Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 8 Issue 3 March 2025

Research Article

Effect of Ami *Bacillus subtilis* on Maydis Leaf Blight Disease of Corn

Joshi Chinmay[1], Zala Viren[1], Pandya Amit[1], Zala Harpal[1], Zala Vibhakshi[1], Zala Prakash[1] and Trivedi Nidhi S[2]*

[1]*Ami Agri Bioscience Pvt ltd, Ahmedabad, Gujarat, India*

[2]*Department of Microbiology, BioAgro Innovators LLP, Gandhinagar, Gujarat, India*

***Corresponding Author:** Joshi Chinmay, Ami Agri Bioscience Pvt ltd, Ahmedabad, Gujarat, India.

DOI: 10.31080/ASMI.2025.08.1492

Received: December 11, 2024

Published: February 12, 2025

Abstract

This study aimed to evaluate the effectiveness of Ami Bacillus subtilis as a biocontrol agent for controlling maydis leaf blight in maize and consider its potential to improve crop growth and yield. Field and greenhouse experiments were conducted at the Ami Experimental Farm in Ahmedabad, Gujarat, from 2020 to 2021. Ami *Bacillus subtilis* was applied as a soil treatment at a rate of 1 kg per acre, with seed treatments applied at varied intervals (weekly, bimonthly, and every three weeks). Disease severity was determined using the Area under Disease Development Curve (AUDDC), and growth characteristics such as root length, plant height, and yield were also measured. Statistical analyses using ANOVA and Tukey's test showed that seed treatment with Ami *B. subtilis* significantly improved root length (17.0 cm) and plant height (183.4 cm) compared to untreated maize seedlings. AUDDC values for disease severity decreased from 8.1 in untreated controls to 4.0 under weekly Ami *B. subtilis* applications. The application of Ami *B. subtilis* also drastically increased plant height (191.3 cm) and resulted in a 46.2% increase in maize yield per ear. Ami *Bacillus subtilis* showed the ability to encourage beneficial microbial communities in soil, thereby increasing overall soil health. The potential of Ami *Bacillus subtilis* as a long-term and environmentally friendly alternative to chemical pesticides represents an effective way to enhance maize production.

Keywords: *Bacillus subtilis;* Maydis Leaf Blight; Corn; Biocontrol; Sustainable Agriculture; Disease Management

Abbreviation

AUDDC: Area under Disease Development Curve; WAP: Weeks After Planting; CFU: Colony Forming Unit; ANOVA: Analysis of Variance; DAP: Days After Planting

Introduction

Maize (*Zea mays L.*) is among the most significant cereal crops, functioning as a staple food source and a critical raw material for various industrial applications such as bioethanol production, starch processing, and bio plastics manufacturing [1]. It grows well in temperate, tropical, and subtropical climates due to its versatility in agro climatic conditions, contributing to global food security and industrial supply chains [2]. Maize-derived products, such as high-fructose corn syrup, starch, and other sweeteners, further enhance its value in the food processing, livestock feed, and biofuel industries [3]. Maize is a critical resource not only as a staple food crop but also as a primary component in animal feed, biofuel production, and various industrial processes [4]. Its economic significance is underscored by its versatility and role in sustaining agricultural economies while addressing the nutritional requirements of an increasing global population [5]. Consequently, maintaining optimal maize yields and ensuring crop health is essential for supporting food security and industrial sustainability [6].

Maize production is affected by various biotic challenges, most notably maydis leaf blight, and a disease caused by the fungal pathogen *Cochliobolus heterostrophus* that, if left untreated, may seriously damage yield and crop quality [7]. Studies have shown that, depending on the severity of the infection, this disease can reduce yields by 30% to 80% [8]. For example, natural infection situations have been observed to cause grain yield losses of 56-70%, whereas artificial inoculation has resulted in even major losses, reaching 79-90% in some cases [8]. The disease affects both the grain and non-grain components of the maize plant [9]. This poses a serious threat to food security and industries reliant on maize. In response, sustainable farming practices particularly those using beneficial microbes like *Bacillus subtilis* are gaining attention as a solution to mitigate these losses. *Bacillus subtilis* is recognized for its ability to promote plant growth and enhance resilience to various biotic and abiotic stresses [10]. As an effective biological control agent, it has the potential to mitigate the impact of diseases on crops such as maize [11].

