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Evaluating Fungicides, Biocontrol Agents and Soil Amendments against *Fusarium* Wilt of China Aster: Towards Integrated Disease Control

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To assess several fungicides and bioagents against wilt of China aster, caused by *Fusarium oxysporium* f. sp. *callistephi*, research was conducted at the Division of Plant Pathology, Faculty of Agriculture, SKUAST-K in 2021 and 2022. Symptoms appeared 21 days post inoculation, progressing to whole plant wilting by day 36. Morphological analysis revealed globose to subglobose chlamydospores, sickle-shaped macroconidia with 3-8 septa, and cylindrical microconidia with 0-1 septum. In vitro experiments for non-systemic fungicides revealed that mancozeb 75 WP was most effective against the test pathogen, with a mean mycelial growth suppression of 92.46%. Conversely, carbendazim 50 WP showed the highest mean mycelial growth inhibition of 93.49% among systemic fungicides. *Trichoderma viride* exhibited the highest mean mycelial inhibition of 78.55% among the bioagents. An integrated disease management study found that combinations of soil amendments, including vermicompost used at 100g per pot, along with *Trichoderma viride* used at 25 ml suspension (2.1×10^7 cfu/ml) per pot, carbendazim 50 WP as seed treatment, and seedling dip. This combination also significantly reduced disease incidence (4.15%), while showing the highest average number of flowers per plant, the highest average flower diameter, and the highest average leaf area.

Keywords : Bioagents, China aster, *Fusarium oxysporum*, IDM, *Trichoderma*

INTRODUCTION

China aster or *Callistephus chinensis* (L.), is a member of the Asteraceae family and is indigenous to China (Krishna *et al*. 2019). China aster is a short growth season crop that adapts to a variety of agroclimatic conditions (Jogi *et al*. 2020). According to Pratiksha *et al*. (2017), farmers in Karnataka, Tamil Nadu, Andhra Pradesh, Maharashtra, and West Bengal are projected to be cultivating China aster commercially across an area of 3500 ha in India.

However, China aster is vulnerable to a variety of diseases and pests. The most devastating disease of China aster is wilt, which is brought on by *Fusarium oxysporum* f. sp. *callistephi* (Horita and Mc Govern 2016). According to reports, *Verticillium alboatrum*, *Acrostagimus vilmorinii*, and *Fusarium oxysporum* f. sp. *callistephi* are the aster wilt causing pathogens (Elmer and Mc Govern 2013). In Jammu and Kashmir, *Fusarium* wilt of aster has been documented by Koul and Ghani (1989). Plants that have this disease wilt, wither, and eventually die at any phase of plant growth. The outside of afflicted branches may show signs of orange, white, or pink fungal growth, particularly in damp conditions (Ajigbola and Babalola 2013). The indications of an infection consist of mild vein clearing on the outer portion of new leaves and epinasty on older leaves.

Many disease control strategies have been tested against *Fusarium* spp. Using a food poisoning technique, Bashir *et al*. (2017) assessed the *in vitro* effectiveness of many fungicides against *F. oxysporum* f. sp. *capsici*. Fungicide alliete outperformed all other fungicides in terms of maximal colony growth, measuring 1.93 cm. Additionally, biological control has emerged as a sustainable approach to safeguarding crop health

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while preserving environmental integrity and supporting ecosystem functions (Bandara and Kang, 2024). Sheath blight of rice diseae caused by *Rhizoctonia solani* has been effectively managed through an integrated approach combining *Trichoderma* treatments and a single fungicide spray of propiconazole, achieving 54.4% disease control while minimizing chemical use (Pal and Mandal, 2025). Gupta and Misra (2009) assessed the volatile compounds and culture filtrates of eight bioagents against guava wilt caused by *Fusarium oxysporum* f. sp. *psidii* and *Fusarium solani*. The growth of *F. solani* and *F. oxysporum* f. sp. *psidii* was considerably inhibited by all bioagents. However, *Trichoderma* spp. used in dual culture against *F. oxysporum* f. sp. *psidii* (61-69%) and *F. solani* (58-68%) showed a considerably lower rate of growth.

