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Bio-Efficacy and Phytotoxicity of Novel Fungicide Isotianil 7% + Fosetyl Al 70% WG in Pomegranate Against Bacterial Blight (*Xanthomonas axonopodis* pv. *punicae*) Disease

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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

The experiment was conducted at Horticultural Instructional Farm, C. P. College of Agriculture, S. D. Agricultural University, Sardarkrushinagar during *Kharif*-2022 & *Kharif*-2023, respectively to evaluate the bio-efficacy and phyto-toxicity on the bacterial blight diseases as well as effect of Isotianil 7% + Fosetyl Al 70% WG on the yield of the pomegranate. The disease Intensity per cent of bacterial blight diseases was recorded, before spraying (Pre-treatment) and at 5 and 10 days

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after each spray (post-treatment). Total pomegranate fruit yield was recorded and converted in to the hectare basis and phytotoxicity observation for yellowing, stunting, necrosis, epinasty and hyponasty, *etc*. were recorded 1, 3, 5, 7, and 10 days after second spray. The results revealed that the treatment Isotianil 7% + Fosetyl Al 70% WG @ 200 gm per 100 lit. of water and Isotianil 7% + Fosetyl Al 70% WG @ 150 gm per 100 lit. of water showed significantly higher efficacy against bacterial blight disease and gives the higher yield of pomegranate over other treatments during the two consecutive seasons *Kharif* 2021 and *Kharif* 2022. The two years data on the Phytotoxicity studies revealed that none of the treatments exhibited any of the phytotoxicity symptoms.

Keywords: Pomegranate; Punica granatum L.; Xanthomonas axonopodis pv. Punicae; bacterial blight.

1. INTRODUCTION

The pomegranate (*Punica granatum* L.) is a fruit-bearing deciduous shrub in the family *Lythraceae*, subfamily *Punicoideae*, in India it has a long history and has become an important horticultural crop due to its adaptability to various climatic conditions especially in the arid and semi-arid regions, nutritional benefits, and economic value. The area under pomegranate cultivation in India is increasing day by day owing to its high demand, hardy nature, low maintenance cost, high yield, better storage quality and therapeutic values. Iran, India, China, Turkey and the USA are major producing countries which contributes around world's 76% of the total production. It is also grown in Afghanistan, Bangladesh, Myanmar, Vietnam, Thailand, Kazakhstan, Turkmenistan, Armenia, Georgia, Morocco, Tunisia, Egypt, Israel, Syria, Lebanon, Greece, Cyprus, Italy, France, Spain, Portugal, Mexico, Argentina and Chile. Maharashtra is the leading state in pomegranate production, known for its high-quality fruits, especially in the regions of Solapur, Sangli, Nashik, and Ahmednagar. In Karnataka, important areas include Bagalkot, Belgaum and Bijapur. In Gujarat prominent regions are Kutch, Sabarkantha, and Banaskantha. Andhra Pradesh and Telangana: Anantapur, Chittoor, and Kurnool are major cultivation areas. Tamil Nadu, Rajasthan, and Madhya Pradesh also contribute to pomegranate production.

Pomegranates are high in fiber and anthocyanins, which are strong antioxidants and responsible for their beautiful ruby red colour. Pomegranate juice is beneficial for people with type 2 diabetes, inflammatory conditions, and other health issues. It may also boost digestion, memory and help prevent cancer [1]. One pomegranate (282 g) provides 234 calories, 4.7 g of protein, 52.7 g of carbohydrates and 3.3 g of fat. Pomegranate seeds, or arils are a very good source of fibre and rich in potassium, phosphorus, magnesium, and calcium [2]. In 2021-22, India produced around 3.21 million metric tons (MMT) of pomegranates, which is about 3% of the world's total fruit production of 107.10 MMT. India ranks seventh in the world for pomegranate production, and the total area under cultivation is around 275,500 hectares. Maharashtra is the leading producer of pomegranates in India, accounting for about 54.85% of the total area and 66% of total production (1,763.99 thousand tonnes). In 2021-22, Gujarat produced 684.32 thousand tonnes with a 21.28% of total production of pomegranates in India [3].