It produces a variety of antibacterial compounds, enzymes, and growth-promoting metabolites that reduce plant infections and improve nutrient availability and uptake, thereby improving plant health [12]. *Bacillus subtilis* generate systemic resistance in plants, providing some proactive defence against future pathogen infections and environmental stressors [13]. Integrated pest management methods provide a sustainable alternative to traditional pesticides, supporting long-term agricultural productivity and protecting environmental health [14].

Despite the beneficial effects of *Bacillus subtilis*, maize remains increasingly threatened by various diseases, including maydis leaf blight caused by *Cochliobolus heterostrophus* [15]. Symptoms of Maydis leaf blight include elongated lesions on the leaves, which can result in premature leaf mortality and a reduction in photosynthetic capacity [16]. Implementing effective management strategies is essential to reduce its impact on maize crops and maintain crop yields.

Bacillus subtilis as a biocontrol agent acts as an effective biopesticide against maydis leaf blight of corn [17]. Unlike chemical pesticides, which can harm non-target organisms, lead to pest resistance, and contribute to environmental contamination, biopesticides like *Bacillus subtilis* offer a safer and more sustainable alternative [18]. They use natural mechanisms to produce antimicrobial chemicals, compete with pathogens, and induce systemic plant resistance [19].

Biopesticides are biodegradable and leave no harmful residues, reducing risks to human health and the environment [20]. Biopesticides help protect the microbes in the soil, which are important for soil health, while chemical pesticides can harm or reduce the number of these helpful microbes [21]. Farmers can use biopesticides such as *Bacillus subtilis* to control diseases effectively. This approach helps protect the environment and supports sustainable farming practices.

This study seeks to evaluate the role of *Bacillus subtilis* in controlling maydis leaf blight disease, assessing its efficacy as a biocontrol agent and its impact on corn plant growth and production. It will concentrate on the interactions between *Bacillus subtilis*, the corn plant, and the pathogen, focusing on resistance development and microbial community transformation in the rhizosphere. Additionally, this research will explore the potential of bio pesticide formulation *Bacillus subtilis* in determining the proper concentration and frequency of application to control maydis leaf blight disease and its effect on the corn crop.

Materials and Method

Experimental site and duration

The study was conducted from 2020 to 2021 at the Ami Experimental Farm in Ahmedabad, Gujarat. This site was selected because its favourable agro-climatic conditions are well-suited for growing corn (*Zea mays* L.), which makes it ideal for testing disease management methods.

Treatment application protocol

Ami *Bacillus subtilis* was applied to the soil at a dosage of 1 kilogram per acre as a soil treatment. The inoculant was uniformly mixed into the soil throughout the experimental field to ensure uniform exposure of the crop to the treatment.

Greenhouse experiment design

In a greenhouse trial, a randomized factorial design with three replications was used to study the effects of Ami *Bacillus subtilis* seed treatment and varying application frequencies. The seed treatment factor included two levels: untreated seeds (B0) and treated seeds (B1). Application frequency was tested across four treatments: no application (A0), weekly application (A1), biweekly application (A2), and application every three weeks (A3).

Seed treatment

In this method 100 grams of seeds were coated with 3 grams of Ami *Bacillus subtilis* and subsequently allow it to settle for two hours before planting. Untreated seeds were planted simultaneously and sown in polybags, each containing 10 kg of soil, with three plants per polybag. To induce Maydis leaf blight, a fungal spore suspension at a concentration of 10^6 spores/mL was uniformly sprayed onto the plant foliage two weeks after planting.

The method involved applying a 10^8 CFU/mL solution of Ami *B. subtilis* to each plant to ensure even coverage. The process of fertilization was performed 15 days following inoculation with a urea/NPK combination. The primary parameters assessed were root length at three weeks after planting (WAP), plant height at eight weeks after planting, and disease severity, which was recorded weekly following inoculation. Disease severity was evaluated based on the extent and intensity of leaf lesions, while plant dry weight was recorded at harvest.

Field experiment design

The field trial utilized a randomized complete block design with three replications and two main factors: Ami *B. subtilis* application concentration and corn variety. Application concentrations were set at four levels (0g/L, 1g/L, 2g/L, and 3g/L), and corn were tested.

