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Effect of Foliar Sprays of Phenylalanine, Nano-potash and Potassium Sulphate on Fruit Quality Attributes of Apple (*Malus x domestica* Borkh) cv. Ambri

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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 present investigation was carried out at Experimental field of Ambri Apple Research Centre (AARC), Shopian, SKUAST-Kashmir, during the year 2022-2023. Eight treatments, viz; control (water spray), potassium sulphate (5 g per litre of water), phenylalanine (@ 0.1, 0.2 and 0.3%), nano-potash (@ 1, 2 and 3ml/l of water) were selected for colour improvement. The treatments

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were applied 30 days prior to harvest. The experiment was laid in complete randomised block design with 3 replications having 3 trees per replication. The results showed that all the treatments outperformed control in terms of chemical characteristics. The treatment T_4 (Phenylalanine @ 0.3%) significantly excelled in all the treatments applied. Significantly higher anthocyanin content (14.41 mg/100g), antioxidants (75.71 %), total sugars (12.98 %), TSS (16.23 %), fruit chlorophyll (0.59 mg/g fw) and fruit ascorbic acid (23.93 %) was recorded in T_4 (Phenylalanine @ 0.3%) as compared to other treatments. According to the current study, apple cultivar Ambri responded favourably to the application of foliar phenylalanine in improving chemical traits, which otherwise lacks in this cultivar. The application of Phe @ 0.3% , 30 days prior to harvest proved superior in enhancing the quality attributes, notably the anthocyanin content of apple cv. Ambri.

Keywords: Phenylalanine; potassium sulphate; Nano-potash; fruit quality.

1. INTRODUCTION

Apple (*Malus* × *domestica* Borkh.) is a typical temperate fruit belonging to family Rosaceae and sub family Pomoideae. It is the result of an interspecific hybridization [1].Apple is one of the major horticultural products, with China and the US having the largest production rates [2]. The cultivation of temperate fruits is Kashmir's speciality. Among these, apples account for the majority of production and area as well as a significant component of the national economy. Apple is the backbone of the rural economy in the Kashmir division. In India, Jammu and Kashmir is the largest producer of apples with an area of about 1.65 lakh hectares and production of 20 lakh MT [3] contributing more than 70 percent of the total apple production in India.

Ambri apple is the only apple that originates in India and is considered as autochthonous to Kashmir. Ambri apple is appealing with a high keeping quality and is chosen for its sweet taste, crispness and fragrance [4]. But poor fruit quality is a significant problem for the marketing of Ambri apples. The skin color of fruit is often a good indicator of its quality and maturity stage. Red fruit is preferred by consumers. Fruit can therefore be priced more and sold more easily because of its red colour, which plays a significant role in its acceptance. Cyanidin-3-galactoside (Idaein) is the main pigment responsible for the red color in apple, which belongs to the anthocyanin group . Natural substances with health-promoting characteristics that are also antifungal and antioxidants are called flavonoids and anthocyanins [5]. They are produced *via* the phenylpropanoid pathway. It is well known that the red pigment formation process depends mainly on environmental factors such as temperature, light quality and light interception. Cultural practices such as pruning, thinning, fertilization (potassium) and plant growth regulators like Phenylalanine influence anthocyanin formation and other bio-chemical characteristics of fruit. Based on the above propositions, our study entitled "Effect of foliar sprays of phenylalanine, nano-potash and potassium sulphate on fruit quality attributes of apple (*Malus x domestica* Borkh) cv. Ambri'' was undertaken at, Ambri Apple Research Centre (AARC) Phanoo, Shopian during the year 2022 with objectives of improving fruit quality particularly fruit colour of cv. Ambri using various chemicals and thereby increasing its consumer acceptability. Also the effect of these chemical sprays was assessed on various bio-chemical characters of Ambri apple.

