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CHANGES IN ACTIVITIES OF CATALASE, GUAIACOL PEROXIDASE AND SUPEROXIDE DISMUTASE DURING PACKING AND STORAGE IN LOOSE FLOWER OF TUBEROSE (*POLIANTHES TUBEROSA*)

Krishan Pal Singh*, Priya B.B. and Ritu Jain

Division of Floriculture and Landscaping
ICAR- Indian Agricultural Research Institute, New Delhi- 110012, India

*Corresponding author: kpsingh.dfr@gmail.com

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ABSTRACT

Tuberose (*Polianthes tuberosa* Linn.) is one of the most important herbaceous bulbous flowering plants and belongs to the family Agavaceae. It is being commercially cultivated in many sub-tropical and tropical areas of the world including India and is in great demand due to its attractive and fragrant flowers. Its loose flowers retain their freshness only for 1-2 day under ordinary condition. Packaging technology concentrated on longer period of storage of loose flowers, would certainly be benefited. The present investigation was undertaken to study the effect of different packaging materials viz., woven bags, high density polyethylene (HDPE) 51micron thickness bags, low density polyethylene (LDPE) 25 micron thickness bags, cloth bags and bamboo baskets (control-without packing) and two storage conditions i.e., ambient condition ($22\pm 1^{\circ}\text{C}$ temp) and cold storage (low temperature) condition ($5\pm 1^{\circ}\text{C}$ temp and 85-95% relative humidity) on a few biochemical attributes namely, catalase (CAT) activity, guaiacol peroxidase (POD) activity and superoxide dismutase (SOD) activity. The study was conducted on fully developed mature florets (loose flowers) of Single petalled tuberose cv. Arka Prajwal at ICAR-Indian Agricultural Research Institute, New Delhi, India. It was observed that packaging material and storage condition significantly influenced all studied attributes. Among five packaging materials, tuberose loose flowers packed in LDPE 25-micron thickness bags recorded the maximum CAT activity under ambient and low temperature storage (0.81, and 1.04 units/g, respectively). However, the minimum CAT activity was obtained in control treatment (bamboo basket) under both storage conditions (0.17, and 0.25 units/g, respectively). Loose flowers packed in LDPE 25 micron thickness bags expressed the maximum POD activity under ambient and cold storage (0.61 and 0.64 units/g, respectively). The lowest POD activity was obtained in control treatment under both storage conditions (0.20 and 0.27 units/g, respectively). Flowers packed in HDPE 51 micron thickness bags expressed the highest SOD activity at both storage conditions (0.61 and 0.65 units/g, respectively). Whereas the lowest SOD activity was recorded in control under both storage conditions (0.19 and 0.27 units/g, respectively). CAT, POD and SOD activities were reported to increase with increase in storage duration and decreased at the end of its useful life. At ambient storage the maximum CAT, POD and SOD activities were recorded on third day (0.57, 0.48 and 0.52 units/g, respectively). While at low temperature the maximum CAT, POD and SOD activities were obtained on fifth day (0.84, 0.59 and 0.61 units/g, respectively).

High density polyethylene (HDPE) 51-micron thickness bags exhibited significantly higher CAT and POD activities, while SOD activity was recorded higher with LDPE 25-micron thickness bags in packing of loose flowers of tuberose cultivar Arka Parjwal.

Key words: Catalase activity, guaiacol peroxidase activity, loose flower, packaging material, *Polianthes tuberosa*, storage condition, superoxide dismutase activity, tuberose

Introduction

With the availability of varied agro-climatic conditions, cheap and skilled labour, good water source and steady

increasing demand for flowers, floriculture is strengthening its strong roots in India. The loose flowers like rose, tuberose, marigold, jasmine, China aster, etc.

