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Physiological basis of post-harvest ripening and standardization of seed extraction in ash gourd

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ABSTRACT

Manual seed extraction of ash gourd is time and labour extensive, and freshly harvested seeds possess dormancy. Hence, seed extraction protocol was standardized, and physio-biochemical changes associated with post-harvest ripening (PHR) were elucidated in ash gourd cv. Kashi Dhaval. Allowing pulp to ferment for 24 to 48 h gave the best quality seed (germination and vigour) compared to acid, alkali and manual extraction. PHR of fruit for 20-30 days before seed extraction gave the higher seed yield (seeds per fruit and 100-seed weight), higher seed germination and vigour compared to seed obtained from freshly harvested fruits. Physiological analysis showed that PHR increased the seed reserve (total soluble proteins, starch) except total soluble sugars, conceivably due to conversion to starch. Decreased O_2 and H_2O_2 activity in PHR seeds indicated a lower threat to oxidative stress, probably due to decreased seed moisture. Consequently, SOD, CAT and POD activity was reduced in PHR seeds. In conclusion, allowing fruit pulp to ferment for 24 to 48 h gave the best quality seed during seed extraction. Moreover, 20-30 days PHR in ash gourd is recommended because numerous physio-biochemical changes related to dormancy release and germination enhancement occur during PHR.

Keywords: *Benincasa hispida, S*eed extraction, Post-harvest ripening, Seed quality, ROS, Antioxidants, Reserve accumulation

INTRODUCTION

Ash gourd (*Benincasa hispida* (Thunb.) belongs to the *Cucurbitaceae* family. It is commonly known as Petha (Hindi), Kushmanda (Sanskrit), Boodida Gummadi (Telugu), Neer Pooshnikkai (Tamil), Kumbalam (Malayalam), Bhuru Kolu or Safed Kolu (Gujarati) and winter melon/wax gourd (English) (Bimakar *et al*., 2). The traditional medicinal worth of this gourd includes usage as an antidote for alcoholic poisoning, laxative, diuretic, and treatment for internal haemorrhages and constipation. Ash gourd seed is also used for body smoothening, hair treatment, cardio-vascular diseases, brain and liver disorders, as it has anti-angiogenic, anti-tumour, antioxidant and anti-pyretic properties (Bimakar *et al*., 2). Being a fleshy fruit vegetable, seeds in ash gourd remain tightly embedded in the fruit pulp. Therefore, manual extraction of seeds after the maturity and harvesting of fruit becomes time and labour extensive.

Moreover, the alkaline nature of pulp also makes seed extraction difficult as it causes itching and irritation on the skin. Generally, the seed quality depends on several factors, *viz*. soil, climate, cultural practices, stage of harvest as well as the method of seed extraction, seed storage etc. (Manimurugan *et al*., 10). An early harvest leads to more immature or unfilled seeds, whereas delayed harvest results in fruit drop, bird damage, and fast ageing. In fleshy vegetables like cucumber, pumpkin, melons etc., the post-harvest ripening (PHR) of harvested fruit before seed extraction is very beneficial to provide additional time to seed with an early harvest of fruits, as the seeds continue to develop and mature in the fruit even after harvest until seed extraction (Silva *et al*., 19; Gupta *et al*., 7). Besides, seed dormancy is also predominant in freshly harvested ash gourd seed (Ganar *et al*., 6). Therefore, PHR of harvested fruits before seed extraction, in many crops improved seed germination, vigour, and field emergence, allowing immature seeds to achieve maturity (Gupta *et al*., 7). Therefore, this study was carried out to standardize seed extraction and to investigate the physiological changes during post-harvesting ripening in ash gourd under North Indian conditions.

MATERIALS AND METHODS

An experiment was conducted during the *kharif* season in 2020-21 and 2021-22 at ICAR– Indian Institute of Vegetable Research, Varanasi, using ash gourd cv. Kashi Dhaval. Plants were grown on raised beds using a row-to-row spacing of 2.0 m and plant-to-plant spacing of 0.6 m. Mature waxy fruits were harvested and stored at room temperature for post-harvest ripening for 0, 10, 20 and 30 days before the seed extraction. For seed extraction, five fruits,

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each in three replicates, were cut into four parts crosswise and lengthwise using a cutter and the pulp was scooped with the help of a scooper. Then, the pulp was kept with various treatments, *i.e.,* manual extraction, acid extraction using HCl (1%, 2%, 3% each for 30, 60 and 90 min), alkaline extraction using Na_2CO_3 [200 mg/L (pH 9.8), 300 mg/L (pH 10.6) and 400 mg/L (pH 11.0)] for overnight (12-14 h) fermentation (24, 48 and 72 h; average temperature 20°C). In each treatment, the pulp was removed by washing with water and seeds were dried adequately under shade up to 8-10% moisture content.

