



Effects of the foliar cedar tar treatment on the control of the Ascochyta blight caused by *Ascochyta rabiei*

Sedir Katranının Ascochyta rabiei'nin neden olduğu nohut yanıklığının kontrolü üzerine etkisi

Emine Burcu TURGAY^{1*}, Sertan ÇEVİK², Eray ŞİMŞEK³, Kadir AKAN⁴

¹Field Crops Central Research Institute, Pest and Diseases Resistance Unit, Ankara, Türkiye

²Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Harran University, Şanlıurfa, Türkiye

³Department of Plant Protection, Faculty of Agriculture, Harran University, Şanlıurfa, Türkiye

⁴Department of Plant Protection, Faculty of Agriculture, Kirsehir Ahi Evran University, Kirsehir, Türkiye

¹<https://orcid.org/0000-0003-1150-4901>; ²<https://orcid.org/0000-0003-1259-7863>; ³<https://orcid.org/0000-0003-4984-4223>; ⁴<https://orcid.org/0000-0002-1612-859X>

To cite this article:

Turgay, E., Çevik, S., Şimşek, E. & Akan, K. (2025). Effects of the foliar cedar tar treatment on the control of the ascochyta blight caused by *ascochyta rabiei*. Harran Tarım ve Gıda Bilimleri Dergisi, 29(1): 11-22

DOI: 10.29050/harranziraat.1581394

*Address for Correspondence:

Emine Burcu TURGAY

e-mail:

cercospora79@gmail.com

Received Date:

07.11.2024

Accepted Date:

07.02.2024

© Copyright 2018 by Harran University Faculty of Agriculture. Available on-line at www.dergipark.gov.tr/harranziraat



This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

ABSTRACT

Ascochyta blight (*Ascochyta rabiei*) is a significant fungal disease that affects chickpea crops all around the world. Synthetic fungicides, which have harmful environmental effects, are commonly used to control the disease. Alternatively, various plant extracts have been explored for disease management. In this study, the antifungal activity of cedar tar, its potential to control the disease, and its disease prevention mechanism in chickpeas were evaluated. The antifungal activity results indicated that whereas 10%, 25%, and 50% cedar tar application doses had an inhibitory effect greater than 50% but were harmful to the plant, 1% and 5% cedar tar application doses suppressed mycelial growth by less than 50%. Therefore, doses of 0.5 %⁻¹, 1 %, and 2 % were selected to determine the disease prevention potential of cedar tar at different application times. Cedar tar treatment applied 72 hours before *Ascochyta rabiei* inoculation effectively prevented disease development in chickpea plants. This treatment also decreased MDA content, indicating the membrane was protected from pathogen attack. These results suggest that cedar tar can be considered an effective bio-fungicide formulation for future integrated pest management programs.

Keywords: *Ascochyta* blight, *Ascochyta rabiei*, *Cedrus libani*, *Cicer arietinum*

Öz

Ascochyta yanıklığı (*Ascochyta rabiei*), dünya çapında nohutu etkileyen önemli bir fungal hastalıktır. Zararlı çevresel etkileri olan sentetik fungusitler hastalığın kontrolünde yaygın olarak kullanılmaktadır. Alternatif olarak hastalık yönetimi için çeşitli bitki ekstraktları araştırılmaktadır. Bu çalışmada sedir katranının antifungal aktivitesi, hastalığı kontrol etme potansiyeli ve nohutta hastalık önleme mekanizması değerlendirilmiştir. Antifungal aktivite sonuçları, sedir katranının %1 ve %5'lik uygulama dozlarının misel gelişimini %50'den daha az engellediğini, %10, %25 ve %50'lik dozların ise %50'den daha büyük bir inhibisyon etkisine sahip olduğunu ancak bitki için toksik olduğu görülmüştür. Bu nedenle farklı uygulama zamanlarında sedir katranının hastalıkları önleme potansiyelini belirlemek için %0,5, %1 ve %2'lik dozlar uygulanmıştır. *Ascochyta rabiei* aşılmasından 72 saat önce uygulanan sedir katranı uygulaması nohut bitkilerinde hastalık gelişimini etkili bir şekilde önlemiştir. Bu uygulama aynı zamanda MDA içeriğini de azalttığı; bu da zararlı patojen saldırısından korunduğunu göstermiştir. Sonuçlar, sedir katranının gelecekteki entegre mücadele yönetimi programları için etkili bir biyo-fungisit olarak değerlendirilebileceğini göstermektedir.

Anahtar Kelimeler: Nohut yanıklığı, *Ascochyta rabiei*, *Cedrus libani*, *Cicer arietinum*

Introduction

The self-pollinating legume chickpea (*Cicer arietinum* L.) has been farmed for generations in the Mediterranean, Central America, South America, the Far East, and the Near East.

Approximately 10% of cropland worldwide is used for legumes. Chickpeas (*Cicer arietinum* L.) are among the most commonly farmed legumes, behind common beans (*Phaseolus vulgaris* L.) and peas (*Pisum sativum* L.) (FAO 2023; Iqbal et al. 2006). It can be produced in a variety of climates, including subtropical, temperate, desert, and semiarid areas, in at least fifty countries. (Jukanti et al. 2012; Zhang et al. 2020). Chickpea occupies 17.8 million hectares of farmland, representing over 15% of global legume production, with an annual output of 17.2 million tonnes. India contributes approximately 72% of global chickpea production, with other major producers including Turkey, the USA, Canada, Australia, and Mexico. In 2022, global chickpea imports reached approximately 1.89 million tonnes, while exports amounted to approximately 2.05 million tonnes (FAO 2023).

Chickpea production is affected by numerous phytopathogenic factors, with more than 50 pathogens known to affect yield and quality at various levels. *Ascochyta* blight, which is brought on by *Ascochyta rabiei*, is one of the most serious biotic hazards to chickpeas. (Pass.) Labr. (Teleomorph *Didymella rabiei* (Kov.) v. Arx). Crop losses were recorded as early as the 1930s, and the illness was first mentioned in 1867. Over the following two decades, *Ascochyta* blight and its associated losses have been studied by researchers from various countries, including Morocco, Bulgaria, Greece, Pakistan, and Spain (Deokar et al. 2019; Nene 1982; Salotti et al. 2021).

