

Effectiveness of Mycorrhizal Biofertilizer in the Management of *Orobanche* in Tomato (*Lycopersicon esculentum* L.)

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ABSTRACT: The management of *Orobanche* under the infested field condition is very difficult to achieve because of its micro sized seed, high fecundity and asynchronous germination. Thus an attempt was made to evaluate native AMF isolates against *Orobanche* emergence in tomato. The experiment was carried out in *Orobanche* infested soils of Belagavi district during 2018-19. During the investigation different methods of application of mycorrhizal biofertilizer were screened for their ability to suppress of *Orobanche* i.e. (planting of pre mycorrhized seedling; soil application and the combination of both). The results of the present investigations with respect different methods of applications of mycorrhizal biofertilizer on *Orobanche* emergence has revealed that planting of pre colonized tomato seedling with mycorrhizal biofertilizer plus soil application of mycorrhizal biofertilizer at the time of planting suppressed the *Orobanche* emergence (0.00 plot⁻¹), followed by planting of pre colonized seedlings with mycorrhizal biofertilizer (1.44 plot⁻¹) and direct soil application of mycorrhizal biofertilizer at the time of planting (4.66 plot⁻¹) compared to un inoculated control (9.66 plot⁻¹) at 90 DAP. Furthermore, mycorrhizal parameters like spore count and per cent of root colonization were found to be the highest in the plots received mycorrhization in the form of pre colonization followed by pre colonization pulse soil application and direct soil application. However the least number of mycorrhizal spore counts and percent root colonization were observed in the rhizosphere of non mycorrhized tomato plants. Hence the present investigation will form a suitable biotic tool against *Orobanche* in tomato.

Keywords: *Orobanche*; AM Fungi; Pre colonization; Tomato; Mycorrhizal biofertilizer.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) belongs to the genus *Lycopersicon* under *Solanaceae* family. Tomato is an herbaceous extensive growing plant at the height of 1-4 m with woody stem. Tomato is a native to Peruvian and Mexican region. Tomato is one of the most important "protective foods" because of its unique nutritive value. It is one of the most versatile vegetable with wide usage in Indian culinary tradition. Global tomato production is currently around 130 million tons, of which 88 million are destined for the fresh market and 42 million are processed. China stands first in production of tomato followed by Europe, India, US and Turkey. They account for 70% of global production. The major Tomato producing States in the country are Andhra Pradesh (5587.56 tonnes per ha), Madhya Pradesh (2746.77 tonnes per ha), Karnataka (2012.70 tonnes per ha) and Gujarat (1425.97 tonnes per ha). These States are account for 91% of the total production of the country. The production of tomato during the year 2017-18 (Anonymous, 2018). In Karnataka Belagavi, and Kolar

and Koppal district occupies 90 per cent of the area under the tomato cultivation. Our field survey has revealed that the reduction in the yield of tomato in the region of Nipani was due to *Orobanche* infestation. *Orobanche* a complete root parasitic weed robs all nutrients and water from the host, resulting in stunted growth of the plant leading to an extent of yield reduction from six to 50 per cent depending on the intensity of infestation (Dhanapal *et al.*, 1998). The major parasite of tomato is *Orobanche* spp. due to lack of leaf and chlorophyll, *Orobanche* spp. depriving water and nutrition from tomato. *Orobanche* spp. results in fading of tomato, decline in growth and finally caused the death of the crop.

The management of *Orobanche* is very difficult to achieve (Nosratti *et al.*, 2020) because of its micro seed size, high fecundity, asynchronous germination of seed and most important their life cycle occurs below ground near the root zone of host plant. Hence difficult to suppress these parasitic weeds under the field conditions. Thus an integrated approach is needed for the management of these parasitic weeds like cultural

practices, crop rotation Pérez-de-Luque *et al.*, (2010); Goldwasser ; Hayat *et al.*, (2020); Kleifeld, (2004), trap crop (Ye *et al.*, 2020), host plant resistance Aly *et al.*, (2021), chemical and biological treatments Jones *et al.*, (2012); Madhura *et al.*, (2017); Manjunath *et al.*, (2018); Shubha *et al.* (2018); Chandrashekhargowda *et al.*, (2018). Hence, there is a need to develop a comprehensive and eco-friendly management system for their control. The studies have revealed that the Mycorrhizal colonization persuades resistance to plant parasitism by converting strigolactones and orobanchol into mycorradicin (Walter *et al.*, 2011; Shubha *et al.*, 2018) which is assembled in the tomato root zone and thereby reduced accessibility of strigolactones and orobanchol for germination of *Orobanche*. Hence the present investigation was aimed to exploit the mycorrhizal biofertilizer in the management of *Orobanche* a parasitic weed in tomato.

