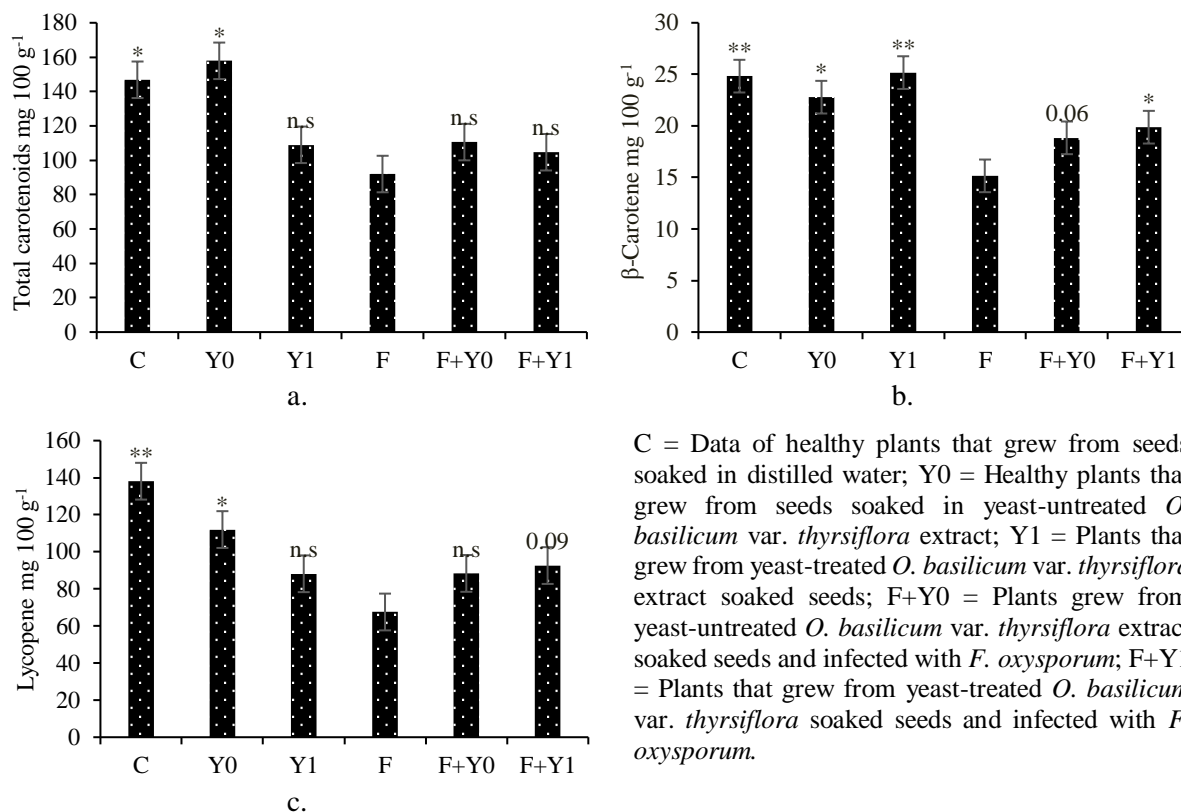
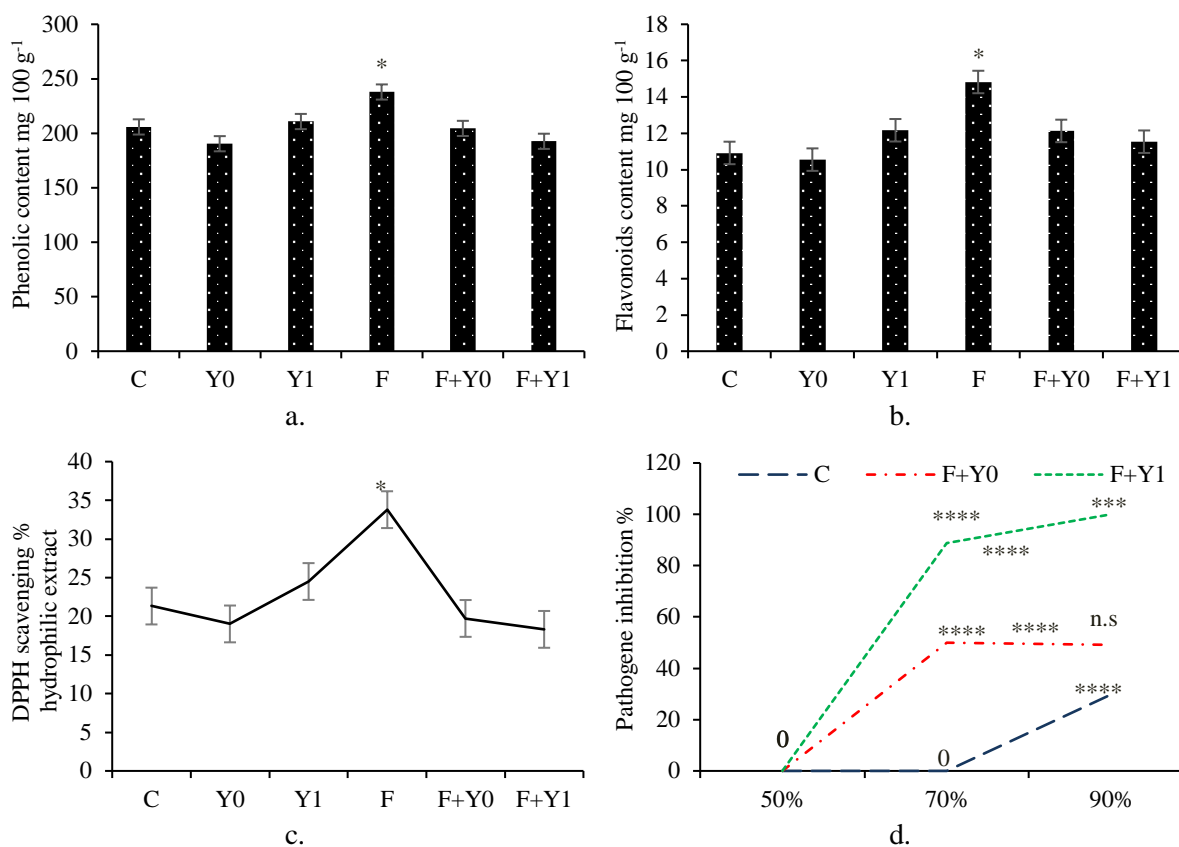


