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Research Article

Floral polymorphism and scanning electron microscopy determination in relation to climatic influence on *Solanum nigrum* L.

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Abstract

Growing concern about climatic influence on plants reproductive biology leads to a recent surge. Climate affects directly floral morphology of plants on this basis current study summarizes climatic effects on floral or reproductive biology of *Solanum nigrum* L. Effect of summer, rainy and winter seasons were recorded on floral morphology, pollens viability & germination, pollen tube growth, fruit-set percentage during investigations which were subjected to one factorial analysis of variance (ANOVA) and least significant differences at $p < 0.05$. Climatic conditions affect floral morphology and produce polymorphism in specific conditions. In rainy and winter seasons, polymorphism was recorded in petals, stamens and pistil which is a first record of climatic influence on polymorphism. Rainy season reported for their maximum flowers numbers which promote a huge fruit-set percentage in open pollination as compared with self and cross pollination. This study confirms the effect of various climates on different floral parts which produce polymorphism along with growth, germination, length, etc. Scanning Electron Microscopy (SEM) study indicated the climatic variations on microscopic observations.

Keywords: climate; floral biology; pollen; polymorphism; *Solanum nigrum* L.

Introduction

Climate change affects the diversity of all living things through a variety of ways (Hughes, 2000; Walther *et al.*, 2002; Parmesan, 2006). Commonly, climate change mainly warming is leading the shift in timing of life history events for many species (Parmesan and Yohe, 2003; Root *et al.*, 2003) and it also affects reproductive success of organism (Sedgley and Griffin, 1889). Many individuals who can adapt to their current climatic conditions but due to change in climate their passed genes to next generation not well adapted (Shivanna, 2003). The process by which the climate-organism interaction is translated into changes in underlying genetic structure of population is natural selection (Raghavan, 2000; Dutkuner *et al.*, 2008). The physiology of reproduction in most flowering plants is markedly influenced by environmental factors (Taiz and Zeiger, 2003) which are generally influence the characters, composition, growth and development of individual plants and plant communities (Lawlor, 2002). In plant reproductive systems, environment exerts considerable influence

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on flowering, pollen fertility, pollen germination and fruiting (Shivanna and Johri, 1985; Shivanna, 2003). Perennial trees interact with environment all-round the year while there flowering and fruiting are closely related to seasonal climatic changes (Sedgley and Griffin, 1989). Extensive studies have been made on the effect of various environmental factors on floral development, pollen fertility, female sterility, flower and fruit abscission including diseases on the development of fruit in plants (Shivanna, 2003). The selected plant, *Solanum nigrum* L. is an annual herb, belonging to family Solanaceae and native of Eurasia (Ogg and Rogers, 1989). It is generally found in disturbed habitats such as roadsides, often on arable land especially, the edges of cultivated fields and plantation and in areas around building and garden; grow well in high nitrogen or phosphorus containing soils (Holm *et al*., 1977). Medicinally this is most important plant and used in hepatitis B virus infection, cardiovascular stimulant, diuretic, anti-inflammatory, anti-oxidative, immunomodulation properties and lowering blood pressure due to breakdown of cholesterol (De Silva *et al*., 2003; Fallah *et al*., 2005). Despite of its high economic and medicinal value limited information is available on its reproductive biology. However, the floral and reproductive biology of *Solanum nigrum* L. has not been studied; hence, present study assesses the extent of climatic conditions in based on floral biology.

