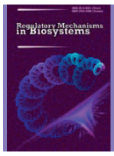

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Effect of *Ascochyta rabiei* on symbiotic efficiency and productivity of *Cicer arietinum*

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The pathological process reduces the intensity of metabolic processes in plants but also negatively affects the symbiotic activity of legumes. Ascochyta blight is the most common disease of chickpeas in Ukraine. The study aimed to determine the influence of *Ascochyta rabiei* (Pass.) on the dynamics of the number and weight of nodules on the root system of chickpeas during the growing season and on the formation of grain yield. Pathogenic microflora had a significant impact on the development and activity of the chickpea symbiotic apparatus. Diseased plants had a smaller number and mass of nodules. A comparative analysis of the results of our experiment showed that plants of the YeS Alunt variety formed a more significant number and weight of nodules. We found that the number and weight of nodules on chickpeas on both experimental varieties were lower: YeS Alunt and Odysei were the lowest, with a strong degree of ascochyta blight damage of 3 points. In the flowering phase (BBCH 60–70), the number of nodules was 86% and 89%, respectively, for the varieties to the control variant. The analysis of the dynamics of the mass of active nodules on chickpea plants showed that in diseased plants this indicator was behind the control by 25% in the budding phase (BBCH 51–59) and by 40% in the flowering phase (BBCH 60–70) in the YeS Alunt variety; by 38% and 28% in the Odysei variety. On average, over the two years of research, the yield was 2.25 t/ha in the YeS Alunt variety and 2.21 t/ha in the Odysei variety. In accordance with the pattern of influence on the symbiotic apparatus, grain yield had a parallel dependence on the degree of ascochyta blight infection of chickpea plants. Chickpea plants with a weak degree of ascochyta blight at harvest showed a decrease in yield by 5.3% (YeS Alunt) and 7.2% (Odysei). The average degree of ascochyta blight damage to chickpea plants led to a shortfall in yield, respectively, by 12.0% and 11.3%. The YeS Alunt variety, in the variant with the most intense damage, showed a decrease in yield by 19.1%, and the Odysei variety by 18.1%.

Keywords: ascochyta blight; chickpea; symbiosis; pathogenic impact; yield loss.

Introduction

Significant warming and an increase in dry growing seasons require the expansion of the area under non-traditional legumes in Ukraine to replace moisture-loving crops (Kalenska et al., 2012; Tkachuk & Telekalo, 2020). The chickpea (*Cicer arietinum* L.) may become one of the most promising legumes for our country, as its sowing is one of the ways to increase soil fertility, solve the problem of food and feed protein production, and establish economic stability of agricultural enterprises (Kalenska et al., 2012; Mazur et al., 2020a; Zhang et al., 2024). However, diseases can be an obstacle to high yields (Pande et al., 2005; Zafar et al., 2021; Fanning et al., 2022).

Despite their microscopic size, pathogens can cause significant losses to farmers. As a biotic factor affecting plant growth, they are a typical problem for the agricultural industry. Pathogenic factors disrupt the normal functioning of plant organisms. The resistance of plants to the harmful effects of pathogens is determined by the accumulation rate of particular substances in the affected area, i.e., phytoalexins. The faster they accumulate, the more resistant the plant becomes to this pathogen (Ferguson & Mathesius, 2014).

When a plant gets infected, its physiological processes change slightly under the influence of pathogens. As a result, it produces lower yields with poorer quality indicators.

In addition to the well-known influence of pathogenic microflora on the physiological functions of plants, it is worthwhile to find out its effect on the symbiotic activity of legumes. Infected plants induce or sharply intensify the production of antibiotic substances, i.e., phytoalexins. This process can prevent legumes from carrying out symbiotic activities with nodule bacteria (Novytska & Barzo, 2013). The formation of root nodules as new morphological structures results from the interaction of two metabolic systems: the host plant and the corresponding strain of microorganisms. These bacteria convert nitrogen in the air into a form available to plants (Didur & Mostovenko, 2021). This complex transformation requires extensive metabolic and physiological changes in both participants (Ryu et al., 2012).