These findings highlight the value of an integrated approach to managing diseases to prevent *Fusarium* wilt. Given the severity of this disease, the current research aimed to look into the etiology and symptomatology of the disease, assess several fungicides and bioagents against the pathogen in vitro, and develop an integrated disease management capsule.

MATERIALS AND METHODS

The experiments were conducted at the Division of Plant Pathology, Faculty of Agriculture, SKUAST-K during 2021 and 2022 to evaluate various fungicides and bioagents against *Fusarium* wilt of China aster and to develop an epilogue designating integrated disease management.

Symptomatology and Etiology

Characteristic symptoms of the disease were recorded on the leaves and stem periodically in the field. The pathogen was isolated using the detached tissue method, and the culture was purified using the single-spore method (Johnston and Booth, 1983). Pure culture of the pathogen was maintained in PDA slants kept at 4 ± 1ºC and mass multiplied on sterilized sorghum sand medium (1 part sorghum grain, 3 parts sand and distilled water to wet the media) for a duration of 15 days at 27 ± 2ºC. Specific characteristics of the isolated pathogen were examined using light microscope at 400x magnification power.

Pathogenicity test

The pathogenicity test was conducted using Nasehi *et al*. (2012) method on China asters (variety Powder Puff) using 7 days old culture of *Fusarium oxysporum* f. sp. *callistephi* with 10^5 conidia/ml of sterile distilled water. After 30 seconds of immersion in a 4% sodium hypochlorite solution, six sensitive China aster seedlings of the Powder Puff variety were transplanted into 15 cm diameter pots with three replications. For four days, the pots were incubated at 27 ± 2ºC. As a control, seedlings were kept in sterile soil without an inoculum. Plants were checked for wilt symptoms and the percentage of disease incidence every 30 days after transplanting (DAT).

In vitro evaluation of fungicides

Using the poisoned food technique of Nene and Thapliyal (1979), four non-systemic fungicides (Mancozeb 75 WP, Copper oxychloride 50 WP, Chlorothalonil 75 WP, and Captan 50 WP) at three concentrations of 500 ppm, 1000 ppm, and 1500 ppm were assessed against the pathogen in vitro. At three distinct concentrations of 100 ppm, 300 ppm, and 500 ppm, four systemic fungicides (Carbendazim 50 WP, Thiophenate methyl 75 WP, Difenoconazole 25 EC, and Kresoxim-methyl 44.3% SC) were also assessed. Using Vincent's (1947) formula, the percentage of test pathogen mycelial growth inhibition was computed as:

$$I=\frac{C-T}{C}\times 100$$

Where I=per cent inhibition, C = colony diameter in control, and T = colony diameter in treatment.

In vitro evaluation of bioagents

The pathogen was assessed using the dual culture technique with various bioagents: *Pseudomonas fluorescence*, *Bacillus subtillis*, *Trichoderma viride*, and *Trichoderma harzianum* (Dennis and Webster 1971). The source of bioagents and AMF (Arbuscular mycorrhizal fungi)

was the Division of Plant Pathology, SKUAST Kashmir, Faculty of Agriculture, Wadura. Vincent's formula (1947), previously discussed, was used to compute the per cent mycelial growth inhibition of the test pathogen.

Integrated Disease Management

An in vivo experiment was carried out in an open field setting utilizing pots that were subjected to various treatments to investigate the efficacy of bioagents, systemic fungicides, and non-systemic fungicides against the wilt disease (Table 1). Thirty-day-old China aster seedlings (variety Powder Puff) were transplanted into pots filled with sick soil of 5-7 days old test pathogen @ 10^5 conidia/ml of sterile distilled water. Twelve treatments, including a control and three replications, were used in the factorial RBD experiment. AMF used was *Glomus* sp., and it was used at the rate of 50 g/pot. The best performing non-systemic fungicide, systemic fungicide, and bioagent under *in vitro* experiments were used in the treatments. Observations were made on a variety of aspects, including the average number of flowers per plant, the average flower diameter (cm), the average leaf area (cm^2), and the percentage of disease incidence.