Pomegranate cultivation challenged by water scarcity and erratic climatic conditions as well as insect pests and diseases outbreaks, among the diseases leaf and fruit spot/rots are the important ones that observed in the orchard during the continuous high moisture condition [4,5]. Cercospora spot generally caused by *Cercospora punicae* (Henn.), this disease causes small, dark brown spots on leaves that can become irregular and almost black. The spots may have a faint halo, and severe infections can cause leaves to turn yellow and fall prematurely. On fruit, the spots resemble bacterial spot disease but are darker, larger, and not cracked or sticky. Infected twigs may also develop raised black lesions and die. Alternaria black spot and fruit rot caused by the fungus *Alternaria alternata* [(Fr.) Keissl.], this disease causes small, reddish-brown or black circular spots on leaves that are surrounded by a green or yellow halo. The spots can enlarge and eventually cause fruit to rot. Anthracnose disease caused by the *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. where sunken brown spots on leaves, flowers, and fruits observed that can eventually lead to rotting. Infected leaves may also turn yellow and drop off [6].

In the field condition the describes this three diseases and bacterial blight disease

[*Xanthomonas axonopodis* pv. *punicae* (Xap)] initiates and develops more or less at similar time period, so that creates the leaf spots and fruit spot/rots disease complex that are threatening the pomegranate cultivation. So, looking to the problems of the farmers the experiment has been conducted to measure the efficacy of new novel fungicide Isotianil 7% + Fosetyl Al 70% WG against bacterial blight diseases of pomegranate. The results will be helpful in advising farmers for managing those diseases through foliar application of this fungicide as scheduled or need based application and can be incorporated in IDM programme in pomegranate cultivation.

2. METHODOLOGY

The experiment was conducted with Randomized Block Design (RBD) at Horticultural Instructional Farm, C. P. College of Agriculture, S. D. Agricultural University, Sardarkrushinagar during *Kharif* season of the year 2022 and 2023, respectively on pomegranate Bhagwa (kesar) variety growing at 4m × 4m distance to evaluate the bio-efficacy on the bacterial blight diseases and phyto-toxicity as well as effect of Isotianil 7% + Fosetyl Al 70% WG on the yield of the pomegranate. four replication per treatment and two trees per replication were considered for the observation. The total of two sprays for each treatment, first at the onset of disease (<1%) and subsequently 1 more spray at 10 days interval were applied in foliar application. The total volume of water was 1000L/ha. sprayed with knapsack sprayer fitted with hollow cone nozzle.

The disease severity (%) on leaves and fruits were recorded separately, before spraying (Pre-treatment) and at 5 and 10 days after each spray (Post treatment). Disease assessment for bacterial leaf & fruit spot were done using standard 0-6 rating scale developed by ICAR-NRCP, [7] considering 5 branches per tree, randomly. Finally, Percent Disease Intensity/ Index [8] and Percent reduction over control (ROC) were calculated. ROC value was calculated based on retransformed value. Total pomegranate fruit yield from all pickings per plot were recorded and converted in to t/ha. Phytotoxicity effects of Isotianil 7% + Fosetyl Al 70% WG on pomegranate were recorded at 1, 3, 5, 7, and 10 days after second spray, the observation for yellowing, stunting, necrosis, epinasty and hyponasty, *etc*. were measured based on 0-10 Phytotoxicity rating scale (0:No Phytotoxicity, 1:0-10, 2:11-20, 3:21-30, 4:31-40, 5:41-50, 6:51-60, 7:61-70, 8:71-80, 9:81-90, 10:91-100.).

Disease Intensity (%) = Sum of individual rating / No. of plant branches examined × Maximum disease scale ×100

Per cent Reduction Over Control (ROC) = Control Mean – Treatment Mean / Control Mean ×100

3. RESULTS AND DISCUSSION

3.1 Efficacy of Isotianil 7% + Fosetyl Al 70% WG Against Bacterial Blight Disease in Pomegranate Leaves during *kharif* 2021

