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Effect of Tank Mix Post-emergence Herbicides on Soil Dehydrogenase Activity and Its Phytotoxicity on Wheat

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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

Aims: The study was conducted to assess the efficacy of tank mix post emergence herbicides on soil dehydrogenase activity and its phytotoxic effect on wheat.
Study Design: The experiment consists of ten treatments and was laid out in randomised complete block design with three replications.
Place and Duration of Study: The present investigation was conducted at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, during *rabi* 2020-21.
Methodology: Weed control and phyto-toxicity ratings at 7, 14 and 21 days after herbicide application were recorded in each treatment on 0-10 scale on the basis of visual observations. Dehydrogenase activity in the soil samples was determined and expressed as µg TPF formed per g soil for 24 hrs.
Results: Post emergent application of metsulfuron-methyl + carfentrazone-ethyl (8.00) recorded good weed control being on par with post-emergence herbicide tank mixture of metsulfuron-methyl + sulfosulfuron (7.83) at 14 DAHA. At 7 DAHA, slight phytotoxic injury to crop was noticed IN T_2

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(0.5), T_3 (0.5), T_7 (0.5), T_4 (1.00) and T_1 (1.00). There was no phytotoxic injury to the crop with any of the post-emergence herbicides at 14 and at 21 DAHA. Soil dehydrogenase activity was found to be higher (41.02 µg TPF g^{-1} day^{-1}) before herbicide application, whereas the activity was reduced upon herbicide application. Among the herbicide treatments, higher dehydrogenase activity of soil was found with the application of metsulfuron-methyl + pinoxaden (34.07 µg TPF g^{-1} day^{-1}).

Conclusion: T_4 was found to be better in controlling weeds and recorded higher weed control rating. The herbicides and their mixtures used in the present study did not cause any injury, hence it has no phytotoxic effect on wheat crop. However, soil dehydrogenase activity was higher before the application of herbicides, whereas herbicide application resulted in reduced dehydrogenase activity.

Keywords: Dehydrogenase; phytotoxicity; post-emergence; wheat.

1. INTRODUCTION

"Wheat (*Triticum aestivum* L.) is considered as the second most important cereal crop after rice in India and is called as king of cereals. Weed infestation stands out as a significant factor affecting wheat yield, particularly in irrigated condition. The vigorous competition between weeds and crop plants for moisture, nutrients, space, and sunlight poses a serious threat to yield potential. The wheat crop is dominated by both grass and broad-leaved weeds which can reduce the grain yield up to 80 per cent" [1]. The primary grassy weeds comprise *Phalaris minor* and various *Avena* species, while broadleaf weeds such as *Chenopodium album*, *Fumaria parviflora*, *Melilotus indica*, *Lathyrus aphaca*, *Anagalles arvensis*, *Cirsium arvense*, and *Vicia sativa* also contribute significantly to weed populations. Chemical weed control is favored due to limited and expensive labor availability, as well as the reduced feasibility of mechanical or manual methods. Herbicide combinations offer several advantages, including broad-spectrum weed control, enhanced herbicide efficiency through synergistic or additive effects, reduced quantities required, lower costs of weed management, prevention of weed shifts, mitigation of herbicide resistance in weeds, and facilitation of overall weed management improvements.

"Soil enzymes are crucial for preserving the physical and chemical properties of soil, thereby safeguarding soil ecology and overall soil health" [2,3]. "The presence of dehydrogenase (DHA) enzyme activity in the soil is intricately connected with microbial functionality, serving as an indicator of alterations in soil properties" [4]. "Dehydrogenase activity is commonly utilized as a general criterion for assessing microbial activity and biomass in soil. Dehydrogenase activity increase with increase in microbial populations following amendments of soils with nutrients" [5]. Considering these factors, the aim of this study was to assess the efficacy of various post-herbicides and herbicide combinations in controlling weeds in winter wheat crops. Additionally, we aimed to achieve broad-spectrum weed control and evaluate their impact on soil dehydrogenase activity, as well as assess any phytotoxic effects of herbicide mixtures on the wheat crop.