To manage *Ascochyta* blight, numerous research groups are currently striving to create disease-resistant genotypes or select resistant ones using a variety of breeding techniques (Gayacharan et al. 2020). However, the genetic basis of resistance to *Ascochyta rabiei* is

extremely complex (Sharma et al. 2016), and the chickpea gene pool is known to be quite limited (Toker et al. 2021; Tekin et al. 2018). Furthermore, breeding efforts have been hampered by the pathogen's great genetic variety (Kumar et al. 2020; Gayacharan et al. 2020). It has been noted that despite the development of cultivars with a certain degree of resistance to this pathogen, fungicides are still required to control *Ascochyta* blight (Kurt et al. 2008; Rani et al. 2020). However, fungicides may occasionally have more negative effects on the environment than positive effects.

The antimicrobial and antifungal properties of various plant extracts, such as monoterpenoids, sesquiterpenoids, and hydrocarbons, obtained from cedar (*Cedrus libani*), have been tested against various plant pathogens (Kızıl et al. 2002; Kurt et al. 2008; Ghanem and Olama 2014; Takci et al. 2021; Venditti et al. 2020). Among these extracts, cedar tar (CT) is known as a byproduct of the distillation of the dry, resinous wood of the Taurus cedar species at high temperatures (Kurt and Işık 2012; Kurt et al. 2008). It has a wide range of applications ranging from controlling harmful insects, pathogenic fungi, and bacteria to combating various parasites (Kurt et al. 2008; Takci et al. 2021). It has been reported that CT can exhibit protective effects against a wide range of pathogenic organisms (Kurt and Işık 2012; Kurt et al. 2008). Additionally, some components of CT's chemical composition have been found to have antifungal properties.

The objective of this study was to determine the potential of cedar tar (CT) treatment to control *Ascochyta* blight (AB) development in chickpea plants. For this purpose, the antifungal activity of CT was investigated using the agar plate method and a pot experimental design. Antioxidant enzyme analyses, proline content and, lipid peroxidation, were also analyzed to understand the disease prevention mechanism of CT. To the best of our knowledge, this is the first study to investigate the efficacy of CT against AB.

Materials and Methods

Preparation of Ascochyta rabiei inoculum and plant material

An isolate was provided as a single spore culture from the collection of *Ascochyta rabiei* pathotype IV, maintained by Assoc. Prof. Kadir AKAN (Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Plant Protection). In previous decades, pathotype IV has been categorized according to variations in the pathogen's virulence (Udupa et al. 1998; Imtiaz et al. 2011). Pathotype I is less aggressive, pathotype II is more aggressive, pathotype III is more aggressive and pathotype IV is extremely aggressive. Therefore, this study employed the pathotype IV group, which is recognized as the most aggressive pathotype of the disease. Chickpea Seed Meal Agar (CSMDA; 40 g of chickpea seed meal, 20 g of dextrose, and 18 g of agar in 1 L of sterilized distilled water) was used to cultivate this isolate for 7–10 days at 20–22°C. Spores were resuspended using a flamed wire loop after the plates were filled with sterile distilled water. According to Trapero-Casas and Kaiser (1992), the suspension's spore concentration was determined using a hemocytometer, adjusted to 5×10^5 pycnidiospores mL^{-1} using sterile distilled water, and treated with 0.15% Tween 20 (polyoxyethylene–sorbitan monolaurate) (MERCK®, Nottingham, UK).

Evaluation of antifungal activity of cedar tar against Ascochyta rabiei in agar plate

The antifungal activity of cedar tar was assessed using the poisoned food approach. To achieve the desired percentage concentrations of cedar tar, a specific amount of tar was dissolved in the PDA medium mix. The concentration of cedar tar diluted with DMSO at a ratio of 50% was considered as 100%, and subsequent dilutions were made accordingly. The preparation of Potato Dextrose Agar (PDA) prepared by autoclaving at 121°C and cooling to 40°C. CT was added to PDA media after being dissolved in ½ of

dimethyl sulfoxide (DMSO). CT doses were changed to 1, 5, 10, 25, and 50% at the end. The mycelial discs were made from the tips of a 7-day-old AB culture using a sterile cork borer with a 5 mm diameter. Once the PDA medium had solidified, they were placed in the middle of each Petri plate. The plates were incubated for 14 days at $25 \pm 2^\circ\text{C}$. Control treatments were prepared without the use of CT extract. Each treatment was set up with two replicates and three replicates. AB's mycelial growth diameters were measured daily. The growth of control plates and mycelial growth in plates with varying doses were compared, and the inhibition rates of various CT doses were computed using the following formula (Deans and Soboda 1990):

$$(C-T)/C \times 100$$

Where C=Length of control hyphae (mm) and T=Length of treated hyphae (mm).

Evaluation of potential of cedar tar treatment against Ascochyta rabiei in-vitro

Different doses of CT (% 0.5, 1 and 2) with varying disease implementation times were applied to determine the potential of CT treatment against *Ascochyta rabiei in-vitro*. In the first treatment, CT and *Ascochyta rabiei* inoculation were applied to the test material simultaneously (T0-D0), while in the second treatment, CT was applied 72 h before the inoculation of *Ascochyta rabiei* (T1-D2). For the third treatment, CT was performed 72 h after the inoculation (T2-D1).

The chickpea cultivar Uzunlu, which is susceptible to *Ascochyta rabiei*, was used in the study. Three seeds were planted in separate pots that contained peat: perlite. (2:1 v/v). The pots were grown under greenhouse conditions with 14 h of light and 10 h of darkness at $25 \pm 5^\circ\text{C}$ until the two-leaf stage. Plants were inoculated with a 5×10^5 pycnidiospores mL^{-1} suspension of spores using a pressure sprayer. A group of plants was treated with only sterile distilled water (negative control) or inoculated only with *Ascochyta rabiei* spores (positive control). A humidifier with a

100% constant humidity setting was then attached to the polythene tent, keeping the plants moist for 48 hours at 18–22°C (12 hours of continuous darkness and 12 hours of light). All plants were then covered with clear polythene. As shown in Table.1, a 0–9 rating scale was used to evaluate the illness response to *Ascochyta rabiei* 14 days after inoculation (Reddy and Singh 1987).

