CONTROL OF SEEDLING MORTALITY IN THE LONG-LEAVED SAUCER-BERRY *CORDIA SINENSIS* LAM. IN KHARGA OASIS, EGYPT: AN IN SITU PHYSIOLOGICAL, CHEMICAL, AND BIOLOGICAL APPROACH

GALAL, T. M.[1*] – KAWEY, S. M. A.[2] – EL-GAMAL, M. A.[3] – HASSAN, L. M.[2] – AL-MUTAIRI, K. A.[4] – EL-BAKRY, A. A.[2]

[1]*Department of Biology, College of Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia*

[2]*Botany and Microbiology Department, Faculty of Science, Helwan University, Cairo, Egypt*

[3]*Soils, Water and Environment Research Institute SWERI, Agriculture Research Center, Giza, Egypt*

[4]*Biology Department, College of Science, University of Tabuk, Tabuk 47512, Saudi Arabia*

**Corresponding author*
e-mail: t.aboseree@tu.edu.sa; phone: +966-57-015-9791; ORCID: 0000-0001-9847-1051

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Abstract. The purpose of this study was to in situ assess the effects of a variety of chemical and biological treatments on the mortality and seedling growth of the Long-leaved saucer-berry (*Cordia sinensis*) in Kharga Oasis, Egypt. Nine plots (5 x 5 m) in the high mortality ruderal habitat were chosen and fenced, where fifteen seedlings per plot were labeled and fixed for every treatment. During the course of six consecutive months, we measured seedling mortality, height, number of leaves per seedling, length and width of leaves as well as the total nitrogen, phosphorus, and potassium (NPK) and pigment content of the apical leaves. In the seedlings' rhizosphere, the total microbial count and plate count of specific beneficial bacteria were enumerated, followed by analysis of their impact on disease incidence. Treating seedlings with abscisic acid (ABA) and synthetic fungicide together was more efficient in mitigating seedling mortality and increasing their growth performance. The highest NPK and pigment contents were also obtained with this combined treatment, followed by treatment with nitrogen-fixing bacteria such as *Azotobacter* spp. On the other hand, the rhizosphere's beneficial bacteria and total microbial population were the lowest in the ABA + fungicide and *Azotobacter* treatments, which also significantly reduced the severity of the pathogenic fungi-induced diseases. The current investigation found that treating seedlings with nitrogen-fixing bacteria. or ABA + fungicide reduced disease and environmental stress, which in turn greatly reduced mortality and improved plant growth metrics showing potential for integrated stress and pest management, thus, will be useful for the in-situ conservation and propagation of the target species for foreseeable commercial benefits.
Keywords: *abscisic acid, fungicides, in situ conservation, medicinal plant, pest management*

Introduction

The genus *Cordia* (Cordiaceae, ex Boraginaceae) comprises roughly 350 species of shrubs and trees that are found in tropical and subtropical forests across Africa, Asia, India, and Central and South America (Galíndez et al., 2019; El-Massry et al., 2021). According to Matias et al. (2015) and Kale and Bhale (2022), a significant number of species are valued primarily for their timber resources, and they are also utilized in restoration projects and for aromatic, medicinal, or ornamental purposes. *Cordia sinensis* Lam. (Long-leaved saucer-berry) is a popular indigenous multipurpose tree species that is useful for fuel wood, timber, fruits, and fodder (Ndung'u, 2018). It is used to treat respiratory and digestive disorders as well as for its anti-inflammatory and antioxidant properties (Marini et al., 2018; Chen et al., 2023). The IUCN Red List of Threatened Species most recently listed it as Least Concern in 2020 (Shaltout and Bedair, 2022). This species is only known from gardens in south-western Egypt, and it seems to be limited to cultivated or naturalized stands in Yemen and Palestine. According to Ndung'u and Kimiti (2017), seedlings are produced using seeds. It grows in clay, clay-sand, or other alluvial soils at elevations up to 1240 m above sea level. *C. sinensis* can also thrive in arid regions on steep hillsides, in limestone cliff crevices, and along wadis (Warfa, 1988).

Biological interactions including competition, mutualism, parasitism, and herbivory have a significant impact on the spatial distribution, abundance, and diversity of species in plant communities (Seiwa et al., 2019). The growth and survival of *C. sinensis* seedlings are influenced by the site physiography and the soil physical and chemical properties (Bergmann et al., 1994). According to Seiwa et al. (2019), the possible reasons of seedling mortality are infections, herbivore grazing, withering (dehydration and uprooting), and/or physical damage. It was difficult to determine the reason of small *C. sinensis* seedling mortality, since pathogens account for the majority of seedling deaths. Pathogens can quickly cause *Cordia* seedlings to perish, but other causes, such as a tortoise-shell beetle's (Chrysomelidae) strong leaf herbivory, can also induce seedling mortality (Augspurger, 1984). Furthermore, Hattori et al. (2013) found that drought in the early post-planting period probably enhances seedling mortality. According to Marod et al. (2002), young seedlings in their first year of growth are probably the stage of life most vulnerable to drought. Moreover, high surface soil compaction (at 10–20 cm depth) impeded lateral root growth and accelerated seedling mortality in the early stages, indicating that soil compaction has a significant impact on plant growth and/or mortality rates (Hattori et al., 2013).

Based on samples taken from several farms in the New Valley Governorate of Egypt, it was shown that *Fusarium oxysporum* and *Rhizoctonia solans* are the two most common soil-borne pathogenic fungi that infect C. sinensis and cause damping off root rot and wilt illnesses (Abdel Kawey, 2016). According to Weller et al. (2002), biological management is a proven environmentally safe and long-term way to shield plants from soil-borne diseases. To combat soil-borne diseases, biological control has recently been employed instead of synthetic fungicides (Nico et al., 2005). In particular, bacteria such as *Bacillus circulans, Bacillus megaterium, Pseudomonas fluorescens*, and *Aspergillus species*, as well as some fungi, such as *Trichoderma, Penicillium,* and *Aspergillus* species, were primarily selected as antagonistic microorganisms in research studies (Gacitua et al., 2009). It has been demonstrated that a number of microorganisms, including bacteria, can promote plant growth in situations where they are associated with roots, and consequently have a direct impact on plant health and soil fertility (Kamal et al., 2009).

