



Fungicidal effect of some non-conventional chemicals for management of alternaria blight disease of mustard

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Abstract: Alternaria blight disease of mustard caused by a necrotrophic fungus *Alternaria brassicae* (Berk.) Sacc. is one of the most important limiting factors, causing yield losses of up to 47% in mustard (*Brassica juncea*). The aim of this present investigation was to evaluate the fungicidal effect of non-conventional chemicals *viz.* calcium sulphate (CaSO₄), potassium chloride (KCl), potassium sulphate (K₂SO₄), zinc sulphate (ZnSO₄) and borax (Na₂B₄O₇·10H₂O) against alternaria blight disease of mustard. The significantly minimum size of spot was recorded in T₁ CaS at 0.5% (1.73) followed by T₃ CaS at 1.5% (1.75) and T₂ CaS at 1.0% (1.78) respectively in comparison to check. The minimum number of average leaf spots/25 mm² leaf area was observed in T₃ CaS at 1.5% (1.78) followed by T₁ CaS at 0.5% (2.26). T₁ CaS @ at 0.5% showed significantly lowest disease index (13.00%) followed by T₁₅ NaB at 0.75% (17.77%) and T₈ KS at 1.0% (18.00%) respectively over check. The average minimum apparent infection rate was recorded in T₁ CaS at 0.5% (0.504) followed by T₉ KS at 1.5% (0.553) and T₃ CaS at 1.5% (0.573) respectively. The AUDPC was significantly minimum in all the treatments of CaSO₄ i.e. in T₁ CaS at 0.5% (32.25), T₂ CaS at 1.0% (33.8) and T₃ CaS at 1.5% (35.55) in comparison to check (77.95). The foliar spray of CaSO₄ at 0.5% concentration induced resistance significantly against alternaria blight and reduce pesticide residue in food and environment.

Keywords: Alternaria blight, *Alternaria brassicae* (Berk.) Sacc., Mustard, Non-conventional chemicals

INTRODUCTION

Mustard (*Brassica juncea* (Linn.) Czern. and Coss.) is an important oil seed crop, grown both in tropical and sub tropical regions of the world. Among the biotic factors, fungal diseases alone are responsible for severe damages to the crop resulting in yield losses up to 70% on a world wide scale. Amongst the major fungal diseases of oilseed brassicas prevalent in India, alternaria blight disease is the most important and destructive disease causing heavy losses all over the world attacking all *Brassica* species (Kolte, 1985; Meena *et al.*, 2010). Alternaria blight disease caused by *Alternaria brassicae* (Berk. and Sacc.) has been reported from all the continents of the world and is one among the important diseases of Indian mustard causing up to 47% yield losses with no proven source of resistance against the disease reported till date in any of the hosts (Meena *et al.*, 2010; Meena *et al.*, 2012). This disease appears on leaves and stems of seedlings and adult plants and also in siliquae during the ripening stage. Dark spots on the leaves and siliquae reduce the photosynthetic capacity and induce immature ripening, which causes reduced

amount of quality seed production in both vegetable and oleiferous brassicas (Kumar *et al.*, 2014).

Alternaria blight disease management mainly relies on fungicide applications. A large number of fungicides have been reported to be effective management of the disease under field conditions *viz.* Baycor (0.2%), Blitox 50 (0.3%), Dithane M45 (0.2%), Dithane Z78(0.2%), Rovral 50 (0.2%), Ridomil MZ (Mancozeb 64% +Metalaxyl 8% WP) etc. (Verma and Saharan, 1994; Khan *et al.*, 2007; Sultana *et al.*, 2009). Although these chemicals found to be effective but leads to residual toxicity, development of resistance in the target organisms and also affect the oil quality in oilseed brassicas (McCartney *et al.*, 1999). The injudicious and unplanned application of fungicides cause health hazards and create environmental pollution. Non-conventional chemicals provide nutrients to the host plant which affect the relationship between crop and pathogen in many ways. It is apparent that plant nutrition is one of the environmental factors which along with others, such as temperature, humidity, moisture and soil reaction may have a measurable effect upon the course of disease development. The present investigations were conducted to find out some

inexpensive, non-toxic, non-conventional chemical compounds as abiotic elicitors of *B. juncea* in relation to activate defence response as possible alternative in the management of alternaria blight.

