Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
https://pjp.pakps.com

RESEARCH ARTICLE

In Vivo Evaluation of Different Fungicides for The Retention of Kinnow Fruit Against Pre-Harvest Pathological Fruit Drop

[a]Malik A. Rehman, [b]Mujahid Ali*, [c]Ahsan Shahzad, [d]Salman Ahmad, [e]Sufian Ikram, [f]Yasir Ali, [d]Nabeel Asghar
[a] *Citrus Research Institute Sargodha, Sargodha, Pakistan.*
[b] *Water Management Research Farm, Renala Khurd, Okara, Pakistan.*
[c] *University of Agriculture, Faisalabad, Pakistan.*
[d] *Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha, Pakistan.*
[e] *Chinese Academy of Agricultural Sciences, Beijing, China.*
[f] *Department of Plant Pathology, Faculty of Agricultural Sciences and Technology, University of Layyah, Layyah, Pakistan.*

Corresponding Author:
Mujahid Ali, Email: mujahidali2263@gmail.com

Article History:
Submitted: March 03, 2024; Revised: August 27, 2024; Accepted for Publication: September 05, 2024.

A B S T R A C T

Pathological fruit drop in citrus is a widespread disease in Punjab causing huge economic losses to kinnow mandarin growers. The disorder was significantly higher during the second fortnight of September and the first fortnight of October in the Punjab. In current research, mature fruit-bearing 10 years old, 28 plants of kinnow mandarin (*Citrus reticulata* L.) grafted on rough lemon (*Citrus jambhiri*) rootstocks were selected. To examine the fruit drop, 6 different fungicides were applied in April, August, and September in a season. Amistar Top @ 1 ml /L of water, Nativo @ 0.5 g/L of water, Topsin M @ 2 g/L of water, Cabrio Top @ 1.5 g/L of water, Rally @ 0.5 g/L of water, Success @ 2 g/L of water were applied on ten years old kinnow plants. It was observed that the significantly (P<0.01) lowest (7.75%) fruit drops were recorded when Topsin. M (Thiophanate methyl) 2 g/litter of water was applied and the highest (39.86%) were dropped was observed when Success (Chlorothalonil+Metalaxyl) @ 2 g/litter of water was applied. The highest fruit yield per plant was recorded in Topsin. M (85.5 Kg) followed by Amistar Top (81.3 Kg), while the lowest was recorded in untreated plants (58.3 Kg). The highest fruit juice was recorded in Topsin M (53%) followed by Amistar Top (52%), while the lowest fruit juice was found in control (44%) where plants were left untreated and treated with Success (43%). Topsin M and Amistar Top were more effective than others in controlling fruit drop and improving the quality of the fruit of kinnow. Similar impact was observed by selected pre-harvest application of fungicide on yield and fruit quality attributes e.g., fruit length, fruit breadth, and fruit weight.

Keywords: Citrus, fruit drop, fruit quality, pathogen, fungicides.

INTRODUCTION

Kinnow mandarin is the largest fruit of Pakistan regarding area and production. The fruit tree is one of the vital positions among citrus types (Khan *et al.,* 2016). Agriculture is the backbone of Pakistan's economy which contributes almost 23.4% (Usman, 2016; Khan *et al.,* 2023). According Ministry of National Food Security & Research, total production of citrus is 2321802 tons on area of 152716 ha. Total annual citrus production in Punjab is 2246473 tons on 14117 ha, Sindh has a total annual production of 34059 tons on 5818 ha KPK has 30383 tons on 3930 ha, while Baluchistan has 10887 tons' annual production on 1790 ha (GOP, 2023).

Citrus, one of the most important fruit crops of the sub-tropical region, is the world's leading tree fruit crop belonging to the family Rutaceae (Bharti *et al.,* 2020.) Citrus fruits constituting approximately 34% of the total fruit production in the country. Notably, over 93% of the cultivated area dedicated to citrus in Punjab is specifically allocated to Kinnow mandarin. The cultivation of Kinnow is primarily driven by its favorable attributes including high yield, superior processing characteristics, suitability for fresh consumption, aromatic flavor profile, and adeptness in adapting to the agro-environmental conditions prevalent in Punjab (Ahmed *et al.,* 2006). Pre-harvest fruit dropping is a common problem in kinnow cultivation that reduces the yield and profitability of the crop. Pre-harvest fruit dropping refers to the premature shedding of fruits from the trees before they are ready to be harvested (Maude and Bouageois, 2013). This can occur due to various factors such as environmental stresses, pests and diseases, nutrient deficiencies, and hormonal imbalances (Ahmad *et al.,* 2024).

Pakistan has natural resources favorable for growing citrus (Cheema and Jamali, 2020). Citrus fruits grown in Pakistan are valued due to their distinctive qualities (Cheem, 2021). With 2.41 metric tons produced annually in an 183849-hectare area, Pakistan ranks 12th among nations that grow citrus (Anonymous, 2018). However, Baluchistan produces 1.3%, Sindh 2.3%, KPK 2.4%, and Punjab 94% of citrus. Punjab has the most significant percentage because of its ideal climate. However, the significant districts that produce kinnow include Sargodha, Toba Tek Singh, and Sahiwal. Nearly 23% of Pakistan's citrus orchards are in Sargodha, the primary citrus-supplying region (Niaz, 2004).