Materials and Methods

Solanum nigrum L. selected as an experimental plant which was grown naturally and evaluated for floral characteristics in three selected seasons; rainy, winter and summer. The metrological data for various seasons were collected from concern metrological department. At the time of flower initiation randomly inflorescences were selected and tagged than number of open flowers recorded daily. Floral morphology, number of pollen grains and ovules were studied by the methods of Kearns and Inouye (Kearns and Inouye, 1993). Anthesis, anther dehiscence and stigma receptivity were recorded by the methods of Shivanna and Rangaswamy (1992). Total number of pollens per anther and per flower was measured by using hemocytometer (Barret, 1985). Pollen viability was assessed by fluorescence - FCR and 1% TTC (2, 3, 5-triphenyltetrazolium chloride) method (Shivanna and Rangaswamy, 1992). *In vitro* pollen germination was checked by hanging drop culture method (Brewbaker and Kwack, 1963) and in different concentration of sucrose solutions (10%, 20% and 30%). *In vivo* pollen germination was checked by Alexander's and aniline blue fluorescence microscopic method (Shivanna and Rangaswamy, 1992). The morphology of different floral parts was studied by Scanning Electron Microscopy (SEM) from LEO-EM-SEM at All India Institute of Medical Sciences (AIIMS), New Delhi. Fruit development was observed from day of pollination to maturation and dehiscence. Mature fruits from plants were harvested and collected both fruits and were collected. Fruit-set and seed-set percentage was calculated by the following formula-

$$\text{Fruit} - \text{set } \% = \frac{\text{No. of fruits/Inflorescence}}{\text{No. of flowers/Inflorescence}} \times 100$$

$$\text{Seed} - \text{set } \% = \frac{\text{No. of seed/Fruit}}{\text{No. of ovules/Pistil}} \times 100$$

For pollination mechanism under selected climatic conditions pollinators, their population, visitation rate and pollination efficiency of different pollinators were recorded by observing pollen load under microscope (Kearns and Inouye, 1993). Breeding behaviors (autogamy, geitenogamy and xenogamy) was tested using control pollination studies in emasculated and bagged flowers (Shivanna and Rangaswamy, 1992).

Statistically, data were presented in mean values ± SD with ten replicates (n=10), one factorial analysis of variance (ANOVA) and least significant differences at $p < 0.05$.

Results

In results, direct effect of climate was observed on vegetative and various floral parts of *Solanum nigrum* L. The observed taxonomic characteristics of plant expressed in Table 1, Figures 1 and 2. The most valuable variations in floral characters during different season were number of flowers, time taken for anthesis, dehiscence and stigma receptivity. Maximum numbers of flowers (7.42) were recorded during rainy season followed by winter (6.43) and summer (5.95); in the case of anthesis at least time (800-830 h) recorded in rainy season while maximum in winter; for the time of anther dehiscence and stigma receptivity, summer and rainy seasons performed similar time (830-900 h) (Table 2). Flower parts mainly petals, stamens, and pistils showed polymorphism during this investigation; main influence on these parts were affected by winter and rainy season; during winter the flower parts increased in their number and size while petals number and size decreased by rainy climate, rest of the investigation flowers performed without polymorphism (Table 3, Figure 3). Scanning electron macroscopy (SEM) of flower (Figure 4), stamen (Figure 5) and pistillate part (Figure 6) clearly shows various patterns of trichomes, necteries, pores, pollen grains etc. in their respective ways.

Table 1. Observations of various common floral characters

Floral Characters	Observations
Type of inflorescence	Extra–axillary scorpioid cyme
Floral architecture	Small, full open, complete, hermaphrodite
Colour of flower	White
Shape of flower	Star shaped
Calyx	5, green, bell shaped, gamosepalous, valvate, persistent
Corolla	5, white, gamopetalous, valvate, rotate
Number of stamens	5, polyandrous
Mode of dehiscence	Apical pores
Number of pistils	1, short
Stigma type	Bilobed, wet, papillate
Style type	Short, white, cylindrical, hairy at base, solid
Ovary type	Bicarpellary, syncarpous, superior, bilocular
Pollen grain size	30–45 µm
No. of pollen/anther	700±11.9
No. of pollen/flower	3500±112.59
No. of ovules/ovary	48±1.32
Pollen ovule ratio	70 : 1
Fruit shape	Oval or Spherical
Fruit colour	Unripe – green, ripe - red
Fruit diameter (mm)	20±1.15
Weight of dry fruit (mg)	61±1.05
Seeds per fruit	35±3.07
Seed colour	Whitish brown
Seed shape	Small, flattened
Seed size (mm)	2.04±0.106
Weight of 100 seeds (mg)	250±2.19
Seed-set	83.33%