A fully randomized design was used to conduct the study. The following formula was used to determine the percentage of disease incidence (PDI) based on the 0–9 rating scale (Sankara and Acharyya 2012).

$$\text{Percentage of Disease Incidence (PDI)} = \frac{\text{Number of diseased plants}}{\text{Total Number of plants observed}} \times 100$$

Table 1. 1-9 scale to be used in reaction evaluation of *Ascochyta rabiei*.

Scale	Disease Intensity	Reactions
0	No minor lesions or symptoms,	Immune (I)
1	Lesions on the apical stems are few and tiny,	Highly Resistant (HR)
2	On certain branches, there are tiny girdling stem lesions deep down,	Resistant (R)
3	One or two branches are broken and lesions up to 5–6 mm in size	Resistant (R)
4	The majority of the plant clearly has lesions (2–5 mm in size), and several of its branches are damaged,	Moderately Resistant (MR)
5	Defoliation has begun, half of the branches are damaged, and there are numerous big lesions,	Moderately Susceptible (MS)
6	More defoliation, damaged stems, and dry branches are among the lesions similar to those in 5.	Susceptible (S)
7	As in 5 and 6, there are several huge lesions with clear defoliation, up to 70% of branches are damaged, and up to 25% of plants are destroyed,	Susceptible (S)
8	As in 7, symptoms include up to 50% of plants being killed.	Highly Susceptible (HS)
9	Symptoms as in number eight, with every plant dead,	Highly Susceptible (HS)

Determination of the malondialdehyde (MDA) content

Malondialdehyde (MDA) content was measured to assess lipid peroxidation (Ohkawa et al. 1979). Five milliliters of a 5% trichloroacetic acid (TCA) solution was used to homogenize 0.5 grams of leaf tissue. Following centrifugation of the homogenate, equal quantities of TCA and thiobarbituric acid solutions were added to the supernatant in the tubes. The tubes were then incubated for 25 minutes at 96°C. The tubes were placed in a cold bath to stop the reaction, and then they were centrifuged for five minutes at 6,000 rpm. A spectrophotometer (Shimadzu Corporation UV-VIS Spektrofotometre UV-1280) was used to measure the absorbance of the final mixture at 532 nm and 600 nm. The extinction coefficient was used to determine the MDA content.

Measuring the amount of free proline

A homogenate of 0.5 g of leaf tissue was mixed with 3% sulfosalicylic acid and centrifuged for 3

minutes at 3,000 rpm. Subsequently, the glacial acetic acid, acid ninhydrin, and supernatant were combined in equal amounts and incubated for one hour at 100°C. Four milliliters of cold toluene were added to the tubes to stop the reaction. After being evaporated, the toluene phase was measured at 520 nm wavelength using a spectrophotometry (Shimadzu Corporation UV-VIS Spektrofotometre UV-1280). A standard curve was used to calculate the proline levels (Bates et al. 1973).

Evaluation of antioxidant enzymes

Catalase (CAT) and superoxide dismutase (SOD) enzyme activity tests were performed using the same extraction technique. Five milliliters of extraction solution comprising 0.1 M potassium phosphate buffer (pH 6.8), 100 milligrams of polyvinylpyrrolidone (PVP), and 0.1 mM ethylenediaminetetraacetic acid (EDTA) were used to homogenize 0.5 grams of fresh leaves. After centrifuging the homogenate for five minutes at 16,000 ×g, the supernatant was

utilized to analyze SOD and CAT. SOD (superoxide dismutase; EC 1.15.1.1) activity was measured as described by Beyer and Fridovich (1987). A solution containing methionine, nitroblue tetrazolium, EDTA, riboflavin, and phosphate buffer (pH: 7.8) was mixed with 200 μL microliters of the extract. The reaction was conducted at 25°C in a chamber illuminated by a fluorescent lamp. The fluorescent lamp was turned on to begin the reaction, then it was turned off after five minutes. At 560 nm, blue formazan—which is created when NBT is photoreduced in the presence of light—was observed. The quantity of enzyme required to block 50% of the NBT was determined to be one SOD unit. Aebi (1983) defined CAT activity (catalase; EC 1.11.1.6) as the consumption of H_2O_2 at 240 nm for 30 s. Three milliliters of a solution containing 50 milliliters of potassium phosphate buffer (pH 7.0) and 20 milliliters of H_2O_2 were mixed with fifty microliters of the enzymatic extract. At 240 nm, the absorbance drop was observed. The extinction coefficient was used to compute the enzyme activity, which was then represented as $\mu\text{mol H}_2\text{O}_2$ oxidized g^{-1} mg protein.

Statistical analysis

Using SAS software, the findings of the agar plate and pot studies were statistically evaluated using one-way ANOVA and post-hoc Tukey's testing with %95 confidence (JMP version 8, SAS Institute Inc.). Two-way factorial analysis of variance (ANOVA) was used to examine the results from the proline content, lipid

peroxidation, and antioxidant enzyme tests, with or without treatment and dose interaction. Descriptive statistics, such as mean, standard deviations, and errors, were calculated using base packages of R version 4.1.2 with RStudio (RStudio Team 2021). The ANOVA of the interaction between all groups and controls was found to be statistically significant ($P < 0.05$). Therefore, Tukey-Kramer test and Only when treatment and dosage interactions were not present, Duncan's multiple range test was performed with %95 confidence to compare the groups. Using the Rcmdr package of the R environment, two-way ANOVA and post-hoc tests were conducted (Fox 2017; R Core Team 2021, RStudio Team 2021).

Results and Discussion

Assessment of cedar tar's antifungal efficacy against Ascochyta rabiei

Using the agar plate method, the effect of five distinct CT dosages on the mycelial development of the fungus was examined. It was determined that the application doses of 25% and 50% completely inhibited mycelial growth, whereas a dose of 10% was found to inhibit mycelial growth by 50%. The doses of 1% and 5% inhibited mycelial growth by less than 50% (Fig.1). The effects of CT applications on mycelial growth inhibition were statistically significant, except for the difference between the doses of 25 % and 50 %.