The phytohormone abscisic acid (ABA) is commonly known to control stomatal closure, slows down shoot growth, and preserves primary root elongation (Finkelstein et al., 2002). According to Finkelstein et al. (2002) and Chen et al. (2006), ABA is essential for taller plants to respond to unfavorable environmental conditions and mitigates the harmful effects of heat stress, which results in seedling mortality. Additionally, it has been noted to slow down the seedlings drying rate, suggesting that

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plants are developing a greater resistance to dehydration (Yang et al., 2007). The synthesis and export of ABA from roots as well as its redistribution within leaves are the two factors contributing to elevated leaf ABA concentrations (Franks and Farquhar, 2001). Investigation of the effect of ABA alone without concomitant decreases in water potential or turgor was made possible by the use of exogenous ABA to chemically replicate dry conditions in hydroponic plants (Franks and Farquhar, 2001). Furthermore, numerous chemical fungicides that work well against soil-borne pathogens is a soil amendment that can also be used to prepare planting material (Akhter et al., 2015). One of these fungicides is Rizolex, which is a contact fungicide that protects against fungal infections transmitted through the soil and seeds that result in seed degeneration and damping-off of seedlings (Abdelhady and Elgohry, 2013).

Information on seed biology and seedling mortality in Cordia species is currently limited and mostly focuses on dormancy and the conditions necessary for seed germination (Baskin and Baskin, 2014; Ndung'u, 2018). The current research was conducted to investigate, in situ, the effect of ABA to alleviate drought stress on *C. sinensis* seedlings and to use biological control agents and chemical inducers to inhibit the growth of the pathogenic organisms *Fusarium oxysporium* and *Rhizoctonia solani*. Determining the effect of ABA, biocontrol agents and chemical inducers on promoting growth and survival of seedlings would lead to decreasing seedling mortality, which will be of potential to use in the propagation and in situ conservation of the target species. Also, the outcome of this study will help in reducing plant mortality, enhancing growth, and pest management.

Materials and methods

Experimental

The experimental site (25° 27′ 24.6 N and 30° 32′ 40.7 E) was laid in Kharga Oasis at the south of the western Egyptian desert. The extreme length of Kharga depression is about 200 km, while its width varies from 20 to 80 km with an area of 7200 km^2. The prevailing climate indicated that the study site lies in a dry rainless part of the Great Sahara with average annual rainfall of 0.0042 mm/year, while the mean annual temperature was 25.5 ºC. Moreover, the mean annual relative humidity was 35.9%, while the annual mean of wind speed was 5.5 Km h^{-1} (NASA–POWER, 2015). Nine plots (1 × 1 m) were selected in the ruderal area of the *C. sinensis* tree to study the seedling mortality. Seventy-five seedlings were chosen in a randomized complete block design; where each treatment was applied three times every 15 days after germination (15 seedling, 5 treatments, 3 replicates). Fifteen seedlings were marked for each of the following chemical and biological treatments: (1) control, sprayed with distilled water (250 ml/plot), (2) abscisic acid (100 mg l^{-1}) for both 250 ml sprayed per plot, (3) Rizolex (T50% wp) a synthetic fungicide obtained from Chemtura, Australia comp. pty Ltd, at a concentration of 3 g l^{-1}, added at a rate of 1 ml/seedling, (4) abscisic acid + Rizolex, and (5) bacteria spp. (*Azospirillum* sp., *Azotobacter* sp., *Bacillus circulans*, *Bacillus megaterium*, and *Pseudomonas fluorescens*) were applied to the soil surface. All bacteria species were grown on their specific medium and enriched on nutrient broth medium (Difco, 1985), then applied as 1×10^8 cfu ml^{-1} at a rate of 2 ml/seedling according to the treatment. The distance between each two seedlings was kept about 10 cm. Data were collected monthly

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during six consecutive months from April to September 2016, and the following criteria were measured:

Seedling mortality

Seedling mortality was determined monthly according to the following equation (Moles and Westoby, 2004):

$$\text{Mortality (\%)} = \frac{\text{Total number of dead seedlings}}{\text{Total number of seedlings}} \times 100 \quad \text{(Eq.1)}$$

Growth parameters

Seedling height, number of leaves per seedling, and leaf length and width for the 4 apical leaves were monthly measured for each treatment. The mean internode length was measured at the 5th and 6th months, where no wide variation between treatments was observed during the early stages.

Physiological parameters

Pigment content

The 1st four apical pairs of fresh leaves from each treatment were harvested for quantitative determination of chlorophyll and carotenoids following Allen (1989). To extract pigments, 2 g fresh materials were homogenized in 85% aqueous acetone for 5 min, the homogenate was centrifuged at 6000 rpm for 10 min and the supernatant was brought to known volume using acetone. The supernatant was measured against a blank of 85% acetone at three wave lengths (452, 645 and 664 nm) using Jenway 6300 spectrophotometer. The concentration of the pigment fractions (chlorophyll a, chlorophyll b and carotenoids) was determined as μ ml^{-1} using the following equations:

$$\text{Chlorophyll a} = 10.3\ \text{E664} - 0.918\ \text{E645} \quad \text{(Eq.2)}$$

$$\text{Chlorophyll b} = 19.7\ \text{E645} - 3.87\ \text{E664} \quad \text{(Eq.3)}$$

$$\text{Carotenoids} = 4.2\text{E452} - (0.0246\ \text{Chla} + 0.426\ \text{Chlb}) \quad \text{(Eq.4)}$$

where E is the absorbance at 663, 644 and 453 nm. Then the values were expressed as (mg g^{-1} fresh wt.).

