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An appraisal in pollen biology and fruit set of some ethnomedicinal angiosperms in Darjeeling Himalaya

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ABSTRACT

The present paper deals with pollen biology in terms of pollen productivity, pollen sterility and pollen viability in the context of fruit set of eight selected ethnomedicinally important angiospermic taxa like *Aconitum heterophyllum* Wall. (Ranunculaceae); *Aeschynanthus sikkimensis* (Cl.) Stapf. (Gesneriaceae); *Curcuma zeodaria* Rosc. (Zingiberaceae); *Nardostachys jatamansi* DC. (Valerianaceae); *Panax pseudoginseng* Wall. (Araliaceae); *Swertia pedicillata* Ban. (Gentianaceae); *Thalictrum foliolosum* DC. (Ranunculaceae) and *Zanthoxylum oxyphyllum* Edgew. **(**Rutaceae) growing in the vicinity of Darjeeling Himalaya. Among the investigated taxa, pollen productivity, sterility and viability were highest in *Curcuma zeodaria* and lowest in *Swertia pedicillata*. The variability and positive correlation of log values between pollen production, sterility, viability and fruit set of selected taxa were noticed. From present finding it is concluded that the pollen biology in terms of productivity, sterility, viability and fruit set showed significant variation and correlation with each other among different selected taxa and even within same taxon. The results are discussed in terms of evolutionary relationship and sexual fitness of selected ethnomedicinal plants.

***Keywords*:** Ethnomedicinal angiosperms, fruit set, pollen production, pollen sterility, pollen viability, stigma receptivity

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1. INTRODUCTION

The major ethnic communities of Darjeeling hills whose descendents continue to live in remote areas are Lepcha, Bhutia and Nepalese. Each of these groups has its own distinct form of worship, culture, language and tradition. All of them are exceedingly generous, light-hearted and law-abiding people bonded together by Nepali language, which is the medium of communication among them. Agricultural practices in these hills are mostly subsistence agriculture, which is characterized by low input, low risk and low yield. Pollen grains, the gold dust of flowering plants, the male reproductive unit play a pre-requisite role in pollination and sexual reproduction of flowering plants. Usually pollen grains encompass three reproductive phases like: development phases, dispersal phase & free living phase. Each phase is governed by physiological & biological functioning of plants and affected by environmental factors. The pollen may leave the anthers as soon as they open or be held in the anther by devices such as pollenkitt, tryphine or elastoviscin (fluids of different viscosity and ontogenesis), viscin threads or sporopollenin filaments (exine extensions that tangle with the pollen). Pollenkitt, tryphine, elastoviscin and viscin threads are all tapetal products. Studies on pollen viability and pollen sterility are very important for pursuing further research on pollen biology. Viability test provide a means of assessing the potential of pollen to germinate on the stigma. Another dehiscence and pollen productivity were studied by Bonner and Dickinson (1999; 2004) in a few member of Solanaceae in relation to the environmental variability and water content in the pollen grains. Humidity stress responses in pollen of different anemophilous and entomophilous species were investigated by Bassani *et al.* (2008). Dafni and Firmage (2014) reported on longevity and viability of different angiospermic pollens in terms of biophysical status and nutrient pool of pollens. This investigation has been done in order to know the pollen production of selected plants reflecting its impact upon the evolutionary relationship and sexual fitness, pollen sterility and its correlation with viability in the context of fruit set of selected taxa.

2. MATERIALS AND METHODS

Darjeeling Himalaya is situated between the 87°59'-88°53'E and 28°31'-27°13'N in the Eastern Himalayan region of India. It is a frontier district running up between Nepal and Bhutan, and stretching from the plains of Bengal in the south to Sikkim in the north. It is bordered by Bhutan in the east and Nepal in the west. The three hill subdivisions of Darjeeling district, Kalimpong, Kurseong and Darjeeling sadar consisting of eight developmental blocks and occupying an area of 2417 km^2 comprise the Darjeeling Himalaya (Fig. 1). The altitudinal range of this hilly region varies from 130 to 3660 m. Due to their great variation, a wide array of climatic zones are available, which favour the luxuriant growth of diversified and rich vegetation. This region is also the abode of many endemic elements and a number of species which have become rare, threatened or endangered. The selected plants are: *Aconitum heterophyllum* Wall. (Ranunculaceae); *Aeschynanthus sikkimensis* (Cl.) Stapf. (Gesneriaceae); *Curcuma zeodaria* Rosc. (Zingiberaceae); *Nardostachys jatamansi* DC. (Valerianaceae); *Panax pseudoginseng* Wall. (Araliaceae); *Swertia pedicillata* Ban. (Gentianaceae); *Thalictrum foliolosum* DC. (Ranunculaceae) and *Zanthoxylum oxyphyllum* Edgew. (Rutaceae). The fresh anthers were collected in the morning after flower anthesis in polythene bags and brought to the laboratory for study. All the investigated plants showed forenoon pattern of flower anthesis