MATERIALS AND METHODS

A field isolate of *Alternaria brassicae* from infected leaves exhibiting typical symptoms of Alternaria spot, usually with concentric rings, was collected from the field-grown plants of highly susceptible *B. juncea* cv. Varuna from Crop Research Centre, Pantnagar. The culture of *A. brassicae* was isolated on potato sucrose agar (PSA) and purified by single spore isolation. The pathogen was maintained on potato sucrose agar (PSA) medium at $20^{\circ} \pm 2^{\circ} \text{C}$, 12 hrs light and 12 hrs dark conditions. Pathogenicity test of the fungal culture was done on one-month-old highly susceptible *B. juncea*.

Alternaria blight susceptible mustard variety varuna was used in the glasshouse experiments. Freshly collected field soil (sandy loam) was mixed with compost in 3 : 1 proportion, sieved through 0.2 mm sieve and was filled in the pot which were used for sowing. Two gram diammonium phosphate (DAP) was mixed in each pot before sowing.

Two-week-old culture on PSA medium was taken and blended with the Blender in 250 ml sterilized water. The concentration was adjusted to 10^4 spores ml^{-1} . The suspension was sprayed on 25-day-old plants using atomizer. Control plants were sprayed with sterilized water. Inoculated plants were incubated for 3 days in the humid chamber at 90-100% R.H. After 3 days, pots were kept outside the glasshouse under normal conditions, at the temperature ranging between minimum of 8°C and maximum of 22°C during the month of January for development of the symptoms. The fungus was re-isolated from infected leaves and maintained on PSA slants and pathogenicity test, as above, was reported twice to confirm the results.

A total of five chemicals viz. CaSO_4 (CaS), KCl (Kl), K_2SO_4 (KS), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (ZnS), and $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ (NaB) were tested as spray treatments with three different concentrations. Two sprays of each chemical of the respective concentrations were given during the entire growth period of plants with an atomizer. Separate atomizer was used for each chemical. First spray of respective chemicals was given on all the leaves of 25-day-old plants i.e. 72 hrs of pre-inoculation and the second spray was given after 72 hrs of post-inoculation.

Size of spot was recorded in randomly selected five spots/leaf was measured in mm including yellow halo, chlorotic area with necrotic brown area in the centre at 10 days interval. The numbers of spots on leaf per 25 mm^2 leaf area were recorded at 10 days interval with the help of a glass slide. Observations were taken on five leaves and average number of spots per 25 mm^2 area was then calculated. Average disease index on leaf due to Alternaria blight was taken at 10 days interval by

use of 0-5 rating scale as 0= no symptom, 1= 1-10%, 2= 11-25%, 3= 26-50%, 4= 51-75% and 5= >75% (Conn *et al.*, 1990). For recording observations, five leaves were selected from each replication randomly and leaves were rated as per the above scale and average disease index was calculated by the following formula (McKinney, 1923):

$$\text{Disease index (\%)} = \frac{\text{Sum of all numerical rating}}{\text{Number of leaves examined} \times \text{Maximum grade}} \times 100$$

Apparent infection rates were calculated from the disease index at different times and subsequent infection rate (r) was calculated by using formula given by Vanderplank (1963).

$$r = \frac{2.3}{t_2 - t_1} \log_{10} \frac{x_2(1 - x_1)}{x_1(1 - x_2)}$$

Where,

r=apparent infection rate

x_1 =disease index at time t_1

x_2 =disease index at time t_2

t_1 =time of initial disease rating (x_1)

t_2 =time of second disease rating (x_2)

For comparative study of disease progress in different treatments, the area under disease progress curve (AUDPC) was calculated for each treatment using formula given by Wilcoxson *et al.* (1975) which are as follows:

$$\text{A-value} = \sum_{i=1}^K \frac{1}{2} (S_i + S_{i-1}) d$$

Where,

S_i =Disease severity at the end of week i

K=Number of successive evaluation of disease

d=Interval between two evaluations.

Area under disease progress curve was plotted by plotting time interval on x-axis and size of spot (mm) on Y-axis in respect of different treatments.