MATERIAL AND METHODS

A field experiment was conducted during *Kharif* 2018 in order to study the “Effectiveness of mycorrhizal biofertilizer in the management of *Orobanche* in tomato (*Lycopersicon esculentum* L.)”. These experiments were carried out in *Orobanche* infested soils of tomato growing areas of Nipani in Belagavi district of northern

Karnataka. An research was carried out to evaluate the three different methods of application of mycorrhizal biofertilizer in the management of *Orobanche viz.*, pre colonization of tomato seedling in nursery; soil application at the time of transplanting and the combination of both methods with the use of following AMF culture UASDAMFT (Isolated from *Orobanche* suppressive soils in tobacco), UASDAMFS (Isolated from *Striga* suppressive soils in sugarcane) and STD AMF Consortium (Department of Agricultural Microbiology, UAS Dharwad). The experiment was laid out in randomized complete design with factorial concept. There were 3 main factors and 3 sub factors consisting of combination of AM fungi and different methods of application and UIC outside the experiment run with RCBD as given below:

A. Application of mycorrhizal cultures:

Pre colonization of the tomato seedlings in the nursery beds with AMF @ 2 kg/m²

Nursery beds were prepared and subjected for solarization (4 to 5 weeks) in order prevents the native AMF infective propagules. AMF culture along with vermicompost @ 2:25 was applied in the furrows prior to the sowing of tomato seeds (Fig. 1).



Pre colonization of the tomato seeds in the nursery beds with AMF @ 2kg/m².



Pre-colonized of the tomato seedlings in the nursery beds at 45 days.

Fig. 1.

Soil application: AMF culture @ 8 kg per acre was applied along with 200 kg of vermicompost at the time of transplanting of tomato seedlings.

Parameters measured

Orobanche parameters

The number of *Orobanche* weed emergence per plot was documented at 60, 90 and 120 days after planting (DAP). The shoot and root portions of *Orobanche* plants were separated and oven dried at 60°C to constant weight. The dry weights were then recorded separately for shoots and roots and average of three were expressed in grams.

Growth parameters of tomato

Plant height: The plant height of tomato was recorded at 60, 90, 120 DAP. The tomato plant height is defined Waratadar *et al.*,

as the average stem distance from the soil to the insertion of the top visible leaf on the stem and were expressed in centimetre (cm).

Relative chlorophyll content (SPAD Reading):

Relative chlorophyll content of the tomato plants were recorded at the intervals 60, 90 and 120, DAP. The single photoelectric analyzing diode (SPAD) meter was used for recording the relative chlorophyll content.

Mycorrhizal parameters: The chlamydo spores in rhizosphere of tomato were determined by wet sieving and decantation method as outlined by Gerdemann and Nicholson (1963). Spores counts were taken under a stereo zoom microscope. Mycorrhizal root colonization was determined as per the procedure proposed by Philips and Hayman (1970).

The percentage of roots colonized by mycorrhizae was calculated by the formula.

$$\text{Percent root colonization} = \frac{\text{Root bits positive for colonization}}{\text{Total number of root bits}} \times 100$$

Statistical analysis and data interpretation: The data collected at different growth stages of crop were subjected to statistical analysis. Based on mean values obtained, analysis and interpretation of data were studied using the Fischer's method of analysis of variance technique as described by Gomez and Gomez (1984). The level of significance used in 'F' and 't' test was $p = 0.05$. Critical difference values were calculated wherever the 'F' test was significant.

RESULTS AND DISCUSSION

In the present investigation different methods of application of AM Fungal cultures *viz.* planting of pre colonized tomato seedling, soil application and the combination of pre colonized seedling plus soil application were tested against *Orobanche*. The outcome of the field investigations has revealed that the germination of *Orobanche* was reduced significantly in the treatment received planting of pre colonized seedling followed by soil application at the time of planting, while the highest number of *Orobanche*

infestation was recorded in the plots not received AMF cultures.