The number of seeds and 100 seed weights were determined from five fruits in triplicate. Seed dimensions were determined using a digital Vernier Calliper from 25 seeds for each treatment. Seed germination was recorded using 100 seeds each in a cabinet germinator at 25°C for eight days in triplicate, and moisture content was determined using the oven method following ISTA (8). Seed vigour indices (vigour index-I and vigour index-II) were computed using 10 normal seedlings per replicate.

The seed leachate's electrical conductance (EC) was measured using 25 seeds in 50 ml MQ water and expressed as µS/cm/g (Pandita and Nagarajan, 13). Dehydrogenase activity was measured using 1% 2,3,5-triphenyl tetrazolium chloride solution as a staining agent (Kittock and Law, 9). Starch, total soluble sugars (TSS) and total soluble proteins (TSP) in seeds were estimated following Gupta *et al.* (7) and expressed as mg/ g DW. Superoxide anions ($O_2^{\cdot-}$) were measured by the ability to reduce nitro blue-tetrazolium (NBT) (Chaithanya and Naithani, 3). Hydrogen peroxide (H_2O_2) was estimated by the formation of titanium-hydro peroxide (Mukherjee and Choudhari, 11). Superoxide dismutase activity (SOD) was assayed by inhibition of photochemical reduction of NBT (Dhindsa *et al*., 4). One unit of SOD was considered the amount of enzyme needed to inhibit NBT reduction by 50%. The catalase activity was measured by quantifying the residual H_2O_2 in the reaction mixture, as catalase quenches the H_2O_2. Peroxidase activity (POD) was assayed by measuring the formation of tetra guaiacol (ϵ= 26.6 mM/cm) from guaiacol (Rao *et al*., 14).

The experiment was carried out in triplicate following a completely randomized design. The percentage values were transformed to arc sine values to normalize the data before statistical analysis. WASP 2.0 software and Microsoft Excel 2019 tool were used for statistical analysis.

RESULTS AND DISCUSSION

The manual extraction of ash gourd seed is time and labour extensive and cumbersome, as the seeds remain tightly impregnated in the fruit pulp. Besides, preliminary analysis at ICAR-IIVR, Varanasi, showed that the alkaline nature of the pulp causes itching and irritation on the skin. Fermentation of seed pulp for 24 - 48 h was significantly superior for seed quality over acid, alkaline, and manual seed extraction. Maximum seed germination (76.0 % and 75.0 %), seedling length (27.5 and 26.8cm), seeding dry weight (0.60 and 0.58 mg), vigour index-I (2090.0 and 2010.0) and vigour index-II (45.6 and 44.5) was recorded in T2 (fermentation for 24 h) and T3 (fermentation for 48 h), respectively (Table 1). Higher seed germination in fermentation treatment may be due to the breakdown of seed dormancy in freshly harvested seeds. Acid and alkaline treatment does not show any significant improvement in seed quality.

PHR had a significant impact on seed yield attributes. The number of seeds per fruit and seed dimensions was consistent among the PHR treatments. Whereas 100-seed weight was significantly increased in 20-day PHR (7.19 g) and 30-day PHR (7.23 g) as compared to non-PHR (0-day) treatment (6.95 g). The seed moisture content (mc %) declined gradually with an increment of PHR duration due to the fruit's natural disintegration and water loss. The highest seed mc % was observed with 0-day PHR (21.58 %), while the lowest seed mc was recorded with 20-day PHR (20.16 %) and 30-day PHR (19.50 %) (Table 2). Similar results of loss in seed moisture with increased PHR period were reported in Cucumber (Gupta *et al.,*7). The seed reserves such as TSS, TSP, and total starch were significantly changed during the PHR. Starch is the central seed storage reserve in ash gourd, and its content in the seed increased (1.07-fold) with advancement in PHR periods from 0 to 30 days. At the same time, TSS depicted a reverse trend of starch accumulation and decreased (1.16 fold) with PHR period from 0 to 30 days. TSS accumulation was negatively related to the PHR duration (r = -0.96), possibly due to its conversion to starch. Similar to starch, TSP content increased (1.09-fold) with increasing PHR period and showed a positive correlation with PHR duration (r = 0.71) (Fig. 1). As a result, it can be deduced that the seed as sink continues to receive accumulations from the fruit as PHR duration advances, resulting in an increase in 100-seed weight and the development of bolder seeds. The increase in total proteins during PHR could be attributed to the intracellular interconversion of free amino acids.