2. MATERIALS AND METHODS

The current study was undertaken at Experimental Fields of Ambri Apple Reseach centre (AARC) Phanoo, SKUAST-Kashmir, Shopian" which is situated at an altitude of 1594 m amsl between 33.747^{o}N, 74.855^{o}E during the years 2021-2022. The trees selected for the present study were of uniform age (15 ± 2) and vigour, grafted on MM106 rootstock and planted at a distance of 2.5 × 3 m. Eight different chemical treatments were selected which included:T_0:control (water spray), T_1: Potassium sulphate @ 5 g/L, T_2: Phenylalanine @ 0.1, T_3: Phenylalanine @ 0.2%, T_4: Phenylalanine @ 0.3%), T_5: Nano-potash (@ 1 ml/L, T_6: Nano-potash (@ 2 ml/L and T_7: Nano-potash (@ 3 ml/L were selected for colour improvement. The treatments were applied 30 days prior to harvest. The experimental design was Randomized Complete Block Design (RCBD) with 3 replications per treatment i,e 8 x 3 = 24 samples. The fruit of each treatment were harvested at optimum maturity and were analyzed for different parameters.The total soluble solids contents (TSS) was decided with the assistance of hand refractometre and expressed in terms of degree brix (^{0}B) [6]. Quantitative determination of ascorbic acid was done by 2,6-dichlorophenol indophenol visual titration method [6].The titrable acidity values were estimated [7].Total sugar were estimated by using the Lane and Eynon method as discussed by [6]. Fruit chlorophyll is estimated by DMSO method by determining the optical density (OD) of known volume of chlorophyll solution at two respective wavelengths (663 and 645 nm) by using spectrophotometer [8]. Anthocyanin was determined according to the method of [9] to determine the optical density at 535 nm, using spectrophotometer. Antioxidant content is measured by 2,20-diphenyl-1-picrylhydrazyl (DPPH) assay method and absorbance was measured at 517nm [10]. The experimental data was then subjected to statistical analysis with one way analysis of varience method as per the standard statistical procedure given by Gomez and Gomez (1984).

3. RESULTS AND DISCUSSION

The data pertaining to (Table 1) shows that the application of potassium sulphate, phenylalanine and Nano-Potash spray at different concentrations had a profound influence on total soluble solids, total sugars and fruit acidity. Maximum TSS (16.23%), total sugars (12.98%) and fruit acidity (0.17%) was found in treatment T_4 (Phe @ 0.3%), followed by treatment T_3 (Phe @ 0.2%) in which total soluble solids, total sugars and fruit acidity of 15.67%, 12.53% and 0.19% was observed respectively. Minimum total soluble solids, total sugars and fruit acidity of 14.10%, 11.28% and 0.31% respectively was found in treatment T_0 (control). The data in Table 1 indicated that, phenylalanine were more effective in increasing fruit total soluble solids content. A viable reason for the increase in TSS may be ascribed to the quick metabolic transformation of starch into soluble compounds and rapid translocation of sugars from the leaves to the developing fruits [11,12]. A higher increase in TSS content with foliar application of potassium is associated to vibrant role played by potassium in the process of translocation of sugar from leaves to fruits [13], which thereby results in fruits of adequate quality in context of total soluble solid. The present data indicated in Table 1 shows, phenylalanine caused a higher effect in increasing total sugars content than other treatments. A possible reason for rise in total sugar content may be due to an increase in mono and disaccharide from the hydrolysis of starch, which owned a simplest form of sugar and this could be one of the main causes for the increase in total sugar content of fruits [12]. The present results are in conformity with the findings of [14] The total sugars of “Red Roomy” grape were increased with increasing the concentration of phenylalanine application [15]. Moreover, Potassium is known to enhance photophosphorylation and dark reaction of photosynthesis resulting in increased accumulation of carbohydrates. Similarly according to the efflux of sucrose to the apoplast is facilitated by potassium availability which thereby increases sugar translocation from source to sink tissues promoting their growth [16].The titratable acidity decreased with the increase in concentration of phenylalanine and potassium but results were more profound with phenylalanine (Table 1). This may primarily be due to the conversion of organic acids into sugars. Phenylalanine also increases membrane permeability(, which speeds up the respiration of acids that are held in cell vacuoles [12] and the role of potassium on cell’s enzymatic activity [17]. These results show a lot of similarity with the results obtained Naiema ,Neilson and Neilson and Anjum in apple [18,19,20].