NPK determination

The apical four pairs of leaves were harvested from each treatment, oven dried at 65°C, and then powdered by grinding in a metal-free plastic mill and then passed through a sieve of 2 mm mesh size. A ground sample of 1 g was digested in a 20 ml tri-acid mixture of HNO_3:$HClO_4$:HF (1:1:2 *v:v:v*). Total nitrogen (N) was assessed by the Kjeldahl method; P was analyzed using molybdenum blue method using spectrophotometer (CECIL CE 1021); and K were analyzed using a flame photometer (CORNING M410). All these procedures for plant analysis were outlined by Allen (1989).

Microbial bioassay

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All bacterial populations were grown on nutrient agar medium (Difco, 1985), while Jensen's agar medium was used for growing the populations of actinomycetes and Dox agar medium for counting of fungal groups (Allen, 1950).

1. *Azospirillum* sp. and *Azotobacter* sp.: Ashby's liquid medium (Ishac and Abdelmalak, 1968) was used for growing *Azotobacter* sp., while Dobereiner (1976) medium was used for *Azospirillum* sp.
2. *Bacillus circulans* was grown according to Alexandro's agar medium (Zahra, 1969).
3. *Bacillus megaterium* was grown according to Pikoveskya's agar medium (Pikoveskya, 1948).
4. *Pseudomonas fluorescens* was grown according to King's agar medium (King et al., 1954). This strain is characterized by producing fluorescent green pigments on the growth medium.
5. Half gram of soil with infected roots was collected from the rhizosphere of diseased seedlings (symptoms of wilting) and transferred directly onto test tubes containing 4-5 ml sterilized tap water. The tubes were shaken vigorously on a vortex for 1 min; then 1 ml of the suspension placed in a sterile Petri dish (3 replicates were used) and Dox agar medium was poured then the plates after solidification were incubated for 5 days at 25°C ± 2 (Allen, 1950). Further purifications were carried out to obtain pure isolates of the pathogenic genera (*Rhizocotonia solani* and *Fusarium oxysporium*).

Effect of different treatments on disease severity

Disease severity and efficacy of the bioagents was evaluated to determine the inhibition of *R. solani* and *F. oxysporium* pathogenicity. The number of symptomatic leaves and dead plants were counted during six months. The disease on each plant was rated numerically as follows: 5 = plant dead; 4 = 76–100% of leaves with symptoms; 3 = 51–75%; 2 = 26–50%; 1 = < 25%; and 0 = no symptoms. Disease severity was calculated as recommended by Liu et al. (1995):

$$\text{\% Disease severity} = \frac{\Sigma nr}{SN} \times 100 \quad \text{(Eq.5)}$$

where n is the number of diseased seedlings in each numerical rate, N is the total number of plants multiplied by the maximum numerical rate, r = s. Disease efficacy was calculated as follows:

$$\text{\% Efficacy} = \frac{\text{Disease severity value of the control} - \text{Disease severity value of the treatment}}{\text{Disease severity value of the control}} \times 100 \quad \text{(Eq.6)}$$

Statistical analysis

Statistical analysis was performed using SPSS software version 15.0 (SPSS, 2012). One-way analysis of variance (ANOVA I) was applied to assess the significance of variations in the growth parameter and physiological variables after checking the data for normality and variance homogeneity with the use of the Shapiro–Wilk and Levene tests, respectively, and, when necessary, the data were log-transformed. A post-hoc test (Duncan's multiple range tests and LSD) was applied when differences are significant.

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Results and discussion

Seedling mortality

Although information on seedling and small tree death rates has been published, it is rather rare in the literature (Vickers et al., 2019). Environmental factors such as cold, drought, desiccation, salt, and mechanical wounds induce the creation of ABA, which speeds up plant acclimatization under stress circumstances (Zainudin and Awang, 2004). Plants and crops often face an increasing number of abiotic and biotic stress combinations as a result of global warming and probable climate irregularities, which have a negative impact on growth and productivity (Mittler, 2006). The combination of abiotic stresses such as drought and heat has been proven to be more damaging to crop productivity than these conditions alone (Ramegowda and Senthil-Kumar, 2015). Our study found a significant percentage ($P < 0.001$) of seedling mortality due to the extreme climatic conditions in the study location, specifically high temperatures and dryness. This finding was consistent with that of Ivanov et al. (2019), who found that draught stress of varying intensities from weak to extremely strong considerably enhanced the mortality of pine and spruce seedlings. In semi-arid areas such as Egypt, seedlings and saplings frequently die (Negussie et al., 2008). Furthermore, Pretreatment of rice seedlings with ABA was shown to promote survival and growth under salt stress (Gurmani et al., 2013) and alkaline environments (Wei et al., 2015).

C. sinensis exists in some environments on the edges of cultivated land and in ruderal areas, therefore it was suggested that pathogenic microorganisms, particularly those with a large host range, could be a cause of seedling mortality. As a result, our approach encompassed the use of chemical and biological treatments to alleviate environmental and disease stresses, either alone or in combination. The data presented in *Figures 1* and *2* demonstrated a significant decrease ($P < 0.001$) in the seedling mortality of *C. sinensis* that was subjected to several chemical and biological treatments. The results show that some common fungi, such as *Fusarium* and *Rhizoctonia* species, cause root rot and damping off in *C. sinensis* seedlings. *Rhizoctonia* and *Fusarium* species are soil-borne plant diseases and disease-causing fungi that can form sclerotium-resistant structures in plant residues (Haryuni et al., 2020) and infect roots, causing vanilla stem rot disease (Agrios, 2005), respectively. The fungicide, *Pseudomonas florescence*, and *Azotobacter* sp. were shown to cause no seedling mortality in *C. sinensis* seedlings until the third month, however a combination treatment of ABA and fungicide did not cause any mortality until the fourth month. ABA + fungicide reduced seedling mortality, which positively influenced the morphological and physiological features of *C. sinensis* seedlings, and ABA can regulate various critical elements of plant growth and development. Furthermore, some earlier studies found that ABA has an antibacterial effect on several fruit trees, including apple and papaya (Qi et al., 2011; Acedo et al., 2012; El-Mogy et al., 2020), as well as altering the gene level of plant adaptation to adverse environments (Franks and Farquhar, 2001). The control treatment showed 100% seedling mortality after six months (*Fig. 3*). Treatments were arranged, based on how well they reduced the death seedling mortality, as follows: ABA + fungicide > *Azotobacter* sp. > *Pseudomonas florescence* > fungicide > *Azospirillum* sp. > *B. circulans* > ABA > *B. megaterium*. Under field conditions, an integrated management strategy that included the use of combined bioagents was found to be much more effective in reducing seedling mortality and improving plant output than individual bioagent applications (Akhter et al., 2015).

