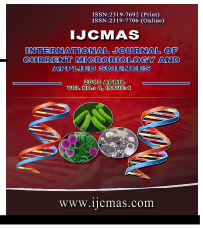
Int.J.Curr.Microbiol.App.Sci (2015) 4(4): 206-215

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 4 (2015) pp. 206-215**
http://www.ijcmas.com



Original Research Article

Effect of Seed Treating Pesticides with *Trichodermaa viridae* on Rhizosphere Mycoflora and Plant Biometrics at 75 DAS of Groundnut (*Arachis hypogeae L.*)

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Keywords

Trichoderma viridae, Pesticides, Rhizosphere Mycoflora, Groundnut (*Arachis hypogeae L.*)

A B S T R A C T

An *in vitro* and *in vivo* experiments were conducted during rabi 2012 and kharif 2013 at department of microbiology, Sri Krishnadevaraya University and Agriculture Research station, Krishi Vignan Kendra Reddypalli, Ananthapuram. With the objective to know the effect of seed treating fungicides and insecticides and their combinations with *Trichoderma viridae* on rhizosphere mycoflora, plant biometrics at 75 days of sowing of ground nut. A total of 13 treatments with required dosages in combination with *Trichoderma viridae* were applied as seed treatment to groundnut crop. Among the treatments T6 (mancozeb + imidacloprid + *Trichoderma viridae*) was found significantly superior to all other treatments. Mean rhizosphere mycoflora observed was 25.7 X 10^{-3}cfu/g and also improved plant biometrics (75 days) like plant height (37.8 cm), leaf weight (2.6 g), root length (9.6 cm), root weight (3.2 g) and number of pods (16.7/plant), pods weight (7.7 g), leaf drop (273), pods left over soil (89) at 75 days of sowing. The correlation studies between rhizosphere mycoflora and plant biometrics at 75 days showed that significantly positive (+ve) correlation. However other plant biometrics like pods left over in the soil and leaf drop were negatively (-ve) correlated with the rhizosphere mycoflora at 75 days.

Introduction

Groundnut (*Arachis hypogaea* L.), is a legume major oil seed crop in the world. Groundnut seeds are valued for oil (40–48%) and protein (22–26%) also contain carbohydrate (26%) fat (3%) and high calcium, thiamine and niacin contents which makes a substantial protein contribution for human and animal nutrition (Maiti *et al.*, 1991.) It is used for extraction of oil, which is an important source of edible oil in Asia and Africa. In leguminous plants rhizosphere microbes play a vital role to improve yield parameters. Rhizosphere refers to the thin layer adhering to the root after the loose soil and lumps removed by shaking, the rhizosphere microbes had great significance in maintaining the fertility and biological control of plant pathogen. The plant species, plant age, the nature of root exudates influence the rhizosphere

mycoflora to a large extent. The fungi static effect in the rhizosphere against soil borne plant pathogens and root pathogens believed due to the microbes releases certain antibiotics. In recent years the search of biological control agents with chemicals used for the management of soil borne diseases besides increasing crop growth and yield has been advocated widely. Among microorganisms that have had a beneficial effects on crop growth and yield and found to be associated to plant rhizosphere, was the species of the genera *Trichoderma* (Sturz and Nowak, 2000). The beneficial effects of the *Trichoderma* species is that it establishes symbiotic rather than parasitic relationships with the plant increasing root growth and productivity, helping to overcome stress stimulations, and improving nutrient absorption (Harman *et al.*, 2004). Combined application of biocontrol agents with commonly used fungicides and insecticides may result either in synergism / antagonism between the two. That biocontrol agent with the chemical agent was applied to seed, soil or both. The use of chemical pesticides arising complexities such as harmful effect on environment and non-target organism including man, domestic animals, beneficial insects, wild life, whereas the use of bio control agent has provides less hazardous method for plant diseases control besides increasing crop growth. By using the pesticides, with biocontrol agent leads to somewhat reduction of harmful on environment besides decreasing pathogens by increasing crop growth. Compatibility of living organisms with modern inputs in plant protection like fungicides, insecticides is a pre-requisite for disease management. Hence, there is need to know the effect of seed treating fungicides and insecticides with biocontrol agent (*Trichoderma viridae)* on rhizosphere mycoflora, plant biometrics at 75 days of sowing of ground nut as one of the objective.

