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Biological management of cumin *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. cumini using antagonistic rhizospheric bacteria *Bacillus licheniformis*

Kavita Yadav[1], T. Damodaran[2]*, Kakoli Dutt[1], Sangeeta Kumari[2], Prasenjith Debnath[2], Alok Shukla[1] and Ram Gopal[3]

*[1]Banasthali Vidyapeeth, Jaipur, Inida. [2]ICAR-Central Institute for Subtropical Horticulture, Lucknow-226101, India. [3]Krishi Vigyan Kendra, ANDUAT, Ayodhya, India. *E-mail: damhort73@gmail.com*

Abstract

The field survey conducted in Rajasthan, India, unveiled the widespread occurrence of *Fusarium* wilt in cumin fields that have been continuously cultivated for approximately 4 to 5 years. The incidence of this disease exceeded 30%, affecting cumin plants at all stages of growth, with severe symptoms ultimately resulting in the complete mortality of the plants. In laboratory studies, a bacterial isolate known as *Bacillus licheniformis* (CSR-D4) exhibited remarkable *in vitro* effectiveness, significantly inhibiting *Fusarium oxysporum* f.sp. cumnini (FOC) mycelial growth by an impressive 79.85%. In controlled pot experiments, cumin plants treated with *B. licheniformis* (CSR-D4) displayed milder symptoms than untreated plants, demonstrating a notably higher tolerance level, with only a 15% disease incidence as opposed to 90% in untreated plants. Further analysis of defense enzymes revealed elevated chlorophyll, carotenoid, peroxidase activity levels, and proline content in cumin plants treated with *B. licheniformis* (CSR-D4). Field assessments confirmed the efficacy of this bacterial isolate, as it successfully suppressed wilt incidence by 60%, significantly increased crop yield by 71.16%, and promoted root and shoot growth. Notably, applying *B. licheniformis* (CSR-D4) did not negatively impact beneficial microorganisms, and no adverse phytotoxic symptoms were observed. This study underscores the considerable potential of *B. licheniformis* (CSR-D4) in managing *Fusarium* wilt, offering an environmentally friendly and highly effective solution to enhance the health and productivity of pea plants.

Key words: *Fusarium* wilt, cumin, *Bacillus licheniformis*, disease incidence, tolerance, crop yield, proline content, defense enzymes

Introduction

Cumin (*Cuminum cyminum* L.) ranks as the world's second most widely used spice, trailing only black pepper (Piri *et al.*, 2020). This versatile spice finds applications in Ayurvedic medicine and as a flavor enhancer in culinary traditions. Cumin seeds, rich in dietary fiber, contain various phytochemicals known for their antioxidant, carminative, and antiflatulent properties (Devi *et al.*, 2019).

Cumin production is hampered by a lack of improved varieties and scientific techniques, as well as its susceptibility to diseases caused by *Alternaria burnsii, Erysiphe polygoni,* and *Fusarium oxysporum* f. sp. cumini (Foc). Cumin wilt, caused by *F. oxysporum* f. sp. cumini (Snyder and Hansen, 1940), poses a significant economic danger to Indian and global cumin production (Özer and Bayraktar, 2015). Under favourable conditions, this disease can cause yield losses of up to 100%, with symptoms including leaf drooping, vascular browning, stunting, and damping off. Recurrent Foc infections frequently cause cumin producers to forgo cumin growing in favour of less sensitive crops.

The management of *Fusarium* wilt necessitates an integrated strategy that combines cultural, biological, and chemical approaches. This strategy encompasses the use of disease-resistant cultivars, quality seeds, adjusted sowing times, crop rotation, seed treatments, fungicide applications, and biocontrol agents. However, variables such as agro-climatic conditions and pathogenic diversity in fungal populations can influence the effectiveness of these management strategies. The limited resistance in cumin germplasm presents a global challenge for the control of cumin wilt (Lodha and Mawar, 2014). Crop rotation, intended to mitigate *Fusarium*'s soil-borne nature, becomes impractical due to the fungus's extended survival in the soil, sometimes exceeding six years without the presence of the host plant (Israel *et al.*, 2005). The use of fungicides and chemical compounds, while common, carries risks of negatively affecting crop physiology, photosynthesis, and the environment (Petit *et al.*, 2012).

Biological management of soil-borne diseases using antagonistic microorganisms, such as endophytic bacteria, offers a promising and eco-friendly alternative to synthetic pesticides (Iftikhar *et al.*, 2020; Giauque *et al.*, 2018). More than 120 species of endophytic bacteria, including *Bacillus*, *Pseudomonas*, *Enterobacter*, and *Agrobacterium*, significantly enhance plant health and defense mechanisms (Afzal *et al.*, 2019). *Trichoderma* spp. are commonly utilized by cumin farmers for wilt disease management, while studies have explored the efficacy of *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Rhizobium* spp. in controlling wilt disease, showing significant inhibition of the cumin wilt pathogen by biological agents such as *Trichoderma harzianum*, *T. viride*, *P. fluorescens*, and *B. subtilis* (Lodha and Mawar, 2014; Chawla *et al.*, 2012). *P. fluorescens* exhibited the highest mycelial inhibition,

underscoring the role of lytic enzymes like chitinase, β-1,3 glucanases, and protease in disease control (Bishi and Vakharia, 2015).

The study screens endophytic bacteria for their biocontrol and growth-promoting abilities against the cumin pathogen *F. oxysporum*, while also examining the physiological responses of cumin plants to bacterial inoculation.