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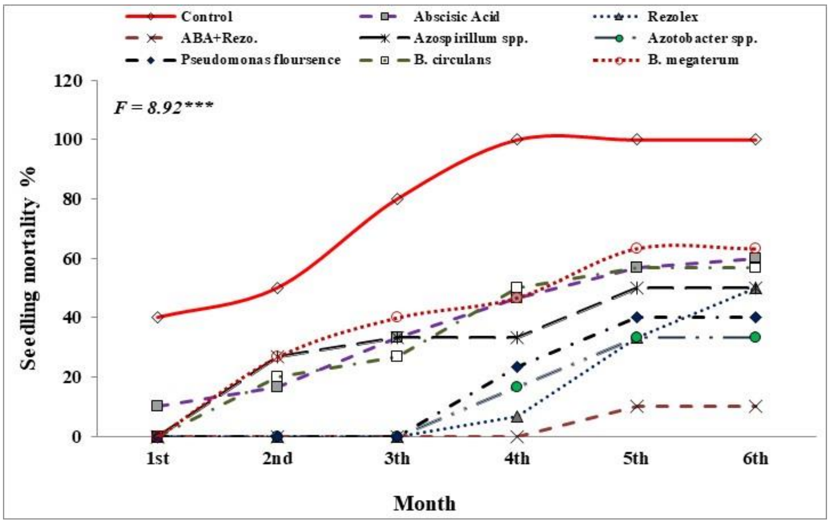

Figure 1. *Effect of different chemical and biological treatments on the monthly seedlings' mortality (%) of Cordia sinensis during six consecutive months. ABA: abscisic acid, Rezo: Rizolex, B. circulans: Bacillus circulans, B. megaterium: Bacillus megaterium. ***P < 0.001*

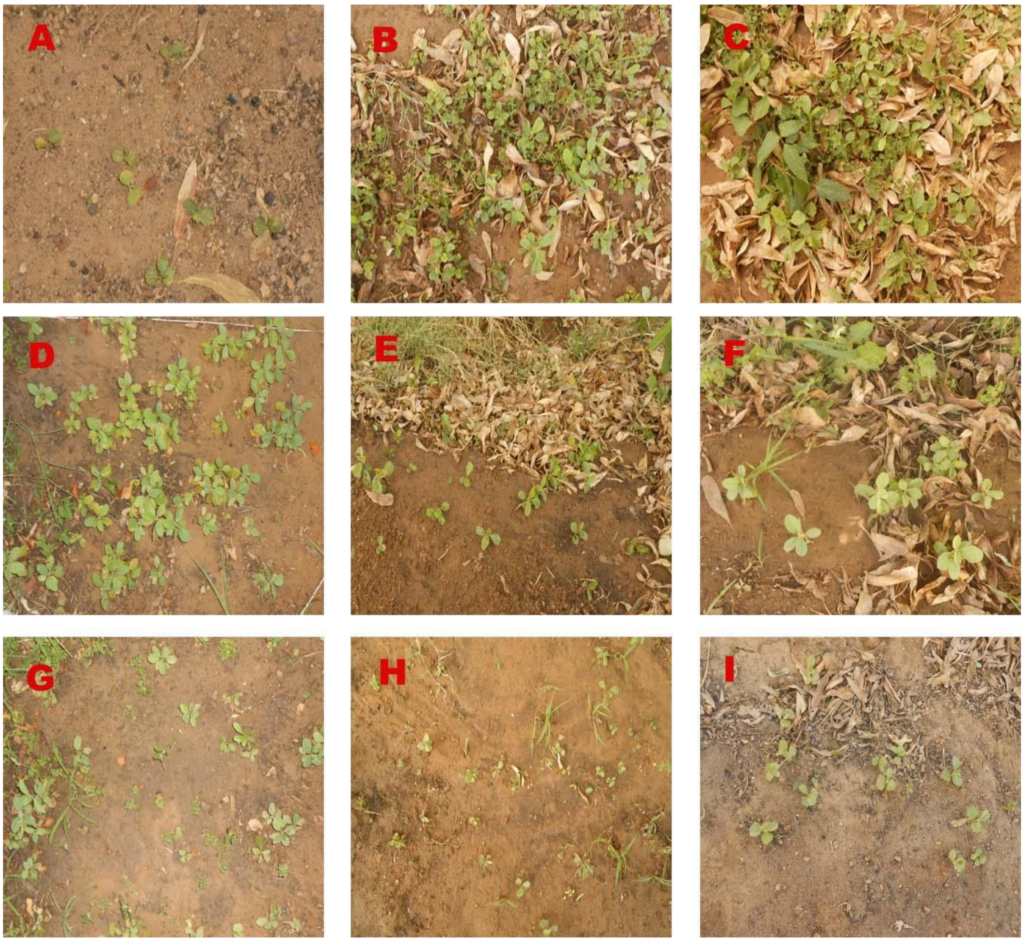

Figure 2. *Cordia sinensis seedling under the effect of treatments after three months: (A) untreated control; (B) Abscisic acid; (C) Fungicide; (D) Abscisic acid + fungicide; (E) Azotobacter sp.; (F) Azospirillum sp.; (G) Pseudomonas fluorescens; (H) Bacillus circulans; (I) Bacillus megaterium*

