

NEEM SEED OILCONTAINING METHYLCELLULOSE AS NEW POSTHARVEST ORGANIC EDIBLE COATING FOR TABLE GRAPES DURING COLD STORAGE

OLORUNSOGBON, S. T.* – ÖZDEN, M. – RILEY, I. T.

Department of Plant Production and Technologies, Faculty of Agricultural Science and Technologies, Nigde Omer Halisdemir University, Nigde, Turkey

**Corresponding author*

e-mail: topesogbon@gmail.com; ORCID: 0000-0002-6488-1332

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Abstract. Table grapes have a high-water content and are non-climacteric. They are prone to degradation and infections. This study reveals the effect of neem seed oil combined with methylcellulose (NSOMC) at (control, 0.5%NSOMC, 2%NSOMC, 8%NSOMC, 32%NSOMC and 1% of methylcellulose (MC) prolonging the postharvest quality of Sultana table grapes during 60 days of storage at 0.5°C. The different concentrations of the NSOMC were found effective compared to the control and 1%MC. NSOMC coated fruits, especially at 32% showed less decay incident (3.1%), weight loss (0.32%) and colour changes (8.49) compared to the other coated fruits and the control. 32%NSOMC coated fruits retained their total soluble content and titratable acidity with the lowest mean pH value (3.43). Phenolic compounds (total phenolic and total flavonoid content) had the highest mean and the antioxidant activity was reduced in 32%NSOMC coated fruits. Therefore, our findings suggested that the neem seed oil combined with methylcellulose is safe and could be used as a commercial treatment to increase the shelf life of table grapes. The higher the concentrations of the neem seed oil, the greater the efficiency of the treatments.

Keywords: *table grapes, neem seed oil, methylcellulose, soluble solid, titratable acidity, weight loss, colour, fruit decay, phenolic compound, antioxidant activity*

Introduction

Table grapes (*Vitis vinifera* L.) are widely grown for consumption and they are mostly esteemed by consumers because of their unique aroma, revitalizing taste and rich in nutrition. Generally grape berries are characterized by their bright colour, juicy taste, and high nutrient and carbohydrate contents. Grapes store a variety of nutritional health benefits such as polyphenols, antioxidants, vitamins, and minerals. However, grapes prone decay as a result of fungus infection and physiological disorders and pathogen infection after harvest (Xu et al., 2019; Zhang et al., 2022). Numerous postharvest fruit treatments such as chemical, biological and physical were used to prolong the quality and retard decay (Ehtesham Nia et al., 2021; Eshghi et al., 2021; Palou et al., 2010). In recent decades most commercial preservation methods of grapes have been inseparable from the use of chemical treatments (Winkler and Jacob, 1925; Xiao et al., 2019). Although chemical treatments (sulphur dioxide) have been effectively proven to retain the post-harvest quality of table grapes by controlling the physiological and biochemical changes (Xue and Yi, 2017; Zhang et al., 2022). However, sulphur dioxide caused unpleasant changes in berry colour and taste because of the chemical residues in the fruits and undesired effect on the environment and consumers' health. The researchers must consider the consumers' health and their environment by using edible products and secure food coatings which warrants more postharvest research.

Edible coatings are natural food products and eco-friendly alternatives that protect food from deterioration and can as well be eaten directly along with the packed food products (Yong and Liu, 2021). Bill et al. (2014), Sivakumar and Bautista-Baños (2014), Sellamuthu et al. (2013) and Tesfay et al. (2017) reported that Edible coatings and essential oils effectively reduced postharvest diseases and prolonged shelf-life of the fruits. Edible coatings include biopolymer polysaccharides and proteins (Diaz-Montes and Castro-Munoz, 2021; Tavares et al., 2021). Neem (*Azadirachta indica*) is a member of the Meliaceae family and a fast-growing tree native to many Indian subcontinents and African countries, with antimicrobial properties attributed to the active compound *Azadirachtin* (Das et al., 2021). Neem seed oil (NSO) is a natural mixture of biologically active ingredients derived from neem tree seeds (Subapriya et al., 2005). The neem extract effectively controlled tomato fruit rot and preserved postharvest quality and shelf life all through storage (Hosea et al., 2017). *Azadirachta indica* oil has been shown to be environmentally friendly and nontoxic to humans. (Shaik et al., 2021)

Methylcellulose (MC) is one of the edible coatings with cheap cost and transparent viscous polymer in aqueous environments (Li et al., 2002). It is also a water-soluble polysaccharide commonly used in the food industry (Filipini et al., 2020). Methylcellulose (MC) is a highly efficient antimicrobial edible coating that is a barrier against oxygen and aroma compounds (Skurtys et al., 2011). Methylcellulose and Carboxymethyl cellulose are both derived from cellulose (polysaccharide) which improves postharvest quality and extends the shelf life of some of the fruits such as peach (Hussain et al., 2016) and grapes (Yinzhe and Shaoying, 2013). Therefore, there should be alternative postharvest treatments that will work with the directive and be effective in reducing postharvest losses. However, there is no literature currently reporting the efficacy of neem seed oil combined with methylcellulose enhancing the quality of the table grapes during cold storage life. Therefore, this study was to evaluate the Neem seed oil (NSO) containing Methylcellulose as a new postharvest organic edible coating for table grapes during cold storage.

Material and methods

Plant material

At a mature stage, the seedless table grapes (*Vitis vinifera* L. cv. Sultana) were harvested from a commercial vineyard in Nigde, Turkey. The grape clusters were immediately transported to the laboratory of Nigde Omer Halisdemir University's Ayhan Sahenk Agriculture and Technologies Faculty. Clusters with no physical or pathological defects and with uniform colour, size, and firmness were randomly selected and disturbed into batches.

Preparation of neem seed oil

The neem seeds were gathered from the Federal University of Technology Akure, Nigeria campus. The seeds were brought to the lab, completely cleansed with distilled water, allowed to dry for 30 days in the air, and then processed into a fine powder using a Binatone electric blender (Model 373). 500 grams of finely ground powder were added to the Soxhlet apparatus, where they were extracted using 1 L of ethanol for 3 h at 50°C. The oil was then exposed to air so that some of the volatile solvent evaporated after redistilling the Soxhlet content using a rotary evaporator.

Postharvest treatment

The fruits were assigned to six postharvest coating treatments, including control, 1% of methylcellulose (MC), 1%MC + 0.5% of neem seed oil (NSO), 1%MC + 2% of neem seed oil (NSO), 1%MC + 8% of neem seed oil and 1%MC + 32% of neem seed oil. Each treatment consisted of five grapes clustered in triple replicate. The grape clusters were dipped in the different concentrations for 5 min before drying for 2 h at room temperature (25°C). The fruits were then placed in cold storage for 60 days at 0.5°C and 90% RH (Fig. 1).



Figure 1. Methylcellulose coated grapes (A). NSOMC coated grapes and uncoated grapes (B). Coated and Uncoated grapes (C) in cold storage

Postharvest fruit quality evaluation

The fruits were evaluated at 15 days intervals for different physical and chemical quality parameters such as fruit decay, weight loss, colour, total solid soluble, titratable acidity, pH, total phenolic, total flavonoid content and antioxidant activity.

Fruit decay, weight loss and colour

The percentage of fruit decay was evaluated by counting numbers of rotten fruits at each sample. The weight loss of the grapes following different cold storage periods was calculated. The weight loss percentage was calculated by estimating the fruits' final and

initial weight. Fruit colour was measured in L* a* b* colour space coordinates with a Minolta CR-200 chromo meter (Minolta Camera Co., Osaka, Japan) and converted to chroma values (Mcguire, 1992) (Fig. 2).



Figure 2. Fruit decay (D). Checking the weight loss (E). Measuring the colour (F)

Total solid soluble, titratable acidity and pH

The fruit juice extracted from each sample was used to evaluate the total solid soluble using a digital refractometer, expressed in percentage. Titratable acidity (TA) was determined by titrating 0.1 N sodium hydroxide (NaOH) solution into 10 ml fruits juice obtained from each sample and then homogenized until the end point of pH 8.2 was attained. The result was expressed as percentage of a tartaric acid. Also, the fruit juices' pH was determined using a pH meter (Atago, Japan; Li et al., 2015; Karimi et al., 2019) (Fig. 3).

Sample extraction preparation

A total of 20 ml of the homogenized seedless grapes (Sultana) was thoroughly mixed in 100 ml of ethanol with 0.1% HCl. The extracts were again sieved, and the filtrate was centrifuged for 15 min at 6000 rpm, 4°C. The filtrate was then extracted using a rotary evaporator set to 50°C and the sample was used to analyse total phenolic, flavonoid contents, and antioxidant activity (Fig. 4).



Figure 3. Determining the total solid soluble, titratable and pH (G & H)



Figure 4. Showing in the samples extraction (I & J)

Total phenolic and total flavonoid content

The Folin-Ciocalteu colorimetric method was used to determine the total phenolic content of grapes (Farhadi et al., 2016). The absorbance was taken at 750 nm by spectrophotometer. Gallic acid (GAE) (20-250 mg/l) was utilised as a calibration standard, and results were indicated as g kg^{-1} GAE of grape berries fresh weight. Total flavonoid content was determined using the aluminium chloride colorimetric method (Farhadi et al., 2016). The absorbance was measured at 510 nm using a spectrophotometer. The catechin equivalent was utilised as a calibration standard and results were indicated as g kg^{-1} CE of grape berries' fresh weight (Fig. 5).

Antioxidant activity

The antioxidant activity was detected by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, and the results were expressed as a percentage of inhibition. 100 μl of sample extract was added with an ethanolic solution of DPPH (2900 μl), then

thoroughly vortex-mixed and kept in the dark for 30 min. The absorbance was taken at 517 nm by using a spectrophotometer (UV-vis, Evolution 201, Thermo Fisher).