and pollen anthesis took place after flower anthesis. Pollen production per anther was studied following the method of Kearns and Inouye (1993) using safranin dye dissolved in 70% alcohol by crushing and mounting the whole anther.

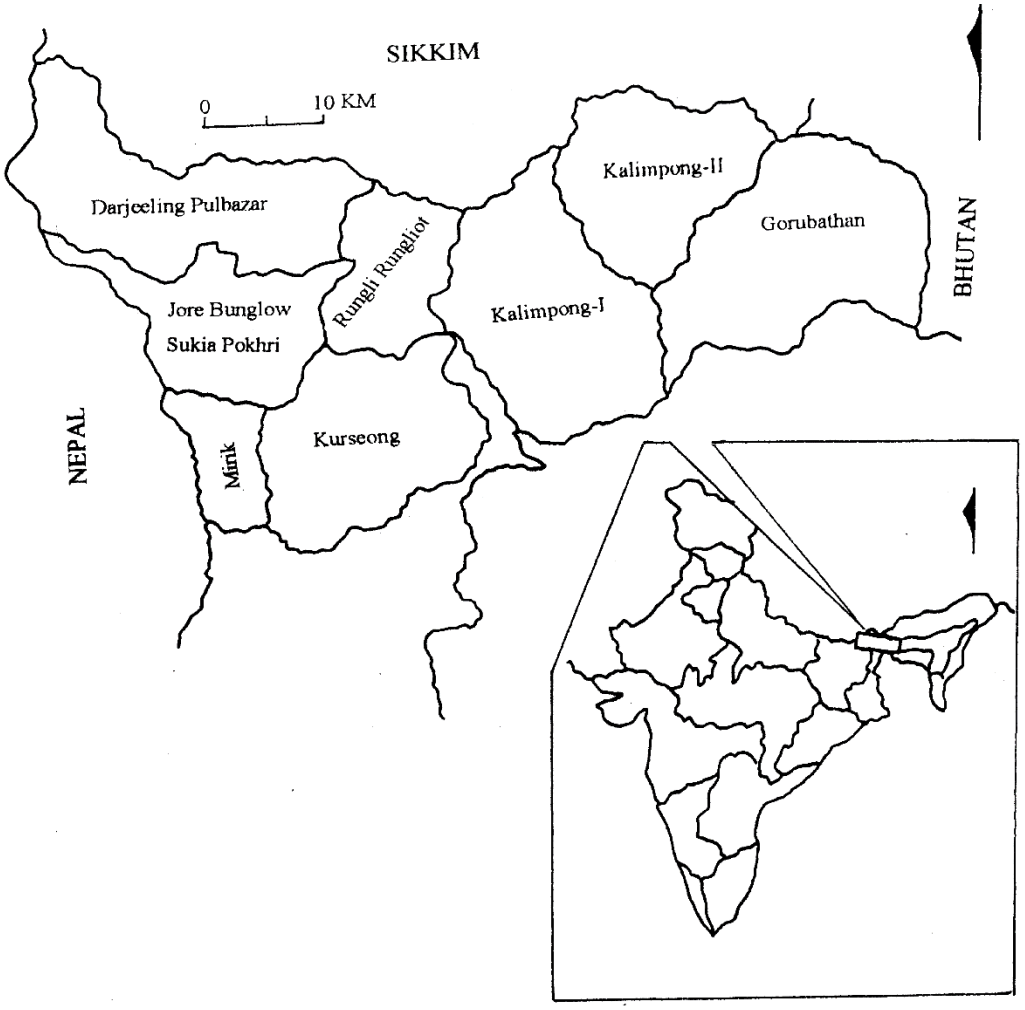

Fig. 1. Map of Darjeeling Himalaya showing eight different blocks

Pollen sterility and viability were investigated by Kearns and Inouye (1993) and Shivanna and Rangaswamy (1992) using the fuchsin dye and 0.5 % TTC solution respectively. Using dye containing malachite green and acid fuchsin, which differentially stains sterile and fertile pollens; malachite green stains cellulose in pollen grains, and acid fuchsin stains the protoplasm. Thus sterile pollens appear green while pollens with protoplasm appear pink. Pollen samples were placed in a drop of this stain, mounted after gently heating and scored under microscope at 400X magnification. A modified staining reagent consisting of 0.5% solution of TTC in 20% sucrose was prepared. A drop of this solution was taken on a microscope slide and pollens added, mounted immediately to exclude oxygen. The slides were incubated at 60 °C for 3 hours, and then scored under microscope at 400X magnification. Pollen grains stained red in presence of reductases, indicating the presence of active enzymes. These

red grains were considered as viable. Statistical analyses were done using SPSS 11.0 version of statistical software. Mean value of collecting pollen samples in each day were considered. The mean values of pollen productivity, sterility and viability were calculated by finding the sum of all the individual observations and then dividing the total by the number of observations in each test. Standard deviations were obtained from the variance of each test by extracting the square root and were expressed in the units in which the measurements were taken. The standard errors of the mean were calculated from the standard deviation of samples, by dividing it by √n (n is sample size). The correlation coefficient value (R) was calculated by dividing the sum of products of deviations from their respective means by the square root of the products of the sums of squares of deviations from the respective means of the variables.

3. RESULTS AND DISCUSSION

