

Evaluation of Bioagents and Phytoextract against *Macrophomina phaseolina* caused by Dry Root Rot of Safflower

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ABSTRACT: Dry Root rot is an important disease of Safflower (*Carthamus tinctorius* L.) caused by *Macrophomina phaseolina* (Tassi) Goid was observed in farmer's field of different districts of Marathwada region which cause severe economic yield loss. The study aimed at minimizing the indigenous usage of chemical fungicides and adopting the strategy of biological control and plant extract for management of this devastating fungus. In this study, applying Dual culture technique and Poisoned food technique and using Potato dextrose agar (PDA) as basal culture medium was used to see how effective various bioagents and plant extract were at managing *M. phaseolina*. Among the tested biocontrol agents against *Macrophomina phaseolina*, *T. virens* (86.42%) was found most effective which resulted in maximum inhibition of mycelial growth of the pathogen followed by *T. koningii* (82.72%), *T. asperellum* (79.77%). Among tested different Phytoextract @ 10, 15 and 20 per cent evaluated against *M. phaseolina*. *Allium sativum* (100%) was found most effective, resulted in complete inhibition of mycelial growth of the pathogen followed by *Z. officinale* (68.16, 75.19 and 80%) and *A. indica* (64.44, 69.52 and 72.22%).

Keywords: Bioagents, biological control, Phytoextract, dry root rot, *Macrophomina phaseolina*, safflower.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.), commonly known as *Kardi* (Marathi), *Kusube* (Kannada), *Kusum* (Hindi) and *Kusumba* (Telugu), is one of the important *Rabi* oilseed crops of the country originated from Abyssinia and Afghanistan. It is drought tolerant, self-pollinated crop belonging to the family *compositae* or *asteraceae*. Safflower crop can be grown in wide range of soils like clay loam, sandy loam, shallow and light textured soils. This crop has a tap root system, which is being cultivated in tropical as well as in sub-tropical conditions with ideal temperature required for this crop being 22° to 35°. It is popular among the farmers due to its hardy nature, short duration and high commercial value.

India ranks first in world in respect area and production of safflower. In India Maharashtra, Karnataka, Andhra Pradesh, Madhya Pradesh, Gujarat, Orissa and Bihar are major safflower growing states. In 2018-19 and 2019-20 India area 46.00 and 52.00 ('000 ha), production 25.00 and 44.00 ('000 Tonne) and productivity 537 and 843 kg/ ha. Maharashtra ranks first in India in respect area and production of safflower. In 2019-20 Maharashtra state area is 21.60 ('000 ha), production 14.93 ('000 Tonne) and productivity 691 kg/ha (Anonymous 2020a & 2020b).

The diseases of safflower, among these diseases, root rot caused by *Macrophomina phaseolina* (Tassi) Goid is a very devastating disease of safflower and causes heavy reduction in total yield (Kore and Deshmukh 1982). It is the major soil borne disease and appears sporadically all over the country (Shambharkar and Indi 1987). Occurrence of this disease on safflower in India was first reported by Amarsingh and Bhowmik (1979) from IARI, New Delhi and later on by others from different parts of the country (Kore and Deshmukh (1982); Lukade, (1992) from Maharashtra; Singh *et al.* (1987) from M.P.).

The crop is being affected by various fungal, bacterial, viral and phytoplasmal diseases. Among the fungal diseases, root rot/charcoal rot/stem rot caused by *Macrophomina phaseolina* (Tassi) Goid., is one of the most devastating diseases, causing approximately 25-30% yield losses in Karnataka (Singh and Bhowmik 1979), 42-45% yield losses in India (Rani *et al.*, 2009). The pathogen being mostly soil borne and sometimes seed borne, cause pre-emergence seed rot as well as post-emergence seedling mortality and also reduction in total plant population per unit area. During drought / water stress conditions and high soil temperature, the safflower crop is more prone to the disease root / charcoal / stems rot, caused by *M. phaseolina*.

MATERIALS AND METHODS

Seven fungal antagonists viz., *Trichoderma asperellum*, *T. harzianum*, *T. hamatum*, *T. koningii*, *T. longibrachiatum*, *T. virens*, *Aspergillus niger* and two bacterial antagonists *Bacillus subtilis* and *Pseudomonas fluorescens* were evaluated *in vitro* against *M. phaseolina* (MpH₃ isolate), applying Dual culture technique (Arora and Upadhyay 1978).