2. MATERIALS AND METHODS

2.1 Field Experiment

A field experiment was taken during *rabi* (winter) 2020-21 at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka. The experiment consists of ten treatments and laid out in randomised complete block design (RCBD) with three replications on *vertisols* with initial pH of 7.21, EC of 0.21 dS/m and available major nutrients of 134, 8 and 145 kg ha^{-1} of N, P_2O_5, K_2O, respectively. "Treatments were tank mixture of T_1 : Metsulfuron-methyl 20% WP + 2,4 – D sodium Salt 80% WP @ 4+ 500 g ha^{-1}, T_2 : Metsulfuron-methyl 20% WP + Pinoxaden 5.1% EC @ 4 + 60 g ha^{-1}, T_3 : Metsulfuron-methyl 20% WP + Sulfosulfuron 75% WG @ 4 + 25 g ha^{-1}, T_4 : Metsulfuron-methyl 20% WP + Carfentrazone-ethyl 20% DF @ 4 + 20 g ha^{-1},while pre mix herbicide *i.e.*, T_5 : Clodinofop-propargyl 15% WP + Metsulfuron-methyl 1% WP @ 60 + 4 g $ha^{-1}$and individual application of T_6 : Metsulfuron-methyl 20% WP @ 4 g ha^{-1}, T_7 : 2,4 – D sodium salt 80% WP @ 2.0 kg $ha^{-1}$and recommended weed management practice (RWMP) of T_8 : Pendimethalin 30% EC 1 kg ha^{-1} as PE + one hand weeding, T_9 : Weed free check and T_{10}: Weedy check. UAS 304 wheat variety at the rate of 150 kg ha^{-1} after treating with *Azospirillium* (1250 g ha^{-1}) were drilled evenly in the furrows at row spacing of 22.5 cm and covered with soil manually. Recommended doses of nitrogen, phosphorus and potassium were given. Half of the recommended dose of N (50 kg ha^{-1}) and full dose of P_2O_5 (75 kg ha^{-1}) and K_2O (50 kg ha^{-1}) were applied as basal and the remaining nitrogen (50 kg ha^{-1}) was applied 30 DAS. Experimental site was uniformly irrigated prior to sowing of seeds and application of fertilizers. Then irrigations were given at an interval of 10-15 days till the crop reaches physiological maturity in order to maintain adequate soil moisture in root zone" [6].

2.2 Herbicide Application

The herbicides were administered 28 days after sowing using a knapsack sprayer, applying at a volume of 500 liters per hectare In T_8, pre-emergence herbicide was sprayed a day after sowing. The weed free plot was maintained by repeated manual weeding. The crop was harvested at physiological maturity the produce from net plots were collected and sun dried for two days.

2.3 Weed Parameter

Weed density was measured before treatment, at 20 days after herbicide application (DAHA), and at 40 DAHA using a 1 m² quadrat. Prior to statistical analysis, the weed density data underwent square root transformation. Weed control rating and Phyto-toxicity ratings (phytotoxic effects of post-emergence herbicides on crop) were recorded at 7, 14 and 21 DAHA (days after herbicide application) in each treatment on 0-10 scale [7] on the basis of visual observations (Tables 1 and 2 respectively).

2.4 Enzymatic Activity

Dehydrogenase activity in the soil samples was determined as per the procedure as described [8]. Moist soil samples (4 g) were placed in 16 × 150 mm² test tubes, to this add 1 ml of 3% aqueous solution of 2,3,5-triphenyl tetrazolium chloride, 40 mg $CaCO_3$ and 2.5 ml distilled water. The contents of each tube were mixed with glass rod and incubated for 4 hrs at 37ºC. Triphenyl formazan (TPF) was extracted by transferring the soil with the help of methanol from each tube to a funnel plugged with cotton as absorbent and the colour intensity determined in a spectrophotometer at a wave length of 485 nm. The dehydrogenase activity was expressed as µg TPF formed per g soil for 24hrs.

