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Postharvest VeSolution treatment mitigates rot in pomegranate (*Punica granatum* L.) fruits

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ABSTRACT

Purpose: Pomegranate is of considerable economic significance, with Maharashtra, Karnataka, and Gujarat serving as the primary cultivation regions. Despite the high production levels, postharvest losses are serious, with certain fruits experiencing fruit cracking, fungal infections, and poor handling during transportation, resulting in postharvest losses of up to 35%. For controlling postharvest infections in a variety of fruits and vegetables, synthetic fungicides are incredibly effective. **Research Method:** VeSolution is a GRAS salt-based formulation with antimicrobial properties developed to assess its efficacy in minimizing fruit rotting. Therefore, the present investigation examined the effectiveness of VeSolution in reducing these postharvest losses. The infected fruits were used to isolate and identify fungal pathogens. Subsequently, the antifungal efficacy of the VeSolution formulation was assessed by both *in vitro* and *in vivo* methodologies. The *in vitro* investigations entailed evaluating the formulation's inhibitory effects on mycelial growth of identified fungal pathogens on PDA plates. In the in vivo evaluation, artificially inoculated pomegranate fruits were subjected to VeSolution treatment to test their effectiveness in mitigating rot advancement. Finally, VeSolution-treated pomegranates were exposed to supply chain conditions to assess their practical efficacy. Critical parameters, including rot advancement, in-transit spoiling, and fruit quality were assessed. **Findings:** *Aspergillus* sp., *Alternaria* sp., and *Coinella* sp. were identified as fungi associated with pomegranate fruit rotting. The results indicated that the growth of the fungal colony was substantially inhibited by the 2% and 5% concentrations of VeSolution. The fruit rot development and progression were effectively restricted by the 2% VeSolution, as confirmed by *in vivo* assessments. Subsequent pilot and large-scale trials demonstrated that 1% VeSolution substantially reduced rot during longer (> 24 h) transportation periods. **Research limitations:** There were no limitations. **Originality/Value:** These results emphasise VeSolution as a viable and non-toxic alternative to conventional synthetic fungicides for maintaining the postharvest quality of pomegranates.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a highly economical fruit due to its wide adaptability to different agroclimatic zones. Indian states including Maharashtra, Karnataka, Gujarat, and Andhra Pradesh are the major contributors to pomegranate production (Jadhav et al., 2023). The varieties like Ganesh, Mrudula, and Bhagwa are commercially cultivated in Solapur, Nashik, Pune, Ahmednagar and Aurangabad districts of Maharashtra. Despite the highest production, India loses 35% of the yield due to cracking, fungal rotting, over-ripening, dehydration, and wound damages during postharvest handling and transportation of fruits (Murthy et al., 2009; Ranjani et al., 2023). Fruit losses are scattered throughout many supply chain stages, including the field, wholesale, and retail locations, which are all connected to transportation (Ambalavanan et al., 2024). In addition to this improper handling of fruits, poor vendor hygiene, unfavourable ambient factors, and sanitary risk may further intensify the market circumstances that encourage postharvest losses. In India, 10% of crop losses occur throughout the cultivation and distribution stages, while an additional 15% occur at the retail stage (Murthy et al., 2009). Fungal rotting including soft rot, anthracnose, black heart rot and gray mold represent about 65% of the total postharvest losses in pomegranate (Mincuzzi et al., 2022).

Minimizing postharvest losses in pomegranate is the prime objective, especially during long-distance transit from source to destination markets. Synthetic fungicides are extremely efficient in managing postharvest infections in a wide range of vegetables and fruits. Waskar et al. (1999) reported that postharvest treatment with fungicides like carbendazim and captan controlled fruit rotting in pomegranate. Similarly, the primary approach to preventing, controlling, or eliminating postharvest pathogens has been the creation of novel synthetic chemicals over the last several decades. Under normal circumstances, fungicides used at postharvest are usually more fungistatic than fungicidal. These fungicides are often applied as fumigants, dips, sprays, treated wraps, and box liners or may be incorporated with waxes and coatings (Ambalavanan et al., 2024).

Although they are effective, these chemicals can disrupt the balance of the ecosystem if used repeatedly (Camele et al., 2010). This may lead to the development of new pathotypes that are resistant to one or more of these chemicals. Additionally, these chemicals may be toxic to organisms that are not the intended target, and they can sometimes accumulate as residues in the food chain, exceeding safe limits. Due to this, alternatives like biocontrol agents and botanicals are gaining commercial importance in the area of crop protection and management (Shricharan et al., 2020). Moreover, owing to health and environmental concerns, the use of postharvest fungicides has been regulated throughout the world.