Figure 5. Showing the total phenolic and flavonoid process (K & L)

Statistical analysis

Data was analysed through a general additive model using R programming (Allaire et al., 2021; R core Team, 2021). Differences were considered significant at $P < 0.001$. All the analyses were performed in triplicate.

Results

Fruit decay

The fruit decay for both coated and uncoated of grape fruits with neem seed oil and methylcellulose (NSOMC) during cold storage at 0.5°C and RH of 90% for 60 days were shown in *Figure 6*. The decay was significantly reduced ($P < 0.001$) in all coated fruits compared with uncoated fruits. The fruits decay decreased mainly based on different concentrations of NSOMC applied compared to those coated with methylcellulose only. The fruits coated with 2%, 8% and 32% of NSOMC had the lowest mean (6.3%, 4.6% and 3.1%, respectively) after 60 days of storage time. The fruits coated with 32% of NSOMC had slowed the decay (0, 0.78% and 2.1%, respectively) as compared to other samples in 15, 30 and 45 days of storage time.

Weight loss and colour

The weight loss and colour (chroma index) of coated and uncoated fruit samples at 0.5°C and RH of 90% for 60 days storage time were shown in *Figure 7* (M and N). As the cold storage time extended, the weight loss of uncoated fruits (control) continuously increased until they reached 1.49% at 60 days of storage time; the weight loss of the fruits coated with 1% of methylcellulose significantly decreased to 0.72% at the same storage time compared to uncoated fruits. The treated fruits with the edible coating

(NSOMC) at 0.5%, 2%, 8% and 32%, respectively, remarkably reduced ($P < 0.001$) the weight loss of the table grapes (0.52%, 0.29%, 0.32% and 0.32%, respectively) when compared with control and other coated fruits with 1% of methylcellulose (0.71%) at 60 days of storage time.

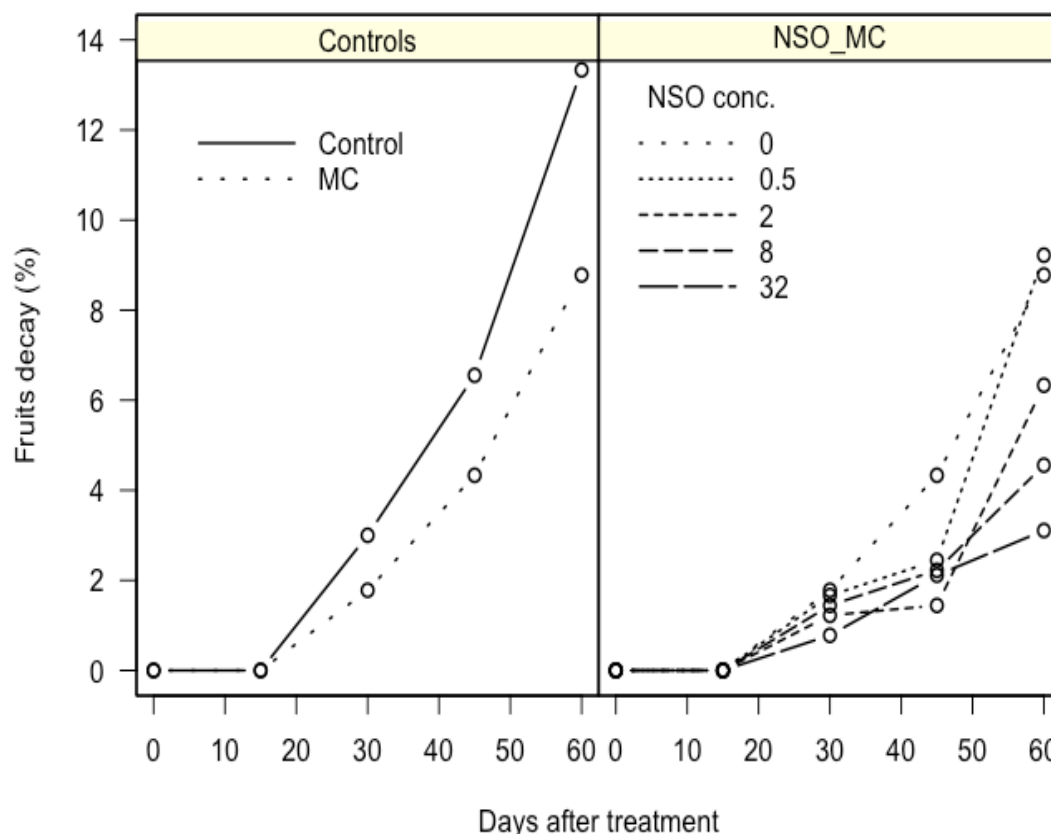


Figure 6. Effect of neem seed oil (NSO) combined with methylcellulose (MC) on fruit decay

Colour (chroma index) significantly declined in uncoated fruits, which was related to more ripening of the fruits surface with storage time. The fruits coated with 1% of methylcellulose (MC) showed diminished in colour during storage but, also less change of colour at 15 and 60 days respectively (8.9 and 7.5), as compared to the uncoated fruits (control). At end of storage time, the fruits coated with 0.5%, 2%, 8% and 32% of NSOMC were able to significantly lesser ($P < 0.001$) the colour changes (7.64, 7.72, 8.06 and 8.49, respectively) when compared to uncoated fruits (6.31%) and 1%MC coated fruits (7.37%). More also, 32% of NSOMC coated fruits were able to maintain their colour at 15 days and 30 days as compared to the zero-day.

Total soluble solid content

The Total soluble solid content in uncoated and coated grapes increased, peaked after 45 days and then rapidly decreased with storage periods as shown in Figure 8. The total soluble solid content in grape fruits coated with 1%MC, 0.5%NSOMC, 2%NSOMC and 8%NSOMC showed significantly lowest mean ($P < 0.001$) compared to the uncoated fruits at 45 days of storage time. The fruits coated with 32%NSOMC at 15, 30 and 45 days respectively, retained the total soluble solid content (17.9%, 18% and 18.1%

respectively) when compared with uncoated (19.9%, 20% and 20.1% respectively) and other coated fruits. The interaction between the different coated fruits and cold storage time for total soluble solid content was significantly shown ($P < 0.001$).

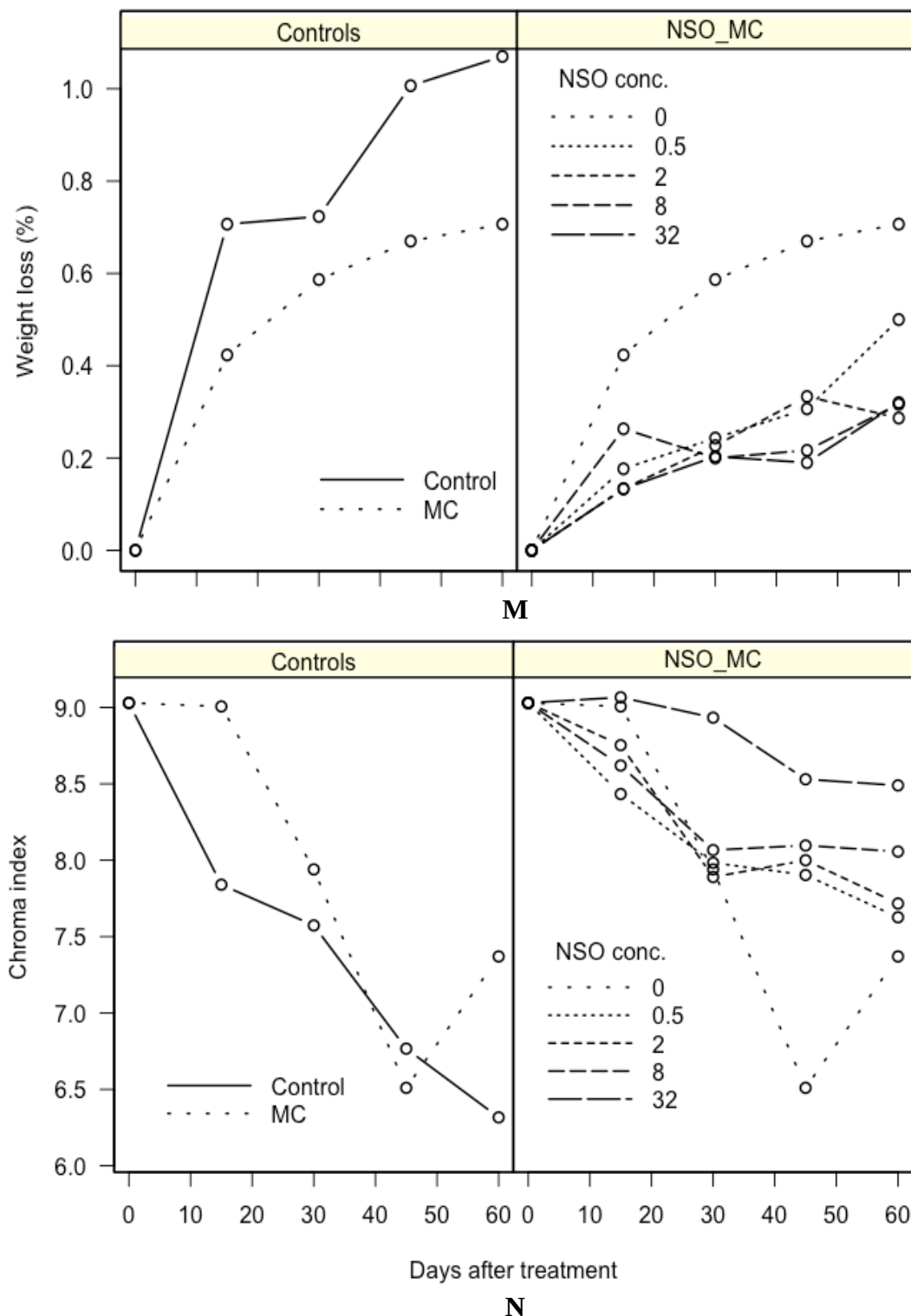


Figure 7. Effect of neem seed oil (NSO) combined with methylcellulose (MC) on weight loss (M) and colour (N)