Materials and methods

Survey and assessment of disease incidence in Jodhpur district: An extensive survey was carried out at village Baori, Anwana, Jalore and Barmer villages of Osian tehsil, Jodhpur, Rajasthan, during "Rabi" season (2019-20) to study the incidence wilt disease of cumin. The selected field, an area of 2.25 m^2 was covered diagonally across the field at five spots, and the observation on incidence of the disease was recorded through counting the disease and the total number of plants per spot. The Percent Disease Incidence (PDI) was calculated by using the formula given by Mayee and Datar, 1986.

Isolation of *F. oxysporum* f.sp. *cumini*: The pathogen was previously isolated from the cumin plants showing the typical symptoms of *Fusarium* wilt that was collected from KVK Banasthali Vidyapith, Jaipur- Rajasthan, which lies between latitude (26° 19' 13.08'' N) and longitude (75° 53' 9.24'' E) respectively (Yadav *et al.*, 2020). The single spore method and hyphal tip method were used to maintain on PDA slants and were stored at 4°C in the refrigerator for further studies (Hilal *et al.*, 2016).

***In vitro* screening of antagonistic potential of *Bacillus licheniformis* (CSR-D4):** The previously isolated *B. licheniformis* (CSR-D4) (Yadav *et al.*, 2021) was assayed *in vitro* for their antagonistic activity against *Foc* on PDA media. A bioassay was conducted by dual culture technique as per Ramanathan *et al.* (2002). Five millimeters of mycelial discs were cut from the young growing edge of the fungus from seven days old culture with a sterilized cork borer and placed at one side of a petri plate. The bacterial isolates were streaked aseptically parallel to the fungus at a 15-20 mm distance and incubated at 28 ± 2ºC for 10 days. Three replications were maintained. The inhibition zone between the two cultures was measured in the nearest millimeter. After 120 hours of the incubation period, the percent inhibition of the fungus was calculated by using the formula:

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

Where, R1- radial growth of the control pathogen; R2- radial growth of treated pathogen

The *in vitro* dual culture assay was carried out in three replicates and the data were statistically analyzed by analysis of variance (ANOVA) and Duncan's multiple range test (DMRT).

Pot evaluation of CSR-D4 **for the suppression of *F. oxysporum* f.sp. *cumini***

B. licheniformis (CSR-D4) isolate was tested against *F. oxysporum* f.sp. *cumini* under pot conditions by growing cumin plants in pathogen infested soil. For this purpose 1 kg of soil was transferred to each plastic pot and moistened suitably 24 hour before soil inoculation with pathogen. *Foc* isolate was multiplied on Potato dextrose broth in an Erlenmeyer flask sterilized at 15 p.s.i for 30 minutes. These flasks containing sterilized media were inoculated with *Foc* isolate and incubated at 26±2°C for 15 days. Mycelial suspension of *Fusarium* isolate was added to soil at 5 g/kg soil and mixed thoroughly. The mycelial suspension was prepared by taking 50g of fresh mycelia in 200ml sterilized distilled water. The harvested fungal mat was macerated and homogenized for 2 minutes, giving 30 gap 20 mL fungal suspension containing five-gram mycelia was added to each pot containing one kg soil and allowed to stabilize to 72 h before sowing of cumin seeds. Ten healthy seeds of cumin were sown in each pot. In the case of control, cumin seeds were sown in pots containing un-inoculated soil. These pots were usually irrigated on alternate days. The plants were observed for any signs of disease, and based on that, disease incidence (DI) and disease severity index were observed (DSI). The following formula was used for the calculation.

$$\text{DSI} = \frac{\Sigma(\text{Number of diseased plants in each grade x Value of relative grade})}{\text{Total number of inspected plants x 5}}$$

Determination of chlorophyll content: Fresh samples (0.25 g) were utilized for chlorophyll content determination in accordance with the method proposed by Lichtenthaler and Buschmann (2001),

Quantification of peroxidase (POD) activity: Peroxidase (POD) activity was assayed by the method suggested by Chance and Maehly (1995) in an alcoholic liquid of the tissue extract (100 µL).

Determination of proline content: Approximately 0.2 g of fresh seedling tissue was homogenized in 10 mL of 3% aqueous sulfosalicylic acid. Proline was determined according to the method described by Bates *et al.* (1973).

Preparation of bio-formulation of application techniques: A loop full of CSR-D4 bacterial isolate was inoculated into the CSR patent-protected culture media (Rai *et al.*, 2012) and incubated in a rotary shaker at 150 rpm for 36 h at room temperature (28 ± 2 ◦C). After 36 h of incubation, the formulation containing 2 × 10^9 spores mL^{-1} was used for drenching the plants. The plants were drenched with 500 mL/plant with 1% of the formulation prepared using the *B. licheniformis* (CSR-D4) bacterial isolate at 2, 4, 6, and 8 months after planting (Damodaran *et al.*, 2019a). The untreated control plants were drenched with the plain culture at the same quantity used in treatment. The wilt disease incidence in the treatments was scored based on a 1–5 disease scale (Ploetz *et al.*, 1999).