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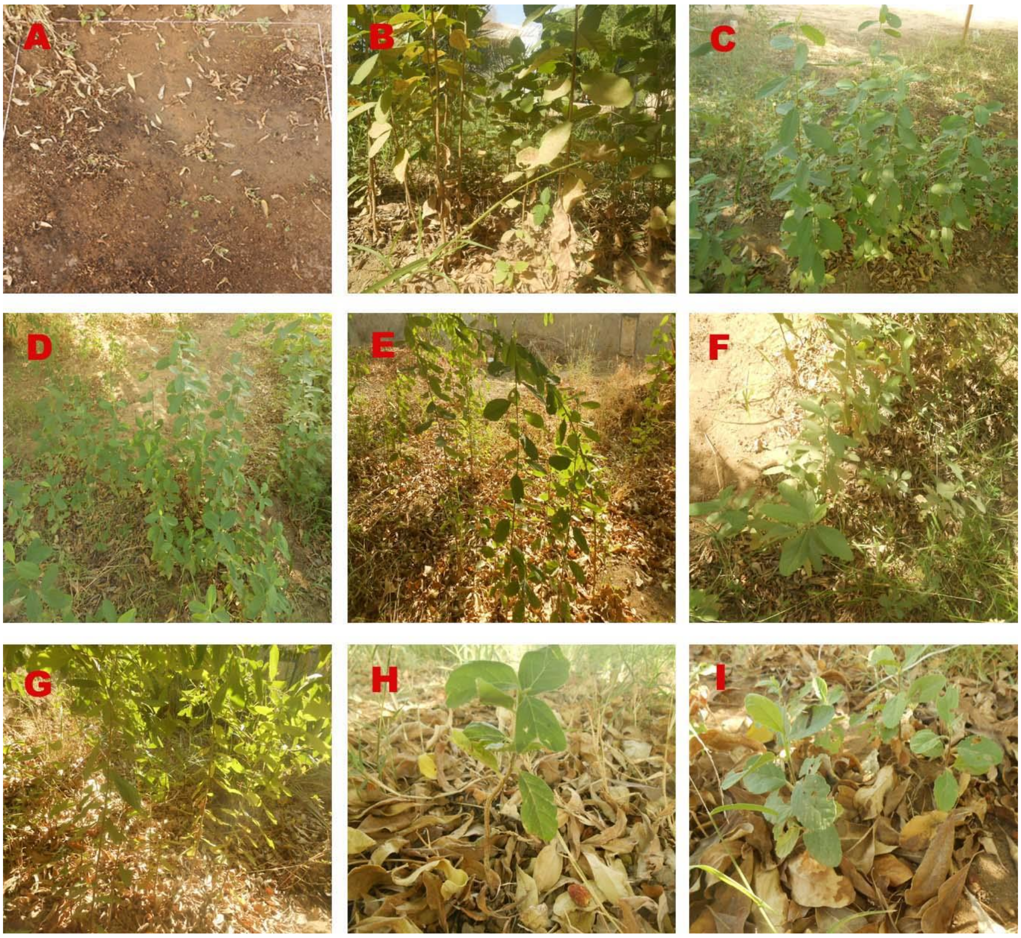

Figure 3. *Cordia sinensis seedling (6 months old) under the effect of different biochemical treatments. (A) untreated control; (B) Abscisic acid; (C) Abscisic acid + fungicide; (D) Fungicide; (E) Azospirillum sp.; (F) Azotobacter sp.; (G) Pseudomonas fluorescens; (H) Bacillus circulans; (I) Bacillus megaterium*

Seedling growth parameters

High levels of mortality during the seed and seedling stages may have an impact on plant abundance and spatial distribution (Alexander and Mihail, 2000). Pathogenic soil microorganisms can induce seed and seedling mortality, as well as slow seedling growth rates in natural communities, and they play a role in population dynamics and community structure. The statistical analysis (ANOVA I) revealed significant changes ($P < 0.05$) in seedling height, number of leaves per seedling, leaf length and width, and internode length among the different biochemical treatments (*Tables 1–3*). Based on their effect on growth measurements, the treatments were arranged as follows: ABA + fungicide > fungicide > ABA > *Azotobacter* sp. > *P. fluorescens* > *Azospirillum* sp. > *B. circulans* > *B. megaterium*.

Seedling height

In the control treatment, the seedling height gradually increased from 4.1 cm at the 1st month until 5.3 cm at the third month, and after that the seedling was died (*Table 1*). Compared with the control, the (ABA + fungicide) was significantly ($P < 0.05$) the

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most efficient in enhancing seedling height, where it started with 9.0 cm length at the 1st month and ended with 67.4 cm at the 6th month.

Table 1. *Monthly variation (mean ± SE) in the Cordia sinensis seedling height (cm) under the effect of different chemical and biological treatments*

