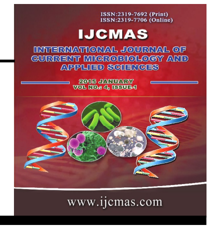

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Original Research Article

Management of damping-off disease of soybean caused by *Pythium ultimum* Trow.

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A B S T R A C T

Damping-off disease of soybean (*Glycine max*) can be common under cool and moist soil conditions caused mainly by *Pythium ultimum* Trow. which is estimated to be associated with 75% of the seed emergence problems. Managing this disease can be difficult due to wide host range of the pathogen and lack of resistant cultivars. Seed treatments are only effective against the soil borne pathogens in the seedling stages. Efficacy of fungicides and bioagents were tested as seed treatment. Among six fungicides tested, Metalaxyl and among five bioagents, *Trichoderma hamatum* seed treatments gave maximum germination percentage in pathogen inoculated soil in pot culture experiments. But seed treatment Metalaxyl + *T. hamatum* had shown better results than Metalaxyl and *T .hamatum* alone. Naturally pathogen infested field trial was conducted to evaluate the effect of Metalaxyl + *T. hamatum* seed treatment. Germination without seed treatment was found to be 45.3% which was significantly increased up to 63.1% in case of seed treatment.

Keywords

Pythium ultimum, Damping-off, Soybean, Metalaxyl, Biocontrol

Introduction

Planting of soybean in cool condition will greatly increase the chances that crops will be damaged by one or more seed or soil-borne disease.

The risk of disease is greatly enhanced by the added stress that cold, wet soils places on germinating seed and young seedling. Pre- and post-emergence damping-off reduce yields by lowering plant populations in all or portions of fields. Stand loss can be so great as to require replanting.

Damping-off is generalized name for diseases caused by a variety of seed-soil-borne pathogens which result in seed rot or death of seedlings prior to emergence (pre-emergence damping-off, fig.1) or death of seedlings following emergence (post-emergence damping-off, fig.2). The most prolific and damaging species which causes damping-off is *Pythium ultimum* Trow. Damage is greatest when soil become saturated soon after planting. Diseased plants are usually distributed in small circular patches, frequently where water has stood. Much larger areas may be affected during extremely rainy weather, especially in fields with poor interior or surface water drainage. *Pythium* species may cause a seed rot or death of young seedlings may occur during or soon after emergence. Affected seedlings will exhibit a soft watery rot, wilt, turn brown and die.

Use of fungicide seed treatments, especially Metalaxyl, is common in regions where pythium injury occurs. Metalaxyl inhibits RNA and protein synthesis in many members of Peronosporales (Fisher and Hayes,1982). It is systemic and has activity against Pythium seed decay and seedling blight, as well as root rot (Hwang *et al*., 2001). There are several reports on the application of fungicidal seed treatment is essential for manage damping-off/seedling/root rot diseases of soybean (Hwang, 2001; Bradley, 2008; Hershman, 2011).

Although, major task now facing scientists is to develop, using a combination approaches, alternatives to chemicals for effective management of crop diseases caused by *Pythium* spp. One such alternative, which has been proposed for biological control of several plant pathogens, involves the introduction of selected microorganisms such as *Trichoderma* spp. (Howell, 2002; Omar, 2007). In India, seed treatment to soybean seeds is not a practice to avoid soil-borne diseases.

The purpose of this research was to determine fungicide and biological seed treatment and their combination effects on controlling damping-off disease of soybean. Also, one hundred germplasm lines, including popularly released varieties were tested against these treatments.

Materials and Methods

Development of sick soil

The soil collected from MAU field was well sieved and was amended with sorghum flour @25g/kg soil. This soil was transferred in gunny bags/cotton cloth bags for sterilization. It was sterilized for 15 lbs pressure/sq. inch for 1 hr. Sterilization was repeated twice at 24 hrs interval. Sterilized soil was transferred to plastic containers. Each container was inoculated with 5mm *Pythium* inoculum disc. These plastic containers were then transferred to plastic trays having 1cm sterile water column at bottom so as to maintain high humidity for multiplication of the pathogen in soil. Trays were covered with polythene sheets. After 4 days of incubation the sick soil was developed.

