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Journal of Food and Nutrition
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ISSN: 2836-2276 RESEARCH ARTICLE

Potential Use of Wild Legume Plant Exudates for the Prevention of Fusarium Wilt Disease (*Fusarium oxysporum*) in Chickpea (Cicer arietinum L.)

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Received Date: 16 August 2023; **Accepted Date:** 04 September 2023; **Published date:** 12 February 2024

Citation: Ozlem Gulmez, Meryem Sengul Koseoglu, Faik Kantar, Omer Faruk Algur. (2024). Potential Use of Wild Legume Plant Exudates for the Prevention of Fusarium Wilt Disease (*Fusarium oxysporum*) in Chickpea (Cicer arietinum L.). Journal of Food and Nutrition. 3 (1); DOI: 10.58489/2836-2276/021

Abstract

The aim of this study was to determine the potential of using wild legume exudates as a biocontrol agent against Fusarium oxysporium which causes wilt in chickpea plants. For this purpose, wild leguminous plants from Erzurum were collected and their exudates were obtained. From wild legume plants from which exudates were obtained in the study, belonging to the genus Onobrychis which It was determined that two of them are endemic and the other three are cosmopolitan species. Chickpea plant was grown in Green house and the plants were infected with *F.oxysporium*. Legume exudates were applied to the infected plants. The data like root stem length, were measured root stem length, chlorophyll, carotenoid, and lipid peroxidation amounts were measured. In addition, antioxidant enzymes were examined.

The result of our finding concluded that exudated from wild legume has been concluded that exudates obtained from wild legume plants can be used as a non-toxic and economically viable fungicide against *F.oxysporium*. In addition, the results ındıcated that both endemic and cosmopolitan Onobrychis exudates can be used as fungicides against *F.oxysporium* in chickpea, which is a protein source in developing countries and is widely consumed in the world, and this is the first study for these species.

Keywords: *Exudate, Fusairium wilt, fungucide, Wild legume*

Introduction

Chickpea is a legume that has been produced and consumed in almost every part of the world since ancient times. Due to its protein content, rich in iron, calcium and other minerals, it is consumed as the main food source of people in developing countries. It is the third most cultivated agricultural plant in the world and Turkey ranks third after India and Pakistan in chickpea cultivation. With the changing climatic conditions and increasing population, the demand for human food and animal feed will increased. This result, in the importance of chickpeas in econmic value as a nurtient and its use as various the importance of legumes such as chickpeas which the economic value of chickpeas will increase in the near future (Meshram et al. 2018; Merga and Haji 2019; Basbagcı et al.2019; Yousef et al. 2020;).

Chickpea production is adversely affected by biotic and abiotic factors. Among factors fungal disease can cause econmic loss in chickpea production. Fungal pathogens prevent the growth and development of the plant by penetrating the vascular tissue of chickpea roots, restricting the transport of water and nutrients to the leaves. Fusarium wilt is one of the most important fungal diseases that cause serious losses in chickpea production. (Kumar et al. 2017; Suthar et al., 2017; Meshram et al., 2018).

Fusarium is the largest filamentous fungus genus common in agricultural areas that causes wilt disease (Khanzada et al., 2016). Many methods have been used to control plant pathogenic fungi. Fungicide applications have proven effective, but they are toxic to humans and other organisms. In addition, it is known that pathogenic fungi develop resistance to fungicides over time (Yoon et al., 2013; Pandey 2015). Therefore, environmentally friendly

approaches such as the use of plant exudates and extracts have been sought and used in recent years (Gurjar et al., 2012). In previous studies, it has been reported that plant extracts used against plant pathogenic diseases reduce the incidence of disease. (Sena et al., 2013; Pandey 2015; Khanzada et al., 2016).

Exudate; is a secretion formed by organic and inorganic molecules released from plant roots (Lica et al. 2018). It is known that root secretions, namely exudates, increase the stability of the soil and provide microbial resistance to plants.(Naveed et al. 2017). Exudates vary considerably between agricultural plants and wild races. This difference gives wild breeds an advantage against pathogenic organisms and other abiotic stresses (Preece, and Peñuelas 2020). In line with this information, it was aimed to determine the potential of using wild legume exudates as a biocontrol agent against *F. oxysporium*, which causes wilt in chickpea plants.

