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Effect of *Pseudomonas fluorescens* and Botanicals against Tikka Leaf Spot Disease (*Cercospora arachidicola, Cercospora personatum*) of Groundnut (*Arachis hypogaea* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop in India. It is susceptible from many diseases, tikka disease is one of them. Tikka disease is due to *Cercospora arachidicola*, *Cercospora personatum* causes much damage to production and pod quality. A survey was conducted during *Rabi* 2020-21 to know the incidence of Tikka disease of groundnut in farmer's fields in Visakhapatnam district of Andhra Pradesh. Five villages were selected in the district and in each village two fields were surveyed. The disease incidence ranged from 29 to 50 per cent irrespective of location surveyed. The disease incidence was least in Taruva village (29%) and highest in Alamanda village (50%) during the month of January to March 2021. To manage the disease an investigation was carried out in farmer's field during *Rabi* 2020-21 in Taruva village, Devarapalle mandal, Visakhapatnam district of Andhra Pradesh to evaluate the efficacy of Bioagent *viz..*, *Pseudomonas fluorescens* (S.T), Botanicals *viz..*, Extract and oil sprays of Neem, Eucalyptus, Clove. Among the treatments the plant height (cm), No. of branches, Root length and Pods per plant at 90 DAS was significantly increased in T3 – S.T with *Pseudomonas fluorescens* +

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neem oil (F.S) (42.8 cm), (6.2), (9.72), (28.51) respectively. The Disease incidence (%) at 90 DAS was significantly decreased in treatment T3 - S.T with *Pseudomonas fluorescens* + neem oil (F.S) (15.5%). The Post parameters i.e., shoot length, Root length, Fresh weight and Dry weight at 90 DAS was significantly increased in T3 – S.T with *Pseudomonas fluorescens* + neem oil (F.S) (42.76 cm), (13.06 cm), (172 gms), (37.96 gms) respectively as compared to control T0.

Keywords: Botanical extracts; Cercospora archidicola; Cercospora personatum; essential oils; fungicides; groundnut; Pseudomonas fluorescens.

1. INTRODUCTION

Groundnut (Arachis hypogaea L.) family, Leguminosae is an important crop among oilseeds and self- pollinated, chromosome no (2n=40) grown in tropical and a sub-tropical region of the world. It is second largest producer and ranks first in acerage throughout the world. The groundnut is particularly valued for its high nutritional value per 100gm i.e. Energy 567k cal, protein-46%, total fat-165%, folates-60%, niacin-75%, thiamin-53%, vit.E-55.5%, zinc-30%, groundnut is also a good source of calcium, copper, phosphorous, iron, selenium and boron. China is the largest producer as well as consumer of groundnut with 18,200 MT as followed by India with 6,000 MT. Groundnut is affected by several fungal, bacterial and viral diseases, of which, spot considerable aroundnut tikka leaf damage, inflicting severe quantitative and qualitative losses. Leaf spot diseases caused by fungus are the major destructive disease of groundnut and could cause a yield loss of up to 50% or more [1]. The disease infects crop directly as well as indirectly and results in huge losses due to leaf defoliation, disruption of photosynthesis and fewer pods that are inferior in quality. Losses are even more when crop is unsprayed [2]. When it is associated with rust (Puccinia arachidis), the losses reached up to 70 per cent [3]. Late leaf spot is almost coexistent with the crop and contributes to significant loss in yield through the world [4]. Different botanicals have been found to inhibit aroundnut disease the of Cercospora arachidicola and Cercospora Personatum [5]. Many research workers have tried to find out safe and economical control of plant diseases by using extracts of different plant parts. Available plant extracts with antifungal activity offer economical, safe and easily available alternative methods for the management of leaf spot in groundnut. Different botanicals have been found to inhibit C. arachidicola and C. Personatum. In view of the above information, it is clear that research work on leaf spot disease

caused by *C. arachidicola* and *C. personatum* which cause serious problem of groundnut cultivation in the country is essential for control by plant extracts and biological control means instead of using chemicals to avoid environmental pollution [5].

