Journal of Advances in Biology & Biotechnology

Volume 27, Issue 11, Page 192-199, 2024; Article no.JABB.126027
ISSN: 2394-1081

In Vitro Antimicrobial Bio-efficacy Assessment of Different Organic Formulations against Root and Stem Rot Disease of Cucumber Incited by *Fusarium oxysporum* f.sp. *radicis-cucumerinum*

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Authors' contributions

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

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i111604

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/126027

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Cite as: *Kumar, Suresh, N. L. Meena, Pokhar Rawal, Amit Trivedi, Virendra Singh, N. L. Panwar, Karan Singh, and Hemant Gurjar. 2024. "In Vitro Antimicrobial Bio-Efficacy Assessment of Different Organic Formulations Against Root and Stem Rot Disease of Cucumber Incited by Fusarium Oxysporum f.Sp. radicis-Cucumerinum". Journal of Advances in Biology & Biotechnology 27 (11):192-99. https://doi.org/10.9734/jabb/2024/v27i111604.*

Original Research Article

Received: 27/08/2024
Accepted: 29/10/2024
Published: 02/11/2024

ABSTRACT

In vitro evaluation of antimicrobial bio-efficacy of different four organic formulations *viz.,* Neem oil (@ 0.5, 1.0, 1.5% con.), Azadirachtin (@ 0.10, 0.15, 0.20% con.), Bijamrut (@ 5, 10, 15% con.) and Jivamrut (@ 5, 10, 15% con.) were evaluated at different three concentrations against *Fusarium oxysporum* f.sp*. radicis-cucumerinum* causing root and stem rot disease of cucumber. Among the four different organic formulations, Azadirachtin was found most effective by showing minimum mycelial growth of 47.48, 31.63 and 21.70 mm and 47.25, 64.86 and 75.89% per cent growth inhibition at 0.10, 0.15 and 0.20% concentrations respectively. The Jivamrut depicted highest mycelial growth 63.88, 55.83, 43.95 mm with 29.03, 37.97 and 51.17% lowest per cent growth inhibition of the pathogen at 5, 10 and 15% concentrations, respectively.

Keywords: Organic formulations; neem oil, azadirachtin; bijamrut; jivamrut; Fusarium oxysporum f. sp. radicis-cucumerinum.

1. INTRODUCTION

"The cucumber (*Cucumis sativus* L.) belongs to family 'Cucurbitaceae' and most important vegetable. Cucumber cultivation is vulnerable to attack of several diseases that interrupt normal physiological process of this crop. Root and stem rot caused by *Fusarium oxysporum* f.sp. *radicis-cucumerinum* is one of them which hamper successful cultivation and causes significant yield losses globally, including India. Use of resistant varieties is considered as key strategy to control *Fusarium* spp. but their main drawback is instability" (Vakalounakis 1996). "Fungicides play a vital role in disease management in various crop ecosystems. Fungicides also prevent infection but use of chemical protectants causes heavy burden to environment pollution. Effective management strategy organic formulations seems to be more appropriate to manage and suppress the cucumber root and stem rot, soil borne disease. Not much information is available on disease controlling properties of jeevamrut. However, literature on disease suppressing activity of other liquid organic formulations *viz.,* panchgavya and beejamrut and other organic products like cow dung, cow urine, cow milk is available. The first time reported that panchgavya was most effective against soil-borne pathogen *F. oxysporum* f.sp. *lycopersici*, a causal agent of tomato wilt. Panchgavya-3 (MPG-3) was superior to carbendazim in reducing the plant disease and in increasing the vigour of plant and yield" (Reddy and Padmodaya 1996). "An experiment on several commercial formulations of botanical extracts and essential oils are being investigated as possible alternatives to soil fumigation for control of *Fusarium* wilt diseases. Soil infested with *F. oxysporum* f.sp. *chrysanthemi* was treated with 1%, 5% and 10% aqueous emulsions of formulated extracts of clove (70% clove oil), neem (90% neem oil), pepper/mustard (chili pepper extract and essential oil of mustard), cassia (extract of cassia tree) and Banrot (a standard fungicide applied at different labeled rates) in separate experiments. Population densities of *F. oxysporum* f.sp. *chrysanthemi* were determined at 0 (before treatment), 1, 3, 7, 14 and 21 days after treatment. Treatment of the soil with 5 and 10 per cent aqueous emulsions resulted in significant ($P < 0.05$) differences among treatment means at each assay date. After 3 days, pepper/mustard, cassia and clove extracts added as 10% aqueous emulsions reduced the population density of *F. oxysporum* f.sp. *chrysanthemi* 99.9, 96.1 and 97.5%, respectively, compared with the untreated control. Neem oil extract increased the population density of *F. oxysporum* f.sp. *chrysanthemi* at all concentrations tested. Banrot did not reduce the population density of *F. oxysporum* f.sp. *chrysanthemi* in any experiment. In a second, related experiment, soil infested with *Fusarium oxysporum* f.sp. *melonis* also was treated with 1%, 5% and 10% aqueous emulsions of formulated extracts, incubated in closed plastic bags for 1 week and planted with muskmelon seeds (cv. Gold Star) in the greenhouse. Treatment of infested soil with 5