Material Method

Collection and Identification of Wild Legumes

Wild leguminous plants were collected from Erzurum province during the flowering period (June to July) and brought to the laboratory in sterile bags. The collected plants were identified.

Obtaining plant exudates

The plants were washed under the tap until the mud and soil were gone, and then washed 3 times with sterile distilled water. The plant samples were then placed in jars containing 1 liter of sterilized distilled water (20 plants in each jar) and sealed with sterile filter papers. These prepared containers were kept at room temperature for 21 days in a place out of direct light. At the end of the process, the plants were taken from the jars, the lids of the jars were closed with sterile lids, and the obtained exudates were kept at +4°C until used.

Growing chickpeas

Chickpea was used in the study Akçin 91; It was obtained from the Eastern Anatolia Agricultural Research Institute. The seeds were washed briefly with 96% alcohol and surface sterilized in 5% Sodium Hypochlorite for 5 minutes, then the seeds were rinsed three times with distilled water and allowed to swell for 5 hours in sterile distilled water. the swollen seeds were then germinated under sterile conditions and then planted in 1 L pots filled with sterilized sand. Germinated seedlings were grown on soil/vermiculite (3:1, v/v) at 25 °C in an environment-controlled chamber at a light intensity of 120 µmol photons m−2 s−1 and a 14/10 h light/dark photoperiod.

Development of *F.oxysporum* and its application to plants

F. oxysporium culture inoculated on Potato Dextrose Agar (PDA) was incubated at 28 C for 7 days. A prepared 5 ml spore suspension was then placed in 100 ml potato dextrose broth (PDB) in 250 Erlenmeyer and incubated at 28°C at 180 rpm for 96 hours. For each pot, 10 ml of mycelium was placed in 10 ml of isotonic water and the micelles were broken up with micelles. Then it was applied to the roots of 5-day old chickpea seedlings. 24 hours after mycelial application, legume exudates were applied to seedling roots at a rate of 50 ml per pot. After the mycelial applications, the seedlings were irrigated with sterile tap water for 10 days. On day 15, the plants were harvested, root, stem lengths, and weights were taken, and the plant parts were stored at -18 °C for further analysis after lyophilization in liquid nitrogen**.**

Determination of lipid peroxidation and antioxidant enzyme activity

Lipid peroxidation was determined by Heath and Packer (1968) method, to determine the activities of antioxidant enzymes, fresh leaves (0.5 g) were ground with a mortar and pestle under chilled conditions in the presence of phosphate buffer (0.1 M, pH 7.5) containing 0.5 mM EDTA. The homogenate was centrifuged at 12,000 g for 10 min at 4 ˚C, and the resulting supernatant was used for the enzyme assay. SOD activity was assayed using the method of Agarwal and Pandey 2004. POX activity was measured according to the method of Zhang and Kirkham (1994). CAT activity was performed according to Qiu et al. (2011.)

Determination of photosynthetic pigments

Fresh leaf tissues (0.1 g) were homogenized in chilled 80 % (v/v) acetone. The homogenate was centrifuged at 8800 g for 10 min at 4 ˚C in dark. The absorbance of the acetone extract was measured at 663, 645, and 470 nm using a spectrometer (Shimadzu UV mini-1240). The contents of chlorophyll a, chlorophyll b, and total carotenoids were calculated according to Arnon 1949.

Statistical analysis

All experiments were performed 6 times and the average of values was presented. The data were analyzed by analysis of variance, and means were compared by using Duncan's Multiple Range Test at $p < 0.05$ significance level.

Results

In the study, the plants obtained from exudates and used against F.oxysporium were diagnosed in the Department of Biology of Atatürk University. According to the diagnostic results, it was determined that two of the plants from which exudates were obtained were endemic and the other three were cosmopolitan species (Table 1).