Materials and Methods

In vitro and *in vivo* experiments were conducted in two successive seasons of rabi 2012 and kharif 2013 at research farm of the Agriculture Research station, Krishi Vignan Kendra Reddypalli and department of microbiology Sri Krishnadevaraya University, Ananthapuram, Andhra Pradesh. To know the effect of seed treating fungicides and insecticides with biocontrol agent (*Trichoderma viridae)* on rhizosphere mycoflora, plant biometrics at 75 days of sowing of ground nut. Recommended concentrations of 13 treatments of fungicides, insecticides, and their combinations with *Trichoderma viridae* were applied to groundnut crop (Fig. 1). For *in vitro* studies soil samples were collected from, rhizosphere of healthy groundnut plants adjacent to stem rot affected plants as described by Sharma and Sen (1991). The soil adhering to the roots was collected and mixed to provide a composite rhizosphere soil. The sampling was done at 75 days after sowing. For isolation of rhizosphere mycoflora the dilution plate method proposed by Aneja (2001) was followed. One gram of soil from each sample was taken in a 250 ml conical flask with 100 ml of sterile distilled water. The sample was agitated to prepare a thorough suspension. Serial dilutions of soil suspensions were prepared. 1: 1000 (10^{-3}) for fungi was poured in sterilized petriplates containing potato dextrose agar media (PDA) and the petriplates were incubated at room temperature (25 – 28^{o}C). Three replications were maintained for each dilution tested. Observations on number of colonies per g of rhizosphere soil, and the number of days taken for appearance of each fungal colony on plates were recorded. The field

experiments were laid out in red soils having good drainage facility, recommended dose of nitrogen, phosphorus, and potassium (20-40-40 kg ha^{-1}) were applied in the farm of urea, single super phosphate and murate of potash as basal application. TMV-2 is selected for this study, which is of 105-110 days duration. observations are done on rhizosphere mycoflora 75 DAS of sowing and plant biometrics studies viz. Plant height, leaf weight, nodule number, nodule weight, root length, root weight, per plant were recorded from five randomly selected plants in each plot replication wise, the correlation studies was done between rhizosphere mycoflora and plant biometrics at 75 DAS.

Statistical Analysis

The data obtained in different experiments were statistically analysed by using CRD (Completely randomized design) and randomized block design (RBD). Results of the various experiments were analysed by following appropriate statistical methods as per the procedure suggested by Panes and Sukhatme (1978).

Figure 1: *In vitro* testing of fungicides, insecticides and their combinations on *Trichoderma viridae*.

Design : CRBD

Replications : 3

Treatments : 12

T1: Mancozeb (@ 3g) 3000 ppm
T2: Carbendazim (@ 1g) 1000 ppm
T3: Tebucoonazole (@ 1g) 1000 ppm
T4: Imidacloprid (@ 2ml) 2000 ppm
T5: Chlorpyriphos (@ 6ml) 6000 ppm
T6: T1 (mancozeb) + T4 (imidacloprid)
T7: T1 (mancozeb) + T5 (chlorpyriphos)
T8: T2 (carbendazim) + T4 (imidacloprid)
T9: T2 (carbendazim) + T5 (chloropyriphos)
T10: T3 (tebucoonazole) + T4 (imidahloprid)
T11: T3 (tebucoonazole) + T5 (chloropyriphos)
T12: *Trichoderma viridae*