Material and methods

According to the research hypothesis and objectives, a two-factor experiment was designed in triplicate. Factor A is the chickpea varieties YeS Alunt and Odysei. Factor B is the intensity of ascochyta blight damage to chickpeas: weak, medium, and strong degrees. Chickpeas were grown in the experimental field of Vinnytsia National Agrarian University. The sown area of the plots was 30 m^2, and the recorded area was 50 m^2. The experiments were carried out in a crop rotation after winter wheat. The primary soil tillage included two stubble peels: the first was 6–8 cm deep, and the second was 10–12 cm. Granular superphosphate and potassium sulfate fertilizers were applied for fall plowing at 60 kg/ha of P_2O_5 and K_2O. Fall plowing was carried out to a depth of 25–27 cm. The soil was leveled in the spring with a KPS-4G continuous tillage cultivator and spike tooth harrow BShN-1.0 to a 10–12 cm depth. Subsequently, ammonium nitrate was applied at a rate of 30 kg of active substance per ha, after which the second cultivation was carried out with the cultivator USMK-5.4V + harrow BZSS-1.0 unit to the seeding depth. The inoculant for the pre-sowing treatment of chickpea seeds, Rizoactyv Legumes, containing a selective strain of bacteria, was used. The consumption rate of the product is two l/t. It contains *Rhizobium cicer*, nitrogen-fixing bacteria symbionts of chickpeas. The bacterization was carried out on the day of sowing using a mechanized method. The preparation is recommended for the pre-sowing treatment of legume seeds, which ensures the formation of symbiotic relationships between plant roots and bacteria to improve nitrogen nutrition through the biological fixation of atmospheric nitrogen. Nodule bacteria are part of the preparation, symbiosis with leguminous plants, fixing molecular nitrogen and converting it into an ammonium form available to plants.

Sowing used a conventional row method with a row spacing of 15 cm. The seeding rate was 0.7 million germinating seeds. The depth of planting of chickpea seeds was 6–8 cm. Chickpea does not bring cotyledons to the soil surface. Therefore, it can be sown in dry conditions, even to a depth of 12 cm. The control was the variant where chickpea was treated with Trichodermin. The consumption rate of the preparation is 20 g per 5 liters of water. This biofungicide contains spores and mycelium of fungi of the genus *Trichoderma* spp. and their biologically active substances. Bioagents of the preparation are microorganisms of bioprotective action, which multiply in the rhizosphere zone of plants and create a protective barrier against phytopathogens throughout the growing season without adversely affecting the symbiosis of chickpeas with nodule bacteria. The need for seed bacterization increases the requirements for seed treatment. Fungicidal protection was not applied to the experimental variants to assess the impact of ascochyta blight on the symbiotic activity of chickpeas with nodule bacteria. Post-emergence harrowing of chickpea crops was performed in the phase of 3–4 leaves for weed protection.

Phenological observations were carried out according to the methodology to detect the phases of chickpea plants' germination, budding, flowering, and technical maturity.

The mathematical processing of the obtained research results was performed by calculating the arithmetic mean with a confidence interval and analysis of variance using MS Office Excel and Statistica computer programs.

The YeS Alunt variety has a growing season of 82 days. The height of the plants reaches 54.0 cm. The weight of 1,000 grains is 410 g. Resistance to ascochyta blight is 8 points. The Odysei variety belongs to the group of mid-season varieties, and its growing season is 97 days. It is used for grain production. Plant height is 38.5–42.7 cm. The weight of 1,000 grains is 366–370 g. It is characterized by resistance to ascochyta blight on a 9-point scale of 8.0–8.5 points.