Lopes *et al.* (2015) conducted a study to evaluate the effectiveness of various antagonistic microorganisms for managing *Colletotrichum acutatum* on preharvest citrus fruits, yielding encouraging outcomes. Additionally, a range of plant extracts, essential oils, gums, resins, and similar substances have demonstrated significant biological activity against fungal pathogens affecting plants, both in laboratory settings (in vitro) and in real-world conditions (in vivo). Consequently, these substances hold potential for utilization as bio-fungicidal agents (Fawzi *et al.,* 2009; Jalili *et al.,* 2010; Romanazzi *et al.,* 2012).

Citrus blooms profusely in the spring, but only a few flowers develop into fruit, which remains on the tree and ripens until harvest. At Kinnow, less than 1.0% of the flowers develop into harvestable ripe fruit (Agustí *et al.,* 2022). Fruit decline at different stages can be caused by fruit flies, pathogen attacks such as late bloom rot, water stress, and hormonal imbalance (Anonymous, 2018; Mahawar *et al.,* 2020). At this stage, a massive fruit drop contributes to around 50% of the fruit yield. All fruits ripen at different times rather than at the same time, and they are all susceptible to several diseases, most of which are fungal diseases. Diplodial stem-end rot of citrus fruits is one of the most common post-harvest diseases (Zhang, 2014). From August until the final harvest, a pre-harvest fruit drop occurred. The fruit drop occurs at the stem-calyx joint, propelling the fruit without the stem. Pre-harvest fruit deterioration in Pakistan is a major threat of less production in the pedigree (Marinho *et al.,* 2005). Keeping in view its importance there was need to it comprehensively. So, present study is designed to evaluate the fungicides against fruit drop, causing twig dieback and stem end rot problems in kinnow.

MATERIALS AND METHODS

This experimental study was conducted in the research area CRI Sargodha 40100. (32° 7' 4.3",72° 40' 35.73") in 2014-2017. Mature fruit-bearing 10 years old, 28 plants of kinnow mandarin (*C. reticulata*) grafted on rough lemon (*Citrus jambhiri*) rootstocks were selected in the research area of Citrus Research Institute (CRI) Sargodha. All the selected plants were given the same horticultural practices during the experiment work.

The experiment was conducted in a Randomized Complete Block Design (RCBD) with one control and six treatments of different fungicides. Each treatment was applied in 4 replications. The fungicides used were Amistar Top (azoxystrobin+difenoconazole) @ 1 ml/L of water; Nativo (Tebuconazole+Trifloxystrobin) @ 0.5 g/L of water, Topsin. M Thiophanate methyl) @ 2g/L of water, Cabrio Top (Pyraclostrobin+metiram) @ 1.5 g/L of water, Rally (Myclobutanil) @ 0.5 g/L of water, and Success (Chlorothalonil+Metalaxyl) @ 2 g/L of water (Table 1). The effect treated plants with funjicides were compared with untreated plant (control).

Each fungicide was applied on four selected plants of kinnow to evaluate the heir effect on stem end root that causes fruit drop. Each treatment was applied three times in a seasonal year. 1st application in 2nd week of April 2nd application in 1st week of August and 3rd time repeat in September of the same year.

Table 1. Detail of fungicides with active ingredients and its dose applied for controlling fruit drop in kinnow

Sr. No	Name of fungicide	Active ingredient	Dose
1	Amistar Top	azoxystrobin+difenoconazole	@ 1 ml/L of water
2	Nativo	Tebuconazole+Trifloxystrobin	@ 0.5 g/L of water
3	Topsin. M	Thiophanate methyl	@ 2 g/L of water
4	Cabrio Top	Pyraclostrobin+metiram	@ 1.5 g/L of water
5	Rally	Myclobutanil	@ 0.5 g/L of water
6	Success	Chlorothalonil+Metalaxyl	@ 2 g/L of water

Fruits on each selected plant were counted before and after fungicide application. The dropped fruits were counted every week from the fungicide application to the harvesting (till the 15th of February). The dropped fruits after chemical application were calculated in percentages given as under: -

$$\text{Percent fruit drop} = \frac{Total\ number\ of\ fruits\ dropped\ per\ tree}{\text{Initial number of fruits per tree}} \times 100$$

An analysis of variance (ANOVA) of means was conducted using Statistix software (10th version), and the least significant difference (LSD) test (P<0.01) was used to compare treatment means (Steel *et al.,* 1997).

RESULTS

A significant difference (*P*<0.01) was observed between pre-harvest fungicide treatments. The highest fruit drop was recorded in control (21.25%) where plants were left untreated. All fungicides reduced the fruit drop. However, among fungicide treatments, the mean comparison showed that Success had the highest fruit drop (15.5%), while Topsin M had the minimum fruit drop (5.75%). However, other treatments are ordered in ascending order i.e., Amistar Top (7.0%), < Nativo (8.25) < Caprio Top (8.75) < Rally (10.5%) (Figure 1).