Testing of fungicides as seed dresser in sick soil

Experiment was planned in RBD with ten treatments and three replications with soybean variety JS-335. Seeds were sown in sick soil treated with different fungicides *viz*. Thiram, Metalaxyl, Cymoxanil, Chlorothalonil, Carbendazim, Captan @ 4g/kg seed and in combination of bioagents (10ml/kg seed) i.e. Metalaxyl + T. hamatum and Cymoxanil + *T. hamatum*. In each plastic container 4 seeds were sown thus forming a replication of 16 seeds. The observations on germination percentage and seed rot were recorded replication wise.

Testing of biocontrol agents as seed dresser in sick soil

Bioagents *viz. T. hamatum, T. harzianum, T. viride, Gliocladium virens, P. fluorescens* were grown in PDB for 15 days. Seeds of JS-335 variety were smeared with respective biocontrol agents before sowing in sick soil. The experiment was conducted in RBD with 10 treatments comprising of biocontrol agents and fungicide checks. Three replications with 16 seeds per replication were sown.

Evaluation of seed treatment under field conditions

Performance of seed treatment was tested on one hundred germplasm lines under field (natural) conditions. Seed treatment comprised of Metalaxyl + *T. hamatum*. The experiment was designed in split plot design. Seed treatment and control without seed treatment served as two main treatments and 100 germplasm lines served as sub treatments.

Five replications with 10 seeds in each replication were maintained for both treatments. Soybean seeds were treated with Metalaxyl first @ 4g/kg of seed and on next day seeds were treated with *T. hamatum* @10 g/kg seed. Seeds were sown in the field. Observations on germination and seed rot and damping-off of seeds were recorded after 12 days.

Result and Discussion

Evaluation of different fungicides as seed dresser in sick soil

In this experiment six fungicides as seed dresser were tested in sick soil. The seeds in treatment of absolute control (Sterilized soil) and inoculated control (sick soil) were not treated with any kind of fungicide.

In each plastic container 4 seeds were sown thus forming a replication of 16 seeds. The observations on germination percentage and seed rot were recorded replication wise. Results are given in Table 1.

From this table, it can be concluded that F_7- Metalaxyl + *T. hamatum* and F_2-Metalaxyl alone were at par and were significantly superior in minimizing the seed rot (%). Cymoxanil + *T. hamatum* and Cymoxanil alone ranked second and were at par. Rest of the fungicides like Captan, Chlorothalonil, Thiram, Carbendazim were at par, however they were significantly superior over inoculated control. Similar trend of efficacy was observed in respect of improvement of germination by different fungicides.

Efficacy of different bioagents as seed dresser against *P. ultimum* in sick soil

From Table 2, it was onserved that all the biocontrol agents have reduced seed rot % significantly over inoculated control. Among the biocontrol agents *T. hamatum* significantly superior in reducing the seed rot % over inoculated control.