Field experiment: The field experiment was conducted on management of cumin wilt using *B. licheniformis* (CSR-D4) as seed treatment and soil application during the cropping season rabi 2019-20 using cumin variety (cv. RZ19) at village Baori, Jodhpur district located between 26º15' N to 26º45' North latitude and 73º00' E to latitude 73º 29' East longitude at an altitude of 231 meter above mean sea level. Based on *in vitro* studies and pot experiment powder formulation *B. licheniformis* (CSR-D4) was used at 2 g/kg seed for seed treatment. Similarly, for soil treatment these were used at the rate of 3%. A uniform amount of farm yard manure was applied in the field soil. In case of control untreated seeds were sown. Observations on wilt incidence were recorded periodically. The shoot length, root length, dry weight and seed yield of cumin plants were recorded at harvest. For recording shoot length, root length and dry weight, the cumin plants were uprooted gently washed in tap water and dried in

oven at 60°C for 24 hour. The shoot length, root length and dry weight of 5 plants for each replication were recorded. The disease incidence percentage was calculated using the formula mentioned below:

$$\text{Disease control (\%)} = \frac{\text{Disease incidence in inoculated control (\%)} - \text{Disease incidence in treated (\%)}}{\text{Disease incidence in inoculated control (\%)}} \times 100$$

Statistical analysis: The data was subjected to analysis of variance (ANOVA) and means were compared using Duncan's Multiple Range Test ($P<0.05$) (Rangaswamy, 1995).

Results

Field survey on wilt disease incidence in Jodhpur district: *Fusarium* wilt was prevalent in cumin fields in Rajasthan, India, especially after 4-5 years of continuous cultivation, with over 30% incidence in marked areas across surveyed villages (Fig. 1a). The disease affected plants at all growth stages, with severity increasing as plants matured. Symptoms appeared around one month, leading to wilting and death in plants reaching 2.5–5.0 cm. Older plants showed a transition from green to yellow leaves, starting from the bottom. Urgent management strategies, like crop rotation and resistant varieties, were necessary to sustain cumin production.

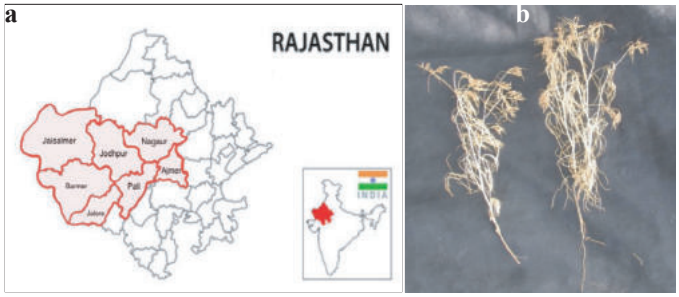

Fig. 1. (a) Showing distribution of the cumin wilt in Jhodpur. The red marked districts indicate the locations with disease incidence of more than 30%, (b) *Fusarium* wilt infected plants of

During severe stages, complete plant mortality occurred, with affected plants easily uprooted, and roots displaying dark brown markings. Plants infected at the flowering stage remained sterile, producing thin, small, and shriveled seeds. Partially wilted plants ceased growth, and their leaves turned pinkish-yellow (Fig. 1b). The survey revealed the highest disease incidence in Baori (25.4%), followed by Barmer (21.25%), Anwana (20.05%), and Jalore (15.19%), respectively (Fig. 2).

***B. licheniformis* (CSR-D4) efficacy *in vitro*:** In assessing *B. licheniformis* (CSR-D4) efficacy *in vitro*, a dual co-culture technique on PDA medium (Fig. 3) significantly reduced *F. oxysporum* f. sp. *cumini* (Foc) mycelial growth (Table 1). After 120 hours, Foc exhibited only 1.47 cm mycelial growth, a substantial reduction from the 6.56 cm in the control group. Notably, the inhibition percentage of Foc increased from 48 to 120 hours after inoculation, reaching an impressive 79.85% inhibition (Fig. 4). This demonstrated a 50% increase in disease inhibition between 24 and 120 hours.

CSR-D4 pot evaluation for *F. oxysporum* f. sp. *cumini* suppression: Two weeks after pathogenic strain inoculation, cumin plants exhibited initial external wilt symptoms. Leaf chlorosis began with older leaves and advanced to younger leaves. In four weeks, treated plants showed recovery, whereas the control group faced severe symptoms and mortality. CSR-D4 treatment resulted in only 15% disease incidence compared to the 90% in the control group (Table 2, Fig. 5 and 6).

Chlorophyll content: Assays of defense enzymes indicated significantly higher total chlorophyll content (15.1 µg/g FW) in plants treated with *F. oxysporum* f. sp.

Table 1. Radial growth of *Fusarium oxysporum* f.sp. *cumini* (*Foc*) in presence of *B. licheniformis* (CSR-D4) under *in vitro* condition

Treatment	Radial growth of *Fusarium* (cm)				
	24 h	48 h	72 h	96 h	120 h
Control (Foc)	0.97 ± (0.06)	2.53 ± (0.25)	3.80 ± (0.56)	4.76 ± (0.41)	6.56 ± (0.05)
CSR-D4	0.60 ± (0.10)	1.03 ± (0.15)	1.43 ± (0.15)	1.47 ± (0.12)	1.47 ± (0.11)
F value	29.978*	77.885*	50.113*	178.550*	5062.339*

Values are the means ± SD of three replicates. *F value* represents the significant value at $P \leq 0.05$.