The highest pollen production per anther, percentage of pollen sterility and viability were recorded in *Curcuma zeodaria* followed in degree of prevalence by *Thalictrum foliolosum, Aeschynanthus sikkimensis, Panax pseudoginseng, Zanthoxylum oxyphyllum, Nardostachys jatamansi, Aconitum heterophyllum* and *Swertia pedicillata* (Table 1). The composition of medium for obtaining optimum *in vitro* germinating pollens varied greatly among different taxa (Table 2). Although, there are significant differences of pollen production (235 – 1185 pollens per anther, df = 13, F = 5.27, $p \leq 0.05$); pollen sterility (14 – 56 %, df = 24, F = 6.38, $p \leq 0.05$); pollen viability (46 – 75%, df = 21, F = 5.95, $p \leq 0.05$) and fruit set (32.5 – 56.8 % in open condition, df = 19, F = 4.18, $p \leq 0.05$; 0 – 42.6 % in bagging condition, df = 19, F = 4.86, $p \leq 0.05$; 2.5 – 39.8 % in netting condition, df = 19, F = 4.25, $p \leq 0.05$) among the selected taxa (Figs. 2 – 5); however a positive correlation between the productivity ($r^2 = 0.009$), sterility ($r^2 = 0.050$) and viability ($r^2 = 0.267$) of pollens individually or in combination were noticed. The logarithmic increase with positive correlation of productivity [$y = 0.072\ln(x) + 2.560$; $R^2 = 0.046$], sterility [$y = 0.100\ln(x) + 1.309$; $R^2 = 0.137$] and viability [$y = 0.045\ln(x) + 1.574$; $R^2 = 0.167$] of pollens with fruit set [$y = 0.052\ln(x) + 1.726$; $R^2 = 0.315$] among the different taxa were observed (Fig. 6). The biological potentiality of an individual flower in an inflorescence could be known by counting the total pollen grains per flower. A great variation of pollen production is noticed but there is a tendency towards logarithmic increase of productivity which might be due to increased selection pressure upon the taxa. The pollen number may differ from individual anther of a single flower. It also differs from anther to another, flower to flower and plant to plant with reference to dispersal (Dafni, 1992), in anther and flower (Faegri and van der Pijl, 1979), in anther, flower and whole plant (Kearns and Inouye, 1993). It also differs in terms of anther number, anther length, filament length, pollen grains size and mode of anther dehiscence (Shivanna and Johri, 1985). The variation in pollen productivity may be due to the difference in the number of pollen grains per anther, anthers per flower, flowers per inflorescence and inflorescences per tree and it is related to evolutionary relationship among taxa. The sterility and viability of pollen are positively correlated with each other and with productivity which might be due to maintaining sexual fitness of taxa. Viability of pollen has been defined as having the capacity to live, grow, germinate or develop. It has been reported that pollen sterility and viability is so liable that it may differ when pollen is collected at different times of the day (Nepi and Pacini, 1993 and Norton, 1996). Pollen collected from flowers in anthesis for one-hour show decreased viability (Buitink *et al.,* 1996).