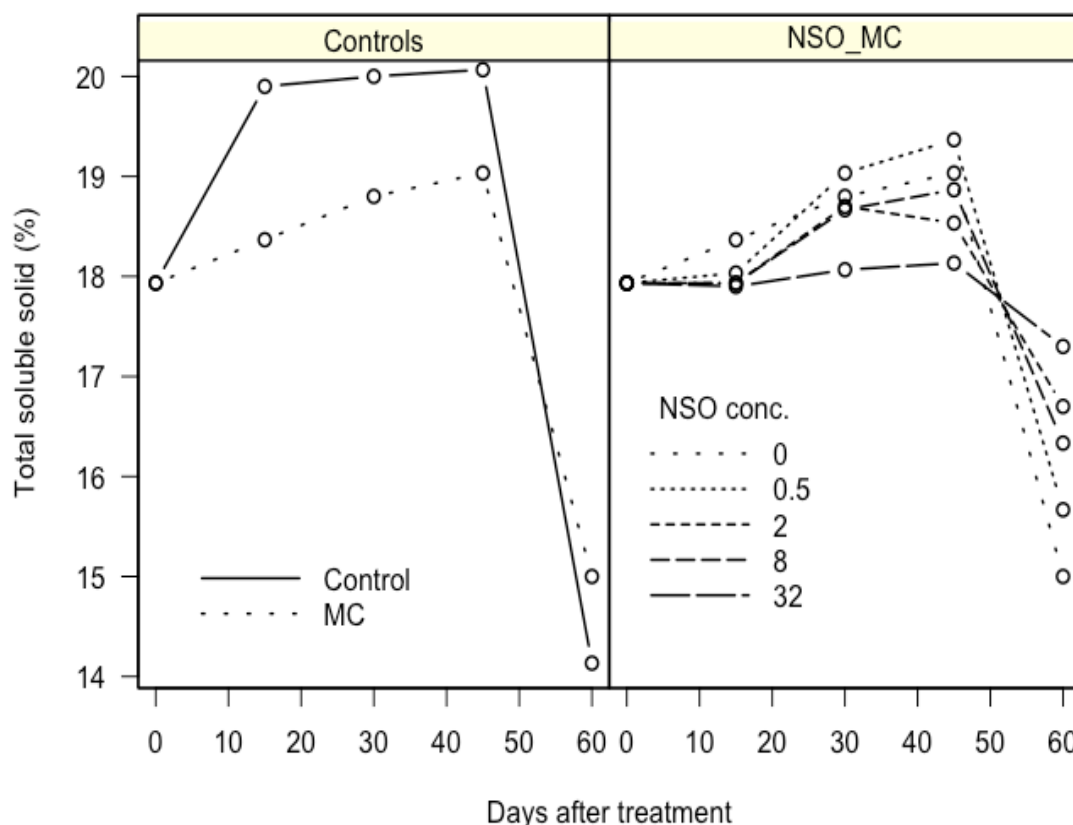


Figure 8. Effect of neem seed oil (NSO) combined with methylcellulose (MC) on Total solid soluble

Titrateable acidity and pH

The titrateable acidity and pH of coated and uncoated fruit samples at 0.5°C and RH of 90% for 60 days storage time were shown in Figure 9. (O and P). All the coated and uncoated fruits displayed a significant decrease in titrateable acidity from 15-60 days of cold storage time. The titrateable acidity of fruits coated with NSOMC (0.5%, 2%, 8% and 32%) significantly ($P < 0.001$) have the highest mean (0.69%, 0.71%, 0.72% and 0.76%, respectively) compared to 1% of methylcellulose {MC (0.63%)} and uncoated fruits {control (0.55%)} at the end of 60 days storage time. 1%MC coated fruits (0.63%) significantly higher ($P < 0.001$) in titrateable acidity as compared to uncoated fruits {control (0.55%)} at the 60 days storage time. Amongst all the coated fruits, the fruits coated with 32%NSOMC had the highest mean from 15 days to 60 days (0.85%-0.75%) in titrateable acidity compared to other coated fruits and uncoated fruits (control). 32%NSOMC coated fruits retained the acid content compared to the zero-day and there was significant interaction different between the coated fruits and storage time.

The pH values of uncoated fruits (control) and 0.5%NSOMC coated fruits increased and slightly decreased at 45 days to maintain a stable pH value at 60 days, moreover the pH value of fruits coated with 2% and 8%NSOMC also increased and slightly decreased at 30 days then increased at 45 and 60 days of storage time. Furthermore, the fruits coated with 1%MC and 32%NSOMC slightly increased at the extension of storage time in pH values except at 60 days of 1%MC coated fruits. Although, there was a significant effect ($P < 0.001$) in the pH values among all the coated fruits as compared to uncoated fruits

(control). Whereas the fruits coated with 32%NSOMC significantly showed ($P < 0.001$) the lowest mean of pH value (3.43) compared to uncoated fruits (3.58%) and other coated fruits (3.49%, 3.48%, 3.47% and 3.47% respectively) at the end of the storage time.

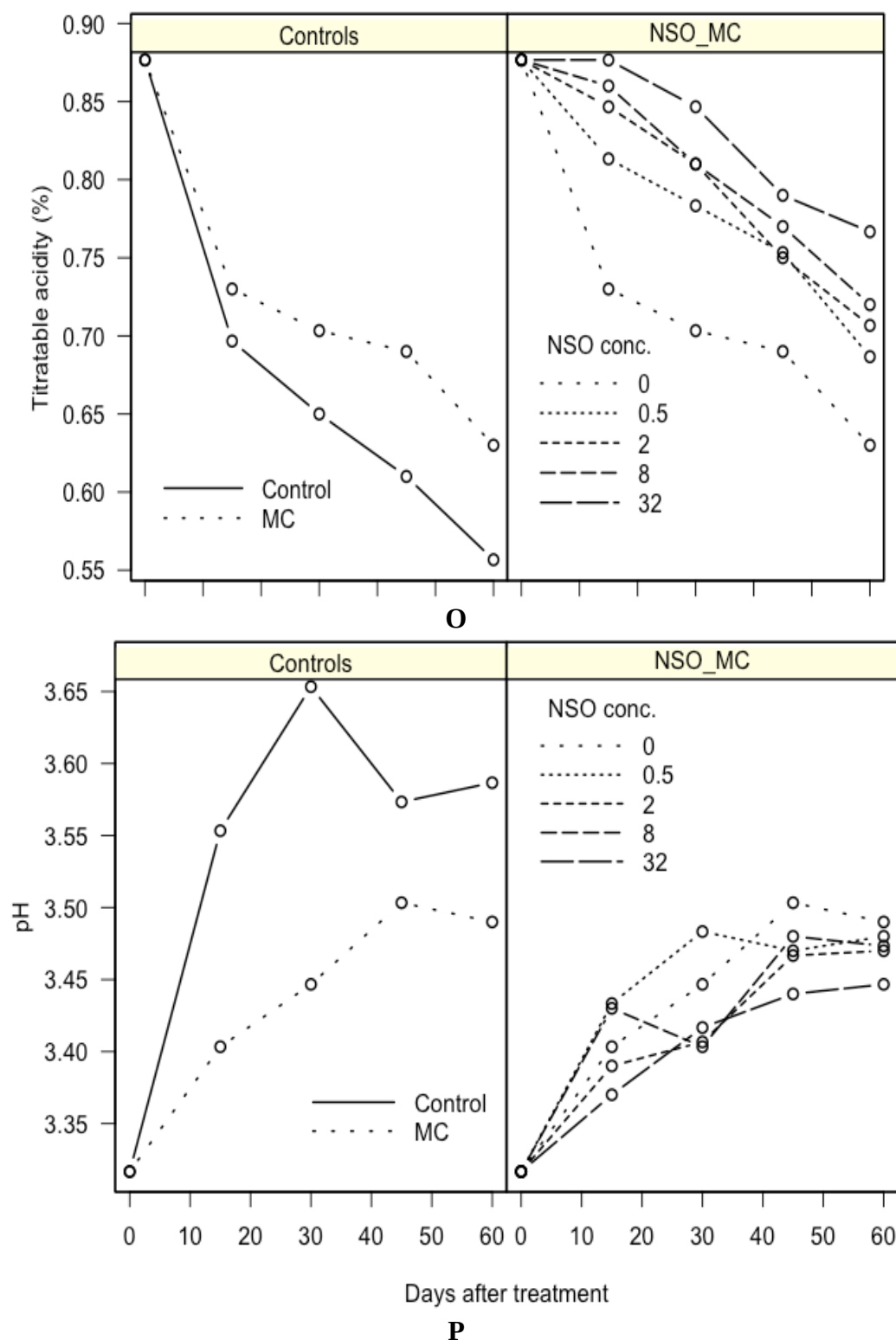


Figure 9. Effect of neem seed oil (NSO) combined with methylcellulose (MC) on titratable acidity (O) and pH (P)

Total phenolic content

The effect of neem seed oil (NSO) combined with methylcellulose (MC) on phenolic content at 0.5°C and RH of 90% for 60 days of storage time was illustrated in Figure 10. The total phenolic content in both coated and uncoated fruits gradually decreased with the storage period. The total phenolic content of coated fruits with 0.5%, 2%, 8% and 32% of neem seed oil combined methylcellulose were significantly ($P < 0.001$) higher (426, 447, 461 and 491 mg/GAE/KgFW, respectively) than uncoated fruits {control (307 mg/GAE/KgFW)} and 1%MC of coated fruits (330 mg/GAE/KgFW) at the end of 60 days. The highest amount (749 mg/GAE/KgFW) of total phenolic content was acquired in 8%NSOMC fruits coated compared to other coated and uncoated fruits at the end of 15 days of storage time. The fruits coated with 1%MC were significantly higher than the control at 30-60 days of storage time.