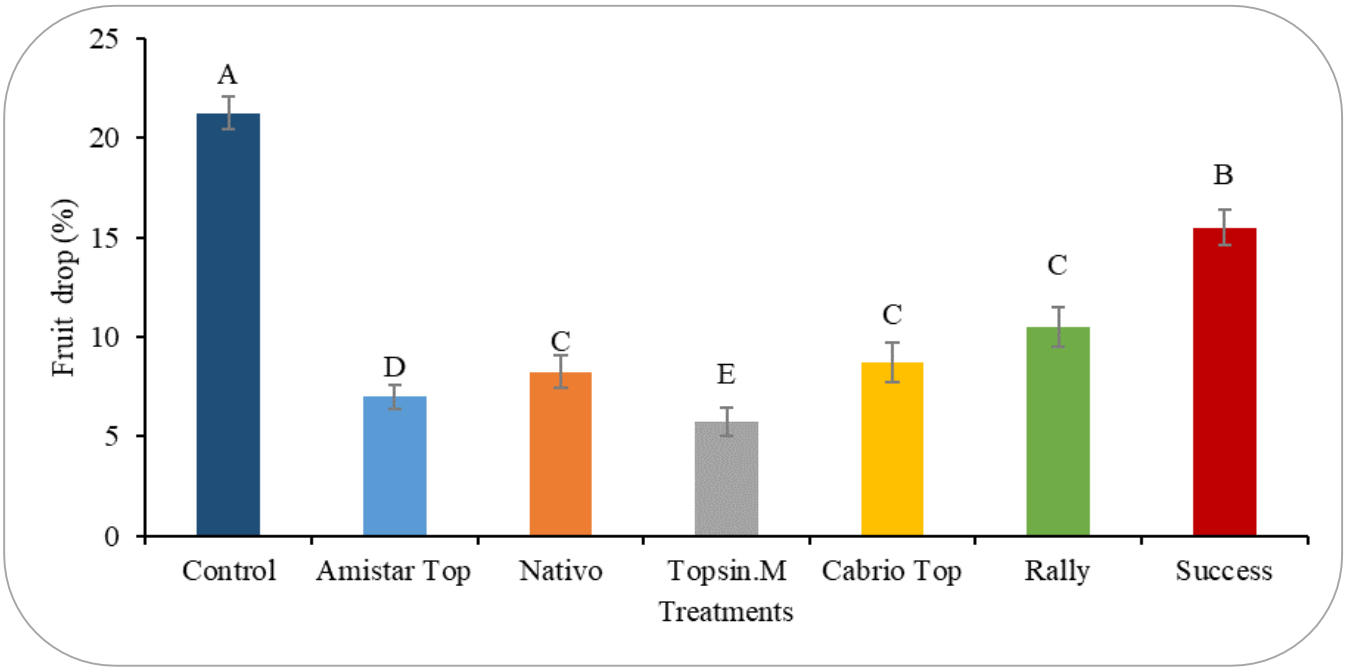

Figure 1. Effect of various fungicides treatment on fruit drop percentage in citrus. The different lettering on error bars shows significance at *P*<0.01

Pre-harvest fungicide treatments showed a significant difference in fruit yield in citrus (*P*<0.01). The highest fruit yield per plant was recorded in Topsin. M (85.5 Kg) followed Amistar Top (81.3 Kg) and by Nativo (80.2 Kg), while the lowest fruit yield was in control (58.3 Kg) where plants were left untreated. However, Cabrio Top, Rally, and Success resulted in 74.3 Kg, 66.5 Kg, and 60.2 Kg, respectively (Figure 2).