Table 1. Pollen productivity, sterility and viability of selected taxa.

Name of the Plants	Number of pollens per anther (± SE)	% Sterile pollens (±SE)	% Viable pollens (±SE)
Aconitum heterophyllum Wall.	294 ± 3.89	19 ± 1.56	56 ± 6.14
Aeschynanthus sikkimensis Stapf.	512 ± 5.70	33 ± 1.32	67 ± 6.93
Curcuma zeodaria Rosc.	1185 ± 22.67	56 ± 5.10	75 ± 7.19
Nardostachys jatamansi DC.	324 ± 8.75	23 ± 1.89	61 ± 5.68
Panax pseudoginseng Wall.	419 ± 7.38	25 ± 0.95	64 ± 4.87
Swertia pedicillata Ban.	235 ± 4.35	14 ± 1.04	46 ± 3.75
Thalictrum foliolosum DC.	760 ± 7.46	41 ± 1.98	70 ± 6.28
Zanthoxylum oxyphyllum Edgew.	397 ± 3.81	30 ± 1.19	66 ± 5.92

Table 2. Medium composition for optimum *in vitro* pollen germination, stigma receptive period and *in vivo* germinating pollen (% ± SE) of selected taxa.

Name of the Plants	Medium composition	Maximum stigma receptive period	*In vivo* germinating pollen (% ± SE)
Aconitum heterophyllum Wall.	10% sucrose + 300 µg ml^{-1} boric acid	Second day after anthesis	66 ± 5.34
Aeschynanthus sikkimensis Stapf.	15% sucrose + 100 µg ml^{-1} boric acid	Third day after anthesis	58 ± 4.95
Curcuma zeodaria Rosc.	10% sucrose + 200 µg ml^{-1} boric acid	First day after anthesis	61 ± 4.39
Nardostachys jatamansi DC.	20% sucrose + 500 µg ml^{-1} boric acid	First day after anthesis	53 ± 2.65
Panax pseudoginseng Wall.	20% sucrose + 300 µg ml^{-1} boric acid	Second day after anthesis	59 ± 3.34
Swertia pedicillata Ban.	15% sucrose + 300 µg ml^{-1} boric acid	Second day after anthesis	52 ± 2.17
Thalictrum foliolosum DC.	20% sucrose + 400 µg ml^{-1} boric acid	Third day after anthesis	61 ± 3.65
Zanthoxylum oxyphyllum Edgew.	10% sucrose + 100 µg ml^{-1} boric acid	Second day after anthesis	58 ± 3.79