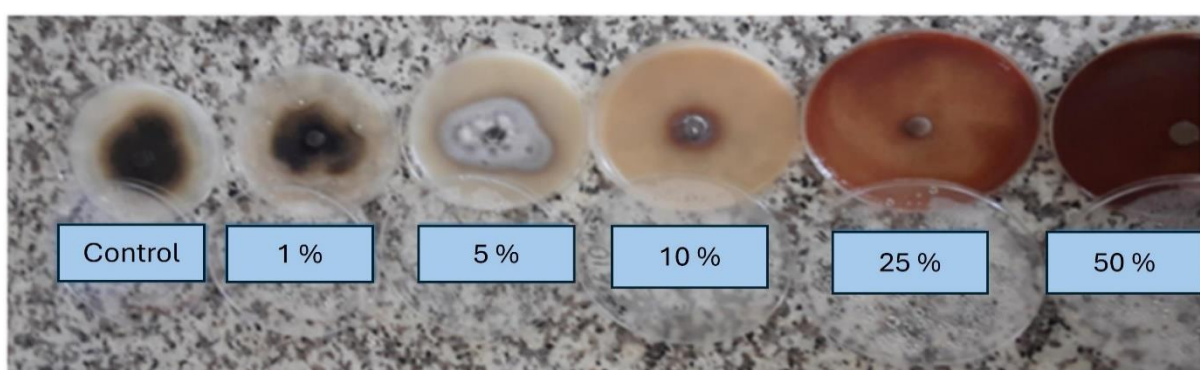


Figure 1. Images of the petri dishes showing the antifungal activity of CT against *Ascochyta rabiei*.

Evaluation of potential of cedar tar treatment against *Ascochyta rabiei* in-vitro

The Chickpea plants were toxically affected by application levels of 5, 10, 25, and 50 %, even though these doses inhibited mycelial growth by below and above 50%, respectively. As a result, studies were carried out *in-vitro* using application dosages between 0.5 % and 2 %. The assessment of AB severity on chickpea plants was assessed

within 14 days of inoculation. Statistical analysis was performed using percentage disease incidence (PDI) results. In the 1st and 2nd experiments, it was observed that AB was notably prevented by CT, with no significant differences between the different doses of CT treatment. In the 3rd experiment, it was determined that none of the CT treatments prevented AB development (Fig. 2).

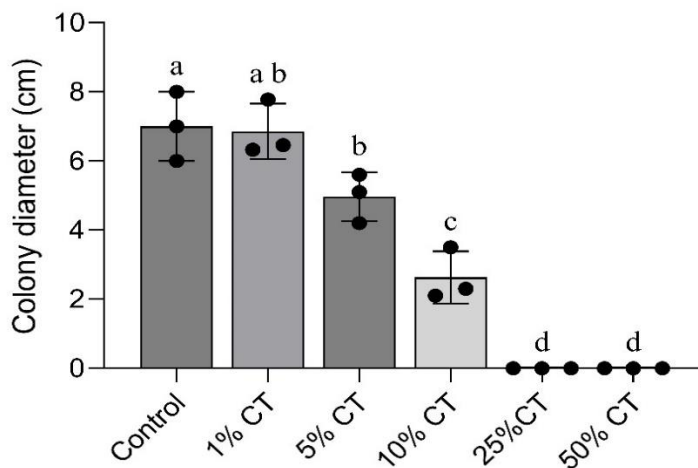


Figure 2. Cedar tar's antifungal effects against *Ascochyta rabiei* in vitro.

The lowercase letters refer to statistical significance among different CT treatments at $p \leq 0.05$.

did not significantly change the proline content, except in the T2-D1 2 % treatment (Fig. 3) in contrast to the control.

Proline and MDA content

Ascochyta rabiei inoculation and CT treatment

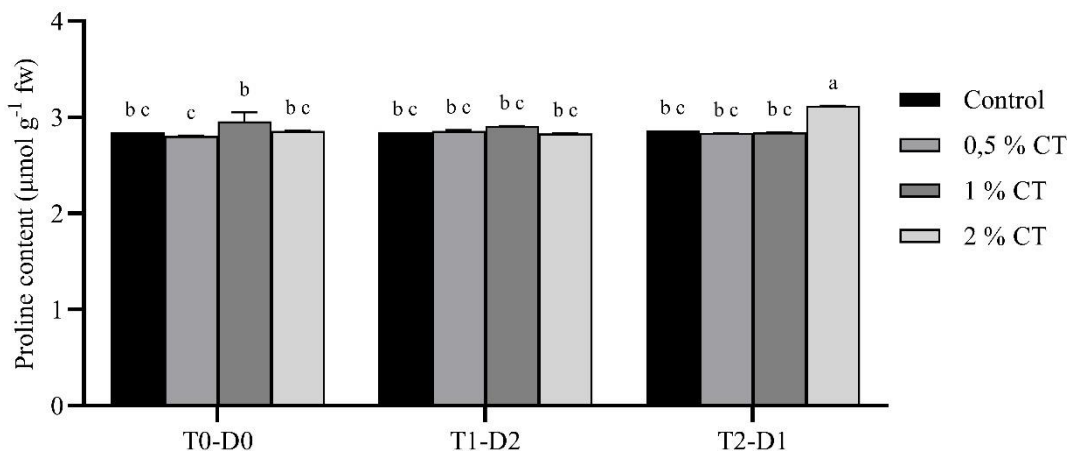


Figure 3. Chickpea plant proline content.

The means of the three plants make up the data. Two-way factorial ANOVA was conducted between the groups. Significant differences ($p <$

0.05) are indicated by asterisks (*).

Inoculation of chickpea plants with *Ascochyta rabiei* increased malondialdehyde (MDA) content

in all CT treatments except in the T1-D2 treatment ($p < 0.05$). It was found that T1-D2's MDA content was comparable to that of the

positive control's. The T2-D1 therapy had the highest MDA content (Fig. 4).