Seven days old cultures of the test bioagents and the test pathogen (*M. phaseolina*) (MpH₃ isolate) grown on agar media was used for the study. The culture disc (5mm) of the test pathogen and bioagent were cutout with sterilized cork borer, from a week old culture. Then two culture discs, one each of the test pathogen and bioagent were placed aseptically at equidistance and exactly opposite with each other on solidified PDA medium petri plates and plates were incubated at 28±2°C. Three replications were maintained. PDA plates inoculated only with culture disc of the test pathogen were maintained as untreated control.

Details of experiment:

Design : CRD
Replications : Three
Treatments : Ten

Fungal antagonists

T₁ : *Trichoderma asperellum*
T₂ : *T. harzianum*
T₃ : *T. hamatum*
T₄ : *T. koningii*
T₅ : *T. longibrachiatum*
T₆ : *T. virens*
T₇ : *Aspergillus niger*

Bacterial antagonists

T₈ : *Bacillus subtilis*
T₉ : *Pseudomonas fluorescens*
T₁₀ : Control

Observations on radial mycelial growth of the fungal pathogen and biocontrol agents were measured and per cent inhibition of the test fungus were calculated by applying formula given by Arora and Upadhyay (1978) as follows.

Colony growth in

$$\text{Percent inhibition (PI)} = \frac{\text{Control plate} - \text{intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

$$\text{Percent inhibition (PI)} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

In vitro evaluation of botanicals/plant extracts.

Aqueous extracts of 11 botanicals were evaluated *in vitro* against *M. phaseolina* (MpH₃ isolate), applying poisoned food technique. Aqueous extracts of the test botanicals were prepared by grinding with mixture-cum grinder. The 100gm held leaves/ bulbs/rhizomes of each of the test botanicals were macerated in 100 ml distilled water (w/v) separately and the acerates obtain were filtered through double layered muslin cloth. Each of the filtrate obtained were further filtered through by G₂ and G₃ filter paper using funnel and volumetric flasks (100 ml caps.). The final clear extracts /filtrates obtained formed the standard aqueous extract of 100 per cent concentration. These were evaluated (@ 10, 15 and 20% each) *in vitro* against *M. phaseolina* (MpH₃ isolate), applying Poisoned food technique (Nene and Thapliyal 1993) and using Potato dextrose agar (PDA) as basal culture medium.

An appropriate quantity of each test aqueous extract (100%) were separately mixed thoroughly with autoclaved and cooled (40°C) PDA medium in conical flasks (250 ml cap.) to obtain desired concentrations (@ 10, 15 and 20%). The PDA medium amended separately with the test aqueous extract were then poured (20 ml/plate) into sterile glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. For each test botanical extract and their respective concentrations, three replications were maintained. All the treatment plates (PDA Amended) were aseptically inoculated by placing in the centre a 5 mm mycelial disc obtained from a week old actively growing pure culture of *M. phaseolina* (MpH₃ isolate). Plates containing plain PDA without any botanical extract and inoculated with mycelial disc of the test pathogen served as untreated control. All these plates were then incubated at 28±2°C temperature for a week or till the untreated control plates were fully covered with mycelial growth of the test pathogen.

Experimental details

Design : CRD
Replications : Three
Treatments : Twelve

Tr. No.	Common Name	Scientific Name	Plant part used	Tr. No.	Common Name	Scientific Name	Plant part used
T ₁	Onion	<i>A. cepa</i>	Bulb	T ₇	Mint	<i>M. spicata</i>	Leaves
T ₂	Garlic	<i>A. sativum</i>	Clove	T ₈	Ginger	<i>Z. officinale</i>	Rhizome
T ₃	Neem	<i>A. indica</i>	Leaf	T ₉	Tulasi	<i>O. sanctum</i>	Leaves
T ₄	Lantana	<i>L. camera</i>	Leaves	T ₁₀	Adulsa	<i>A. vasica</i>	Leaves
T ₅	Turmeric	<i>C. longa</i>	Rhizome	T ₁₁	Shatavari	<i>A. racemosa</i>	Leaves
T ₆	Karanj	<i>P. pinnata</i>	Seed	T ₁₂	Control (Untreated)		