Table 1: Scientific names, distribution and codes of the plants from which exudates are obtained

Codes	Scientific names	Distribution
O1	*Onobrychis cornuta (L.) Desv.*	Cosmopolitan
O2	*Onobrychis montana*	Cosmopolitan
O3	*Onobrychis stenostochya sups. stenostochya*	Endemic
O4	*Onobrychis ornata* (Wild.) Desv.	Endemic
O5	*Onobrychis sativa*	Cosmopolitan

Effect of Exudates on Chickpea Growth

It has been determined that wild legume exudates applied as a curative against *F.oxysporium*, which causes wilt in chickpea plants, increase the root lengths by approximately 14% to 53% compared to *F.oxysporium*. For body lengths, equivalent lengths were measured with the control. At the same time, it was found that the amount of wet weight and dry matter decreased in F.oxypsorium application and increased in exudate applications (Table 2).

Table 2: Root length, stem length, fresh weight, and plant dry matter of chickpea after treatments with Fusarium wilt disease and wild legume plant exudates

Applications	Root Length	Stem Length	Fresh weight	Dry matters (%)
Control	15,63±0,23d	32±0,15a	2.2 ± 0,20d	10,9±0,03e
F.oxysporium	13,13±0,08f	26,83±0,22d	0,83±0,03g	12,0±0,02a
***F.oxysporium*+O1**	20±0,1a	32 ± 0,5 a	1,65±0,50f	10,9±0,06b
***F.oxysporium*+O2**	18,23±0,12b	32±0,0a	2,03±0,25e	10,8±0,12b
***F.oxysporium*+O3**	15 ± 0,14 e	30,33±0,33c	2,09±0,37e	11,0 ± 0 b
***F.oxysporium*+O4**	16,76±0,05c	30 ± 0,17 c	2.39±0,00b	10,0±0,023d
***F.oxysporium*+O5**	18,12±0,38b	31 ± 0,2 b	1,99±0,00e	9,5 ± 0 d

Effects on Leaf Pigment

Application of wild legume exudates significantly (P<0,05) increased leaf total chlorophyll content (mg/ g tissue), leaf total carotinoide content (mg/ g tissue) (Table 3). Total chlorophyll content was higher in all treatments (2,13 mg/ g tissue in *F.oxysporium*+ O1 and 3,54 in *F.oxysporium* +O2 treatment) including control than only F.oxysporium applied treatment (1,93 mg/ g tissue) (Table 3). Total Carotinoide content was also higher in all treatments (8,35 in *F.oxysporium* +O2) including control (5,43 mg/ g tissue) than only *F. oxysporium* applied *F.oxysporium* +O2 treatment (4,66 mg/ g tissue) (Table 3).

Table 3: Chlorophyll and Carotinoide contents

Applications	Total Chlorophyll Content (mg/ g tissue)	Total Carotinoide Content (mg/ g tissue)
Control	2,40 ± 0,01 e	5,43 ± 0,05 f
F.oxysporium	1,93 ± 0,04 g	4,66± 0,024 **i**
***F.oxysporium*+O1**	2,13 ± 0,02 f	5,17 ± 0,017 h
***F.oxysporium*+O2**	3,54 ± 0,05 a	8,35 ± 0,01 a
***F.oxysporium*+O3**	2,94 ± 0,04 b	7,00 ± 0 b
***F.oxysporium*+O4**	2,53 ± 0,03 d	5,91 ± 0,05 e
***F.oxysporium*+O5**	2,24 ± 0,01 f	5,31± 0,03 g

Effect on Lipid peroksidasyon (LPO)

In *F.oxysporium* application, the amount of LPO increased in both root and stem. In almost all exudate applications, LPO levels close to the control were measured. This shows that the applied exudates are an effective agent against wilt (Table 4).