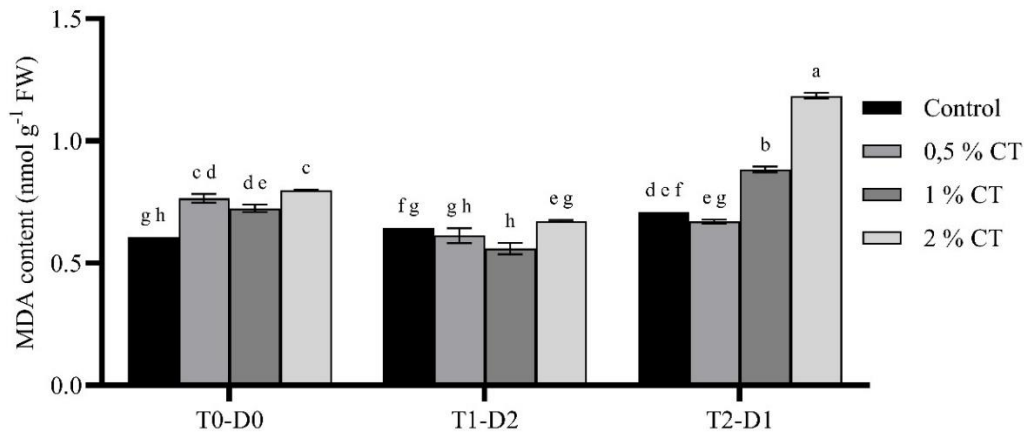


Figure 4. MDA content of chickpea plants

The data represent the mean of three plants. Between groups, a two-way factorial ANOVA was conducted. Significant differences are indicated by asterisks at $p \leq 0.05$ (*) and $p \leq 0.01$ (**).

Antioxidant enzyme analysis

The highest catalase activity was observed in T0-D0 and T1-D2, except in the T2-D1 1 % treatment ($p < 0.05$) (Fig. 5).

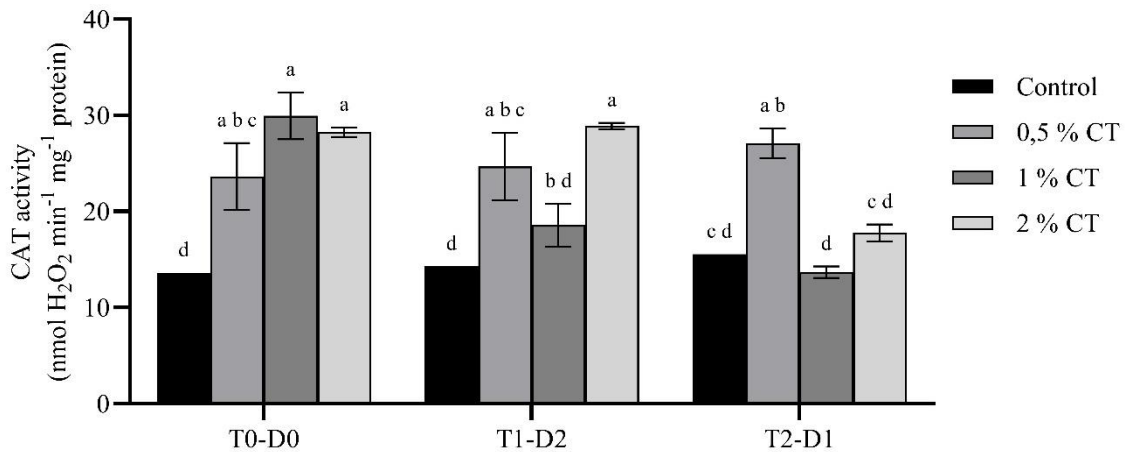


Figure 5. Catalase activity of chickpea plants

The data represent the mean of three plants. Two-way factorial ANOVA was performed between groups. Asterisks denote significant differences at $p \leq 0.05$ (*)

(SOD) activity in all groups increased ($p < 0.05$) in compared to that in the control group. The T1-D2 group exhibited the highest SOD activity (Fig. 6).

Following the inoculation and CT treatment, all groups' superoxide dismutase

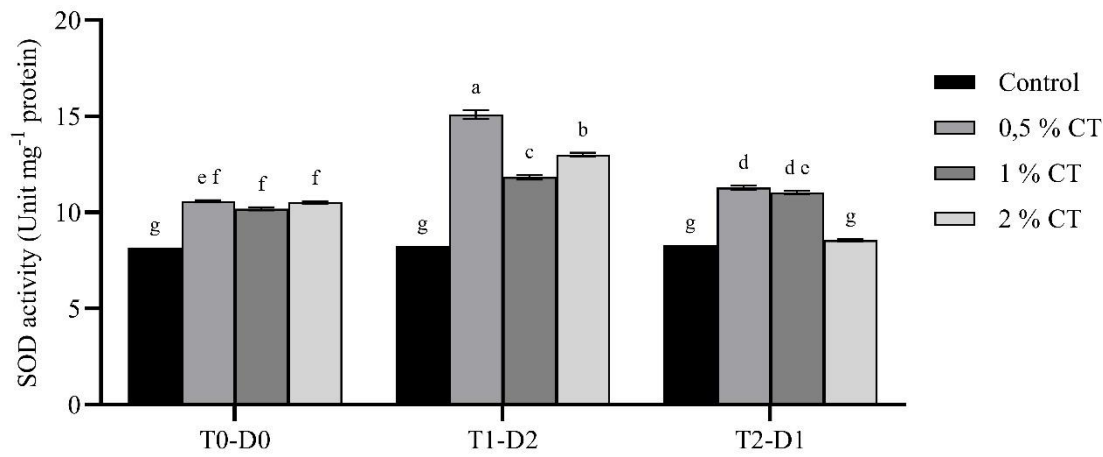


Figure 6. SOD activity of chickpea plants

The data represent the mean of three plants. Two-way factorial ANOVA was performed between groups. Asterisks denote significant differences at $p \leq 0.05$ (*), $p \leq 0.01$ (**)