are commonly and frequently demanded both in the domestic as well as international markets. Among these, tuberose (*Polianthes tuberosa* Linn.) occupies a prime position among commercial loose flower crops, because of its highly fragrant flowers which can be used in various forms. It belongs to the family Agavaceae and originated in Mexico. It is one of the most popular commercial flower crops in tropical and sub-tropical areas of the world including India. It is also grown for garden decoration, in kitchen gardens, pots, beds, borders, etc. It is perennial herbaceous plant usually produces flowering spikes throughout the year but it blooms profusely during summer and rainy seasons (Singh *et al.*, 2010). Waxy white flowering spikes of Single as well as Double petalled cultivars of tuberose impregnate the surroundings with their sweet lingering fragrance and are in great demand for use in different forms of loose and cut flowers and extraction of concrete (Singh *et al.*, 2010). Tuberose is classified as Single petalled, Semi-double petalled and double petalled florets types. The major portion of tuberose flowers consumption is in the various forms of loose flower, followed by cut flower and extraction of essential oils (Knongwir *et al.*, 2017).

The prices of tuberose loose flowers are determined by market demand, quantity of production and their freshness. Production of high-quality flowers is important in fetching up higher prices in market, hence, it is necessary step to handle the flowers properly after harvesting them from field. Tuberose flowers are delicate and perishable in nature. It is estimated that about 50 per cent loss of tuberose flowers occurs during marketing (Bhattacharjee and De, 2005).

Floral weight and its freshness play a very important role in fetching the higher prices in loose flower market. Marketing of flowers without proper postharvest handling leads to reduction in quality (freshness) as well as its quantity (fresh weight) due to damage caused by various factors which reduces the shelf life, storability and deprives the farmers in getting the better prices of their produce. Postharvest technologies like packaging and storage of flowers are helpful to restrict the change in metabolic activities. Packaging is a tool for controlling flower quality in distribution chain. Apart from preventing mechanical damage, the package serves as a barrier between the condition inside and outside the package. It protects the flowers from unfavorable outside condition and enables a micro climate to develop inside the package (Nowak *et al.*, 1991). The main principles of packaging towards long storage life and keeping quality are to lower their rate of transpiration, respiration and cell division during transport and storage. Since flowers are delicate

and highly perishable, they need great attention through suitable technology in packaging to keep them fresh to consumers satisfaction (Bhattacharjee, 1997). Tuberose loose flowers retain their freshness only for 1-2 day in ordinary condition. Due to short span of life of harvested loose flowers of tuberose, growers/sellers/consumers often face the problem of storage. However, very little results, are documented to prolong the storability of loose flowers of tuberose. Keeping these considerations in point of view, the present study was undertaken with an objective to find out the response of packaging materials and storage conditions on changes in a few postharvest biochemical attributes namely, CAT, POD and SOD activities in loose flowers of Single petalled tuberose cv. Arka Pajwal.

Materials and Methods

The study was carried out at the Division of Floriculture and Landscaping, ICAR- Indian Agricultural Research Institute, New Delhi, India, during 2020- 2021. Single petalled tuberose cultivar Arka Parjwal was selected as plant material for this study. At ICAR- Indian Institute of Horticultural Research Bengaluru, Karnataka state, India, after regress screening of tuberose hybrids for vegetative growth, floral and yield characteristics resulted in selection of a hybrid superior to existed ones. It has been named as Arka Prajwal and released during 2001 for commercial cultivation for cut flower and loose flower purposes. Single petalled type florets of Arka Prajwal are borne on straight, tall and study spikes. The florets (flower buds) are attractive with slight pinkish tings at unopened stage. The individual flowers having snow white colour are heavier in weight and larger in size compared to its parents. Tuberose cultivar Arka Prajwal has been adopted widely in different states of India with higher yield potential compared to earlier grown tuberose cultivar Mexican Single (Singh *et al.*, 2010). The fully matured unopened florets of tuberose cultivar Arka Prajwal were hand plucked from flowering spikes of growing plants during early morning hours. Healthy crop was raised under sub-tropical climate condition of plains of north India. The investigation was conducted with five packaging materials namely, P1- Woven bags, P2- High density polyethylene (HDPE) 51-micron thickness bags, P3- Low density polyethylene (LDPE) 25-micron thickness bags, P4- Muslin cloth bags and P5- Bamboo bucket (control). The matured and unopened floret buds (loose flowers) of tuberose having almost uniform shape, size, weight and free from physical damage, insect and disease weighing a quantum of two kilogram in each treatment were prepared. The prepared samples were stored under two storage conditions *i.e.* ambient condition

(22±1°C temp.) and cold storage (low temperature) (with 5±1°C temp and 85-95% R.H.).