Observations on radial mycelial growth and sporulation of the test fungus were recorded at 24 hrs. interval and were continued till growth of the test pathogen in untreated control plate is fully covered. Per cent inhibition of the test pathogen was calculated by applying formula given by Vincent (1927).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C= growth of the test pathogen in untreated control plates (mm)

T= growth of the test pathogen a in treated plates (mm)

RESULT AND DISCUSSION

In vitro evaluation of bioagents

The results obtained on mycelial growth and its inhibition of *M. phaseolina* with the test bioagents / antagonists are presented in Table 1, Plate 1 and Fig. 1.

Results (Table 1 and Plate 1) revealed that all of the bioagents evaluated exhibited antifungal activity against *M. phaseolina* and significantly inhibited its growth over untreated control of the antagonists tested, *T. vires* was found most effective with least colony diameter and highest mycelial growth inhibition (12.22 mm and 86.42 %) of the test pathogen respectively which was significantly superior over all the treatments followed by *T. koningii* (15.55 mm and 82.72 %), *T. asperellum* (18.21 mm and 79.77 %), *T. longibrachiatum* (22.45 mm and 75.06 %), *A. niger* (26.15 mm and 70.94 %), *T. harzianum* (29.20 mm and 67.56 %), *T. hamatum* (33.42 mm and 62.87 %), *Pseudomonas fluorescens* (42.15 mm and 53.17 %) and *Bacillus subtilis* (43.21 mm and 51.99 %).

Table 1: *In vitro* bioefficacy of bioagents against *M. phaseolina* (MpH₃).

Tr. No.	Treatments	Mean Colony Dia.of test pathogen * (mm)	% Inhibition
T ₁	<i>T. asperellum</i>	18.21	79.77(63.27)
T ₂	<i>T. harzianum</i>	29.20	67.56(55.28)
T ₃	<i>T. hamatum</i>	33.42	62.87(52.46)
T ₄	<i>T. koningii</i>	15.55	82.72(65.44)
T ₅	<i>T. longibrachiatum</i>	22.45	75.06(60.04)
T ₆	<i>T. (Gliocladium) vires</i>	12.22	86.42(68.38)
T ₇	<i>Aspergillus niger</i>	26.15	70.94(57.38)
T ₈	<i>Bacillus subtilis</i>	43.21	51.99(46.14)
T ₉	<i>Pseudomonas fluorescens</i>	42.15	53.17(46.82)
T ₁₀	Control	90.00	0.00(0.00)
	SE (m) +	1.13	0.95
	C.D. (P=0.01)	3.36	2.82

*: Mean of three replications, Dia.: Diameter, Figures in parentheses are arcsine transformed values

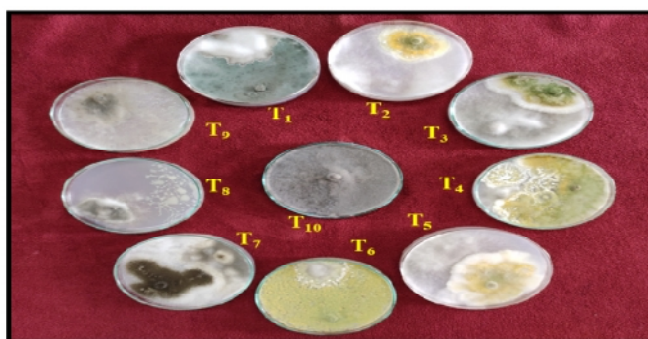


Plate 1. *In vitro* efficacy of bioagents against *M. phaseolina* (MpH₃ isolate).