Basic substances are substances like lecithin, talc, vinegar, chitosan, mustard seed powder, etc. are nontoxic and not predominantly used in plant protection but can be used in plant protection. In recent years, multiple inquiries have been conducted in this domain to explore the antimicrobial characteristics of Generally Recognized as Safe (GRAS) salts (Guimaraes et al., 2019; Martinez-Blay et al., 2020; Allagui et al., 2024). GRAS substances including botanicals, essential oils, inorganic and organic salts including bicarbonates, benzoates, silicates, metabisulphites, etc (Palou et al., 2016) are gaining attention as they can be employed to mitigate postharvest problems due to their exemption from residual limits on all agro-commodities by US FDA (Palou, 2018; Romanazzi et al., 2022). A novel formulation termed VeSolution was developed utilizing GRAS salts (metabisulphite salts) with potent antimicrobial properties. Moreover, studies on controlling fruit rotting particularly for long-distance transportation are lacking in pomegranate.

Therefore the work was carried out with the following objectives (i) to evaluate *in vitro* antifungal activity of different concentrations of VeSolution against the isolated fungi (ii) to assess *in vivo* activity of the most promising concentration(s) of VeSolution to control pathogens associated with fruit rotting, (iii) to study the effectiveness of postharvest dipping of pomegranates in promising concentration(s) of VeSolution to control rotting, (iv) to investigate the impact of VeSolution treatment(s) in reducing rotting under short and long-distance transport conditions.

MATERIALS AND METHODS

VeSolution and fruit sample collection

VeSolution is a formulation (metabisulphite salt + 0.5% Tween 20) (developed by Velabs, Vegrow, Bengaluru, Karnataka) designed to decrease fruit rotting and to maintain the quality of pomegranate fruit. This was developed from an inorganic salt that has been previously recognized as GRAS and reported as an antifungal, antioxidant, and reducing agent in the food industry (Kolaei et al., 2012; Mladenović et al., 2018).

The pomegranate fruit rotting is primarily associated with fungal pathogens. Hence for its isolation, pomegranate fruits (var. Ganesh) that exhibited rotting symptoms as described by Ambalavanan et al. (2024) were collected from fruits received at Vegrow Distribution Centre (DC) (Bengaluru, Karnataka) from Bhuj (Gujarat, India) with a transit length of around 60 to 70 hours. The samples were collected and stored at 4 °C until isolation. The subsequent experimentations and data collection of *in vitro* and *in vivo* were carried out at the in-house R&D facility of Vegrow, Bengaluru, and Karnataka.

Fungal isolation, purification and identification

The symptomatic region from the fruit was cut into small pieces with a sterile blade, surface sterilized in 1% sodium hypochlorite for 1 min, and plated aseptically on PDA pH 5.6±2 supplemented with chloramphenicol 100 µg ml^{-1} to prevent bacterial contamination and incubated at 25±1 °C for 5 days. A pure culture was obtained by single spore method and maintained by sub-culturing the different colonies that developed on PDA plates and incubated at 25±1 °C for 5 days. The colony morphology and slide culture techniques were followed for microscopic examination of fungi. Briefly, a sterilized microscopic slide was placed on a bent glass rod in a sterilized petri plate. About 1×1 cm agar block was cut from a PDA plate and transferred to the glass slide, and the fungi were inoculated using a loop on the top four corners of the agar block. The agar block was covered with a cover slip from the top. The plate was covered and incubated at 25±1 °C for 3-4 days. For microscopic observations, the cover slip from the inoculated agar block was removed and placed inverted on a drop of Lactophenol Cotton Blue (LPCB) stain on a new slide and observed under a microscope for the identification of fungi (Leck, 1999).