During the course of experimentation data on following biochemical attributes were collected time to time: Catalase (CAT) activity (units/g), guaiacol peroxidase (POD) activity (units/g) and superoxide dismutase (SOD) activity (units/g). Catalase catalyze the reduction of hydrogen peroxide to water and molecular oxygen. The enzyme was extracted and estimated following standard method given by Aebi (1983). It is mainly based on the absorbance of H₂O₂ at 240 nm in UV-range and decrease in the absorbance is recorded over a time period.

Enzyme assay: 3.0 ml reaction mixture consisted of the following:

- Potassium phosphate buffer 50 mM (1.5ml of 100 mM buffer, pH 7.0)
- Hydrogen peroxide 12.5 mM (0.5 ml of 75 mM H₂O₂)
- Enzyme 50 µl.
- Water to make up volume to 3.0 ml.

Adding H₂ O₂ started reaction and decrease in absorbance of 240 nm was recorded for 1 minute. The activity of catalase was expressed as change in absorbance per minute per gram tissue.

Activity of enzyme was computed by calculating the amount of H₂ O₂ decomposed. By comparing with a standard curve drawn with known concentrations of hydrogen peroxide initial and final contents of hydrogen peroxide are calculated. Quantity of hydrogen peroxide reduced= Initial reading- Final reading per min per mg protein.

Guaiacol peroxidase also referred as non- specific peroxidase/ guaiacol- peroxidase catalyse the reduction of hydrogen peroxide with a concurrent oxidation of a substrate



Guaiacol peroxidase activity was estimated by standard method given by Shannon *et al.*, (1966). It is analysed as increase in optical density due to the oxidation of guaiacol to tetra guaiacol.

Enzyme assay: Reaction mixture contain:

- Phosphate buffer (50 mM, pH 6.1) 1.0 ml of 100 mM.
- Guaiacol (16mM) 0.5 ml of 96 mM.
- H₂ O₂ (2mM) 0.5 ml of 12 mM
- Enzyme 0.1 ml.

- Water 0.4 ml, to make final volume of 3.0 ml.

Absorbance due to the formation of tetra guaiacol was recorded at 470 nm and enzyme activity was calculated as per extinction coefficient of its oxidation product, tetra guaiacol = 26.6 mM/cm.

Enzyme activity is expressed as µ mol tetra guaiacol formed per minute per g flower weight (1 unit= µ mole/ minute).

Superoxide dismutase (SOD) catalyze the dismutation of superoxide radical (O₂⁻) to hydrogen peroxide (H₂O₂)



Assay is based on formation of blue coloured formazone by nitro-blue tetrazolium and O₂ radical, which absorbs at 560 nm and the enzyme (SOD) decreases absorbance due to reduction in the formation of O₂ radical by enzyme.

Enzyme assay: Three ml of reaction mixture consist

- 13.33 mM methionine (0.2 ml of 200 mM)
- 75 µM nitroblue tetrazolium chloride (NBT) (0.1 ml of 2.25 mM)
- 0.1mM EDTA (0.1 ml of 3 mM)
- 50 mM phosphate buffer (pH 7.8) (1.5 ml of 100 mM)
- 50 mM sodium carbonate (0.1 ml of 1.5 M)
- 0.05 to 0.1 ml enzyme
- 0.9 to 0.95 ml of water (to make a final volume of 3.0ml)

By adding 2 mM riboflavin (0.1ml) reaction was started then placing the tubes under two 15 W fluorescent lamps for 15 min. Control is a complete reaction mixture without enzyme, which gave the maximal colour. Switching off the light and putting the tubes into dark stopped the reaction. Blank is non irradiated complete reaction. For total SOD separate controls (without enzymes) were used. Absorbency was recorded at 560 nm and 1 unit of enzyme activity was taken as that amount of enzyme, which reduced the absorbency reading to 50% in comparison with tubes lacking enzyme.

$$\text{One unit (of enzyme)} = \frac{\text{Blank Sample}}{\text{Blank}/2}$$

Data on required observations were recorded with the help of essential tools and equipments available with ICAR-IARI, New Delhi. The statistical design of experiment was followed factorial completely randomized design (FCRD) in which packaging materials and storage conditions were two factors and all treatments were replicated four times. Recoded data were subjected to

Table 1. Influence of packaging material, duration and their interaction on catalase activity (units/g) of tuberose cv. Arka Prajwal loose flowers under ambient and cold storage conditions.