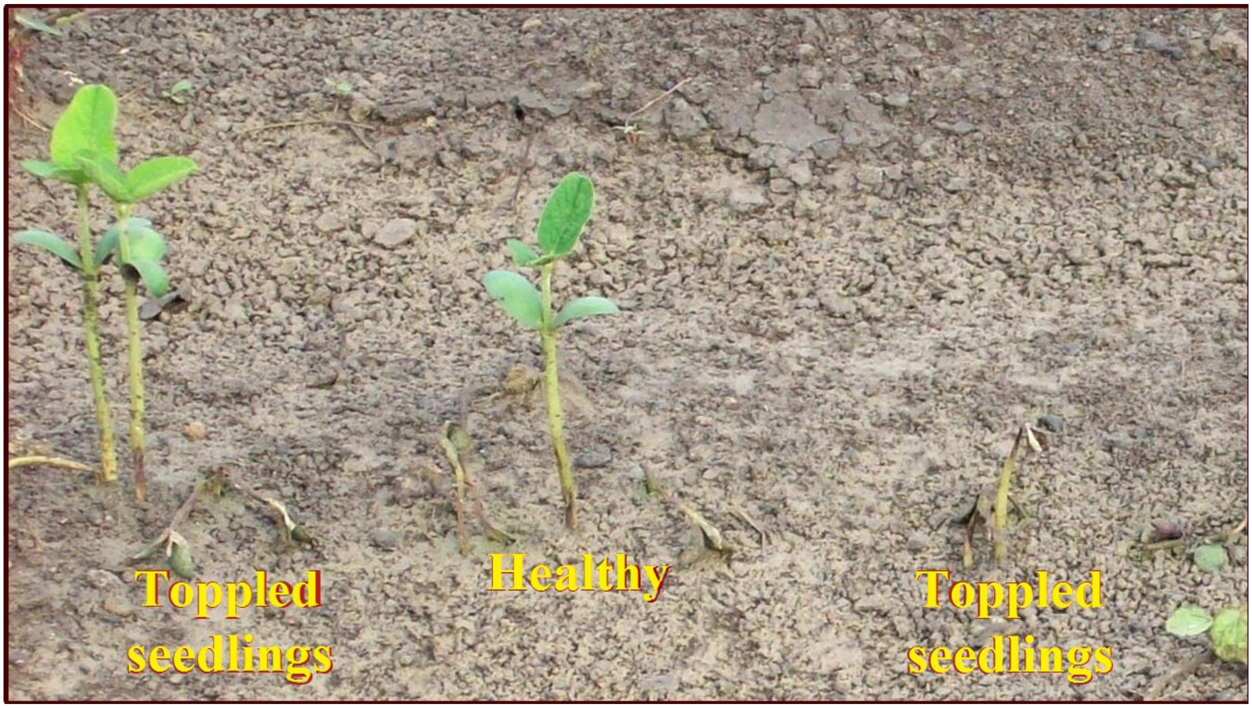

Fig.1 Post-emergence damping-off symptoms caused by *Pythium ultimum*

Fig.2 Symptoms showing pre-emergence mortality

Table.1 Effect of seed treatment fungicides on pre-emergence damping off in soybeancaused by *P. ultimum*

		Seed rot			
Sr. No.	Treatments	(%)	√x+1 Trans.	(√x+1)² Trans.	Arc sin value
1	Thiram	54.16	7.42	55.09	33.44[bcd]
2	Metalaxyl	27.08	5.28	27.98	16.25[fg]
3	Cymoxanil	39.58	6.36	40.50	23.90[e]
4	Chlorothalonil	58.33	7.68	59.23	36.42[bc]
5	Carbendazim	52.08	7.28	53.03	32.04[cd]
6	Captan	62.50	7.94	63.38	39.67[b]
7	Metalaxyl + *T. hamatum*	16.66	4.14	17.60	10.15[g]
8	Cymoxanil+ *T. hamatum*	33.33	5.81	34.22	20.08[ef]
9	Inoculated Control	100.00	10.04	100.8	89.98[a]
10	Absolute Control (Uninoculated)	4.16	2.12	5.15	2.95[h]
	SE±	3.57	0.34	3.55	2.34
	C.D. at 5%	10.62	1.02	10.57	7.11
	C.V. (%)	13.82	9.33	13.49	13.59

* Figures with common letters are statistically at par.

Table.2 Effect of biocontrol agents as a seed treatment option on per cent seed rot of soybean

Sr. No.	Treatment	Seed rot (%)
1	*T. hamatum*	56.25 (35.03)[d]
2	*T. harzianum*	60.41 (37.82)[cd]
3	*T. viride*	75 (49.56)[b]
4	*Gliocladium virens*	68.75 (44.28)[bc]
5	*P. fluorescens*	66.66 (42.65b)[cd]
6	Metalaxyl + *T. hamatum*	18.75 (11.40)[f]
7	Cymoxanil +*T. hamatum*	31.25 (18.83)[ef]
8	Thiram + *T. hamatum*	37.5 (22.61)[e]
9	Inoculated Control	100 (89.98)[a]
10	Absolute Control(Uninoculated)	2.08 (1.76)[g]
	SE±	3.79 (2.57)
	C.D. at 5%	11.28 (7.81)
	C.V. (%)	12.72 (12.87)

Note : Figures with common letters are statistically at par.
: Figures in parenthesis are arc sin transformations.