RESULTS AND DISCUSSION

The present investigations were conducted to find out the fungicidal effect of some inexpensive, non-toxic, non-conventional chemical compounds as possible alternative in the management of alternaria blight disease of mustard. Size of alternaria leaf spot due to some non-conventional chemicals was measured from 30 to 50 days after sowing at 10 days interval. The size of leaf spot increased from 30 Days after sowing (DAS) to 50 DAS in all the treatments. Minimum size of spot (1.73 to 1.78 mm) was found significant in all three treatments of CaSO_4 . The maximum reduction of size of spot was recorded in T_1 CaS (52.20) followed by T_3 CaS (51.65) and T_2 CaS (50.82%), respectively over check. The maximum average size of leaf spot was observed in check (3.62 mm). The interactions between different treatments and observation intervals was also found significant (Table 1).

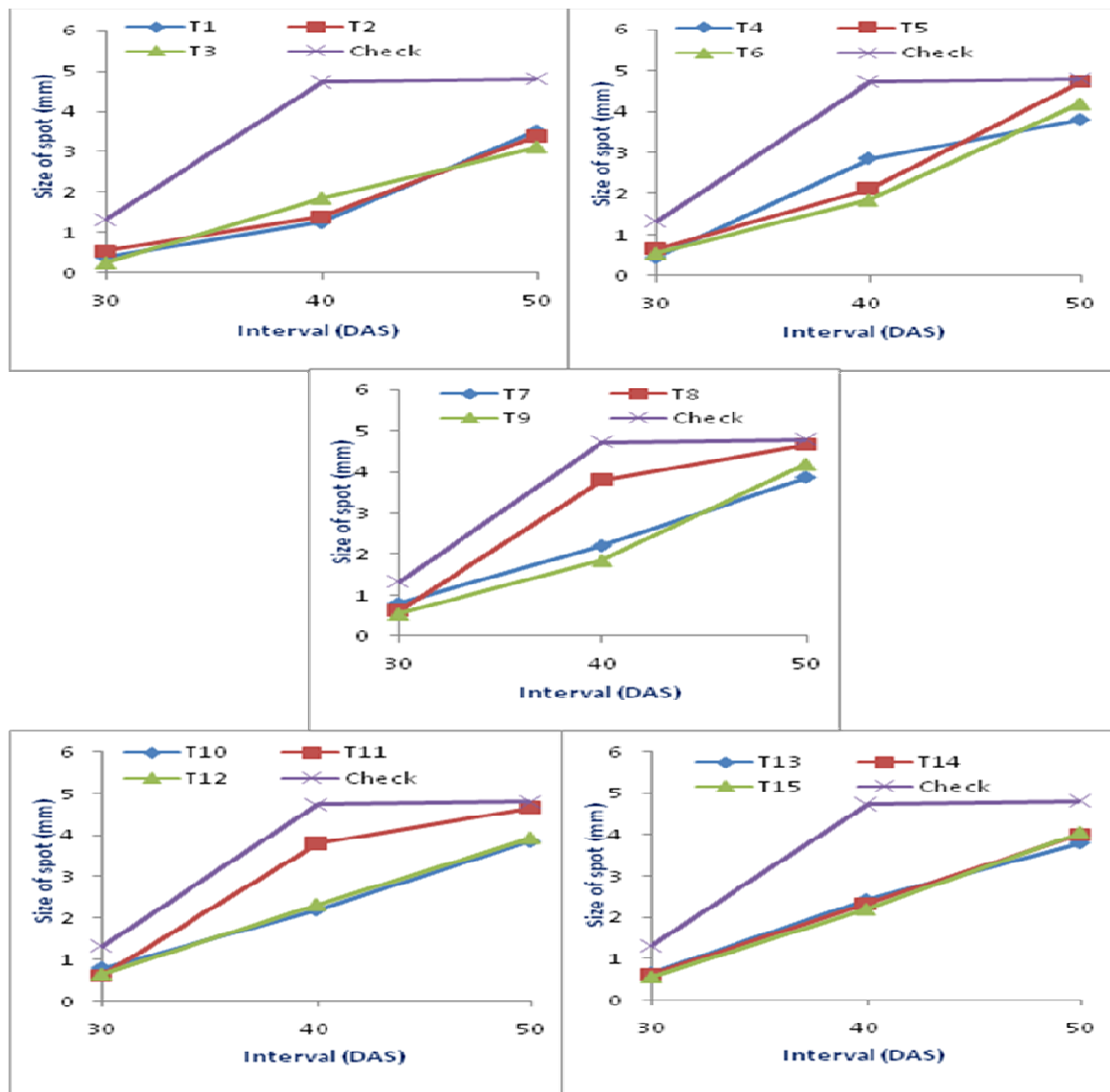


Fig. 1. Effect of some non-conventional chemicals on AUDPC of *A. brassicae* in relation to size of spot (mm) in mustard.

Average number of leaf spots/25 mm² leaf area also progressively increased from 30 to 50 DAS in all the treatments (Table 2). The increase of number of leaf spots per 25 mm² leaf area was found to be highly significant at 1% level among the treatments and observation intervals. The interactions among these variable were also highly significant. The significantly minimum number of average leaf spots/25 mm² leaf area was observed in T₃ CaS at 1.5% (1.78) followed by T₁ CaS at 0.5% (2.26) in comparison to check (5.06).

The leaf disease index was found to be highly significant among the treatments as well as between observation intervals and their interactions. Among the treatments, T₁ CaS at 0.5% showed lowest disease index (13.00%) followed by T₁₅ NaB at 0.75% (17.77%) and T₈ KS at 1.0% (18.00%) respectively over check at 5% level of significance. The maximum disease index was observed in T₁₀ ZnS at 0.25% (25.00%) followed by T₁₂ ZnS at