The number of *Orobanche* weed emergence per plot was documented at 60 and 90 DAP: The results of the present investigations have revealed that the treatment received UASDAMFT had reduced the emergence of *Orobanche* (0.66 plot^{-1}) compared to STD AMF (0.88 plot^{-1}) and UASDAMFS (1.61 plot^{-1}). The results with respect different methods of applications of AMF on *Orobanche* numbers revealed that, planting of pre colonized tomato seedling plus soil application at the time of planting suppressed the *Orobanche* emergence (0.00 plot^{-1}) compared to planting of pre colonized seedlings (1.00 plot^{-1}) and direct soil application of AMF cultures at the time of planting (2.16 plot^{-1}). The results pertaining to the interactive effect between mycorrhizal cultures in conjunction with the methods of application of AMF cultures significantly reduced the population of *Orobanche* with the treatment received planting of pre colonized seedling along with soil application of UASDAMFT, UASDAMFS and STD AMF recorded zero emergences of *Orobanche*. However the highest numbers of weeds were recorded in the treatment received zero application of AMF cultures (5.66 plot^{-1}) at 60 DAP (Table 1) Fig. 2. Similar trend were also recorded at 90 DAP.

Table 1: Effect of AMF fungal cultures on *Orobanche* emergence in tomato.

Treatment	60 DAP				90 DAP			
	Method of application				Method of application			
AM Fungi	S ₁	S ₂	S ₃	Mean	S ₁	S ₂	S ₃	Mean
M ₁	0.00	2.00	0.00	0.66	0.00	5.66	0.00	1.88
M ₂	1.66	3.16	0.00	1.61	3.66	7.00	0.00	3.55
M ₃	1.33	1.33	0.00	0.88	0.66	1.33	0.00	0.66
Mean	1.00	2.16	0.00		1.44	4.66	0.00	
UIC				5.66				9.66
	S.Em±		C D at 5 %		S.Em±		C D at 5 %	
M	0.055		0.218		0.320		1.259	
S	0.090		0.279		0.252		0.778	
M at S	0.157		0.484		0.437		1.347	
UIC	0.228		0.678		0.522		1.551	

AM Fungi	Method of application
M ₁ : UASDAMFT (tobacco native)	S ₁ : pre-colonized
M ₂ : UASDAMFS (sugarcane native)	S ₂ : soil application
M ₃ : STD AMF	S ₃ : pre-colonized + soil application
UIC: Uninoculated control	



UASDAMFT (Pre cononized + Soil application).



UASDAMFT + Pre colonized.



Un inoculated control.

STDAMF Pre colonized + Soil application.

Fig. 2. Effect of mycorrhization on *Orobanche* emergence in tamato at 60 DAP.

The biomass of *Orobanche* per plot was documented At 120 DAP (Table 2), The results with respect to the interactive effect between mycorrhizal cultures and their methods of application significantly reduced the biomass of *Orobanche* with the treatment received planting of pre colonized seedling along with soil application at the time of planting with UASDAMFT, UASDAMFS and STD AMF recorded zero biomass of

Orobanche at 120 DAP compared to uninoculated control (41.66 g plot⁻¹). This might be due to the reduced availability of signaling molecules in mycorrhized roots like strigolactones and orobanchol as reported by Walter *et al.* (2011) and Vierheilg *et al.*, 2004 Regulatory mechanisms during the plant-arbuscular mycorrhizal fungus interaction.

Table 2: Effect of AMF fungal cultures on dry biomass of *Orobanche* at 120 DAP.

Treatment	Biomass (g/plot)			
	Method of application			
AM Fungi	S ₁	S ₂	S ₃	Mean
M ₁	0.00	10.83	0.00	3.61
M ₂	15.66	21.00	0.00	12.22
M ₃	15.50	10.58	0.00	8.69
Mean	10.38	14.13	0.00	
UIC				41.66
	S.E.m±		C D at 5 %	
M	0.42		1.68	
S	0.41		1.28	
M at S	0.72		2.23	
UIC	1.22		3.65	