Table. 3(a) Effect of fungicidal seed treatment on per cent germination of soybean seed

Germination (%) : Arc sin value											
Code	Name	ST_0	ST_1	Mean	Code	Name	ST_0	ST_1	Mean	Main Tr.	
G_1	MAUS-68-2	50.10	51.38	50.74	G_{26}	KB-230	62.70	79.65	71.17		
G_2	Himso-1598	39.88	64.14	52.01	G_{27}	MAUS-142	41.39	47.90	44.65		
G_3	MAUS-199	70.07	77.44	73.76	G_{28}	Himso-1579	59.73	69.31	64.52		
G_4	NRC-15	30.08	58.00	44.04	G_{29}	PK-1222	79.65	84.82	82.23		
G_5	Pusa-97-03	26.14	55.32	40.73	G_{30}	MAUS-101	42.91	53.12	48.01		
G_6	MAUS-34	70.07	84.82	77.44	G_{31}	NRC-18	59.73	74.48	67.11		
G_7	IC-1183	50.10	90.00	70.04	G_{32}	EC-37055	39.88	55.32	47.60		
G_8	G_5P_2	41.39	67.86	54.63	G_{33}	MAUS-164	37.13	61.94	49.54		
G_9	G_5P_{14}	67.86	74.48	71.17	G_{34}	MAUS-155	31.36	55.32	43.34		
G_{10}	EC-1247	44.65	51.84	48.24	G_{35}	Dsb-4	39.88	50.10	45.00		
G_{11}	Indra Soya	30.17	47.90	39.03	G_{36}	MAUS-144	46.16	64.14	55.15		
G_{12}	G_3P_{10}	53.58	61.94	57.76	G_{37}	MAUS-163	46.16	64.14	55.15		
G_{13}	JS-8021	35.76	49.64	42.70	G_{38}	IC-118443	41.39	55.79	48.59		
G_{14}	EC-116343	53.12	79.65	66.38	G_{39}	EC-251417	38.51	46.16	42.33		
G_{15}	MAUS-136	32.83	53.58	43.21	G_{40}	G_2P_{18}	41.39	44.42	42.91		
G_{16}	LN-617	30.08	46.16	38.12	G_{41}	H_2P_2	30.00	49.64	39.81		
G_{17}	MAUS-123	34.11	49.64	41.88	G_{42}	EC-281462	44.65	51.38	48.01		
G_{18}	JS-87-14	64.14	90.00	77.06	G_{43}	EC-333866	67.11	79.65	73.38		
G_{19}	IC-209	74.48	84.82	79.65	G_{44}	EC-16-116	47.90	57.53	52.71		
G_{20}	Kalitur	74.48	90.00	82.23	G_{45}	MAUS-60	67.11	84.82	75.96		
G_{21}	MACS-304	53.58	74.48	64.03	G_{46}	Ankur	84.82	90.00	87.40		
G_{22}	Himso-1587	84.82	90.00	87.40	G_{47}	SL-528	24.91	55.79	40.35		
G_{23}	H_2P_6	46.16	64.14	55.15	G_{48}	MAUS-17	26.14	64.14	45.14		
G_{24}	EC-33940	49.64	64.14	56.89	G_{49}	Himso-1554	36.86	62.70	49.78	SE±	C.D. @0.05
G_{25}	JS-9988	79.65	84.82	82.23	G_{50}	JH-SH-92-93	55.32	84.82	70.07		
	Mean	45.30	63.08	54.19		Mean	45.30	63.08	54.19	0.36	1.43
Sub Tr.					Sub Tr.						
SE±				2.47	SE±				2.47		
C.D.@0.05				7.13	C.D.@0.05				7.13		
Interaction (ST×G) :											
SE±	3.49										
C.D.@0.05	10.08										

Table. 3(b) Effect of fungicidal seed treatment on per cent germination of soybean seed