Table 1. Qualitative description of treatment effects on weeds

Effect	Rating	Weed
None	0	No control
Slight	1	Very poor control
	2	Poor control
	3	Poor to deficient Control
Moderate	4	Deficient control
	5	Deficient to moderate Control
	6	Moderate control
Severe	7	Control
	8	Good control
	9	Good to excellent
Complete	10	Complete control

Table 2. Qualitative description of treatment effects on crop (Phytotoxicity rating) in the visual scoring scale of 0 to 10

Effect	Rating	Crop
None	0	No injury, normal
Slight	1	Slight stunting, injury or discoloration
	2	Some stand loss, stunting or discoloration
	3	Injury more pronounced but not persistent
Moderate	4	Moderate injury, recovery possible
	5	Injury more persistent, recovery doubtful
	6	Near severe injury, no recovery possible
Severe	7	Severe injury stand loss
	8	Almost destroyed, few plants surviving
	9	Very few plants alive
Complete	10	Complete destruction

2.5 Statistical Analysis

Analysis of variance was conducted in order to examine the impact of herbicides on soil dehydrogenase and phytotoxicity rating. Data obtained were statistically analysed using the F-test procedure as given by [9]. The level of significance used in "F" test was P=0.05. Standard error (SE) and critical difference (CD) were worked out for comparing treatment means of the studied variables of crop and weeds.

3. RESULTS AND DISCUSSION

3.1 Effect of Herbicides on Weed Control Rating

The weed group comprised both broad-leaved and narrow-leaved weeds. The post-emergence herbicides were applied at 28 days after sowing (DAS), and the herbicide's impact on crop stand and growth was assessed at 7, 14, and 21 days after herbicide application in each respective treatment using a visual score scale ranging from 0 to 10 (Table 2).

At 7 DAHA, T_8 recorded good weed control (9.17). Among the post-emergence herbicide treatments, T_4 (6.00) followed by T_3 (4.83) were found to be better in controlling weeds. These two treatments found to be superior over post emergence tank mixture application of Metsulfuron-methyl + 2,4-D sodium salt (4.00), Metsulfuron-methyl + Pinoxaden (2.83) and pre mix of Clodinofop-propargyl + Metsulfuron-methyl (2.17). All the herbicide mixtures recorded good weed control rating compared to individual application of Metsulfuron-methyl (2.17). However, weed free check recorded statistically superior weed control rating (10.00) and in contrast weedy check recorded lower rating (0.00).

At 14 DAHA, weed free check recorded statistically superior weed control rating (10.00). T_8 recorded higher weed control rating (8.83). Among the post-emergence herbicide tank mixtures, Metsulfuron-methyl + Carfentrazone-ethyl (8.00) recorded good weed control being on par with post-emergence herbicide tank mixture of Metsulfuron-methyl + Sulfosulfuron (7.83) and pre mix Clodinofop-propargyl + Metsulfuron-methyl (7.33). These treatments found to be superior over post emergence tank mixture of Metsulfuron-methyl + 2,4-D sodium salt (7.00), Metsulfuron-methyl + Pinoxaden (6.83). All the herbicide mixtures recorded higher weed control rating compared to individual application of Metsulfuron-methyl (6.00). However, weedy check treatment (0.00) recorded least weed control compared to all other treatments.

Table 3. Weed control rating (0-10) as influenced by weed management practices in wheat

Treatments	Weed Control Rating (0-10)		
	7 DAHA	**14 DAHA**	**21 DAHA**
T_1 : Metsulfuron-methyl 20% WP + 2,4 – D Sodium salt 80% WP (Tank Mix) @ 4 + 500 g ha^{-1} PoE at 25-30 DAS	4.00	7.00	6.83
T_2 : Metsulfuron-methyl 20% WP + Pinoxaden 5.1% EC (Tank Mix) @ 4 + 60 g ha^{-1} PoE at 25-30 DAS	2.83	6.83	6.50
T_3 : Metsulfuron-methyl 20% WP + Sulfosulfuron 75% WG (Tank Mix) @ 4 + 25 g ha^{-1} PoE at 25-30 DAS	4.83	7.83	7.50
T_4 : Metsulfuron-methyl 20% WP + Carfentrazone-ethyl 20% DF (Tank Mix) @ 4 + 20 g ha^{-1} at 25-30 DAS	6.00	8.00	7.83
T_5 : Clodinofop-propargyl 15% WP + Metsulfuron-methyl 1% WP (Pre mix) @ 60 + 4 g ha^{-1} PoE at 25-30 DAS	2.17	7.33	6.83
T_6 : Metsulfuron-methyl 20% WP @ 4 g ha^{-1} PoE at 25-30 DAS	2.17	6.00	5.67
T_7 : 2,4 – D Sodium salt 80% WP @ 2.0 kg ha^{-1} PoE at 25-30 DAS	2.00	6.83	5.83
T_8 : Pendimethalin 30% EC 1 kg ha^{-1} as Pre emergence + one hand weeding (RWMP)	9.17	8.83	8.00
T_9 : Weed free check	10.00	10.00	10.00
T_{10} : Weedy check	0.00	0.00	0.00