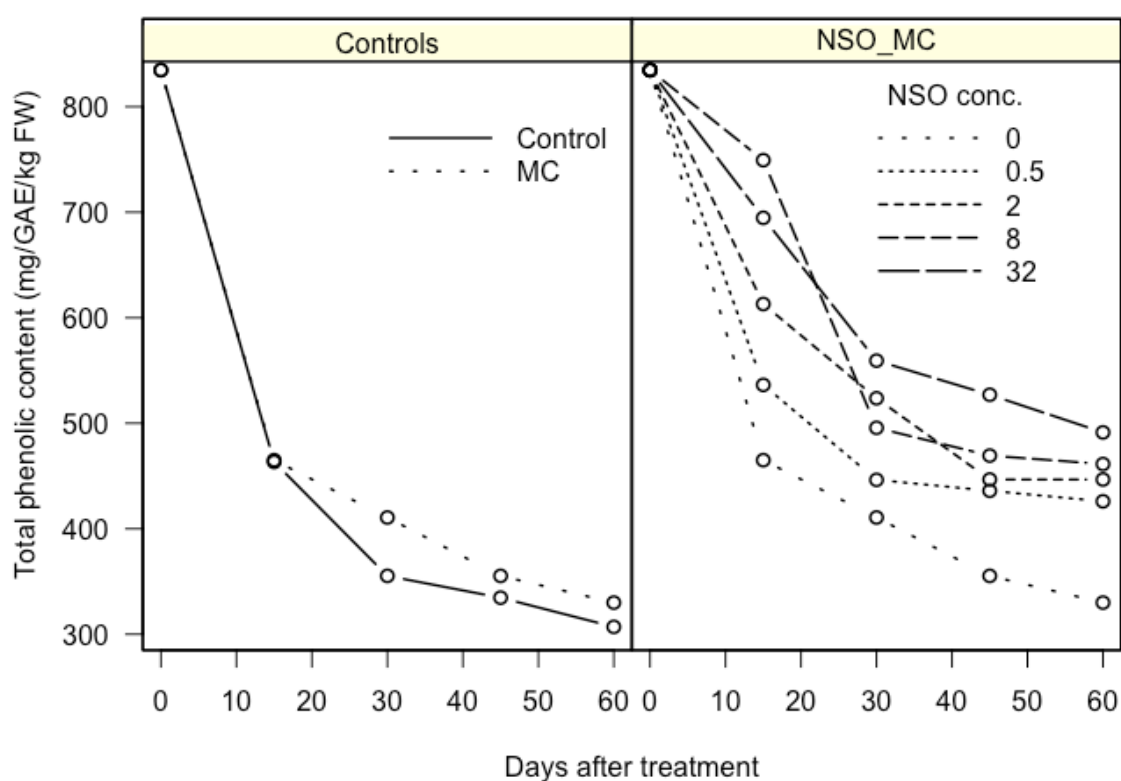


Figure 10. Effect of neem seed oil (NSO) combined with methylcellulose (MC) on total phenolic content

Total flavonoid content

The effect of neem seed oil combined with methylcellulose (NSOMC) on flavonoid content showed in Figure 11. The total flavonoid content in both coated and uncoated fruits gradually decreased with the storage period. The total flavonoid content of the fruits coated with 0.5%, 2%, 8%, and 32% of methylcellulose combined neem seed oil was significantly ($P < 0.001$) higher (473, 492, 524, and 552 mg/Catechin/KgFW, respectively) than uncoated fruits {control (242 mg/Catechin/KgFW)} and 1%MC of coated fruits (331 mg/Catechin/KgFW) at the end of 60 days storage time. The highest amounts (915 and 805 mg/Catechin/KgFW, respectively) of total flavonoid content

were acquired in 32%NSOMC coated fruits compared with other coated and uncoated fruits at the end of 15 and 30 days of storage time. The fruits coated with 1%MC were significantly higher than the control at 45-60 days of cold storage.

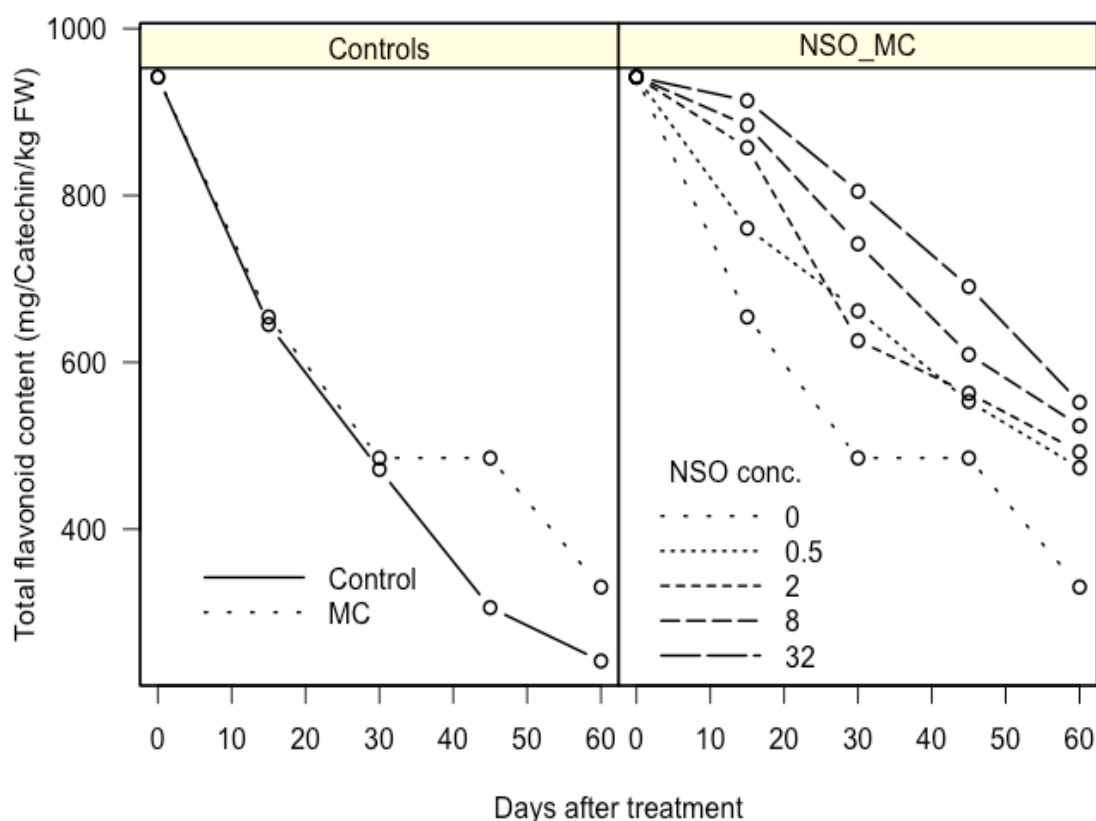


Figure 11. Effect of neem seed oil combined (NSO) with methylcellulose (MC) on Total flavonoid content

Antioxidant activity

Figure 12 represents the change in antioxidant activity of fruits coated and uncoated during cold storage. The antioxidant activity increased gradually in fruits coated and uncoated during cold storage. At 60 days of storage period, the antioxidant activity decreased in all coated fruits compared to the uncoated fruits (control). Moreover, the antioxidant activity significantly reduced in the fruits coated with 0.5%, 2%, 8% and 32%NSOMC (67%, 66%, 66% and 65%, respectively) when compared with 1%MC of coated fruits (69%) and uncoated fruits {control (71%)} at the end of 60 days of storage period.

Discussion

Fruit decay

Postharvest decay of grape berries is one of the traits of grape ageing. Decay increases in table grape industry speed up the quality degradation, lessen commercial values and shorten the storage time. Increase in respiration and transpiration rates during postharvest cause moisture loss in grape berries (Min et al., 2001; Lo'ay and

Dawood, 2017). In this study, 32%NSOMC (Neem seed oil and methylcellulose) effectively reduced fruit decay, similar to the effect of Kombucha on grape (Xian et al., 2019) and the research of Palei and Dash (2017) reported that 15% and 20% of neem leaf extract on mango fruits showed no spoilage after cold storage.

