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Synchronous fruit splitting in nutmeg using plant growth regulators

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Abstract

In this study, a simple technique with hormone treatment was developed for synchronous splitting (ripening) of nutmeg fruits. The methodology involves harvesting physiologically mature fruits, dipping the harvested fruits in 500 ppm ethrel (2-chloroethylphosphonic acid) solution for 10 minutes, and then storing them in shade. By this method, 90 to 100 per cent fruits split in 18 to 20 hours. Width of the split which helps in easy separation of nut from fruit pericarp was on par with that of naturally split fruits. The dry recovery, nut to mace ratio and fresh and dry weight of the nut and mace of the treated fruits were comparable with naturally split fruits. The intrinsic quality *i.e.*, oil, oleoresin and moisture content of nut and mace of treated fruits were on par with that of naturally split fruits. This indicates that nut and mace of treated fruits had similar physical and intrinsic quality parameters as that of naturally split fruits. The advantage of the method is that it is very effective in preventing aflatoxin (mycotoxin) contamination of nut and mace due to soil contact of naturally split fruits that fall on the ground. The method for synchronous fruit splitting in nutmeg is very simple and can be easily practiced by farmers. It also saves time, labour and money both for harvesting and processing of nutmeg. The cost of ethrel treatment would be around ₹ 800 per ton of fruit. This is the first report on the induction of synchronous and uniform fruit splitting (ripening) of pre-split harvested fruits in nutmeg.

Keywords: Aflatoxin, ethrel, fruit splitting, mace, *Myristica*, oleoresin

Introduction

Nutmeg (*Myristica fragrans* Houtt.) is the only tropical fruit which yields two different spices (nut and mace). In India, it is cultivated in Kerala, Tamil Nadu and Karnataka. The period from flower to fruit ripening takes around 6 to 9 months (Flach and Cruickshank, 1969). Generally, nutmeg starts flowering in 5th or 6th year and may reach peak yield in 20 years. The yield varies from tree to tree. The average yield is around 1500 nuts tree^{-1} year^{-1} but a single tree can yield up to 10000 fruits per year also.

Nutmeg (both seed and mace) is used for flavouring many dishes in countries like India, Indonesia, Japan, Middle East, Europe, USA *etc*. In Indonesia, nutmeg is used in various dishes (many spicy soups) including gravy for meat dishes and in sweet preparation, manisan. In India, nutmeg is used in many sweet and savoury dishes. Nutmeg rind is also used to make juice, pickles and chutney in Kerala. Both nutmeg and mace are used mainly in potato dishes and processed meat products in Europe. Japanese use nutmeg as one of the ingredients in curry powders. Nutmeg is known as the main pumpkin pie spice in US (https://en.wikipedia.org/wiki/Nutmeg) and is also used as folk medicine for treating various ailments.

Though flowering and fruiting is noticed throughout the year in nutmeg, the main fruiting season extends for around three months from June to August which is due to asynchronous flowering. Nutmeg fruits are harvested when the fruit is split at the bottom exposing the inner red mace which serves as an indicator of harvest. Often farmers do not harvest the split fruits from the tree, instead they pick up the ripened (split) fruits fallen and in most of the cases, nut and mace will be separated from the fruit and would have come in contact with the

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soil which increases the chances of *Aspergillus* (aflatoxin) contamination. Also, farmers need to visit each tree daily to collect the fallen fruits, separate the nut and mace and dry them separately each day which consumes time, labour and energy. Hence, it is ideal to harvest all physiologically mature fruits together and induce synchronous splitting which can save time, labour and energy of the farmer besides preventing aflatoxin contamination of nut and mace. Natural fruit splitting takes place when the fruit is fully mature which can otherwise be treated as fruit ripening. Ethylene, known as ripening hormone, plays a key role in the ripening process in many fruits (Carvalho *et al.*, 2003, Tomas *et al.*, 2012, Zhang *et al.*, 2012). It is also used to induce artificial fruit ripening in many crops. Naphthalene acetic acid (NAA) is also used to induce ripening in a few fruits (Leila *et al.*, 2011). If physiologically mature nutmeg fruits are harvested and treated with such chemicals, it may help inducing synchronous splitting of nutmeg fruits. Once the splitting is induced like natural splitting, extraction of mace and nut can be done easily for further processing. So far, no reports are available on harvesting of physiologically mature fruits and inducing synchronous splitting in nutmeg. The objectives of the present study were to induce synchronous fruit splitting through the use of plant growth regulators, to compare the quality of induced split fruits with that of naturally split fruits and to work out the economics of hormone treatment

Materials and methods

Naphthalene acetic acid (99% purity) was procured from HiMedia, Ethrel (Ethephon 39% S.L) was procured from Bayer Crop Sciences Limited and acetone (99% pure) from Merck.