Furthermore, the reduced activity of hydrolytic enzymes (proteases and amylases) aids the fruit, and thus the seeds, in accumulating proteins and

Table 1. Effect of seed extraction treatments on seed quality in ash gourd.

Code	Treatment	Germination (%)	Seedling length (cm)	Seedling dry weight (g/seedling)	Vigour index-I	Vigour index-II
T1	Manual extraction	51.0	23.2	0.37	1305.6	20.5
T2	Fermentation- 24 h	76.0	27.5	0.60	2090.0	45.6
T3	Fermentation- 48 h	75.0	26.8	0.58	2010.0	44.5
T4	Fermentation- 72 h	71.0	25.0	0.56	1777.8	39.8
T5	1% HCl (pH- 2.8) - 10 ml/l -30 min	48.0	24.5	0.41	1174.6	19.7
T6	1% HCl (pH- 2.8) - 10 ml/l -60 min	68.0	23.5	0.52	1597.3	35.4
T7	1% HCl (pH- 2.8) - 10 ml/l -90 min	60.0	24.4	0.37	1465.2	22.2
T8	2% HCl (pH- 2.1) - 10 ml/l -30 min	60.0	26.4	0.45	1585.2	27.0
T9	2% HCl (pH- 2.1) - 10 ml/l -60 min	60.0	26.1	0.42	1566.6	25.2
T10	2% HCl (pH- 2.1) - 10 ml/l -90 min	56.0	24.5	0.40	1370.3	22.4
T11	3% HCl (pH- 1.3) - 10 ml/l -30 min	55.0	20.1	0.28	1107.2	15.4
T12	3% HCl (pH- 1.3) - 10 ml/l -60 min	48.0	18.7	0.33	899.5	15.8
T13	3% HCl (pH- 1.3) - 10 ml/l -90 min	44.0	26.0	0.46	1144.0	20.2
T14	Na_2CO_3 - 200 mg/l overnight	48.0	26.9	0.44	1288.8	21.1
T15	Na_2CO_3 - 300 mg/l overnight	36.0	23.1	0.42	832.0	15.1
T16	Na_2CO_3 - 400 mg/l overnight	28.0	23.9	0.30	668.4	8.4
CD (0.05)		3.2	1.6	0.04	103.5	1.2
CV (%)		4.8	3.9	2.6	5.5	6.4

Table 2. Effect of Post-harvest ripening (PHR) period on seed quality in ash gourd.

Treatments	Seed length (mm)	Seed breadth (mm)	Seed moisture content (%)	100 seed weight (g)	Germination (%)	Seedling length (cm)	Seedling dry weight (g/ seedling)	Vigour index-I	Vigour index-II
0 day PHR	12.29	7.13	21.58	6.95	38	23.6	0.39	896.8	14.82
10 day PHR	12.24	7.11	20.69	7.06	63	24.9	0.43	1568.7	27.09
20 day PHR	12.30	7.14	20.16	7.19	75	26.1	0.45	1957.5	33.75
30 day PHR	12.27	7.12	19.50	7.23	73	25.9	0.46	1890.7	33.58
40 day PHR	12.22	7.11	21.55	6.99	67	24.9	0.41	1668.3	27.47
CD (0.05)	0.08	0.04	0.6	0.09	4.1	1.1	0.03	134.3	1.4
CV (%)	2.1	2.6	5.6	2.6	4.5	5.3	3.8	3.1	4.0

starch during the later stages of ripening. Therefore, accumulation may increase during PHR due to assimilate transfer from pulpy fruit to seeds. These results confirmed with Gupta *et al*. (7) in cucumber. However, a decline in seed biomass during PHR as seeds tend to respire and use the food reserves is also reported.