In vitro assay of VeSolution

VeSolution was initially tested under *in vitro* conditions against the isolated pathogens. PDA medium was amended with concentrations of VeSolution ranging from 2%, 5%, 10%, and 20% before autoclaving at 121 °C for 20 mins. The PDA without VeSolution served as a negative control (CK-ve) while the PDA with 0.1% Fludioxonil served as a positive control (CK+ve). A mycelial plug (4 mm diameter) was taken using a cork borer from 7 to 10 days old pure culture and placed on the center of the PDA plates with pH 5.6±2. The plates were then incubated at 25±1 °C. The radial mycelial growth was determined in each Petri plate by measuring two perpendicular diameters (in mm) of the fungal colony. The

measurements were taken once when the mycelial completely covered the plate in CK-ve. The assay was conducted in triplicates and the results were expressed as percentage inhibition of radial mycelial growth (IRMG) using the formula as described by Bouhlali et al. (2021) (1).

$$IRMG\ (\%) = [(dc - dt)/dc] \times 100 \qquad (1)$$

Where, dc: average diameter of fungal colony in control plates (mm); dt: average diameter of fungal colony (mm) in assayed petri plates.

***In vivo* curative assay of VeSolution in pomegranate fruits**

The pomegranate fruits for *in vivo* assay were procured directly from farms around Karnataka. The healthy fruits were selected without wounds, and randomly divided into 4 sets, and their surface was sterilized by dipping them in 1% sodium hypochlorite solution for 1 min, rinsed twice with sterile water, and air dried. For fruit inoculation, the spores were harvested by adding 0.05% Tween 20 to the pure cultures, scraped with a sterile rod and filtered through 3 layers of cheesecloth. The spores were counted with a hemocytometer under a microscope and diluted to the concentration of 1×10^5 spores/ml with 0.05% Tween 20. The surface sterilized pomegranates were wounded aseptically with a 3 mm cork borer and each wounded site of the fruit was inoculated with 20 µl spore suspension (the suspension was mixed by vortexing before inoculation).

The curative assay was carried out by dipping the fruits for 2 mins in a predetermined promising concentration of VeSolution after 24 h of inoculation with spore suspension. The fruits dipped in sterile water were considered as CK-ve and fruits dipped in 0.1% fludioxonil served as CK+ve. The experiment was conducted with three replications with 10 fruits per replication. All the treated fruits were stored in plastic crates at room temperature for 10 days. The rot diameter on each fruit was measured (in mm) perpendicularly on the 10^{th} day and expressed as rot inhibition % using the formula as described by Allagui and Ben Amara (2024) (2).

$$Rot\ inhibition\ (\%) = [(dc - dt)/dc-] \times 100 \qquad (2)$$

Where, dc- is the rot diameter (mm) of CK-ve and dt is the rot diameter (mm) of the treated fruits.

Pilot scale and implementation trial in transit conditions

Based on the preliminary trial, the pilot scale trial of postharvest treatment of VeSolution was conducted from Lingsugur, Karnataka to different destinations in trucks under ambient conditions. The details of the shipment including the source, destination, and sample quantity are given in Table. 1. Based on the results from the pilot trial, this process was implemented on a large scale from different source locations to the destination markets with transit hours ranging from 15 h to 84 h (Table 2). The rotting percentage was estimated (as described earlier) in both pilot and implementation trials once the shipment reached the destination markets.

Statistical analysis

The experiment was conducted in CRD and the analysis of variance (ANOVA) was carried out in WASP 2.0 (Web Agri Stat Package 2) statistical tool (www.icargoa.res.in/wasp2/index.php). The variables were transformed for normality and the graphs were developed in GraphPad Prism version 9.2.0 for Windows, GraphPad Software, Boston, Massachusetts USA.

Table 1. Details of pilot shipments and the treated quantity in 1% and 2% VeSolution.

Shipment No.	Source	Destination	Total (Kg)	Control (Kg)	Treated (Kg) in 1% VeSolution	Treated (Kg) in 2% VeSolution
1	Lingsugur	Delhi	70	20	0	50
2	Lingsugur	Bengaluru	50	20	30	0
3	Lingsugur	Bengaluru	80	30	50	0
4	Lingsugur	Bengaluru	80	30	50	0
5	Lingsugur	Bengaluru	90	18	36	36
6	Lingsugur	Bengaluru	90	18	36	36

Table 2. The process implementation of postharvest treatment from source to destination and their transit time.