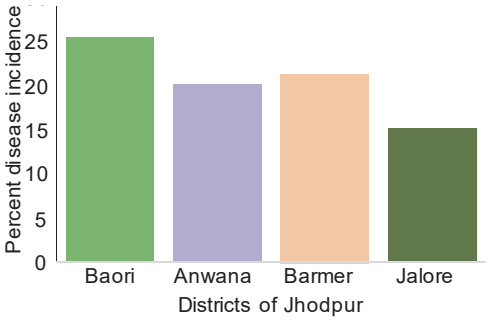

Fig. 2. Percent disease incidence of *Fusarium* wilt of cumin in different districts of Jhodhpur

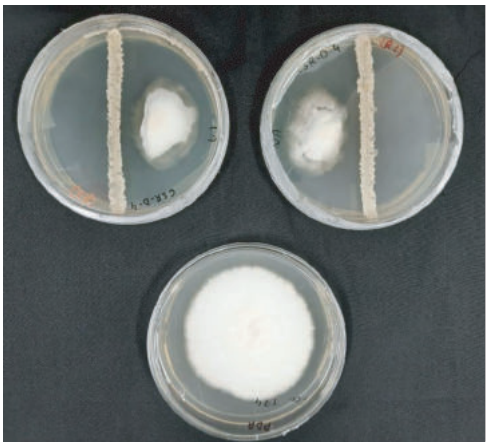

Fig. 3. Showing antagonistic activity of *B. licheniformis* (CSR-D4) against *F. oxysporum* f. sp. cumini

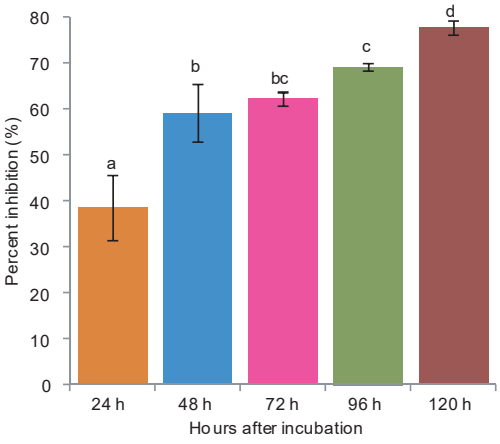

Fig. 4. Graphical presentation of percent inhibition of *B. licheniformis* (CSR-D4) bacterial isolate against *Fusarium oxysporum* f.sp. *cumini* (*Foc*) under *in vitro* condition. Values are the means of three replicates. Means followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$.

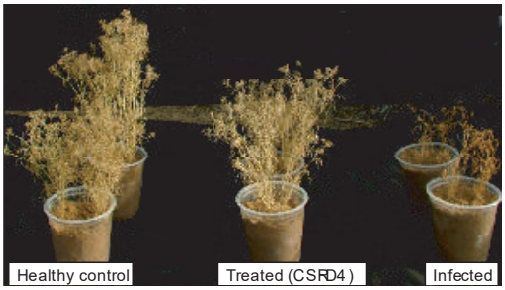

Fig. 5. Differences in the morphological characters of cumin plants observed in treated *B. licheniformis* (CSR-D4), Healthy control and infected treatments

Table 2. Effect of *B. licheniformis* (CSR-D4) bacterial isolate on *Fusarium* wilt incidence and growth of cumin plant grown under greenhouse pot experiment

Treatment	Plant height (cm)	Branches/ plant (No.)	Yield/ pot (gm)	Disease severity index (%)
TF	$20.90^{a} \pm (1.52)$	$6.60^{a} \pm (1.35)$	$1.60^{a} \pm (0.49)$	$82.00^{b} \pm (1.67)$
TFD4	$27.54^{b} \pm (0.89)$	$13.00^{b} \pm (1.41)$	$60.24^{c} \pm (0.97)$	$0.00^{a} \pm (0.00)$
THC	$30.40^{c} \pm (1.46)$	$13.60^{b} \pm (1.62)$	$55.88^{b} \pm (0.82)$	$0.00^{a} \pm (0.00)$
F value	68.372*	35.028*	8703.566*	12007.143*

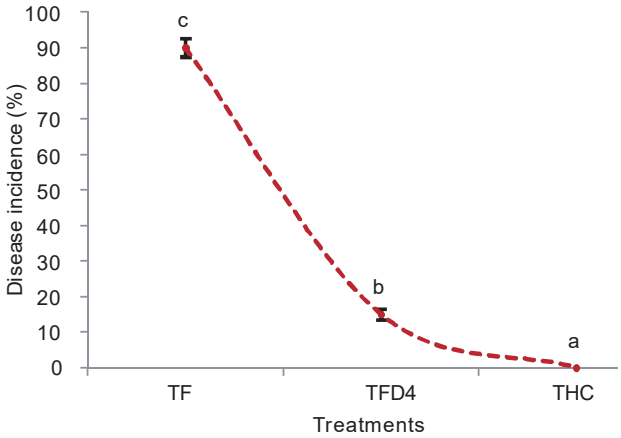

Fig. 6. Disease incidence analysis of *Fusarium* wilt in cumin plants grown under greenhouse pot experiment. Treatments include: TF- treatment with *Fusarium oxysporum* f.sp. *cumini*; TFD4 – treatment with *Fusarium oxysporum* f.sp. *cumini* and CSR-D4 (*Bacillus licheniformis* isolate); THC – treatment healthy control (without any inoculation). Means followed by the same letter are not significantly different according to Duncan's