Figure 1. Floral biology of *S. nigrum*. **a.** extra axillary scorpioid cyme; **b.** anthers; **c.** persistent calyx

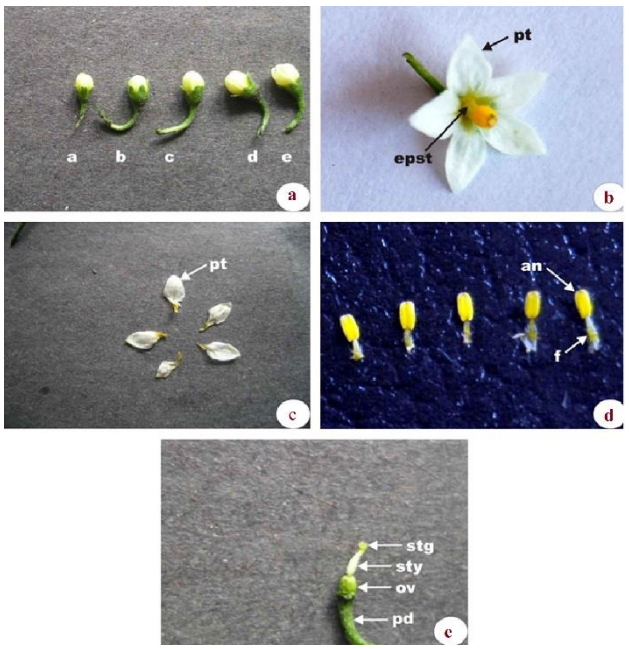

Figure 2. Flower development and arrangement of floral parts. **a.** stages of flower development; **b.** epipepatalous (epst) condition; **c.** petals (pt); **d.** dithecous anther (an) with filament (f); **e.** different parts of pistil

Table 2. Variation in some floral characters under different season

Floral characters	Seasons		
	Winter	Summer	Rainy
Flower/inflorescence	6.43±2.09	5.95±0.04	7.42±1.34
Time of anthesis (h)	0930-1000	0830-0900	0800-0830
Time of anther dehiscence (h)	0930-1000	0830-0900	0830-0900
Time of stigma receptivity (h)	0930-1000	0830-0900	0830-0900

Table 3. Floral polymorphism in *Solanum nigrum* L. under different seasons

Seasons	Month	Temp (°C)		Humidity (%)	Numbers			Petal's length (mm)	Flowers (%)
		Max	Min		Petal	Stamen	Pistil		
Winter	Dec	25.9	11.4	82	No Polymorphism (NP)				
	Jan	21.4	7.6	81	10	10	2	9.44±4.96	10
	Feb	27.8	10.4	80	9	10	2	8.44±4.96	15
	Mar	31.2	17.0	79	No Polymorphism (NP)				
Summer	April	33.4	19.6	60	No Polymorphism (NP)				
	May	38.0	27.0	57	No Polymorphism (NP)				
	June	40.5	24.5	58	No Polymorphism (NP)				
Rainy	July	31.6	24.9	95	No Polymorphism (NP)				
	Aug.	28.6	25.6	96	4	NP	NP	4.08±0.07	40
	Sep.	32.0	24.0	95	4	NP	NP	4.08±0.07	40
	Oct	33.2	18.5	88	No Polymorphism (NP)				

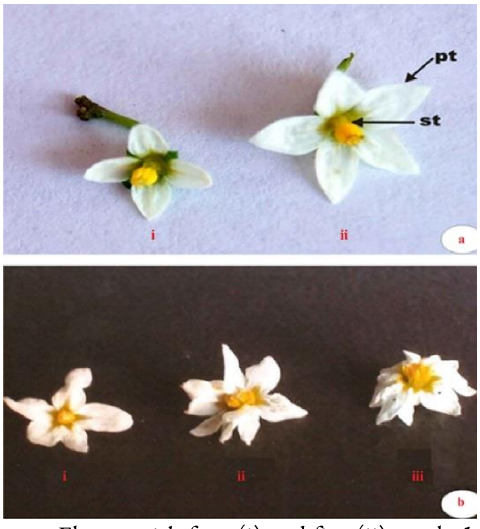

Figure 3. Floral polymorphism **a.** Flower with four (i) and five (ii) petals; **b.** flower with five large petals and five stamens (i), nine petals, ten stamens and two pistils (ii), ten petals, ten stamens and two pistils (iii)

