

## RESPONSE OF SWEET CHERRY (*PRUNUS AVIUM* L.) POLLEN GRAINS TO VEHICULAR EXHAUST POLLUTION AT QUETTA, BALOCHISTAN, PAKISTAN

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**Abstract.** The aim of this study was to assess the effect of air pollution on road side fruit plants (*Prunus avium* L.) palynology. Flower samples were collected from polluted (1-2 m away from the edge of Quetta to Hunna Urak road) and for the comparison from non-polluted sites (250-300 m away from the same collection site). During the investigation different pollen parameters including pollen umbers/production, pollen size, pollen tube growth, pollen viability, pollen regularity and pollen protein contents were used through different methods. Results indicated that pollen grains of *Prunus avium* L. were very sensitive to automobile air pollution. Consequences showed significant reduction in numbers and sizes of pollen grains, pollen tube length and pollen viability of polluted site with respect to the control site. Maximum irregularity in pollen morphology and low pollen protein contents were also recorded from the polluted site. Moreover, results also designated that vehicular pollution increases the resting period of pollen grains for 1 to 3 h. So the different attributes of pollen grains can be used as sensitive bioindicators of adverse factors in botanical environment and this leads to the idea that reduction in the development and potential of pollen grains in response to various poisonous environmental pollutants can be exploited as a good index of air purity.

**Keywords:** *sweet cherry, road side contamination, flowers, soil and statistical analysis*

### Introduction

Growing *human* population and technologies are producing one of the important problems which we are facing today i.e., air pollution. Plant fertility and precise insemination of plant mostly depend on the pollen grains. In severe air pollution conditions, the fertility of plant decreases because of the straight and subsidiary effects on the propagative mechanism. Air pollution due to vehicle exhaust causes irregularity in anthers, decreases the number and masculine infertility (Rezanejad, 2007). The chemical pollutants produced by industries and traffic emissions not only put damaging effects on morphological and physiological parameters of plants but also comprise alterations and chromosomal impairment. According to Pfahler (1981) these irregularities are contingent on ecological and hereditary issues and may eventually go to alteration in propagative capability of plant species. Between several features that add to air contamination,

locomotive emissions are problems of growing dimensions. Vehicular emissions enhance large quantities of dust particulate matters, smolder, toxic gases, heavy metals and biological molecules in the environment all over the world. All these air contaminants produced adverse effects on the health of humans, animals and vegetation (Rezaei et al., 2010; Atkinson et al., 2012). Majd et al. (2004) reported that air pollution due to automobile emission enhances pollen allergenicity and increases the problem of asthma among people residing along road sides. According to Shannigrahi et al. (2004) outdoor vegetation growing is the main and primary target of all types of air contamination.

Several investigations have indicated vehicular emission effects on plant leaves stomatal apertures, viability of pollen and development of plants (Iannotti et al., 2000; Verma et al., 2006; Leghari et al., 2015). Other researchers such as Iannotti et al. (2000), Duro et al. (2013), Kaur et al. (2016) indicated that air pollution mainly effects the size, shape, viability and exine sculpturing of pollen grains. Kaur et al. (2016) also reported that developed pollen grains when released from the flower buds in the contaminated air absorbed humidity and some pollutants, which influenced their viability henceforth plants reproductive system. The pollutants such as heavy metals, fluorides, pesticides gather on the pollen grains surfaces and make them lively and sensitive bioindicators of atmospheric pollution (Noori et al., 2009; Yousefi et al., 2011). To perceive and assess the poisonous materials in the atmosphere, biomonitoring techniques can be engaged.