The territory of the experimental field has a flat relief. Its soil cover is gray forest medium loamy soils. The soil density was 1.32–1.40 g/cm^3 during the growing season. According to the agrochemical survey of the experimental field, the topsoil has the following physicochemical parameters: humus content (according to Tiurin) is 2.23%, alkaline hydrolyzed nitrogen (according to Kornfield) is 64 mg/kg, mobile phosphorus and exchangeable potassium (according to Chyrykov) are 198 and 89 mg/kg of soil, respectively, and the pH of the salt extract is 5.4. Hydrolytic acidity is 1.7 mg-eq./100 g of soil. Hydrothermal conditions in the region were favorable for chickpea cultivation.

The analysis of weather conditions for the growing season in 2022 and 2023 and their level of variability was based on calculating the hydrothermal coefficient of moisture (HCT) according to Equation 1 and the aridity index (AI) according to Equation 2:

$$\text{HCT}= \frac{\Sigma R}{0,1x\Sigma t}, \quad (1)$$

where ΣR is the amount of precipitation (mm) for the period with a temperature above 10 °C; Σt is the sum of effective (above 10 °C) temperatures for the same period.

$$\text{AI} = \frac{12x\text{Lp}}{\text{T}av+10}, \quad (2)$$

where Lp i T_{av} – precipitation and average air temperature in the given month.

Table 1
Hydrothermal supply in the growing season of chickpea

Years	April		May		June		July	
	hydrothermal coefficient of moisture	aridity index	hydrothermal coefficient of moisture	aridity index	hydrothermal coefficient of moisture	aridity index	hydrothermal coefficient of moisture	aridity index
2022	0.56	57.4	1.43	31.3	1.50	36.1	0.90	22.4
2023	1.54	91.5	0.08	1.90	1.64	38.9	1.41	35.8

According to the table, in April 2022, chickpeas had rather dry starting conditions (hydrothermal coefficient of moisture HCT = 0.56; aridity index AI = 57.4). However, in May and June, it was quite humid for the plants, and the activity of ascochyta blight pathogens. Plants need much moisture to fill grain, so its presence in the soil will positively affect yield. However, the disease affects the plants, leading to the deterioration of metabolic processes and yield reduction.

In 2023, April was excessively wet (hydrothermal coefficient of moisture HCT = 1.54), but May was very dry (hydrothermal coefficient of moisture HCT = 0.08; aridity index AI = 1.9). The high drought tolerance of the selected varieties allowed the plants to survive this difficult period. Then, weather conditions in terms of temperature and precipitation were quite favorable for chickpeas.

The data were statistically analyzed using ANOVA and criterion of significant differences of Tukey's test for average values. The results are expressed as mean and standard error (x ± SE). Differences between the data were considered significant at $P < 0.05$.

Results

The nodules protect bacteria from external adverse factors and supply them with nutrients as plant photoassimilates. The bacteria provide plants with nitrogen fixation products necessary for the growth of the plant organism. If cultivators can provide the most favorable conditions for such cooperation between chickpea plants and nodule bacteria, this will ensure a high grain yield formation.

Observations showed that the formation of nodules on the roots of chickpea plants began 18–20 days after emergence. At the same time, the number and weight of nodules gradually increased, and from the beginning of seed filling, these indicators remained at the achieved level until the grain ripening. Fixation of atmospheric nitrogen by nodule bacteria begins immediately after nodule formation, but at the beginning of the growing season, nitrogen fixation is slow. This process continues until the plants mature, and its highest activity is observed from flowering to bean formation. Only light pink nodules were counted because they have a high degree of nitrogen fixation activity. They are located mainly on the main root of the chickpea and its first-order branches in the soil layer at a depth of 0–15 cm.

The intensity of chickpea damage by ascochyta blight is expressed in points on the following scale: 0 points – no damage; 1 point – a weak degree, single leaves or beans are affected; 2 points – medium degree, up to 1/3 of the leaves or beans are affected; 3 points – severe damage, up to 2/3 or more of the leaves and beans are affected.

Abundant precipitation and moderate temperature conditions create favorable conditions for developing ascochyta blight. High air temperature prevents the disease from progressing.