To ensure proper inoculation, corn seeds were coated with 3 grams of Ami *Bacillus subtilis* per 100 grams of seed and allowed to settle for two hours prior to planting. Seedlings were planted in plots measuring 3.75 m by 5 m, with one plant per planting hole, spaced 75 cm by 25 cm. Fertilizers (urea and NPK at 300 kg/ha) were applied at three and four WAP. Plants were inoculated with *Cochliobolus heterostrophus* spores at four WAP, and the Ami *B. subtilis* formulation was applied 15 days after inoculation, using treatment-specific concentrations.

Disease assessment

Disease severity was assessed weekly and calculated based on the Townsend and Heüberger formula:

$$DS = \frac{\sum (ni \times vi)}{Z \times N} \times 100\%$$

DS= disease severity

ni = number of plant infected to i.

vi = score with category infection to i.

Z = the highest score.

N = number of plant observed.

Disease progression over time was quantified using the Area under the Disease Development Curve (AUDDC).

Statistical analysis

Analysis of variance (ANOVA) was conducted using the General Linear Model (GLM) procedure [22] with a significance threshold of 5%, and Tukey's test was used for mean comparisons [23] at P = 0.05. All data analyses were performed using STAR 2.0.1 statistical software.

Result and Discussion

Effect of Ami *Bacillus subtilis* on corn seedling growth

The effect of Ami *Bacillus subtilis* formulation on the root length and plant height of corn seedlings is presented in figure 1. The results indicate that seed treatment with Ami *Bacillus subtilis* had improved both root length and plant height compared to no seed treatment. Specifically, seedlings treated with Ami *Bacillus subtilis* had an average root length of 17.0 cm at 21 days after planting (DAP) and a plant height of 183.4 cm at 56 DAP. In contrast, seedlings without seed treatment had a shorter root length of 11.3 cm at 21 DAP and a plant height of 148.4 cm at 56 DAP. Similarly, maize plants treated with *Bacillus subtilis* inoculated grains exhibited the highest root length, measuring 34.66 ± 2.08 cm. [24] In other experiment, Seed treatment by using with Bacillus subtilis TM4 increased corn height by 43.19 cm two weeks after planting (WAP) [25].

Influence of seed treatment and application frequency on plant growth at 56 DAP

The study examined whether seed treatment and various application frequencies of an Ami *Bacillus subtilis* formulation affect plant height 56 days after planting (DAP). The results in figure 2 indicate that seed treatment and the amount of Ami *B. subtilis* used had a statistically significant effect on plant height. Plants with treated seeds and no additional applications (tp 0 x) had an average height of 142.1 cm (±0.23). Five treatments (tp 5x) increased plant height to 151.8 ± 0.65 cm, while three applications (tp 3x) resulted in 159.3 ± 0.34cm.

Two applications (tp 2x) resulted in the highest plant height of 167.3 ± 0.11cm. Similarly, untreated seeds showed growth tendencies with increased application frequency. Plants with untreated seeds and no applications (p 0x) reached an average height

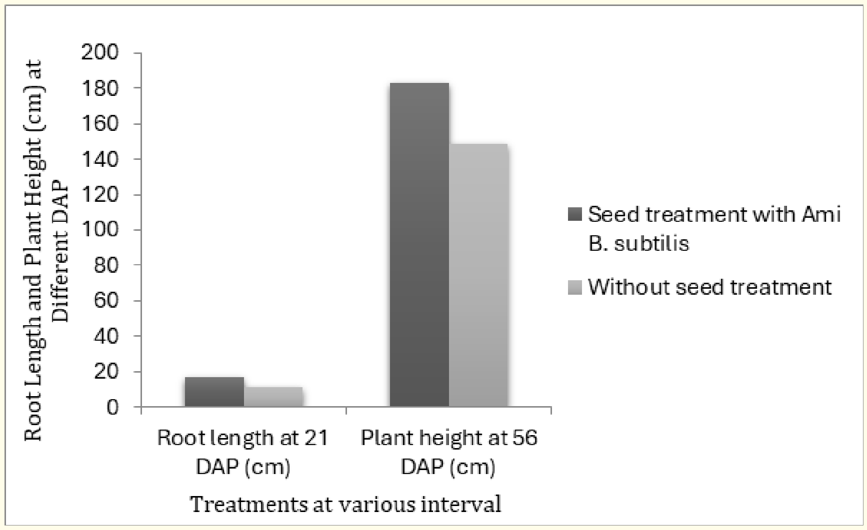

Figure 1: The effect of Ami *B. subtilis* formulation to root length and plant height of corn seedling.