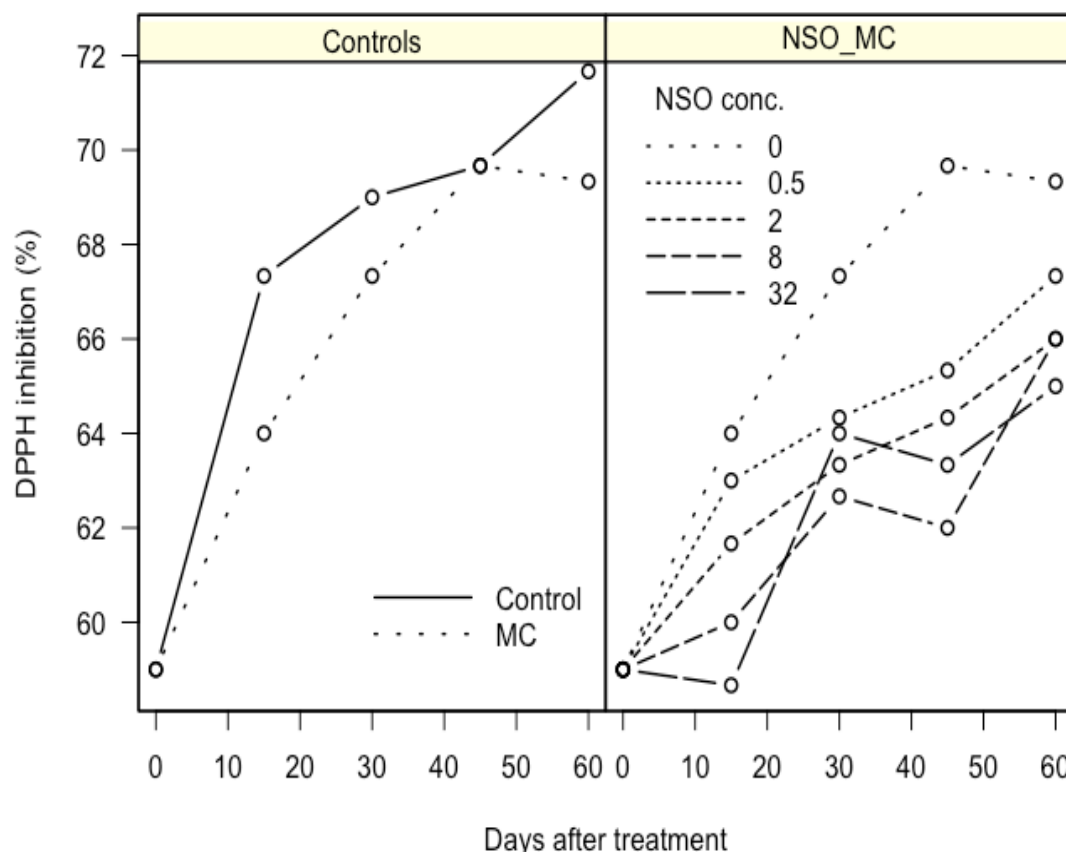


Figure 12. Effect of edible coating composite with neem seed oil (NSOMC) on antioxidant activity using DDPH (2,2-diphenyl-1-picrylhydrazyl)

Weight loss

Weight loss also impinges on other qualities of fruits, such as fruit appearance and moisture. The result of the study revealed that the fruits treated with edible coating (NSOMC) at 0.5%, 2%, 8% and 32%, respectively, remarkably reduced the weight loss compared to the control and 1% of methylcellulose after 60 days. In the current study, the treatments reduced the weight loss by moulding films that inhibit respiration in the grapes (Min et al., 2001; Lo'ay and Dawood, 2017). The effect of edible coating (NSOMC) on the table grape was related to the effect of the chitosan on grapes (Lo'ay and El-khateeb, 2018). Moreover, the result recorded in this study also agreed with Maftoonazad and Ramaswamy (2005) who reported the avocados treated with methylcellulose reduced the moisture loss.

Colour

The colour change is one of the attributes of maturation in many fruits which also determine the quality criteria used by the grower and consumers. The result of this study

showed that 0.5%-32% of NSOMC were able to lessen the change in colour of the table grapes after 60 days of storage time and 32% of NSOMC at 15 and 30 days of storage were able to maintain the colour of the table grapes due to inhibition in respiration and rate of ethylene production of the fruits. This result was similar to the report of Fagunde et al. (2014) that used Hydroxypropyl methylcellulose beeswax coating with different food preservatives to reduce the respiration rate in cherry tomatoes which produced no changes in the peel colour of coated cherry tomatoes and also similar to the chitosan and methylcellulose films that were able to maintain the colour of fresh cut cantaloupe over the storage time (Sangsuwan et al., 2008).

Total soluble solid (TSS), titratable acidity (TA) and pH

The effect of neem seed oil combined with methylcellulose was positively shown on the examined biochemical properties (TSS, TA, and pH) of grape berries during storage. The changes in the TSS, TA, and pH content were different among varieties of grapes during storage (Champa et al., 2014; Mirdehghanand and Rahimi, 2016). The change in TSS content could be because of water loss and the destruction of starch to glucose, fructose, and sucrose during storage (Duan et al., 2011). The fruits coated with 32%NSOMC significantly retained the TSS content during storage because the treatment retarded the rate of respiration, production of ethylene and the conversion of starch into sugars (Bautista-Banos et al., 2006). Similarly, the preharvest application of chitosan and postharvest Aloe vera gel coating preserve TSS content (Ehtesham Nia et al., 2021) and more also the result is similar to the report of Chaucha and Joshi (1990) that botanical extract performs better in retaining the total soluble solid in Ratna cv. Of mango.

The fruits coated with 32%NSOMC exhibited highest level of titratable acidity during cold storage, possibly slower the degrees of fruit ripening because of the slowdown in the rate of metabolism of organic acids as a substrate during respiration process in coated fruits (Diaz-Mula et al., 2012). These finding are in accordant with the results of the study conducted by Asgarian et al. (2022) reported that grape treated with 40 mM GABA (Gamma-Aminobutyric Acid) had shown significant effect on TA of the grape fruits during cold storage and also similar to the result obtained from influence of carboxymethyl cellulose that exhibited higher TA in the cold stored (Baswal et al., 2020). The lowest pH value observed in fruit coated with 32%NSOMC as results of lower degradation of organic acids. Wijewardane and Guleria (2013) have reported that neem seed oil extract plays an essential role in maintaining and controlling the pH of the cell during postharvest period. The coating treatments had also slowed the metabolism of fruits as these have been reported to preserve higher CO₂ and lower O₂ levels inside the coated fruits (Kader et al., 1989), and this explained the retention of higher acidity and lower pH value.

Total phenolic, total flavonoid content and antioxidant activity

The grape phenolic compounds comprise colour, aroma, taste, firmness and senescence are involved in fruit quality. These compounds have antioxidant activity that makes them essential crude materials in the oxidation of grape juice (Karimi et al., 2017). Flavonoids which also part of phenolic compounds and enzymatic antioxidant systems and many of these phenolic compounds in grapes trap free radicals (Karimi et al., 2017). The higher stability of total phenolic and flavonoid content in NSOMC

coated fruits grape had shown a positive effect on amino acids that increase the nutritional and antioxidant of coated fruits during storage (Asgarian et al., 2022). These could be the results of the neem active ingredients that possess antimicrobial and a thin film of neem oil surrounding treated fruits that reduced the evapotranspiration, respiration rate and antioxidant rate (Singh et al., 2000). Eshghi et al., 2021 reported that the depletion of flavonoids and phenolics during cold storage, ageing and the involvement of these compounds in metabolism is because of enzymatic and non-enzymatic reactions during cold storage. The flavonoids and phenolic are important in fruits because they function in the defence system.

Antioxidant activity

The antioxidant activity of grape determined using DDPH (2,2-Diphenyl-1-picrylhydrazyl). The antioxidant activity of grapes relate to the amount and quality of phenolic compounds in the samples (Karimi et al., 2017). Persistent with the results of this study, the postharvest application of GABA in grapes (Asgarian et al., 2022), NSOMC preserved the antioxidant capacity of the fruits compared to the uncoated fruits.

Conclusion

The result of this study showed the positive of neem seed oil combined with methylcellulose (NSOMC) as an edible coating and healthy compound on the retaining the Sultana table grape postharvest quality and as well preservation of phenolic compounds and antioxidant activity during cold storage (60 days). The different concentrations of the NSOMC were found effective, the more neem seed oil more effectiveness of the treatments was. Therefore, postharvest application of NSOMC is safe and could be used as a commercial treatment for prolonging the shelf life of harvested table grapes.

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Conflict of interests. There is no potential conflict of interests reported.

Data availability. The data set analysed during this study are available from the corresponding author on request.