The infected plants had small necrotic dots, which later increased in size on the affected stems, leaves, and petioles. They were more rounded and eventually merged on the leaves. The spots were elongated and reached several centimeters in length on the stems. They were gray-brown in color and surrounded by a brown border. When counting the number of nodules in chickpea plants and analyzing their weight, we found that these indicators varied in the studied varieties depending on the phase of crop development and the degree of ascochyta blight damage.

Healthy chickpea plants had the highest symbiotic activity of nodule bacteria. The growth and development of plants, their resistance to stress, nitrogen supply, and the yield of legumes will depend on the effectiveness of this symbiosis. The highest number of them was observed in the flowering phase. With increasing degrees of disease damage, the number of nodules on chickpea plants of both varieties decreased. A comparative analysis of the results of our experiment showed that plants of the YeS Alunt variety formed a more significant number and weight of nodules.

The effect of ascochyta blight on the weight of active nodules in the second half of the growing season was also significant (Table 3). At a higher degree of disease damage, plants of both varieties had the lowest nodule weight. In the phase of technical ripeness of chickpea, there was a decrease in the number and weight of nodules, which is explained by the biological aging of plants and probably also by soil compaction. The prevalence of the disease was 12% in 2022 and 11% in 2023 due to the relatively high resistance of the experimental varieties to ascochyta blight.

Table 2
The nodule number dynamics depending on the condition of the chickpea plants (pcs./plant, average for 2022–2023, x ± SD, n = 3)

Variety (factor A)	Degree of disease damage (factor B)	Growth and development phases of chickpeas			
		budding BBCH 51–59	flowering BBCH 60–70	filling grain BBCH 71–79	full ripeness BBCH 81–89
YeS Alunt	0 points (control)	19.1 ± 1.22	34.2 ± 1.66*	32.1 ± 1.67	25.9 ± 1.47
	1 point	18.7 ± 1.21	31.8 ± 1.54	30.5 ± 1.55	24.1 ± 1.34
	2 points	17.2 ± 1.19	30.5 ± 1.51	29.4 ± 1.50	23.0 ± 1.31
	3 points	16.3 ± 1.17	29.4 ± 1.48	28.1 ± 1.47	21.7 ± 1.30*
Odysei	0 points (control)	18.6 ± 1.18	32.9 ± 1.49	30.8 ± 1.42	24.4 ± 1.54
	1 point	18.0 ± 1.16	31.6 ± 1.45	28.9 ± 1.37	23.7 ± 1.46
	2 points	17.4 ± 1.14	30.4 ± 1.41	28.0 ± 1.35	21.4 ± 1.39
	3 points	16.2 ± 1.15	29.3 ± 1.37	27.1 ± 1.32	20.8 ± 1.31*

Table 3
Dynamics of active nodule weight depending on the condition of the chickpea plants (g/plant, average for 2022–2023, x ± SD, n = 3)

Variety (factor A)	Degree of disease damage (factor B)	Growth and development phases of chickpeas			
		budding BBCH 51–59	flowering BBCH 60–70	filling grain BBCH 71–79	full ripeness BBCH 81–89
YeS Alunt	0 points (control)	0.32 ± 0.06	0.60 ± 0.07*	0.51 ± 0.05	0.36 ± 0.06
	1 point	0.30 ± 0.05	0.56 ± 0.06	0.48 ± 0.05	0.31 ± 0.05
	2 points	0.28 ± 0.05	0.49 ± 0.05	0.42 ± 0.05	0.26 ± 0.06
	3 points	0.24 ± 0.04	0.36 ± 0.05	0.34 ± 0.05	0.20 ± 0.04*
Odysei	0 points (control)	0.29 ± 0.05	0.54 ± 0.06*	0.45 ± 0.05	0.29 ± 0.05
	1 point	0.24 ± 0.04	0.50 ± 0.05	0.41 ± 0.05	0.26 ± 0.05
	2 points	0.20 ± 0.04	0.44 ± 0.05	0.34 ± 0.05	0.19 ± 0.04
	3 points	0.18 ± 0.03	0.39 ± 0.04	0.27 ± 0.04	0.17 ± 0.03*