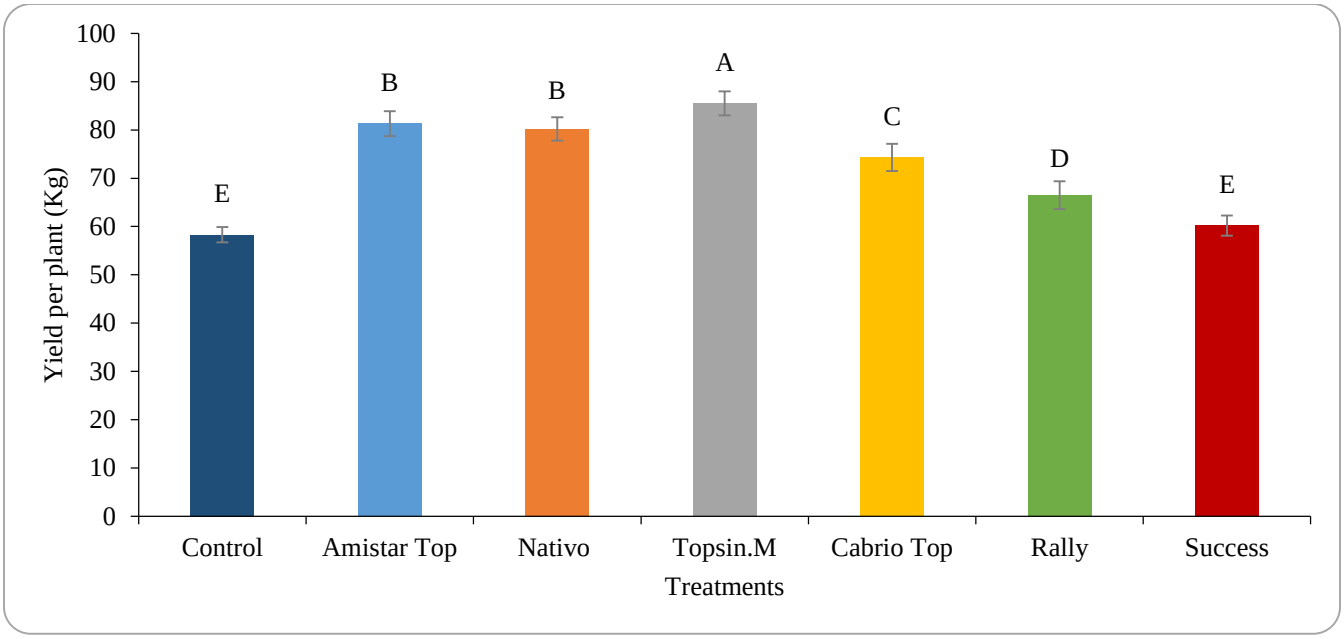

Figure 2. Effect of various fungicides application on fruit yield per plant in citrus. The different lettering on error bars shows significance at $P<0.01$

A significant difference was observed in fruit weight by pre-harvest fungicide treatments ($P<0.01$). The highest fruit weight was recorded in Amistar Top (188 g) and Topsin M (184 g). However, among treatments mean comparison showed two groups success (168 g) and control (166 g) had the least fruit weight (Figure 3).

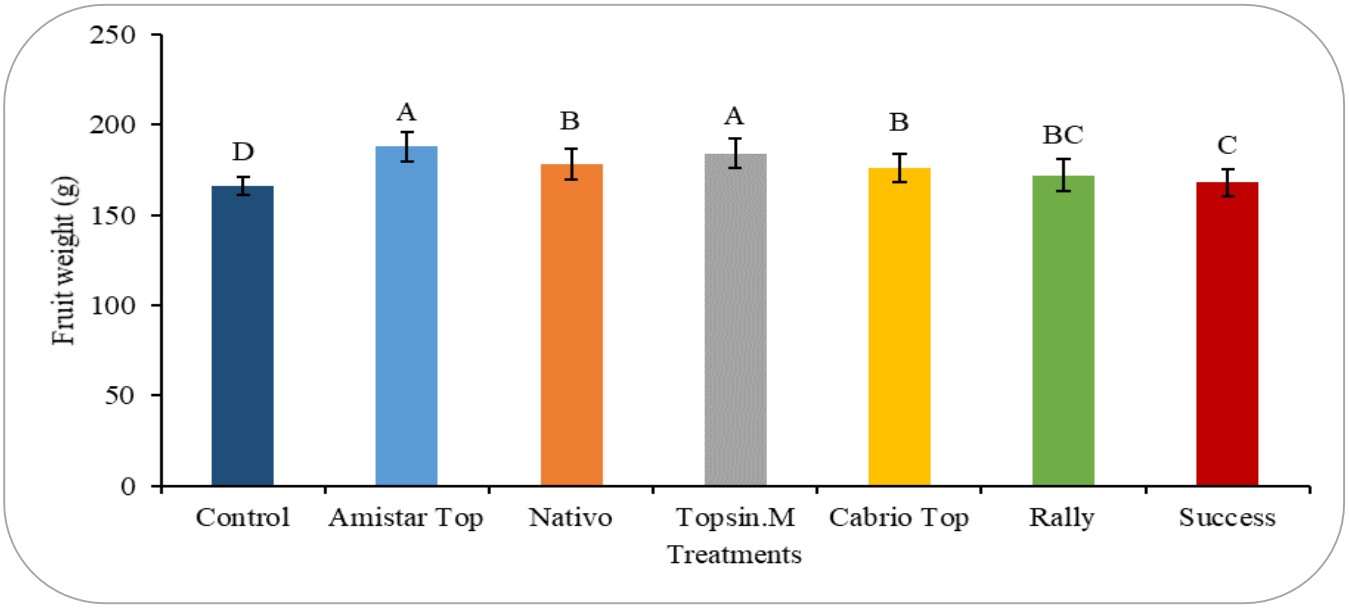

Figure 3. Effect of various fungicides application on fruit weight in citrus. The different lettering on error bars shows significance at $P<0.01$

Pre-harvest application of fungicide showed a significant difference in fruit breadth (mm) in citrus ($P<0.01$). The highest fruit breadth was recorded in Topsin M. (72 mm) followed by Amistar Top (71 mm), while the lowest fruit breadth (59 mm) was found in control (58 mm) where plants were left untreated. Among other treatments, the fruit breadth was Nativo, Cabrio Top, Rally, and Success, respectively (Figure 4).