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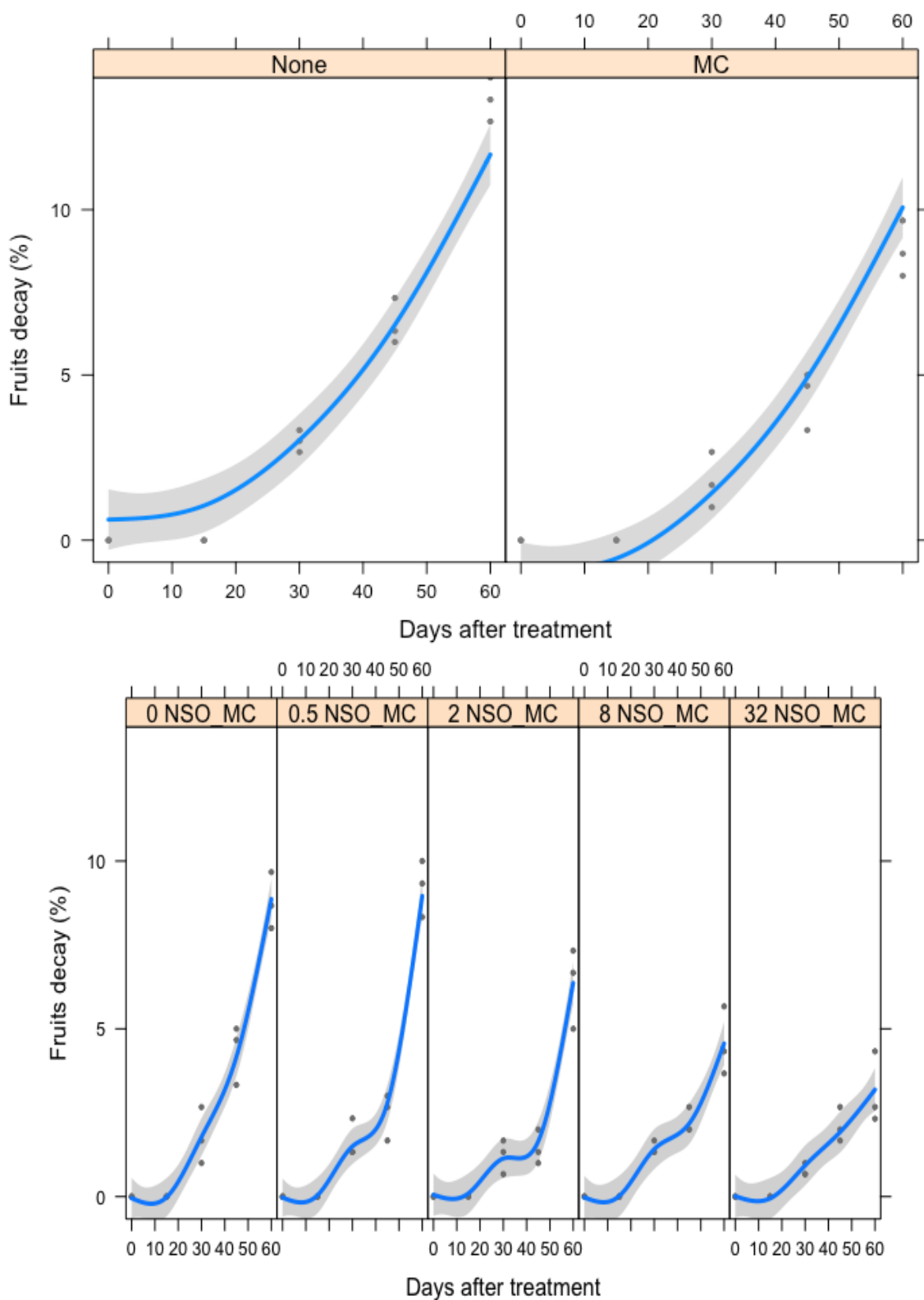
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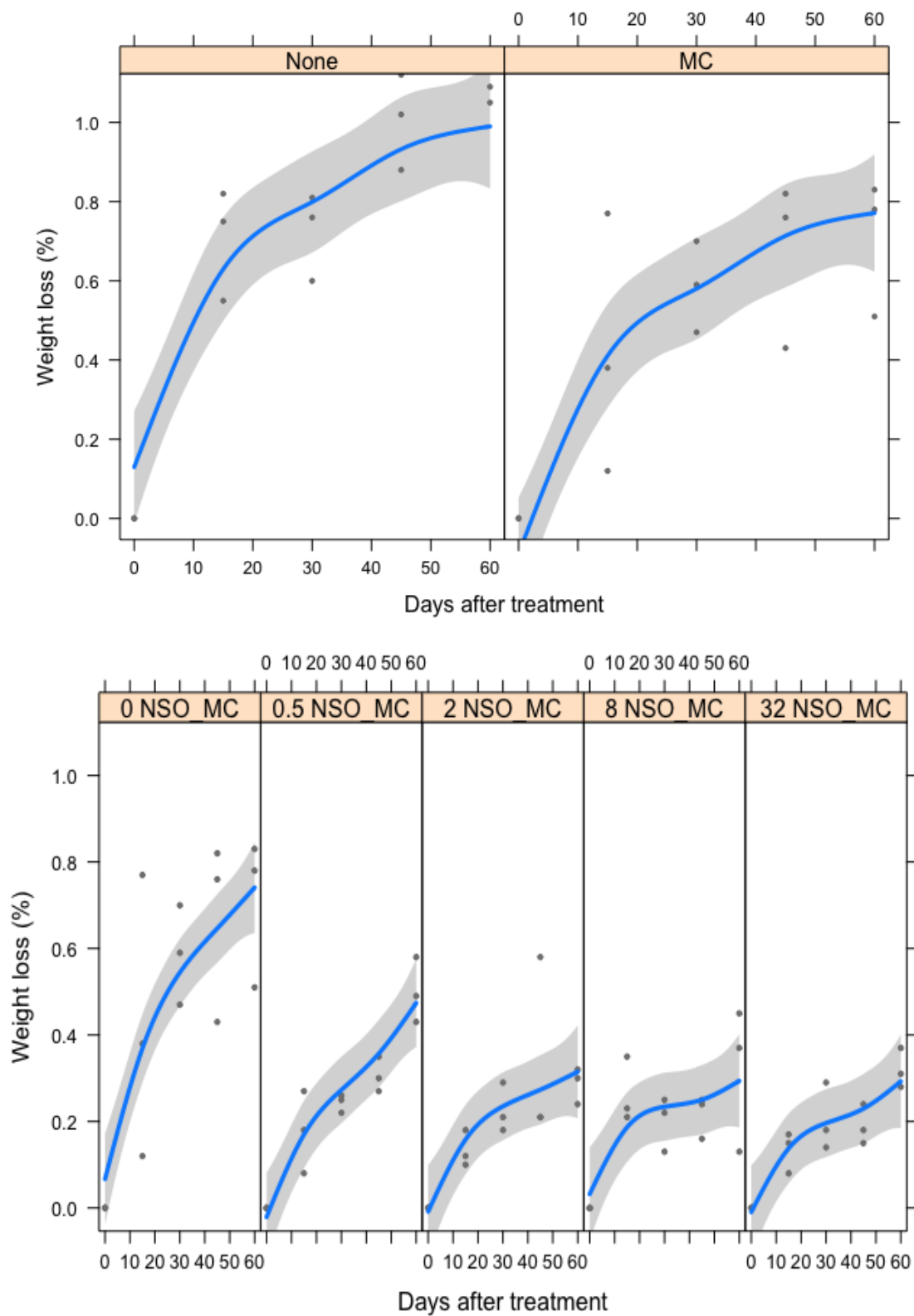
APPENDIX