0.75% (23.55%) and T₄ KI at 0.5% (23.11%) respectively over check (Table 3).

The Apparent infection rate progressively increased between 30-40 DAS and 40-50 DAS in all the treatments. The maximum apparent infection rate was observed in check within 30-40 and 40-50 DAS i.e. 0.661 and 0.796, respectively. Based on overall mean of apparent infection rate, the minimum apparent infection rate was recorded in T₁ CaS at 0.5% (0.504) followed by T₉ KS at 1.5% (0.553) and T₃ CaS at 1.5% (0.573), respectively over check (Table 4).

The area under disease progress curve (AUDPC) was measured for different treatments (Fig.1). It was observed that the AUDPC was significantly minimum in all the treatments of CaSO₄ i.e. in T₁ CaS at 0.5% (32.25), T₂ CaS at 1.0% (33.8) and T₃ CaS at 1.5% (35.55) in comparison to check (77.95) (Table 5). The maximum AUDPC was recorded in both treatments T₈ KS at

Table 1. Effect of some non-conventional chemicals on size of alternaria leaf spot at different stages of growth of mustard.

Treatment	Concentration (%)	Size of leaf spot (mm)			Mean	Reduction over check (%)
		30 DAS	40 DAS	50 DAS		
T1 CaS	0.5	0.40	1.26	3.53	1.73	52.20
T2 CaS	1.0	0.56	1.40	3.40	1.78	50.82
T3 CaS	1.5	0.26	1.86	3.13	1.75	51.65
T4 KI	0.5	0.46	2.86	3.80	2.37	34.53
T5 KI	1.0	0.66	2.13	4.73	2.51	30.66
T6 KI	1.5	0.56	1.86	4.20	2.21	38.95
T7 KS	0.5	0.80	2.20	3.86	2.28	37.01
T8 KS	1.0	0.63	3.80	4.66	3.03	16.29
T9 KS	1.5	0.56	1.86	4.20	2.21	38.95
T10 ZnS	0.25	0.80	2.20	3.86	2.28	37.01
T11 ZnS	0.50	0.63	3.80	4.66	3.03	16.29
T12 ZnS	0.75	0.66	2.33	3.93	2.31	36.18
T13 NaB	0.25	0.66	2.43	3.80	2.30	36.46
T14 NaB	0.50	0.63	2.33	4.00	2.32	35.91
T15 NaB	0.75	0.56	2.20	4.06	2.27	37.29
T check	–	1.33	4.73	4.80	3.62	–
Mean C.D. at 5%		0.63	2.45	4.04		
Treatment Interval					0.46	
Interaction					0.19	
					0.79	

Table 2. Effect of some non-conventional chemicals on number of alternaria leaf spots at different stages of growth of mustard.