and 10% aqueous emulsions of the botanical extracts resulted in differences among treatments after 5 to 6 weeks. The pepper/mustard, cassia and clove extracts suppressed disease development in repeated experiments (80 to 100% healthy plant stand) compared with the untreated infested soil (< 20% stand). The observed reduction in the pathogen population and increased healthy plant stand in the greenhouse indicates that these extracts could have important roles in biologically based management strategies for control of *Fusarium* wilt diseases" (Bowers and Locke 2000). "The antifungal potential of panchgavya against *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani, Sclerotinia sclerotiorum* and *Phytophthora colocasiae* and advocated that the mycelial bits dipped for 6 hours in panchgavya caused complete suppression of mycelial growth of *R. solani* and the growth inhibition ranged between 88.1 to 92.3 per cent in other pathogens (Sugha 2005). The effect of crude extracts of neem (*Azadirachta indica*) leaf, neem seed and garlic (*Allium sativum*) at concentrations ranging from 5 to 30% of the material in 100 ml of potato dextrose agar on mycelial growth of *F. oxysporum* f.sp. *lycopersici* was assessed. All the extracts inhibited mycelial growth at various levels. Dry neem seed extract gave 100% inhibition of mycelial growth. Fresh neem leaf extract reduced mycelial growth with increasing concentration while in garlic there were no differences in growth inhibition among the various concentrations used. However garlic extracts decreased sporulation with increasing concentration and cultures grown on extract amended agar plates remained viable" (Ogechi et al. 2006). "Soaked pigeon pea seeds in 10 per cent cow urine, vermiwash, neem leaf extract, biogas slurry, cow dung slurry, homa farming ash and cow dung slurry (10 per cent) + homa farming ash (10 per cent) for 6 hr. All the treatments significantly enhanced seed germination, shoot length, root length and vigour index. Amendment of soil with cow dung compost caused more than 50 per cent reduction in *Fusarium, Sclerotinia* & *Phytophthora* and 26 per cent reduction in *Rhizoctonia solani"* (Sharma and Deshpande 2006*)*. Application of Jeevamrut to soil not only improves the soil considerably but has been reported as an effective organic disease management input. It also encourages microbial activity in the soil. Jeevamrut has to be applied once in 15 days @ 50-200 litres $acre^{-1}$ during vegetative stage, flowering stage and grain filling stage and can also be applied alone or along with irrigation water (Ramprasad et al. 2009).

2. MATERIALS AND METHODS

2.1 Evaluation of Different Organic Formulations (Poison Food Technique)

In vitro evaluation of four different organic formulations was evaluated at three different concentrations against *F. oxysporum* f.sp*. radicis-cucumerinum* by using poison food technique (Nene and Thapliyal 1993).