In this study, the potential use of CT against AB, which causes foliar infection in chickpea, was analyzed. The efficacy of different doses of CT as an antifungal agent against the pathogen *Ascochyta rabiei* was examined. Antifungal activity test findings unequivocally demonstrated that CT treatment prevented *Ascochyta rabiei* from growing mycelially. Exogenous CT treatment also inhibited the development of the disease. Our results showed that CT can have a potential effect in delaying disease symptoms and reducing susceptibility to *Ascochyta rabiei* in chickpea. This beneficial impact of CT is prominent in both consecutive and simultaneous applications. This is the first report to reveal the antifungal activity of CT against *Ascochyta rabiei*, and it is promising that the plant extract used in this study could be an effective approach against a new and highly virulent *Ascochyta rabiei* isolate (pathotype IV). Limited information is available on the antifungal activity of different plant extracts in reducing AB in chickpea. *Ascochyta rabiei* was evaluated to determine the antifungal efficacy of various concentrations of *Chenopodium album* leaf methanolic extract (1%, 2.5%, 4%, 5.5%, and 7%). The highest fungal biomass reduction (68%) was noted with a 7% dose of *C. album* methanolic extract (Sherazi et al. 2016).

Withania somnifera, a weed species, was used

to control the AB. According to the findings, pathogenic fungal growth was effectively suppressed by a 0.2% dose of *W. somnifera* leaf extract in methanol (Javaid et al. 2020). Tests against five distinct isolates of *Ascochyta rabiei* were carried out *in-vitro* to determine the antifungal activity of *Mentha spicata* L. essential oils. Consequently, it was discovered that a 10 μ L dose of *Mentha spicata* essential oil inhibited the fungus's mycelial growth (Bayar 2018). According to the study's findings, CT might include a number of antifungal substances that can successfully stop *Ascochyta rabiei* from growing.

No significant change was observed in proline content after *Ascochyta rabiei* inoculation and CT treatment in any of the groups. However, increases in proline levels in chickpea plants following *Ascochyta rabiei* inoculation have been demonstrated by various researchers (Hasanian et al. 2020; Üstün and Dolar 2001). Additionally, there has been an increase in the expression of genes linked to proline synthesis that help plants tolerate *Ascochyta rabiei* (Coram and Pang 2006). Although proline's function as an osmoprotectant is its most well-known characteristic (Chun et al. 2018), it also has direct or indirect antioxidant functions (Szabados and Saviouré 2009). In addition, chickpea cultivars that are more tolerant to *Ascochyta rabiei* have a lower proline content (Hasanian et al. 2020). The finding of proline content in this study, which is close to that of the positive control in all treatments, suggests that CT treatment may lessen the adverse effects of the

disease (Hasanian et al. 2020).

MDA, one of the end products of lipid peroxidation, is a good indicator of the level of membrane damage caused by oxidative stress (Çevik 2021). An increase in MDA content after *Ascochyta rabiei* inoculation was shown by Bahmani et al. (2020). In the same study, tolerant cultivars had lower MDA contents than sensitive cultivars under *Ascochyta rabiei* inoculation. CT treatment before *Ascochyta rabiei* inoculation decreased MDA content in the T1-D2 group. Low MDA content under pathogen attack indicates that CT treatment helps preserve the membrane integrity of the plant. Providing this protection with pre-disease treatment is also a positive outcome for the potential field application of CT. The PDI of the T2-D1 group was much higher than that of the T1-D2 and T0-D0 groups (Fig. 2); this result was also consistent with the MDA results. Considering the MDA results, it can be concluded that pre-disease CT treatment protects plant membranes from the effects of the disease.

Following stress and CT treatments, CAT and SOD activity increased in every group. Although the T0-D0 group showed the highest catalase activity, the T1-D2 group had the highest overall catalase activity. The two crucial components of the plant antioxidant systems are CAT and SOD. The antioxidant system's first line of defense is SOD, which catalyzes the conversion of superoxide radicals into H_2O_2 and O_2 . The transformation of H_2O_2 into H_2O and O_2 is catalyzed by CAT (Kaur et al. 2021). In addition to a significant increase in CAT under disease pressure (Hasanian et al. 2020), upregulated defense enzyme activities have also been observed against *Ascochyta rabiei* inoculation (Kaur et al. 2021). These investigations unequivocally demonstrated that, in an infected environment, resistant genotypes exhibit lower MDA concentrations and higher antioxidant enzyme activities than susceptible genotypes. Higher antioxidant enzyme activities following tar treatment in response to AB may indicate the suppression of oxidative stress caused by the pathogen. However, isozyme analysis is are

required to better understand the effects of tar treatment on the antioxidant system during infection.

The cost performance of CT was also evaluated comparatively with that of a fungicide used to control AB. The "partial budgeting" method was preferred, based on the principle that all expenditures are the same except for the cost of the fungicide and CT. The approximate cost of 500 mL of a fungicide containing "25% Boscalid, 12% Pyraclostrobin" is 37 USD, while the approximate cost of 500 mL of CT is 4 USD. It was calculated that the cost of supplying CT is 90% lower than the fungicide. Fungicide application is recommended at a dose of 500 mL ha⁻¹, and the cost per hectare is calculated to be 37 USD. It was calculated that 100 L of water would be used for a one-hectare area, and 2 mL of CT is sufficient for the 2% application dose, with the approximate cost for this area being 8 USD. The unit area cost of the 2% application dose of CT is 78% lower than the fungicide. Considering that large areas are cultivated; greater profit will be achieved owing to the reduction of input costs by 90% for the initial supply cost and by 78% for the unit area. In fact, if the profit margin is reduced slightly reduced, the probability of finding customers in the market is quite high. In this case, it is predicted that this will improve the production cost performance (Jukanti et.al. 2012; Shuping. et al. 2017; Wani et.al.2022)

Fungicides are the main methods for controlling AB in chickpeas. The potential for new fungicide-resistant races to emerge or possible shifts in the pathogen's population structure present the greatest obstacles to both chemical control techniques and resistance breeding research. Using CT can assist in reducing on the use of fungicides or serve as an alternative to them. The findings of the study demonstrated that CT has a great potential for use against infections.

Author information

Acknowledgment: Prof. Dr. Yusuf KURT is thanked

for their help in this study.