2. MATERIALS AND METHODS

The survey was conducted to understand the severity of Tikka disease of groundnut in farmers area in Visakhapatnam district of Andhra Pradesh during Rabi 2020. Five villages have been decided with in each village two fields were surveyed. In each field, the plants were selected in zigzag way and the incidence of Tikka disease of groundnut. So all the samples are collected at different time periods and shown difference in incidence of disease. An experiment was conducted at the farmer's field at Taruva village, Devarapalle mandal, Visakhapatnam (district), Andhra Pradesh during Rabi season 2021. The site selected was uniform, cultivable with typical sandy loam soil having good drainage. The total given area was divided into sub-plots and each treatment were replicated three times with plot size of 2×1 m² and specified in randomized block design (RBD). The seeds were treated with bio-agent-Pseudomonas fluorescens and sown in field with spacing of 30×10 cm. For treated control check- Saaf (Carbendazim 12% + Mancozeb 63% WP) is used for seed treatment. Neem leaf extract applied - 3ml/lt water, Eucalyptus leaf extract - 2-5ml/lt water, cloves - 3ml/lt water (15-20cloves used). Spraying done 3 times from 25 days of sowing. Neem oil was sprayed @2-4ml/lt water, Eucalyptus oil was sprayed @ 3ml/lt, Clove oil was sprayed @ 3ml/lt water was mixed. For mixing oil and water, emulsifier (liquid soap) is used. These sprayed 3 times from 80 days of sowing. Disease incidence of Tikka disease was recorded at 30. 60, 90 DAS and plant growth parameters were also recorded at 30, 60, 90 DAS.

The disease start appearing about 30-40 days after sowing. Plants showing typical symptoms

in the field, that is identified as infected plant part of groundnut. The disease materials were brought to the lab for further investigation. Examining of the fungal colony characteristics was done through microscopic examination by following the technique of Aneja, 2015. Using a sterile needle, a small portion of the infected plant part (leaf) was taken and placed on a sterile glass slide. It was stained using lacto phenol and cotton blu e and covered with the cover slip. Then, the microscope was used for the examination of morphological characteristics of fungal structures [6]. Cercospora spp. consists of both external and internal hyphae. The internal hyphae are both intercellular and intracellular. The haustoria are absent. Asexual reproduction takes place by means of long, cylindrical, hyaline and multiseptate conidia. Conidiophores are dark, straight to slightly

curved, produced in acropetal succession from short sympodially extending dark condiophores. Conidiophores arise in tufts form a stroma lying in a sub stomata cavity and emerge by rupturing the overlying epidermis. The conidiophores are geniculate (knee joint like) and 1-2 septate.

2.1 Preparation of Aqueous Leaf Extracts

The fresh leaves were collected and cleaned under running tap water and grounded in a pestle and mortar by using sterile distilled water. The extract was filtered through double layered muslin cloth and made to the required concentration by adding distilled water Hasan et al. [7] and Pande [8]. Neem leaves, eucalyptus leaves, clove extract is used as foliar spray.



Disease incidence (%) is calculated by using the following formula

Disease incidence (I = $\sum x/N$) was the proportion of diseased plants, which consisted of the number of diseased plants (x) divided by the total number evaluated (N).

Disease incidence (%) = No. of infected plants / Total no. of plants × 100

2.2 Observations Been Recorded

Observations of the characters under study were recorded for comparing the effect of bio- agents, botanicals and fungicides for each observation, five randomly selected plants were tagged from each plot and used further observations. The mean values of the recorded data were taken as the actual values of the respective characters [7].

Pre-harvest growth parameters were Plant height (cm), No. of branches, Disease incidence recorded at 30,60,90 DAS and post-harvest growth parameters were Pods per plant, Root length of the plant (cm), Shoot length (cm), Fresh weight of the plant (gms), Dry weight of the plant (gms).

3. RESULTS

Survey for Tikka disease (*Cercospora arachidicola, Cercospora personatum*) of groundnut was carried out in Devarapalle mandal, Visakhapatnam district, Andhra Pradesh for the duration of *Rabi*

2020-21 season to find out the severity of the disease as defined in the materials and methods and the villages wise disease incidence has been presented in Table 1. The disease incidence ranged from 29 to 50 per cent

irrespective of location surveyed. The disease incidence was least Taruva village with 29% during January 2021 and highest in Alamanda village with 50% during the month of March 2021. The data on survey, revealed the incidence varied from locality to locality, because of type of variety grown, environmental conditions, cropping pattern and development of inoculum. The average disease incidence varied in various locations of different villages in Visakhapatnam district owing to varied agroclimatic conditions and also different cultivers used.