RESULTS AND DISCUSSION

Symptomatology and Etiology

China aster plants were sowed in the first week of May and transplanted to pots in the first week of June. The plants were subjected to periodic observations every five days on symptomological development. The disease symptoms were observed on tagged leaves and stems. On 21st day after transplantation, 25 per cent yellowing of leaves on China aster plants was noted as a result of *Fusarium* wilt. 50 per cent of the leaves turned yellow on the 26th day after inoculation and transplantation (DAIT), and 75 per cent of the leaves yellowed on the 31st DAIT. Vascular browning in the roots and complete plant wilting and drying up were noted on the 36th day. According to Horita and McGovern (2016) and Bose and Yadav (1989), plants infected with *Fusarium* wilt show yellowing, curving, bending, leaves drying up, and eventually dying of older plants. Cuts made longitudinally from diseased stems reveal vascular discolouration. Specific characteristics of the isolated pathogen were examined in relation to the cultural traits. Colony characteristics included a grey to pinkish colour, a regular form, a quick growth rate (growing 90 mm in 7 days), a fluffy texture, a greyish-pink edge, and a dark violet to pinkish underside. The isolated pathogen's morphological characteristics were examined by examining a culture established on potato dextrose agar (PDA) at a temperature of 25 ± 1ºC (Fig. 1). Table 2 presents observations about the morphological features of several structures, including mycelium, macroconidia, microconidia, and chlamydospores. Based on these studies the pathogen was identified as *Fusarium oxysporium* f. sp. *callistephi.*

Fusarium species produced both macroconidia and microconidia (from slender phialides), as noted by Okungbowa and Shittu (2012). Hyaline, fusiform to sickle-shaped, with two to multiple celled, elongated apical and pedicellate basal cells are the most common features of macroconidia. Microconidia are hyaline, pyriform, fusiform to ovoid, straight or curved, and have one to two cells. The isolates' morphology and culture displayed a great degree of variation. A pathogenicity test was conducted in China aster using Powder Puff variety after which the plants began to droop 21 days after the inoculation.

In vitro evaluation of Fungicides

Through the poisoned food technique, relative effectiveness of four non-systemic and four systemic fungicides in inhibiting mycelial growth of *Fusarium oxysporium* f. sp. *callistephi* was assessed in vitro. The results for non-systemic fungicides showed that mancozeb (75 WP) was most effective as it inhibited mycelial development by 92.46% compared to the control, followed by captan (50 WP) with 86.11% inhibition and chlorothalonil (75 WP) with 60.71% inhibition (Fig. 2). The least effective treatment, however, was copper oxychloride (50 WP), which inhibited mycelial development by 52.88%.

Overall, as concentrations of test fungicides grew, so did their inhibitory effects. At 500 ppm, a minimum of 56.25% inhibition was obtained, and

Table 1: Treatment details for integrated disease management studies in China aster

Treatment No.	Treatment Code	Details of treatment	
		Soil Amendments	Seed treatment and seedling dip
T_1	SA_0STSD_0	Nil	Nil
T_2	SA_1STSD_0	AMF	Nil
T_3	SA_2STSD_0	Vermicompost + Bioagent	Nil
T_4	SA_0STSD_1	Nil	Non-systemic fungicide
T_5	SA_1STSD_1	AMF	Non-systemic fungicide
T_6	SA_2STSD_1	Vermicompost + Bioagent	Non-systemic fungicide
T_7	SA_0STSD_2	Nil	Systemic fungicide
T_8	SA_1STSD_2	AMF	Systemic fungicide
T_9	SA_2STSD_2	Vermicompost + Bioagent	Systemic fungicide
T_{10}	SA_0STSD_3	Nil	Bioagent
T_{11}	SA_1STSD_3	AMF	Bioagent
T_{12}	SA_2STSD_3	Vermicompost + Bioagent	Bioagent

Factor 1: Soil amendments = 03
SA_0=No soil amendment.
SA_1=Soil amendment with AMF.
SA_2=Soil amendment with vermicompost + best bioagent.
Factor 2: Seed and seedling treatments = 04
$STSD_0$=No seed and seedling treatment.
$STSD_1$=Seed and seedling treatment with best effective non systemic fungicide found in vitro
$STSD_2$=Seed and seedling treatment with best effective systemic fungicide found in vitro
$STSD_3$=Seed and seedling treatment with best effective bioagent found in vitro

