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Original Research

Effect of Oxalic Acid on Vase Life and Antioxidative Activities of 'Mero Star' Cut Lily Flowers

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ABSTRACT

Lily (*Lilium* L. hybrids) is sub-tropical cut flower that can be grown in open place or in green house and has consumer demand in local as well as in offshore markets. Short vase life, postharvest decay of flowering petals, activities of oxidative enzymes, malondialdehyde contents, electrolyte leakage and low water ratio are the main problems in marketing of cut lilies. This study was carried out to investigate the effect of oxalic acid (OA) treatments on vase life (12 days) and postharvest quality of 'Mero Star' cut lily flowers. The flowering stems were placed in 2, 4 and 6% aqueous solution of OA for 12 days at 20±2ºC with 70±5% relative humidity. Flowers placed in 6% OA solution exhibited longest vase life and flower diameter with higher relative water content and increased fresh weight and reduced flower decay. Reduced malondialdehyde contents and electrolyte leakage with higher activities of catalase, superoxide dismutase and peroxidase enzymes were also noted in 6% OA-treated flowers. In conclusion, exogenous postharvest application of 6% OA extended vase life and maintained postharvest quality of cut flowers by reducing electrolyte leakage and malondialdehyde contents with higher antioxidative enzymes activities.

Keywords: *Antioxidative enzymes, lily, oxalic acid, vase life.*

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INTRODUCTION

The economic value and marketing demand of cut flowers is directly associated with the postharvest quality and vase life (Liao et al., 2013). Pre-harvest plant nutritional condition (Azad et al., 2008), harvest maturity and harvesting season (Fanourakis et al., 2013), activities of anti-oxidative enzymes (Zeng et al., 2014), stability of membrane (Saeed et al., 2014) and postharvest temperature and water balance (Pompodakis et al., 2005) are the most important factors that influence the vase life and postharvest quality of cut flowers. Pre-storage exogenous applications of certain chemical substances like 1-methycyclopropene (Nergi and Ahmadi, 2014), salicylic acid (Zamani et al., 2011), hydrogen rich water (Ren et al., 2017), humic acid (Fan et al., 2015) and sucrose (Arrom and Munné-Bosch, 2012) have also been reported to extend the vase life of flowers. Hybrid lilies are one of the most significant bulbous crops with typical fragrance and trumped shaped flowers. There is high economic value and consumer demand of cut flower lilies in local as well as in offshore distant markets (Burchi et al., 2010). Vase life, postharvest decay due to microbial activities and membrane leakage are the main constraints in the export and marketing window of cut flower lilies (Van Doorn and Han, 2011). To increase the vase life of lilies, many chemicals have been reported by different researchers but there is a gap regarding the role of antioxidative enzymes activities in relation to the vase life of lily.

Recent studies have revealed the anti-oxidative properties of oxalic acid (OA) that have been used for its defensive role against physiological responses in many plants to avoid the aging and senescence (Serna-Escolano et al., 2021). Postharvest exogenous OA application has been reported to lower the incidence of browning, delayed electrolyte leakage with higher phenolic contents in lotus (Ali et al., 2020). Pre-harvest exogenous OA application also lowered the risk of drought, salinity, and temperature stress in cut flowers (Jabeen and Ahmad, 2013). Postharvest OA application enhanced the shelf life of tomato by reducing the activities of antioxidative enzymes. There was delay in MDA content and electrolyte leakage (Kant et

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al., 2013). Pre-harvest OA application has lowered the disease incident, postharvest decay and improved the quality attributes in peach (Jin et al., 2014).

Keeping in view the marketing demand and economic importance of lily, this study was carried out to assess the influence of exogenous OA application on vase life, quality attributes and anti-oxidative enzymes activities of cut 'Mero Star' lily.

MATERIALS AND METHODS

Flower material

The flowering stems of 'Mero Star' lily were harvested early in the morning at green and tight bud stage from a commercial farm (Aziz Nursery Farm, Pattoki, Pakistan). Flowers of uniform size were kept in distilled water and immediately transported to the Floriculture Laboratory, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan through reefer van.