Physiologically mature (210 to 250 days after flowering with pale yellow to yellow colour) fruits were harvested from trees with peduncle intact, pooled and were washed well in tap water. The treatment solutions consisted of 1000 ppm ethrel, 500 ppm ethrel, 50 ppm naphthalene acetic acid (NAA), 25 ppm NAA and tap water. Fruits with and without peduncle/stalk were separated and both the group of fruits were dipped in treatment solution for 10 minutes. Another set of fruits as such with or without peduncle without any treatment was maintained as control. Naturally split fruits were used as absolute control. Each treatment was replicated thrice with 25 fruits in each replication. The experiment with CRD design was repeated three times to confirm the results.

The treated fruits were shade dried and maintained at room temperature (32 ^{o}C overnight). Observation on fruit split percentage and split width were recorded twenty hours after the treatment. Later, mace and nut were separated from the fruits, dried in an oven at 55 ^{o}C to constant weight. Nut and mace dry recovery as well as nut to mace ratio were worked out in all the treatments.

Nut dry recovery (%) was estimated using the formula,

Nut dry recovery = (Nut dry weight/Nut fresh weight) x 100.

Mace dry recovery was estimated using the formula,

Mace dry recovery = (Mace dry weight/Mace fresh weight) x 100.

Nut to mace ratio (per fruit) was worked out using the formula,

Nut/Mace ratio = (Mace dry weight / Nut dry weight) x 100.

Oleoresin and oil were estimated both from nut as well as mace as per ASTA (ASTA, 1968) procedure.

Solvent percolation method was followed for oleoresin estimation. Nuts from ethrel, NAA and water treatments were pooled separately. Dried and powdered nut weighing 10 g was loaded in to a column of 20 mm diameter and 250 mm length. Non-absorbent cotton was used to plug the bottom end of the column. Pure acetone (50 mL) was poured over the loaded column and maintained as such overnight. Later, the column containing the solvent (acetone) was drained in to a pre-weighed beaker. Additional 25 mL acetone was poured over the column containing the residue, left overnight and then drained the solvent to the same beaker. The content of the beaker was then dried at room temperature (32 ^{o}C) to remove the excess solvent and the left over oleoresin was weighed with the beaker and the dry weight was calculated after deducting the beaker weight and expressed as per cent oleoresin. Three replications were maintained for all the treatments.

Mace oleoresin was also estimated as explained above. Samples from ethrel, NAA and water treatments were pooled separately before analysis. Mace oleoresin was extracted from ten gram mace sample with 3 replications from each treatment.

Nut oil and mace oil was estimated by hydro-distillation method after pooling the nut and mace samples separately from all treatments. Dried and powdered nut (50 g) and mace (25 g) were for respective analysis.

Statistical analysis (two way ANOVA) was conducted using mstatc package.

Results and discussion

Fruit splitting and split width

All the treatments induced fruit splitting in 18 to 20 hours after treatment. Significant differences in fruit splitting percentages were noticed among the treatments. Fruit splitting percentage was highest for ethrel treatment followed by NAA treatment (Table 1). Fruits with and without stalk showed similar splitting percentage. Fruits treated with 1000 ppm ethrel showed 98.6 per cent while those treated with 500 ppm ethrel showed 96.6 per cent splitting (when fruits with and without stalk were considered together). Fruit split percentage of all the ethrel treatments were on par. NAA at 25 ppm induced 80.3 per cent and NAA at 50 ppm induced 77.0 per cent fruit splitting and were on par. Dipping in water induced 52.5 and 46.6 per cent (with and without stalk) and control (fruits kept as such after harvest) induced 45.4 and 54 per cent (with and without stalk) fruit splitting.