The data presented in Table 3 revealed that there was significant difference observed among the treatments in terms of bacterial blight disease intensity on leaves over untreated control. Isotianil 7% + Fosetyl Al 70% WG @ 200 gm per 100 lit. of water recorded significantly lower mean disease intensity after first spray application (17.31%) and second spray application (18.12%), respectively which was found at par with Isotianil 7% + Fosetyl Al 70% WG @ 150 gm per 100 lit. of water with mean disease intensity of 19.28% and 19.20% at first and second spray application, respectively. Streptomycin Sulphate 90% + Tetracycline Hydrochloride 10% SP @ 100 ppm per 100 lit. of water treatment show mean disease intensity of 20.82% and 22.74% at first spray application and second spray application, respectively which was found at par with Isotianil 7% + Fosetyl Al 70% WG @ 100 gm per 100 lit. of water with mean disease intensity of 20.47% and 22.28% at first spray application and second spray application, respectively. Isotianil 200g/L SC @ 70 ml per 100 lit. of water having mean disease intensity of 23.66% and 25.52% at first spray application and second spray application, respectively which was found at par with the treatment Fosetyl Al 80% WP @ 175 gm per 100 lit. of water (23.26% and 25.17%). Isotianil 7% + Fosetyl Al 70% WG @ 200 gm per 100 lit. of water observed highest percent of reduction over control after first spray application (73.48%) and second spray application (84.49%), respectively which was followed by Isotianil 7% + Fosetyl Al 70% WG @ 150 gm per 100 lit. of water recorded with 71.06% and 82.62% reduction over control after first and second spray application, respectively.

Table 1. Disease rating scales (0-6)

Disease score	Lesion area on leaves (%)	Lesion area on Fruit (%)
0	0.00	0.00
1	Up to 1	Up to 1
2	>1-10	>1-10
3	>10-20	>10-20
4	>20-40	>20-40
5	>40-70	>40-70
6	-	>70-100

Table 2. Treatment details

Tr. No.	Treatments	Dosage (per 100 lit. of water)	
		a.i. (gm)	Formulation (gm or ml)
T_1	Untreated Control	--	--
T_2	Isotianil 7 % + Fosetyl Al 70 % WG	7 + 70	100 g
T_3	Isotianil 7 % + Fosetyl Al 70 % WG	10.5 + 105	150 g
T_4	Isotianil 7 % + Fosetyl Al 70 % WG	14 + 140	200 g
T_5	Isotianil 200g/L SC	14	70 ml
T_6	Fosetyl Al 80 % WP	140	175g
T_7	Streptomycin Sulphate 90 % + Tetracycline Hydrochloride 10 % SP	--	100 ppm
T_8	Isotianil 7% + Fosetyl Al 70 % WG	28+280	400g

Table 3. Efficacy of Isotianil 7% + Fosetyl Al 70% WG against bacterial blight in pomegranate leaves during Kharif 2021 & 2022

Tr. No	Treatments	Dosage /100 litre of water		Before spray	2021 PDI Mean	% ROC	2022 PDI Mean	% ROC
		g a.i	Formulation					
T_1	Untreated Control	--	--	17.51 (9.13)	55.46 (62.49)	00.00	53.11 (63.74)	00.00
T_2	Isotianil 7% + Fosetyl Al 70%WG	7+70	100g	17.09 (8.80)	22.28 (14.42)	76.92	22.55 (14.77)	48.97
T_3	Isotianil 7% + Fosetyl Al 70%WG	10.5+105	150g	17.54 (9.12)	19.20 (10.86)	82.62	19.61 (11.41)	82.09
T_4	Isotianil 7% + Fosetyl Al 70%WG	14+140	200g	17.18 (8.82)	18.12 (9.69)	84.49	18.33 (9.92)	84.43
T_5	Isotianil 200g/L SC	14	70 ml	17.41 (9.10)	25.52 (18.60)	70.23	25.57 (18.66)	70.72
T_6	Fosetyl Al 80% WP	140	175g	17.17 (8.74)	25.17 (18.12)	71.00	25.24 (18.21)	71.43
T_7	Streptomycin Sulphate 90% + Tetracycline Hydrochloride 10% SP	-	100 ppm	17.43 (9.08)	22.74 (15.00)	63.61	22.78 (15.06)	76.37
S. Em. ±				0.90	0.87	-	1.00	-
C. D. (P=0.05)				NS	2.61	-	3.02	-
CV%				6.54	7.44	-	8.47	-