Treatments and storage

Analytical grade OA was purchased from Sigma-Aldrich (St. Louis, USA). Flowers were graded for uniform size and were segregated in 16 main groups including 4 treatments replicated four times and 4 sampling durations with 240 (4 × 4 × 15) flowers in each main group. After division, flowers were placed in 2, 4 and 6% OA aqueous solution for 12 days at 20±2 °C with 70±5% relative humidity. Control flowers were placed in distilled water only. The mouth of vases was properly wrapped with plastic cover to maintain the OA concentrations and minimum evaporation. Flowers were assessed after 3 days interval for quality parameters and vase life. Leaf relative water content and electrolyte leakage was determined using fresh samples. The leaf samples were frozen with liquid nitrogen and stored at -60 °C for the determination of Malondialdehyde (MDA) content, activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) enzymes. This study was carried out under Completely Randomized Design was used for this study (as there were similar conditions for all treatments) replicated four times (15 flowers in each replication).

Flower diameter and vase life

Flower diameter was measured with digital Vernier Calliper and expressed in centimetres till the vase life of flowers. Vase life of flowers is defined as the time period from first day of stem placement in solution to become curled with wilted flower. It was determined in days.

Determination of flower decay and flower fresh weight

Flower decay was determined by the ratio of decayed flowers with total number of flowers in each replication and expressed as percent. The fresh weight of cut flower was recorded quickly after stem cutting and before placement in OA solution. After 3 days interval, the fresh weight of flower was determined. The change (%) in flower stem was assessed as;

$$\text{Fresh weight increase} = \frac{Wi - Ws}{Ws} \times 100$$

Where W_i was fresh weight on interval and W_s was fresh weight before storage.

Relative water content (RWC) of cut flower leaves

Relative water content (RWC) of cut flower leaves was determined by the method used by Ren et al. (2017) with some modifications. After each interval, the leaves from cut flower were defoliated by hand and quickly weighed using digital weight balance. The leaves were dipped in water for 4 hours and weighed after air drying. These leaves were placed in oven for 60 h at 48°C and weighed. RWC was estimated by using the formula,

$$\text{RWC (\%)} = [(W_f - W_d)/(W_e - W_d)] \times 100$$

Where W_e was turgid weight, W_d was dry weight and W_f is the fresh weight of leaves.

Leaf electrolyte leakage and malondialdehyde (MDA) content

Leaf discs of cut flower were immersed in Mannitol (0.3 mmol L^{-1}) solution at room temperature for 30 min. The electrolyte leakage was estimated by using conductivity meter (HI-98305, Hanna Instruments Inc., Mauritius) from the solution. The solution along with discs was heated for 15 min at 98ºC and cooled at ambient temperature. The final reading of electrolyte leakage was determined on cooling and presented in percent as proposed by Jiang and Chen (1995) with some modifications. Malondialdehyde (MDA) content was estimated as described by Shah et al. (2017) by the incubation of one gram leaf sample in 10% trichloroacetic acid. The solution containing leave sample (1 g) was centrifuged for 20 min at 10,000 × *g*. The supernatant (2 mL) was heated in water bath at 100ºC for 15 min with 0.6% 2-thiobarbituric acid (2 mL). The final MDA content was determined by noting absorbance at 450, 532 and 600 nm, respectively, and expressed as nmole kg^{-1} on fresh weight basis.

Activities of CAT, POD and SOD enzymes

For the assessment of enzymes assay, 1 g ground leave tissues and 2 mL citrate buffer (pH 4) having polyvinylpyrrolidone were homogenized in chilled mortar and pestle followed by centrifugation at 4 °C for 10 min at 10,000 × *g*. The supernatant was collected to determine the activities of following enzymes. The method proposed by Ali et al. (2016) was used to assess the activity of CAT enzyme (EC 1.11.1.6). In short, freshly prepared 100 µL H_2O_2 (5.9 mmol L^{-1}) was reacted with 100 µL crude enzyme extract. The absorbance was noted at 240 nm and enzyme activity was expressed as µmol s^{-1} kg^{-1}. POD (EC 1.11.1.7) enzyme activity was monitored by reacting 100 µL enzyme extract with mixture containing 100 µL guaiacol as substrate, 100 µL H_2O_2 (40 mmol L^{-1}), and 800 µL phosphate buffer [50 mmol L^{-1} (pH 50)]. The final POD enzyme activity was monitored at 470 nm and expressed as µmol s^{-1} kg^{-1} (Ali et al., 2016). SOD (EC 1.15.1.1) enzyme assay was determined by the method proposed by Ali et al. (2016) and expressed in µmol s^{-1} kg^{-1}. The bovine serum albumin was used as previously proposed method of Bradford (1976) for standard protein content.