Table 4: Lipid peroxidation (LPO) of roots and stems of chickpea after treatments with Fusarium wilt disease and wild legume plant exudates

Application	LPO (nmol. g^{-1} tissue) Root	LPO (nmol. g^{-1} tissue) Stem
Control	1,26 ± 0,24 b	1,25 ± 0 c
F.oxysporium	**2,00 ± 0,03 a**	**2,12 ± 0,08 a**
***F.oxysporium*+O1**	1,43 ± 0,015 de	1,36 ± 0,05 b
***F.oxysporium*+O2**	1,35 ± 0,015 c	1,37 ± 0,02 b
***F.oxysporium*+O3**	**1,27 ± 0,02 b**	**0,84 ± 0 e**
***F.oxysporium*+O4**	**1,25 ± 0 b**	**1,00 ± 0,03 d**
***F.oxysporium*+O5**	1,39 ± 0,09 d	1,27 ± 0,16 c

Effects on Enzyme Activity

In the study, antioxidant enzymes in the root and stem were examined separately. Although CAT enzyme activity was higher in *F.oxysporium* application compared to control, it was observed that it could not cope with wilt, which is the abiotic stress factor. CAT enzyme activities increased in both root and stem in all exudate applications. However, the highest activity was observed in O3 (Onobrychis stenostochya) exudate application (Table 5).

Table 5: CAT enzyme activity

Application	CAT (U.mg^{-1} protein) Root	CAT (U.mg^{-1} protein) Stem
Control	2.11±0 g	1.45 ± 0 f
F.oxysporium	3.73±0,36f	2.23 ± 0,08 e
***F.oxysporium*+O1**	5.86± 0,27c	3.57 ± 0,05 c
***F.oxysporium*+O2**	6.85±0,5b	3.4 ± 0,02 c
***F.oxysporium*+O3**	**8.6 ±0,5a**	**4.27± 0 a**
***F.oxysporium*+O4**	5.21±0,2d	3.78 ± 0,03 b
***F.oxysporium*+O5**	4.75 ±0,12e	2.67± 0,16 d

SOD enzyme, which is one of the most important enzymes in the fight against stress factors, decreased in both root and stem in F.oxysporium application. In exudate applications applied as a curative, the highest O5 (Onobrychis sativa) application in the root, and in the stem; It was detected in O2 (Onobrychis montana) application (Table6).

Table 6: SOD enzyme activity

Applications	SOD (U.mg^{-1} protein) Root	SOD (U.mg^{-1} protein) Stem
Control	17,76 ± 0,23 e	15,14 ± 0,07 d
F.oxysporium	12.84± 0,06 f	13,4 ± 0,018 e
***F.oxysporium*+O1**	21 ± 0,25 d	17,91 ± 0,05 b
***F.oxysporium*+O2**	22,30 ± 0,04 c	**19,5 ± 0,02 a**
***F.oxysporium*+O3**	21,16 ± 0,05 d	16,00 ± 0,06 c
***F.oxysporium*+O4**	23,90 ± 0,01 b	11,00 ± 0,05 f
***F.oxysporium*+O5**	**27,60 ± 0,3 a**	16,3 ± 0,15 c

Another enzyme that increases stress tolerance, POX; It increased in all applications, including F:oxysporum application (Table 7).

Table 7: POX enzyme activity

Applications	POX (U.mg^{-1} protein) Root	POX (U.mg^{-1} protein) Stem
Control	2843± 2,64 g	4910 ± 4,00 f
F.oxysporium	7878 ± 2,0 c	5287 ± 1,42 e
***F.oxysporium*+O1**	6124 ± 2 e	9412 ± 2,12 b
***F.oxysporium*+O2**	4747± 3,45 f	5590 ± 2,82 d
***F.oxysporium*+O3**	6539 ± 5 d	11119 ± 1,5 a
***F.oxysporium*+O4**	9871 ± 0,0 b	5591 ± 3,50 d
***F.oxysporium*+O5**	11151 ± 5 a	6955 ± 2,82 c

Discussion

F.oxysporium, which prevents legumes production worldwide and causes great economic losses, is difficult to control because it has the ability to survive for a long time even in the **soil** (Lv et al.2020). Many methods have been used to combat Fusarium wilt. Some of these are growing resistant plant species (banana, cucumber, tobacco) and the use of chemical fungicides. These methods are not economical and the chemicals used accumulate in plants and soil and adversely affect human health. In recent years, plant exudates with natural strong fungicidal effects have been used against fusarium wilt instead of chemical fungicides (Zuo et al.2015).