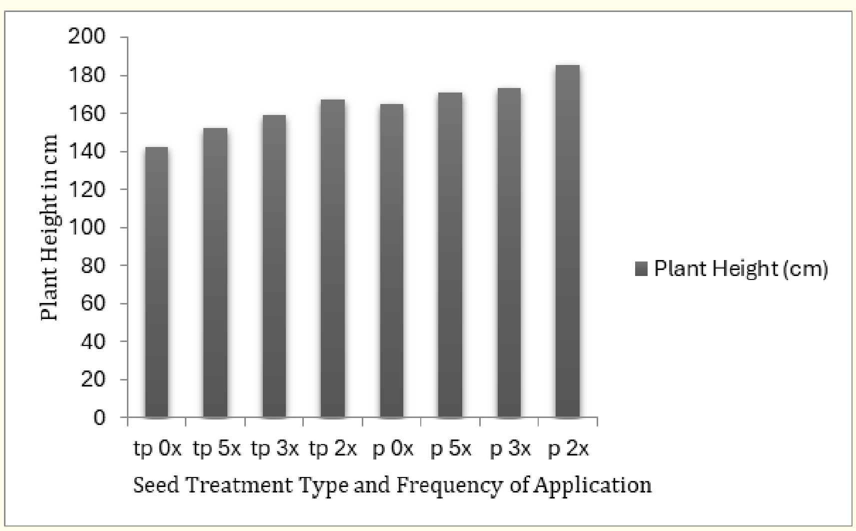

Figure 2: Interaction between seed treatment with the frequency of application of Ami *B. subtilis* formulation to plant height at 56 DAP. The relationship between seed treatment and the frequency of ami *B. subtilis* application in disease development (AUDDC).

tp = no seed treatment;

p = with seed treatment;

Application frequency is every 1-3 weeks.

of 165.1 ± 0.45 cm, whereas those with five applications (p 5x) reached 171.0 ± 0.78 cm. Three treatments (p 3 xs) boosted height to 173.6 cm (±0.22), whereas two applications (p 2 xs) resulted in a maximum height of 185.2 ±0.43 cm. In another study on leaf blight disease in corn, the application of *Bacillus subtilis* combined with biochar inoculate resulted in a maize plant height of 95 cm [26].

Influence of Ami *Bacillus subtilis* application frequency on maydis leaf blight disease progression

Maydis leaf blight is measured using the area under the disease development curve (AUDDC). The AUDDC was highest at 8.1 without the use of Ami *B. subtilis* treatment, indicating disease progression as shown in figure 3. Applying Ami *B. subtilis* weekly resulted in a decrease in AUDDC to 5.8. Similarly, a two-week application resulted in an AUDDC of 6.4, indicating modest disease suppression but slightly increased disease presence compared to weekly applications. However, a weekly application resulted in the lowest AUDDC value of 5.7, indicating that this interval was the most effective in reducing maydis leaf blight development among the studied intervals. This result is in line with other research. The application of *Bacillus subtilis* RB14 has been reported to prevent dampness in tomato plants by up to 80 percent due to the antifungal action of its lipopeptide A content [27].