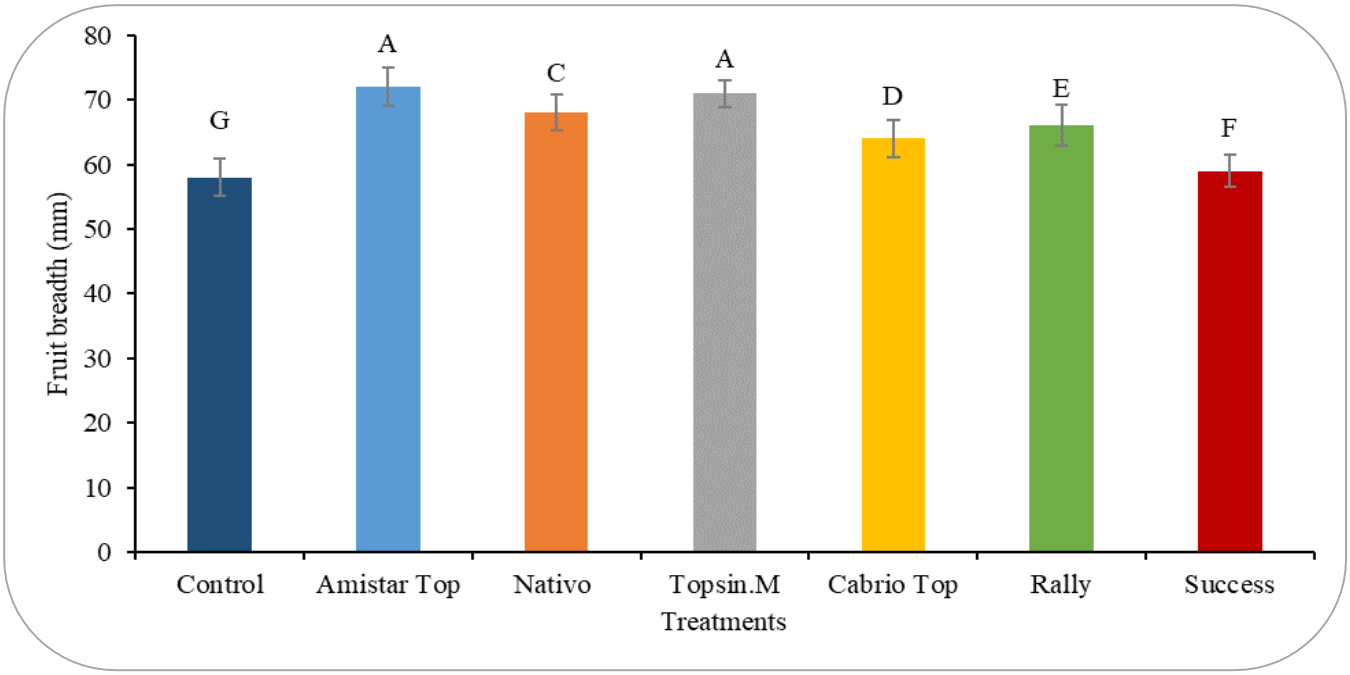

Figure 4. Effect of various fungicides application on fruit breadth in citrus. The different lettering on error bars shows significance at $P<0.01$

A significant difference was observed between pre-harvest fungicide treatments ($P<0.01$). The highest fruit length was recorded in Amistar Top (79 mm) and Topsin M. (69 mm). However, success (57 mm) and control (57 mm) had the least fruit length (Figure 5).

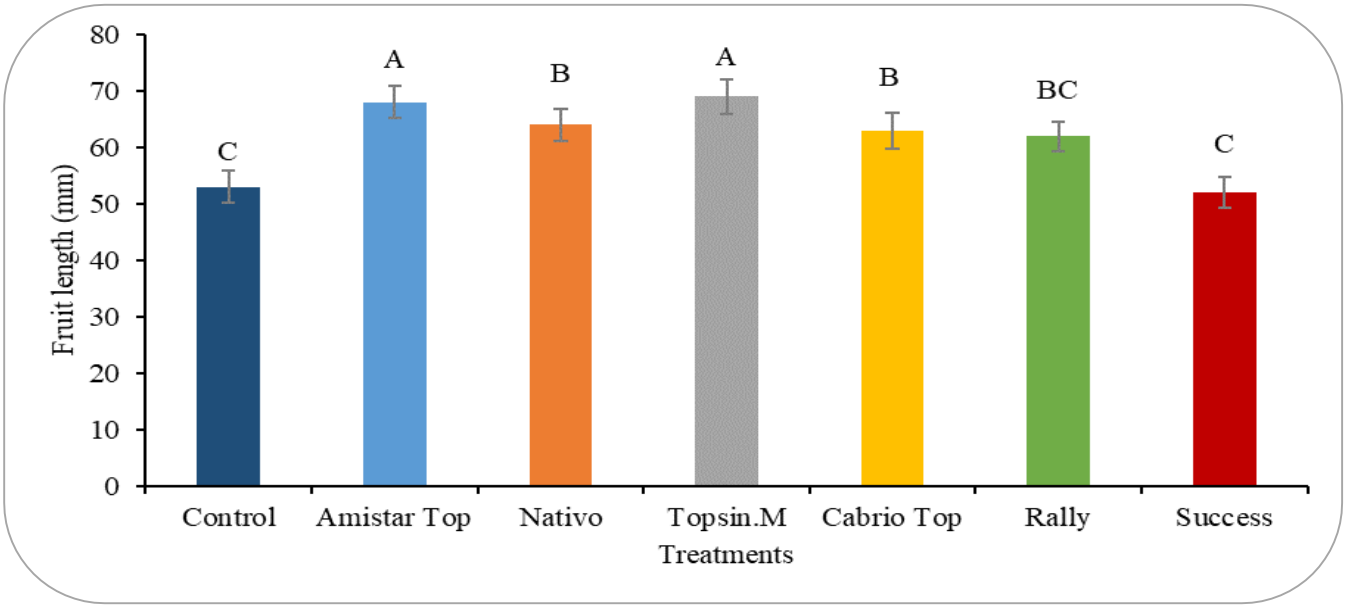

Figure 5. Effect of various fungicides application on fruit length in citrus. The different lettering on error bars shows significance at $P<0.01$

Pre-harvest application of fungicide showed a significant difference in fruit juice in citrus ($P<0.01$). The highest fruit juice was recorded in Topsin. M (53%) followed by Amistar Top (44%), while the lowest fruit juice was found in control where plants were left untreated and treated with Success (43%) (Figure 6).