Table 2: Morphological characters of the isolated pathogen

Fungal structure	Shape	Colour	Size(µm)	Septation
Mycelium	Filamentous and branched	Hyaline	W = 0.92-4.60	Septate
Macrconidia	Long Sickle shaped	Hyaline	L = 14.2-28.40 B =1.80–6.30	3–8; Mostly 3 septate
Microconidia	Short cylindrical	Hyaline	L = 5.30–9.90 B = 1.35–7.50	0–1 Mostly non septate
Chlamydospores	Globose to subglobose	Hyaline	D = 7–11	1-4 Mostly 2 septate

W = Width, L= Length, B= Breadth and D= Diameter

Table 3: *In vitro* inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *callistephi* by non-systemic fungicides

Fungicides	Inhibition of mycelial growth (%) Concentrations (ppm)			
	500	1000	1500	Mean
Mancozeb 75 WP	87.80	92.27	97.32	92.46
Copper oxychloride 50 WP	26.78	55.65	76.19	52.88
Chlorothalonil 75 WP	34.82	61.01	86.31	60.71
Captan 50 WP	75.60	89.29	93.16	86.01
Mean	56.25	74.55	88.24	

Table 4: *In vitro* inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *callistephi* by systemic fungicides

Fungicides	Inhibition of mycelial growth (%) Concentrations (ppm)			
	100	300	500	Mean
Carbendazim 50 WP	89.66	93.10	97.70	93.49
Thiophenate methyl 75WP	86.50	92.81	96.26	91.86
Difenoconazole 25 EC	78.74	84.77	94.54	86.02
Kresoxim-methyl 44.3 % SC	61.78	69.25	87.93	72.99
Mean	79.17	84.98	94.11	

Table 5: The effectiveness of different bioagents i*n vitro* on the mycelial growth of *Fusarium oxysporum* f. sp. *callistephi*

Bioagents	Mycelial growth inhibition over check (%)
Trichoderma viride	78.45 (52.13)*
Trichoderma harzianum	73.17 (50.05)
Bacillus subtilis	21.34 (31.624)
Pseudomonas fluorescens	23.17 (32.42)
C.D. (p≤0.05)	1.26

*The values in parenthesis are arcsine transformed values

Table 6: Integrated disease management of China aster against *Fusarium oxysporum* f. sp. *callistephi*

S. No.	Treatment	Disease incidence (%)	No. of flowers/ plant	Flower diameter (cm)	Leaf area (cm^2)
1	Carbendazim 50 WP (STSD)**	35.56 (5.96)*	7.73	4.86	11.12
2	AMF @ 50g/pot (SA)*** + Carbendazim 50 WP (STSD)	8.89 (3.11)	10.93	6.76	14.43
3	Vermicompost @ 100g/ pot + *Trichoderma viride* @ 25ml suspension (2.1×10^7 cfu/ml) per pot (SA) + Carbendazim 50 WP (STSD)	4.45 (2.18)	11.47	7.36	15.56
4	Mancozeb 75 WP (STSD)	44.45 (6.74)	6.67	4.76	10.57
5	AMF (SA) + Mancozeb 75 WP (STSD)	15.56 (3.98)	10.13	5.80	13.40
6	Vermicompost + *Trichoderma viride* (SA) + Mancozeb 75 WP (STSD)	11.11 (3.45)	10.67	6.33	13.75
7	*Trichoderma viride* (STSD)	60.00 (7.80)	4.80	4.13	9.81
8	AMF (SA) + *Trichoderma viride* (STSD)	20.00 (4.54)	9.07	4.90	11.72
9	Vermicompost + *Trichoderma viride* (SA) + *Trichoderma viride* (STSD)	15.55 (4.05)	10.13	5.76	12.53
10	AMF (SA)	24.45 (5.04)	9.60	4.56	10.06
11	Vermicompost + *Trichoderma viride* (SA)	17.32 (4.01)	8.30	5.23	12.08
12	Control	88.89 (9.47)	1.33	3.66	9.24
	C.D. ($p\leq0.05$)	1.22	0.17	0.08	0.01

*The values in parenthesis are Square root transformed values
**STSD = Seed treatment and seedling dip ***SA = Soil amendments

at 1500 ppm, it progressively climbed to 87.24%. The data also shows that at 1500 ppm concentration, mancozeb (75 WP) provided 97.32% mycelial inhibition, making it the most effective treatment (Table 3). Mancozeb (75 WP) @ 1500 ppm was likewise determined to be the most effective non-systemic fungicide by Nisa *et al*. (2011).