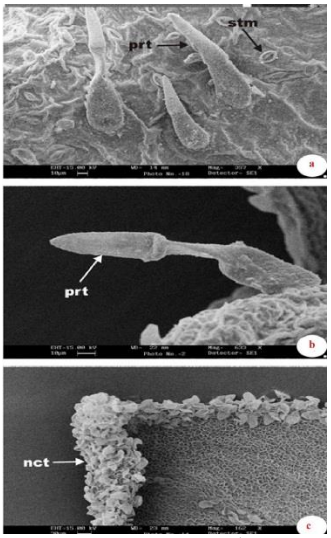

Figure 4. Trichomes on various floral parts **a.** stomata (stm) and procumbent type of trichome (prt) on dorsal surface of sepal 357X; **b.** magnified view of procumbent trichomes (prt) 633X; **c.** nacteries (nct) on upper portion of petal 162X

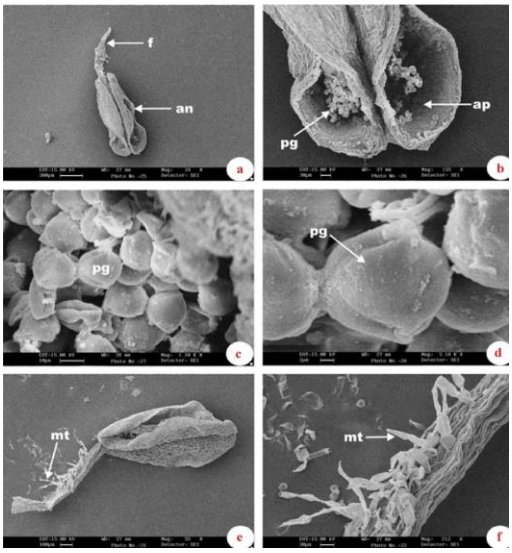

Figure 5. Scanning electron photomicrographs of staminate parts **a.** dithecous condition 34X; **b.** dehiscence through apical pore (ap) and anther lobe with pollen grain (pg) 155X; **c.** tricolporate pollen grain with reticulate exine sculpturing 1.10 KX; **d.** magnified view of pollen grain (pg) 3.18 KX; **e.** filaments showing multicellular trichome (mt) 55X; **f.** magnified view of multicellular trichome 212 X

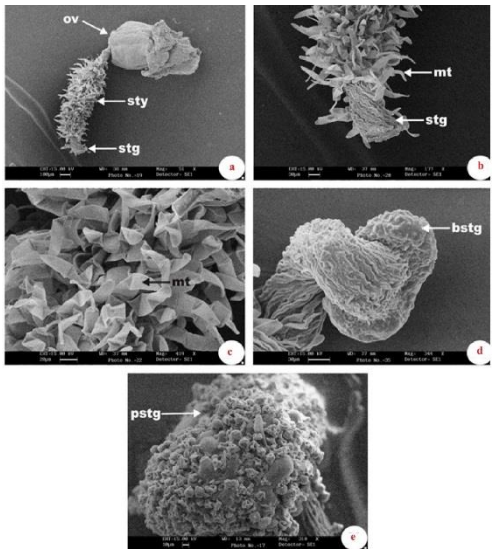

Figure 6. Scanning electron photomicrographs of pistillate parts **a.** pistil 51X; **b.** multicellular trichome (mt) on stylar surface 177X; **c.** magnified view of multicellular trichomes (mt) 419X; **d.** bilobed stigma (bstg) 344X; **e.** compact arranged papillae on stigmatic surface 310X