Packaging material (P)	Storage condition													
	Ambient storage							Cold storage						
	Duration (D)							Duration (D)						
	0	1	2	3	4	Mean	0	1	2	3	4	5	6	Mean
P ₁ : Woven bag	0.49	0.57	0.62	0.67	0.66	0.60	0.52	0.63	0.68	0.72	0.78	0.84	0.73	0.70
P ₂ : HDPE 51-micron bag	0.56	0.64	0.68	0.78	0.74	0.68	0.59	0.67	0.72	0.82	0.93	1.04	0.95	0.82
P ₃ : LDPE 25 micron bag	0.66	0.72	0.86	0.95	0.89	0.81	0.68	0.77	0.88	0.94	1.18	1.46	1.38	1.04
P ₄ : Muslin cloth bag	0.15	0.22	0.25	0.29	0.26	0.23	0.25	0.29	0.40	0.46	0.52	0.56	0.52	0.43
P ₅ : Control (bamboo basket)	0.12	0.17	0.20	0.19	0.17	0.17	0.14	0.19	0.25	0.27	0.34	0.31	0.28	0.25
Mean	0.39	0.46	0.52	0.57	0.54	–	0.44	0.51	0.59	0.64	0.75	0.84	0.77	–
	C.D.(P=0.05)							C.D.(P=0.05)						
Packaging material (P)	0.02							0.02						
Duration (D)	0.02							0.02						
Interaction (P × D)	0.05							0.05						

statistical analyses. Compilation of mean, standard error of mean (S.E.m.) and critical difference (C.D.) were used for all comparison where significant f-probability ($P < 0.5$) were found using OPSAT version 5.1 software for analysis of variance (ANOVA).

Results and Discussion

Catalase activity (units/g)

Data presented in Table 1 indicate that under ambient condition, there was a significant difference among packaging material, duration and their interaction on catalase (CAT) activity of loose flowers of tuberose. Among five packaging materials, loose flowers packed in LDPE 25-micron thickness bags recorded the maximum catalase activity (0.08 units/g) which was significantly higher with other packaging materials, whereas the minimum catalase activity (0.17 units/g) was recorded in control treatment (bamboo basket). The maximum catalase activity (0.57 units/g) was obtained on third day which was higher with other days, while the minimum catalase activity (0.39 units/g) was recorded on zero day. Interaction effect of packaging material and duration indicates that the maximum catalase activity (0.5 units/g) was obtained in control treatment on zero day.

Table 1 also indicates that under low temperature storage condition, we obtained a significant difference among the packaging material, duration and their interaction. Flowers packed in LDPE 25-micron thickness bags recorded the maximum catalase activity (1.04 units/g) which was higher with other packaging materials, however, the minimum (0.25 units/g) was recorded in control (bamboo basket). The maximum mean of catalase activity (0.84 units/g) was obtained on fifth day, which was higher with other days, while the minimum (0.44 units/g) was obtained on zero day. Interaction effect of

packaging material and duration indicates that significantly the maximum catalase activity (1.46 units/g) was recorded in tuberose flowers packed in LDPE 25micron thickness bags on fifth day, while the minimum (0.14 units/g) was recorded in control flowers on zero day, which was statistically *at par* with control treatment on first day.