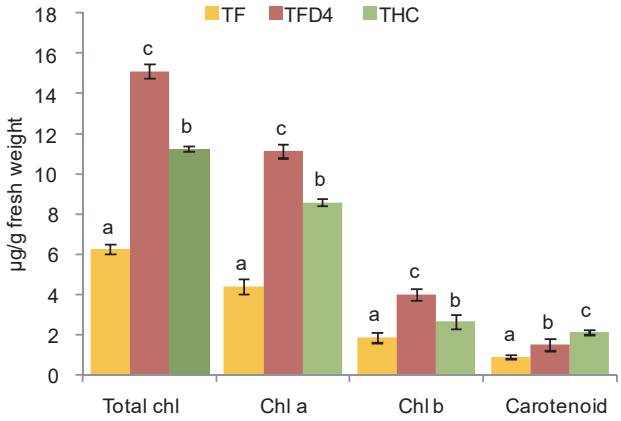

Fig. 7. Graphical representation of chlorophyll and carotenoid content in cumin plants grown in pot experiment. Where, Total chl- total chlorophyll, Chl a – chlorophyll a, Chl b- chlorophyll b, and carotenoid. Treatments include: TF- treatment with *Fusarium oxysporum* f.sp. *cumini*; TFD4 – treatment with *Fusarium oxysporum* f.sp. *cumini* and CSR-D4 (*Bacillus licheniformis* isolate); THC – treatment healthy control. Means followed by the same letter within the treatment are not significantly different according to Duncan's multiple range test at $P \leq 0.05$.

cumini and CSR-D4 compared to infected (6.24 µg/g FW) and healthy control plants (11.25 µg/g FW). Carotenoid induction (1.5 µg/g FW) in CSR-D4 treatment surpassed infected (0.91 µg/g FW) and healthy control plants (2.13 µg/g FW) (Fig. 7).

Peroxidase (POD) and proline activity: Peroxidase activity reached its highest (11.41 U/mg protein.min) in CSR-D4-treated plants, followed by healthy control (9.51 U/mg protein.min) and *F. oxysporum* f. sp. *cumini* treated plants (5.78 U/mg protein.min) (Fig. 8a). CSR-D4-treated plants also showed significantly higher proline content (310 µmol/g FW) compared to healthy control (327.77 µmol/g FW) and *F. oxysporum* f. sp. *cumini* treated plants (223.34 µmol/g FW) (Fig. 8b).

***Field evaluation of B. licheniformis* (CSR-D4)**

Efficacy (Cumin Experiment, 2019-20): Field evaluations indicated a significant reduction in wilt incidence following seed and soil treatment with *B. licheniformis* (CSR-D4) (Fig. 10). CSR-D4-treated plants yielded 5.71 quintals per hectare compared to the control's 2.02 quintals per hectare, a 71.16% increase. Disease incidence reduced to 32%, with a 60% disease control rate (Table 3). The application had no adverse effects on beneficial microbes in the cumin rhizosphere at all dosage levels.

Table 3. Effect of bio-control *B. licheniformis* (CSR-D4) on wilt incidence, disease control, seed yield and yield over control of cumin under field conditions

Treatments	Control	CSR-D4
Disease incidence (%)	80.68 (63.94)	32.00 (34.43)
Disease control (%)	--	60.29
Seed yield (q/ha)	2.02	5.71
Increase over control (%)	--	71.16

Root and shoot lengths significantly improved with CSR-D4 treatment, recording maximum lengths of 12.18 cm and 26.50 cm. Control plants had shorter root lengths of 7.34 cm and shoot lengths of 13.60 cm. CSR-D4-treated plants exhibited higher dry weight at 1.52 grams, while control plants had 0.89 grams (Table 4).

Table 4. Effect of biocontrol agent CSR-D4 *B. licheniformis* on dry weight of plants and seed yield of cumin under field conditions in year 2019-2020

Treatments	Root length (cm)	Shoot length (cm)	Dry weight (g/plant)
Control	7.34 ± (2.86)	13.60 ± (3.52)	0.89 ± (0.50)
Treated (CSR-D4)	12.33 ± (2.22)	26.50 ± (5.00)	1.52 ± (0.48)
F value	12.072*	30.141*	5.299*

Treatments include control- untreated plants and, treated (CSR-D4)- infected plants treated with CSR-D4 (*B. licheniformis*) isolate. Values are the means of ten replicates. Values in the parentheses indicate the standard deviation of the mean

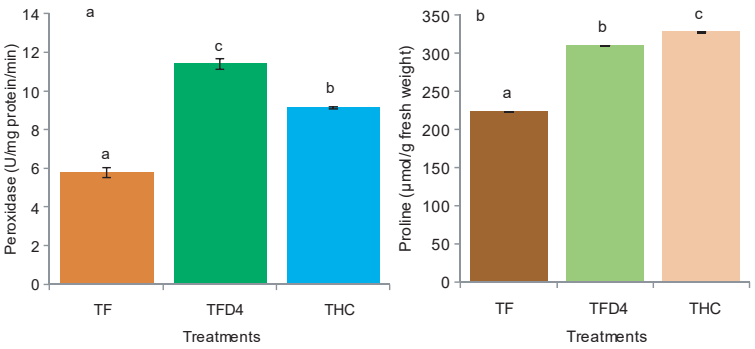

Fig. 8. Graphical representation of (a) Peroxidase, and (b) Proline content in cumin plants grown in pot experiment. Treatments include: TF- treatment with *Fusarium oxysporum* f.sp. *cumini*; TFD4 – treatment with *Fusarium oxysporum* f.sp. *cumini* and CSR-D4 (*Bacillus licheniformis* isolate); THC – treatment healthy control.Values are the means of three replicates with the sample size n=5. Means followed by the same letter treatment are not significantly different