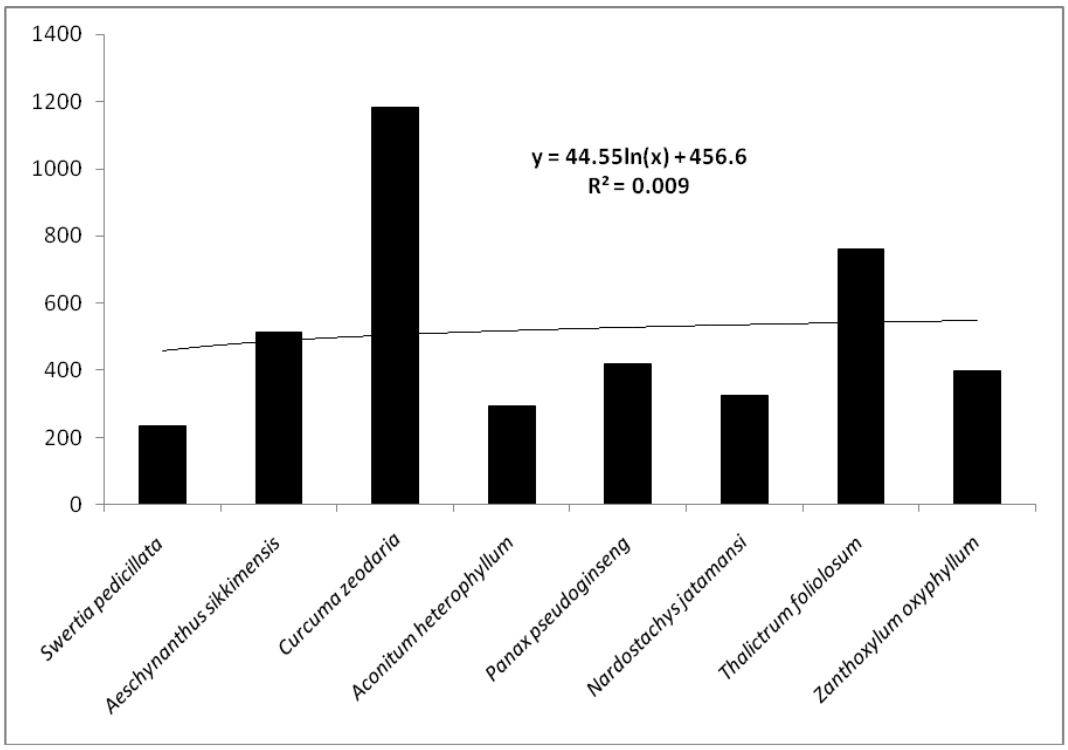

Fig. 2. Pollen production and its' correlation among taxa

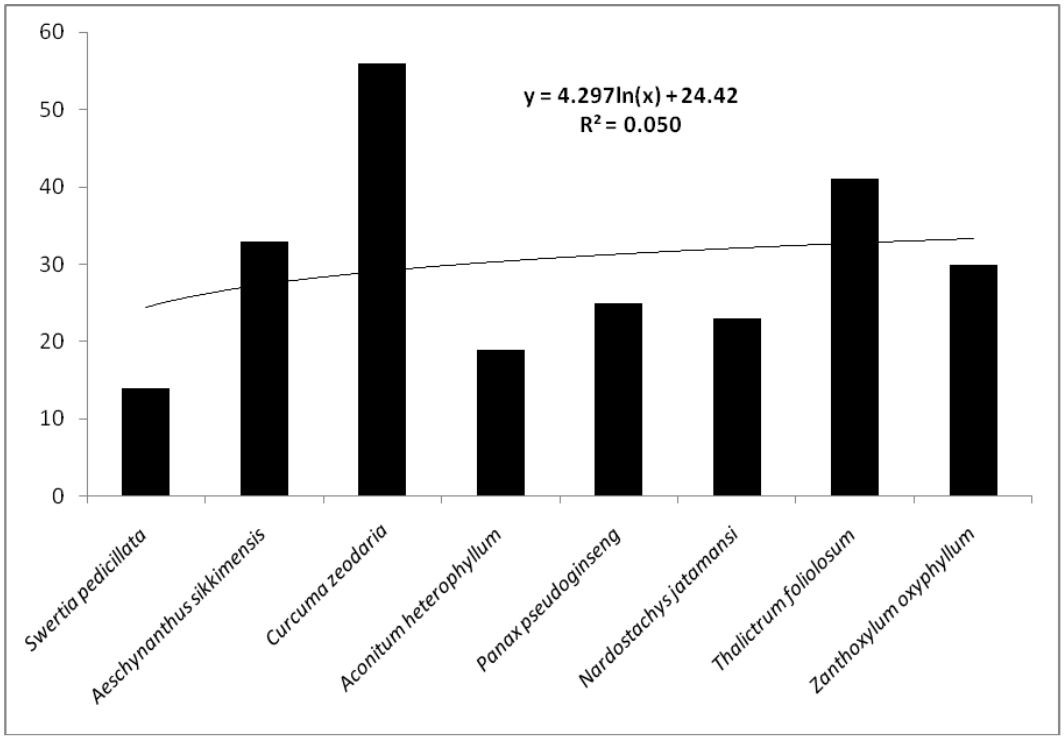