Examination of data (Fig. 1) reveals that application of potassium sulphate, phenylalanine and Nano-Potash at different concentrations has a profound influence on fruit ascorbic acid content. The maximum ascorbic acid content (23.93 mg/100g) was noted in case of the treatment T_4 (Phe @ 0.3%).The treatment T_3 (Phe @ 0.2%) was statistically at par with T_2 (Phe @ 0.1%) having a fruit ascorbic acid content of 23.46 and 22.98 mg/100g respectively and treatment T_2 (Phe @ 0.1%) was statistically at par with treatment T_1 (K_2SO_4 @ 5g/l) having a fruit ascorbic acid content of 22.98 and 22.39 mg/ 100g respectively. The minimum fruit ascorbic acid content (19.48 mg/ 100g) was observed in case of treatment T_0 (control). The maximum ascorbic acid content was observed in phenylalanine. This increase in the ascorbic acid content by phenylalanine application may be attributed to the higher synthesis of some metabolic intermediary substances which promoted the greater synthesis of the precursor of ascorbic acid *i.e* Glucuronate [12]. Similar results were obtained by Ommol [21], who found an increase in the ascorbic acid content of plum with applications of phenylalanine’s, increased the activity of antioxidant enzymes and the accumulation of ROS scavenging agents, such as ascorbic acid.

As evident from Fig. 2, application of potassium sulphate, phenylalanine and Nano-Potash sprays at different concentrations had a pronounced influence towards fruit antioxidant content. In this context, maximum antioxidant content (75.71 mg/ 100g) was observed in T_4 (Phe @ 0.3%) which was followed by T_3 (Phe @ 0.2 %) and T_2 (Phe @ 0.1%), whereas the least antioxidant content (46.76 mg/ 100g) was observed in control (T_0). Phenylalanine, the precursor of the phenylproponoid pathway, is an aromatic amino acid existing naturally in plants and derived from the shikimate pathway. Phenylalanine increase secondary metabolites such as anthocyanins, flavonoids, phenols which act as antioxidants, through the phenylproponoid pathway [22]. Similar findings were made by Ommol [21] who discovered that applying phenylalanine to plum increased its antioxidants level. The positive effect of potassium on antioxidant can be ascribed to the fact that potassium improves primary metabolites in plants that could influence the biosynthesis of some antioxidant compounds [23].

Perusal of data presented in (Fig. 3) reveals that the application of potassium sulphate, phenylalanine and Nano-Potash spray at different concentrations had pronounced influence on chlorophyll of fruit. Lowest fruit chlorophyll (0.59 mg/g fw) was observed in T_4 (Phe @ 0.3%) followed by T_3 (Phe @ 0.2%) and T_2 (Phe @ 0.1%). The highest fruit chlorophyll (1.08 mg/g fw) was found in treatment control (T_0). This may be attributed to the fact that phenylalanine might be involved in the activation of chlorophyllase enzyme that break down chlorophyll and increases anthocyanin content [24]. Similar results were obtained by Fanyuk [22] who found that chlorophyll fluorescence decreased in almost all the phenylalanine treated apples and almost all treatments presented statistical significance. Potassium, however, has a beneficial effect on chlorophyll since it slightly raises the carotenes content in fruit skin, which lowers the amount of chlorophyll in fruit [25].

Data presented in Fig. 4 divulges that applying potassium sulphate, phenylalanine and nano-potash sprays at different concentrations had positive influence on anthocyanin content in fruit. Highest anthocyanin content (14.41mg/ 100g) was observed in treatment T_4 (Phe @ 0.3%) and the lowest (7.88 mg/100g) was observed under treatment T_0 (control). The treatment T_3 (Phe @ 0.2%) exhibited a profound effect on anthocyanin content and was second highest (13.88 mg/100g) after treatment T_3. The increased amount of anthocyanin by phenylalanine could be attributed to their influence on anthocyanin biosynthesis since phenylalanine is the primer compound in the biosynthesis pathway of anthocyanin. The presence of phenylalanine may stimulate the activity of the enzyme phenylalanine ammonia lyase (PLA), which is essential for the production of anthocyanins [12,22]. These results were found to be more in harmony with those obtained by Ommol [21] and [15] who found that anthocyanin pigment was increased with increasing the concentration of phenylalanine applications in “Red Roomy” cultivar. High levels of potassium in tissues improve red pigment formation in apples by enhancing the anthocyanin biosynthesis. It seems that K is an important element in the pathway of anthocyanin and could be a cofactor playing a vital role in the activation of specific enzymes, similar to UDP galactose: flavanoide-3-o-glicosiltransferase [26]. These results are in close proximity to the observations of Mosa [27] in Anna apple.