The formation of the crop and its quality indicators is influenced not only by the biological characteristics of the crop but also by environmental conditions. They determine the nature and intensity of physiological and biochemical processes occurring in plants. Since the agronomist can change some of the factors that determine the yield level, the share of their influence on this process can also change. The optimal combination of all productivity elements ensures the growth of chickpea yields. Chickpea plants maximize their genetic potential only when their biological needs are fully met. This is possible with a favorable combination of soil, climatic, and technological factors. Chickpea yields can vary widely depending on growing conditions. However, it should be noted that, unlike other legumes, chickpea is extremely drought-resistant; its plants do not lodge, it is characterized by smooth ripening, the beans do not crack, and the grain does not fall off.

Table 4
Chickpea yield depending on plant condition (t/ha, x ± SD, n = 3)

Variety	Degree of disease damage	2022 year	2023 year
YeS Alunt	0 points (control)	2.18 ± 0.510*	2.32 ± 0.551*
	1 point	2.05 ± 0.463	2.21 ± 0.534
	2 points	1.91 ± 0.314	2.04 ± 0.482
	3 points	1.76 ± 0.289*	1.87 ± 0.413*
Odysei	0 points (control)	2.13 ± 0.492	2.28 ± 0.508
	1 point	2.01 ± 0.453	2.08 ± 0.469
	2 points	1.94 ± 0.395	1.97 ± 0.457
	3 points	1.78 ± 0.321	1.84 ± 0.444

Deviations from the control variant are significant since they exceed the least significant difference of the experiment. The deterioration of the photosynthetic apparatus and the symbiotic activity of chickpeas caused a decrease in the yield of infected plants.

The weather conditions in 2023 were more favorable for the growth and development of chickpea plants than in 2022, which affected its yield. Chickpea grains affected by ascochyta blight were smaller and had a flatter shape. The lowest yields were recorded for those chickpea plants that had a severe degree of ascochyta blight damage. About 2/3 of their leaves and beans were damaged during harvest. The YeS Alunt variety reduced the yield by 0.43 t/ha on average over the two years of research, and the Odysei variety by 0.40 t/ha.

Discussion

Due to various factors, the symbiosis process can deviate from the natural program (Nascimento et al., 2012; Mazur et al., 2020b). In particular, it can be affected by the reaction of the soil solution, lack of nutrients or moisture, and the vital activity of pathogens in the plant (Tkachuk et al., 2023; Ben Gaied et al., 2024).

On soils with a low pH value, the permeability of membranes in plant cells changes, so rhizobia cannot penetrate them and attach to root hairs (Chabaniuk & Brovko, 2017; Didur et al., 2019).

In addition, the lack of carbohydrates in legumes leads to a decrease in nitrogen fixation activity (Foresto et al., 2023). Therefore, it causes necrotic processes in the nodules (Chabaniuk & Brovko, 2017; Plett et al., 2021; Zhang et al., 2024).

Some researchers suggest that the symbiosis period can be extended by liming the soil and foliar fertilizing with microelements (Didur & Mostovenko, 2021). The duration of symbiosis and nodule weight will determine the indicators of both total (TSP) and active (ASP) symbiotic potentials (Mostovenko et al., 2022; Mazur et al., 2023).

Nodules on plants that have suffered from drought cannot resume their activity after water metabolism is normalized. New smaller nodules can form on the root hairs. They will have a lower nitrogen fixation level than those formed earlier but lose viability (Chabaniuk & Brovko, 2017, Didur et al., 2020).

In Ukraine, the chickpea is threatened mainly by fungal diseases such as ascochyta blight, fusarium wilt, white rot (or sclerotinia), and gray rot (Kyryk & Pikovskyi, 2020).