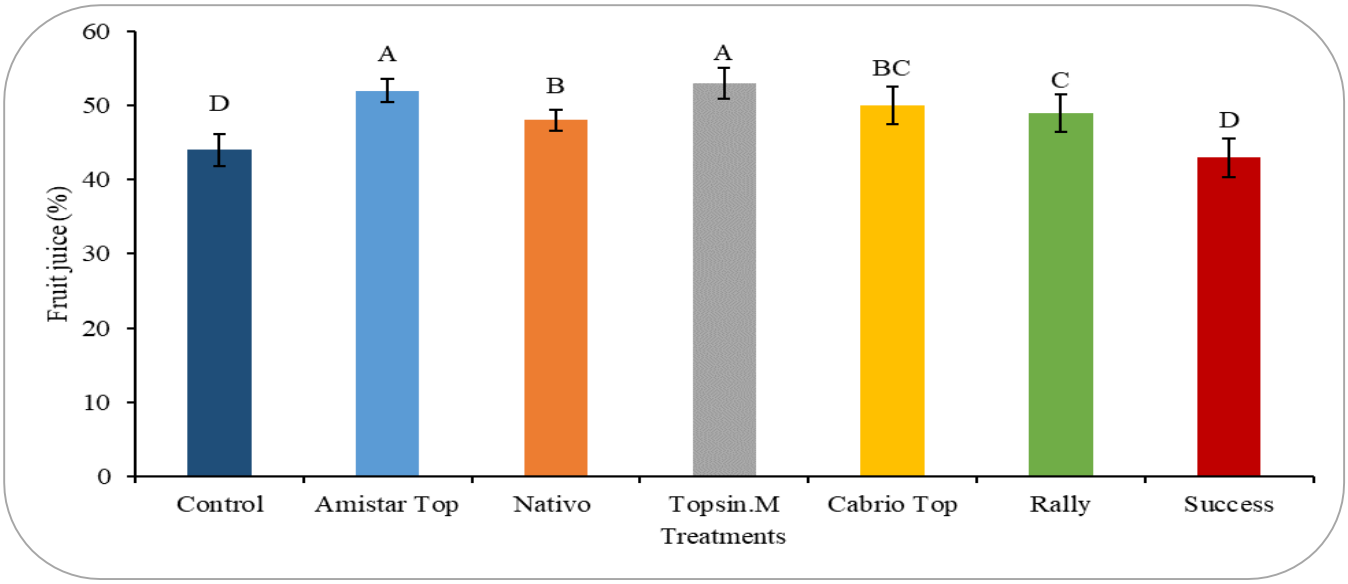

Figure 6. Effect of various fungicides application on fruit length in citrus. The different lettering on error bars shows significance at $P<0.01$

Correlation between fruit drop percentage and fruit yield in citrus: The correlation analysis showed a highly negative correlation (r=-0.825) between fruit drop percentage and fruit yield in citrus. Fruit drop and fruit yield showed an inverse relation as fruit drop percentage increases the total fruit yield decreases.

Table 1. Pearson's correlation analysis among fruit drop percentage and fruit yield in citrus

	Fruit drop%	*Yield*
Fruit drop%	1	
Yield	-0.82586	1
	P-value 0.000	

DISCUSSION

Selection and time of application of fungicides are vital for control of preharvest fruit drop in citrus (Silva-Junior *et al.,* 2014). The incidence of pre-harvest fruit drops due to pathological factors reached its peak between September and December, resulting in a decline in both yield and quality of the harvested crops. Hence, the implementation of integrated management strategies is crucial for efficiently addressing fruit drop sustainably. Consequently, it can be inferred that integrated plant disease management (IPDM) represents an ecologically and economically feasible approach to augment both the quantity and quality of crops (Bharti *et al.,* 2020).

Various preharvest fungicides application impact the post-harvest decay, shelflife and various post-harvest attributes of citrus (Feliziani *et al.,* 2013). Thiophanate methyl has been proven to be very effective in previous studies in alleviating fruit drop in citrus which is used in the present study (Kaur *et al.,* 2015). The Kinnow mandarin has emerged as a dominant citrus cultivar within the citrus industry, owing to its high productivity and superior fruit attributes. Consequently, researchers endeavored to assess the impact of multiple applications of specific fungicides and plant growth promotors on fruit drop in Kinnow mandarin. It was determined that the foliar application of plant growth regulators proved to be an efficacious approach for enhancing both the fruit set and quality of Kinnow mandarin (Atiq *et al.,* 2022). Notably, foliar sprays of Topsin M. and Amistar Top and other fungicides exhibited maximal effectiveness in promoting fruit set and fruit retention, respectively. Furthermore, fruits of superior quality were observed in terms of both size and biochemical characteristics (Kaur *et al.,* 2022).