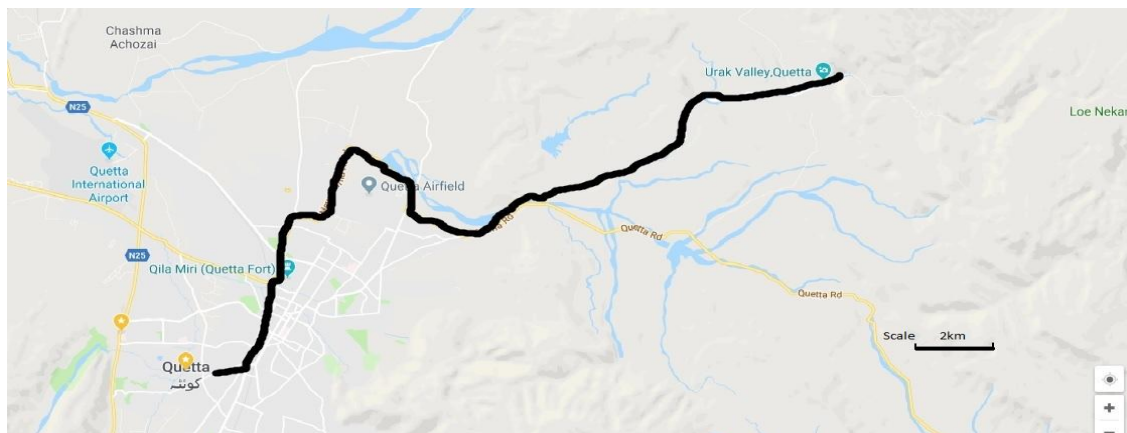
In the current ages several researchers of diverse fields give their attention to the application of palynology in various fields. In palynology the development of pollen grains, pollen grain viability and pollen tube evolution are the superficial bio-pointers of adverse atmospheric pollution. Vehicular traffic adds up different kinds of contaminants like heavy metals, polyaromatic hydrocarbons, gaseous toxins etc. to the atmosphere. Pollen grains of different plants have been found to be sensitive to these pollutants, which show varied effects on their viability, and size and hence can be used as bioindicators of air pollution (Kaur and Nagpal, 2017). This research directed to the knowledge that the downcast growth and developmental potential of pollen in reply to many poisonous environmental pollutants released by traffic can be introduced as a good directory of air cleanliness. This current research study, therefore, was carried out to understand the harshness of vehicular exhaust pollution on the fruit plant at Quetta, Balochistan and delivers some reasonable ideas to decrease vehicular contamination. The present study was conducted on *Prunus avium* L. through different attributes of pollen grains.

## Materials and methods

### Study area

A heavily trafficked road from Quetta city to Urak valley (*Fig. 1*) located in Balochistan, Pakistan was selected in order to evaluate the effect of air pollutants released by vehicles on *Prunus avium* L. fruit crop pollen grains. The road between Quetta city and Urak valley is about 21 km segment and lined on either side with wild roses, medicinally important plants and fruit orchards. Peaches, plums, apricots, cherries and apples of many varieties are grown in this valley. There is no industry on this road and the major source of pollution is from the vehicular emissions. One beautiful water fall is located at the end of Urak valley which makes an interesting picnic spot. The surrounding area of this picnic spot is full of orchards of apple, sweet cherry and apricot, gathering large number of visitors from all over the country to visit the beautiful valley especially during pleasant summer/flowering season. Before sampling in the

target area, a walk through survey of the study area was made and on the basis of small and large vehicular traffic density, the emissions were expected very high along the road sites.



**Figure 1.** Google roads map of study area. (Black colour showing road from Quetta city to Urak valley)

### ***Climate of the study area***

Study area (Quetta district) lies between 300-03' and 300-27' N and 660-44' and 670-18' E. It is situated at an altitude of 1,700 m above sea level. The weather is extremely dry and mean relative humidity of this area mostly remains in range of 15-25% throughout the year. The winter is very cold and the minimum temperature ranges between, -15 to -7 °C and summer is relatively mild and the maximum temperature ranges between 32 to 35 °C, July is generally the hottest month. The district lies outside the range of the monsoon currents and the rainfall is scanty and irregular. The long term 127 year average annual rainfall for Quetta city is 220 mm (BARDC annual report 2017), whereas in the Hanna Urak area, the average is about 312 mm which is mostly recorded in winter months (December –March). (Source: Directorate of Minerals G.O.B Quetta 2017).

### ***Plant material collected***

For the investigation of vehicular exhaust emission effects on physiological parameters of pollen grains such as; production, viability and size of pollen along with germination rate and growth of pollen tube, flower samples were collected from polluted (1-2 m away from the edge of the heavily trafficked road) and comparatively non-polluted areas (about 250-300 m away from the same collection site) having the same soil characteristics (*Table 1*) during the year 2017, as was carried out by Ma et al. (2009). Five sampling sites on the heavily trafficked road were designated after a walk through inspection of various sites and on the basis of assessment of traffic density, level of visible auto emissions fumes/smoke, road dust. Collection of flowers for anther was done by random selection of 5 plants from the polluted (P) and control (C) sites separately at each location from the base, middle and upper portion of the plant around all four sides (north, south, east and west) for the accuracy. The average of the results obtained was used for statistical analysis

and interpretation of results. The flower sample collection was done on the same day and time from both sites (polluted and control).