Plant affected by different pathogenic fungi and insect pests which reduce yield. These include pathogenic fungi, bacteria, viruses, nematodes, and arthropods. Especially pathogenic organisms affect plant growth and health (Baetz and Enrico Martinoia 2014). It also causes product loss economically. Plants naturally have self-defense mechanisms against pathogens with root secretions (Xu et al.2015). Root exudates contain primary metabolites (sugars, amino acids) and secondary metabolites, phenolic compounds, terpenoids, aromatic acids and enzymes. These compounds have antibacterial and antifungal effects (Xiao et al. 2013; Lambordi et al. 2018; Lv et al. 2020). *F. oxysporum* includes numerous strains that cause vascular wilt diseases and damage economically important crops worldwide (Gordon 2017). Root, stem length and wet dry weight, which are the first parameters to be checked in plant pathogen applications. In a study, they stated that the root stem lengths decreased in *F.oxysporium* applications in cucumber plants (chen et al. 2019). Root stem lengths and wet-dry weights measured in this study also decreased in *F.oxysporium* applications. It was observed that these rates increased in wild legume applications (Table 2). It has been reported in various studies that it reduces the amount of chlorophyll in plants exposed to pathogen attacks (Baghbani et al. (2019)). In our study, the amount of chlorophyll in the chickpea plant exposed to the pathogen decreased significantly compared to the control. Chlorophyll and carotenoid amounts increased in plant exudate applications with *F.oxysporium* (Table 3). *F.oxysporium*, which is a pathogen in many plants, increases H_2O_2 reactive oxygen species and lipid peroxidation in plants. As it is known, ROS cause disruption of cellular metabolism and DNA damage. In addition, the increase in the amount of ROS is accepted as an indicator of the presence of stress factors in biological organisms. (Kunstler et al. 2015; Camejo et al. 2016; Sewelam et al. 2016). Plants, like many organisms, have defense mechanisms to cope with these stress factors. Catalase (CAT) and peroxidase (POX) enzyme and superoxide enzyme that breaks down superoxide anion are the main elements of this defense mechanism (Jayamohan et al. 2018). Fusarium wilt is one of the major biotic stresses that reduce chickpea yield. In this study, *F.oxysporium* applied to the chickpea plant caused stress and significantly increased the LPO amount in both root and stem compared to the control. The plant extracts we use to combat pathogens have somewhat reduced the amount of LPO. especially *F.oxysporium*+O3 and *F.oxysporium*+O4 applications brought this rate to the level of the control group.(Table 1). Considering the LPO amounts, the antioxidant enzyme system elements SOD, CAT and Pox amounts; There is a significant increase in the amount of *F.oxysporium* applied plants and plants in the control group (Tables 5, 6 and 7). The applied plant exudates contributed to the defense system of the chickpea plant with the primary and secondary metabolites they contained and gained resistance against *F.oxysporium*. Were et al. (2021), in their study, reported that they inhibited the development of F.oxysporim by using the exudates of 2 types of leguminous plants, and they determined that this feature was the phenolic substances in the exudates. Many studies have reported that plant exudates have antifungal effects against *F.oxysporium* (Zuo et al.2015; Zhao et al. 2015; Lombardi et al.2018; Were et al.2021). This study coincides with the literature information. In addition, each plant exudate had different effects. In this study, the antifungal effect of wild legume strains against F. oxysporium was evaluated and it was seen that the endemic species O3 and O4 were more effective. This is thought to be due to the fact that they are grown in different regions and their contents are different (Table4,5,6and 7).

As a result, it was concluded that the exudates obtained from wild legume plants can be used against *F.oxysporium* and as a non-toxic and economically suitable fungicidal. In addition to a preliminary study used against F.oxysporim, which is a pathogen in chickpea and many plants, of the exudates of both endemic species and cosmopolitan species of Onobrychis legumes.

Conclusions

In this study, it was determined by evaluating the morphological and physiological parameters that the exudates of Onobrychis species can be used as a natural and effective biocontrol agent against *F. oxysporium*.

Disclosure statement

No potential conflict of interest was reported by the authors

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