The results of the systemic fungicide test indicated that carbendazim (50 WP) was the most effective, inhibiting mycelial growth by 93.49%. Thiophenate methyl (75 WP) showed 91.86% inhibition, while difenoconazole (25 EC) showed 86.02% inhibition (Table 4). The least effective, however, was kresoxim-methyl 44.3% SC, which showed a 72.99 % reduction of mycelial development.

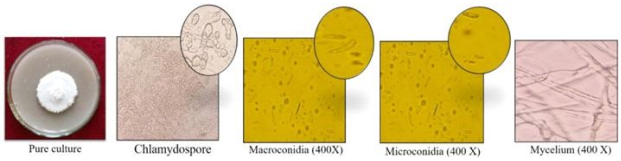

Fig. 1: Morphological characteristics of *Fusarium oxysporum* f. sp. *callistephi*

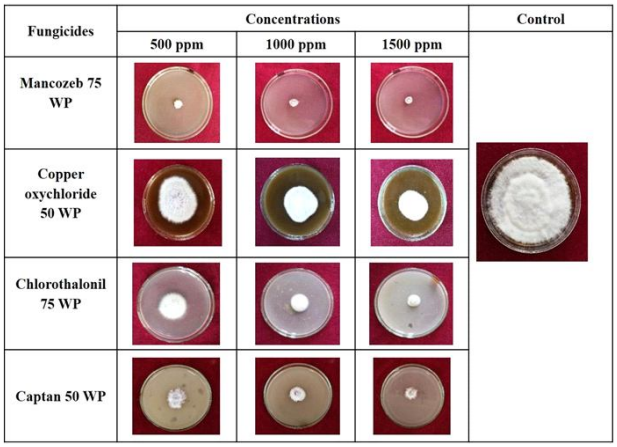

Fig. 2: *In vitro* tests of different non-systemic fungicides *Fusarium oxysporum* f. sp. *callistephi*

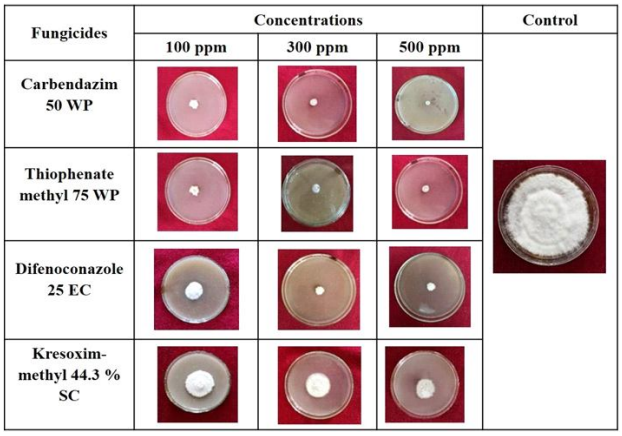

Fig. 3: *In vitro* tests of different systemic fungicides *Fusarium oxysporum* f. sp. *callistephi*

As concentrations of test fungicides grew, so did their inhibitory effect. At a dosage of 500 ppm, the minimum inhibition was 94.11%, progressively rising to 94.11% at 100 ppm (Fig. 3). Carbendazim (50 WP) at 500 ppm concentration demonstrated the highest level of effectiveness, outperforming all other test fungicides and delivering 96.70% mycelial inhibition at this concentration. Among the systemic fungicides tested for *Fusarium* wilt, carbendazim (50 WP) was also found to be efficacious by Dahal and Shrestha (2018), Niwas *et al*. (2020), and Yerukala *et al*. (2021).

Fig. 4: *In vitro* inhibition of mycelial development of *Fusarium oxysporum* f. sp. *callistephi* by various bioagents

In vitro evaluation of Bioagents

Employing dual culture technique, two bacterial bioagents, *Pseudomonas fluorescens* and *Bacillus subtilis*, and two fungal bioagents, *Trichoderma viride* and *Trichoderma harzianum*, were evaluated in vitro against *Fusarium* wilt pathogen (Fig. 4). The data (Table 5) shows that all bioagents were antagonistic against *F. oxysporium* f. sp. *callistephi*, and that *T. viride* outperformed other treatments with a growth inhibition of 78.45 %. *B. subtilis* was found to be least effective, inhibiting 21.34% of mycelial growth.