**Table 1.** Summary of polluted and control sites soil samples

Experimental sites	Soil parameters			
	Soil pH	Specific conductivity	Organic matters	Soil texture
Polluted site	7.7 ±0.06	$5.29 \times 10^{-4} \pm 0.01$	1.0 ±0.2	Sandy loam
Control site	7.4 ±0.2	$5.26 \times 10^{-4} \pm 0.02$	1.2 ±0.06	Sandy loam

±: standard deviation

### **Determination of pollen grain count/production per anther**

For the investigation of pollen grain count/production 36 normal anthers were taken from each plant species of *Prunus avium* L. and put into a 1.5 ml Eppendorf tube at 25 °C, 1 ml of 50 g L<sup>-1</sup> sodium polyphosphate solution was added, and the tube was capped and shaken. Small quantities of the pollen suspension were placed in a hemocytometer counting chamber and the chamber was placed on different microscopic stages to count the number of pollen grains. The number of pollen grains in one anther was calculated as the number of pollen grains per square area × 10 × 36 × 1000/36, with three replications (Shi et al., 2015).

### **Analysis of pollen grains regularity and size**

Regularity of pollen grains was evaluated on the basis of percentage of well-regulated and normal pollen grains. At least 500 pollen grains were investigated from each site (polluted and control). Pollen size was determined by micrometry method. A visual micrometer was fixed on light microscope and measurements of pollen grain size were determined under the magnification of 400×, as per procedure carried out by (Kaur and Nagpal, 2017). A minimum of 500 pollen grains were examined from each plant species. The investigation was repeated thrice and an average was expressed in µm (Iyer and Bholay, 2015).

### **Pollen viability**

Pollen grain viability were examined by using 2, 3, 5 - triphenyl tetrazolium chloride (TTC) staining technique by small alterations as described by Iannotti et al. (2000). By adding 1% solution of TTC in 60% sucrose solution, TTC stain was made stain and saved in dark bottles for the process (making slides). Ten anthers from ten randomly selected appropriate size flower buds of the respective plant species were removed by a shrill needle and placed on a glass slide for examination adding one drop of TTC. After removing air bubbles, the slides were covered by cover slip and airtight by didutylphthalate xylene (DPX) and hatched in the daylight for 2-3 h. These slides were prepared with-in no time after sampling from both sites (polluted and control). Prepared slides were examined under light microscope at the exaggeration of 400× separately. Pollen grain viability was determined by noting/counting the viable and non-viable pollen grains under following categories: the yellowish pollen grains with shrunken configuration were designated as non-viable, whereas the red marked and deep pink pollen grains,

which were rounded in shape, were counted as viable pollen grains. At least 500 pollen grains were studied by each plant (separately from polluted and control sites) and the experiment was repeated for three times and an average was calculated (Kaur and Nagpal, 2017). Further the viability of pollen grains was also tested by the germination of pollen grains (Iyer and Bholay, 2015) and percentage of viable pollen grains was calculated by the following formula:

$$\text{Pollen viability (\%)} = \frac{\text{Number of viable pollen grains}}{\text{Total number of counted pollen grains}} \times 100$$

### ***Rate of pollen grain germination and pollen tube growth***

Pollen grains propagation probability and pollen tube growth was determined by marking the different stages of pollen grain development on flower buds of different sizes. At the same time open flowers were pulled and the succeeding flowers series were marked as; F-0, F-24, F-48 and F-72, where; F-0 was designated for flower bud at the time of dehiscence of anthers, F-24 was for flower buds needed 24 h to open, F-48 was for the flower buds required 48 h to open and F-72 was for the flower buds required 72 h to open. Potential of pollen grains germination, the in-vitro experiment was performed by the method of standing drop in Brewbaker and Kwack's culture medium in the laboratory of Botany department in suitable relative humidity (Brewbaker and Kwack, 1963). Pollen germination and tube growth rate were noted by setting the cultures in one hour intervals that was extended for 12 h. The germination tests were conducted in triplicates and average data were displayed in the results.

### ***Rate of decrease in pollen grain propagation and pollen tube growth***

It contracts by the capability of the pollen grains to persist feasible in the detached successive flowers under natural conditions after the dehiscence of the anthers. The decreasing rate of pollen grain germination and pollen tube development was also deliberated time to time throughout storing via setting cultures medium at 3 h intermissions. These cultures were sustained for 15 h (Nair and Rastogi, 1963).