Split width is an indicator of ease with which the nut can be separated from the pericarp. More width indicates easier separation. Split width was higher in ethrel treatments (64 mm average) compared to other treatments and it was on par with split width of naturally split fruits (68 mm). Split width of the ethrel treatments were also on par. Lowest split width was noticed in treatment with NAA (40 mm average) (Table 1).

Almost 100 per cent fruit splitting was observed in ethrel treatment, after 18-20 hrs while it was 50 per cent in control and about 75-80 per cent in NAA treatment. Ethrel at 500 and 1000 ppm were on par suggesting that 500 ppm ethrel is sufficient to induce synchronous ripening of nutmeg fruits. Had the fruits not treated with ethrel and harvested, it would have taken at least another 4 to 6 weeks for all the fruits to split open naturally on the tree, after which they would have been harvested/fallen on the ground and collected which requires daily supervision. But in pre-split harvested trees, as most of the mature fruits are already harvested, farmers need not visit each tree looking for fallen fruits or split fruits on the tree, collecting them, separating nut and mace, wash and dry them each day, thus saving time, money and labour for collecting/harvesting fruits each day and drying them separately. Fruits from 210 to 250 days after flowering with pale yellow to yellow colour were harvested and fruit splitting was induced. Hence, it can be said that ethrel treatment advanced ripening by around 30-40 days in nutmeg as it takes around 250 days after flowering to attain natural fruit splitting on the tree (full ripening). It has been shown in many crops that ethrel induces uniform ripening and also advances maturity. For instance in coffee, pre-harvest application of ethephon provided both uniformity and 15 to 30 days advancement of maturation of fruits (Carvalho *et al.*, 2003). Post-harvest dipping of date fruits of Helali cultivar at mature stage in ethrel (4.2 mL L^{-1}) and

Table 1. Fruit split percentage and split width as influenced by different treatments

Treatment	Split percentage ± SE	Split width (mm) ± SE
E1000 - P	98.0 ± 0.90	70 ± 6.76
E1000 + P	99.2 ± 0.40	64 ± 6.03
E500 - P	96.0 ± 1.24	63 ± 6.23
E500 + P	97.2 ± 1.05	60 ± 7.81
NAA50 - P	82.4 ± 2.10	43 ± 7.22
NAA50 + P	71.5 ± 2.84	36 ± 4.65
NAA25 - P	82.0 ± 2.45	35 ± 5.50
NAA25 + P	78.6 ± 2.64	46 ± 6.62
Control - P	45.4 ± 3.44	38 ± 6.62
Control + P	54.0 ± 3.75	46 ± 5.52
Water dip - P	52.5 ± 2.95	56 ± 9.28
Water dip + P	46.6 ± 3.16	50 ± 7.53
Natural splitting	-	68 ± 5.77
CD at 5 %	3.3	10.8

-P = no pedicel, +P = with pedicel, E1000 = ethrel 1000 ppm, E500 = ethrel 500 ppm, NAA25 = NAA 25 ppm, NAA50 = NAA 50 ppm

abscisic acid (1.0 mM) significantly enhanced ripening, compared to the control. Immersion of fruit in water for 10 h increased fruit ripening significantly compared to the control, but to a lesser extent (Awad, 2007). Ethephon hastened the maturity in persimmon fruits by 13 to 22 days (Kim *et al.*, 2004). On the contrary, ethephon did not affect harvest date and gum exudation in almond (Leonel *et al.*, 2011). In sugarcane, Moddus (ripening hormone) affected growth processes in both the stalk and leaf canopy above a certain concentration, which influenced ripening efficacy (Van Heerden *et al*., 2015). In mango, fruits harvested with petiole and treated with ethrel showed better ripening percentage (personal communication). It was found that fruits with and without stalk had similar split percentage in all the treatments, indicating retention of stalk had no specific advantage in enhancing fruit split percentage in nutmeg.