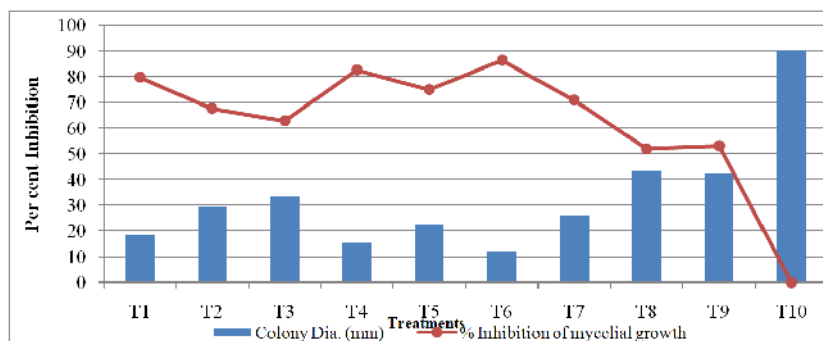


Fig. 1. *In vitro* bioefficacy of bioagents against *M. phaseolina* (MpH₃).

The fungistatic/antifungal action exerted by the fungal and bacterial antagonists against *M. phaseoliona*, the cause of safflower dry root rot may be attributed to the various mechanisms such as competition, lysis, antibiosis, production of volatile/non-volatile substances and production of cellulolytic/pectolytic enzymes, by the antagonistic organisms.

Fungal bioagents viz., *T. virens*, *T. koningii* and *T. asperellum* were reported efficient antagonists against these *M. phaseoliona* results are in consonance with the findings of several workers Gojiya *et al.* (2016) they reported maximum mycelial growth inhibition with *T. harzianum*-II (76.84%) followed by *T. harzianum*-III (72.31%), *T. koningii* (65.13%), *T. viride* (63.89%); Maruti *et al.* (2017b) reported that *T. viride* resulted with maximum mycelia growth inhibition (77.20 %), followed by *T. virens* (75.76 %), *T. harzianum* (73.91 %) and *P. fluorescens* (38.12 %); (Ushamalini, *et al.*, 1997; Malathi and Doraisamy 2003; Thombre *et al.*, 2013; Shahare, 2014; Wadhav and Navgire 2014; Aravind and Brahmhatt 2018; Thombre and Kohire 2018a)

In vitro evaluation of plant extracts/botanicals.

Aqueous extracts of 11 botanicals were evaluated *in vitro* (each @ 10, 15 and 20%) against *M. phaseoliona* and the results obtained on its mycelial growth and inhibition are presented in the Table 2 and depicted in the Plate 2. A, B, C and Fig. 2. Results (Table 2) revealed that all the 11 botanicals extracts tested were fungistatic/antifungal to *M. phaseoliona*, which significantly reduced mycelial growth and increased its inhibition over untreated control. The mycelial growth was found to be decreased and its inhibition was increased with increase in concentrations of the botanicals tested.

Mycelial growth. At 10, 15 and 20 per cent, (Table 2, Plate 2A, B, C and Fig. 2) radial mycelial growth of the test pathogen was ranged from *A. sativam* (0.00, 0.00 and 0.00 mm) to *M. spicata* (82.43, 79.33 and 69.00 mm) as against 90.00 mm in untreated control

respectively, however, significantly least mycelial growth was recorded with *A. sativam* (0.00, 0.00 and 0.00 mm) which was significantly superior over all the treatments followed by the botanicals viz., *A. cepa* (18.33, 13.33 and 9.66 mm), *Z. officinale* (28.66, 22.33 and 18.00 mm), *A. indica* (32.00, 27.43 and 25.00 mm), *O. sanctum* (38.00, 35.66 and 28.33 mm), *C. longa* (42.33, 40.33 and 33.56 mm), *A. vasica* (51.93, 48.43 and 42.33 mm), *P. pinnata* (64.56, 58.00 and 53.36 mm), *L. camera* (70.16, 62.43 and 55.33 mm), *A. racemosa* (79.23, 71.00 and 62.66 mm) and showed highest mycelial growth on *M. spicata* (82.43, 79.33 and 69.00 mm).

Mycelial growth inhibition. Results obtained on mycelial growth inhibition of the test pathogen with the botanicals tested at various concentrations are presented in the Table 2 and depicted in the Plate 2A, B, C. Results (Table 2) revealed that all the botanicals tested (@each 10, 15 and 20%), significantly inhibited mycelial growth of the test pathogen over untreated control. Further, it was found that percentage mycelial growth inhibition of the test pathogen was increased with increase in concentrations of the botanicals tested (Plate 2A, B, C).