PE : Pre-emergence herbicide
PoE : Post emergence herbicide
DAS: Days after sowing
DAHA: Days after herbicide application

At 21 DAHS, T_8 recorded higher weed control rating (8.00) and was found to be on par with post-emergence tank mixture of Metsulfuron-methyl + Carfentrazone-ethyl (7.83) and Metsulfuron + Sulfosulfuron (7.50). These two treatments were found to be superior over tank mixture of Metsulfuron-methyl + 2,4-D sodium salt (6.83), Metsulfuron-methyl + Pinoxaden (6.50) and pre mix of Clodinofop-propargyl + Metsulfuron-methyl (6.83). All the herbicide mixtures recorded higher weed control rating compared to application of Metsulfuron-methyl (5.67) alone. However, weed free check recorded statistically superior weed control rating (10.00) and in contrast weedy check recorded lower ratings of weed control (0.00).

The better weed control was found in T_4, this is because Carfentrazone-ethyl, a member of the aryl triazolinone family, has demonstrated effectiveness in managing broadleaf weeds (BLWs) and sedge weeds in cereal crops. It achieves this by obstructing the activity of protoporphyrinogen oxidase within the chlorophyll biosynthesis pathway. Metsulfuron-methyl being systemic herbicide, effective in killing broad leaf weeds and some annual grasses. Consequently, combining these herbicides in a tank mixture leads to comprehensive weed control across a broad spectrum. Similar findings were also observed by [10]. he application of Clodinafop + metsulfuron-methyl herbicide combination has demonstrated high efficacy against diverse weed populations, effectively controlling both grassy and broad-leaved weeds at a rate of 95 percent [11]. Similar results were obtained as reported in [12], showing reduced weed populations in herbicide-treated plots compared to control plots. Furthermore, the tank mix application of Clodinafop propargyl + Carfentrazone-ethyl demonstrated the highest total weed control efficiency (84.9%), attributed to Carfentrazone-ethyl's superior efficacy in controlling broadleaved weeds compared to Clodinafop propargyl [13].

3.2 Effect of Post-Emergent Herbicides on Phytotoxicity Rating on Wheat

Crop toxicity rating recorded at 7, 14 and 21 DAHA (Table 4) based on visual observation of symptoms on wheat crop and it is rated from 0-10 (Table 2). At 7 DAHA, slight phytotoxic injury to crop was noticed in T_2 (0.5), T_3 (0.5), T_7 (0.5), T_4 (1.00) and T_1 (1.00). There was no phytotoxic injury to the crop with any of the post-emergence herbicides at 14 and at 21 DAHA. The treatments *viz*., T_2, T_3, T_7, T_4 and T_1 did not cause any injury to the crop from 14 to 21 DAHA.

Table 4. Phytotoxicity rating (0-10) as influenced by weed management practices in wheat