Catalase (CAT) catalyze the reduction of hydrogen peroxide to water and molecular oxygen. Catalyses as an antioxidant enzyme whose activity indicates the ameliorative on oxidative stress (Jhangi and Dhatt, 2022). In the present study loose flowers packed in LDPE 25micron thickness bags recorded the maximum catalyses activity. Also catalyses activity revealed a steady increase from zero, fourth and sixth day of storage under both ambient and cold storage conditions of loose flowers. This might be due to the modified atmospheric condition created within the package. High carbondioxide (CO₂) might have reduced the damage caused by the external harmful conditions to the antioxidant system. Our results are well supported with the findings of Yamene *et al.* (1999); Jhangi and Dhatt (2022) in gladiolus florets, Droillard *et al.*, (1887) in cut carnation (*Dianthus caryophyllus*) flowers and Singh *et al.*, (2023) in tuberose loose flowers.

Guaiacol peroxidase activity (units/g)

As evident from Table 2 that under ambient condition, there was a significant difference among packaging material, duration and their interaction on guaiacol peroxidase (POD) activity of loose flowers of tuberose. Loose flowers packed in LDPE 25micron thickness bags recorded the maximum guaiacol peroxidase activity (0.61 units/g) which was higher with other packaging materials, whereas the minimum (0.20 units/g) was recorded in control treatment flowers. The maximum guaiacol

Packaging material (P)	Storage condition													
	Ambient storage						Cold storage							
	Duration (D)						Duration (D)							
	0	1	2	3	4	Mean	0	1	2	3	4	5	6	Mean
P ₁ : Woven bag	0.35	0.49	0.52	0.54	0.43	0.47	0.34	0.46	0.41	0.53	0.60	0.66	0.62	0.52
P ₂ : HDPE 51-micron bag	0.46	0.57	0.59	0.63	0.59	0.57	0.45	0.53	0.58	0.61	0.67	0.73	0.71	0.61
P ₃ : LDPE 25 micron bag	0.48	0.59	0.63	0.69	0.66	0.61	0.46	0.55	0.60	0.64	0.72	0.79	0.75	0.64
P ₄ : Muslin cloth bag	0.13	0.32	0.39	0.26	0.23	0.27	0.26	0.24	0.38	0.44	0.56	0.43	0.33	0.38
P ₅ : Control (bamboo basket)	0.10	0.17	0.29	0.24	0.19	0.20	0.11	0.13	0.28	0.35	0.37	0.34	0.29	0.27
Mean	0.30	0.43	0.47	0.48	0.42	–	0.32	0.38	0.45	0.51	0.59	0.59	0.54	–
	C.D.(P=0.05)						C.D.(P=0.05)							
Packaging material (P)	0.01						0.01							
Duration (D)	0.01						0.01							
Interaction (P × D)	0.02						0.02							

the minimum (0.32 units/g) was recorded on zero day. Interaction effect of packaging material and duration shows that the maximum guaiacol peroxidase activity (0.79units/g) was obtained in flowers packed in LDPE 25micron thickness bags on fifth day, while the minimum (0.11 units/g) was recorded in control treatment on zero day.

The antioxidant enzymes such as catalase activity and guaiacol peroxidase activity are considered as an adaptive response to defend cells against oxidative stress (Jhangei and Dhatt, 2022). The increased activity of both enzymes with increase in storage duration in the present study explains the higher extent of oxidative stress leading to tissue damage or senescence that leads to decline in postharvest parameters of flowers with increase in storage period. Bhaskar *et al.*, (2006) reported a marked increase and then a subsequent decrease in the peroxidase activity, which explains the onset of senescence in rose *Rosa hybrida* petals. Our results

Packaging material (P)	Storage condition													
	Ambient storage						Cold storage							
	Duration (D)						Duration (D)							
	0	1	2	3	4	Mean	0	1	2	3	4	5	6	Mean
P ₁ : Woven bag	0.39	0.46	0.52	0.58	0.52	0.49	0.35	0.44	0.49	0.54	0.62	0.67	0.52	0.52
P ₂ : HDPE 51-micron bag	0.49	0.54	0.64	0.75	0.64	0.61	0.46	0.59	0.61	0.68	0.79	0.80	0.66	0.65
P ₃ : LDPE 25 micron bag	0.45	0.49	0.58	0.63	0.57	0.54	0.42	0.47	0.54	0.59	0.66	0.74	0.64	0.58
P ₄ : Muslin cloth bag	0.25	0.31	0.41	0.44	0.44	0.37	0.14	0.28	0.38	0.45	0.48	0.46	0.45	0.38
P ₅ : Control (bamboo basket)	0.15	0.18	0.23	0.21	0.20	0.19	0.09	0.16	0.23	0.30	0.38	0.36	0.34	0.27
Mean	0.34	0.40	0.48	0.52	0.47	—	0.29	0.39	0.45	0.51	0.59	0.61	0.52	—
	C.D.(P=0.05)						C.D.(P=0.05)							
Packaging material (P)	0.02						0.01							
Duration (D)	0.02						0.01							
Interaction (P × D)	0.04						0.02							