### ***Determination and analysis of protein***

Protein contents from *Prunus avium* L. pollen grains were determined by the methods used by Rezanejad (2007). Pollen extracts were made by incubating pollen grains in 0.1 M phosphate buffered saline (PBS) pH 7.4 in 15% quantity through inspiring at 4-8 °C for 8 h. Postponements were centrifuged at 10000 × g for 50 min and the supernatants were removed. Protein concentration in pollen grain extracts was recorded in 595 nm as per method of Bradford (1976). Sample protein and standard protein were unglued via SDS-PAGE patterns at 80 V continuous powers for 1-2 h at 15 °C, similar to the modified procedure of Laemmli (1970). Proteins were detected by using a mixture of methanole; acetic; distilled water (1:1:8) with Coomassie brilliant blue R 250.

### Soil analysis

The soil samples were collected from the areas, where under investigated plant species were grown (polluted and control sites) and examined to know the range of soil pH, electrical conductivity (E.C.), contents of organic matters (O.M.) and soil texture. Electrical conductivity and pH were determined by using the conductivity and pH meters respectively. Soil texture and soil organic matter were determined by using Atomic absorption spectrophotometer as the methods described by ICARDA (2001).

### Vehicle count

The vehicles passing along the selected road were counted for 12 peak hours from 8 am to 8 pm for three consecutive times of the season at 5 sites each on target study road. Busses, trucks, vans, cars, motor bikes, rickshaws, container and loaders were counted and classified into three groups (2 and 3-wheelers, 4-wheeler and  $\geq$  6-wheelers (Leghari et al., 2013; Kalid et al., 2017).

### Statistical analysis

All the observation of investigated parameters were calculated as an average and standard deviation. T-test of significance was used for the statistical analysis of data by using self-coded software on Microsoft excel 2007. Comparison between two means (polluted and control) was also intended for the determination of relative stimulatory and inhibitory effects of air pollution on the different attributes (Iyer and Bholay, 2015).

### Results and discussion

The rapid increase in automobile activities and industrial processes, are the main causes of environmental pollution which have adverse effects on plant growth, fertility and productivity. In several contents it has been recommended that the pollen grains have capacity as bioindicators of environmental pollution. In this study, observations recorded show that the plants growing along the road side exhibited considerable damage to their pollen grains in response to automobile exhaust emission as compared to the control site while having the same soil characteristics (Table 1). All results regarding the pollen grains parameters are illustrated in Figure 2a-j and Tables 2 to 5.

**Table 2.** Effect of vehicular exhaust pollution on the rate of decrease ( $\mu\text{m}$ ) in pollen germination of successive flowers of *Prunus avium* L.

Time in hrs.	Successive flowers				
	F-0			F-24	
	Control site	Polluted site	Sig. (p<0.05)	Polluted site	Control site
00	95.3 $\pm$ 0.8	67.5 $\pm$ 2.22	**	Ng	Ng
03	85.9 $\pm$ 1.3	55.1 $\pm$ 2.13	**	Ng	Ng
06	77.1 $\pm$ 0.7	47.4 $\pm$ 0.97	*	Ng	Ng
09	67.2 $\pm$ 0.9	38.8 $\pm$ 0.79	*	Ng	Ng
12	60.4 $\pm$ 0.4	31.3 $\pm$ 0.41	*	Ng	Ng
15	55.7 $\pm$ 0.4	21.4 $\pm$ 0.52	*	Ng	Ng

$\pm$ : standard deviation, Ns: non-significant, \*: slightly significant, \*\*: significant, Ng: no germination

**Table 3.** Effect of vehicular exhaust pollution on the rate of decrease pollen tube length ( $\mu\text{m}$ ) of successive flowers of *Prunus avium* L.

Time in hrs.	Successive flowers				
	F		Sig. (p<0.05)	F-24	
	Control	Polluted		Polluted	Control
00	814.8 $\pm$ 54.4	710.1 $\pm$ 18.9	**	Ng	Ng
03	751.8 $\pm$ 38.3	633.7 $\pm$ 57.6	*	Ng	Ng
06	667.8 $\pm$ 40.3	547.4 $\pm$ 30.9	*	Ng	Ng
09	567.9 $\pm$ 28.2	440.6 $\pm$ 21.0	Ns	Ng	Ng
12	524.5 $\pm$ 23.5	365.5 $\pm$ 28.1	*	Ng	Ng
15	452.3 $\pm$ 22.5	215.3 $\pm$ 24.4	**	Ng	Ng

$\pm$ : standard deviation, Ns: non-significant, \*: slightly significant, \*\*: significant, Ng: no germination

**Table 4.** Effect of vehicular exhaust pollution on the rate of pollen tube length ( $\mu\text{m}$ ) of successive flowers of *Prunus avium* L.