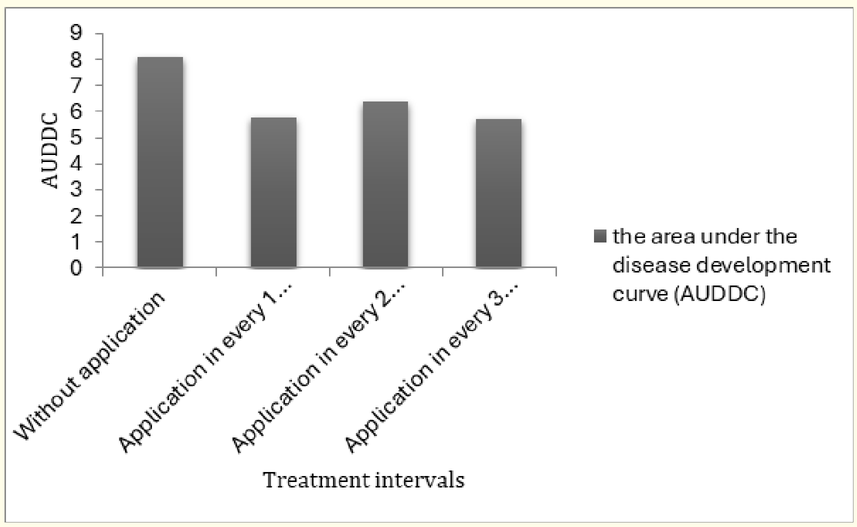

Figure 3: Effect of application frequency of Ami *B. subtilis* on the development of maydis leaf blight disease.

Interaction between seed treatment and application frequency of *Bacillus subtilis* on disease development (AUDCC)

Seed treatment with Ami *Bacillus subtilis* and the frequency of application had a substantial effect on disease spread, as determined by the Area Under the Disease Development Curve (AUDDC) in figure 4. The experiment examined two primary conditions: (1) no seed treatment (denoted as tp) and (2) seed treatment with Ami *Bacillus subtilis* (denoted as p). Ami *Bacillus subtilis* was applied at varied frequencies for each treatment, ranging from zero (0x) to five (5x), with intervals of one to three weeks between each application.

These results indicate that in the absence of seed treatment (tp), the lack of Ami *Bacillus subtilis* (tp 0x) resulted in the highest AUDDC score of 8.7, signifying disease progression. The interaction between seed treatment and the frequency of Ami *Bacillus subtilis* applications affected disease development, as per AUDDC measurements. For treatments without seed inoculation (tp), five applications (tp 5x) lowered AUDDC to 5.3, three applications (tp 3x) to 6.8, and two applications (tp 2x) to 5.6. In contrast, The AUDDC for the seed treatment group (p), even without subsequent use (p 0x), was 5.2. Using Ami *Bacillus subtilis* further improved disease control, with five applications (p 5x) decreasing the AUDDC to 4.0, the lowest value reported among all treatments. Three applications (p 3x) resulted in an AUDDC of 6.9, whereas two applications (p 2x) produced a slightly higher value of 6.8, indicating moderate control of the illness. Similar results have been reported in other experiments, indicating that seed treatment and biological agents sprayed at various frequencies can increase plant height [28].

Impact of Ami *B. subtilis* treatment on maydis leaf blight severity in the field

Applying Ami *Bacillus subtilis* at a rate of 1 kg per acre effectively reduced the growth of maydis leaf blight under field conditions

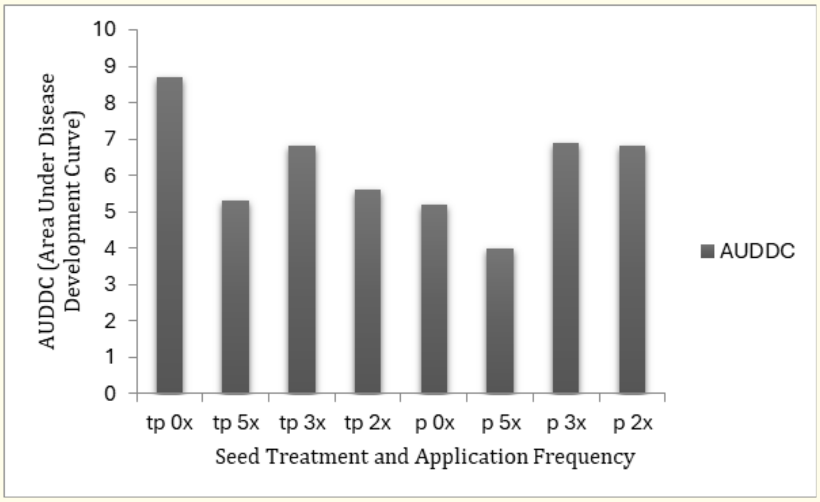

Figure 4: The interaction between seed treatment and the frequency of application of Ami B. subtilis To the Development of disease (AUDDC).

tp = no seed treatment.

p = with seed treatment.

application frequency in every 1-3 weeks.