Statistical analysis

All treatments were replicated four times with 15 flowers in each replication. Completely Randomized Design (CRD) was used to arrange the flowers in experiment. The data collected was subjected to analysis of variance by using window software Statistix 8.1® and mean ± SE was expressed. Fisher's LSD at $P \leq 0.05$ was used for least significant difference test where *F*-test was significantly different.

RESULTS

Flower diameter was increased in those flowering stems which were placed in OA concentrations (Fig. 1a). Maximum flower diameter was observed in flowers stored in 6% and 4% OA solution that was 1.32- and 1.2-folds higher as compared with control (Fig. 1a). Postharvest exogenous OA application increased the vase life of flowers on an average as compared with control (Fig. 1b). Maximum vase life was noted in 6% OA treated flowers followed by 4% and 2% treatments. Compared with the control, vase life was about 1.53-folds higher in flowers placed in 6% aqueous solution of OA (Fig. 1b). With the advancement in the storage period, flower decay was increased in all treatments (Fig. 2a). In comparison, this increase was significantly higher in control as compared to the OA treatments. After 12 days, minimum decay was noticed in 6% OA-treated flowering stems followed by 4% and 2% OA-treated flowers, respectively (Fig. 2a). As far as the fresh weight increase was concerned, it showed continuous increase up to 6 days (Fig. 2b). There was significant decrease in fresh weight increase after 6 days storage in all the treatments. However, this decline in fresh weight was minimum in 6% OA-treated flowers followed by 4% and 2% OA-treated flowers, respectively (Fig. 2b). However, no data was available for control on day 12 as the vase life was about 9 days.

There was substantial rise in RWC for first 3 days of storage;

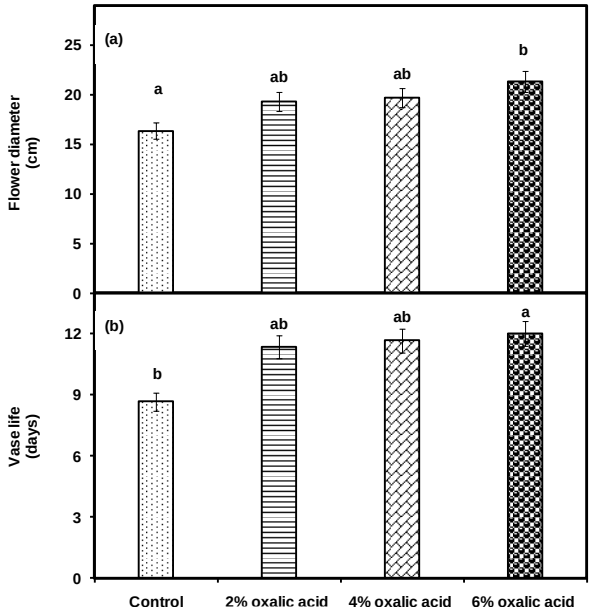

Figure 1: Effect of postharvest application of oxalic acid on flower diameter (a) and vase life (b) of 'Mero star' lily. Mean values in graphs with different letters are significantly different by Fisher's least significant difference (LSD) test ($P \leq 0.05$). Vertical bars represent ± SE of mean and bars are invisible where values are less than symbols. n = 5.