Integrated Disease Management

Pot culture experiment results are shown in Table 6. In this experiment, disease incidence ranges from 4.45 to 88.89%. The combination of vermicompost + *Trichoderma viride* (SA–Soil amendment) + carbendazim 50 WP (STSD–Seed and seedling dip) gave the lowest disease incidence (4.45%) and outperformed all other treatments (88.89%). Additionally, data in Table 6 showed that every treatment combination significantly affected the average number of flowers per plant during the check. Vermicompost + *Trichoderma viride* (SA) + carbendazim 50 WP (STSD) produced highest average number of flowers per plant (11.47), making it the best combination. The average flower diameter over check was significantly impacted by every treatment combination. Vermicompost +

Trichoderma viride (SA) + carbendazim 50 WP (STSD) showed the largest average flower diameter (7.36 cm), making it the best combination. Similar results were achieved by Madhavi and Bhattiprolu (2011) against *Fusarium* wilt of chilli, where the integrated treatment of carbendazim (root dip), vermicompost, fungicide drench (carbendazim + mancozeb), and *T. viride* soil application showed the lowest plant mortality (5.83%).

The information in Table 6 also demonstrated that all treatment combinations had a noteworthy impact on the average leaf area (cm^2) compared to the control group. Of these combinations, vermicompost + *Trichoderma viride* (SA) + carbendazim 50 WP (STSD) demonstrated the highest average leaf area (15.56 cm^2). Prior research has shown that biocontrol agents promote key growth parameters, including root-shoot length, biomass, and plant height. For example, *Trichoderma parareesei* has been linked to improved growth in tomato (Rubio *et al*. 2012), and *T. harzianum* and *T. atroviride* have enhanced seed germination and root biomass in peppers (Colla *et al*. 2015). These plant–fungal interactions lead to physiological and genetic changes that improve both growth and defense responses. Besides, Sahane *et al*. (2021) evaluated the effectiveness of mycelial growth inhibition by fungicides against *Fusarium oxysporum* f. sp. *ciceri* in pot culture as well as compatible isolates of *Pseudomonas fluorescens*. Carbendazim 50 WP + PF5 (80.73%) demonstrated the highest disease control percentage, according to the results. The present study findings highlight the significance of combining fungicide, a potential microbial antagonist, and soil amendments as seed treatments and seedling dips. These findings align with earlier studies showing that integrated disease management effectively controls *Fusarium* wilt in gladiolus (Anand and Gautam, 2006), and soil-borne diseases in vegetable nurseries (Steven *et al*., 2003).Hossain *et al*. (2013) evaluated the combined effectiveness of *T. harzianum* isolate T-75, *Azadirachta indica* leaf extract, and Provax-200 seed treatment as an integrated management strategy for *Fusarium* wilt of chickpea. This method seemed to be much better at both increasing seed output and minimizing *Fusarium* wilt.

CONCLUSION

In vitro studies conducted on *Fusarium* wilt of China aster, caused by *Fusarium oxysporium* f. sp. *callistephi*, revealed that the most effective non-systemic fungicide was mancozeb 75 WP, with a mean mycelial growth inhibition of 92.46%. Carbendazim 50 WP showed the maximum mean mycelial growth inhibition of 93.49% among systemic fungicides. *Trichoderma viride* exhibited the maximum mean mycelial inhibition of 78.55% among the bioagents. Vermicompost + *Trichoderma viride* as (SA) and carbendazim 50 WP as (STSD) significantly reduced disease incidence to 4.45%, enhanced the highest average number of flowers per plant, the highest average flower diameter, and the highest average leaf area when compared to the control, according to an integrated disease management capsule. The study showed that mitigating the *Fusarium* wilt of China aster can be accomplished with the application of bioagents and fungicides in addition to soil amendments. As antimicrobial resistance is becoming a bigger threat to crop sustainability, integrated disease control is crucial. It is an essential tool for long-term, successful, and sustainable management methods.

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DECLARATION

Conflict of Interest. The authors declare no conflict of interest.

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