Figures in parentheses are retransformed values; those outside are arc sin transformed value
NS: Non Significant, ROC: Reduction Over Control, PDI: Per cent Disease Incidence

3.2 Efficacy of Isotianil 7% + Fosetyl Al 70% WG Against Bacterial Blight Disease in Pomegranate Leaves during *Kharif* 2022

The data presented in Table 3 revealed that there was significant difference observed among the treatments in terms of bacterial blight disease intensity on leaves over untreated control. Isotianil 7% + Fosetyl Al 70 % WG @ 200 gm per 100 lit. of water recorded significantly lower mean disease intensity after first spray application (17.35%) and second spray application (18.33%), respectively which was found at par with Isotianil 7% + Fosetyl Al 70% WG @ 150 gm per 100 lit. of water with mean disease intensity of 18.60% and 19.61% at first and second spray application, respectively. Streptomycin Sulphate 90% + Tetracycline Hydrochloride 10% SP @ 100 ppm per 100 lit. of water treatment show mean disease intensity of 20.89% and 22.78% at first spray application and second spray application, respectively which was found at par with Isotianil 7% + Fosetyl Al 70% WG @ 100 gm per 100 lit. of water with mean disease intensity of 20.71% and 22.55% at first spray application and second spray application, respectively. Isotianil 200g/L SC @ 70 ml per 100 lit. of water having mean disease intensity of 23.70% and 25.57% at first spray application and second spray application, respectively which was found at par with the treatment Fosetyl Al 80% WP @ 175 gm per 100 lit. of water (23.31% and 25.24%). Isotianil 7% + Fosetyl Al 70% WG @ 200 gm per 100 lit. of water observed highest percent of reduction over control after first spray application (74.20%) and second spray application (84.43%), respectively which was followed by Isotianil 7% + Fosetyl Al 70% WG @ 150 gm per 100 lit. of water recorded with 70.22% and 82.09% reduction over control after first and second spray application, respectively.

3.3 Efficacy of Isotianil 7% + Fosetyl Al 70% WG Against Bacterial Blight Disease in Pomegranate Fruits during *kharif* 2022

The data presented in Table 4 revealed that there was significant difference observed among the treatments in terms of bacterial blight disease intensity on fruits over untreated control. Isotianil 7% + Fosetyl Al 70% WG @ 200 gm per 100 lit. of water recorded significantly lower mean disease intensity after first spray application (17.26%) and second spray application (18.18%), respectively which was found at par with Isotianil 7% + Fosetyl Al 70% WG @ 150 gm per 100 lit. of water with mean disease intensity of 18.45% and 19.29% at first and second spray application, respectively. Streptomycin Sulphate 90% + Tetracycline Hydrochloride 10% SP @ 100 ppm per 100 lit. of water treatment show mean disease intensity of 21.02% and 22.96% at first spray application and second spray application, respectively which was found at par with Isotianil 7% + Fosetyl Al 70% WG @ 100 gm per 100 lit. of water with mean disease intensity of 20.45% and 22.45% at first spray application and second spray application, respectively. Isotianil 200g/L SC @ 70 ml per 100 lit. of water having mean disease intensity of 23.85% and 25.69% at first spray application and second spray application, respectively which was found at par with the treatment Fosetyl Al 80% WP @ 175 gm per 100 lit. of water (23.43% and 25.35%). Isotianil 7% + Fosetyl Al 70% WG @ 200 gm per 100 lit. of water observed highest percent of reduction over control after first spray application (77.09%) and second spray application (84.62%), respectively which was followed by Isotianil 7% + Fosetyl Al 70% WG @ 150 gm per 100 lit. of water recorded with 73.96% and 82.60% reduction over control after first and second spray application, respectively.