Pathological fruit drop in citrus, a prevalent ailment in Punjab, causes substantial economic detriment to Kinnow mandarin cultivators (Atiq *et al.,* 2023). The affliction peaks notably in the latter half of September and the initial half of October (Kaur *et al.,* 2015). The malady is attributed to two fungal pathogens, specifically *Colletotrichum gloeosporioides* and *Diplodia natalensis*, which have been identified as linked to the fruit drop syndrome. A significant difference was observed between pre-harvest fungicide treatments (Usman *et al.,* 2023).

The highest fruit weight was recorded in Amistar Top and Topsin M. However, among treatments mean comparison showed two groups success and control had the least fruit weight. The outcomes aligned with those reported by Nawaz *et al.* (2008), indicating significant effects on fruit weight from growth regulators 2, 4-D, GA3, and their respective combinations. The positive impact of Topsin M in controlling fruit drop has been previously recognized by Iqbal *et al.* (2020) which might be due to management of *Phytophthora* species by its foliar application.

Pre-harvest application of fungicide showed a significant difference in fruit juice in citrus. The highest fruit juice was recorded in Topsin M. followed by Amistar Top, while the lowest fruit juice was found in control where plants were left untreated and treated with Success. The findings about juice percentage corresponded with previous studies by Zhang and Timmer (2007) and Matthew *et al.* (2002), demonstrating that the utilization of growth regulators during flowering and preharvest stages resulted in a significant augmentation of juice percentage across diverse citrus species. Current findings in line with the study of preharvest fungicides application on oranges by Besil *et al.* (2019).

CONCLUSION

Fom above results and discussion it could be concluded that fruit drop could be best control by the selection of fungicide. Topsin M having an active ingredient of Thiophanate methyl with a dose of 2 g/L of water followed by Amistar Top having an active ingredient of Azoxystrobin + difenoconazole with a dose of 1 ml/L of water was the most effective in mitigating fruit drop, improving yield and fruit quality of kinnow.

REFERENCES

Agustí, M., C. Reig, A. Martínez-Fuentes and C. Mesejo. 2022. Advances in citrus flowering: A review. Frontiers in Plant Science, 13: 868831 https://doi.org/10.3389/fpls.2022.868831.

Ahmad, H., N.A. Rajput, M. Atiq, G.A. Kachelo, M. Usman, H. Tariq and M. Wahab. 2024. Detection of *Phytophthora nicotiana* induced citrus gummosis by the loop mediated isothermal amplification. Pakistan Journal of Botany, 56(5): 1-11.

Ahmed, W., M. A. Pervez, M. Amjad, M. Khalid, C. M. Ayyub and M. A. Nawaz. 2006. Effect of stionic combinations on the growth and yield of Kinnow Mandarin (*Citrus reticulata* Blanco). Pakistan Journal of Botany, 38(3): 603-612

Anonymous. 2018. Fruit, vegetables and MINFAL condiment statistics of Pakistan, Government of Pakistan, Ministry of Food, Agriculture and Live Stock (Economic Wing) Islamabad, p.1-2.

Atiq, M., M.Z. Talib, N.A. Rajput, S.T. Sahi, M.A. Khan, M. Usman, A. Jabar, M.J. Arif, M.D. Gogi, M.A. Khan and A. Akram. 2023. New-fangled tactics towards cotton leaf curl virus disease A review. Journal of Natural Fibers, 20 (2): 2217364.

Atiq, M., N.A. Rajput, S.T. Sahi, A. Akram, M. Usman, G.A. Kachelo, H. Ahmad, A.Q. Khan, H. Tariq, S. Ramzan, Z.B. Tahir and M.J. Matloob. 2022. A way forward towards the management of chilli anthracnose -a review. Agricultural Science Journal, 4(1): 1-10.

Besil, N., Pérez-Parada, A., Bologna, F., Cesio, M. V., Rivas, F. and H. Heinzen. 2019. Dissipation of selected insecticides and fungicides applied during pre-harvest on mandarin and orange trees in Uruguay. Scientia Horticulturae, 248: 34-40

Bharti, A., K. Karuna, H. Mir and S. K. Shashikala. 2020. Studies on pre-harvest fruit drop and improvement in physical parameters of Kinnow mandarin through exogenous application of plant growth regulators. International Journal of Chemical Studies, 8(1): 1036-1040

Cheem, I. 2021. Growth of Citrus Fruits in Pakistan. Revista Amazonia Investiga, 9: 74-81. 10.34069/AI/2020.35.11.6

Cheema, I. A., and H. K. Jamali. 2020. Growth of citrus fruits in Pakistan. Revista Amazonia Investiga, 9(35): 74-81

Fawzi, E. M., A. A. Khalil and A. F. Afifi. 2009. Antifungal effect of some plant extracts on *Alternaria alternata* and Fusarium oxysporum. African Journal of Biotechnology, 8(11): 2598-2603

Feliziani, E. and G. Romanazzi. 2013. Preharvest application of synthetic fungicides and alternative treatments to control postharvest decay of fruit. Stewart Postharvest Review, 9(3): 1-6

GOP. 2023. Fruit, Vegetables and Condiments Statistics of Pakistan 2021-22. Economic Wing Government of Pakistan Ministry of National Food Security & Research Islamabad.