The technique includes cultivation of test organism on a medium that contains the test organic formulation. PDA was employed as the basal medium in all the studies. The required quantity of each organic formulation at three different concentrations was incorporated aseptically in 100 ml PDA in 250 ml flasks at the time of pouring the media in petri plates. The medium was vigorously shaken to ensure that the organic formulation was distributed evenly. After that 20 ml of medium was poured in each petri plate aseptically and allowed to solidify. Five mm diameter mycelial disc was cut from periphery of 10 days old fungus cultures and inoculated into petri plate. The mycelial disc was inverted in the center of the plates to establish direct contact with organic formulation medium and incubated for 7-8 days at 28±1 ^{0}C. In Factorial Complete Randomized Design (FCRD) four replications of each treatment were kept. At the same time a control was also maintained by growing on organic formulation free PDA. Observations on linear growth were taken, when the fungus reached maximum development in control petri plate.

The per cent inhibition of the mycelial growth of the fungus in each treatment was calculated by using formula (Vincent 1947).

$$I = \frac{C - T}{C} X100$$

Where, I = Per cent inhibition
C = Area of test fungus in control (mm)
T = Area of test fungus in respective treatment (mm)

2.2 Statistical Analysis

The data from various experiments were subjected to analysis for coefficient of deviation. For laboratory, completely randomized design was followed. Means of the experiments were used to compare for efficacy of treatments.

List 1. List of treatments used for the study

Treatments	Concentrations
T_1 - Neem oil	C_1-0.5, C_2-1.0, C_3-1.5%
T_2 - Azadirachtin	C_1-0.10, C_2-0.15, C_3-0.20%
T_3 - Bijamrut	C_1-5, C_2-10, C_3-15%
T_4 - Jivamrut	C_1-5, C_2-10, C_3-15%
T_5 - Control	

3. RESULTS

3.1 Evaluation of Different Organic Formulations

In vitro evaluation of different four organic formulations *viz.,* Neem oil (@ 0.5, 1.0, 1.5% con.), Azadirachtin (@ 0.10, 0.15, 0.20% con.), Bijamrut (@ 5, 10, 15% con.) and Jivamrut (@ 5, 10, 15% con.) were evaluated at different three concentrations against *F. oxysporum* f.sp. *radicis-cucumerinum* by using poison food technique. Among the four different organic formulations, Azadirachtin was found most effective by showing minimum mycelial growth of 47.48, 31.63 and 21.70 mm and 47.25, 64.86 and 75.89 per cent growth inhibition at 0.10, 0.15 and 0.20% concentrations, followed by Neem oil with 55.68, 45.75, 33.90 mm mycelial growth and 38.14, 49.17 and 62.33% growth inhibition 0.5, 1.0 and 1.5% concentrations. Further, Bijamrut showed 59.68, 48.93 and 39.53 mm mycelial growth with 33.69, 45.64, 56.08% growth inhibition at 5, 10 and 15% concentrations. Whereas, Jivamrut depicted highest mycelial growth 63.88, 55.83, 43.95 mm with 29.03, 37.97 and 51.17% lowest per cent growth inhibition of the pathogen at 5, 10 and 15% concentrations, respectively. Results are presented in Table 1 & Fig. 1 and Plate 1.

Table 1. Evaluation of different organic formulations against *Fusarium oxysporum* f.sp. *radicis-cucumerinum* in *In vitro* condition

Sr. No.	Treatments	Mycelial growth/Colony diameter at different concentrations (mm*)				Per cent growth inhibition at different concentrations			
		C_1	C_2	C_3	Mean	C_1	C_2	C_3	Mean
1.	T_1 - Neem oil	55.68	45.75	33.90	45.11	38.14 (38.14)	49.17 (44.52)	62.33 (52.14)	49.88 (44.93)
2.	T_2 - Azadirachtin	47.48	31.63	21.70	33.60	47.25 (43.42)	64.86 (53.65)	75.89 (60.60)	62.67 (52.56)
3.	T_3 - Bijamrut	59.68	48.93	39.53	49.38	33.69 (35.48)	45.64 (42.50)	56.08 (48.49)	45.14 (42.16)
4.	T_4 - Jivamrut	63.88	55.83	43.95	54.55	29.03 (32.59)	37.97 (38.04)	51.17 (45.67)	39.39 (38.77)
5.	T_5 - Control	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Concentration Mean		**63.34**	**54.43**	**45.82**	**54.53**	**29.62 (29.93)**	**39.53 (35.74)**	**49.09 (41.38)**	**39.41 (35.68)**
		S. Em±	**C. D. at 5%**	**CV (%)**		**S. Em±**	**C. D. at 5%**	**CV (%)**	
Treatments		**0.30**	**0.86**			**0.30 (0.18)**	**0.86 (0.51)**		
Concentrations		**0.23**	**0.67**			**0.23 (0.14)**	**0.67 (0.40)**		
T × C		**0.53**	**1.50**	**1.93**		**0.58 (0.35)**	**1.66 (1.00)**	**2.96 (1.96)**	