At 10, 15 and 20 per cent, (Table 2, Plate 2A, B, C and Fig. 2) mycelial growth inhibition was ranged from *M. spicata* (8.41, 11.86 and 23.33 %) to *A. sativam* (100, 100 and 100 %) respectively, however, significantly highest mycelial growth inhibition was recorded with *A. sativam* (100 %) each concentration which was significantly superior over all the treatments followed by the botanicals viz., *A. cepa* (79.63, 85.19 and 79.27 %), *Z. officinale* (68.16, 75.19 and 80.00 mm), *A. indica* (64.44, 69.52 and 72.22 %), *O. sanctum* (57.78, 60.38 and 68.52 %), *C. longa* (52.97, 55.19 and 62.71 %), *A. vasica* (42.30, 46.19 and 52.97 %), *P. pinnata* (28.27, 35.56 and 40.71), *L. camera* (22.04, 30.63 and 38.52 mm), *A. racemosa* (11.97, 21.11 and 30.38 %) and least inhibition in *M. spicata* (8.41, 11.86 and 23.33 %) at respective concentration.

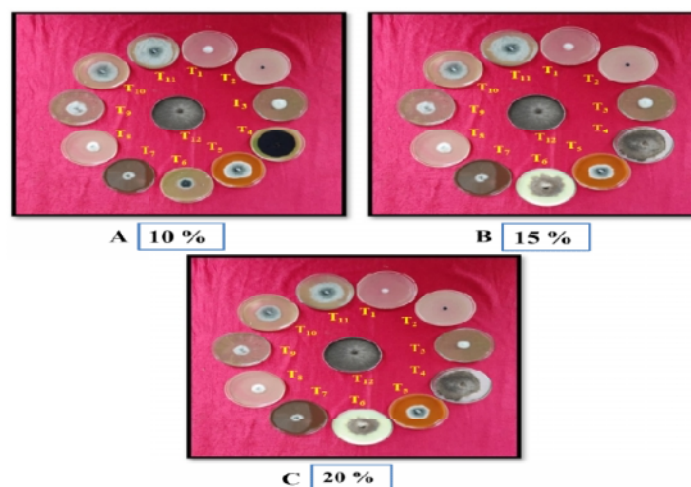


Plate 2. A, B, C. *In vitro* efficacy of plant extract/botanicals at 10%, 15% and 20% Conc. on growth and inhibition of *M. phaseoliona* (MpH₃).

Table 2: *In vitro* efficacy of different botanicals/plant extracts against mycelial growth and inhibition of *Macrophomina phaseolina* (MpH₃).

Tr. No.	Treatments	Mean colony Dia.(mm)* at Conc.			% Inhibition		
		10%	15%	20%	10%	15%	20%
T ₁	Onion (<i>Allium cepa</i>)	18.33	13.33	9.66	79.63 (63.17)	85.19 (67.37)	89.27 (70.88)
T ₂	Garlic (<i>Allium sativum</i>)	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T ₃	Neem (<i>Azadirachta indica</i>)	32.00	27.43	25.00	64.44 (53.40)	69.52 (56.49)	72.22 (58.19)
T ₄	Lantana (<i>Lantana camera</i>)	70.16	62.43	55.33	22.04 (28.00)	30.63 (33.61)	38.52 (38.36)
T ₅	Turmeric (<i>Curcuma longa</i>)	42.33	40.33	33.56	52.97 (46.70)	55.19 (47.98)	62.71 (52.36)
T ₆	Karanj (<i>Pongamia pinnata</i>)	64.56	58.00	53.36	28.27 (32.12)	35.56 (36.60)	40.71 (39.65)
T ₇	Mint (<i>Mentha spicata</i>)	82.43	79.33	69.00	8.41 (16.86)	11.86 (20.14)	23.33 (28.88)
T ₈	Ginger (<i>Zingiber officinale</i>)	28.66	22.33	18.00	68.16 (55.65)	75.19 (60.13)	80.00 (63.43)
T ₉	Tulasi (<i>Ocimum sanctum</i>)	38.00	35.66	28.33	57.78 (49.47)	60.38 (50.99)	68.52 (55.87)
T ₁₀	Adulsa (<i>Adho todavastica</i>)	51.93	48.43	42.33	42.30 (40.57)	46.19 (42.81)	52.97 (46.70)
T ₁₁	Shatavari (<i>Asperagus racemosa</i>)	79.23	71.00	62.66	11.97 (20.24)	21.11 (27.35)	30.38 (33.45)
T ₁₂	Control (Untreated)	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SE (m) +		0.28	0.44	0.31	0.18	0.19	0.20
C.D. (P= 0.01)		0.84	1.30	0.90	0.56	0.55	0.59