Treatment	Concentration (%)	Number of leaf spots/ 25 mm ² area			Mean	Reduction over check (%)
		30 DAS	40 DAS	50 DAS		
T1 CaS	0.5	0.66	1.33	5.40	2.26	55.33
T2 CaS	1.0	1.36	2.20	5.06	2.87	43.28
T3 CaS	1.5	0.56	1.26	3.53	1.78	64.82
T4 KI	0.5	0.73	2.66	6.20	3.20	36.75
T5 KI	1.0	1.70	3.53	5.93	3.72	26.48
T6 KI	1.5	1.23	3.00	5.86	3.36	33.59
T7 KS	0.5	1.03	2.53	6.66	3.41	32.60
T8 KS	1.0	1.06	2.00	4.86	2.64	47.82
T9 KS	1.5	1.06	2.20	5.40	2.88	43.08
T10 ZnS	0.25	1.43	4.46	5.53	3.81	24.70
T11 ZnS	0.50	1.16	2.00	5.53	2.90	42.68
T12 ZnS	0.75	1.70	3.40	5.86	3.65	27.86
T13 NaB	0.25	1.30	2.93	4.40	2.87	43.28
T14 NaB	0.50	1.60	3.26	4.66	3.17	37.35
T15 NaB	0.75	1.23	2.60	3.86	2.56	49.40
T check	–	2.06	6.13	7.00	5.06	–
Mean C.D. at 5%		1.20	2.84	5.36		
Treatment Interval					0.74	
Interaction					0.32	
					1.28	

Table 3. Effect of some non-conventional chemicals on disease severity of alternaria blight on leaf at different stages of growth of mustard.

Treatment	Concentration (%)	Disease index (%)			Mean	Percent increase or decrease over check
		30 DAS	40 DAS	50 DAS		
T1 CaS	0.5	3.00 (19.34)	8.00 (16.40)	28.00 (31.79)	13.00 (19.18)	59.51
T2 CaS	1.0	4.00 (11.47)	11.00 (19.35)	44.00 (41.53)	19.66 (24.12)	38.77
T3 CaS	1.5	5.66 (13.75)	10.00 (18.37)	38.66 (38.44)	18.11 (23.52)	43.60
T4 KI	0.5	1.66 (6.03)	15.66 (23.30)	52.00 (46.15)	23.11 (25.16)	28.02
T5 KI	1.0	3.33 (10.40)	10.66 (19.04)	46.66 (43.08)	20.22 (24.17)	37.02
T6 KI	1.5	4.66 (12.41)	18.66 (25.59)	44.00 (41.54)	22.44 (26.51)	30.11
T7 KS	0.5	3.00 (9.88)	12.00 (20.22)	42.66 (40.77)	19.22 (23.62)	40.14
T8 KS	1.0	3.33 (10.49)	10.66 (19.04)	40.00 (39.21)	18.00 (22.91)	43.94
T9 KS	1.5	3.00 (9.88)	8.00 (16.40)	45.33 (42.32)	18.77 (22.87)	41.54
T10 ZnS	0.25	11.00 (19.32)	22.66 (28.42)	41.33 (40.00)	25.00 (29.24)	22.14
T11 ZnS	0.50	5.00 (12.87)	14.66 (22.49)	44.00 (41.55)	21.22 (25.64)	33.91
T12 ZnS	0.75	4.00 (11.47)	10.66 (18.98)	56.00 (48.45)	23.55 (26.30)	26.65
T13 NaB	0.25	7.00 (15.31)	14.00 (21.93)	33.33 (35.26)	18.11 (24.17)	43.60
T14 NaB	0.50	5.00 (12.74)	14.66 (22.36)	35.33 (36.46)	18.33 (23.86)	42.91
T15 NaB	0.75	6.66 (14.95)	14.66 (22.47)	32.00 (34.42)	17.77 (23.95)	44.65
T check	–	11.00 (19.32)	30.00 (33.17)	55.33 (48.06)	32.11 (33.52)	–
Mean		5.08 (12.48)	14.12 (21.72)	42.41 (40.56)		
C.D. at 5%						
Treatment					2.71	
Interval					1.17	
Interaction					4.70	

1.0% and T₁₁ ZnS at 0.5% (64.44 mm²) followed by T₄ KI at 0.5% (49.90 mm²) in comparison to check (77.95 mm²).