Discussion

Cumin stands as one of the world's most widely used spices, trailing only behind black pepper (*Piper nigrum*) in global popularity (Piri *et al.*, 2020). This spice, extensively used in Ayurvedic medicine and culinary applications, is characterized by its dietary fiber content and numerous phytochemicals, including antioxidants and carminatives, which

contribute to its esteemed status (Devi *et al.*, 2019). India, a major player in cumin production, consumption, and exports, dedicates an extensive area for its cultivation, particularly in arid and semi-arid regions across the Indian subcontinent. The country dominates the global market with an annual production of approximately 0.15 million tonnes of cumin seeds (Bhatnagar *et al.*, 2013). However, the cumin crop faces significant challenges, notably the emergence of cumin wilt disease, which

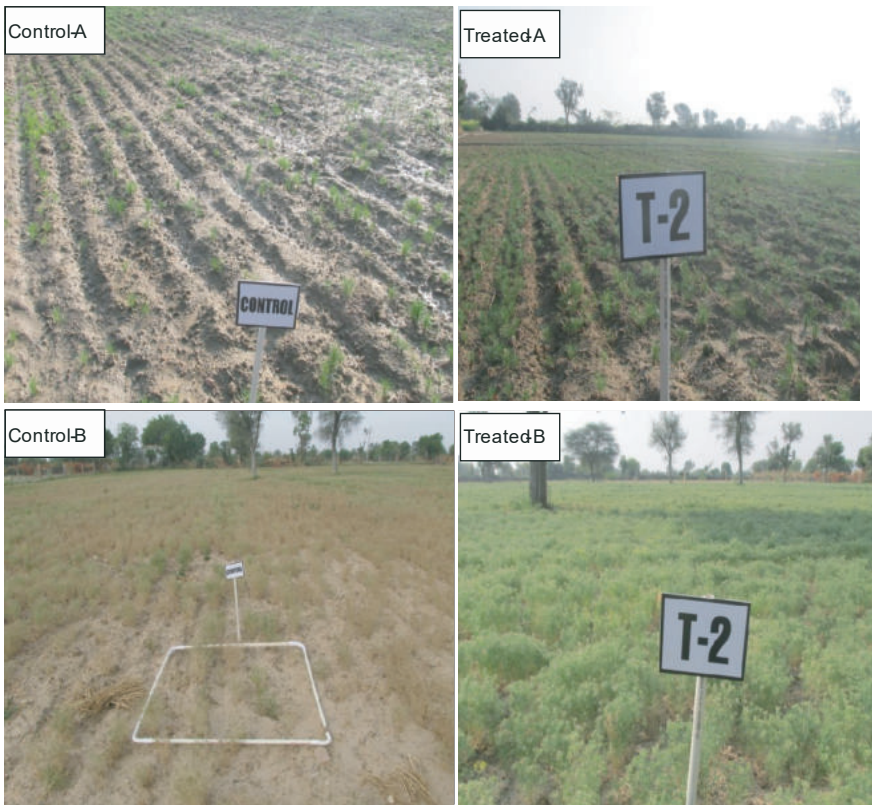

Fig. 9. Assessment of efficacy of *B. licheniformis* (CSR-D4) under field evaluation of cumin experiment year 2019-20 (a) initial observation (b) final observation

considerably threatens its production.

Cumin wilt disease, attributed to *F. oxysporum* f. sp. *cumini* (Foc), is recognized as a major concern for cumin cultivation. Recent studies suggest that even wilt-resistant cumin varieties, such as JC-2000-21 and JC-2000-22, have shown susceptibility to this ailment (Talaviya *et al.*, 2017). The disease primarily impacts the vascular system of cumin plants, leading to wilting and eventual plant death. Extensive surveys conducted across different districts in Rajasthan, India, have highlighted the substantial incidence of cumin wilt disease, particularly after continuous cultivation for 4-5 years, underscoring the gravity of the issue even in well-drained sandy or loamy soils. This problem becomes more pronounced during the rabi season (Vyas and Mathur, 2002). *Fusarium* wilt can affect cumin plants at all stages of growth, with the severity intensifying as the plants mature. Symptoms typically manifest around one month after planting, characterized by wilting and eventual plant death. Older plants display yellowing from the bottom to the top, leading to complete plant mortality. Affected roots exhibit dark brown markings, and in some instances, partial wilting occurs. The disease's wide-reaching impact is exemplified by survey data showing high disease incidence in areas such as Baori (25.4%), Barmer (21.25%), Anwana (20.05%) and Jalore (15.19%) (Sharma *et al.*, 2013). Notably, environmental factors, including humidity and water availability, are closely associated with the occurrence of this disease. High post-flowering humidity levels have been identified as a significant contributing factor to cumin wilt disease incidence (Khosh-Khui and Bonyanpour, 2010). This susceptibility of cumin to *Fusarium* wilt is particularly notable in arid and semi-arid regions, where water scarcity poses a significant constraint to crop production, particularly in major cumin-growing areas in Rajasthan and Gujarat (Lodha and Mawar, 2007). In this study, we explored the antagonistic effects of *Bacillus* strains, with particular emphasis on *B. licheniformis* (CSR-D4), against *F. oxysporum* f. sp. *cumini* (Foc), the pathogenic fungus responsible for cumin wilt disease. Our findings demonstrated that *B. licheniformis* (CSR-D4) significantly hindered the growth of Foc, aligning with previous research (Chawla and Gangopadhyay, 2009; Khan and Gangopadhyay, 2012). The potential of *Bacillus* species as biocontrol agents against various fungal pathogens, including *F. oxysporum*, is well-documented (Abo-Elyousr *et al.*, 2022). Research has shown that *Bacillus* strains can suppress *Fusarium* infections by producing hydrolytic enzymes and cyclic lipopeptides (Mardanova *et al.*, 2017). Moreover, *B. licheniformis* has been recognized for its beneficial characteristics, serving as both a fungal antagonist and a promoter of plant growth and abiotic stress tolerance (Yadav *et al.*, 2021; Abo-Elyousr *et al.*, 2022). It has been reported to protect various crops, such as maize and faba beans, against infections by different pathogens (Wang *et al.*, 2009). The culture filtrate of *B. licheniformis* has also been found to inhibit the growth of several fungal pathogens, including *F. oxysporum* (Karthika *et al.*, 2022). This illustrates that *B. licheniformis* strains not only possess antimicrobial properties but also have the potential to enhance plant growth and improve plant tolerance to abiotic stress.