Source	Destination	Transit time (h)
Nashik	Guwahati	84
Nashik	Lucknow	65
Pune	Bhubaneswar	50
Pune	Bengaluru	18
Sangola	Hyderabad	15

RESULTS

Morphological and microscopic characterization of the isolated fungal pathogens

The identification was done after purification of the colonies isolated from the rotten samples of pomegranate. The fungal colonies were identified by observing the colony growth and morphology on potato dextrose agar (PDA) plates and after microscopic examination. The initial isolated fungi colony exhibited white growth with a complete margin, which was subsequently transformed to black pigmentation as a result of spore production. Proper development of septate hyphae was evident in their extensive branching. The isolated fungi was confirmed as *Aspergillus* sp. due to the globose conidia and brown to black conidial head, which were similar to the *Aspergillus* characterised by Romero-Cortes et al. (2019) and Shricharan et al. (2020). The subsequent colony was initially light grey in colour, but it transitioned to a dark brown to black colour with a white margin. Conidiophores were present on the hypahe, which were pale brown in colour. Conidia were dark brown in pigmentation and consisted of 3 to 4 transverse septa. Their morphology was typically ovate to obclavte. The fungi were identified as *Alternaria* sp. based on the characteristics reported by Yu et al. (2016) and Saleem and El-Shahir et al. (2022). The third colony was initially white and was transformed to brown as it grew and developed pycnidia. Hayline, single-celled, elongated, ellipsoid to fusiform conditions were observed. The observations were consistent with those of Uysal et al. (2018) and Mahadevakumar et al. (2019) and were subsequently identified as *Coinella* sp.

VeSolution inhibited the growth of fungal colonies under *in vitro* condition

In general, the radial mycelial growth of all three fungal colonies was inhibited by VeSolution, and the percentage of inhibition increased as the concentration of VeSolution (VS) increased. The control (CK+ve) plates exhibited the highest percentage of inhibition in all three fungal colonies, while the maximal colony diameter was observed in the CK-ve plates. In particular, the colony growth was not significantly affected by 10% VS, 15% VS, or 20% VS, while 2% VS and 5% VS exhibited a significant growth inhibition as compared to CK+ve plates (Fig. 1). Subsequently, the toxicity effect of these concentrations of VeSolution

was checked on the pomegranate surface. Pomegranate fruits exhibited toxicity and skin burn symptoms at concentrations exceeding 5% (data not presented). Therefore, considering the fungal growth inhibitory properties of VeSolution and the fruit appearance, 2% VS and 5% VS were chosen as prospective interventions for *in vivo* fruit inoculation studies.

Curative VeSolution treatments reduced the fungal rot progression in pomegranate fruits

Pomegranate fruits that were inoculated with spore suspension exhibited rot progression symptoms 3 days after the curative VeSolution treatment. Initially, the inoculated spores germinated and sporulated, resulting in the visible growth of sporulated fungal growth on CK-ve fruits. The fungal growth had penetrated the fruits and was beginning to infect the arils by six days after treatment (DAT). Notably, the highest diameter (in mm) of rot progression was observed in CK-ve fruits. Whereas, the highest inhibition in rot progression percentage was observed in CK+ve on the 10th day of curative treatment, followed by 2% VS and 5% VS treatments (Fig. 2). Although both the 2% VS and 5% VS treatments significantly reduced rot progression in comparison to CK+ve, there was no significant difference in VeSolution concentrations. The 2% VS treatment was chosen for further investigation due to its apparent ability to impede fungal development (Fig. 3 & Fig. 4).

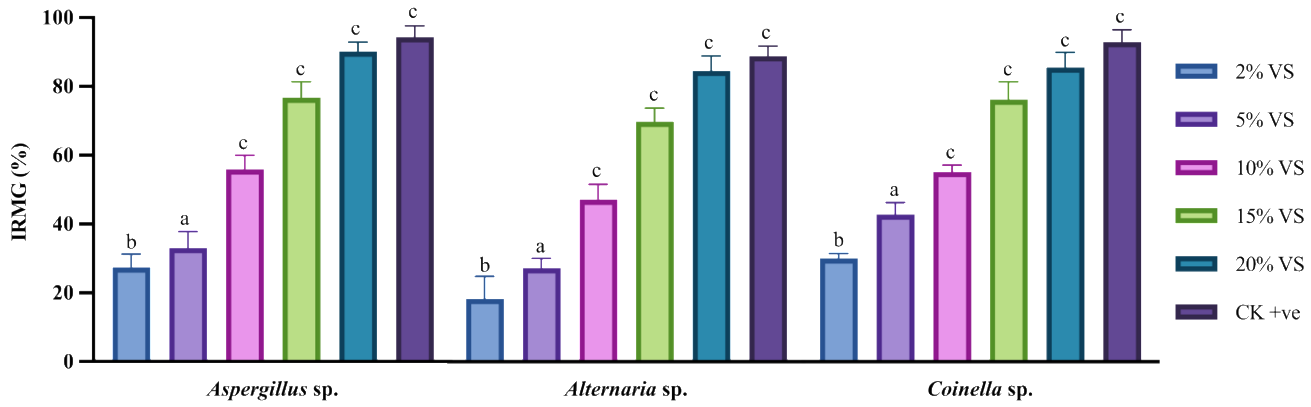

Fig. 1. The inhibition of radial mycelial growth by VeSolution on *Aspergillus* sp., *Alternaria* sp. and *Coinella* sp. under *in vitro* conditions. The bars with different letters are statistically significant ($P \leq 0.05$).