Fanyuk reported that Phenylalanine, the precursor of the phenylpropanoid pathway, is an aromatic amino acid, is generally recognised as a harmless material that can improve the nutritive value and resistance to disease of fruits and vegetables [28]. that means they exists naturally in plants and can be found in three different forms (L, D and DL-Phenylalanine) [29,30,31]. It is derived from the shikimate pathway [32,33], where anthocyanins are one of the secondary metabolites of downstream pathway and help to accumulate red pigment in fruit peel. Fruit must be red in order to be marketable. Fruit with more red colour can both reduce food loss and improve consumer health [34,35]. The secondary metabolites known as anthocyanins are linked to the red colour of apple and other fruit peels. Through the flavonoid pathway, which begins with phenylalanine, anthocyanins and flavonols are produced. Anthocyanins and flavonols have antioxidant, antifungal and health-promoting properties. In diverse apple cultivars, preharvest treatment of phenylalanine with solar radiation boosted the phenylpropanoid production pathway, which increased the coverage and intensity of red-colored peel fruit. The stimulation of the phenylpropanoid pathway by Phenylalanine treatment raised the phenolic and flavonoid contents, which in turn led to the manufacture of anthocyanins, which directly contribute to the red colour of the peel and have health advantages [36,37].

Table 1. Effect of different chemical sprays on TSS (%), Total Sugars (%) and Fruit Acidity (%) of Apple cv. Ambri

Treatment	Total soluble solids content (%)	Total Sugars (%)	Fruit acidity (%)
T_0 (Control)	14.10	11.28	0.31
T_1 (K_2SO_4 @5g/l)	14.67	11.58	0.24
T_2 (Phenylalanine @ 0.1 %)	15.04	12.03	0.21
T_3 (Phenylalanine @ 0.2 %)	15.67	12.53	0.19
T_4 (Phenylalanine @ 0.3 %)	16.23	12.98	0.17
T_5 (Nano-Potash @ 1ml/l)	14.13	11.36	0.28
T_6 (Nano-Potash @ 2ml/l)	14.41	11.39	0.28
T_7 (Nano-Potash @ 3ml/l)	14.56	11.52	0.29
SE(d)	0.041	0.181	0.027
LSD = CD (p≤ 0.05)	**0.090**	**0.372**	**0.058**

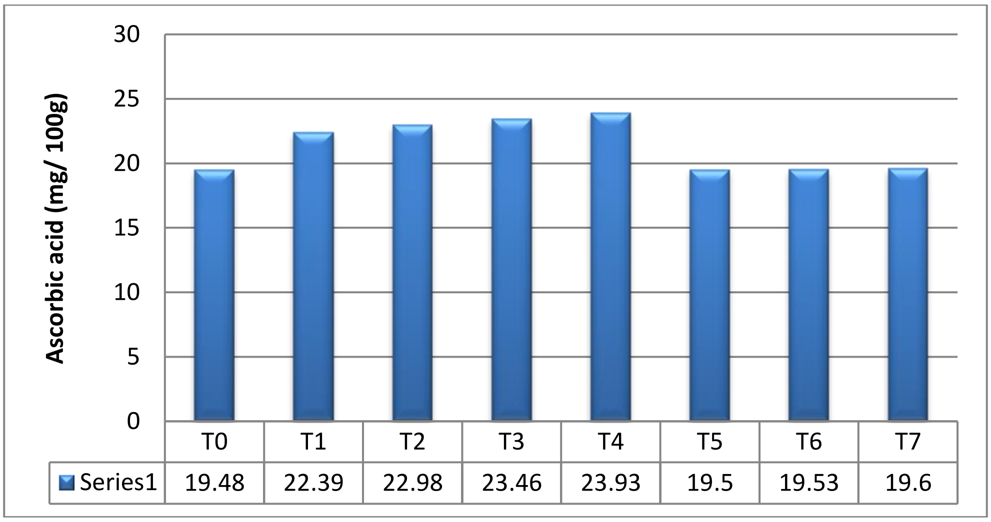

Fig. 1. Effect of different chemical sprays on fruit ascorbic acid (mg/100 g) of Ambri Apple