are in close agreement with the findings of Kumar *et al.*, (2018); Singh *et al.*, (2023) on postharvest studies in tuberose (*Polianthus tuberosa*) and Yamane *et al.*, (1999); Jhangi and Dhatt (2022) in gladiolus cut flowers.

Superoxide dismutase (SOD) activity (units/g)-

Table 3 reveals that under ambient condition there was a significant difference among packaging material, storage and their interaction on superoxide dismutase (SOD) activity of loose flowers of tuberose. Among five packaging materials, loose flowers packed in HDPE 51micron thickness bags expressed the maximum SOD activity (0.61 units/g) which was superior with other packaging materials, whereas the minimum SOD activity (0.19 units/g) was obtained in control flowers. The maximum SOD activity (0.52 units/g) was recorded on third day which was higher with other days, while the minimum SOD activity (0.34 units/g) was recorded on zero day *i.e.*, initial day. Interaction effect of packaging material and duration shows that the maximum SOD activity (0.75 units/g) was recorded in flowers packed in HDPE 51micron thickness bags on third day, while the minimum SOD activity (0.15 units/g) was recorded in control treatment (bamboo basket) on zero day and was statistically *at par* with control treatment (bamboo basket) on first day.

Table 3 also reveals that under cold storage condition there was a significant difference among packaging material, duration and their interaction. Flowers packed in HDPE 51micron thickness bags recorded the maximum SOD activity (0.65 units/g) which was higher over other packaging, whereas the minimum SOD activity (0.27 units/g) was recorded in control flowers (kept in bamboo basket). The maximum SOD activity (0.61 units/g) was recorded on fifth day which was higher with other days and *at par* with fourth day, while the lowest SOD activity (0.29 units/g) was recorded on zero day. Interaction effect of packaging material and duration indicates that the maximum SOD activity (0.80 units/g) was reported in flowers packed in HDPE 51-micron thickness bags on fifth day which was *at par* with P₂ treatment on fourth day, while the minimum SOD activity (0.09 units/g) was recorded in control flowers on zero day. In the present study, tuberose loose flowers packed in HDPE 51micron thickness bags recorded the maximum SOD activity. This might be due to modified atmospheric nature of HDPE with high CO₂ and low O₂ which reduces the oxidative stress caused during senescence and preventing the accumulation of superoxide radicals in the floret cells with increased enzyme activity during storage (De Pascale *et al.*, 2005).

According to Kellogg and Fridovich (1975); Dhindsa

et al., (1981) superoxide dismutase (SOD) and catalase (CAT) are essentials for the survival of aerobic cells. They catalytically scavenge superoxide radicals and H₂ O₂, respectively and their activities determine the abundance of O₂ H₂ O₂, OH and singlet oxygen, there by controlling the level of lipid per oxidation. SOD is a key enzymatic antioxidant, which has been shown respond to a range of stresses. Our results are in close conformity with the findings of Yamane *et al.*, (1999) and Droillard *et al.*, (1987) in gladiolus and carnation cut flowers, respectively.

From the present investigation it may be concluded that the maximum SOD activity was obtained in loose flowers of tuberose cultivar Arka Prajwal packed with HDPE 51micron under thickness bags under ambient and cold storage conditions. The maximum catalase activity and guaiacol peroxidase activity were found in loose flowers packed with LDPE 25-micron thickness bags under ambient and cold storage conditions. Catalase activity, guaiacol peroxidase activity and SOD activity parameters were found to increase with increase in storage duration and decreased at the end of its useful life.

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