Treatments	Phytotoxicity Rating (0-10)		
	7 DAHA	**14 DAHA**	**21 DAHA**
T_1 : Metsulfuron-methyl 20% WP + 2,4 – D Sodium salt 80% WP (Tank Mix) @ 4 + 500 g ha^{-1} PoE at 25-30 DAS	1	0	0
T_2 : Metsulfuron-methyl 20% WP + Pinoxaden 5.1% EC (Tank Mix) @ 4 + 60 g ha^{-1} PoE at 25-30 DAS	0.5	0	0
T_3 : Metsulfuron-methyl 20% WP + Sulfosulfuron 75% WG (Tank Mix) @ 4 + 25 g ha^{-1} PoE at 25-30 DAS	0.5	0	0
T_4 : Metsulfuron-methyl 20% WP + Carfentrazone-ethyl 20% DF (Tank Mix) @ 4 + 20 g ha^{-1} at 25-30 DAS	1	0	0
T_5 : Clodinofop-propargyl 15% WP + Metsulfuron-methyl 1% WP (Pre mix) @ 60 + 4 g ha^{-1} PoE at 25-30 DAS	0	0	0
T_6 : Metsulfuron-methyl 20% WP @ 4 g ha^{-1} PoE at 25-30 DAS	0	0	0
T_7 : 2,4 – D Sodium salt 80% WP @ 2.0 kg ha^{-1} PoE at 25-30 DAS	0.5	0	0
T_8 : Pendimethalin 30% EC 1 kg ha^{-1} (PE) + one hand weeding (RWMP)	0	0	0
T_9 : Weed free check	-	-	-
T_{10} : Weedy check	-	-	-

PE : Pre-emergence herbicide
PoE : Post emergence herbicide
DAS: Days after sowing
DAHA: Days after herbicide application

Table 5. Dehydrogenase activity of soil as influenced by weed management practices in wheat

Treatments	Soil dehydrogenase activity (µg TPF g^{-1} day^{-1}) at 45 DAS
T_1 : Metsulfuron-methyl 20% WP + 2,4 – D Sodium salt 80% WP (Tank Mix) @ 4 + 500 g ha^{-1} PoE at 25-30 DAS	21.33
T_2 : Metsulfuron-methyl 20% WP + Pinoxaden 5.1% EC (Tank Mix) @ 4 + 60 g ha^{-1} PoE at 25-30 DAS	34.07
T_3 : Metsulfuron-methyl 20% WP + Sulfosulfuron 75% WG (Tank Mix) @ 4 + 25 g ha^{-1} PoE at 25-30 DAS	23.77
T_4 : Metsulfuron-methyl 20% WP + Carfentrazone-ethyl 20% DF (Tank Mix) @ 4 + 20 g ha^{-1} at 25-30 DAS	32.40
T_5 : Clodinofop-propargyl 15% WP + Metsulfuron-methyl 1% WP (Pre mix) @ 60 + 4 g ha^{-1} PoE at 25-30 DAS	21.37
T_6 : Metsulfuron-methyl 20% WP @ 4 g ha^{-1} PoE at 25-30 DAS	24.97
T_7 : 2,4 – D Sodium salt 80% WP @ 2.0 kg ha^{-1} PoE at 25-30 DAS	24.00
T_8 : Pendimethalin 30% EC 1 kg ha^{-1} as Pre emergence + one hand weeding (RWMP)	18.60
T_9 : Weed free check	48.28
T_{10} : Weedy check	50.33
S. Em. ±	0.77
C. D. at 5%	2.28

PE : Pre-emergence herbicide
PoE : Post- emergence herbicide
DAS: Days after sowing

In the current study, neither the individual herbicides nor the herbicide mixtures employed caused any discernible injury to the wheat crop at both the 14 and 21 days after herbicide application (DAHA). But at 7 DAHA, T_1 and T_4 showed moderate yellowing and recovered after few days of herbicide application with no reduction in crop yields, this suggests that all the herbicides tested are safe for use, as evidenced by the absence of any detrimental effects on wheat crop growth, as indicated in Table 4. Similar observations on phytotoxicity on Carfentrazone-ethyl and Metsulfuron-methyl were observed in wheat [10,14]. Pinoxaden at the recommended rate caused 1.5% average wheat phytotoxicity [15]. There was no phytotoxicity recorded in T_8 (Table 4). Similar effect of herbicide was reported in Soybean [16]. The autumn application of post-emergent 2,4-D amine and Dichlorprop plus 2,4-D ester resulted in notable visual injury, with significant effects observed at 24, 26, 28, and 31 weeks following herbicide application, compared to the non-treated control. Moreover, crop injury escalated with higher doses of 2,4-D amine or Dichlorprop plus 2,4-D ester [17]. By the 28th day after herbicide application (DHA), wheat plants treated with 2,4-D, diclofop, iodosulfuron, clodinafop and pyroxsulam displayed no symptoms [18]. Additionally, the post-emergence herbicide combination of mesosulfuron-methyl + iodosulfuron-methyl exhibited minor phytotoxic effects (score 2) on wheat crops, characterized by temporary stunting and leaf discoloration observed in the initial days, followed by full recovery thereafter, with no impact on the final wheat yield [19].