SE(d) 0.087
C.D(p≤ 0.05) 0.174

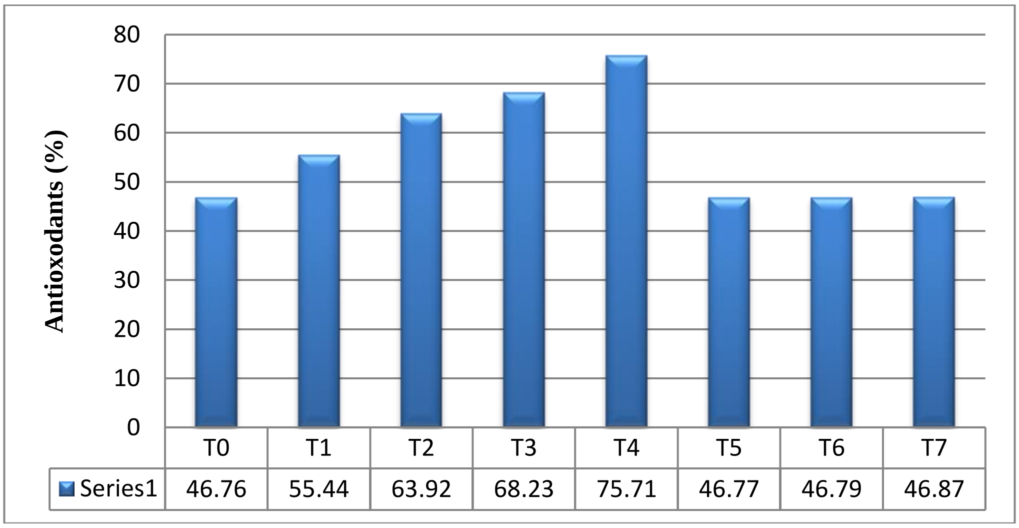

Fig. 2. Effect of different chemical sprays on fruit Antioxidants (peel) (%) of Ambri apple

SE(d) 1.068
C.D(p≤ 0.05) 2.136

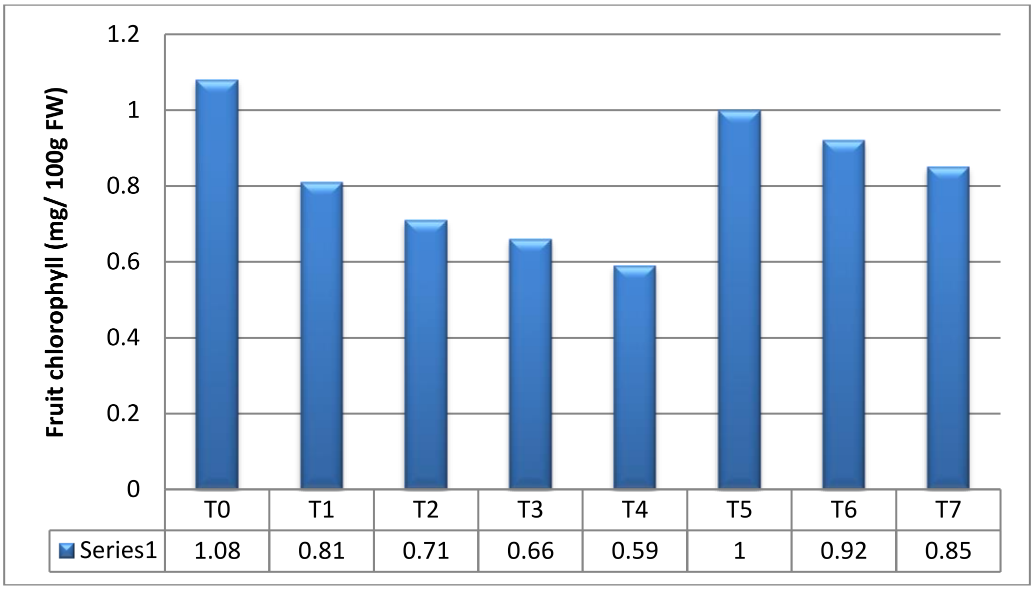

Fig. 3. Effect of different chemical sprays on fruit chlorophyll (peel) (mg/100 g fw) of Ambri apple