In respect with various climatic conditions maximum pollen viability was recorded during rainy season followed by winter in all the selected tests. Alexander test performed much better in all the seasons; during rainy season it had 98.04% pollen viability followed by FCR test (70%) and TTC test (62.70%) (Table 4, Figure 7). For *in vitro* pollen germination, maximum pollens germinated during rainy season. Brewbaker and Kwack's Medium performed maximum germination with maximum tube length of pollens; among the various concentration of sucrose solution pollen germination and tube length increased as increasing solution concentration (Table 5, Figure 8). *In vivo* study of pollen germination and tube elongation on stigmatic surface, Alexander's multiple staining method performed better germination and elongation while aniline blue fluorescence test showed only high pollen load (Figure 9).

Table 4. Pollen viability in different tests under various seasons

Viability Test	Season		
	Winter	**Summer**	**Rainy**
Alexander test (%)	84.32	69.35	98.04
TTC test (%)	40.50	18.50	62.70
FCR test (%)	66.60	51.00	70.00

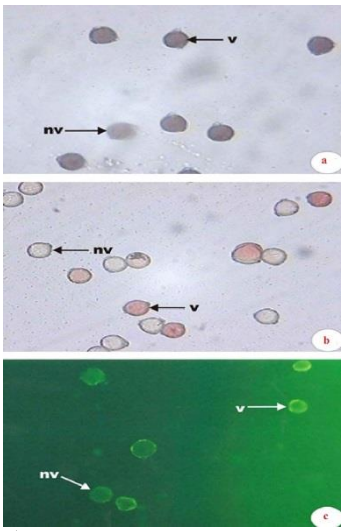

Figure 7. Viable (v) and non-viable (nv) pollens in various tests **a.** Alexander's stain test; **b.** TTC test; **c.** Fluorochromatic reaction (FCR) test

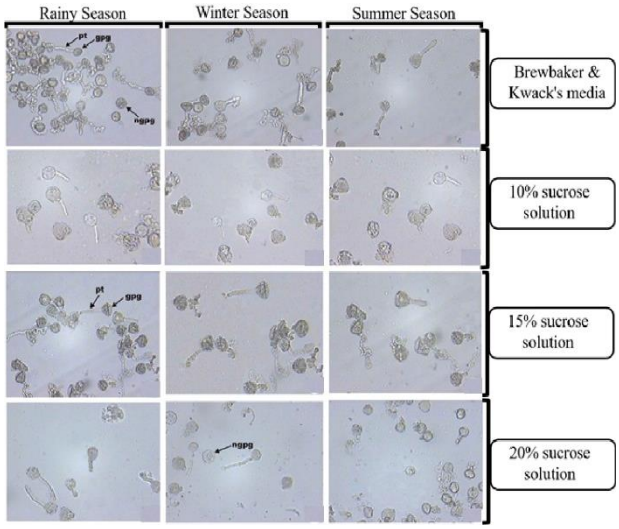

Figure 8. *In vitro* pollen germination in different media under various seasons

Table 5. *In vitro* pollen germination in different media under various seasons

Season	Brewbaker & Kwack's Medium		Sucrose solution					
			10%		15%		20%	
	PG (%)	TL (µm)	PG (%)	TL (µm)	PG (%)	TL (µm)	PG (%)	TL (µm)
Winter	57.3	125.01±8.79	32.0	50.1±1.29	48.0	51.45±5.39	53.0	109.79±8.43
Summer	52.0	118.05±4.25	30.0	49.2±3.45	45.0	51.00±9.01	50.0	105.0±11.20
Rainy	60.0	128.30±7.45	34.0	51.6±7.45	50.0	53.30±7.49	56.0	113.33±7.45

(PG = Pollen Germination, TL = Tube Length)