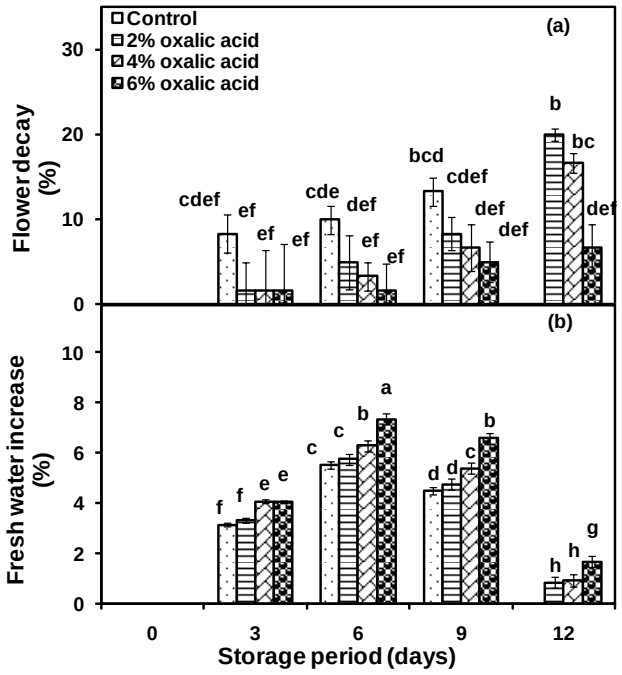

Figure 2: Effect of postharvest application of oxalic acid on flower decay (a) and fresh weight increase (b) of 'Mero star' lily. Mean values in graphs with different letters are significantly different by Fisher's least significant difference (LSD) test ($P \leq 0.05$). Data regarding flower decay and fresh weight increase was not present for control on 12 days as vase life was only 9 days. Vertical bars represent ± SE of mean and bars are invisible where values are less than symbols. n = 60 (15 flowers × 4 replications). n = 5 for fresh weight increase.

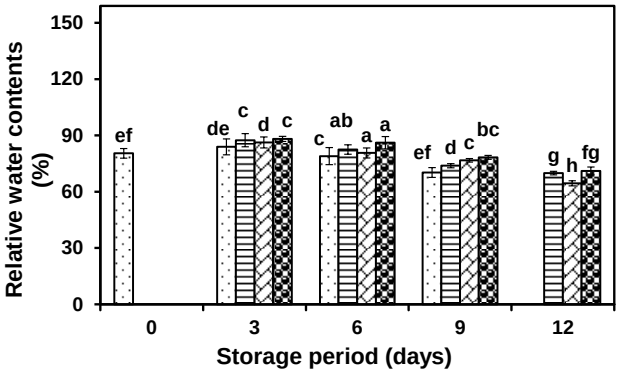

Figure 3: Effect of postharvest application of oxalic acid on relative water content of 'Mero star' lily. Mean values in graphs with different letters are significantly different by Fisher's least significant difference (LSD) test ($P \leq 0.05$). Data regarding relative water content was not present for control on 12 days as vase life was only 9 days. Vertical bars represent ± SE of mean and bars are invisible where values are less than symbols. n = 5.

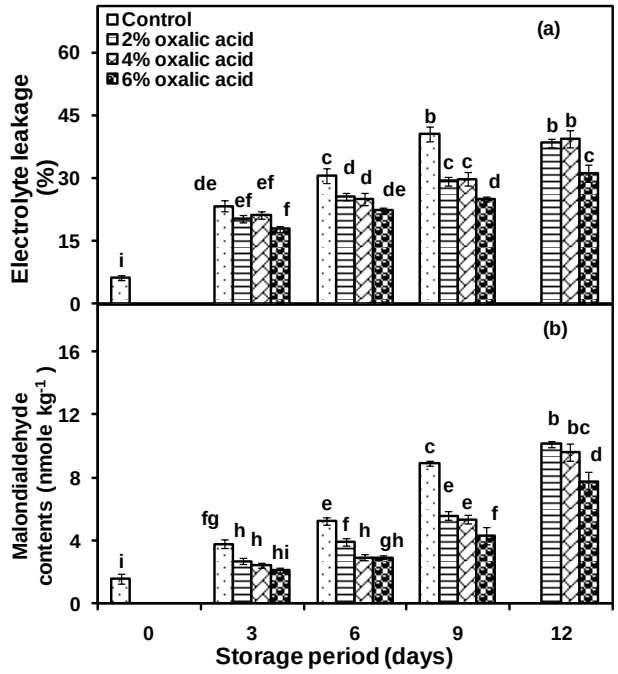

Figure 4: Effect of postharvest application of oxalic acid on leaf electrolyte leakage (a) and malondialdehyde contents (b) of 'Mero star' lily. Mean values in graphs with different letters are significantly different by Fisher's least significant difference (LSD) test ($P \leq 0.05$). Data regarding electrolyte leakage and malondialdehyde content was not present for control on 12 days as vase life was only 9 days. Vertical bars represent ± SE of mean and bars are invisible where values are less than symbols. n = 5.