3.1 Plant Height (cm) and No. of Branches Affected by Different Treatments at 90DAS

All treatments significantly increased the plant height (cm) of groundnut plant as compared to control either as treatment. Maximum plant height was recorded in T3 – Seed treated with P. fluorescens @5gm/kg seed + Neem oil spray @1% (42.8).The second best treatment was T2 - P. fluorescens (S.T) @ 5gm/kg seed + Clove oil spray @1% (40.56), which was followed by T6- P. fluorescens (S.T) + Eucalyptus oil spray @1% (39.6), T4- P. fluorescens (S.T) + Neem leaf extract @5% (36.4), T7- P. fluorescens S.T) clove extract @5% + (34.4),

Sr.no	Village	Farmers	Variety	Farmer	Stage of the	Mean	
	-	name	-	field	crop	Disease Incidence (%)	
1.	Taruva	Ramaiah	Kadiri-9	Field-1	Flowering phase	39%	
		Madhav reddy	Kadiri-7	Field-2	Peg initiation	29%	
2.	Tenugupudi	Subba raju	Kadiri - 7	Field-3	Maturity phase	35.6%	
		Mallikarjuna	Kadiri-9	Field-4	Peg intiation	39%	
3.	Marepalli	Raja ram naidu	Kadiri - 7	Field-5	Pre-flowering phase	45%	
		Viswanath Reddy	Greeshma	Field-6	Maturity phase	32%	
4.	Kothapalli	Prasad rao	Kadiri – 7	Field-7	Pod formation phase	40%	
		Sudarshan rao	Greeshma	Field-8	Peg intiation	45%	
5.	Alamanda	Shiva sankar	Greeshma	Field-9	Pod formation phase	35%	
		Amarnath Reddy	Kadiri-7	Field-10	Pod formation phase	50%	

 Table 1. Survey of tikka disease (Cercospora arachidicola, Cercospora personatum) of groundnut in parts of Visakhapatnam district, Andhra Pradesh during Rabi 2020-21

(T5 - P. fluorescens (S.T) + Eucalyptus leaf extract @5% (30.43) as compared to T1 CHECK- Seed treatment with carbendazim (12%) + mancozeb (63% WP) @2gm/kg seed (45.43) and untreated control T0 (28.33). The treatments significantly increased the plant height was found in T1 and T3 as compared with other treated plots. Among the treatments (T0, T5), (T7, T4,), (T6, T2) are non-significant from each other.

All treatments significantly increased No. of branches of groundnut plant as compared to control either as treatment. Maximum plant height was recorded in T3 - Seed treated with P. fluorescens @5gm/kg seed + Neem oil spray @1% (6.2). The second best treatment was T2 -P. fluorescens (S.T) @ 5gm/kg seed + Clove oil spray @1% (5.93), which was followed by T6- P. fluorescens (S.T) + Eucalyptus oil spray @1% (5.73), T4- P. fluorescens (S.T) + NLE @5% (5.46), T7- P. fluorescens (S.T) + clove extract @5% (5.26), T5 - P. fluorescens (S.T) + Eucalyptus leaf extract @5% (4.86) as compared to T1 CHECK- S.T with carbendazim (12%) + mancozeb (63% WP) @2gm/kg seed (6.5) and untreated control T0 (4). The treatments significantly increased the no. of branches was found in T1 and T3 as compared with other treated plots. Among the treatments (T7, T4), (T4, T6), (T6, T2), (T2, T3) are nonsignificant from each other.