Result and Discussion

Rhizosphere mycoflora at 75 DAS of sowing, the mean total number of cfu/g of red soil varied from 22.7 x 10^{-3} to 25.8 x 10^{-3} and significant difference was observed among the treatments. Highest colonies of 25.8 x 10^{-3} cfu/g of soil was recorded in T1 (mancozeb + *Trichoderma viridae*) which was significantly superior than other treatments except T6 (mancozeb + imidachloprid + *Trichoderma viridae*) 25.7 x 10^{-3} cfu/g, T2 (carbendazim + *Trichoderma viridae*) 24.8 x 10^{-3} cfu/g, T3 (tebuconazole + *Trichoderma viridae*) 24.7 x 10^{-3} cfu/g, T4 (imidachloprid + *Trichoderma viridae*) 24.5 x 10^{-3} cfu/g and T12 (*Trichoderma viridae*) 24.3 x 10^{-3} cfu/g which are on par. While lowest cfu/g of soil was recorded in T7 (mancozeb + chloropyriphos + *Trichoderma viridae*) 20.1 x 10^{-3} cfu/g treatment followed by T9 (carbendazim + chloropyriphos + *Trichoderma viridae*) 22.7 x 10^{-3} cfu/g, T10 (tebuconazole + imidachloprid + *Trichoderma viridae*) 22.9 x 10^{-3} cfu/g, T5 (chloropyriphos + *Trichoderma viridae*) 23.3 x 10^{-3} cfu/g, T13 (Control, No seed treatment) 23.3 x 10^{-3} cfu/g, T11 (Tebuconazole + chloropyriphos + *Trichoderma viridae*) 23.4 x 10^{-3} and T8 (carbendazim + imidachloprid + *Trichoderma viridae*) 23.7 x 10^{-3}. However, all the treatments are on par with each other (Table 1). Sudha Mall (1979) reported that considering the age of the plant the mycoflora of root zone increased with advancement of growth and after attainment

of peak there was a progressive decline and again a flaring up of flora at senescent obviously because of saprophytic colonization. The reduction of various species of soil fungi was more extensive in first twenty days in comparison with the latter half of the experimental period. The fungicidal effect of both fungicides decreased with increase of time and it resulted in reappearance of certain fungi after a certain period of time.

Regarding the effect of fungicides on sunflower growth and phosphorus content, it was found that as the concentration of the fungicides increased, the growth decreased and minimum growth was observed in 1% concentration of Bavistin as well as of Dithane M-45. Both fungicides had deleterious effect on mycorrhizal spore number and percentage mycorrhizal root colonization (Ashok Aggarwal *et al.,* 2005). Hence, in the present study more number of fungal colonies was observed may be due to more leaf drop. This helped as food base or mulch in colonization of mycoflora, besides increased soil temperature and soil moisture.

Plant biometrics at 75DAS

The results indicated that among all the treatments T6 (mancozeb + imidacloprid + *Trichoderma viridae*) significantly superior to all other treatments and improved plant biometrics viz. germination percentage (90.3), initial plant population (433), final plant population (390), plant height (37.8 cm), root length (9.6 cm), leaf weight (12.6 g), number of nodules (53.0), root weight (3.2 g), number of branches (5.1) followed by the treatments T1 (mancozeb + *Trichoderma viridae*), plant germination percentage (90.5), initial plant population (431), final plant population (392), plant height (36.5 cm), root length (8.5 cm), leaf weight (11.6 g), number of nodules (58), root weight (2.8 g), number of branches (5.3 g), T2 (carbendazim + *Trichoderma viridae*) germination percentage (82.5), initial plant population (389), final plant population (296), plant height (34.2 cm), root length (7.0 cm), leaf weight (10.0 g), number of nodules (48.8), root weight (2.3 g), number of branches (3.7), T3 (tebuconazole + *Trichoderma viridae*) germination percentage (85.0), initial plant population (407), final plant population (314), plant height (34.0 cm), root length (7.0 cm), leaf weight (10.0 g), number of nodules (46.5), root weight (2.3 g), number of branches (3.9), T4 (imidachloprid + *Trichoderma viridae*) germination percentage (88.2), initial plant population (415), final plant population (365), plant height (34.7 cm), root length (7.0 cm), leaf weight (10.0 g), number of nodules (54.5), root weight (2.3 g), number of branches (4.8) and T12 (Control, *Trichoderma viridae*) germination percentage (86.5), initial plant population (423), final plant population (329), plant height (35.1 cm), root length (7.8 cm), leaf weight (10.8 g), number of nodules (47.2), root weight (2.7 g), number of branches (4.6) which are on par.

While lowest plant biometrics were recorded in T7 (mancozeb + chloropyriphos + *Trichoderma viridae*) germination percentage (86.0), initial plant population (411), final plant population (351), plant height (35.8 cm), root length (8.5 cm), leaf weight (11.5 g), number of nodules (50.8), root weight (2.8 g), number of branches (3.8) followed by T9 (carbendazim + chloropyriphos + *Trichoderma viridae*) germination percentage (81.5), initial plant population (390), final plant population (294), plant height (31.6 cm), root length (6.3 cm), leaf weight (9.3 g), number of nodules (44.5), root weight (2.1 g), number of branches (3.7).