(Table 1). Disease progression was measured using the Area under the Disease Development Curve (AUDDC), with lower values indicating less disease severity. The treatment with Ami *B. subtilis* produced an AUDDC of 15.1, indicating that the formulation effectively reduced the problem to some extent. In comparison, the untreated control group had a higher AUDDC (22.4), indicating rapid disease progression. Different studies reported similar views, highlighting that *Bacillus subtilis* was determined to be an efficient biocontrol agent on maize, considerably reducing fumonisin concentration by 0.29-0.77 ppm when treated with it [29].

Treatments	AUDDC
Ami *B.subtilis* Treated (1kg/Acre)	15.1
Control	22.4

Table 1: The effect of Ami *B. subtilis* formulation to development of maydis leaf blight in the field.

Effect of Ami *B. subtilis* formulation on plant height at 56 days after planting

The data in Table 2 show that treated plants had considerably higher plant height than untreated controls. Specifically, plants that received the Ami *B. subtilis* formulation had an average height of 191.3 cm, while the control plants, which did not receive any treatment, averaged a height of 165.1 cm. This result is consistent with previous research, as the application of *Bacillus subtilis* formulation significantly increased maize plant height at 28 days after planting (DAP), with treated plants reaching an average height of 53.5 cm compared to the control. [30]

Impact of Ami *B. subtilis* on ear dry weight

The application of Ami *B. subtilis* formulation significantly increased the dry weight of ears in field conditions. Table 3 illustrates that plants treated with Ami *Bacillus subtilis* had a dry weight of 2.3 kg per ten ears, whereas the untreated control group had 1.6

kg per ten ears. Accordingly, the dry weight increased by 0.7 kg as an outcome of the treatment. In a related study treatment with *Bacillus subtilis* resulted in a seedling dry weight of 2.3 g after three months, confirming its effectiveness in promoting seedling growth and contributing to integrated corn disease management [31].

Effect of Ami *B. subtilis* formulation on ear number and yield per plant

The effect of Ami *B. subtilis* formulation on yield is presented in table 4. The results show that the treated group produced fewer ears and a lower yield per 10 plants than the control group. The control group, without treatment, produced 35 ears and a total yield of 5.6 kg per 10 plants. In contrast, the treated group, which received the Ami *B. subtilis* formulation, produced only 25 ears with a total yield of 3.2 kg per 10 plants. Although the yield in the treated group is lower, the data indicate a 46.2% increase in yield compared to the untreated control when adjusting for the number of ears. In another study, treatment with *Bacillus subtilis* resulted in a fruit yield of 0.17 kg per plant, showing its efficiency as a biocontrol agent against root rot caused by *Fusarium solani* in tomatoes [32].

Treatments	Plant height at 56 DAP(cm)
Control Without application	165.1
Treated Ami *B. subtilis* applied	191.3

Table 2: Effect of application n of Ami *B. subtilis* formulation on plant height.

Treatments	The dry weight of ear (Kg/10 ear)
Control Without application	1.6
Treated Ami *B. subtilis* applied	2.3

Table 3: Effect of concentration of Ami *B. subtilis* formulation to dry weight of ear in the field.

Conclusion

The study emphasizes the potential of *Bacillus subtilis* (Ami formulation) as an effective biocontrol agent for managing *Cochliobolus heterostrophus*-induced Maydis leaf blight in corn. The results show that seed treatment with *Bacillus subtilis* enhanced plant growth, with significant improvements in root length and plant height. The formulation was most effective in reducing disease severity when applied weekly, as shown by the lowest Area under Disease Development Curve (AUDDC) values. Field experiments confirmed that *Bacillus subtilis* helped slow disease progression and lower AUDDC compared to untreated controls. Although the formulation did not increase ear number, it improved yield by 46.2%, emphasizing its potential for sustainable maize production. These results show that Ami Bacillus subtilis is an effective alternative to chemical pesticides in integrated pest management systems. Farmers that use this product may benefit from better soils, less reliance on chemical pesticides, and potentially increased crop resilience and yields. Further research is needed to enhance application methods and investigate the long-term effects of *Bacillus subtilis* on soil health and beneficial microbial populations.

Treatments	Number of ears	Yield	
		(kg/10 plant)	Percentage increase
Control Withoutapplication	35	5.6	0.0
Treated Ami Bacillus *Subtilis*applied	25	3.2	46.2

Table 4: Effect of application of Ami *B. subtilis* formulation to the yield.

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