Fresh and dry weights and nut:mace ratio

Table 2 shows fresh and dry weights of nut and mace and also mace:nut ratio for different treatments. Fresh weight of nuts varied from 6.70 to 8.03 g and dry weight from 4.52 to 5.56 g. Significant differences among the treatments were noticed for fresh and dry weights of both nut and mace while mace:nut ratio was on par among the treatments. Highest fresh weight (8.03 g) of nuts was noticed in 500 ppm ethrel treatment (7.55 g pooled average for ethrel treatments) followed by 7.95 g in 25 ppm NAA treatment (7.41 g pooled average for NAA treatments) while it was 7.53 g in natural splitting treatment and all these treatments were statistically on par. Highest nut dry weight of 5.56 g was noticed in 500 ppm ethrel treatment (5.35 g pooled average for ethrel treatments) while it was 5.15 g in naturally split fruits and these two treatments were on par. Mace dry weight was maximum in ethrel treatments (1 g pooled average) which was on par with naturally split fruits (0.96 g). These results indicate that fruit splitting using ethrel did not affect nut or mace weight as nut and mace weight of ethrel treated fruits were on par with that of naturally split fruits. This suggests that both nut and mace would have attained maximum weights at physiological maturity stage and retention of these fruits on the tree till natural splitting would not have increased nut and mace weights further.

Dry recovery

Dry recovery of a constituent is its ratio between dry weight to fresh weight. Nutmeg fruit has three parts, pericarp (rind), nut (seed) and the mace. The dry recovery of each part varies depending on its moisture content. Since nut and

Table 2. Nut and mace weight and mace:nut ratio in different treatments

Treatment	Nut FW (g)	Nut DW (g)	Mace FW (g)	Mace DW (g)	Mace: Nut DW basis
E1000 - P	6.89	4.98	2.29	1.01	0.21
E1000 + P	7.54	5.40	2.35	1.03	0.19
E500 - P	8.03	5.49	2.29	1.00	0.18
E500 + P	7.73	5.56	2.16	0.97	0.18
NAA50 - P	6.72	4.83	1.94	0.90	0.18
NAA50 + P	7.62	5.46	2.11	0.91	0.17
NAA25 - P	7.95	5.42	2.31	0.92	0.17
NAA25 + P	7.36	5.19	2.01	0.93	0.18
Control - P	7.81	5.27	2.06	0.92	0.17
Control + P	7.68	5.34	2.12	0.92	0.17
Water dip - P	7.00	5.07	2.11	1.05	0.19
Water dip + P	6.70	4.52	1.82	0.80	0.17
Natural splitting	7.53	5.15	2.17	0.96	0.18
CD at 5 %	0.86	0.74	0.26	0.11	NS

-P = no pedicel, +P = with pedicel, E1000 = ethrel 1000 ppm, E500 = ethrel 500 ppm, NAA25 = NAA 25 ppm, NAA50 = NAA 50 ppm

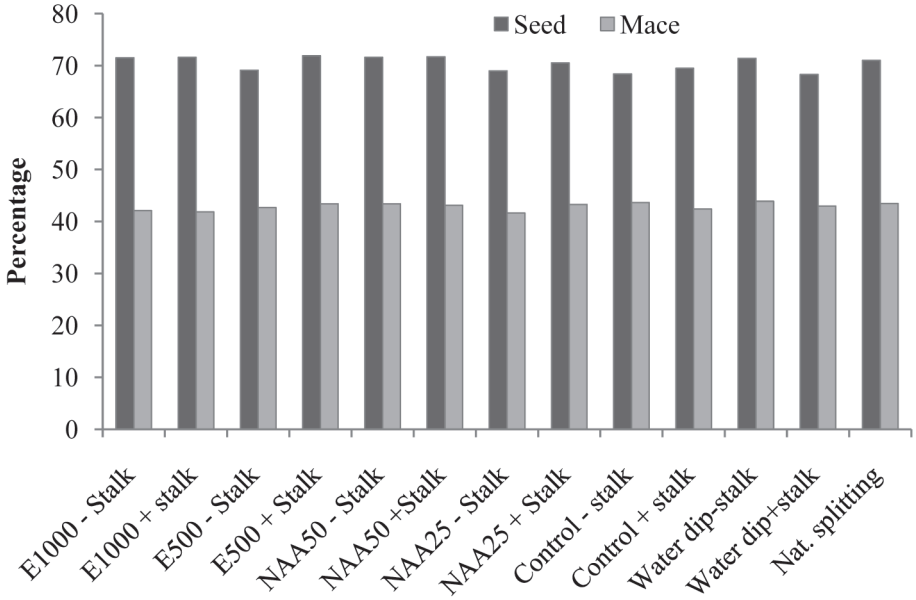

Fig. 1. Dry recovery of nut and mace in different treatments (Each value is a mean of seven individual values)