PHR registered significant differences in physical and physiological seed quality. The higher mean values for seed germination (75.0 and 73.0 %), seedling growth (26.1 and 25.9 cm), seedling biomass (0.45 and 0.46 g/seedling), seed vigour index-I (1957.5 and 1890.7) and seed vigour index-II (33.8 and 33.6) were recorded with PHR treatment for 20 and 30 days, respectively (Table 2). At the same time, the least mean values for seed germination (38.0 %), seedling length (23.6 cm), seedling biomass (0.39 g/ seedling), vigour index-I (896.8), and vigour index-II (14.8) were recorded with 0-day PHR (Table 2) which may be associated with the presence of dormancy in freshly harvested seed (Gupta *et al*., 7; Ganar *et al*., 6). Higher seed reserve accumulation resulted in bolder seeds, improving seed quality during PHR. These results are consistent with Gupta *et al*. (7) in

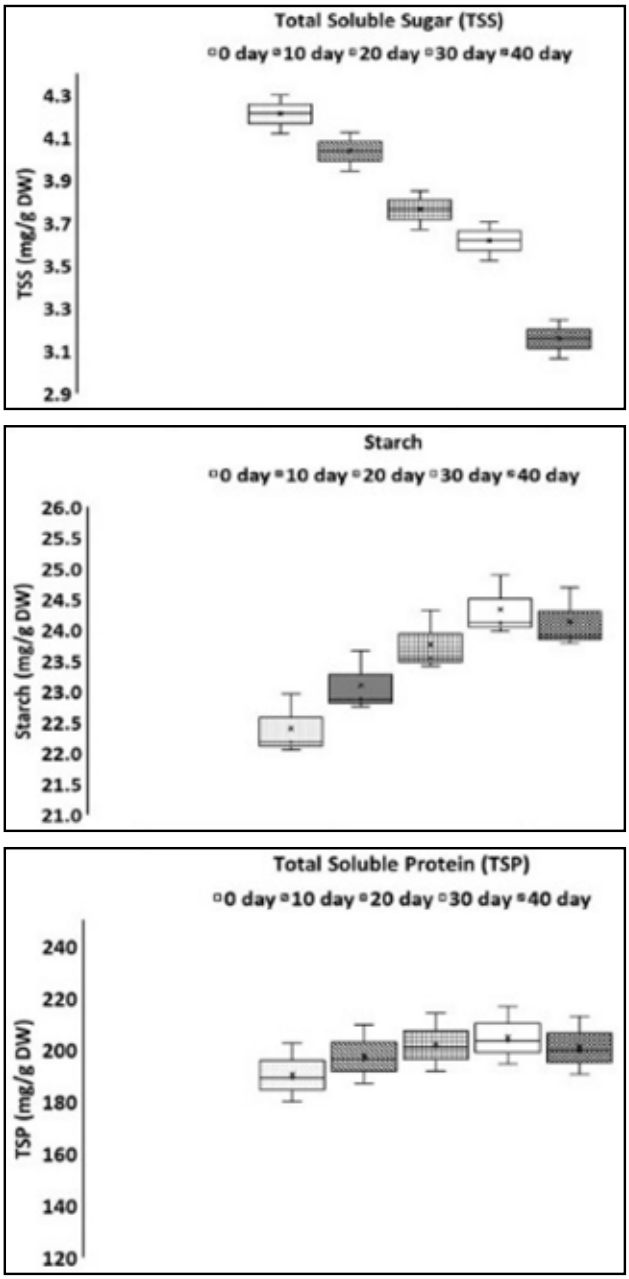

Fig. 1. Changes in seed storage reserves in ash gourd during post-harvest ripening