AM Fungi	Method of application
M ₁ : UASDAMFT (tobacco native)	S ₁ : pre-colonized
M ₂ : UASDAMFS (sugarcane native)	S ₂ : soil application
M ₃ : STD AMF	S ₃ : pre-colonized + soil application
UIC: Uninoculated control	

Furthermore, our research findings are in accordance with the finding of Yoneyama *et al.*, (2007); Lenzemo *et al.*, (2005) Ai-Rong *et al.*, (2012); Lopez-Raez *et al.*, (2011); Casadesús and Munné-Bosch (2021); Elsakhawy *et al.*, (2020) where in inoculation of mycorrhizal fungi significantly reduced the haustorial formation, there by controlling the parasitic weeds. Cardoso *et al.*, (2011), revealed that strigolactones are secondary metabolites produced in root zones of host plant under the nutrient stress conditions which also act as stimulant for both AMF and parasitic weed.

Plant height of tomato: Plant height of tomato as influenced by the method of application of three different native mycorrhizal fungi and their interactive studies indicated that, planting of pre colonized tomato seedling of UASDAMFT at the time of planting recorded significantly the highest plant height, (136.67

cm) followed by the treatment received pre colonized tomato seedling along with soil application STD AMF (134.33 cm) and pre colonized UASDAMFS plus soil application (130.33 cm). However reduced plant height was noticed in uninoculated control (109.67 cm) at 120 DAP (Table 3). The reduction in plant height with non mycorrhizal plots due to depletion of strigolactones which leads to suicidal germination of *Orobanche* seeds, as initially reported by Olakojo *et al.*, (2001) in maize genotypes. The positive interactions between native isolates AMF and host plants indicated an increased plant height in sugarcane by several workers Madhura *et al.* (2017); Manjunath *et al.*, (2018); Shubha *et al.*, (2018); Asif *et al.*, (2020) reported that the tomato plants pre colonized with native AM fungal cultures enhanced the plant height compared to non mycorrhizal tomato plants under *Orobanche* infested soils.

Table 3: Plant height of tomato as influenced by AMF fungal cultures in *Orobanche* infested soil.

Treatment	Plant height (cm)											
	60 DAP				90 DAP				120 DAP			
	Method of application				Method of application				Method of application			
AM Fungi	S ₁	S ₂	S ₃	Mean	S ₁	S ₂	S ₃	Mean	S ₁	S ₂	S ₃	Mean
M ₁	71.87	68.30	62.22	67.46	102.00	95.33	100.00	99.11	136.67	129.33	130.00	132.00
M ₂	61.80	60.39	69.56	63.92	99.33	95.33	100.00	98.22	127.67	124.00	130.33	127.33
M ₃	66.12	63.03	71.51	66.88	96.00	96.67	101.00	97.89	120.33	119.33	134.33	124.67
Mean	66.60	63.91	67.76		99.11	95.78	100.33		128.22	124.22	131.56	
UIC				59.89				85.67				109.67
	S.Em±		C D at 5 %		S.Em±		C D at 5 %		S.Em±		C D at 5 %	
M	1.04		4.11		0.69		2.71		1.45		5.73	
S	1.27		3.93		1.73		5.34		2.12		6.55	
M at S	2.21		6.81		3.00		9.26		3.68		11.36	
UIC	2.122		6.31		2.92		8.68		4.17		12.40	

AM Fungi	Method of application
M ₁ : UASDAMFT (tobacco native)	S ₁ : pre-colonized
M ₂ : UASDAMFS (sugarcane native)	S ₂ : soil application
M ₃ : STD AMF	S ₃ : pre-colonized + soil application
UIC: Uninoculated control	

Relative chlorophyll content (SPAD reading): At 120 DAP (Table 4), Among the interactive studies, the highest relative chlorophyll content was recorded in the treatment received pre colonization tomato seedling UASDAMFT (44.60), at the time of planting significantly superior to the treatment received pre colonized tomato seedling with plus soil application STD AMF (43.66).

However the lowest relative chlorophyll content recorded in the non mycorrhized treatment (38.38). Abdel and Mohamedin (2000) documented an increased in the physiological parameter like relative chlorophyll content due to the mycorrhizal inoculation in buffalo grass. This may be due to the enhanced uptake of major nutrients like K and P in mycorrhized plants.

Table 4: Relative chlorophyll content as influenced by AM fungi in tomato.