T10 (tebuconazole + imidachloprid + *Trichoderma viridae*) germination percentage (82.8), initial plant population (397), final plant population (326), plant height (31.9 cm), root length (6.3 cm), leaf weight (9.3 g), number of nodules (47.9), root weight (2.2 g), number of branches (3.8), T5 (chloropyriphos + *Trichoderma viridae*) germination percentage (81.8), initial plant population (393), final plant population (293), plant height (34.4 cm), root length (7.1 cm), leaf weight (10.1 g), number of nodules (41.3), root weight (2.4 g), number of branches (3.7), T13 (Control, No seed treatment) germination percentage (73.2), initial plant population (352), final plant population (246), plant height (28.4 cm), root length (5.6 cm), leaf weight (8.6 g), number of nodules (39.0), root weight (1.9 g), number of branches (3.7), T11 (tebuconazole + chloropyriphos + *Trichoderma viridae*) germination percentage (80.7), initial plant population (394), final plant population (294), plant height (33.3 cm), root length (6.3 cm), leaf weight (9.3 g), number of nodules (44.2), root weight (2.1 g), number of branches (3.9) and T8 (carbendazim + imidachloprid + *Trichoderma viridae*) germination percentage (81.5), initial plant population (391), final plant population (317), plant height (32.1 cm), root length (7.1 cm), leaf weight (10.1 g), number of nodules (44.5), root weight (2.6 g), number of branches (3.9). However, all the treatments are on par with each other (Table 2).

Gupta *et al.* (2002) reported that peanut seeds bacterized with *Pseudomonas* GRC2 showed a significant increased germination (83%). Studies have been confirmed in case of *Trichioderma harzianum* and *Trichioderma viridae* to enhanced seed germination root and shoot length (Dubey *et al.*, 2007) as well as increasing the frequency of healthy plants, and boosting yield (Rojoa *et al.*, 2007). These results were similar with the findings of Davut Soner *et al.*, (2011) who reported that fungicides decreased the germination disorders caused by some fungal agents (*Aspergilus spp., Rhizopus sp., Penicillium spp.*) in and/or on the surface of the seeds significantly and treatments provided an increase in germination ratio of seeds by 64-96%.

Manoranjitham *et al.* (2000) noticed that *Pseudomonas fluorescens* and *T. viridae* either individually or in combination significantly increased the growth of chilli seedlings after 14 days using rolled paper towel method, suggesting that growth promotion was due to production of growth promoting substances by bioagents. Maiti and Sen (1985) reported that, a combination of calcium ammonium nitrate + antagonist, *T. harzianum* was more effective in reducing stem blight of groundnut caused by *S. rolfsii* than urea + antagonist although all treatments were significantly effective.

They also reported that *Trichoderma* inoculum when added along with nitrogenous fertilizers, and a combination with calcium ammonium nitrate @ 20 kg N ha^{-1} significantly increased the population of *Trichoderma* in 30 days.

The results of the present study indicate that combined application of selected fungicide or insecticide and biocontrol agent *Trichoderma viridae* increases the growth-promoting activity, solubilisation and sequestration of inorganic plant nutrient, production of unidentified growth promoting chemicals jointly.

Table.1 Effect of fungicides and insecticides with *Trichoderma viridae* and their combinations on rhizosphere mycoflora cfu/g of dry soil at 75 DAS