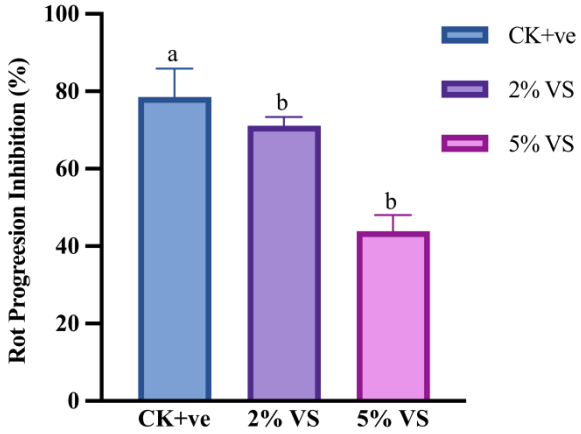

Fig. 2. The Inhibition of rot progression (%) by VeSolution on pomegranate fruits under *in vivo* conditions. The bars with different letters are statistically significant ($P \leq 0.05$).

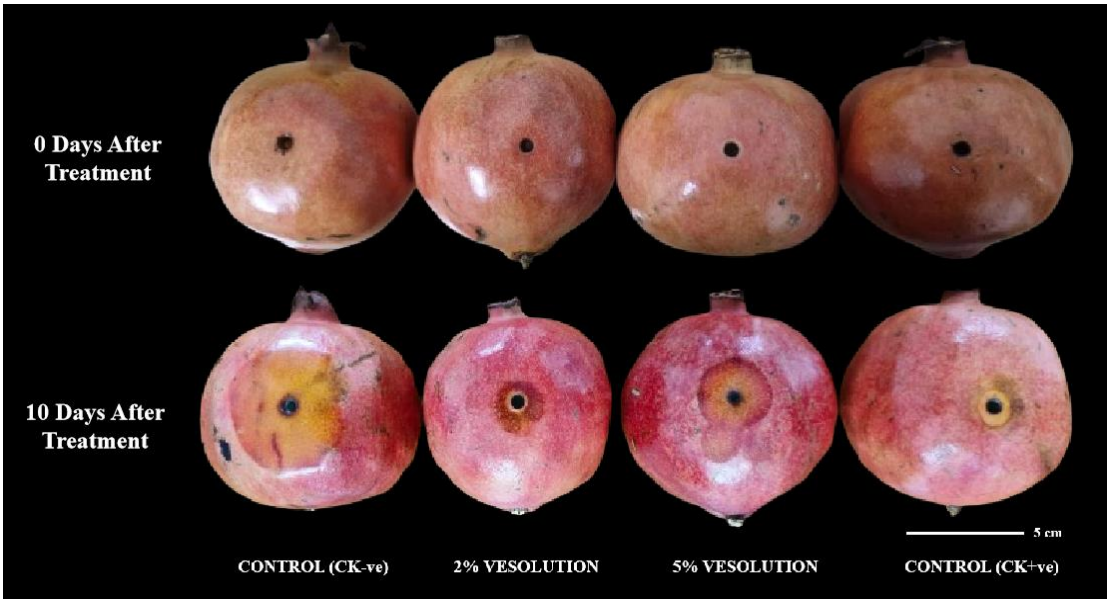

Fig. 3. The progression of fruit rot in pomegranate inoculated with fungal spores and with post curative VeSolution treatment. The treated fruits exhibit variations in rot progression over time demonstrating the treatments efficacy in reducing rot progression compared to control (CK-ve) fruits.