Time in hrs.	Successive flowers				
	F		Sig. (p<0.05%)	F-24	
	Polluted	Control		Polluted	Control
01	Ng	152.2 $\pm$ 23.3	-	Ng	Ng
02	Ng	253.3 $\pm$ 28.1	-	Ng	Ng
03	Ng	341.3 $\pm$ 44.5	-	Ng	Ng
04	164.9 $\pm$ 27.0	453.2 $\pm$ 57.8	*	Ng	Ng
05	244.3 $\pm$ 35.1	555.7 $\pm$ 52.1	*	Ng	Ng
06	323.9 $\pm$ 43.7	650.8 $\pm$ 65.7	**	Ng	Ng
07	400.4 $\pm$ 38.5	736.4 $\pm$ 60.2	*	Ng	Ng
08	480.2 $\pm$ 57.1	832.3 $\pm$ 45.0	*	Ng	Ng
09	550.2 $\pm$ 75.2	920.5 $\pm$ 42.8	*	Ng	Ng
10	611.2 $\pm$ 99.6	1013.4 $\pm$ 36.9	*	Ng	Ng
11	660.5 $\pm$ 60.1	1141.7 $\pm$ 50.4	**	Ng	Ng
12	707.2 $\pm$ 36.2	1237.4 $\pm$ 95.3	*	Ng	Ng

$\pm$ : standard deviation, Ns: non-significant, \*: slightly significant, \*\*: significant, Ng: no germination

**Table 5.** Average traffic density at 5 study sites

Average number of vehicles per hour			
2 and 3 wheelers	4 wheelers	$\geq 6$ wheelers	Sum
530.5 $\pm$ 160.31	324.23 $\pm$ 140.45	50.2 $\pm$ 10.4	904.93

$\pm$ : standard deviation

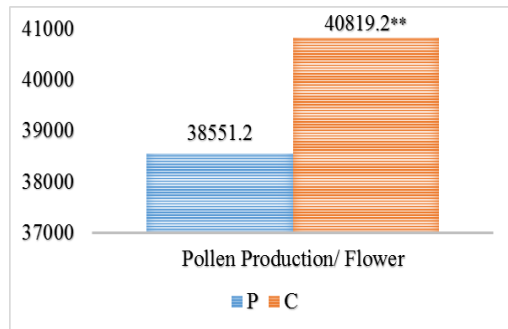
In this study the consequences of pollen production, pollen size, pollen viability and pollen regularity are shown in *Fig. 2a, b, c, d, e*, respectively. Statistics exhibited slightly to significant ( $p < 0.05$ ) decrease in pollen grains production, pollen size (breadth and length), pollen regularity and pollen viability at polluted site as compared to the non-polluted site. The number of pollen grains per flower from polluted site was found 38551.2, whereas pollen numbers were found 40819.2 from control site which is significantly ( $p < 0.05$ ) high (*Fig. 2a*). During the study of pollen grain size; pollen breadth before and after the dehiscence from anther was noted 35 and 47  $\mu\text{m}$  at control site, while at polluted site its breadth decreased by 20 and 31  $\mu\text{m}$  respectively. Similarly pollen length: before and after dehiscence from anther was 45 and 60  $\mu\text{m}$  at control site, whereas it decreased by 32 and 46  $\mu\text{m}$  at the polluted site. Pollen size and length showed slightly to significantly ( $p < 0.05$ ) reduction at polluted site (*Fig. 2b*). Slightly significant ( $p < 0.05$ ) differences in pollen viability were also found between polluted and control sites samples. The percentage of pollen viability at polluted site was recorded 76.2% (T.T.C method) and 78.7% (germination method) with respect to the control site samples 85.6 and 85.5 %, respectively (*Fig. 2c, d*). The data shown in *Figure 2e* indicate that air pollution has significant effect on pollen structure. The air pollution caused a significant ( $p < 0.05$ ) increased number of irregular and breakable pollen grains with respect to control site pollen grains. At control site sample 90% pollen grains were regular, while at polluted site pollen grains regularity reduced with the percentage of 70%. So the data disclosed in *Fig. 2a-e* indicate that the investigated plant species exhibited inhibition in its production of pollen grains, pollen viability, pollen regularity and pollen size due to auto exhaust pollution. Therefore, palynology of any plant can be used as vehicular pollution indicators. Similar results regarding inhibition in pollen production, pollen viability, and pollen size and pollen regularity were also reported by Iyer and Bholay (2015) and Rezanejad (2009). Pollen grain fertility reduction due to vehicular emission was also reported by Bharadwaj and Chauhan (1998) and this effect extends to the heredities development of plants and generative ecology which was highly influenced by the pollen grains propagation (Jain et al., 1997). Rezanejad (2007) also verified that air contamination produced abnormality in anthers, decrease of pollen grains, pollen numbers and male infertility of *Spartium junceum* L. (Spanish broom).