Treatment	Chlorophyll content (SPAD reading)											
	60 DAP				90 DAP				120 DAP			
	Method of application				Method of application				Method of application			
AM Fungi	S ₁	S ₂	S ₃	Mean	S ₁	S ₂	S ₃	Mean	S ₁	S ₂	S ₃	Mean
M ₁	49.24	41.55	44.58	45.12	49.60	42.83	43.29	45.24	44.60	38.54	42.42	41.85
M ₂	38.83	38.08	45.41	40.77	41.95	40.06	46.00	42.67	39.27	38.60	42.97	40.28
M ₃	41.42	43.81	47.15	44.13	41.83	43.00	48.73	44.52	42.37	38.43	43.66	41.49
Mean	43.16	41.15	45.72		44.46	41.96	46.01		42.08	38.53	43.02	
UIC				32.40				38.20				38.38
	S.Em±		C D at 5 %		S.Em±		C D at 5 %		S.Em±		C D at 5 %	
M	1.60		6.31		0.86		3.39		0.86		3.41	
S	0.90		2.80		0.94		2.90		0.96		2.98	
M at S	1.57		4.85		1.63		5.02		1.67		5.17	
UIC	1.85		5.51		1.52		4.53		1.55		4.62	

AM Fungi	Method of application
M ₁ : UASDAMFT (tobacco native)	S ₁ : pre-colonized
M ₂ : UASDAMFS (sugarcane native)	S ₂ : soil application
M ₃ : STD AMF	S ₃ : pre-colonized + soil application
UIC: Uninoculated control	

Mycorrhizal spore & root colonization: At 90 DAP (Table 5), Spore counts was maximum in the treatment received pre colonization seedlings of UASDAMFT (492.67/50 g of soil) and second highest was recorded in the treatment received pre colonized tomato seedling with STD AMF plus soil application at the time of

planting (426.33/50 g of soil) followed by pre colonized tomato seedling UASDAMFS plus soil application (413.33/50 g of soil). However the least number of mycorrhizal spore counts were observed in the rhizosphere of non mycorrhized tomato plants (103.67/ 50 g of soil).

Table 5: Mycorrhizal spore load as influenced by AM fungi in tomato rhizosphere.

Treatment	Number of spores /50g of soil											
	45 DAP				60 DAP				90 DAP			
	Method of application				Method of application				Method of application			
AM Fungi	S ₁	S ₂	S ₃	Mean	S ₁	S ₂	S ₃	Mean	S ₁	S ₂	S ₃	Mean
M ₁	379.67	268.33	210.67	286.22	441.00	314.67	225.33	327.00	492.67	393.00	295.67	393.78
M ₂	294.33	183.67	318.67	265.56	315.67	203.33	320.67	279.89	378.67	232.67	413.33	341.56
M ₃	199.33	305.00	319.67	274.67	220.67	318.67	378.33	305.89	241.00	341.33	426.33	336.22
Mean	291.11	252.33	283.00		325.78	278.89	308.11		370.78	322.33	378.44	
UIC				63.33				78.67				103.67
	S.Em±			C D at 5 %	S.Em±			C D at 5 %	S.Em±			C D at 5 %
M	5.01			19.67	2.20			8.65	7.63			29.97
S	4.78			14.74	5.51			16.98	9.63			29.69
M at S	8.28			25.53	9.54			29.41	16.69			51.43
UIC	7.93			23.58	8.26			24.55	15.20			45.18

AM Fungi	Method of application
M ₁ : UASDAMFT (tobacco native)	S ₁ : pre-colonized
M ₂ : UASDAMFS (sugarcane native)	S ₂ : soil application
M ₃ : STD AMF	S ₃ : pre-colonized + soil application
UIC: Uninoculated control	

At 90 DAP (Fig. 3.), the highest mycorrhizal root colonization was recorded pre colonized tomato seedling of UASDAMFT alone (80.81%), followed by the treatment received pre colonized UASDAMFS plus soil applications (75.79 %) and the treatment received pre colonized tomato seedling with STD AMF plus soil application at the time of planting (75.74 %).

However least percent of root colonization was observed in the roots of non mycorrhized tomato plants (43.89%) Table 6. The inoculation of sugarcane plants with AM fungal cultures increased the root colonization and spore number was recorded with mycorrhization compared to non mycorrhizal plants Jones *et al.*, (2012); Madhura *et al.*, (2017); Shubha *et al.*, (2018).