3.3 Effect of Post-Emergent Herbicides on Soil Dehydrogenase Activity

The dehydrogenase activity of soil markedly differed with different weed management treatments (Table 5). Soil enzymes are crucial for biochemical processes and are integral to organic matter decomposition, mineralization, and nutrient transportation within soil systems. Enzymes also serve vital functions by catalyzing essential reactions for the life processes of microorganisms in soils. Moreover, they contribute to stabilizing soil structure, facilitating nutrient cycling, and fostering the formation of organic matter. These enzymes undergo continuous synthesis, accumulation, and deposition within the soil. The dehydrogenase activity is most commonly used as an indicator of soil biological activity. This enzyme oxidizes soil organic matter by mediating the transferring electrons and protons from substrates to acceptors. These processes are the major part of respiration pathways of soil micro-organisms. It is an indicative of the potential status of the soil to support biochemical processes.

Prior to the application of post-emergence herbicides, the soil dehydrogenase activity was measured at a higher level, registering at 41.02 µg TPF g^{-1} day^{-1}. Application of herbicides reduced the dehydrogenase activity, further post-emergence herbicides have less impact on soil dehydrogenase activity than pre-emergence herbicides (Table 5). The application of herbicides to the soil resulted in a notable decrease in dehydrogenase activity compared to untreated control soil samples [20]. Among the treatments, weedy check recorded the highest dehydrogenase activity at 45 DAS (50.33 µg TPF g^{-1} day^{-1}) found on par with weed free treatment (48.28 µg TPF g^{-1} day^{-1}). This is mainly because there was no herbicidal effect on soil micro-organisms in these treatments. Among the herbicidal treatments, higher dehydrogenase was found with Metsulfuron-methyl + Pinoxaden (34.07 µg TPF g^{-1} day^{-1}) followed by Metsulfuron-methyl + Carfentrazone-ethyl (32.40 µg TPF g^{-1} day^{-1}) indicating that these two herbicides have less impact on dehydrogenase activity. The increase in dehydrogenase activity was mainly due decrease in the effect of herbicides over lapse of time period. But, T_8 (18.60 µg TPF g^{-1} day^{-1}) recorded lower dehydrogenase activity compared to other treatments. This could be attributed to the application method of pre-emergence herbicides, which involves direct spraying onto the soil surface. This practice can impact soil microorganisms, thereby influencing soil dehydrogenase activity. Whereas, post-emergence herbicides are usually sprayed on the foliage which results in the less contact of herbicides with soil surface and these post-emergence herbicides are usually sprayed at 25-30 DAS, where there will be sufficient buildup of soil micro-organisms leading to higher dehydrogenase activity. Also, the herbicides used at recommended dosage were non-inhibitory on dehydrogenase activity [21]. On the contrary the adverse effect of metsulfuron-methyl herbicide on soil dehydrogenase activity was observed initially at 4 g ha^{-1}, with the increase in incubation period there was improved soil dehydrogenase activity, slow recovery at the higher recommended dose of the herbicide was seen [22].

"The increase in soil dehydrogenase activity in herbicides treated soil from 7th to 28th day of incubation was observed which might be due to increase in the microbial community composition which have the capability of utilizing the herbicides as carbon source" [20,23].

4. CONCLUSION

Among the various chemical treatments, T_4 applied post emergence in a tank mix at rates of 4 + 20 g ha^{-1}, was observed to be particularly effective in weed control, resulting in superior weed control ratings. T_1 and T_4 showed little yellowing and recovered after few days of herbicide application, this suggests that the herbicide mixtures are safe and do not exhibit any phytotoxic effects on the growth of wheat crops. Greater dehydrogenase activity was observed in the weed-free check, followed by the weedy check, while the application of herbicides led to a decrease in soil dehydrogenase activity. This experiment revealed the adverse impact of herbicides on soil microbiomes. Moreover, the tank mixture of new generation herbicides showed no signs of phytotoxicity on wheat.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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