Effects of vehicular emission on pollen grains in the form of reduction in pollen germination and pollen tube length considerably affect the yield of plants. Many other researchers, including Gottardini et al. (2004), Higashitani (2013), Rezanejad (2013), Paupiere et al. (2014), have concluded that the vehicular emission and other atmospheric generated pollutants significantly influence the pollen grain production, pollen size, structure and its viability of different plant species. Kaur and Nagpal (2017) reported inversely proportion between number of traffic and pollen viability for *Alstonia scholaris*, *Nerium oleander* and *Tabernaemontana divaricata* at selected study sites.

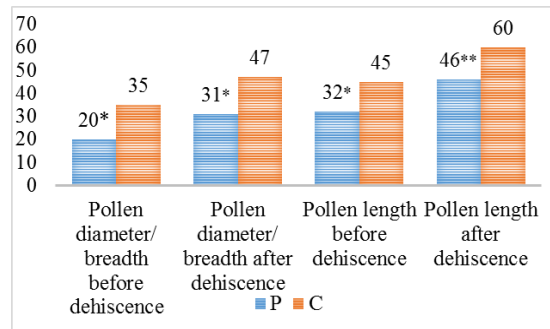
Results presented in *Table 4* indicate that vehicular pollution increases the resting period of pollen grains in examined plant species of F-series by 1, 2 and 3 h, because the pollen grains of *Prunus avium* L. were pretentious due to the perpetuation of the resting time of F-series from 1 to 3 h at polluted site sample. At polluted site the pollen grains started their growth after 3 h and at 4<sup>th</sup> hour the growth was recorded only 164  $\mu\text{m}$  as compared to control site sample 453.2  $\mu\text{m}$ , while on the other hand pollen grain started its growth at 152.2  $\mu\text{m}$  within one hour at control site. These results indicate that the pollen grains were under the stress of vehicular pollution. Data in



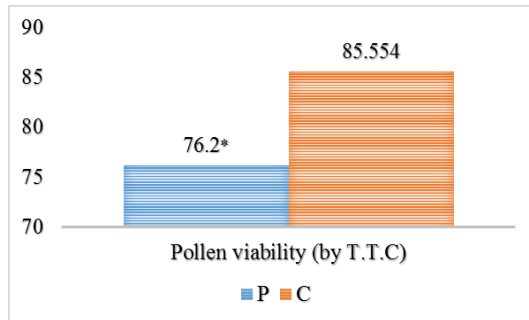
Table 4 also shows that the pollen grains of *Prunus avium* L. exhibited no propagation in F-24 series at all in both sites samples, including polluted and control. The results of this study are in conformity with observation recorded by Iyer and Bholay (2015) who found serious effects of air pollution on pollen grains of *Peltophorum ferrugineum*. They found resting period elongation to 5 h in F-series along with no germination in F-24 series due to air pollution.