Author contributions: Conceptualization, EBT and SÇ; methodology, SÇ; software, EBT, EŞ, SÇ; resources, KA; review and editing, EBT, SÇ, EŞ; writing-original draft preparation, EBT, SÇ, EŞ; writing review and editing, EBT, SÇ, EŞ. All authors have read and agreed to the published version of the manuscript. Authorship must be limited to those who have contributed substantially to the work reported.

Funding: This research received no external funding.

Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

All data available within the manuscript.

References

- Aebi, H. (1983). Catalase in Vitro. *Met Enzy.* 105:121-126. [http://dx.doi.org/10.1016/S0076-6879\(84\)05016-3](http://dx.doi.org/10.1016/S0076-6879(84)05016-3)
- Akhtar, R., Javaid, A., Qureshi, M.Z. (2020). Bioactive constituents of shoot extracts of *Sisymbrium irio* against *Fusarium oxysporum* f. sp. *cepae*. *Planta Daninha.* <https://doi.org/10.1590/S0100-83582020380100008>
- Aydin, M.H, Oğuz A., Erdemci, İ., Karademir, Ç. (2016). Control of *Ascochyta* Blight (*Ascochytha rabiei*) in Chickpea in Winter Sowing in Southeastern Anatolia. *J Turk Phyto.* 45:2-3:87-96
- Bahmani, M., Maali-Amiri, R., Javan-Nikkhah, M., Atghia, O., Rasolnia, A. (2020). Enhanced Tolerance to *Ascochyta* Blight in Chickpea Plants via Low Temperature Acclimation. *Russian. J Plant Physiol.* <https://doi.org/10.1134/S1021443720040020>
- Bates, L.S, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil.* <https://doi.org/10.1007/BF00018060>
- Bayar, Y. (2018). Determination of Antifungal Activity of *Mentha spicata* L. Essential Oils Against Different Isolates of Chickpea Blight Disease [*Ascochyta rabiei* (Pass) Labr.] *Turk J Agric Res.* <https://doi.org/10.19159/tutad.346569>
- Beyer, W.F, Fridovich, I. (1987). Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal Biochem* 161:559-566
- Çevik, S (2021). Effects of Severe Drought Stress on Some Physiological and Biochemical Parameters of AMF Inoculated *C. arietinum*. *YYU J AGR SCI.* <https://doi.org/10.29133/yyutbd.870384>
- Chun, S.C., Paramasivan, M., Chandrasekaran, M. (2018). Proline accumulation influenced by osmotic stress in arbuscular mycorrhizal symbiotic plants. *Front Microbiol.* <https://doi.org/10.3389/fmicb.2018.02525>
- Coram, T.E., Pang, E.C. (2006). Expression profiling of chickpea genes differentially regulated during a resistance response to *Ascochyta rabiei*. *Plant Biotechnol J.* <https://doi.org/10.1111/j.1467-7652.2006.00208.x>
- Deokar, S.D., Girase, V.S., Patil, S.G., Bhavsar, V.V. (2019). Assessment and Implication of Selection Indices in F₂ Generation of Chickpea (*Cicer arietinum* L.). *IJCMAS.* <https://doi.org/10.20546/ijcmas.2019.810.109>
- Food and Agriculture Organization (2023). FAOSTAT Statistical Database
- Fox, J. (2017). Using the R Commander: A Point-and-Click Interface for R. Chapman and Hall/CRC Press.
- Gayacharan, R.U., Singh, S., Basandrai, A.K., Rathee, V.K., Tripathi, K., Singh, N., Dixit, G.P., Rana, J.C., Pandey, S., Kumar, A., et al. (2020). Identification of novel resistant sources for *ascochyta* blight (*Ascochytha rabiei*) in chickpea. *PLoS ONE* <https://doi.org/10.1371/journal.pone.0240589>
- Ghanem, S., & Olama, Z. (2014). Antimicrobial potential of Lebanese cedar extract against human pathogens and food spoilage microorganisms. *EJBSP 1:13-26.*
- Hasanian, S., Sofalian, O., Zare, N., Tarinejad, A., Davari, M. (2020). Effect of *Ascochyta* blight disease on antioxidant enzymes activities, amount of proline and carbohydrate in some chickpea genotypes. *IJGPB.* <https://doi.org/10.30479/IJGPB.2021.15181.1292>
- Iqbal, A., Ateeq, N., Khalil, I.A., Perveen, S., Saleemullah, S. (2006). Physicochemical characteristics and amino acid profile of chickpea cultivars grown in Pakistan. *J Foodserv*17, 94–101
- Imtiaz, M., Abang, M.M., Malhotra, R.S., Ahmed, S., Bayaa, B. (2011). Pathotype IV, a new and highly virulent pathotype of *Didymella rabiei*, causing *Ascochyta* blight in chickpea in Syria. *Plant Dis.* <https://doi.org/10.1094/PDIS-04-11-0333>.
- Javaid, A., Afzal, R., Shoaib, A. (2020). Biological management of southern blight of chili by *Penicillium oxalicum* and leaves of *Eucalyptus citriodora*. *IJAB.* <https://doi.org/10.17957/IJAB/15.1263>.
- Jukanti, A.K., Gaur, P.M., Gowda, C.L.L., Chibbar, R.N. (2012). Nutritional quality and health benefits of