In pot experiments, cumin plants exposed to the Foc displayed severe wilt symptoms, leading to death within four weeks. However, plants treated with the bacterium *B. licheniformis* along with Foc showed only mild symptoms and recovered completely by the fourth week. These treated plants had a low Disease Index (DI) of 15%, in contrast to 90% in solely Foc-infected plants. Analysis indicated that CSR-D4 treatment improved levels of chlorophyll, carotenoids, peroxidase activity, and proline, supporting prior research that *Bacillus* species can act as effective biocontrol agents and boost plant health.

The study exploring the antagonistic capabilities of the endophytic biocontrol agent *B. lichenibacillus* against *Fusarium* wilt in cumin yields critical insights into potential disease management strategies. Our investigation underscores the pronounced antagonistic impact of *B. lichenibacillus* on *Fusarium* wilt, substantially curtailing disease prevalence. In both, dual culture tests and pot experiments, this strain showcased its prowess in effectively suppressing the growth of *F. oxysporum* f.sp. *cumini*, demonstrating its promise as a robust biocontrol agent.

In conclusion, our findings underscore the potential of *B. lichenibacillus* as a potent biocontrol agent in combatting *Fusarium* wilt in cumin. This presents an eco-friendly and sustainable approach to disease management

in cumin cultivation. By harnessing the power of biocontrol agents like *B. lichenibacillus,* we can protect cumin crops, ensure their continued productivity, and reduce reliance on chemical interventions.

References

Abo-Elyousr, K.A.M., M.M. Saad, A.D. Al-Qurashi, O.H.M. Ibrahim and M.A.A. Mousa, 2022. Management of cumin wilt caused by *Fusarium oxysporum* using native endophytic bacteria. *Agronomy*, 12: 2510.

Afzal, I., Z.K. Shinwari, S. Sikandar and S. Shahzad, 2019. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. *Microbiol. Res*., 221: 36-49.

Bates, L.S., R.P. Waldren, and I. Teare, 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39(1): 205-207.

Bhatnagar, K., S.K. Tak, R.S. Sharma, V.L. Majumdar and R.L. Meena, 2013. Management of cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini* through chemical and biological agents. Indian *Phytopath.*, 66(1): 101-102.

Bishi , S. and D. Vakharia, 2015. Secondary metabolites and lytic enzymes produced by fluorescent pseudomonads determine *in vitro* antagonistic potential against *Sclerotium rolfsii* in groundnut (*Arachis hypogaea* L.). *J. Pure Appl. Microbiol.,* 9: 3283-3293.

Chance, B and A. Maehly, 1955. Assay of catalases and peroxidases. https: //doi.org/10.1016/S0076-6879(55)02300-8

Chawla, N. and S. Gangopadhyay, 2009. Integration of organic amendment and bioagents in suppressing cumin wilt caused by *Fusarium oxysporum* f.sp. *cumini.* Indian *Phytopath.,* 62: 209-16.

Chawla, N., S. Gangopadhyay and B. Dhaka, 2012. Eco-friendly approach for suppressing *Fusarium* wilt of cumin. *Plant Dis. Res.*, 27: 127-133.

Damodaran, T., S. Rajan, R. Gopal, A. Yadav, K. Yadav and P.K. Shukla, 2019a. Successful community-based management of banana wilt caused by *Fusarium oxysporum* f. sp. *cubense* Tropical race-4 through ICAR- FUSICONT. *J. Appl. Hortic,* 21: 37-41.

Devi, K., M.I. Thangjam, and W. Singh, 2019. Phytochemical screening of selected twelve medicinal plants commonly used as spices and condiments in manipur, north-east India. *Int. J. Curr. Res. Life Sci,* 8: 2945-2947.

Giauque, H., E.W. Connor and C.V. Hawkes, 2018. Endophyte traits relevant to stress tolerance, resource use and habitat of origin predict effects on host plants. *New Phytol*., 221: 2239-2249.

Hilal, A., I. Ismail and E. Ghebrial, 2016. *Fusarium oxysporum* f. sp. *cumini,* the Causal of Cumin Wilt in Egypt: Vegetative Compatibility Groups, Virulence and Geographical Distribution. *Egypt. J. Phytopathol*., 44: 241-259.

Iftikhar, Y., A. Sajid, Q. Shakeel, Z. Ahmad and Z.U. Haq, 2020. Biological antagonism: a safe and sustainable way to manage plant diseases. In: Plant disease management strategies for sustainable agriculture through traditional and modern approaches *Springer,* 83-109.