3.4 Efficacy of Isotianil 7% + Fosetyl Al 70% WG Against Bacterial Blight Disease in Pomegranate Fruits during *kharif* 2022

The data presented in Table 4 revealed that there was significant difference observed among the treatments in terms of bacterial blight disease intensity on fruits over untreated control. Isotianil 7% + Fosetyl Al 70% WG @ 200 gm per 100 lit. of water recorded significantly lower mean disease intensity after first spray application (17.86%) and second spray application (18.39%), respectively which was found at par with Isotianil 7% + Fosetyl Al 70% WG @ 150 gm per 100 lit. of water with mean disease intensity of 18.74% and 20.31% at first and second spray application, respectively. Streptomycin Sulphate 90% + Tetracycline Hydrochloride 10% SP @ 100 ppm per 100 lit. of water treatment show mean disease intensity of 21.58% and 22.64% at first spray application and second spray application, respectively which was found at par with Isotianil 7% + Fosetyl Al 70% WG @ 100 gm per 100 lit. of water with mean disease intensity of 20.78% and 21.56% at first spray application and second spray application, respectively. Isotianil 200g/L SC @ 70 ml per

100 lit. of water having mean disease intensity of 23.79% and 25.45% at first spray application and second spray application, respectively which was found at par with the treatment Fosetyl Al 80% WP @ 175 gm per 100 lit. of water (22.96% and 24.17%). Isotianil 7% + Fosetyl Al 70% WG @ 200 gm per 100 lit. of water observed highest percent of reduction over control after first spray application (73.06%) and second spray application (84.29%), respectively which was followed by Isotianil 7% + Fosetyl Al 70% WG @ 150 gm per 100 lit. of water recorded with 70.54% and 82.00% reduction over control after first and second spray application, respectively.

3.5 Effect of Isotianil 7% + Fosetyl Al 70% WG on Phytotoxicity During 2020-21 & 2021-22

The two years data on the Phytotoxicity studies of Isotianil 7% + Fosetyl Al 70% WG evaluated at two doses *i.e*. @ 200 gm and 400 gm per 100 lit. of water, respectively along with untreated control, revealed that none of the treatments exhibited any of the Phytotoxicity symptoms on pomegranate during *kharif* 2021 and *kharif* 2022, respectively for yellowing, stunting, necrosis, epinasty, hyponasty *etc.*on the basis of 0-10 Phytotoxicity rating scale (PRS) scale.

3.6 Effect of Isotianil 7% + Fosetyl Al 70% WG on Yield of Pomegranate During 2020-21 & 2021-22

The data presented in Table 5 revealed that Isotianil 7% + Fosetyl Al 70% WG @ 200 gm per 100 lit. of water recorded higher yield of pomegranate 18.25 ton/ha and Isotianil 7 % + Fosetyl Al 70 % WG @ 150 gm per 100 lit. of water (17.96 t/ha) remained at par with Isotianil 7 % + Fosetyl Al 70 % WG @ 100 gm per 100 lit. of water (16.74 t/ha) during first year of experiment (*kharif* 2021). The data presented in Table 4. revealed that Isotianil 7% + Fosetyl Al 70% WG @ 200 gm per 100 lit. of water recorded highest yield of pomegranate 19.45 ton/ha which was followed by the treatment Isotianil 7 % + Fosetyl Al 70 % WG @ 150 gm per 100 lit. of water (18.29 t/ha) whereas the treatment Isotianil 7 % + Fosetyl Al 70 % WG @ 100 gm per 100 lit. of water (16.89 t/ha) remained at par with Streptomycin Sulphate 90 % + Tetracycline Hydrochloride 10 % SP @ 100 ppm per 100 lit. of water (16.78 t/ha) during second year of experiment (*kharif* 2022).

Table 4. Efficacy of Isotianil 7% + Fosetyl Al 70% WG against bacterial blight in pomegranate fruit during Kharif 2021 & 2022

Tr. No	Treatments	Dosage /100 litre of water		Before spray	2021 PDI Mean	% ROC	2022 PDI Mean	% ROC
		g a.i	Formulation					
T_1	Untreated Control	--	--	17.81 (9.44)	52.75 (63.42)	00.00	53.38 (64.24)	00.00
T_2	Isotianil 7% + Fosetyl Al 70%WG	7+70	100g	16.94 (8.55)	22.45 (14.63)	76.93	21.56 (13.54)	78.92
T_3	Isotianil 7% + Fosetyl Al 70%WG	10.5+105	150g	16.43 (8.07)	19.29 (11.03)	82.60	20.31 (11.56)	82.00
T_4	Isotianil 7% + Fosetyl Al 70%WG	14+140	200g	16.78 (8.35)	18.18 (9.75)	84.62	18.39 (10.09)	84.29
T_5	Isotianil 200g/L SC	14	70 ml	16.88 (8.56)	25.69 (18.83)	70.30	25.45 (18.53)	71.15
T_6	Fosetyl Al 80% WP	140	175g	17.29 (8.86)	25.35 (18.37)	71.03	24.17 (16.85)	73.77
T_7	Streptomycin Sulphate 90% + Tetracycline Hydrochloride 10% SP	-	100 ppm	17.38 (8.99)	22.96 (15.28)	75.90	22.64 (14.9)	76.80
S. Em. ±				0.67	0.97	-	1.15	-
C. D. (P=0.05)				NS	2.92	-	3.31	-
CV%				5.78	7.84	-	7.41	-