mace are economically important, dry recovery of these two parts were estimated. Nut dry recovery indicates dry weight to fresh weight ratio of nut and similarly mace dry recovery indicates dry weight to fresh weight ratio of mace Dry recovery of nut ranged from 68.4 (control-stalk) to 71.9 (E500+stalk) per cent in different treatments. Nuts from naturally split fruits recorded 71.0 per cent dry recovery. Similarly, dry recovery of mace varied from 41.5 to 43.5 per cent (Fig. 1). Pooled average dry recovery of all ethrel treatments and all NAA treatments were on par with that of naturally split fruits for both nut as well as mace. This implies that dry recovery of nut and mace were not affected by ethrel and NAA treatments.

Split width was maximum with ethrel treatment and naturally split fruits had a similar split width. This may indirectly hint on the fruit firmness being reduced by ethrel treatment. Increased split width has advantages like it is easy to separate seed from fruit. The only disadvantage noticed with some treated fruits (either ethrel/NAA/control) was the slight difficulty in separating mace from seeds because of stickiness which was not noticed in naturally split fruits. Influence of ethrel treatment on fruit firmness is well documented in many studies. Zhang *et al.* (2012) reported that ethephon could be applied for ripening and maintaining the physico-chemical and quality attributes of kiwifruit. Ethephon decreased firmness and titratable acidity and increased respiration rates significantly in kiwifruit. In tart cherry, ethephon (applied 22 days before harvest at a rate of 3.5 L ha^{-1}) enhanced exocarp color in 'Bing' by 27 per cent, and reduced firmness in both 'Bing' (-19%, 22 days prior to harvest) and 'Chelan' (-15%, 20 days prior to harvest). This proves the potential of ethephon for use in mechanical harvest of fresh market quality sweet cherry fruit (Smith and Whiting, 2010). In persimmon, ethephon (200 mg L^{-1}) decreased quality fruits percentage and firmness of fruits (Kim *et al.*, 2004). In mango fruits also, ethephon reduced firmness (Tomas *et al.*, 2012).

Quality parameters

Oil content in the nut varied from 14.7 to 16.4 per cent. Oleoresin content varied from 26.6 to 28.2 per cent and moisture from 9.5 to 10.3 per cent. All the treatments were on par for oil, oleoresin and nut moisture (Fig. 2.). This suggests that ethrel or NAA treatment did not influence the oil or oleoresin content of nuts.

Mace oil ranged from 13.6 to 15 per cent and mace oleoresin from 24.5 to 26.3 per cent. Moisture

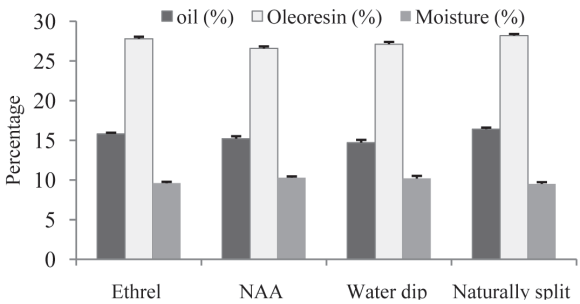

Fig. 2. Quality parameters of nut under different treatments

content ranged from 9.9 to 10.7 per cent. Statistically, all treatments were on par (Fig. 3.) indicating that the mace quality was not affected by ethrel or NAA treatments.

In the present study, though ethrel induced advanced fruit maturity (fruit splitting), the quality of the fruits in terms of oil and oleoresin content of both nuts and mace were on par with the nuts and mace of naturally split fruits. This suggests that ethrel treatment did not affect the quality of the nut or the mace. In coffee also, ethephon treatment did not influence either beverage quality or coffee classification (Carvalho *et al.*, 2003). Pre-harvest application of ethephon increased total soluble solids, reduced titratable acidity, increased TSS:acid ratio, and enhanced fruit quality in mango, (Tomas *et al.*, 2012). Ethephon did not affect pistil length and nut quality in almond (Leonel *et al.*, 2011).