The above results underline the importance of mineral nutrition as a component of disease management practices (Agrios, 2005; Hossain and Mian, 2005; Sugimoto *et al.*, 2008; Meena *et al.*, 2011). The present investigation showed that foliar application of calcium sulphate showed maximum fungicidal effect against the alternaria blight of mustard. The size of spot, disease index, apparent infection rate and area under disease progress

curve were recorded minimum by foliar application of calcium sulphate at 0.5% concentration. Several studies have reported that calcium applications can suppress diseases caused by several pathogens (Volpin and Elad, 1991; Conway *et al.*, 1992; Yamazaki and Hoshina, 1995; Biggs *et al.*, 1997).

Calcium has critical roles in cell division, cell development, carbohydrate movement, neutralization of cell acids, cell wall deposition and formation of pectate salts in the middle lamella (Huber and Arny, 1985). It has

Table 4. Effect of some non-conventional chemicals on apparent* infection rate (r) at different stages of growth of mustard.

Treatment	Concentration (%)	Apparent infection rate (r)		Mean
		30-40 DAS	40-50 DAS	
T1 CaS	0.5	0.361	0.648	0.504
T2 CaS	1.0	0.440	0.744	0.592
T3 CaS	1.5	0.430	0.716	0.573
T4 KI	0.5	0.450	0.780	0.615
T5 KI	1.0	0.427	0.755	0.591
T6 KI	1.5	0.554	0.748	0.651
T7 KS	0.5	0.447	0.738	0.592
T8 KS	1.0	0.427	0.724	0.575
T9 KS	1.5	0.361	0.746	0.553
T10 ZnS	0.25	0.609	0.736	0.672
T11 ZnS	0.50	0.507	0.746	0.626
T12 ZnS	0.75	0.434	0.792	0.613
T13 NaB	0.25	0.504	0.690	0.597
T14 NaB	0.50	0.507	0.702	0.604
T15 NaB	0.75	0.513	0.682	0.597
T check	–	0.666	0.796	0.731

Table 5. Effect of some non-conventional chemicals on AUDPC of alternaria blight of mustard.

Treatment	Concentration (%)	AUDPC (A) (mm ²)
T1 CaS	0.5	32.25
T2 CaS	1.0	33.80
T3 CaS	1.5	35.55
T4 KI	0.5	49.90
T5 KI	1.0	48.25
T6 KI	1.5	42.40
T7 KS	0.5	45.30
T8 KS	1.0	64.45
T9 KS	1.5	42.40
T10 ZnS	0.25	45.30
T11 ZnS	0.50	64.45
T12 ZnS	0.75	46.25
T13 NaB	0.25	46.60
T14 NaB	0.50	46.45
T15 NaB	0.75	45.10
T check	–	77.95

* Apparent infection rate computed on the basis of disease index.

been noted that the Ca²⁺ ion signal is one of the earliest events in challenged cells, and the signal is essential for the activation of plant defense responses such as phytoalexin biosynthesis, induction of defense-related genes, and hypersensitive cell death (Knight *et al.*, 1991). The extent of disease reduction was related to increased calcium uptake by plants, suggesting that calcium was the

effective element in reducing phytophthora stem rot in soybean (Sugimoto *et al.*, 2008).

Conclusion

Manipulation of crop nutrition gives the farmer a valuable tool for managing crop health as well as reduced public pressure over pesticide residue in food and in the environment.

This study showed that applications of non-conventional chemicals *i.e.* foliar spray of nutrient solution greatly influenced the reduction of alternaria blight disease of mustard. The foliar application of CaSO_4 at 0.5% concentration induced resistance to alternaria blight significantly in comparison to different concentration of KCl , K_2SO_4 , ZnSO_4 , and $\text{Na}_2\text{B}_4\text{O}_7$. The findings of this work may lead to effective field strategies for the management of alternaria blight disease of mustard.

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