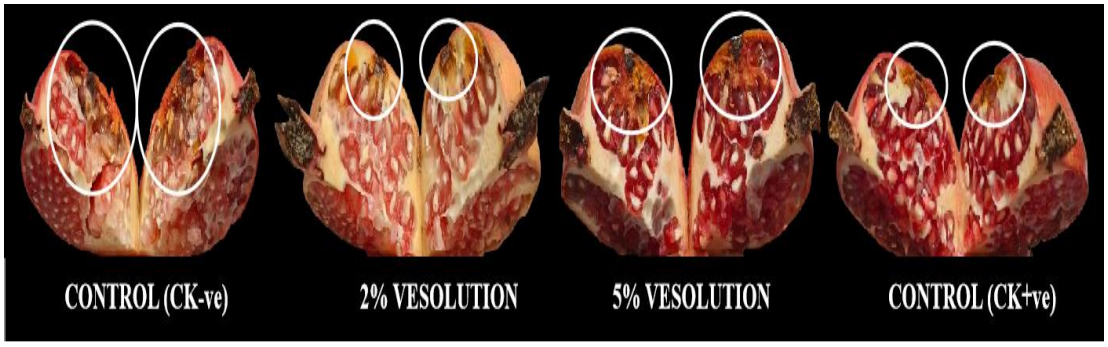

Fig. 4. The severity of rot progression inside pomegranate fruits at 10 DAT. The progression of fungal sporulation and infection within the peel and arils are highlighted within circles.

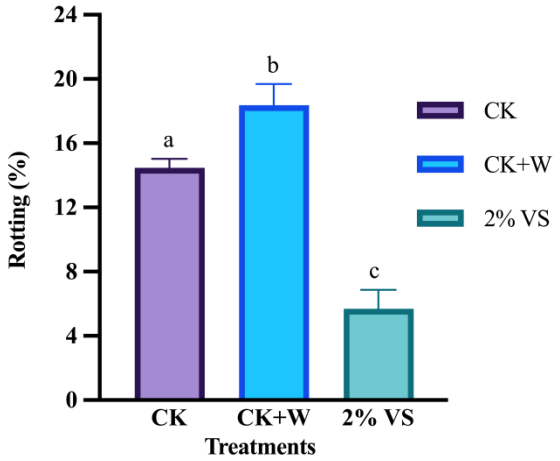

Fig. 5. The effect of postharvest treatment of 2%VS on rotting with controls (dry and wet). The bars with different letters are statistically significant ($P \leq 0.05$).

Significant reduction in rotting percentage achieved with 2% VeSolution treatment

Based on the above investigations, the decaying percentage was significantly reduced by the usage of 2% VeSolution at 6 days after treatment (DAT) in comparison to the control (CK) and water-treated control (CK+W). In CK+W, the highest decaying percentage was observed (Fig. 5), suggesting that moisture is a critical factor in the proliferation of pathogens. This is because the presence of water fosters pathogen growth and infection. In contrast, the dry control (CK) demonstrated a lower decaying percentage, but it was not as effective as the 2% VeSolution treatment. This implies that 2% VeSolution not only effectively regulates fruit spoilage but also preserves the fruit's overall quality and appearance.