3.2 No. of Pods Per Plant and Root Length Affected by Different Treatments at 90DAS

The No. of pods significantly increased in T3 -Seed treated with Ρ. fluorescens @5gm/kg seed + Neem oil sprav @1% (28.51). The second best treatment was T2 - P. fluorescens (S.T) @5gm/kg seed + Clove oil spray @1% (24.15), which was followed by T6-P. fluorescens (S.T) + Eucalyptus oil spray @1% (22.2), T4- P. fluorescens (S.T) + NLE @5% (20.98), T7- P. fluorescens (S.T) + clove extract @5% (19.63), T5 - P. fluorescens (S.T) + Eucalyptus leaf extract @5% (18.7) as compared to T1 CHECK- S.T with carbendazim (12%) + mancozeb (63% WP) @2gm/kg seed (30.8) and untreated control T0 (17.3). Among the treatments (T0, T5), (T5, T7), (T7, T4), (T4, T6) are non-significant from each other.

The root length of groundnut significantly increased in T3 – Seed treated with P. *fluorescens* @5gm/kg seed + Neem oil spray

@1% (9.72).The second best treatment was T2 – *P. fluorescens* (S.T) @5gm/kg seed + Clove oil spray @1% (9.44) which was followed by T6- *P. fluorescens* (S.T) + Eucalyptus oil spray @1% (8.51), T4- *P. fluorescens* (S.T) + NLE @5% (8.31), T7- *P. fluorescens* (S.T) + clove extract @5% (8.02), T5 – *P. fluorescens* (S.T) + Eucalyptus leaf extract @5% (7.74) as compared to T1 CHECK- S.T with carbendazim (12%) + mancozeb (63% WP) @2gm/kg seed (11.58) and untreated control T0 (6.76). Among the treatments (T5, T7, T4, T6), (T2, T3) are non-significant from each other.

3.3 Post-shoot Height, Fresh Weight and Dry Weight Influenced by Different Treatments

All treatments significantly increased the Post shoot height (cm) of groundnut plant as compared to control either as treatment. Maximum plant height was recorded in T3 -Seed treated with P. fluorescens @5gm/kg seed + Neem oil spray @1% (42.76). The second best treatment was T2 - P. fluorescens (S.T) @5gm/kg seed + Clove oil spray @1% (41.78), which was followed by T6- P. fluorescens (S.T) + Eucalyptus oil spray @1% (40.66), T4- P. fluorescens (S.T) + Neem leaf extract @5% (36.93), T7- P. fluorescens (S.T) + clove extract@ 5% (33.36), T5 - P. fluorescens (S.T) + Eucalyptus leaf extract @5% (32.06) as compared to T1 CHECK- Seed treatment with carbendazim (12%) + mancozeb (63% WP) @2gm/kg seed (44.43) and untreated control T0 (29.53). The treatments significantly increased the plant height was found in T1 and T3 as compared with other treated plots. Among the treatments (T5, T7), (T6, T3, T2), (T3, T1,) are non-significant from each other.

All treatments significantly increased the Post shoot height (cm) of groundnut plant as compared to control either as treatment. Maximum plant height was recorded in T3 -Seed treated with P. fluorescens @5gm/kg seed + Neem oil spray @1% (42.76). The second best treatment was T2 - P. fluorescens (S.T) @5qm/kg seed + Clove oil spray @1% (41.78), which was followed by T6- P. fluorescens (S.T) + Eucalyptus oil spray @1% (40.66), T4- P. fluorescens (S.T) + Neem leaf extract @5% (36.93), T7- P. fluorescens (S.T) + clove extract @5% (33.36), T5 - P. fluorescens (S.T) + Eucalyptus leaf extract @5% (32.06) as compared to T1 CHECK- Seed treatment with carbendazim (12%) + mancozeb (63% WP) @