Supplementary data sets

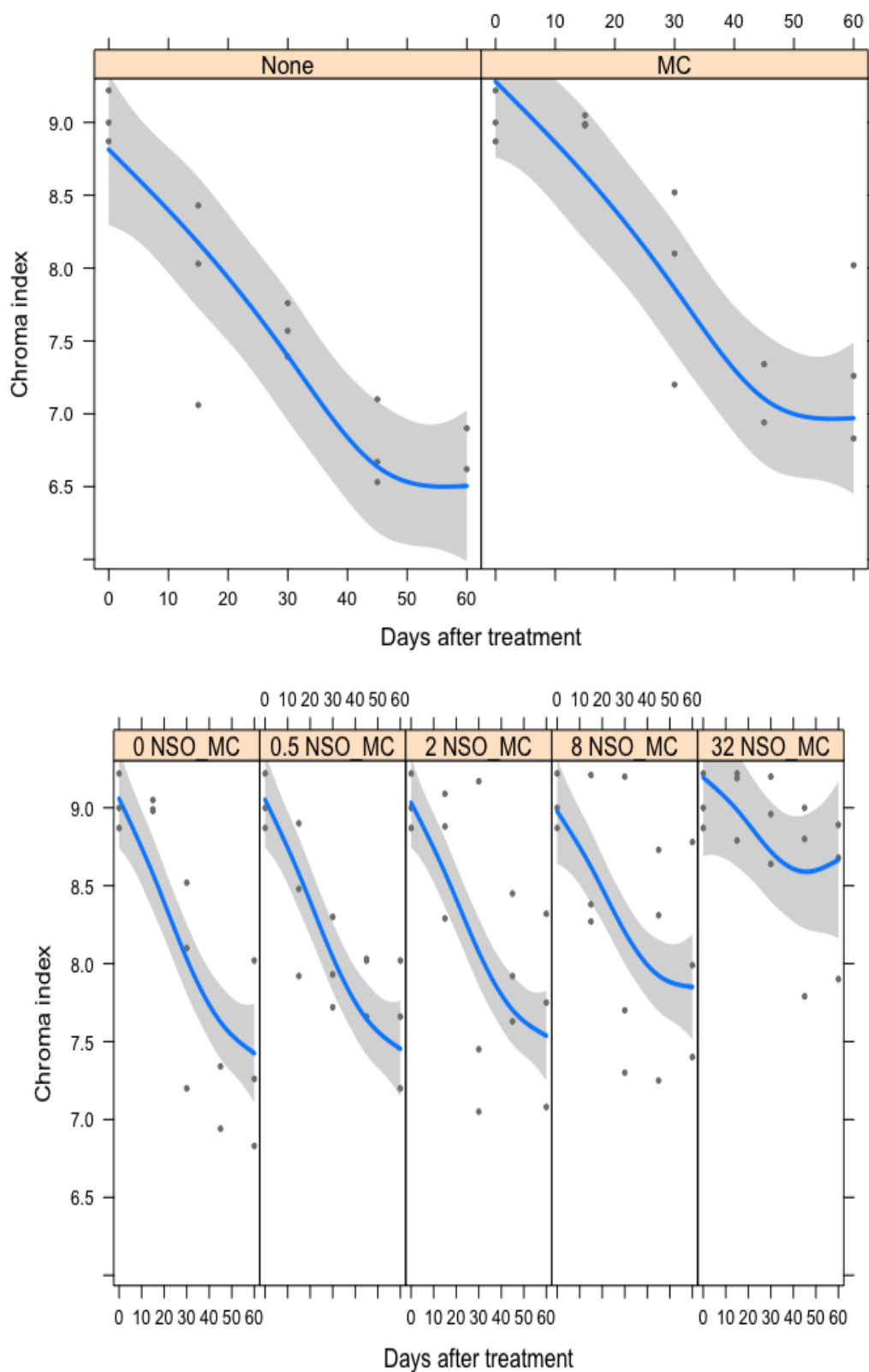
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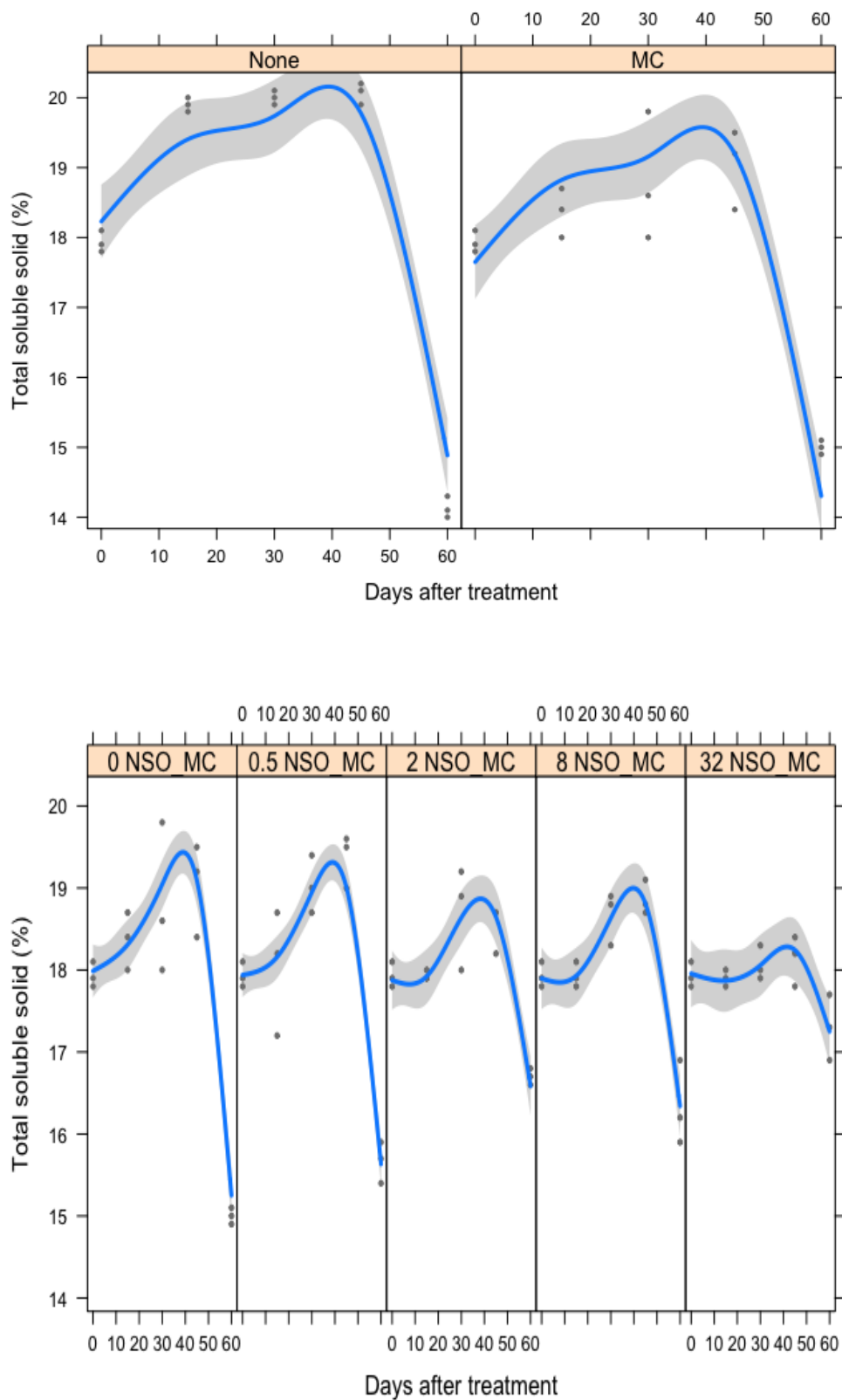
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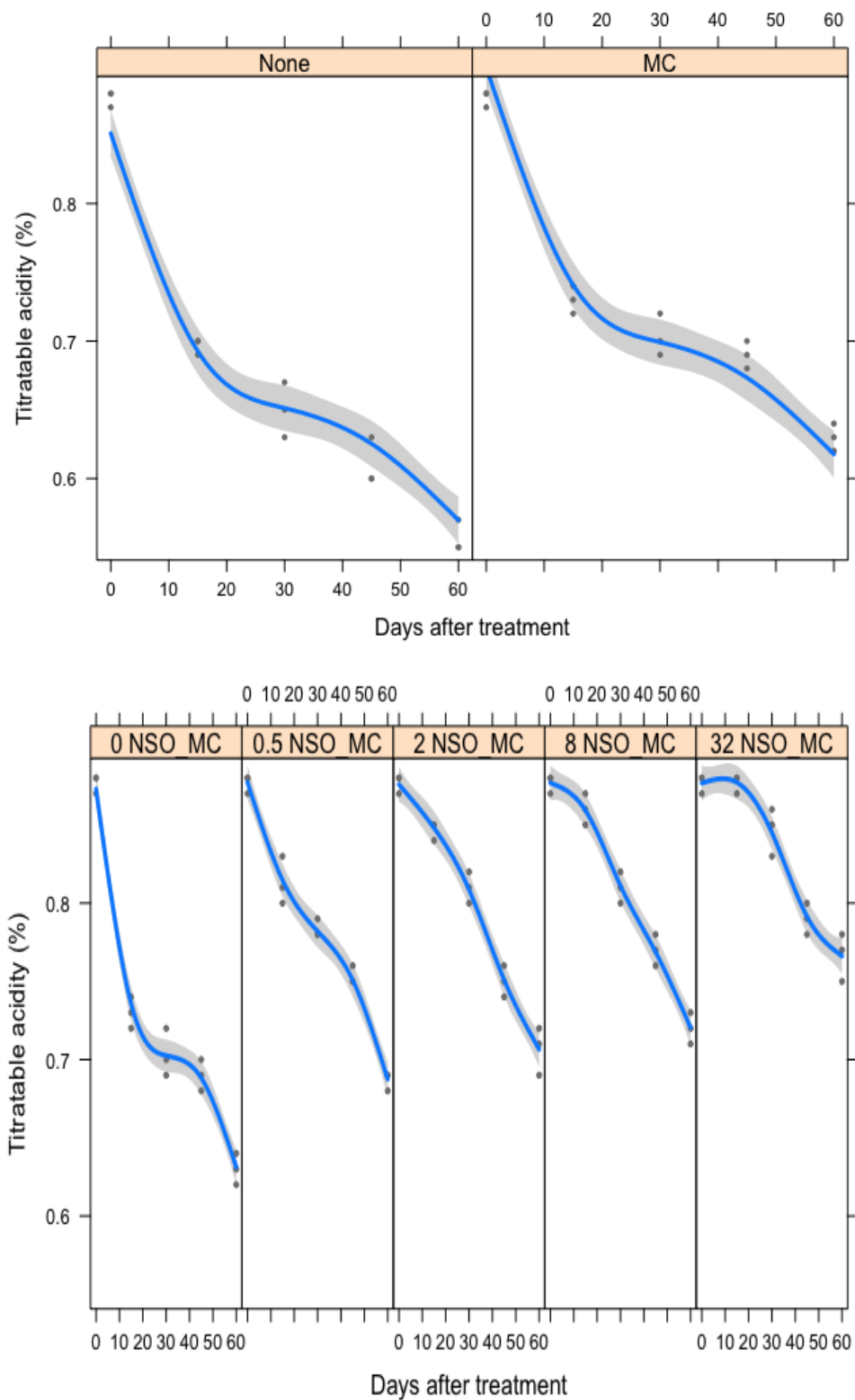
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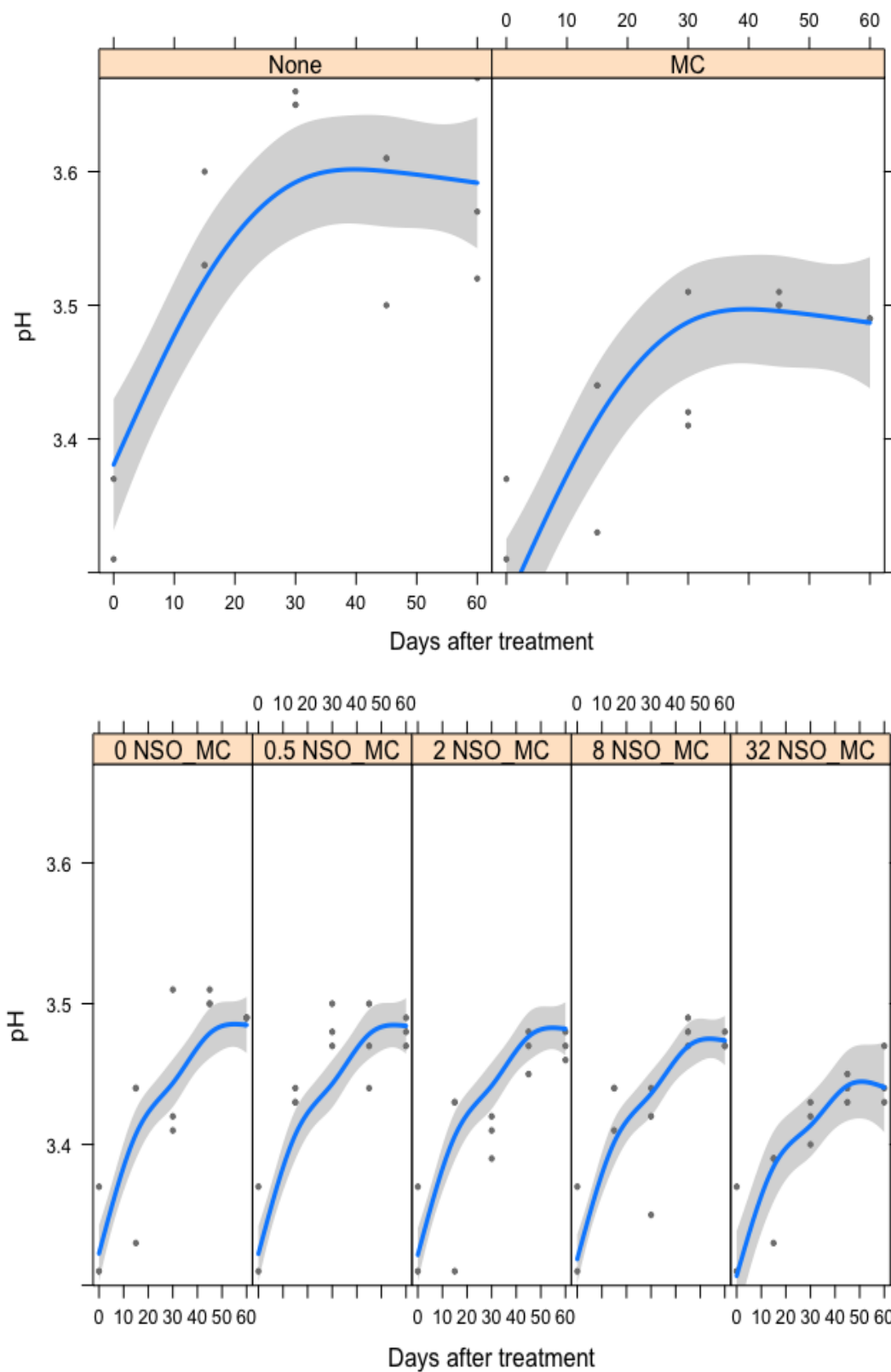