Treatments	**Month**					
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**
Control	4.1 ± 0.2^d	5.3 ± 0.3^d	5.3 ± 0.3^g	0.0^g	0.0^f	0.0^f
Abscisic acid (ABA)	6.5 ± 0.3^b	8.5 ± 0.4^b	10.7 ± 0.4^c	19.9 ± 1.1^c	29.8 ± 4.1^c	33.6 ± 4.5^d
Fungicide	4.9 ± 0.3^c	6.7 ± 0.5^c	8.9 ± 0.5^de	22.2 ± 0.8^b	42.4 ± 0.6^b	49.1 ± 0.5^b
ABA + fungicide	9.0 ± 0.3^a	11.4 ± 0.3^a	17.7 ± 0.7^a	35.2 ± 1.4^a	55.2 ± 1.4^a	67.4 ± 1.3^a
Azospirillum sp.	3.6 ± 0.2^de	6.9 ± 0.3^c	8.5 ± 0.4^e	13.8 ± 0.4^e	15.0 ± 0.4^d	30.2 ± 0.8^d
Azotobacter sp.	6.1 ± 0.3^b	8.8 ± 0.4^b	12.7 ± 0.4^b	17.3 ± 0.4^d	18.5 ± 0.4^d	39.3 ± 0.6^c
Pseudomonas fluorescens	5.0 ± 0.2^c	7.4 ± 0.2^c	10.2 ± 0.3^cd	15.4 ± 0.3^de	16.6 ± 0.3^d	34.9 ± 0.8^cd
Bacillus circulans	2.9 ± 0.2^e	5.1 ± 0.2^d	7.0 ± 0.2^f	10.4 ± 0.7^f	11.6 ± 1.0^e	15.5 ± 1.7^e
Bacillus megaterium	2.9 ± 0.2^e	5.1 ± 0.2^d	6.5 ± 0.1^f	9.6 ± 1.0^f	11.2 ± 1.3^e	12.6 ± 2.1^e
LSD at 5%	**0.76**	**1.1**	**1.5**	**2.3**	**5.6**	**5.7**

Maximum and minimum values are underlined. Means with different letters are significantly different at $p < 0.05$ according to Duncan's test

Number of leaves

The number of leaves per seedling in the control treatment gradually increased from 5.4 ± 0.5 at the 1st month until 7.5 ± 1.1 at the third month (*Table 2*). After six months of seedling growth, ABA + fungicide combined treatment had the greatest effect on the number of leaves per seedling (45.1 ± 0.7), while *B. megaterum* had the lowest (6.1 ± 1.3 leaves/seedling). Wei et al. (2017) found that ABA administration significantly ($P < 0.05$) reduced leaf withering by 22%-50% and seedling death by 11%-17% compared to the control treatment. Besides, all biological treatments showed slight increase, except *B. megaterum* that recorded decline in the number of leaves. The current study found that the bioagents had a considerably ($P < 0.05$) higher stimulating effect on plant development features than the control. Plant growth-promoting bacteria, such as *Azotobacter* and *Azospirillum*, have been shown to minimize fungal disease in numerous plants by creating secondary metabolites and antimicrobial compounds that are hostile to many phytopathogenic fungi and bacteria that cause plant diseases. These bioagents showed biocontrol ability against a variety of fungal diseases, including root rot disease caused by *Fusarium* and *Rhizoctonia* spp. (Kamal et al., 2009).

Leaf morphology

The statistical analysis of the morphometry of *C. sinensis* seedling leaves, during the 5th and 6th months of growth, showed considerable variation ($P < 0.05$) among the different applied treatments (*Table 3*). The combined treatment (ABA + fungicide) gave the optimum results of leaf length, width, and petiole length (7.0, 5.6 and 9.7 cm, respectively) after the 6th month, while treatment with *B. megaterium* showed the lowest (1.9, 1.5, and 1.3 cm). The combination treatment of ABA and fungicide had

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the most stimulating effect (*P < 0.05*) on seedling height, number of leaves per seedling, leaf length and width, and internode length, while *B. megaterum* had the least. Furthermore, the ABA spray greatly improved drought resistance, with a survival percentage of 100% following rehydration against 35% for water-sprayed plants (Khoshniat et al., 2023).

Table 2. *Monthly variation (mean ± SE) in the number of leaves per seedling of Cordia sinensis under the effect of different chemical and biological treatments*

Treatments	**Month**					
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**
Control	5.4 ± 0.5 d	7.3 ± 0.5 e	7.5 ± 1.1 f	0.0 e	0.0 g	0.0 g
Abscisic acid (ABA)	8.1 ± 0.6 c	9.9 ± 0.6 d	11.8 ± 0.7 d	16.8 ± 0.8 c	26.5 ± 3.5 c	26.5 ± 3.5 c
Fungicide	8.4 ± 0.5 c	10.5 ± 0.5 cd	10.5 ± 0.5 e	19.5 ± 0.5 b	36.3 ± 0.6 b	36.3 ± 0.4 b
ABA + fungicide	10.4 ± 0.4 ab	12.4 ± 0.4 b	22.3 ± 0.6 a	31.1 ± 1.0 a	44.5 ± 1.1 a	45.1 ± 0.7 a
Azospirillum sp.	6.4 ± 0.4 d	12.7 ± 0.5 b	14.0 ± 0.4 c	15.7 ± 0.4 c	15.7 ± 0.4 e	15.7 ± 0.4 e
Azotobacter sp.	11.5 ± 0.6 a	14.5 ± 0.4 a	17.9 ± 0.4 b	19.7 ± 0.4 b	19.7 ± 0.4 d	21.2 ± 0.3 d
Pseudomonas fluorescens	9.2 ± 0.5 bc	11.5 ± 0.5 bc	15.5 ± 0.5 c	17.5 ± 0.5 c	17.5 ± 0.5 de	17.5 ± 0.3 e
Bacillus circulans	6.0 ± 0.4 d	7.3 ± 0.3 e	9.3 ± 0.3 e	8.5 ± 0.7 d	7.9 ± 0.9 f	8.9 ± 0.9 f
Bacillus megaterium	6.3 ± 0.4 d	7.7 ± 0.3 e	8.5 ± 0.3 e	8.0 ± 0.8 d	7.2 ± 1.0 f	6.1 ± 1.3 f
LSD at 5%	**1.7**	**1.3**	**1.8**	**2.0**	**4.1**	**4.1**

Maximum and minimum values are underlined. Means with different letters are significantly different at *p < 0.05* according to Duncan's test

Table 3. *Variation (mean ± SE) in the leaf length and width (cm) as well as the internode length (cm) of the 5th and 6th old Cordia sinensis seedlings under the effect of different chemical and biological treatments*