Tr No.	Treatment	Plant ht.	Branches /plant	No. of pods /plant	Root length	Post shoot ht.	Fresh Wt.	Dry wt.
T0	CONTROL	28.33	4	17.3	6.76	29.53	176.66	24.85
T1	CHECK- S.T with carbendazim (12%) + mancozeb (63% WP) @ 2gm/kg seed	45.43	6.5	30.8	11.58	44.43	175.96	39.56
T2	S.T with <i>Pseudomonas fluorescens</i> @ 5gm/kg seed + Clove oil spray @1%	40.56	5.93	24.15	9.44	41.78	166.6	36.11
Т3	S.T with <i>Pseudomonas fluorescens</i> @ 5gm/kg seed + Neem oil spray @1%	42.8	6.2	28.51	9.72	12.76	172	37.96
T4	S.T <i>Pseudomonas fluorescens</i> @ 5gm/kg seed + Neem leaf extract @5%	36.4	5.46	20.98	8.31	36.93	154.13	29.86
Τ5	S.T with <i>Pseudomonas fluorescens</i> @ 5gm/kg seed + Eucalyptus leaf extract @5%	30.43	4.86	18.7	7.74	32.06	135.6	26.40
Т6	S.T with <i>Pseudomonas fluorescens</i> @ 5gm/kg seed + Eucalyptus oil spray @ 1%	39.6	5.73	22.2	8.51	40.66	160.53	34.66
T7	S.T with <i>Pseudomonas fluorescens</i> @ 5gm/kg seed + clove extract @ 5%	34.4	5.26	19.63	8.02	33.36	142.46	27.73
	S.Ed. (±)	0.982	0.129	0.65	0.394	1.16	3.76	1.029
	C.D. (5%)	2.1	0.27	1.40	0.84	2.491	8.06	2.150

Table 2. Effect of plant growth parameter of groundnut

2gm/kg seed (44.43) and untreated control T0 (29.53). The treatments significantly increased the plant height was found in T1 and T3 as compared with other treated plots. Among the treatments (T5, T7), (T6, T3, T2), (T3, T1,) are non-significant from each other.

Maximum fresh weight (gms) was recorded in T3 - Seed treated with P. fluorescens @5gm/kg seed + Neem oil spray @1% (172gms).The second best treatment was T2 - P. fluorescens (S.T) @5gm/kg seed + Clove oil spray @1% (166.6), which was followed by T6- P. fluorescens (S.T) + Eucalyptus oil spray @1% (160.53), T4- P. fluorescens (S.T) + Neem leaf extract @5% (154.13), T7- P. fluorescens (S.T) + clove extract @5% (142.46), T5 - P. fluorescens (S.T) + Eucalyptus leaf extract @5% (154.13) as compared to T1 CHECK- S. T with carbendazim (12%) + mancozeb (63% WP) @2gm/kg seed (175.96) and untreated control T0 (126.66). The treatments significantly increased the plant height was found in T1 and T3 as compared with other treated plots. Among the treatments (T5, T7), (T4, T6), (T6, T2,), (T2, T3), (T3, T1) are non-significant from each other.

Maximum dry weight was recorded in T3 – Seed treated with *P. fluorescens* @5gm/kg seed + Neem oil spray @1% (37.96gms).The second best treatment was T2 – *P. fluorescens* (S.T) @5gm/kg seed + Clove oil spray @1% (36.11), which was followed by T6- *P. fluorescens* (S.T) + Eucalyptus oil spray @1% (34.66), T4- *P.*

fluorescens (S.T) + Neem leaf extract @5% (29.86), T7- *P. fluorescens* (S.T) + clove extract @5% (27.73), T5 – *P. fluorescens* (S.T) + Eucalyptus leaf extract @5% (26.40) as compared to T1 CHECK- S.T with carbendazim (12%) + mancozeb (63% WP) @2gm/kg seed (39.56) and untreated control T0 (24.85). The treatments significantly increased the plant height was found in T1 and T3 as compared with other treated plots. Among the treatments (T0, T5), (T5, T7), (T7, T4), (T6, T2), (T2, T3), (T3, T1) are non-significant from each other.

3.4 Disease Incidence Percent at 90DAS

The disease incidence of Cercospora leaf spot of groundnut significantly decreased in treatment T3 – S.T with *P. fluorescens* + Neem oil spray @1% (15.5) followed by T2 -S.T with P. fluorescens @ 5gm/kg seed + Clove oil spray @1% (20.5), T6- S.T with P. fluorescens + Eucalyptus oil spray @1% (27.3), T4- S.T with P. fluorescens + NLE @5% (32.16), T7- S.T with P. fluorescens + clove extract @5% (37.16), T5 -S.T with *P. fluorescens* + Eucalyptus leaf extract @5% (43.66), when compared to T1 CHECK-S.T with carbendazim (12%) + mancozeb (63%) WP) @ 2gm/kg seed (11.16) and untreated control T0 (45.9). The treatments significantly decreased the disease incidence was found in T1 and T3 as compared with other treated plots. Among the treatments (T1, T3), (T6, T4,), (T5, T0) are non-significantly differ from each other.