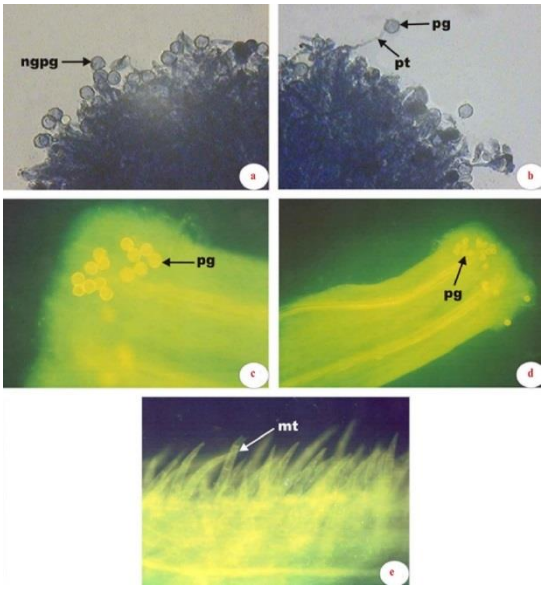

Figure 9. *In vivo* pollen germination on stigmatic surface **a & b.** Alexander's multiple staining method; **c.** aniline blue fluorescence method; **d.** high pollen load on stigmatic surface; **e.** multicellular trichomes (mt) on stylar surface

Figure 10. Different pollinators and visitors in *S. nigrum* plant

In the study of fruit set percentage under different mode of pollinations, open pollination performed a maximum 90% and self-pollination 80% while cross-pollination performed 15% in geitenogamy and 7% in xenogamy (Table 6). Different pollinators and visitors during this investigation have been shown in Figure 10. Number of fruits and fruit set percentage in different seasons were studied, maximum percentage was recorded during rainy season followed by winter and summer; a maximum 5.88% and 85% fruiting and fruit set recorded respectively during rainy season while minimum 3.79% and 56% during summer (Table 7).

Table 6. Percentage of fruit-set under different mode of pollination

Mode of pollination	Open pollination	Self-pollination	Cross-pollination	
			Geitonogamy	Xenogamy
Fruit set (%)	90	80	15	7

Table 7. Number of fruits and fruit set percentage in different season

Parameters	Season		
	Winter	Summer	Rainy
Fruits/inflorescence	4.11±1.93	3.79±0.06	5.88±1.66
Fruit set (%)	72.2	56.0	85.0

Discussion

Floral biology considered the study of flower parts which includes tepals, androecium and gynoecium; individually the study of reproductive part is reproductive biology. In this study, anthesis, anther dehiscence and stigmatic receptivity occurred simultaneously in all the seasons; pollens per anther and flower were 700 and 3500 respectively; pollen and ovule ratio were 70:1. Similar observation recorded in *Withania somnifera* by Jha (2005), mechanism of anther dehiscence, anthers opening and release of pollen grains varies in different plants (Arey and Keating, 1996). Under different climatic conditions the flowers of *S. nigrum* exhibited various interesting polymorphic features, as length and numbers of petals, anthers and pistil. It was interesting to note that all polymorphic features present on single plant as well as different plants during investigation. In normal, flower have five petals, five stamens and one pistil while here some showed polymorphism with four, nine and ten petals; ten stamens and two pistils during various climatic influences. Similar polymorphic observations were recorded in different plants during various previous investigations; as in *Tecoma stans* (Singh and Chauhan, 1994), *Cassia tora* (Sharma *et al*., 2006), *Pyrostegia venusta* (Singh *et al*., 2009). Stylar polymorphism recorded in various plants of Solanaceae by many of the studies; as in *Solanum ovigerum* (Saha and Datta, 2018), *S. hirutum, S. melanospermum* (Diggle and Miller, 2004). The sexual polymorphisms increase the precision of cross pollination and reduce lost mating opportunities associated with self-interference, especially geitonogamy (Barrett *et al*., 2000).