SE(d) 0.003
C.D (p≤ 0.05) 0.007

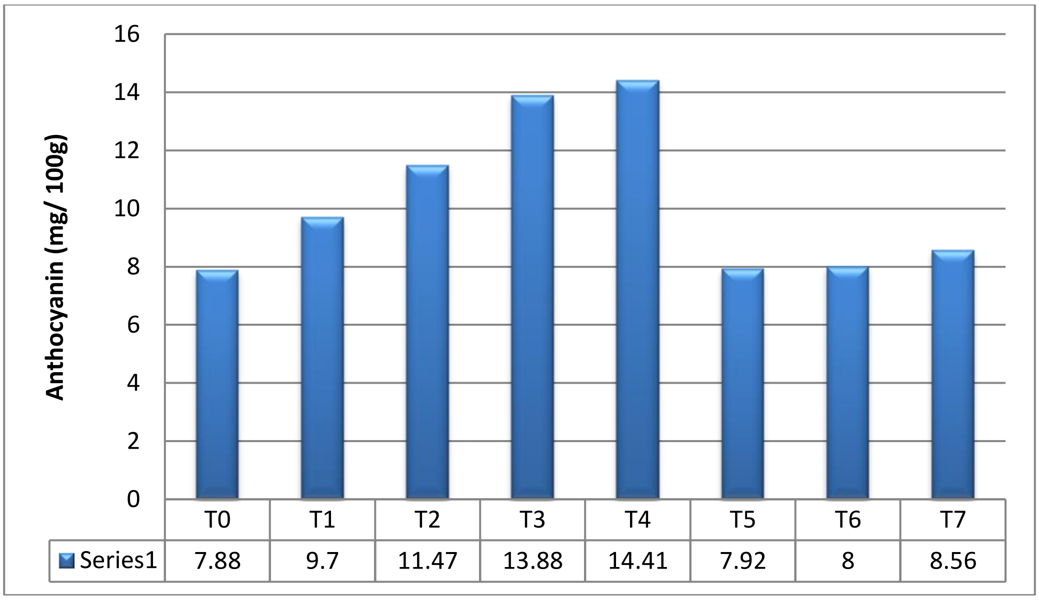

Fig. 4. Effect of different chemical sprays on fruit anthocyanin (peel) (mg/100 g) of Ambri apple

SE(d) 0.327
C.D (p≤ 0.05) 0.708

4. CONCLUSION

The efficacy of three different chemicals (potassium sulphate, phenylalanine and nano-potash) on anthocyanin, TSS, total sugars, ascorbic acid, chlorophyll and antioxidant content were investigated. Phenylalanine and potassium sulphate treatments gave evidence of improvement in fruit quality. It was concluded that foliar application of phenylalanine @ 0.3%, 4 weeks before harvest is very much effective in overcoming anthocyanin development problems in Ambri besides helps in obtaining higher quality fruit. In conclusion, the study investigated the impact of foliar sprays of phenylalanine, nano-potash, and potassium sulphate on the fruit quality attributes of apple. Our findings reveal significant enhancements in various quality parameters such as fruit size, color, firmness, sugar content, acidity, and shelf life. The application of phenylalanine exhibited promising results in improving fruit color and sugar content, while nano-potash contributed positively to fruit size, firmness, and shelf life. Additionally, potassium sulphate treatment showed notable improvements in acidity levels. These findings suggest the potential of foliar sprays as a viable strategy for enhancing apple fruit quality. Further research is warranted to explore optimal application methods, dosage levels, and long-term effects on orchard productivity and environmental sustainability. Overall, the findings contribute to the advancement of apple cultivation practices, aiming for improved fruit quality and market competitiveness.

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 manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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