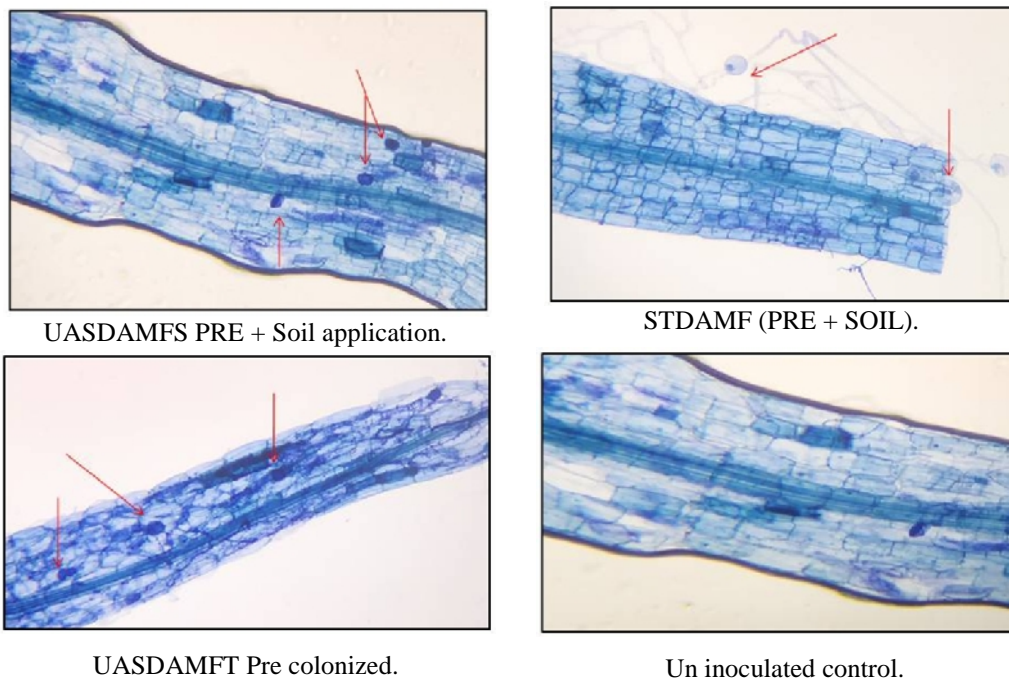


Fig. 3. Mycorrhizal root colonization in tamato.

Table 6: AMF root colonization in tomato as influenced by AM fungi.

Treatment	Percentage (%)							
	60 DAP				90 DAP			
	Method of application				Method of application			
AM Fungi	S ₁	S ₂	S ₃	Mean	S ₁	S ₂	S ₃	Mean
M ₁	77.64	68.49	72.14	72.76	80.81	71.69	58.93	70.48
M ₂	56.85	51.59	72.16	60.20	72.02	52.77	75.79	66.86
M ₃	72.07	53.27	73.88	66.41	52.75	72.89	75.74	67.13
Mean	68.85	57.79	72.73		68.53	65.78	70.15	
UIC				39.63				43.89
	S.Em±		C D at 5 %		S.Em±		C D at 5 %	
M	1.423		5.586		1.158		4.548	
S	1.186		3.655		1.323		4.076	
M at S	2.055		6.331		2.291		7.060	
UIC	2.103		6.25		2.205		6.55	

AM Fungi	Method of application
M ₁ : UASDAMFT (tobacco native)	S ₁ : pre-colonized
M ₂ : UASDAMFS (sugarcane native)	S ₂ : soil application
M ₃ : STD AMF	S ₃ : pre-colonized + soil application
UIC: Uninoculated control	

CONCLUSION

The suppression of *Orobanche* by AM fungi is chiefly known to be due to depletion of strigolactones by them in the rhizosphere of the host plants. Strigolactones are signaling compound that play a vital role as germination stimulants of the parasitic *Orobanche*. Hence research of the present investigation is a promising strategy to develop a bio herbicide against parasitic weeds with a special reference to *Orobanche* in tomato and other solanaceous crops like tobacco, brinjal and others.

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Conflict of interest. Nil.

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