Germination (%) : Arc sin value											
Code	Name	ST_0	ST_1	Mean	Code	Name	ST_0	ST_1	Mean	Main Tr.	
G_{51}	PK-1029	36.86	59.73	48.30	G_{76}	JS-9467	42.91	51.84	47.37		
G_{52}	IC-26936	55.32	84.82	70.07	G_{77}	Birsa	57.53	90.00	73.76		
G_{53}	ACPS-147	51.38	77.44	64.41	G_{78}	B 5-41	31.36	44.42	37.89		
G_{54}	IC-49860	35.62	53.12	44.37	G_{79}	MAUS-38	23.57	49.64	36.60		
G_{55}	MAUS-173	30.08	38.65	34.36	G_{80}	MAUS-26	75.24	90.00	82.61		
G_{56}	JS-8981	41.39	51.84	46.62	G_{81}	US-1	23.57	47.90	35.73		
G_{57}	EC-11820	34.25	46.39	40.32	G_{82}	NRC-12	46.16	72.27	59.22		
G_{58}	JS-SH-97-14	23.57	53.58	38.58	G_{83}	MAUS-104	61.94	90.00	75.96		
G_{59}	JS-SH-1343	35.62	53.12	44.37	G_{84}	JS-2000	59.73	84.82	72.27		
G_{60}	MAUS-110	46.16	59.73	52.95	G_{85}	JS-9212	59.73	90.00	74.86		
G_{61}	IC-118059	22.35	32.74	27.54	G_{86}	SL-637	49.64	53.12	51.38		
G_{62}	NRC-52	38.51	44.87	41.69	G_{87}	Dsb-6-1	53.12	69.31	61.22		
G_{63}	DS-8414	36.86	53.12	45.00	G_{88}	RKS-30	22.35	35.49	28.92		
G_{64}	JS-SH-9016	21.12	51.84	36.48	G_{89}	MAUS-1116	67.11	90.00	78.55		
G_{65}	MAUS-175	41.39	64.14	52.77	G_{90}	Moneta	61.94	90.00	75.96		
G_{66}	MAUS-96	22.35	47.90	35.12	G_{91}	MAUS-98	51.38	74.48	62.93		
G_{67}	PK-1259	51.38	59.73	55.56	G_{92}	MAUS-201	23.69	47.90	35.79		
G_{68}	Pusa-99-01	19.90	51.84	35.87	G_{93}	MAUS-305	24.91	49.64	37.28		
G_{69}	MAUS-120	31.36	55.32	43.34	G_{94}	Sel-9	59.73	74.48	67.11		
G_{70}	EC-39754	49.64	67.86	58.75	G_{95}	PBNS-104	61.94	84.82	73.38		
G_{71}	MAUS-1	38.37	64.90	51.64	G_{96}	G_3P_7	22.41	42.91	32.66		
G_{72}	JS-9821	18.67	47.90	33.29	G_{97}	MAUS-172	44.65	44.42	44.53		
G_{73}	IC-21012	34.11	47.90	41.00	G_{98}	EC-34094	55.32	59.73	57.53		
G_{74}	NRC-59	30.00	44.42	37.20	G_{99}	MRSB-342	37.13	59.73	48.43	SE±	C.D.
G_{75}	Dsb-1	32.74	46.16	39.45	G_{100}	G_1P_{11}	54.05	69.31	61.68		@0.05
	Mean	45.30	63.08	54.19		Mean	45.30	63.08	54.19	0.36	1.43
Sub Tr.					Sub Tr.						
SE±				2.47	SE±				2.47		
C.D.@0.05				7.13	C.D.@0.05				7.13		
Interaction (ST×G) :											
SE±	3.49										
C.D.@0.05	10.08										