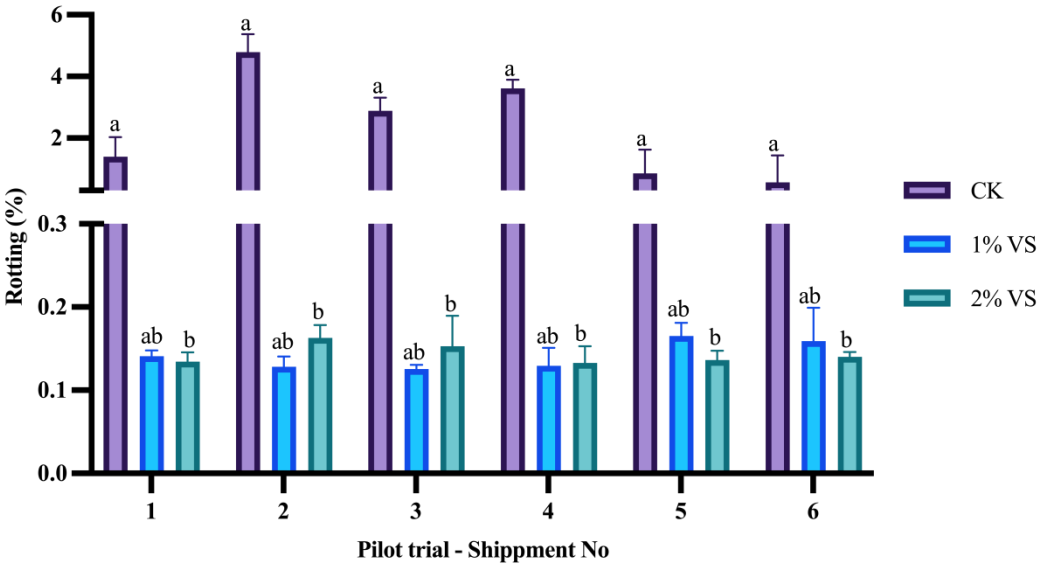

Fig. 6. The effect of postharvest treatment of 1%VS and 2%VS on rotting % during 6 pilot trial shipments. The bars with different letters are statistically significant ($P \leq 0.05$).

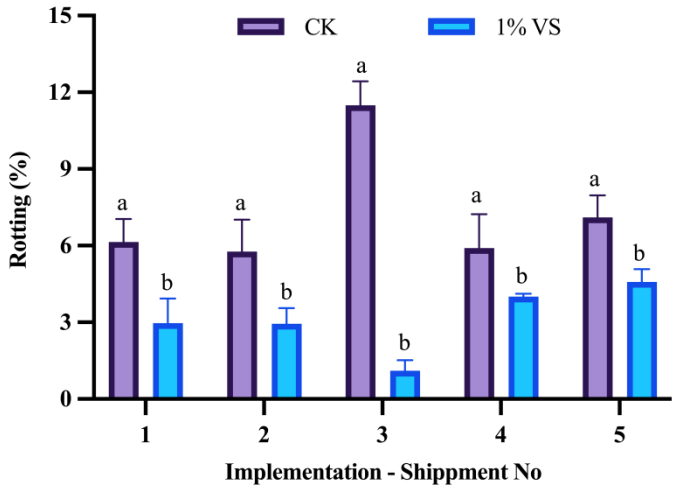

Fig. 7. The effect of postharvest treatment of 1%VS implementation on rotting % during long distance transportation. The bars with different letters are statistically significant ($P \leq 0.05$).

Postharvest VeSolution treatment controlled pomegranate fruit rotting under long distance transit conditions

Eventually, a pilot trial was conducted in which the shipments were dipped in 1% (not optimized earlier) and 2% VeSolution. The results were obvious that CK showed the highest % of rotting while both 1% and 2% VeSolution showed significantly least % of rotting in all shipments to Bengaluru and Delhi from Lingsugur (Fig. 6). In particular, 1% VeSolution showed only the least significant variation as compared to 2% VeSolution. Therefore the process of postharvest treatment with 1% VeSolution was implemented in large shipments with transit time ranging from 15 h to 84 h approximately. The results revealed that CK showed the highest rotting % in all shipments while 1% VeSolution significantly reduced the rotting % as compared to CK (Fig. 7). Particularly, 1% VeSolution was effective in controlling rotting till 84 h with the highest efficacy (91%) in Pune to Bhubaneswar shipment with a transit time of 50 h.

DISCUSSION

The isolated fungal colonies were identified based on its growth, morphology and microscopic characters based on the previous reports on *Aspergillus* (Romero-Cortes et al., 2019; Shricharan et al., 2020), *Alternaria* (Yu et al., 2016; Saleem & El-Shahir, 2022) and *Coinella* (Uysal et al., 2018; Mahadevakumar et al., 2019). Furthermore, the effect of GRAS sodium salt on the spore number and hyphae morphology was reported by Lyousfi et al. (2023). The microscopic observations showed a decrease in spore number and tight aggregations of hyphae with abnormal bulges, disruptures, and swellings when treated with GRAS salts while control samples showed normal morphology. Similarly, morphological damage was detected in *B cinerea* hyphae treated with GRAS salts compared to the control. The GRAS salt-treated hyphae had shriveled and unusual bulges on the surface as compared to the control (Youssef et al., 2019).