S. No	Treatments	2012				2013				Pooled Mean	% increase / Decrease over control
		R1	R2	R3	Mean	R1	R2	R3	Mean		
T1	Mancozeb @ 3g + *Trichoderma viridae* 8g / kg seed.	26.8	26.6	25.3	26.2 (30.8)	26.8	23.3	26.2	25.4 (30.3)	25.8(30.5)*	5.5.5
T2	Carbendazim @ 1g + *Trichoderma viridae* 8g / kg seed	24.8	24.1	25.5	24.8 (29.9)	25.1	24.5	24.8	24.8(29.9)	24.8(29.9)	3.5
T3	Tebucoonazole @ 1g + *Trichoderma viridae* 8g / kg seed	24.2	25.1	24.0	24.4 (29.6)	24.6	25.8	24.5	25.0(29.9)	24.7(29.8)	3.5
T4	Imidachloprid @ 2 ml + *Trichoderma viridae* 8g / kg seed	24.8	25.3	23.8	24.6 (29.7)	24.1	25.2	23.9	24.4(29.6)	24.5(29.7)	3.0
T5	Chlorpyriphos @ 2 ml + *Trichoderma viridae* 8g / kg seed	24.2	22.2	22.4	22.9 (28.6)	23.8	23.6	23.4	23.6(29.1)	23.3(28.8)	-
T6	T1 + T4 + *Trichoderma viridae* 8g / kg seed.	25.8	26.6	25.2	25.9 (30.6)	26.6	25.2	24.8	25.5(30.3)	25.7(30.5)	6.0
T7	T1 + T5 + *Trichoderma viridae* 8g / kg seed	22.1	22.8	22.3	22.4 (28.2)	23.8	22.3	22.6	22.9(28.6)	22.7(28.4)	-2.0
T8	T2 + T4 + *Trichoderma viridae* 8g / kg seed	24.0	25.9	21.1	23.7 (29.1)	25.9	21.1	24.0	23.7(29.1)	23.7(29.1)	1.0
T9	T2 + T5 + *Trichoderma viridae* 8g / kg seed	26.5	22.1	20.6	23.1 (28.7)	22.1	19.0	25.8	22.3(28.1)	22.7(28.4)	-2.0
T1	T3 + T4 + *Trichoderma viridae* 8g / kg seed	22.8	22.1	24.6	23.2 (28.8)	22.6	23.6	21.8	22.7(28.4)	22.9(28.6)	-1.0
T11	T3 + T5 + *Trichoderma viridae* 8g / kg seed	21.6	24.8	22.8	23.1 (28.7)	25.6	23.8	21.8	23.7(29.1)	23.4(28.9)	0.0
T12	*Trichoderma viridae* 8g / kg seed	24.2	23.8	25.0	24.3 (29.5)	23.8	25.0	24.2	24.3(29.5)	24.3(29.5)	2
T13	Control (No seed treatment)	24.5	23.4	22.6	23.5 (28.9)	23.4	22.6	23.5	23.2(28.8)	23.3(28.9)	--
	CD (5%)				NS				NS	1.1	
	CV (%)				3.2				3.5	3.2	

CD: Critical difference between treatments CV: Coefficient of variation between replications. NS: Non Significant. * Figures in the parenthesis are angularly transformed values.

Table.2 Effect of fungicides and insecticides with *Trichoderma viridae* and their combinations on plant biometrics at 75 DAS sowing of ground nut

Treatments	Germi Nation (%)	Initial plant popula tion	Final plant popula tion	Plant height (cm)	Haulm weight (g)	Root length (cm)	No. of nodules	Root weight (g)	No. of branches	No. of pods	Pods weight (g)	Pods left over in soil	Leaf drop
T1	90.5 (72.1)	431	392	36.5	8.5	11.6	58.0	2.8	5.3	16.4	7.5	90.5 (72.1)	431
T2	82.5 (63.9)	389	296	34.2	7.0	10.0	48.8	2.3	3.7	12.2	5.5	82.5 (63.9)	389
T3	85.0 (67.3)	407	314	34.0	7.0	10.0	46.5	2.3	3.9	11.5	4.9	85.0 (67.3)	407
T4	88.2 (68.5)	415	365	34.7	7.0	10.0	54.5	2.3	4.8	16.2	7.5	88.2 (68.5)	415
T5	81.8 (64.8)	393	293	34.4	7.1	10.1	41.3	2.4	3.7	9.7	4.3	81.8 (64.8)	393
T6	90.3 (72.1)	433	390	37.8	9.6	12.6	53.0	3.2	5.1	16.7	7.7	90.3 (72.1)	433
T7	86.0 (68.0)	411	351	35.8	8.5	11.5	50.8	2.8	3.8	10.7	4.8	86.0 (68.0)	411
T8	81.5 (64.5)	391	317	32.1	7.1	10.1	44.5	2.6	3.9	10.7	4.7	81.5 (64.5)	391
T9	81.5 (64.5)	390	294	31.6	6.3	9.3	44.5	2.1	3.7	9.4	4.3	81.5 (64.5)	390
T1	82.8 (65.5)	397	326	31.9	6.3	9.3	47.9	2.2	3.8	9.2	4.1	82.8 (65.5)	397
T11	80.7 (65.3)	394	294	33.3	6.3	9.3	44.2	2.1	3.9	8.8	4.1	80.7 (65.3)	394
T12	86.5 (69.9)	423	329	35.1	7.8	10.8	47.2	2.7	4.6	12	5.9	86.5 (69.9)	423
T13	73.2 (58.8)	352	246	28.4	5.6	8.6	39.0	1.9	3.7	8.5	3.9	73.2 (58.8)	352
CD (5%)	1.7	22.9	12.3	2.6	0.8	0.7	10.6	0.3	0.7	1.5	0.7	1.7	22.9
CV (%)	3.2	7.4	7.8	8.7	9.5	6.3	19.3	10.6	13.8	10.7	10.2	3.2	7.4