Treatments	**Leaf length**		**Leaf width**		**Internode length**	
	5th month	**6th month**	**5th month**	**6th month**	**5th month**	**6th month**
Control	0.0 e	0.0 e	0.0 e	0.0 e	0.0 f	0.0 g
Abscisic acid (ABA)	4.5 ± 0.5 c	4.6 ± 0.5 c	3.8 ± 0.4 c	3.8 ± 0.4 c	4.8 ± 0.2 c	4.8 ± 0.1 d
Fungicide	5.5 ± 0.08 b	5.6 ± 0.08 b	4.8 ± 0.06 b	4.8 ± 0.05 b	6.8 ± 0.2 b	7.7 ± 0.2 b
ABA + fungicide	6.8 ± 0.06 a	7.0 ± 0.07 a	5.5 ± 0.08 a	5.6 ± 0.08 a	8.5 ± 0.2 a	9.7 ± 0.2 a
Azospirillum sp.	4.2 ± 0.1 c	4.1 ± 0.1 c	3.3 ± 0.1 c	3.2 ± 0.1 c	5.3 ± 0.2 c	5.3 ± 0.2 d
Azotobacter sp.	5.6 ± 0.1 b	5.6 ± 0.1 b	4.8 ± 0.1 b	4.8 ± 0.1 b	6.3 ± 0.2 b	6.5 ± 0.3 c
Pseudomonas fluorescens	5.0 ± 0.09 b	5.1 ± 0.1 b	4.4 ± 0.09 b	4.4 ± 0.09 b	5.3 ± 0.1 c	5.3 ± 0.2 d
Bacillus circulans	2.3 ± 0.2 d	2.3 ± 0.2 d	1.9 ± 0.2 d	2.0 ± 0.2 d	2.2 ± 0.2 d	2.2 ± 0.2 e
Bacillus megaterum	2.0 ± 0.3 d	1.9 ± 0.3 d	1.7 ± 0.3 d	1.5 ± 0.3 d	1.4 ± 0.2 e	1.3 ± 0.2 f
LSD at 5%	**0.83**	**0.91**	**0.75**	**0.75**	**0.85**	**0.97**

Maximum and minimum values are underlined. Means with different letters are significantly different at *p < 0.05* according to Duncan's test

Physiological parameters

A wide range of bacteria, including *Pseudomonas*, *Azospirillum*, *Azotobacter*, and *Bacillus*, have been shown to increase plant growth (Gao et al., 2020) and to be effective against soil-borne plant diseases (El-Kazzaz et al., 2022). Except for *Bacillus* spp., all of the tested biochemical agents stimulated plant physiological activities such as pigment content and nutrient accumulation potential, with the combined treatment (ABA + fungicide) being the most effective. The statistical assessment (ANOVA I) indicated significant differences (*P < 0.05*) in the effect of the applied treatments on the

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pigments and nutrients content of *C. sinensis* seedling leaves, after six months. The combined treatment (ABA + fungicide) showed the highest effect on chlorophyll a, b, carotenoids, and total pigment contents (2.88, 2.28, 0.53, and 5.69 mg/g, respectively) (*Table 4*). In addition, it significantly ($P < 0.05$) contributed to the highest seedling N, P, and K (1.97, 1.76, and 2.04%, respectively), while *B. circulans* and *B. megaterium* treatments contributed to the lowest N (1.03 and 1.08%, respectively) and K (1.22 and 1.15%) contents (*Table 5*).

Table 4. *Effect of different chemical and biological treatments (mean ± SE) on the pigments content of the 6th months old seedling of Cordia sinensis*

Treatments	**Pigment (mg g^{-1} dry weight)**			
	Chla	**Chlb**	**Carotenoid**	**Total pigments**
Control	0.84 ± 0.06^{d}	0.71 ± 0.04^{d}	0.09 ± 0.003^{c}	1.64 ± 0.1^{e}
Abscisic acid (ABA)	2.14 ± 0.2^{b}	1.59 ± 0.1^{bc}	0.36 ± 0.09^{b}	4.09 ± 0.09^{b}
Fungicide	1.71 ± 0.03^{c}	1.62 ± 0.1^{b}	0.33 ± 0.05^{b}	3.66 ± 0.2^{cd}
ABA + fungicide	2.88 ± 0.08^{a}	2.28 ± 0.2^{a}	0.53 ± 0.02^{a}	5.69 ± 0.2^{a}
Azospirillum sp.	1.61 ± 0.2^{c}	1.33 ± 0.09^{c}	0.31 ± 0.02^{b}	3.25 ± 0.3^{d}
Azotobacter sp.	1.86 ± 0.1^{bc}	1.72 ± 0.09^{b}	0.38 ± 0.01^{b}	3.96 ± 0.1^{b}
Pseudomonas fluorescens	1.80 ± 0.06^{bc}	1.65 ± 0.05^{b}	0.37 ± 0.01^{b}	3.82 ± 0.1^{bc}
Bacillus circulans	0.90 ± 0.09^{d}	0.66 ± 0.08^{d}	0.11 ± 0.03^{c}	1.67 ± 0.09^{e}
Bacillus megaterium	0.91 ± 0.04^{d}	0.59 ± 0.02^{d}	0.08 ± 0.002^{c}	1.58 ± 0.02^{e}
LSD at 5%	**0.35**	**0.25**	**0.14**	**0.47**

Maximum and minimum values are underlined. Means with different letters are significantly different at $p < 0.05$ according to Duncan's test

Table 5. *Effect of different chemical and biological treatments (mean ± SE) on the nutrients content of the 6th months old Cordia sinensis seedlings*