Figure 3. The effect of *O. basilicum* var. *thyriflora* extract on pigments content: (a) Total carotenoids, (b) β -carotene, and (c) Lycopene (mg 100 g⁻¹ dry weight (DW)) of tomato fruits of healthy plants and *F. oxysporum*-infected plants

Note: A multiple ANOVA is conducted using ordinary one-way ANOVA multiple comparisons in order to compare each treatment with *F. oxysporum* infection treatment. The significance is designated as follows: * $p < 0.05$, ** $p < 0.01$



C = Data of healthy plants that grew from seeds soaked in distilled water; Y0 = Healthy plants that grew from seeds soaked in yeast-untreated *O. basilicum* var. *thyrsoiflora* extract; Y1 = Plants that grew from yeast-treated *O. basilicum* var. *thyrsoiflora* extract soaked seeds; F+Y0 = Plants grew from yeast-untreated *O. basilicum* var. *thyrsoiflora* extract soaked seeds and infected with *F. oxysporum*; F+Y1 = Plants that grew from yeast-treated *O. basilicum* var. *thyrsoiflora* soaked seeds and infected with *F. oxysporum*

Figure 4. The effect of *O. basilicum* var. *thyrsoiflora* extract on: (a) Phenolic content, (b) Flavonoids content (mg 100 g⁻¹ dry weight (DW)) of tomato fruits, (c) Percentage of DPPH scavenging of healthy plants, and (d) Representative the inhibition effect of *O. basilicum* var. *thyrsoiflora* extract on *F. oxysporum* *in vitro*

Note: A multiple ANOVA is conducted using ordinary one-way ANOVA multiple comparisons in order to compare each treatment with *F. oxysporum* infection treatment. The significance is designated as follows: * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$

Pathogen inhibition test *in vitro*

Figure 4d illustrates the percentage of pathogen inhibition with botanical extract *in vitro*. The increasing inhibition proportion with Thai basil extract in the pattern was obtained. Aqueous extract from yeast-treated Thai basil created inhibition activity in the 50%, 70% and 90% concentrations. Meanwhile, the yeast-untreated plant extract is only active in 70% and 90% concentrations. The excessive phytochemicals in Thai basil extract prove its remarkable antifungal potency (Avetisyan et al., 2017).

Moreover, the 90% extract of yeast-treated Thai basil showed total inhibition against fungal growth. This 100% inhibition is connected to enhancing active component linalool with biotic stress (yeast infection) (Nassar et al., 2015), which

massively contributes to higher suppression of fungus growth. Unfortunately, not as expected, both of the extracts *in vivo* brought no different effects. This result is possibly affected by the metabolism processes of the plant and the uncontrolled conditions of plant cultivation in the field (Wens and Geuens, 2022).

The application of plant extract as a biological control for pests and diseases is important since the results of these applied studies parallel sustainable agriculture trends (Sales et al., 2016; Ngegba et al., 2022). Plant extract utilization in the plant protection sector has several advantages, such as reducing toxicity to humans and the environment, enhancing the resistance of cultivated plants to stress, increasing the quality and the quantity of crops, and reducing the use of

chemical fertilizers and pesticides (Godlewska et al., 2021). The promising result of the current study promotes the possibility of utilizing botanical extract as an alternative fungicide against *Fusarium* fungus that infected tomatoes and other crops. The *O. basilicum* var. *thyrsoiflora* is easy to cultivate since it can grow in warm and temperate areas. Moreover, obtaining a crude extract from a Thai plant for pest control is cheap and straightforward. It can be extracted by water maceration or distillation (Umerie et al., 1998; Akladios et al., 2015). The practical study of Thai basil extract application as a biological pesticide is still a special demand in sustainable agriculture. Although the essential oil of *O. basilicum* has a broad spectrum of activity and seems a typical candidate for fruit and vegetable preserving (Li and Chang, 2016; Kačániová et al., 2022), the investigation of long-term effects of Thai basil extract on plant health and potential side effects is required.

CONCLUSIONS

O. basilicum var. *thyrsoiflora* extract has good antifungal potency to control *F. oxysporum* infection in tomato plants. Presoaking tomato seeds with Thai basil extract recovered the passive impact of *Fusarium* infection on tomato plants. It also enhanced the physical and chemical parameters of tomato fruits. The pathogen inhibition test *in vitro* showed high activity of the extract against fungi from yeast-treated plants, which displayed complete pathogen inhibition proportion. Thus, the researchers recommend conducting a comparative study between chemical fusariumicides and Thai basil extract to better understand both effect differences on infected plants in terms of growth, recovery, taste and phytochemicals content.

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