The fresh and dry weights of nut and mace and mace:nut ratio were analysed mainly to ascertain the maturity status of the pre-split harvested fruits. The results revealed that fresh and dry weights of nut and mace and mace:nut ratio of ethrel treated fruits were comparable with those of naturally split fruits indicating that the harvest did not affect the maturity of fruit and they were mature indeed. Had the fruits not fully matured, then the mean nut and mace weight of treated fruits would have been lower than the nut and mace weights of naturally split fruits. This suggests that physiologically mature fruits can be harvested and ethrel can be safely used for inducing synchronous and uniform splitting of these fruits. Leila *et al*. (2011) reported that NAA at 20 or 40 mg L^{-1} increased fruit weight but ethephon did not influence fruit weight in apricot. In the present study also, nuts from ethrel treated fruits had similar fresh (7.55 g) and dry weights (5.35 g) as that of nuts from naturally split fruits (7.53 and 5.15 g respectively). It is justified also as we know that weight gain is a slow process and that organs are not expected to gain weight within minutes after treatment (as fruits were dipped in ethrel for 10 minutes only and the fresh weights were recorded after 20 hours immediately after recording the observation on fruit split percentages).

Cost of ethrel treatment

About 200 litres of 500 ppm ethrel is required to treat 500 kg harvested nutmeg fruits. The solution can be reused for the second time. Hence 200 litres is sufficient to treat 1000 kg fruits. To prepare 200 litres of 500 ppm, 250 mL of 39 per cent ethrel is required, the cost of which is around ₹ 550. About 0.5 labour is required for dipping the fruits and put them for shade drying. Considering that the labour charge is ₹ 500 per day (in Kerala), the labour charges will be ₹ 250 per ton. Hence, the total cost of ethrel treatment per ton of fruit will be around ₹ 800.

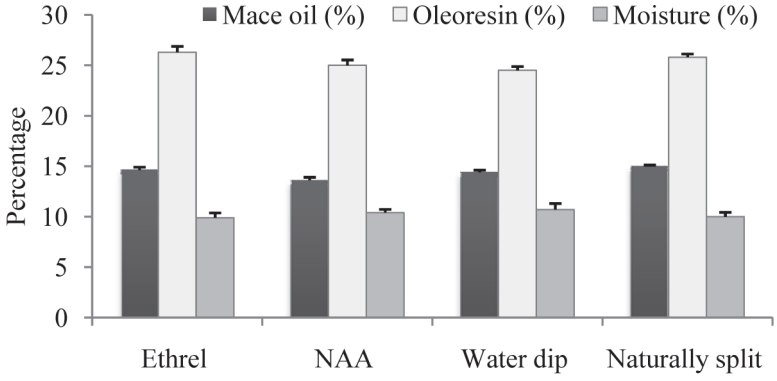

Fig. 3. Quality parameters of mace

There are other advantages of ethrel treatment and pre-split harvest of nutmeg fruits apart from inducing synchronous fruit splitting. In practice, farmers generally do not harvest nutmeg fruits from the tree. Ripened fruits are split on the tree exposing the red mace. These fruits then fall down on the ground and these fallen fruits are collected, mace and nut are separated from the fruits and then dried. Hence, farmers need to visit all the trees almost daily looking for fallen fruits, separate nut and mace from fruits and dry them. This is laborious and time consuming. Also drying has to be done separately each day which also consumes time, labour and money. The other major risk is that since farmers collect fallen fruits with exposed mace and seed which come in contact with soil, chances of aflatoxin contamination is very high. Aflatoxin is highly toxic when consumed and in international market, most often nutmeg and mace are rejected by importing countries due to aflatoxin contamination. The major advantage of artificial ripening of pre-split harvest nutmeg fruits is the prevention of aflatoxin contamination Another problem generally faced by farmers when fruits are split open on the tree exposing red mace is the damage of mace by rodents and birds. This can be avoided by pre-split harvesting.

Conclusion

In this study, a hormone treatment was developed for synchronous splitting of nutmeg fruits and separate nut and mace by harvesting the physiologically mature fruits and treating them with ethrel, reducing the risk of aflatoxin contamination, which normally occurs when ripened fruits fall on soil. The dry recovery, nut to mace ratio, intrinsic quality and fresh and dry weights of the nut and mace of the treated fruits were comparable with that of naturally split fruits. The treatment is cost effective, simple and very effective to induce fruit splitting. This technique, being the first report to induce synchronous fruit splitting of physiologically mature nutmeg fruits using ethrel/NAA could induce uniform ripening of fruits without compromising its quality.

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