Additionally, the evaluation on fungal growth under different concentration of VeSolution showed growth inhibition properties with increase in concentration. Similarly, the antifungal properties of 17 GRAS salts were evaluated against postharvest fungal diseases of citrus fruit. The results revealed that 1% sodium silicate showed 100% inhibitory action against every tested fungus. Conversely, sodium carbonate (1%) had no efficacy against *G. citri-aurantii* or *G. gloeosporioides* but was 100 % effective against *P. digitatum* and *P. italicum* (Zhao et al., 2023). In context to this, Lyousfi et al. (2023) studied the *in vitro* effect of a few organic and inorganic food additive salts against *Monilina fructigena.* The majority of the additives demonstrated a notable reduction in mycelial growth, however, the percentage of inhibition varied depending on the additives and their concentration. Sodium bicarbonate, sodium carbonate, and copper sulphate were the most effective with ammonium carbonate and citric acid showing the least effectiveness. Furthermore, similar results were reported by Allagui and Ben Amara (2024) on *the in vitro* efficacy of sodium metabisulfite (SMB), ammonium bicarbonate, sodium bicarbonate and potassium dihydrogen orthophosphate against *Alternaria alternata, Botrytis cinerea, Penicillium italicum* and *Penicillium digitatum.* Results revealed that 0.2% SMB showed complete inhibition of all the fungal species. While bicarbonate salts of ammonium and sodium were least effective at 0.2% in inhibiting fungal growth.

In addition, numerous research has been carried on studying the antifungal activity of GRAS salts against postharvest fungal pathogens responsible for fruit rotting in citrus (Guimarães et al., 2019; Soto-Munoz et al., 2020), stone fruit (Martinez-Blay et al., 2021), grapes (Youssef & Roberto, 2014), pomegranate (Palou & Taberner, 2022), kiwi

(Türkkan et al., 2017), banana (Alvindia & Natsuak, 2007), and mango (Kalupahana et al., 2020).

Although, *in vitro* tests may be used to determine the potential of GRAS salts to reduce postharvest rotting pathogens. However, *in vivo* bio assays are still required to validate the *in vitro* findings on the fruit. Our present study results on *in vivo* fruit inoculation showed notable significant decrease in rot progression on the surface and inside the fruit surface treated with 2% and 5% VeSolution as compared to controls. Similar results were also reported by Youssef et al. (2012) that potassium sorbate (GRAS salt) reduced rotting in citrus. Comparably, metabisulphite, sulphite derivates, and citric acid were reported to be effective in controlling molds (Sgroppo et al., 2010).

In a related study, Lyousfi et al. (2023) reported the use of 2% sodium sulfate in controlling brown rot in apples. Chloride and carbonates of calcium treatments minimized stem end rot in mangoes (Montecalvo et al., 2023). Interestingly, Pedrozo et al. (2024) optimized the compatible combinations of biocontrol yeast (*Metschnikowia pulcherrima*) with GRAS salt (sodium bicarbonate) and reported their ability to control blue rot in table grapes.

However, the above reports concentrated on the use of GRAS substance to improve storage while its effect on in-transit fruit rotting has not been studied. Conversely, the results from our present study demonstrated the effectiveness of postharvest dipping of fruit in 1% VeSolution in significantly controlling rotting under long-distance transit conditions. Additionally, the studies related to MAP and CA for managing rotting under prolonged transport and storage were reported earlier in pomegranate (Fuchs et al., 2007), Indian gooseberry (Singh et al., 2023), cherry (Cabañas et al., 2023), guava (Yadav et al., 2022) and the role of 1-MCP in inducing postharvest disease resistance in fruits to improve shelf life was reviewed by Ranjani et al. (2023).

CONCLUSION

The VeSolution presents a promising solution for reducing rotting during transportation in pomegranates. Initially, *Aspergillus* sp., *Alternaria* sp. and *Coinella* sp. were purified after isolation from the rotten pomegranate samples. The primary *in vitro* and *in vivo* studies with different concentrations of VeSolution demonstrated efficiency in minimizing the growth of fungal pathogens associated with pomegranate fruit rotting. However, concentrations above 5% showed toxicity symptoms on the fruit surface. The pilot trials revealed the effectiveness of 1% VS in minimizing pomegranate rotting and the same was implemented in larger shipments which ensured minimal rotting up to 84 h of transit. This implementation offers a practical strategy for long-distance fruit transportation. Overall, this study underscores the potential of using VeSolution as an alternative to traditional fungicides in minimizing postharvest losses and maintaining fruit quality during transportation, thus enhancing the economic viability of pomegranate production.

Conflict of interest

The authors declare no conflicts of interest.

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Conceptualization of research work and designing of experiments (SS, ARD); Execution of lab and shipment trial experiments and data collection (SS, ARD); Analysis of data and interpretation (SS, BV); Preparation of initial draft of the manuscript (SS); Contributed to

writing through review and editing (KJH, AAB). All authors read and approved the final version of the manuscript.

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