Iqbal, Z., S. Ahmad, M. Asim, M.A. Rehman, A. Rehman, W. Raza, M. Raza, M. S. Bilal and H. U. Abid. 2020. Management of *Phytophthora* species associated with citrus decline in Pakistan. International Journal of Botany Studies, 5(1): 98-103

Jalili-Marandi, R, A. Hassani, Y. Ghosta, A. Abdollahi, A. Pirzad and F. Sefidkon. 2010. *Thymus kotschyanus* and *Carumcopticum* essential oils as botanical preservatives for table grape. Journal of Medicinal Plants Research, 4: 2424- 2430

Kaur, N., R. Singh and A. Ughareja. 2022. Effect of 2, 4-D

with the combination of fungicides to reduce the pre-harvest fruit drop and quality Improvements in Mandarins cv. Kinnow (*Citrus nobilis* x *Citrus deliciosa* L.). The Pharma Innovation Journal, 11(6): 895-900

Kaur, S., K. B. Bardhan, A. Kumar and T. S. Chahal. 2015. Prevalence of pre-harvest fruit drop disease of citrus in Punjab and in vitro evaluation of fungicides against pathogen. Plant Disease Research, 30(1): 40-45

Khan, A. 2023. Major Problems of Agriculture Sector of Pakistan. Economics and Education Blog. Retrieved from: https://ahsankhaneco.blogspot.com/2012/04/major-problems-of- agricultural-sector.html

Khan, F. U., N. Khan and N. Anjum. 2016. Farmers perception about yield losses of kinnow (*Citrus reticulate*) during its harvesting and post-harvesting operations: case study of tehsil Sargodha. Pakistan Journal of Rural Development and Agriculture, 1(1): 12-19

Lopes, M. R., K. Nadjara, F. L. Pompeo, A. C. Silva and K. C. Kupper. 2015. Saccharomyces cerevisiae: A novel and efficient biological control agent for *Colletotrichum acutatum* during pre-harvest. Microbiololgy Research, 175: 93-99

Mahawar, M. K., Jalgaonkar, K., Bibwe, B., Bhushan, B., Meena, V. S., and R. K. Sonkar. 2020. Post-harvest processing and valorization of Kinnow mandarin (*Citrus reticulate* L.): A review. Journal of Food Science and Technology, 57: 799-815

Marinho, C. S., L. Oliveira, J. C. Serrano and J. Carvalho. 2005. Effects of gibberellic acid and fungicides on post-bloom fruit drop in Tahiti acid lime. Laranja, 26(1): 47-57.

Matthew, W. F. and F. S. Davis. 2002. Gibberellic acid application timing affects fruit quality of processing oranges. HortScience, 37: 353-357.

Maude, L. and G. Bouageois. 2013. Effect of preharvest weather conditions on firmness of McIntosh apples at harvest time. HortScience, 48: 474–480

Nawaz, M. A., W. Ahmad, S. Ahmad and M. M. Khan. 2008. Role of growth regulators on preharvest fruit drop, yield and quality in Kinnow mandarin. Pakistan Journal of Botany, 40(5): 1971-1981

Niaz, C. M. 2004. History and origin of Citrus fruits. Proceedings of the International Symposium on Citrus. Agriculture Foundation of Pakistan Islamabad. pp. 7-10.

Romanazzi, G., A. Lichter, F. M. Gabler and J. L. Smilanick. 2012. Recent advances on the use of natural and safe alternatives to conventional methods to control postharvest gray mold of table grapes. Postharvest Biology and Technology, 63: 141-147

Safdar, A., N. Javed, S.A. Khan, H.U. Khan, A. Rehman and I.U. Haq. 2010. Survey and investigation of different citrus growing areas for citrus sudden death syndrome. Pakistan Journal of Phytopatholgy, 22(2): 71-78.

Silva-Junior, G. J., M. B. Spósito, D. R. Marin and L. Amorim. 2014. Efficacy and timing of application of fungicides for control of citrus post bloom fruit drop. Crop Protection, 59: 51-56

Steel, R. G., J. H. Torrie and D. A. Dickey. 1997. Principles and Procedures of Statistics: A biological approach. McGraw-Hill., 3rd Edition, New York, USA

Usman, M. 2016. Contribution of agriculture sector in the GDP growth rate of Pakistan. Journal of Global Economics, 4(2): 1-3.

Usman, M., M. Atiq, N.A. Rajput, S.T Sahi, M. Shad, N. Lili, S. Iqbal, A.M Arif, U. Ahmad, K.S. Khan, M. Asif and F.U. Haider. 2024. Efficacy of green synthesized silver-based nanomaterials against early blight of tomato caused by *Alternaria solani.* Gesunde Pflanzen, 76(1): 105-115.

Zhang, J. 2014. Lasiodiplodia theobromae in citrus fruit (Diplodia stem-end rot). In Postharvest Decay (pp. 309-335). Academic Press.

Zhang, J. and L. W. Timmer. 2007. Preharvest application of fungicides for postharvest disease control on early season tangerine hybrids in Florida. Crop Protection, 26(7): 886-893.

Contribution of Authors:		
Malik A. Rehman	:	Designed, supervised and facilitated in the conduct of research
Mujahid Ali	:	Conducted the research experiments and wrote the results
Ahsan Shahzad	:	Assisted in research experiments
Salman Ahmad	:	Wrote the introduction and discussion of the results
Sufian Ikram	:	Wrote the abstract and helped in results writing
Yasir Ali	:	Wrote the conclusion section and formatted the reference style according to journal style.
Nabeel Asghar	:	Overall compiled the manuscript.