Israel, S., R. Mawar and S. Lodha, 2005. Soil solarisation, amendments and bio-control agents for the control of *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *cumini* in aridisols. *Ann. Appl. Biol*., 146: 481-491.

Karthika, S., M. Remya, S. Varghese, N.D. Dhanraj, S. Sali, S. Rebello, S.M. Jose, M.S. Jisha, 2022. *Bacillus tequilensis* PKDN31 and *Bacillus licheniformis* PKDL10 –As double headed swords to combat *Fusarium oxysporum* f. sp. *lycopersici* induced tomato wilt. *Microbial Pathogenesis,* 172: 105784.

Khan M.A. and S. Gangopadhyay, 2012. Effect of soil inhabiting antagonistic microflora against *Fusarium oxysporum* f.sp. *ciceri,* incitant of wilt in chickpea. *J. Myco. Plant Path.*, 42: 341-6.

Khosh-Khui, M. and A.R. Bonyanpour, 2010. Effects of some variables on seed germination and seedling growth of cumin (*Cuminum cyminum* L.). *Int. J. Agric. Res.*, 5: 365-369.

Lichtenthaler, H.K. and C. Buschmann, 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Current Protocols in Food Analytical Chemistry*, 1(1): F4-3.

Lodha, S. and R. Mawar R., 2014. Cumin wilt management: A review. *J. Spice Aromat. Crop*, 23: 145-155.

Lodha, S.K. and R. Mawar, 2007. Integrated disease management in seed spices. In: Production, development, quality and export of seed spices: Mahotra S.K. and Vashistha B.B. Eds. National Research Centre on Seed Spices, Ajmer, India, pp. 119-138.

Mardanova, A.M., G.F. Hadieva, M.T. Lutfullin, I.V. Khilyas, L.F. Minnullina, A.G. Gilyazeva, L.M. Bogomolnaya and M.R.Sharipova, 2017. *Bacillus subtilis* strains with antifungal activity against the phytopathogenic fungi. *Agric. Sci.*, 8: 1-20.

Mayee, C.D. and V.V. Datar, 1986. Phytopathometry Technical Bulletin-1. Marathwad Agricultural University, Parabhani, pp. 25.

Özer, G. and H. Bayraktar, 2015. Determination of fungal pathogens associated with *Cuminum cyminum* in Turkey. *Plant Prot. Sci.,* 51: 74-79.

Petit, A.N., F. Fontaine, P. Vatsa, C. Clément and N. Vaillant-Gaveau, 2012. Fungicide impacts on photosynthesis in crop plants. *Photosynth Res.,* 111: 315-326.

Piri, R, A. Moradi and H. Balouchi, 2020. Improvement of salinity stress in cumin (*Cuminum cyminum*) seedling by inoculation with Rhizobacteria. Indian *J. Agric. Sci.,* 90(2): 371-5.

Ploetz, R., J. Haynes and A. Vazquez, 1999. Responses of new banana accessions in South Florida to Panama disease. *Crop Protec*, 18: 445-449.

Rai, R.B., T. Damodaran, R. Kannan, A.P. Rathore, D.K. Sharma and V.K. Mishra, 2012. Low cost multiplication technology of salt tolerant bio-growth enhancers (*Bacillus, Pseudomonads* and *Trichoderma*) for increasing productivity of agri-horti crops in normal and sodic soils. *J. Plant Interact.,* 9: 577-584.

Ramanathan , A., V. Shanmugam, T. Raguchander and R. Samiyappan, 2002. Induction of systemic resistance in ragi against blast disease caused by *Pseudomonas fluorescens*. *Ann. Plant Prot. Sci*., 10: 313-318.

Rangaswamy, R. 1995. A Textbook Book of Agricultural Statistics. New Delhi: New Age Int. (P) Ltd.

Sharma, Y., K. Kant, R. Solanki and R. Saxena, 2013. Prevalence of cumin diseases on farmer's field: A survey of Rajasthan and Gujarat states. Int. *J. Seed Spices*, 3. 46.

Snyder, W.C. and H.N. Hansen, 1940. The species concept in *Fusarium*. *Am. J. Bot.*, 27: 64-67.

Talaviya, J.R., I.B. Kapadiya, C.M. Bhaliya and S.V. Lathiya, 2017. Screening of Cumin Varieties/Lines against Wilt Disease. *Int. J. Curr. Microbiol. App. Sci.,* 6(6): 3173-3176.

Vyas R.K. and K. Mathur, 2002. Distribution of *Trichoderma* Spp. In Cumin Rhizosphere and Their Potential In Suppression Of Wilt. Indian *Phytopath*, 55(4): 451-457.

Wang H, K. Wen, X. Zhao, X. Wang, A. Li and H. Hong, 2009. The inhibitory activity of endophytic *Bacillus* sp. strain CHM1 against plant pathogenic fungi and its plant growth-promoting effect. *Crop Prot.*, 28: 634-9.

Yadav, K., T. Damodaran, K. Dutt, A. Singh, M. Muthukumar, S. Rajan, R. Gopal and P.C. Sharma, 2021. Effective biocontrol of banana *Fusarium* wilt tropical race 4 by a *Bacillus* rhizobacteria strain with antagonistic secondary metabolites. *Rhizosphere*, 18: 100341.

Yadav, K., T. Damodaran, N. Kumari, K. Dutt, R. Gopal and M. Muthukumar, 2020. Characterization of *Trichoderma* isolates and assessment of antagonistic potential against *Fusarium oxysporum* f. sp. *cumini*. *J. Appl. Hortic.,* 22: 38-44.

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