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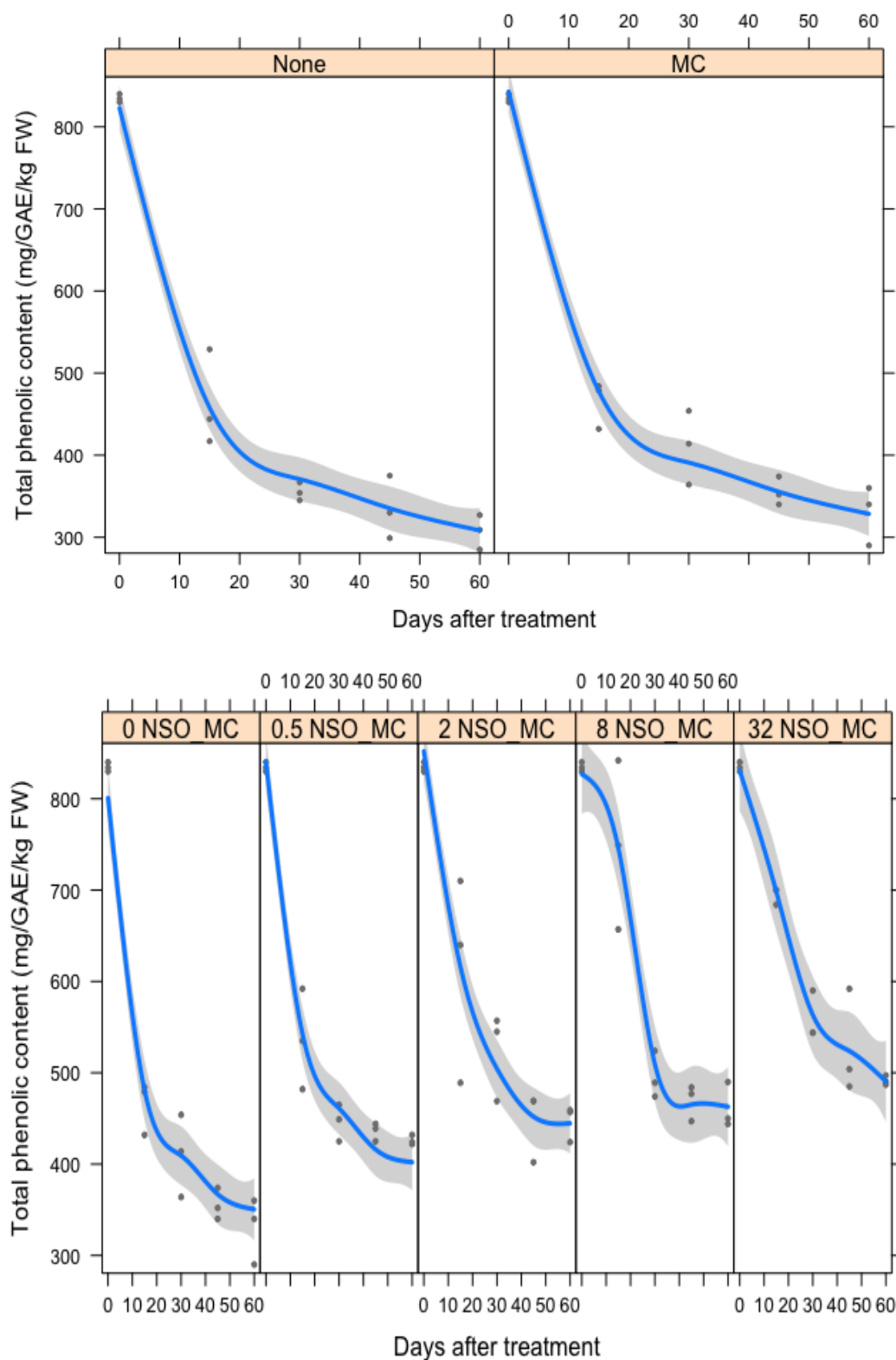
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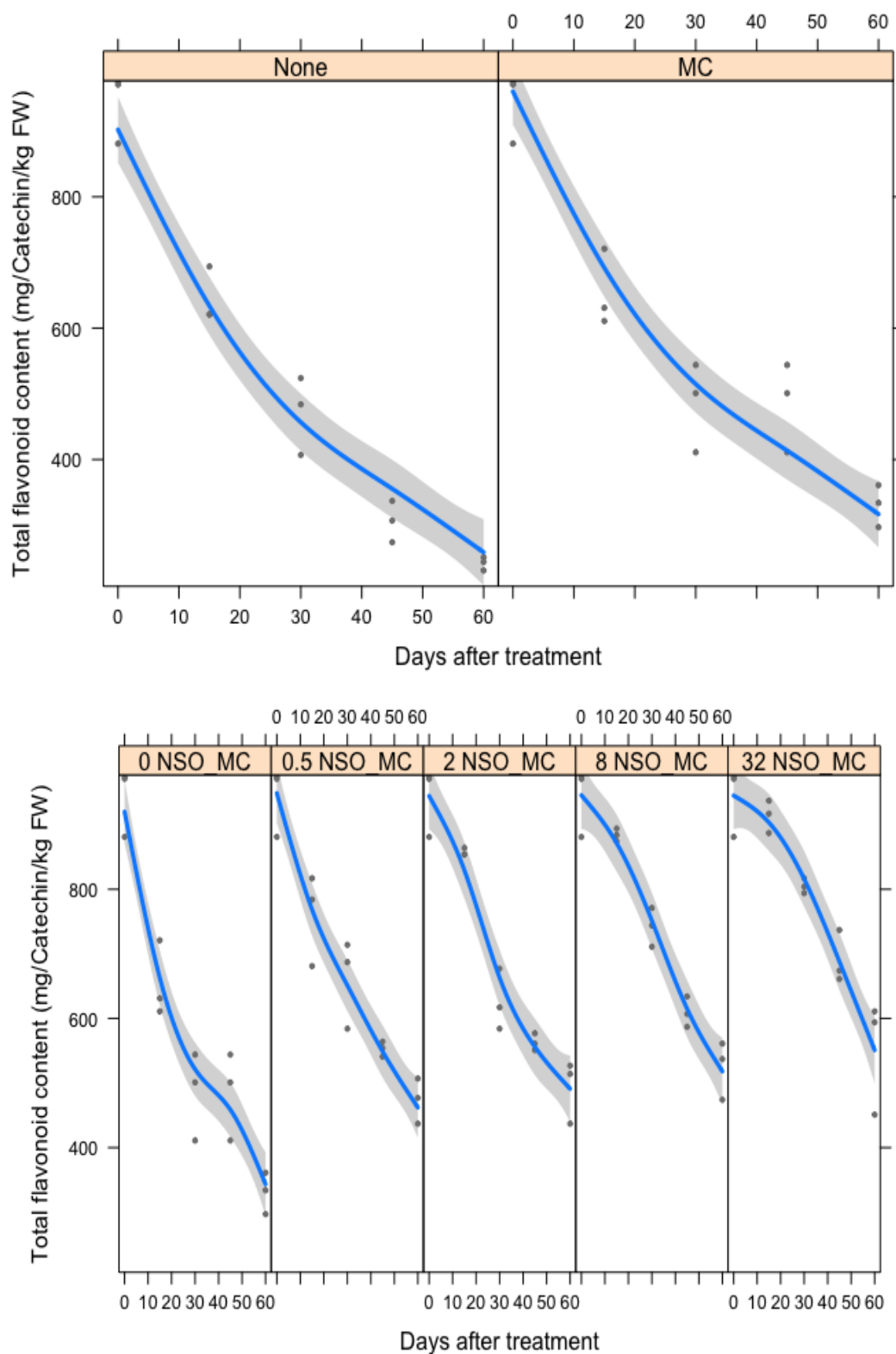
Supplement to Figure 9 P



Supplement to Figure 10



Supplement to Figure 11



Supplement to Figure 12