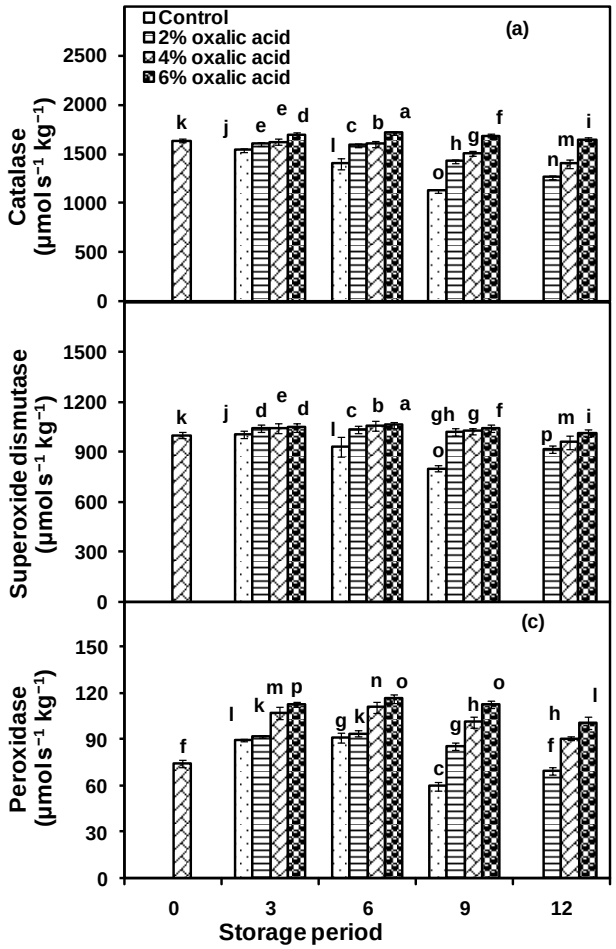

Figure 5: Effect of postharvest application of oxalic acid on the activities of catalase (a), superoxide dismutase (b) and peroxidase (c) enzymes of 'Mero star' lily. Mean values in graphs with different letters are significantly different by Fisher's least significant difference (LSD) test ($P \leq 0.05$). Data regarding activities of catalase, superoxide dismutase and peroxidase was not present for control on 12 days as vase life was only 9 days. Vertical bars represent ± SE of mean and bars are invisible where values are less than symbols. n = 5.

however, there was decline in RWC with the increase in storage duration up to 12 days (Fig. 3). Maximum RWC was exhibited by those flowers placed in 6% OA solution at the end of 12 days storage followed by 2% and 4% OA-treated flowers, respectively (Fig. 3). Regardless of the treatments, linear and continuous rise in the electrolyte leakage and MDA content of leaves was noticed with the progress in storage period that was more pronounced in control, compared to OA treatments (Fig. 4a, b). After 9 days storage, electrolyte leakage and MDA content was significantly less (1.61- and 2.06-folds, respectively) in 6% OA-treated leaves, compared with control. Moreover, 2% and 4% OA treated flowers also exhibited reduction in electrolyte leakage and MDA content (Fig. 4a, b). However, no data was available for control on day 12 as the vase life was about 9 days.

Irrespective of the treatments, CAT and SOD enzyme activities were declined with the increase in storage period (Fig. 5a, b). On an average, this decline was significantly higher in untreated flowers. In contrast to control, flowers stored in 6% OA solution maintained 1.49- and 1.16-folds higher CAT and SOD enzymes activities, respectively, after 9 days storage (Fig. 5a, b). Activity of POD enzyme exhibited a linear increase up to 3 days storage; however, there was linear and continuous decline in all treatments till 12 days storage (Fig. 5c). Maximum decline was noted in those flowers that were stored untreated. After 9 days storage, flowers stored in 6 and 4% OA solution maintained about 1.88- and 1.69-folds higher POD enzyme activity as compared with the control (Fig. 5c). As the vase life of untreated flowers were 9 days so no data was available about the activities of CAT, SOD and POD enzymes on day 12.