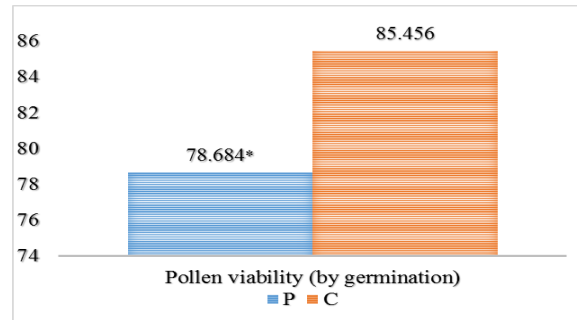
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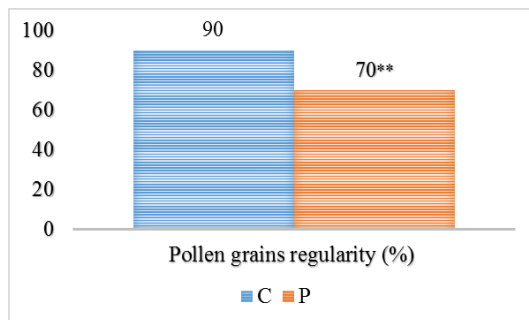
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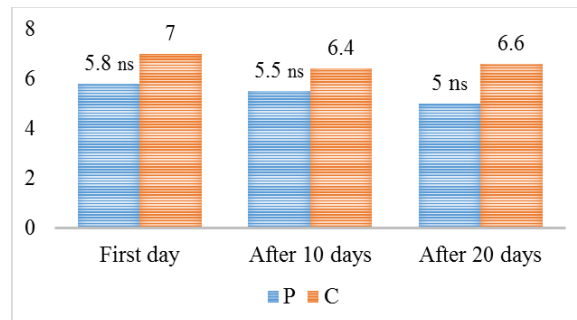
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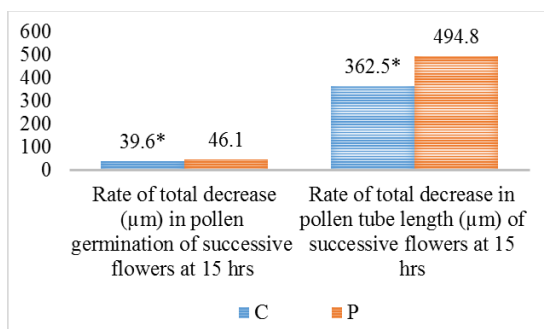
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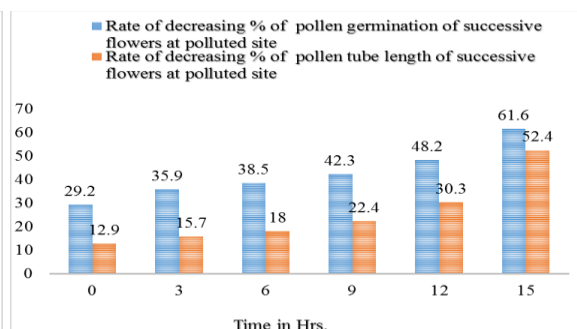
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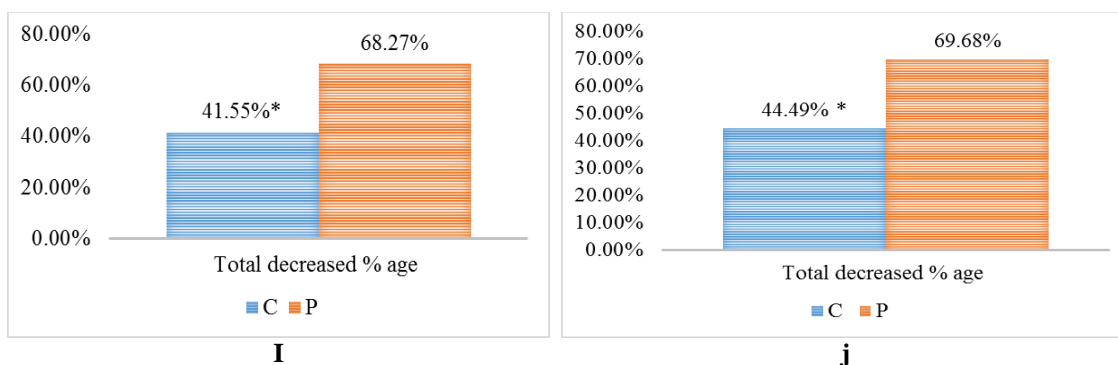
**f**



**G**



**h**



**Figure 2.** *a* Effect of pollution on pollen production. *b* Effect of air pollution on pollen size ( $\mu\text{m}$ ). *c* Effect of pollution on pollen viability (T.T.C). *d* Effect of pollution on pollen viability (by germination). *e* Effect of air pollution on pollen grains regularity (%). *f* Effect of air pollution on protein contents (mg/g dw) of pollen grain in different days. *g* Effect of vehicular exhaust pollution on the rate of total decrease ( $\mu\text{m}$ ) in pollen germination and pollen tube length ( $\mu\text{m}$ ) of successive flowers of *Prunus avium* L. at 15 h. *h* Effect of vehicular exhaust pollution on the rate of decreasing % of pollen germination and pollen tube length of successive flowers. *i* Total decreased % age in the rate of pollen grains germination of successive flowers at 15 h with respect to 0 h due to effect of vehicular exhaust pollution. *j* Total decreased % age in the rate of pollen tube length in F-series of successive flowers at 15 h with respect to 0 h due to effect of vehicular exhaust pollution. (C: control site or non-polluted site, P: polluted site, ns: non-significant, \*: slightly significant, \*\*: significant)