**Mean of four replications*

Figures are in parentheses are √arcsine per cent angular transformed values

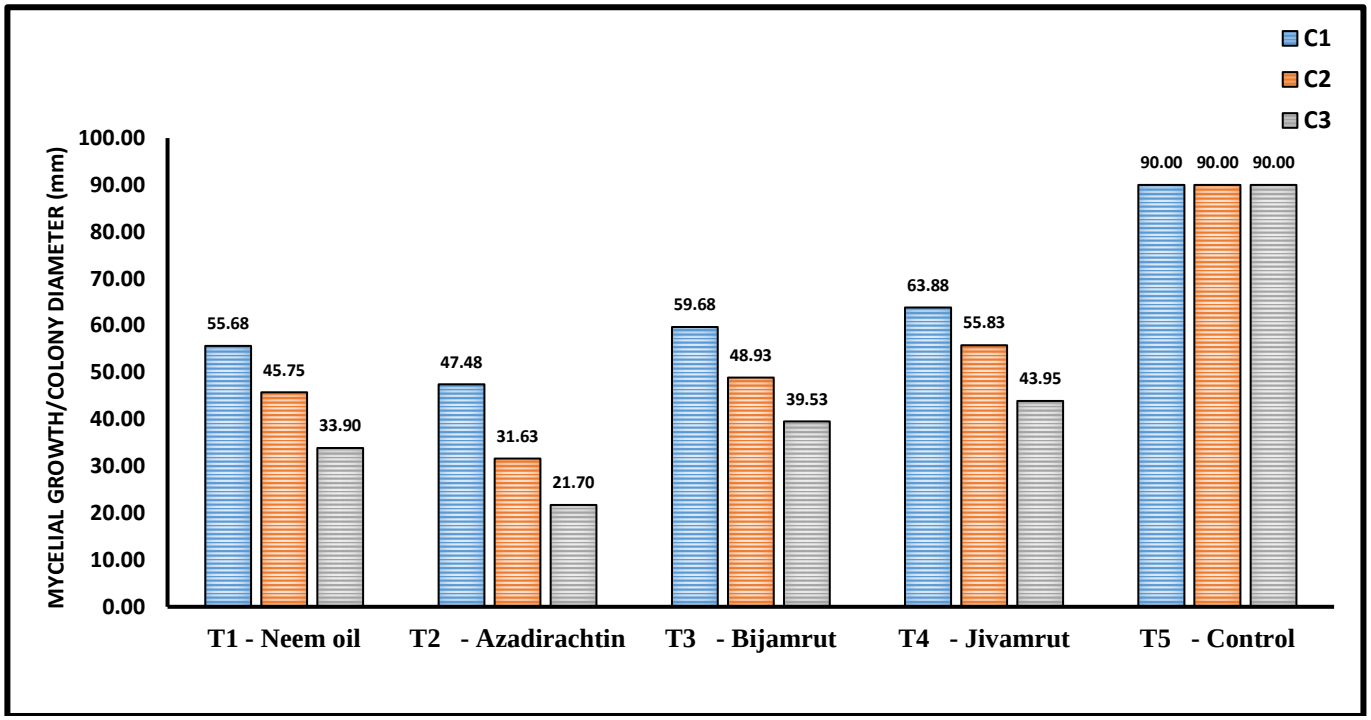

Fig. 1. Evaluation of different organic formulations against *Fusarium oxysporum* f.sp. *radicis-cucumerinum* in *In vitro* condition