Treatments	**Nutrient (%)**		
	N	**P**	**K**
Control	1.10 ± 0.08^{e}	1.14 ± 0.08^{c}	1.27 ± 0.03^{d}
Abscisic acid (ABA)	1.35 ± 0.05^{d}	1.54 ± 0.08^{b}	1.80 ± 0.03^{bc}
Fungicide	1.52 ± 0.01^{bc}	1.58 ± 0.03^{ab}	1.81 ± 0.03^{bc}
ABA + fungicide	1.97 ± 0.02^{a}	1.76 ± 0.02^{a}	2.04 ± 0.04^{a}
Azospirillum sp.	1.46 ± 0.03^{cd}	1.68 ± 0.02^{ab}	1.67 ± 0.04^{c}
Azotobacter sp.	1.60 ± 0.06^{b}	1.74 ± 0.03^{a}	2.03 ± 0.04^{a}
Pseudomonas fluorescens	1.45 ± 0.03^{cd}	1.68 ± 0.03^{ab}	1.94 ± 0.04^{ab}
Bacillus circulans	1.03 ± 0.02^{e}	1.21 ± 0.05^{c}	1.22 ± 0.10^{d}
Bacillus megaterium	1.08 ± 0.01^{e}	1.21 ± 0.04^{c}	1.15 ± 0.08^{d}
LSD at 5 %	0.14	0.14	0.21

Maximum and minimum values are underlined. Means with different letters are significantly different at $p < 0.05$ according to Duncan's test

Synthetic fungicides, such as Rizolex, are the most often employed disease control measure by farmers worldwide on diverse crops (Haryuni et al., 2020). Although both

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ABA and fungicide play an important role in inhibiting the action of *Fusarium* and *Rhizoctonia* in healthy *C. sinensis* seedlings, which have the best morphological and physiological growth parameters, biocontrol agents such as *Azotobacter* outperformed the control and had a slightly lower effect than the fungicide and abscisic acid combined treatment. These findings were consistent with those reported by Castro del Ángel et al. (2019) for the bean crop. Furthermore, these bacteria could produce cytokinin, which is involved in many physiological processes in plant cell division, including breaking the period of hibernation in buds, stimulating seed germination, improving root and leaf growth, and delaying senility (Yuan et al., 2013; Alsaady et al., 2020).

Microbiological assay

Bioagents are typically selected for phytopathogen management because they are environmentally safe and leave no known hazardous residues in food chains, whereas chemical fungicides might cause soil contamination or other negative effects (Gaigole et al., 2011). Several biocontrol bacteria, including *Bacillus* and *pseudomonas* species, have been demonstrated to have antagonistic effects on *F. oxysporum* (Abdulkareem et al., 2014). Many studies have been conducted to support our current findings that biological control is a realistic and reliable approach against soil-borne diseases (Khare et al., 2010). The effect of different chemical and biological treatments on the disease severity of soil borne fungi (*F. oxysporium* and *R. solani*) showed that the control treatment had both pathogens that caused complete seedlings' deformation and death with 100% disease severity (*Fig. 4*). Besides, all investigated treatments notably ($P < 0.05$) reduced the disease severity with different efficiency, where ABA + fungicide, fungicide, *Azotobacter* sp., and *P. fluorescens* significantly had the highest efficacy (84, 80, 78, and 70%, respectively). *Azotobacter* spp., *Pseudomonas* and *Bacillus* genera in bacteria, and *Trichoderma* in fungal groups are the most viable biocontrol microorganisms used to suppress several pathogenic species, which are known to reduce the incidence of root rot and damping-off diseases caused by these pathogens (Dubey et al., 2007). Moreover, *B. circulans* treatment significantly had the least efficacy (35%) to prevent seedling mortality.

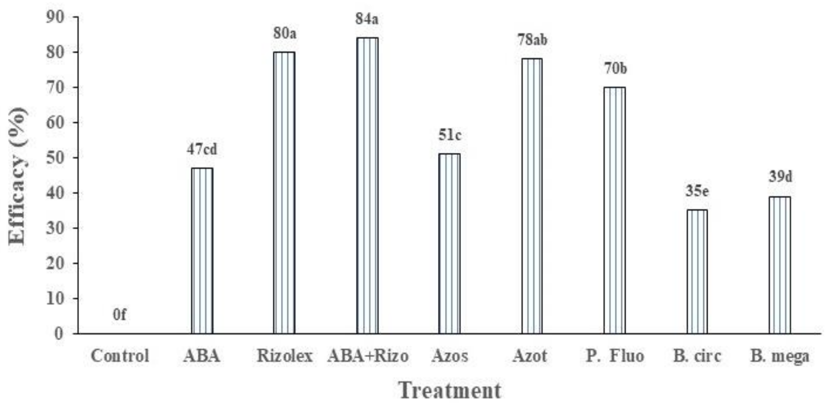

Figure 4. *Effect of different chemical and biological treatments on the diseases' severity of the bioagents of Cordia sinensis seedlings. ABA: abscisic acid, Rizo: Rizolex, Azos: Azospirillum sp., Azot: Azotobacter sp., P. fluo: Pseudomonas fluorescens, B. circ: Bacillus circulans, B. mega: Bacillus megaterium. Means with the same letters are significantly different at P < 0.05 according to Duncan's test*

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Conclusion

The bioagent-based formulations utilized in this investigation demonstrated potential efficacy against the fungus *F. oxysporum* and *R. solani* in terms of disease severity, as well as growth metrics. Biocontrol approaches are developing as substantial, ecologically acceptable alternatives to chemical pesticides for disease management in field crops (Nagaraja et al., 2022). The current study discovered that treating seedlings with *Azotobacter* spp. or ABA + fungicide reduced disease and environmental stress, resulting in lower mortality and higher plant growth metrics showing potential for integrated stress and pest management. The outputs of this research can be applied on a large scale in the natural habitats for the in-situ conservation and propagation of the target species, with foreseeable commercial benefits. However, care should be taken for the potential long-term impacts of chemical treatments on soil health and microbial communities.

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Conflict of interests. The authors declare that they have no competing interests.

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