cucumber. At the cellular level, higher dehydrogenase activity and lower EC values from seed leachates indicate better germinability in the seeds (Gupta *et al*., 7). A significant difference in electrical conductance (EC) and dehydrogenase activity was registered in different PHR treatments. The minimal values for EC were recorded with 20-day PHR (106.2 µS/cm/g) followed by 30-day PHR (109.1 µS/cm/g). Whereas maximal values for EC were registered, with 0-day PHR (142.3 µS/cm/g) (Fig. 2). EC from the seed leachates significantly reduced with advancement in PHR periods, which may positively correlate with TSS and TSP content in seed leachates (Gupta *et al*., 7). Higher dehydrogenase activity was observed in 20-day (3.28 µg/g) and 30-day (3.20 µg/g) PHR seeds, lowest activity was noticed in 0-day PHR seed (3.16 µg/g). Higher vigour indices, dehydrogenase activity and lower EC reflected improved seed vigour in PHR-seeds, which may imply enhanced seed longevity or storability (Gupta *et al*., 7). However, increased mc and EC, and reduced dehydrogenase activity in 40-day PHR seeds were caused by the rotting of the fruit. During PHR, seeds usually remain impregnated in fruit mass, with water content near to imbibition and adequate oxygen, but with restricted germination. But extended PHR period may lead to reduced seed quality and yield due to decomposition of fruits and seeds, and precocious germination (Gupta *et al*., 7).

The significant differences for Reactive Oxygen Species (ROS) ($O_2^{\cdot-}$ and H_2O_2) and antioxidants, Super oxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activity were observed in different PHR treatments. Normally, the level of ROS is under the stringent control of antioxidants, which decides the seed status during germination, dormancy or ageing. Overall, the PHR seeds registered lower activity of ROS. PHR for 20- and 30-days showed a decrease in O_2 activity of 1.06 and 1.05 folds, respectively. However, H_2O_2 activity was 1.03 folds and 1.05 folds, respectively, in PHR and non-PHR (0-day PHR) seeds (Fig. 3). The correlation analysis gave a negative relation ($r = -0.71$ to -0.99, $p < 0.05$) between ROS ($O_2^{\cdot-}$ and H_2O_2) activity and different PHR durations except 40-day PHR (Fig. 3). Whereas, rotting of fruit in 40-day PHR seeds showed a sudden increase in $O_2^{\cdot-}$ and H_2O_2 activity. Remarkably, the antioxidant activity decreased significantly with an increase in PHR durations. The activity of SOD, CAT and POD recorded a decrease of 1.09-folds, 1.23-folds and 1.34-folds) in the 30-

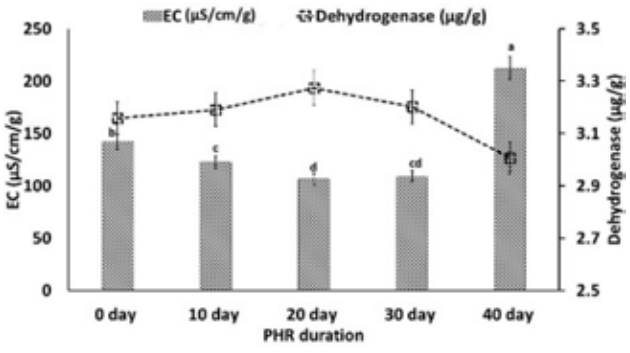

Fig. 2. Electrical conductance and dehydrogenase activity in ash gourd seed during PHR.