Figures in parentheses are retransformed values; those outside are arc sin transformed value
NS: Non Significant, ROC: Reduction Over Control, PDI: Per cent Disease Incidence

Table 5. Effect of Isotianil 7% + Fosetyl Al 70% WG on yield of pomegranate fruit during *kharif* - 2021 and *kharif 2022*

Tr. No	Treatments	Dosage/ 100 liter of water		Pomegranate fruit Yield (t/ ha) 2021-22	Pomegranate fruit Yield (t/ ha) 2022-23
		g a.i	Formulation		
T_1	Untreated Control	--	--	12.83	12.90
T_2	Isotianil 7% + Fosetyl Al 70%WG	7+70	100g	16.74	16.89
T_3	Isotianil 7% + Fosetyl Al 70%WG	10.5+105	150g	17.96	18.29
T_4	Isotianil 7% + Fosetyl Al 70% WG	14+140	200g	18.25	19.45
T_5	Isotianil 200g/L SC	14	70 ml	15.38	15.45
T_6	Fosetyl Al 80% WP	140	175g	15.76	15.86
T_7	Streptomycin Sulphate 90% + Tetracycline Hydrochloride 10% SP	-	100ppm	16.53	16.78
S. Em. ±				0.38	0.30
C. D. (P=0.05)				1.08	0.89

Isotianil was discovered by Bayer in 1997 Toquin et al., [9]. Isotianil is a systemic fungicide, belonging to the chemical class of thiadiazol carboxamide that activates the plant's defence mechanisms against a wide range of fungal and bacterial pathogens. As a plant activator, isotianil does not exhibit antimicrobial activity directly against the pathogenic microorganisms but instead it induces systemic acquired resistance in the host plant. Isotianil can significantly reduce the impact of Fusarium wilt of banana and protect different banana cultivars. The elicitor isotianil is able to induce the formation of tyloses in cortex vascular tissues of corms, preventing pathogen from root entering the corms. It can activate the three major systems of ISR, SAR, and starch granule synthesis in the corms, and inhibit the diffusion of pathogen in the corms, so as to reduce the effect of Fusarium wilt of banana [10]. Fosetyl (and its aluminium salt) is systemic fungicides with protectant action against a number of oomycete and ascomycete fungi and some plant pathogenic bacteria in a range of fruit, vegetables and ornamental crops. It is rapidly absorbed through both leaves and roots and exhibit both acropetal and basipetal translocation. It is act as inhibiting germination of spores and by blocking development of mycelium, competing with phosphate as allosteric regulator of several enzymes. In addition, it can induce direct or indirect production of phytoalexin and pathogenesis-related proteins leading to an induction of plant defence mechanisms against fungal or bacterial pathogens [11]. Bultreys et.al. [12] studied the antibacterial activity of fosetyl-Al, ethyl-phosphite and phosphite against Pseudomonas syringae on plant surfaces and in vitro. According their findings growth of *P. syringae* can be inhibited by the phosphite that it releases from ethyl-phosphite.

4. CONCLUSION

The treatments Isotianil 7% + Fosetyl Al 70% WG @ 200 gm per 100 lit. of water and Isotianil 7% + Fosetyl Al 70% WG @ 150 gm per 100 lit. of water showed significantly higher efficacy against bacterial blight disease as well as yield of pomegranate over other treatments during the two consecutive seasons *Kharif* 2021 and *Kharif* 2022. The two years data on the Phytotoxicity studies revealed that none of the treatments exhibited any of the phytotoxicity symptoms.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares 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 manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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