In the study of SEM, dorsal surface of sepal shows the presence of procumbent glandular trichomes and stomata, extra floral nectaries in abaxial surface of the corolla and multicellular trichomes on various floral parts; similarly, extra floral necteries were observed on the corolla of *Solanum cunninghamii, S. diocum* and *S. tudununggae* (Gregory and David, 1985). Pollen morphology is of great significance in taxonomy, phylogeny, palaeobotany, aeropalynology and pollen allergy. Pollen identification on the basis of palynology is based exclusively on pollen morphology (Shivanna, 2003). Gentry (1986) examined the pollens morphology in thirteen genera (*Cestrum* L., *Coeloneurum* Radlk., *Fabiana* Ruiz & Pav., *Goetzea* Wydler., *Juanulloa* Ruiz & Pav., *Markea* Rich., *Metternichia* J.C. Mikan, *Nicotiana* L., *Nierembergia* Ruiz & Pav., *Petunia* Juss., *Rahowardiana* D'Arcy, *Sclerophylax* Miers and *Vestia* Willd.) and twenty species of cestreae tribe (solanaceae) by using SEM where he observed, majority of the taxa were characterized by tricolporate, monad pollen grains and exine sculpturing in striate, regulate, foveolate, scabrate, echinate and reticulate. In the case of stigma's SEM study, it showed twisted bilobed, presence of small and loosely arranged papillae on the stigmatic surface while stylar surface showed the presence of multicellular trichomes. Similar type of stigmatic characters was reported in *Withania somnifera* (Kaul *et al*., 2005), *Duranta repens* L. (Sharma and Rana, 2009) and *Solanum ovigerum* (Saha and Datta, 2018).

Pollen viability and germination were maximum during rainy season followed by winter and summer in various tests (Alexander, TTC and FRC). Similar results were obtained in *Withania somnifera* (Kaul *et al*., 2005) and *Solanum ovigerum* (Saha and Datta, 2018). The viability mainly affected by temperature and humidity; many previous studies clearly indicated their effect on pollen viability (Kaul, 1970; Gupta and Nanda, 1973; Chauhan and Kumar, 1980; Jain and Chauhan, 1985). On the other hand, pollen viability is one of the limiting factors that affect seed-formation (Koltunone *et al*., 1990; Goldberg *et al*., 1993; Yang and Sundaresan, 2000). In the report of Shivanna and Johri (1985) the pollen germination and tube growth were

highly sensitive to climatic factors. The pollen germination and growth increased with increasing sugar concentration similar as the study of Tupy (1960).

Pollination is a most important process for plants which creates the initiation of progeny. The attractants (flowers, inflorescence, floral architecture and floral density) and rewards (quantity of pollens and nectar) of *S. nigrum* L., invites a wide variety of insects during different season. Many pollinators or visitors as black ants (*Componotus campestris*), small bees, bug (*Nezara viridala*), beetle (*Epilachna* spp.), *Lygaeus* sp. and *Diabrotica* sp. etc. visited on plants. Among all the pollinators; black ant was identified as most efficient pollinator. Pollen grains were observed on the mouth parts, legs and wings of these visitors which create results of pollen load, visitation rate and fruit-set. Fruit-set varies with seasons, maximum occurred during rainy season followed by winter and summer. Autogamy performed higher percentage of fruit-set while other modes have very small percentage. Similarly, Kaul *et al.* (2005) observed maximum 80-85% of fruit-set in *Withania somnifera* by autogamy. Xenogamic cross gave only 2-4% fruit-set which is similar as in *Jatropha gossypifolia* L. (Gupta, 2004) and *Withania somnifera* (Jha, 2005).

Conclusions

In nutshell, this study confirms the effect of various climates on vegetative as well as floral parts. During different climates flower showed polymorphism as recorded in petals, stamens and pistil. Among various climates rainy season was better for all selected parameters followed by winter, hence, this study concluded that the rainy season provides most suitable climate to the *Solanum nigrum* L. In this respect, this study might be helpful for the climate related biologist due to huge change in climate day by day.

Authors' Contributions

Both authors conceived and designed this work. CK conducted experiments and wrote the manuscript. IPS analyzed data and finalizes the draft.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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