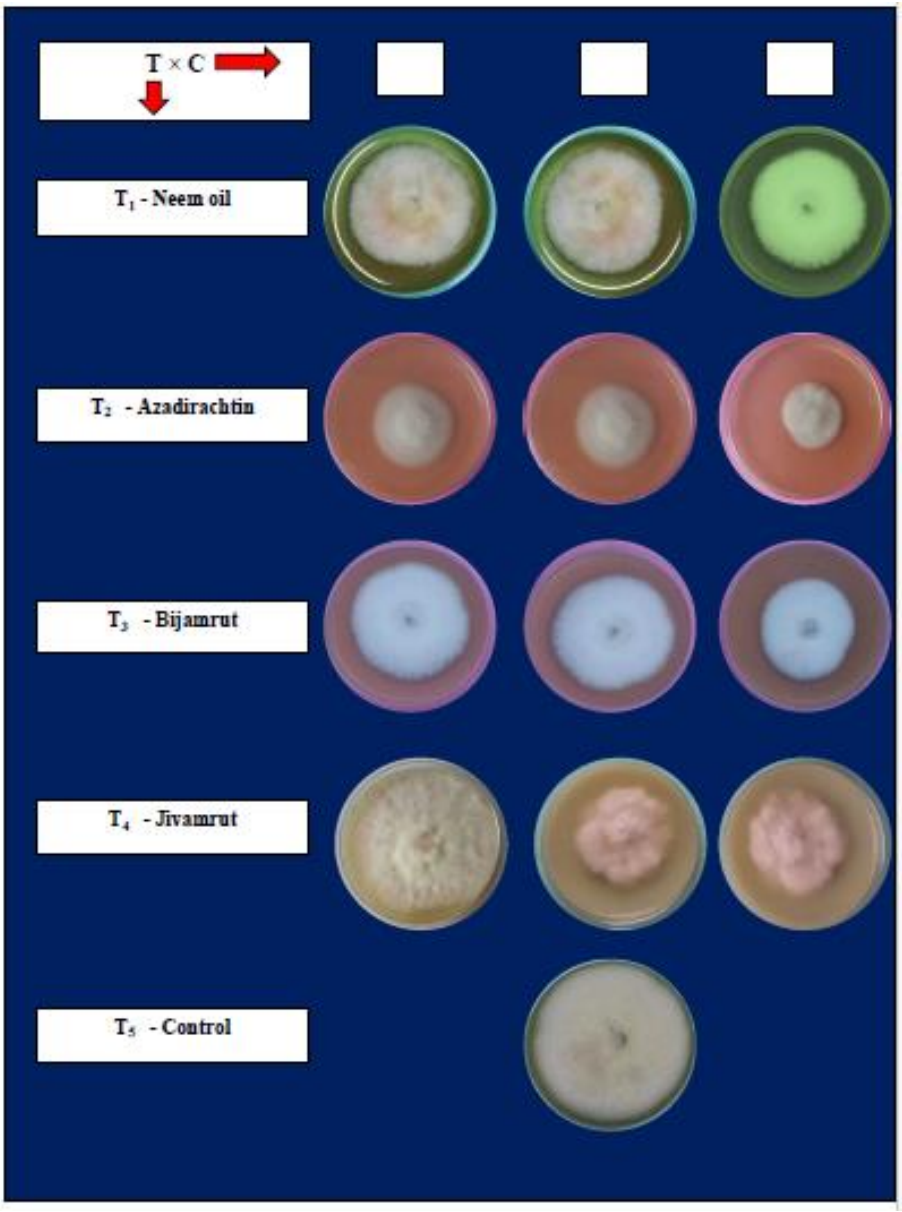

Plate 1. Evaluation of different organic formulations against *Fusarium oxysporum* f.sp. *radicis-cucumerinum* in *In vitro* condition