Fig. 3. Pollen sterility (%) and its' correlation among taxa

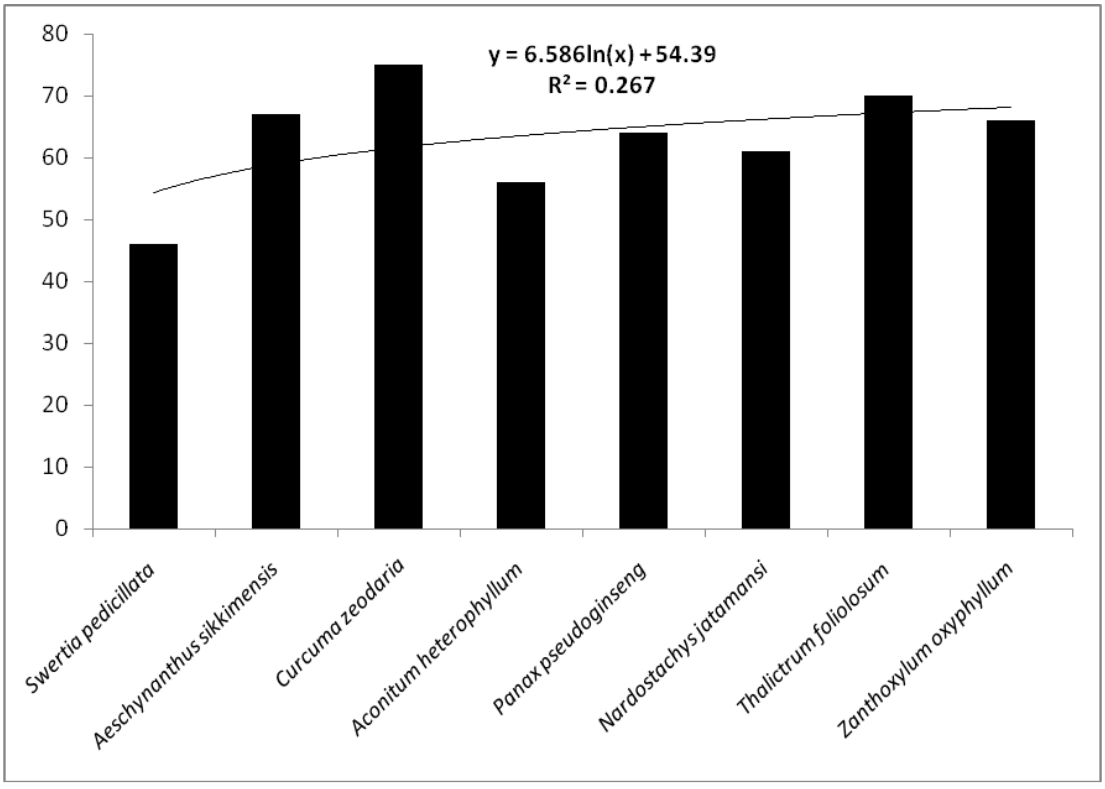

Fig. 4. Pollen viability (%) and its' correlation among taxa.