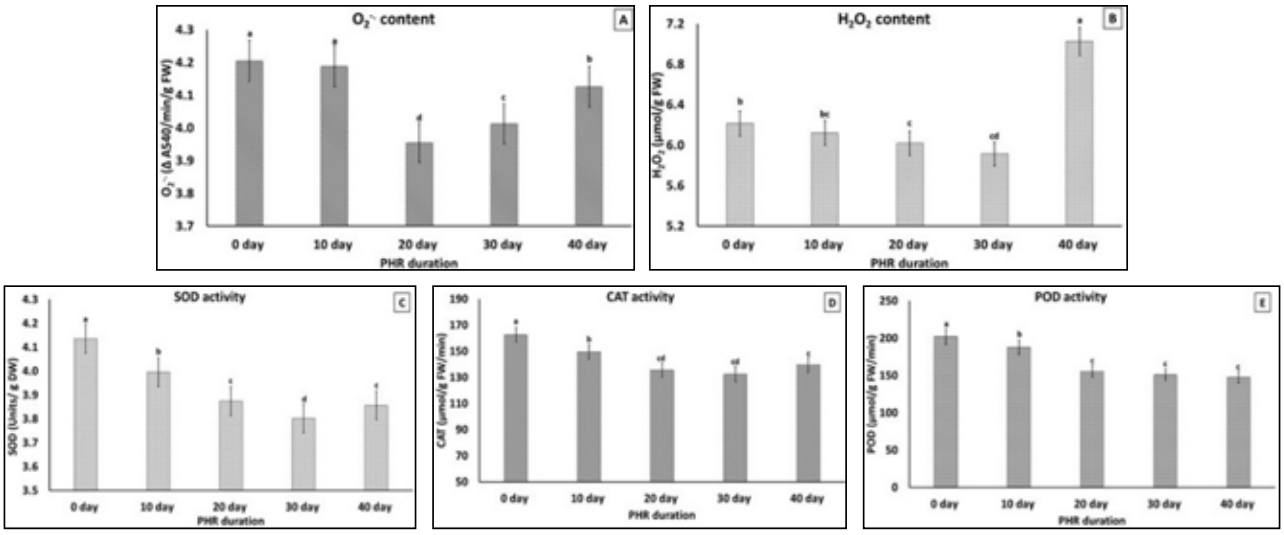

Fig. 3. ROS ($O_2^{\cdot-}$ and H_2O_2) and antioxidants (SOD, CAT and POD) activity in ash gourd seed during post-harvesting ripening.

day PHR seed over the 0-day PHR seed (Fig. 3). Regression analysis showed a strong but negative correlation [r = -0.93 to -0.98 ($p < 0.05$)] between antioxidants with PHR durations up to 30-day PHR (Fig. 3). Generally, ROS is produced under stringent regulation of antioxidants, as the ratio of ROS and antioxidants plays a crucial role in the regulation of seed development and maturation, seed germination and dormancy and seed ageing (Bailly, 1). In the present study, reduced ROS activities in PHR seeds may be due to reduced seed moisture, which imposes a lower potential threat to the seed and reduces antioxidants (SOD, CAT and POD). These results are consistent with Silva *et al.* (15) in pumpkin and Gupta *et al.* (7) in cucumber. However, the higher activity of antioxidant enzymes in non-PHR seeds of ash gourd may be to eliminate the higher free radicals. These antioxidants work interdependently in close association; SOD dismutates the $O_2^{\cdot-}$ anion into H_2O_2 and oxygen. Further, H_2O_2 is detoxified by CAT and POD into water. Irrespective of PHR durations, results demonstrated an overall decrease in the activity of ROS and antioxidant enzymes. Our findings emphasize that SOD, CAT and POD are important in maintaining the cellular homeostasis.

The first possibility could be the release of dormancy during PHR. Germination inhibitors and abscisic acid sensitivity mediated seed dormancy may break during PHR (Finch-savage and Leubner-Metzger, 5). Besides, ROS and antioxidant activity changes during PHR are also expected to regulate seed dormancy. Secondly, the increased accumulation of food reserves in seeds during PHR may lead to improved seed germination. The third possibility is that the improved growth potential of the embryo and the activity of endosperm cell wall degradation enzymes enhanced the ability of radicle emergence by penetrating the physical barrier of seeds. The fourth possibility could be the acquisition of desiccation tolerance during PHR (Murugesan and Vanangamudi, 12). Another reason could be the improvement in the rate of water uptake during germination and altered gene expression pattern of several functional genes.

In conclusion, allowing fruit pulp to ferment for 24 to 48 h gave the best quality seed (germination and vigour) during seed extraction. PHR of ash gourd fruit allows for an early harvest of fruits and an additional period of seed maturation since the seed continues to receive the storage reserve until extraction. Furthermore, lower EC, increased dehydrogenase activity, and balanced ROS and antioxidant production during PHR resulted in improved seed germination and vigour. On the other hand, PHR allows the immature seed to mature within the fruit and prevents drying damage, resulting in reduced ROS generation and, as a result, reduced antioxidants. Thus, 20-30 days PHR in ash gourd is recommended because numerous physio-biochemical changes related to dormancy release and germination enhancement occur during PHR.

AUTHORS' CONTRIBUTION

Conceptualization of research (NG, PMS and RK); Designing of the experiment (NG, PMS, RK, TC and VS); Contribution of experimental materials (NG and TC); Execution of field/lab experiments and data collection (NG, RK, TC and VS); Analysis of data

and interpretation (NG and PMS); Preparation of the manuscript (NG).

DECLARATION

The authors declare no conflict of interest.

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