CD: Critical difference between treatments CV: Coefficient of variation between replications. * Figures in the parenthesis are angularly transformed values

Table.3 Correlation coefficient between rhizosphere mycoflora and plant biometrics of groundnut at 75 DAS

	Rhizosphere mycoflora	Germination	Initial plant population	Final plant population	Plant height	Haulm weight	Root length	No. of nodules	Root weight	No. of branches	No. of pods	Pods weight	Pods left over in soil	Leaf drop
Rhizosphere mycoflora	1.000													
Germination	0.593*	1.000												
Initial plant population	0.608*	0.998**	1.000											
Final plant population	0.605*	0.924**	0.922**	1.000										
Plant height	0.624*	0.901**	0.914**	0.833**	1.000									
Haulm weight	0.606*	0.815**	0.836**	0.841**	0.899**	1.000								
Root length	0.598*	0.811**	0.832**	0.836**	0.899**	0.999**	1.000							
No. of nodules	0.652*	0.835**	0.829**	0.925**	0.758**	0.706**	0.698**	1.000						
Root weight	0.559*	0.793**	0.816**	0.818**	0.858**	0.986**	0.988**	0.643*	1.000					
No. of branches	0.782**	0.777**	0.783**	0.844**	0.677**	0.714**	0.706**	0.801**	0.688**	1.000				
No. of pods	0.861**	0.771**	0.777**	0.852**	0.754**	0.740**	0.735**	0.865**	0.692**	0.918**	1.000			
Pods weight	0.846**	0.766**	0.774**	0.843**	0.744**	0.730**	0.725**	0.859**	0.685**	0.937**	0.996**	1.000		
Pods left over in soil	-0.684**	-0.925**	-0.928**	-0.931**	-0.856**	-0.817**	-0.810**	-0.843**	-0.796**	-0.916**	-0.843**	-0.854**	1.000	
Leaf drop	-0.641*	-0.957**	-0.952**	-0.930**	-0.833**	-0.786**	-0.779**	-0.838**	-0.779**	-0.846**	-0.786**	-0.788**	0.967**	1.000

* Significant at 5% ** Significant at 1%

Correlation

The correlation analysis between rhizosphere mycoflora and yield attributes like germination (0.593), initial plant population (0.608), final plant population (0.605), plant height (0.624), haulm weight (0.606), root length (0.598), No. of nodules (0.652), root weight (0.559), No. of branches (0.782), No. of pods (0.861) and pods weight (0.846) showed significantly positive (+ve) correlation. However yield attribute like pods left over in soil (-0.684) and leaf drop (-0.641) were negatively (-ve) correlated with rhizosphere mycoflora at 75 DAS (Table 3).

In conclusion, combination of mancozeb @ 3g + imidachloprid @ 2ml with *Trichoderma @ 8*g was found significantly superior to all other treatments and improved the plant biometrics as well as rhizosphere mycoflora at 75 days of sowing of ground nut. Correlation studies between rhizosphere mycoflora and plant biometrics at 75 days showed that significantly positive (+ve) correlation. However other plant biometrics like pods left over on soil and leaf drop were negatively (-ve) correlated with the rhizosphere mycoflora at 75 days.

Acknowledgement

P.Vasundra, greatful to Department of Microbiology, Sri Krishnadevraya University, Anathapuram, for grant from of BSR-JRF.

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