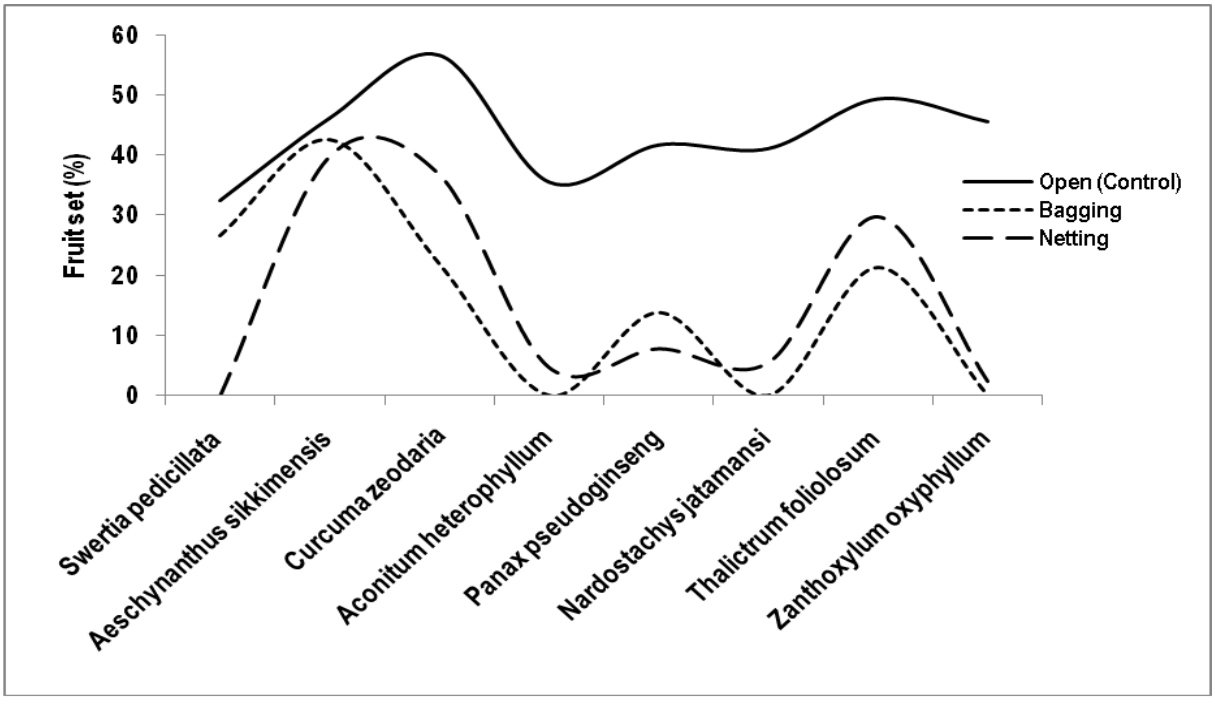

Fig. 5. Fruit set (%) of selected taxa.