*Mean of three replications; Dia: Diameter; Figures in parenthesis are arc sine transformed value.

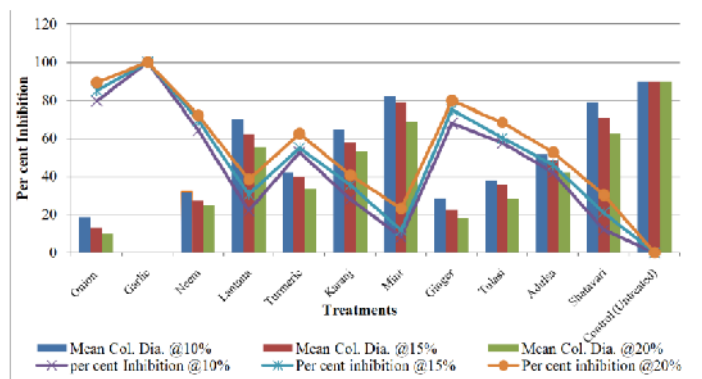


Fig. 2. *In vitro* efficacy of different botanicals/plant extract against mycelial growth and inhibition of *M. phaseolina*.

Results of the present study on antifungal activity of the botanicals are in conformity with those reported earlier by several workers. Botanicals/plant extracts viz., *A. sativum*, *A. cepa*, *Z. officinale*, *A. indica*, *O. sanctum*, *C. longa*, *A. vasica*, *P. pinnata*, *L. camera*, *A. racemosa* and *M. spicata* reported antifungal/fungistatic against *M. phaseolina*, earlier by several workers Gawande *et al.*, (2018) reported that botanicals viz., *A. sativum*, *A. cepa* and *Z. officinale* is most effective and per cent inhibit at concentrations 10, 15, 20% (78.14, 84.07 and 88.51) against *M. phaseolina*; Magar *et al.*, (2011) reported that @ 10% garlic (*A. sativum*) is superior treatment and recorded maximum inhibition (88.15%). (Upadhyaya and Gupta 1990; Sundarraj *et al.*, 1996; Sindhan *et al.*, 1999; Jha *et al.*, 2000; Tandel *et al.*, 2010; Dhingani, 2013; Khaire *et al.*, 2018).

CONCLUSION AND FUTURE SCOPE

The ability of polyphagous, soil inhabiting *R. bataticola* to survive for longer periods as sclerotia in the soil makes its management a difficult chore. However, the current study found that using bioagents and plant extract aids in the effective management of safflower dry root rot disease. *Trichoderma virens* was found to be the most effective bioagent, followed by *T. koningii* with mycelial growth inhibition percentage of 86.42 and 82.72 respectively. *Bacillus subtilis*, on the other hand, was shown to be the least effective against pathogen. Also different Phytoextract @ 10, 15 and 20 per cent evaluated against *M. phaseolina*. *Allium sativum* (100%) was found most effective, resulted in complete inhibition of mycelial growth of the pathogen followed by *Z. officinale* (68.16, 75.19 and 80%)

respectively, *M. spicata* on the other hand, was shown to be the least effective against pathogen.

Use of bioagents and plant extract greatly lower the disease incidence. Farmers benefit from the use of bioagents and phytoextract not only in terms of lowering cultivation costs, but also in terms of increasing yields. The current study advises more trials to be undertaken in naturally infected safflower fields to encourage the use of bioagents and plant extract as an eco-friendly technique for the management of the dry root rot disease and thereby, lower the cost of cultivation by avoiding unsustainable chemical practices.

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Conflict of Interest. None.

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