Table 3. Effe	ect of treatments	on disease	incidence of	groundnut
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Tr. no	Treatment	Disease incidence @ 90DAS
Т0	CONTROL	45.9
T1	CHECK- S.T with carbedazim (12%) + mancozeb (63% WP) @ 2gm/kg seed	11.16
T2	S.T with <i>P. fluorescens</i> @ 5gm/kg seed + Clove oil spray @1%	20.5
Т3	S.T with <i>P. fluorescens</i> @ 5gm/kg seed + Neem oil spray @1%	15.5
T4	S.T with <i>P. fluorescens</i> @ 5gm/kg seed + Neem leaf extract @5%	32.16
T5	S.T with <i>P. fluorescens</i> @ 5gm/kg seed + Eucalyptus leaf extract @5%	43.66
Т6	S.T with <i>P. fluorescens</i> @ 5gm/kg seed + Eucalyptus oil spray @ 1%	27.3
Τ7	S.T with <i>P. fluorescens</i> @ 5gm/kg seed + clove extract @ 5%	37.16
F-test		S
S.Ed. (±)		1.993
C.D. (5%)		4.90

4. DISCUSSION

Probable reasons for this result is due to Neem oil contains active compound such as Azadirachtin, as well as salanin, meliantriol, nimbin. Constituents such as Nimbin, Nimbidin, Nimbolide, Limonoids controls the disease. Quercetin and β -sistosterol were polyphenolic flavanoids purified from neem leaves show antifungal and antibacterial activities. The major constituents of Eucalyptus leaves are 1,8-cineol (49.07 to 83.59%) and α -pinene (1.27 to 26.35%). Clove contains Eugenol (80-95%), acetyl eugenol (1-5%), and β -caryophyllene (4-12%). Besides this, it has Gallotannic acid, Oleanolic acid, Vannilin, and Eugenin also. Antimicrobial activity (antibacterial and antifungal) of clove oil is directly related to the presence of a high concentration (85.3%) of Eugenol. Indeed, the extracts contained alkaloids, tannins and phenolic compounds. They contain antifungal properties which retarded or inhibited the activity of the fungi leading to a decrease in disease incidence and disease severity. Mainly the formation of trans-membrane pores or ion channels on the cellular membrane leading to the leakage of essential metabolites and the disruption of cell wall structure, interfering with cell wall synthesis. This could have led to an increase in photosynthetic activity which enhanced vegetative growth, net assimilation and dry matter accumulation and results in more yield. The findings of this study support the report by Hasan, [7].



Fig. 1. Effect of bio-control agent, botanical extracts and essential oils on plant growth parameters and disease incidence

Pseudomonas fluorescens are known to have important traits in bacterial fitness such as the ability to adhere to soil particles and to the rhizoplane, motility and prototrophy, synthesis of antibiotics, and production of hydrolytic enzymes. An increase in activity of phenyl alanine ammonia lyase one day after application of bacterial antagonist was seen, and maximum activity was observed 3 days after treatment. The anti-fungal metabolite 2,4-diacetyl phloroglucinol play a major role in bio-control capabilities of P. fluorescens. Moreover, benefits of P. fluorescens also possesses plant growth-promoting traits fixation. such nitrogen phosphate as solubilization, iron chelation, and phyto-hormone

production and promoting plant disease resistance. This strain improved seed germination, nodulation, dry weight and pod yield as well as protected plants. The findings of this study support by Ganeshan, [9].

5. CONCLUSION

The in vivo results revealed that plant height, No. of branches, Pods per plant, Root length of plant, Shoot length, fresh and dry weight of plant significantly increased and disease incidence (%) in groundnut significantly decreased in the treatment T3 - P. fluorescens (S.T) + Neem oil (F.S) [10,11].

Applying Bio-agent, Botanical extracts, Essential oil in the field will be considered as beneficial and eco-friendly. However, the present study was limited to one crop season i.e., to substantiate the present result more trails are needed for 2-3 seasons for further recommendations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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