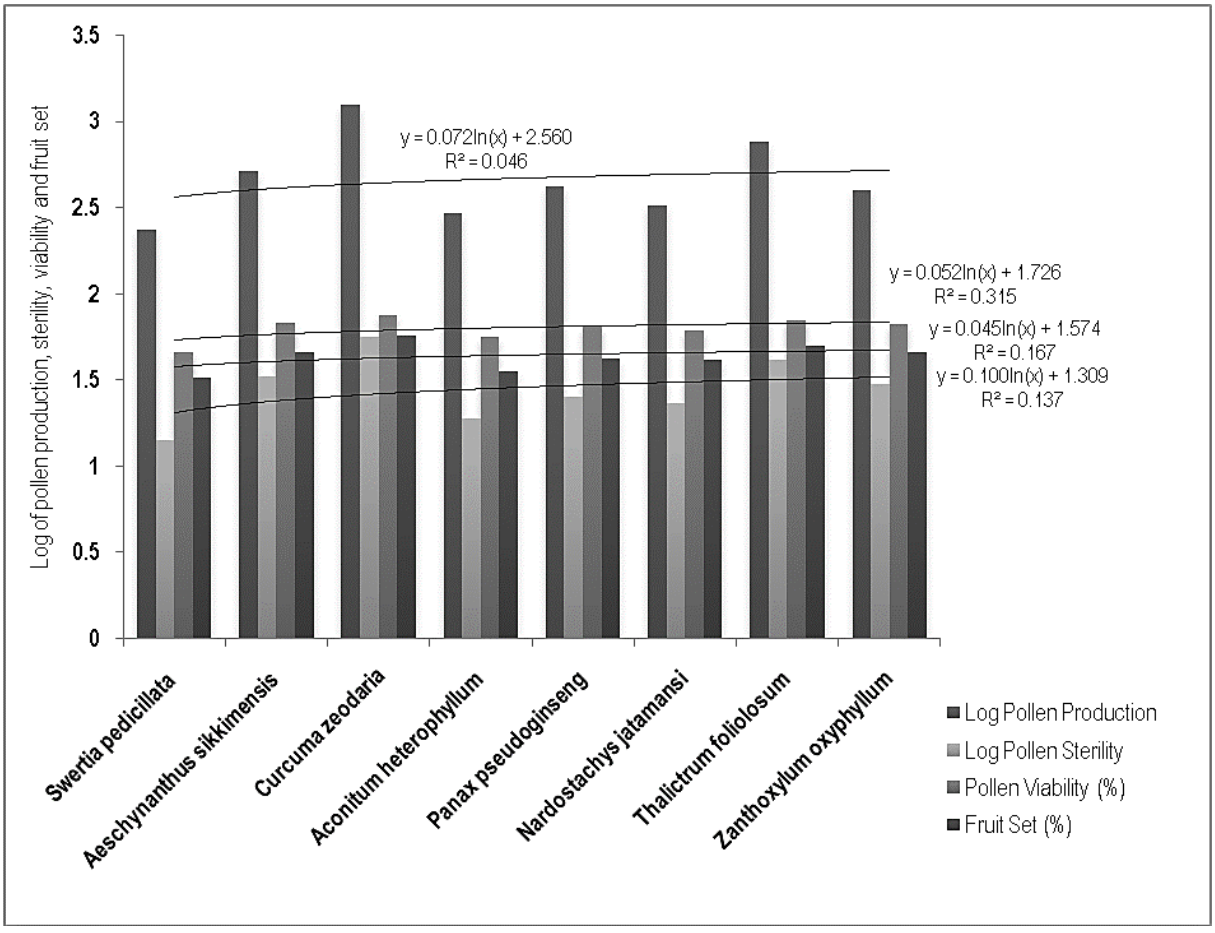

Fig. 6. Correlation of log values between pollen production, sterility, viability and fruit set.

Pollen sterility and viability has a genetic component; results may be different depending on the genetic variability of individuals used as donors (Franchi *et al.*, 1996). The use of malachite green and acid fuchsin in present investigation may have led to overestimation of pollen sterility since staining capacity depends not on the sterility but on protoplasm content of the pollen grains. So, this measure of pollen stain ability may depart considerably from real value of pollen sterility. The proportion of both sterile and viable pollen grains steadily increases in selected taxa. Pollen may express genetically based traits during its development, maturation and free dispersal phases. Reproductive effort, physiological stress, resource availability may be the factors for variation in pollen sterility and viability. Populations of out crossing plants are far from being genetically uniform (Pacini, 1994) and may constitute important sources of variability.

The biological potentiality of individual flowers in an inflorescence could be calculated by counting the total pollen grains per flower, but it is very difficult to estimate absolute pollen production. However, pollen per flower and per ovule is related to fertilisation rate. Among the selected taxa, a moderate number of pollen per flower indicates xenogamous nature which is genetically superior. Pollen competition may occur during post pollination period and this competition might be influenced by number of pollen which in turn may have an effect on pollen

tube development and fruit formation. The low fruit production in the natural population of selected taxa may be due more to pollen competition that reflects strong xenogamy. The *in vitro* effect of sucrose suggests that sucrose has an increasing influence in pollen germination which is directly proportional to the concentrations of sucrose.

The medium composition for best pollen germination of different taxa varied greatly which might be attributed to the fact that sucrose alone is necessary for proper pollen nutrition, osmotic control and possibly for other reasons but sucrose in combination with H_3BO_3 promoted pollen germination because boron makes a complex with sucrose which may be easily translocable rather than sucrose alone. Boron may enhance the sucrose uptake and stimulate germinating ability. Stigmas receptivity also varied greatly among different taxa. Generally receptivity reaches a maximum soon after anthesis but the period of receptivity may vary from species to species. In few taxa the delayed receptive period indicates suppots cross pollination strategy. Less fruit production as compared to flowers may be due to delayed receptivity or unavailability of pollinators or pollen competition. Accumulation of somatic mutations in different natural plant population might be a concept to our understanding of the pollen sterility and viability. This view gets support from the findings of Shivanna and Johri (1985); Trognitz (1991); Pacini (1994); Rodriguez-Riano and Dafni (2000) and it is concomitant with the earlier works investigated by Faegri and van der Pijl, 1979; Shivanna and Rangaswamy, 1992; Kearns and Inouye, 1993; Armbruster and Rogers, 2004; Bassani *et al*., 2008; Irwin *et al.*, 2010 Mandal and Bhattacharya, 2011; 2012; Pettengill and Moeller, 2012; Brothers *et al.*, 2013; Zhao and Huang, 2013; Bhattacharya, 2014; Dafni and Firmage, 2014 and Bhattacharya and Subba, 2015.

4. CONCLUSION

From present investigation it is concluded that the pollen biology in terms of productivity, sterility and viability in the context of fruit set showed significant variation and positive correlation with each other in different selected taxa with an impact of these parameters upon the sexual fitness of plants with reference to fruit set. The flowers produced sufficient high quality pollen. Although the flowers have viable pollen, self and cross pollination with other flowers may results in a significant increase in fertile and more viable pollens which is yet to be studied. Finally, it is recommended that at least four different tests for pollen viability should be followed for more accurate and effective results.

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