4. DISCUSSION

"Among the four different organic formulations, Azadirachtin was found most effective by showing minimum mycelial growth of 47.48, 31.63 & 21.70 mm and 47.25, 64.86 and 75.89% per cent growth inhibition at 0.10, 0.15 and 0.20% concentrations. While, Jivamrut depicted highest mycelial growth 63.88, 55.83, 43.95 mm with 29.03, 37.97 and 51.17% lowest per cent growth inhibition of the pathogen at 5, 10 and 15% concentrations. Similarly, as per previous research also reported highest inhibition of radial growth of *F. udum* with *Azadirecta indica* (67.8%)" (Singh et al. 2010). "Efficacy of botanicals against *F oxysporum* f.sp. *dianthi In vitro* conditions. The extracts of different botanicals were tested against *F. oxysporum* f.sp. *dianthi* by poisoned food technique *In vitro*. Least growth of pathogen was recorded in extracts of neem leaf extract showing excellent inhibitory effect of (78.19 %) reduction over control. Next best in order of merit was eucalyptus extract (75.87%) followed by ashoka extract (72.48%) and calotropis extract (65.22%) and least by others. Among the neem oil cake and Datura extract maximum growth inhibition of (62.09%) and (60.27%) over control,

respectively" (Raut et al. 2017). "The use of agro-chemicals has caused environmental problems and toxicity to humans, so natural alternatives for disease control during harvest and post harvest have been evaluated. The aim of this study was to evaluate cinnamon essential oil, neem oil and black sapote fruit extract for *In vitro* inhibition of fungi isolated from chayote fruit. The extracts were applied at 300, 350 and 400 ppm in petri dishes and the mycelial growth of *Fusarium oxysporum*, *Fusarium solani, Goetrichum* sp. and *Phytophthora capsici* was evaluated for 7 days and the percentage of mycelial growth inhibition per day was calculated. Cinnamon oil showed a fungicidal effect at all concentrations. Neem oil at 400 ppm showed a 42.3% reduction in the growth of *F. solani* and 27.8% reduction in the growth of *F. oxysporum*, while at 350 ppm it inhibited the mycelial growth of *Phytophthora capsici* by 53.3% and of *Goetrichum* sp. by 20.9% finally, the black sapote extract at 400 ppm inhibited 21.9 to 28.6% of the growth of all fungi. The growth of post harvest fungi on chayote fruit could be prevented or reduced by applying the plant extracts evaluated at adequate concentrations" (Garcia et al. 2023). "The antifungal activity of twelve botanicals including commercial formulations of neem and garlic at 1, 2, 5 and 10% concentrations was tested against *Fusarium oxysporum* (*i.e*., Isolate Fo8) under *In vitro* conditions. The botanicals revealed marked reduction in mycelial growth and sporulation of the *F. oxysporum* isolate. Growth inhibition of *F. oxysporum* increased linearly with an increase in concentration of the botanicals. Among the botanicals, neem oil formulation (Nemazal) and garlic oil exhibited significant effect on the test fungus. The neem oil (Nemazal) and garlic oil at 10 per cent concentration completely inhibited the mycelial growth" (Singh et al. 2017). "Organic enriched composts *viz.,* himcompost, vermicompost, farm yard manure, poultry manure & NADEP and organic formulations namely cow urine, beejamrit, panchgavya, tamarlassi, vermiwash and biosol were evaluated against Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* under *In vitro* conditions. Among all the organic formulations, panchgavya showed maximum mycelial inhibition of 92.22 per cent followed by Biosol *i.e.,* 72.22 per cent at 10 per cent concentration against the pathogen whereas, tamar lassi was found least effective with 15.56 per cent mycelial inhibition. The extracts of all five organic composts showed antifungal properties against the pathogen even at 5 per cent concentration. At 25 per cent concentration Himcompost yielded maximum mycelial inhibition of 71.11 per cent followed by Vermicompost *i.e.,* 65.27 per cent against the pathogen whereas, NADEP was found least effective *i.e.,* 44.17 per cent inhibition. Hence, Himcompost and Panchgavya can be used as soil amendment and seed dresser, respectively for ecofriendly management of Fusarium wilt of chickpea" (Rana et al. 2020).

5. CONCLUSION

Among the different organic formulations, Azadirachtin was found most effective by showing minimum mycelial growth of 47.48, 31.63 & 21.70 mm and 47.25, 64.86 and 75.89% per cent growth inhibition at 0.10, 0.15 and 0.20% concentrations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/126027