Observations about the rate of decrease in pollen grains germination and pollen tube length in F-series of successive flowers are displayed in *Tables 2* and *3*. These consequences show reverse proportion to the storage period in F-series of successive flowers. At 0 h, rate of decrease in pollen grain germination was 95.3 and 67.46  $\mu\text{m}$  for control and polluted site respectively, while at 15<sup>th</sup> hour the rate of decrease was 55.7 and 21.4  $\mu\text{m}$ , respectively (*Table 2*). Total decrease rate of pollen grains germination after 15 h was 39.6  $\mu\text{m}$  (control) and 46.06  $\mu\text{m}$  (polluted site) with respect to the 0 hour (*Fig. 2g*). The total decreasing percentage was 41.55 and 68.27%, at control and polluted site samples respectively (*Fig. 2i*). Similarly the pollen tube length at 0 h was 814.8  $\mu\text{m}$  (control site) and 710.1  $\mu\text{m}$  (polluted site) in successive flowers, while at 15 h it was decreased by 452.3 and 215.3  $\mu\text{m}$  respectively (*Table 3*). The total decreased rate of pollen tube length at 15 h was 362.5 and 494.8  $\mu\text{m}$  for control and polluted sites respectively (*Fig. 2g*) with respect to 0 hour. The total decreasing percentage in pollen tube length at control and polluted sample after 15 h was 44.49 and 69.68 %, respectively (*Fig. 2j*). Observations reported by Iyer and Bholar (2015) in *Peltophorum ferrugineum* are also coinciding with our results. Significant reduction in both parameters was also noted for each interval of time (0 to 15 h), respectively in the samples of polluted site with respect to the control site (*Fig. 2h*). From these results, it is concluded that the low rate of pollen grains propagation and pollen tube length in the plant species was might be due to greater rate of air pollution in city area. The decrease or inhibition in pollen grain germination and pollen tube length through auto exhaust pollution was also reinforced by other researchers, Farkhondeh (2009) who also found reduction in pollen of *Thuja orientalis* L. (Cupressaceae). Similarly destructive properties of automobile pollutants on vegetation have long been documented (Carhart,

1995; Agrawal and Tiwari, 1997; Singh et al., 2002; Byers et al., 2008; Mashitha and Pise, 2008; Leghari et al., 2013; Laghari et al., 2015) and can be utilized as a bioindicator of air pollution.

Data about soluble protein contents in pollen grain extract is shown in *Figure 2f*. These results conclude that the total contents of protein in polluted site sample was low (5.8 mg/g dw) as compared to the control site sample (7.0 mg/g dw). The results also indicate that as exposing time to the air pollution increased the level of protein contents decrease, which was non-significant. After 20 days at control site sample the pollen protein concentration was recorded at 6.6 mg/g dw but in control site sample it was only 5 mg/g dw (*Fig. 2f*). Similar results were revealed by Hjelmroos et al. (1994) and Parui et al. (1998) who found decrease in Bet v 1 concentration in air contaminated sample. Our results indicate that the difference in protein contents between the sample of polluted and non-polluted sites was non-significant. Similarly, non-significant variation was also noted by Rezanejad (2007) in soluble pollen proteins of *Spartium junceum* L. (Fabaceae) in the samples of polluted and control sites. Observations reported by several other researchers are found in confirmation to our results. Helender et al. (1997) did not perceive any important changes in the bands of protein of control and polluted site samples. Investigation on *lagerstroemia indica* exhibited reduction in total protein concentration and succeeding lower intensity of proteins in air contaminated conditions (Rezanejad et al., 2003).

## Conclusion

This study concludes that the pollen grains are sensitive to air pollution. Vehicular exhaust pollutants prolonged the resting period of pollen grain of F-series by 1 to 3 h in the species study. *Prunus avium* L. exhibited potentiality of pollen grain germination only in F-series and showed no germination at all in F-24 series. Air pollution inhibited pollen tube length, pollen structure, viability and growth of pollen tube and their growth was also found inversely proportionate to the storage time. It is concluded that air contamination seems proficient in altering the pollen grain fertility, development, viability and biochemical and physiological properties of the airborne pollen, therefore imperiling fertilization, reproduction ability, seed setting, plant breeding and possibly allergenicity. So it may be concluded that pollen grains can give significant data about the organic impact of air impurities and can be used as decent applicants as bioindicators of environmental pollution. This type of research may be supportive in exploration of tolerance or sensitivity level of different roadside plant species against air pollution.

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