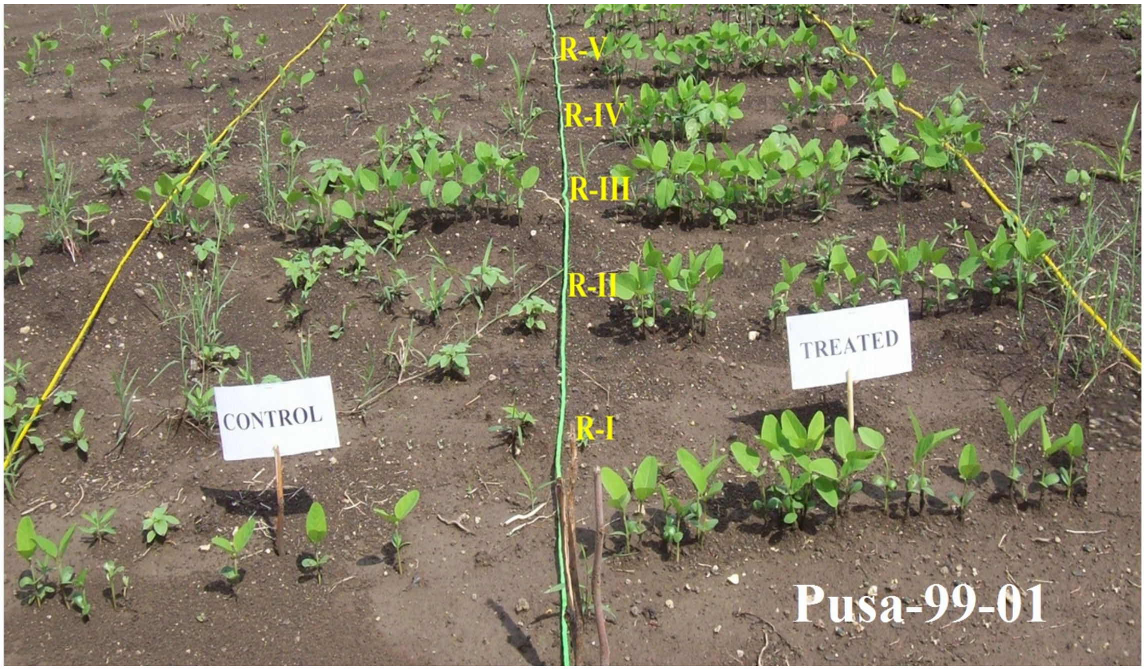

Fig.3 Metalaxyl + *T. hamatum* treated seeds showing maximum germination of seedlings

T. hamatum, *T. harzianum* were at par. *P. fluorescens* and *Gliocladium virens* were at par and were superior to *T. viride*. Significantly highest reduction in seed rot % was given by *T. hamatum* + Metalaxyl. *T. hamatum* + Cymoxanil and *T. hamatum* + Thiram which were at par. Similar trend was observed in respect of germination.

Field testing of seed treatment on germplasm lines

The experiment was conducted during Kharif 2012 in heavy black cotton soil. As the year had unique higher rainfall with frequent natural flooding which created the epidemic conditions for testing the seed treatment under stagnant and high natural inoculum level created in heavy black cotton soil. The observations on germination were taken replication wise after 12 days of sowing.

The data on germination per cent is given in Table 3 (a, b). Improvement in the germination of germplasm lines was expressed with Fig.3.

From this table, under field conditions the mean germination without seed treatment was found to be 45.3%. Seed treatment with Metalaxyl + *T. hamatum* significantly improved mean germination raised to 63.1%. The germplasm lines also differ significantly in respect of germination ranging from 38.12 to 87.40%. The interaction seed treatment × germplasm line was also significant. The untreated control had germination range 21.12 to 84.82% while treated germplasm lines had germination range from 44.5 to 90%.

Seed treatments of soybean with different contact fungicides like Thiram and Captan has been practiced since fifties. The present investigations with different fungicides was indicated that Metalaxyl + *T. hamatum* and Metalaxyl alone were significantly superior over rest of the fungicides tested. These findings are supported by Griffin, 1990 and Bradley, 2008 in case of soybean. They showed beyond doubt the efficacy of Metalaxyl seed treatment in improving germination and plant stand by reducing pre- and post-emergence damping-off in soybean and improving yields by raising plant stands greatly. The present findings indicated significant improvement in germination from 45% to 64% in most of the soybean germplasm lines tested. However, the response of seed treatment with Metalaxyl + *T. hamatum* differed with germplasm lines. These results support earlier studies by the scientists from various soybean growing countries where fungicide seed treatment (specially metalaxyl) to soybean seeds is recommended *viz*. Laura *et.al*., 2008 (Columbia), Lawrence and Darrel, 2010 (South Dakota), Hershaman, 2011(U.K.), Kim *et. al*., 2014 (North Dakota) and many scientists from IOWA, North Carolina, Virginia, Ohio State Universities (U.S.).

The results of this study demonstrated the efficacy of certain bioagents, as seed treatments, for controlling the pathogen either under greenhouse and field conditions. The bioagents *T. hamatum* was the best antagonist that significantly reduced seedling damping-off in soybean. This result support earlier studies that certain biocontrol agents are promising factors for controlling soil-borne diseases on various plants (Roberts *et.al*., 2005; Harman, 2006; Sahar *et.al*., 2009). However, all tested fungicides and bioagents showed positive effects in reducing pre- and post-emergence damping-off disease in pathogen infested soils, that may lead to more seed germination and ultimately more crop yield.

Acknowledgments

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