Among the latter, one of the most widespread is ascochyta blight. Caused by *Ascochyta rabiei*, it can be detected in leaves, roots, and other plant tissues and can lead to total yield losses or significantly reduced quality in vulnerable, untreated cultivars (Foresto et al., 2023).

Ascochyta blight is characterized by brownish-dark spots of various sizes and shapes on chickpea plants, a promising crop for Ukraine. Its first visible signs appear during germination in the form of oblong, elliptical, slightly depressed dark brown spots. Leaves turn yellow, wither, and fall off with severe infection. Spotting on the stems can lead to fractures and wilting of the plant (Pande et al., 2005).

The symbiosis of legumes and rhizobia is very sensitive to pesticides. All treatments inhibit nodule formation and reduce nitrogen-fixing activity to varying degrees. The combination of therapy with nitrogenization of seeds delays the onset of nodule formation and reduces their number by 1.3–7.5 times, biomass by 1.5–3.3 times, and nitrogenase activity by 1.2–3.8 times (Kolesnikov & Kadyrov, 2022).

According to Logosha et al. (2023), it was established that *Mesorhizobium ciceri* ND-64 strain exhibits the highest cytokinin activity in the bioassay. Cytokinins in the total amount of 174.94 μg/g of completely dry biomass were detected in the culture medium of *M. ciceri* ND-64, which is 53% higher than that of *M. ciceri* ND-101 strain and 99% higher than that of *M. ciceri* H-12 strain.

The overall effect of the symbiosis and its longevity are strongly dependent on many regulatory factors during the plant's growing season (Wanjofu et al., 2022; Gabilondo et al., 2023; Ben Gaied et al., 2024). Symbiotic relationships between plants and microorganisms play an essential role in their life (Rafique et al., 2022; Zhang et al., 2024). In particular, rhizosphere bacteria can synthesize various phytohormones and enzymes that improve plant growth and reduce the stressful effects of unfavorable environmental conditions (Ben Gaied et al., 2024).

As a result of biological nitrogen fixation, plants are fed with cheap and environmentally safe nitrogen because bacteria from the air fix it. For a nitrogen-fixing (legume-rhizobial) system to be formed, it is necessary to carry out the process of nitrogenization, which is the presowing treatment of chickpea seeds with a biological product of a selective strain of nodule bacteria. As a result, this system will provide cultivated plants with molecular nitrogen from the air, allowing for a higher level of chickpea yield. The number and weight of nodules in symbiotic activity depend on many factors (Belay et al., 2022). In our research, we have examined in detail the impact of *Ascochyta rabiei* on chickpeas' symbiotic efficiency and productivity.

It is necessary to provide optimal conditions for legume-rhizobial symbiosis to increase the efficiency of biological nitrogen fixation in chickpeas (Zafar et al., 2021). Plant health, optimal soil moisture and density, and favorable temperature in the first half of summer are essential conditions for nodule formation and active nitrogen fixation (Rowson et al., 2024). An increase in rhizobia in the presence of a plant can be caused by both specific and nonspecific stimulation of microbial populations by root exudates (Plett et al., 2021). As metabolic processes in infected plants change, their root exudates' chemical composition and flow into the soil also change (Yang et al., 2022). This, in turn, will affect the symbiotic activity of the legume and nodule bacteria (Zafar et al., 2021).

Conclusions

The number of nodules on the root system of chickpea was lower in infected plants, from budding to full ripeness, than in the control variant. The lowest number of nodules was observed at a high degree (3 points) of ascochyta blight damage on both experimental varieties.

The analysis of the dynamics of active nodule mass of chickpeas showed that the highest rate was recorded in the flowering phase (BBCH 60–70) in all experimental variants. With a substantial degree of ascochyta blight damage to chickpeas, plants of the YeS Alunt variety had 40% less weight, and the Odysei variety had 27.8%.

The more severe the degree of ascochyta blight damage, the lower the grain yield. Plants of the YeS Alunt variety with a lesion intensity of 3 points at harvest showed a decrease in yield by 19.1%, and the Odysei variety decreased yield by 18.1%.

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