DISCUSSION

Postharvest quality and vase life are one of the most important parameters in marketing of cut flowers. In the present study, flower diameter and vase life were increased by OA treatments (Fig. 1a, b). Flower diameter is the quality indicator for lily and there was minimum reduction in the flower diameter due to OA application (Zhang et al., 2011). OA plays a defensive role against many physiological responses (Serna-Escolano et al., 2021). In present study, the extended vase life of cut flower lily might be due to the reduced flower decay and higher activities of

antioxidative enzymes. Previously, OA extended the shelf life of plum (Wu et al., 2011) and mango (Ding et al., 2007). *Botrytis cinerea* and *Botrytis elliptica* are fungus species that are responsible for the flower decay in lilies (Doss et al., 1988). In the present study, reduced flower decay (Fig. 2a) in OA-treated flower might be correlated with anti-fungal activities of OA as reported by Shimazono and Takubo (1952). The increased fresh weight of OA-treated flowers was noticed throughout the storage period that might be ascribed with improved water uptake by 'Mero Star' lilies (Fig. 2b).

RWC is one of the most important parameters for the maintenance of cut flower quality (Shaheen et al., 2015). Reduction in RWC is closely associated with the water loss due to membrane leakage and production of MDA content. In current study, higher RWC in OA-treated flowers might be credited to the reduced MDA content and maintained membrane integrity for longer period (Fig. 3). Membrane permeability often expressed in the terms of membrane leakage is the critical index for the maintenance of quality in horticultural commodities that is directly associated with the senescence (Bajji et al. 2002). In the present study, the OA treatments inhibited the senescence of flowers that lowered the incidence of membrane leakage (Fig. 4a). Previously, OA has been reported to reduce the membrane leakage in 'Bayuecui' peach (Zheng et al., 2007) and 'Mollar de Elche' pomegranate fruits (Sayyari et al., 2010). In the present study, reduction in MDA content was noted in OA-treated flowers (Fig. 4b). MDA content is the major by-product of lipid peroxidation stimulated by the production of reactive oxygen species (ROS) especially H_2O_2 (Shewflet and del Rosario, 2000). However, the activities of anti-oxidative enzymes lower the incident of lipid peroxidation by reducing the production of ROS. Owing to the less oxidative damage and increased anti-oxidative enzymes activities MDA content OA treatments had low MDA content, compared with the control (Fig. 4B). Similar results have been reported in 'Zill' mango (Ding et al., 2007) and 'Baifeng' peach (Jin et al., 2014).

Higher activities of CAT, POD and SOD enzymes were assessed in current study. Higher activities of CAT and SOD were maintained with delayed senescence and ROS scavenging (Jimenez et al., 2002). SOD rapidly dismutase O^{2-} in H_2O_2; while CAT then decompose it in O_2 and H_2O (Mittler, 2002). OA-treated flowers exhibited higher CAT and SOD enzymes activities that might be ascribed by the reduced senescence. POD is anti-oxidative enzyme that is involved in the ROS scavenging by the conversion of H_2O_2 in O_2 and H_2O (Xue and Liu, 2008). Moreover, membrane integrity is also a critical index that maintains higher POD enzyme activity. The higher POD enzyme activity in present study might be due to the maintained membrane integrity. Similar results have been reported by Ding et al. (2007) in 'Zill' mango fruit.

CONCLUSION

In conclusion, postharvest exogenous application of OA (6% and 4%) increased vase life, flower diameter, relative water content and reduced the incidence of flower decay during storage. Moreover, OA-treated flowers also maintained higher membrane integrity and reduced MDA content. Activities of anti-oxidative enzymes (CAT, POD and SOD) were also higher in 6% OA-treated flowers at the end of 12 days storage.

AUTHOR CONTRIBUTION STATEMENT

Hafiz Farooq Anwar: Conceptualization, data collection, research conduction, methodology and lab work. **Hafiz Muhammad Shoaib Shah:** Writing, review, analysis, data arrangement and validation. **Abdul Waheed**: Methodology, analysis. **Mudassar Anwar Butt:** Methodology writing, review, and analysis. **Hafiz Zafar Ul Qasim:** Writing, assisted in results and review. **Asim Bari:** Performed formal analysis, software analysis.

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