- chickpea (*Cicer arietinum* L.): A review. *Br. J. Nutr.* 108, S11–S26
- Kaur, K., Grewal, S.K., Singh, S., Rani, U., Bhardwaj, R.D. (2021). Timing and intensity of upregulated defensive enzymes is a key factor determining resistance in chickpea to *Ascochyta rabiei*. *Phy Mol Plant Pathol.* <https://doi.org/10.1016/j.pmpp.2021.101645>
- Kızıl, M., Kızıl, G., Yavuz, M., Aytekin, Ç. (2002). Antimicrobial activity of resins obtained from the roots and stems of *Cedrus libani* and *Abies cilicia*. *Appl Biochem Microbiol.* <https://doi.org/10.1023/A:1014358532581>
- Kumar, A., Perween, S., Kumar, R.R., Kumar, S., Kumar, M., Ranjan, R.D. (2020). Chickpea Biotic Resistance Breeding in The Genomic Era: Progress and Prospects. *TTPP* 1:307-329
- Kurt, Y., & Isık, K. (2012). Comparison of tar produced by traditional and laboratory methods. *Stud Ethno-Med* 6(2):77-83
- Kurt, Y., & Kaçar, M.S., Isık, K. (2008). Traditional tar production from *Cedrus libani* A. Rich on the Taurus Mountains in Southern Turkey. *Eco Bot.* <https://doi.org/10.1007/s12231-008-9023-x>
- Nene, Y.L. (1982). A Review of *Ascochyta* Blight of Chickpea. *Trop Pest Manag.* <https://doi.org/10.1080/09670878209370675>
- Ohkawa, H., Ohishi, N., Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Annual Biochem.* [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
- R Core Team (2021) R: A language and environment for statistical computing Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>
- Rani, U., Singh, S., Basandrai, A.K., Rathee, V.K., Tripathi, K., Singh, N., Singh, K. (2020). Identification of novel resistant sources for *ascochyta* blight (*Ascochyta rabiei*) in chickpea. *PloS one.* <https://doi.org/10.1371/journal.pone.0240589>
- Reddy, M.V., & Singh, K.B. (1984). Evaluation of a world collection of chickpea germplasm accessions for resistance to *Ascochyta* blight. *Plant Dis.* <https://doi:10.1094/PD-68-900>
- RStudio Team (2021). *rstudio: Integrated Development for R.* Boston, MA: RStudio Inc. <http://www.rstudio.com/>
- Salotti, I., & Rossi, V.A. (2021). Mechanistic Weather-Driven Model for *Ascochyta rabiei* Infection and Disease Development in Chickpea. *Plants.* <https://doi.org/10.3390/plants10030464>
- Sankara, R.K., & Acharyya, P. (2012). Incidence of yellow vein mosaic virus disease of okra [*Abelmoschus esculentus* (L.) Moench] under summer and rainy environments. *Int. J. Curr. Res.* 4;518 – 21
- Sharma, M., & Ghosh, R. (2016). An update on genetic resistance of chickpea to *Ascochyta* blight. *Agron J.* <https://doi.org/10.3390/agronomy6010018>
- Sherazi, A.Z., Jabeen, K., Iqbal, S., Yousaf, Z. (2016). Management of *Ascochyta rabiei* by *Chenopodium album* extracts. *Planta Daninha.* <https://doi.org/10.1590/S0100-83582016340400007>
- Shuping, D.S.S., & Eloff, J.N. (2017). The use of plants to protect plants and food against fungal pathogens: a review. *Afr J Tradit Complement Altern Med.* <https://doi.org/10.21010/ajtcam.v14i4.14>. eCollection 2017
- Szabados, L., & Savouré, A. (2010). Proline: a multifunctional amino acid. *Trends Plant Sci.* <https://doi.org/10.1016/j.tplants.2009.11.009>
- Takci, H.A.M., Turkmen, F.U., Sari, M. (2021). Effect of Cedar (*Cedrus libani* A. Rich) Tar on Bacterial Growth. *J Microbiol Biotechnol Food Sci.* <https://doi.org/10.15414/jmbfs.2020.9.4.805-808>
- Tekin, M., Sari, D., Catal, M., Ikten, C., Smykal, P., Penmetsa, RV., Von Wettberg, E.J., Toker, C. (2018). Ecogeographic distribution of *Cicer isauricum* .P.H. Davis and threats to the species. *Gen Res Crop Evol.* <https://doi.org/10.1007/s10722-017-0509-1>
- Toker, C., Berger, J., Eker, T., Sari, D., Sari, H., Gokturk, R.S., Von Wettberg, E.J. (2021). *Cicer turcicum*: A new *Cicer* species and its potential to improve chickpea. *Front Plant Sci.* <https://doi.org/10.3389/fpls.2021.662891>
- Trapero-Casas, A., & Kaiser, W.J. (1992). Development of *Didymella rabiei*, the teleomorph of *Ascochyta rabiei*, on chickpea straw. *Phytopathol* 82:1261-1266
- Udupa, S.M., Weigand, F., Saxena, M.C., Kahl, G. (1998). Genotyping with RAPD and microsatellite markers resolves pathotype diversity in the *ascochyta* blight pathogen of chickpea. *Theory Apply Gen.* <https://doi:10.1007/s001220050899>
- Üstün, A.S., & Dolar, S. (2001). *Ascochyta* Yanıklığı (*Ascochyta rabiei* (Pass.) Labr.)'na Dayanımları Farklı Nohut Çeşitlerinde Oransal Su, Kuru Madde ve Prolin Miktarlarındaki Değişimler. *J Agri Sci.* https://doi.org/10.1501/Tarimbil_0000000266
- Venditti, A., Maggi, F., Saab, A.M., Bramucci, M., Quassinti, L., Petrelli, D., Vitali, L.A., Lupidi, G., Samrani, A., Borgatti, M., Bernardi, F., Gambari, R., Abboud, J., Saab, M.J., Bianco, A. (2020). Antiproliferative, antimicrobial and antioxidant properties of *Cedrus libani* and *Pinus pinea* wood oils and *Juniperus excelsa* berry oil. *Plant Biosys Inter J Deal Asp Plant Biol.* <https://doi.org/10.1080/11263504.2020.1864495>
- Wani, F.F., Wani, T.A., Shah, T.A., Ayoub, L., Amin, Z., Manzoor, T., Bhat, T.A. (2022). Efficacy of different fungicides, plant extracts and bioagents against *Phoma exigua* causing *Ascochyta* blight of common bean (*Phaseolus vulgaris* L.). *Ind Phytopathol.*

75(4), 1191-1195

Zhang, J., Chen, W., Shang, Y., Guo, C., Peng, S., Chen, W. (2020). Biogeographic distribution of chickpea rhizobia in the world. In *Molecular Aspects of Plant*

Beneficial Microbes in Agriculture; Sharma, V., Salwan, R., Al-Ani, K.L.T., Eds.; Academic Press: Chennai, India, pp. 235–239