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RESEARCH

# Effects of light and nutrient on flower formation and vegetative growth of *Viola collina*

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**Background:** Mixed breeding herb *Viola collina* Besser, which produces both chasmogamous and cleistogamous flower, has limited habitats under closed canopy and short and early flowering timing, making it relatively more vulnerable to climate change. To better understand the effect of light and nutrient on the flower formation and vegetative growth of *V. collina*, a mesocosm experiment was conducted. Two-by-two factorial treatments of two light conditions (100% and 60% of natural light) and two fertilizer treatment conditions (fertilized and not fertilized) were applied in the mesocosm experiment.
**Results:** The number of flowers, including chamogamous and cleistogamous flowers, was highest (5.65/pot) under 60% light and fertilized condition and lowest (1.41/pot) under 100% light and not-fertilized condition. However, above ground vegetative growth was highest (2.89 g/pot) under 100% light and fertilized condition and lowest (2.38 g/pot) under 60% light and not-fertilized condition. Above ground biomass to belowground biomass ratio was highest (1.50) under 60% light and fertilized condition and lowest (1.26) under 100% light and fertilized condition.
**Conclusions:** This study showed that high light and nutrient are responsible for the vegetative growth , though the effect of fertilizer was reduced due to allocation and retainment of nutrients. In addition, the low light is necessary to make flowers, especially chasmogamous flowers.

**Keywords:** chasmogamous flower, cleistogamous flower, plasticity, shade

## Introduction

*Viola collina* Besser, distributed among Northeast Asia including Korea, is often seen in mountainous area. Among the 52 known *Viola* species in Korea, *V. collina* is known to have an earlier flowering timing of March to April (Lee 2003). Not only *V. collina* has the early and short blooming period, but also has the limited habitat under closed canopy of deciduous forests. *Viola collina* is a mixed breeding herb and produces both chasmogamous and cleistogamous flowers. Chasmogamous flowers are big, showy, open and out-crossing which are usually visited by insect pollinators. Cleistogamous flowers start to be produced in later part of the flowering season, resemble small buds, closed and self-pollinate (Culley and Klooster 2007). This unique reproductive system could be beneficial compared to other reproductive strategies, and could be the key to its survival as habitat change occurs (Yang and Kim 2016). Several studies have shown modified production of chasmogamous and cleistogamous flowers under various environmental conditions, such as light intensity and soil moisture content (Bell and Quinn 1987; Sternberger et al. 2020; Waller 1980), and nutrient availability (Corff 1993).

Climate change, especially global warming, makes trees to sprout early and forest canopy to close earlier (Nam and Kim 2020). These environmental changes could impact more significantly to *V. collina* due to its early and short blooming period added to their limited habitat. Mt. Odae, known habitat of *V. collina*, has been evaluated to be high on current and future climate change vulnerability (Kim and Kim, 2016). This vulnerability was estimated using temperature and precipitation factors, which indicate that habitat of *V. collina* would face temperature rise and relatively dry condition. As climate change is known to threaten one in six species (16%) to extinct (Urban 2015), *V. collina* could be more vulnerable. Along the other species in genus *Viola*, *V. collina* is capable of producing both chasmogamous and cleistogamous flowers.

Although there are several previous studies about *V. collina*, most of them have focused on its taxonomic characters (Hodálová et al. 2008), seed dispersal (Beattie and Lyons 1975), and seed germination (Lee and Hwang 2006). In spite of the importance of this particular species, little is known about the growth of *V. collina*. However, it is important to understand the effect of shade and nutrient on the growth and reproduction for sustainable restoration or conservation of *V. collina*.

In this study, we focused on independent and potentially interacting effects of two principal environmental factors for the growth of mountainous herbaceous plants, shade and soil fertility, to understand the ecological characteristics of *V. collina* growth. Therefore, we conducted a mesocosm experiment to determine 1) the effects of shade and nutrient on reproduction, especially the production of chasmogamous flowers and cleistogamous flowers, and 2) the effects of shade and nutrient on the vegetative growth of *V. collina*.

# Materials and Methods

## Origin of plants used in the experiments

*Viola collina* plants were collected at Jogaedong-neup (N37°27′ E126°57′, 120 m a.s.l.) and Sohwangbyeongsan-neup (N37°46′, E128°40′, 1,170 m a.s.l.) in Mt. Odae and at Mt. Gwanak (N37˚27′ E126˚57′, 120 m a.s.l.) (Fig. 1). They were individually transplanted into flowerpots (Φ 12.5 cm, height 12 cm) filled with the mixture of agricultural topsoil 1 and sand 3 in May 2016. The mesocosm experiment was conducted at Seoul National University (N37˚27′ E126˚57′, 120 m a.s.l.), Seoul, Korea from May 2016 to 31 August, 2017.

## Design of the mesocosm experiments

The effects of shade and fertilizer on the growth of *V. collina* were assessed with a 2 × 2 factorial experiment (i.e., two levels each of light and nutrient). Collected samples were situated in experimental sets with two light conditions of 100% natural light (light, group L) and 80% natural light using black polyester net (shade, group S) and two soil fertility conditions of fertilizer treated (fertilized, group F) and fertilizer not-treated (not fertilized, group N). For group F, 0.24 g of fertilizers (Won Ye Bak Sa; Dong-Bu High-Tech®, Bucheon, Korea) of a granule type, which gradually release N-P-K (11-10-8) and Mg-B-Ca-Si-S (2-0.2-11-5-4), was applied five times since 30th June 2016 for fifty-one weeks, which is a recommended dose by manufacturers in agronomical usage. Each group has 17 replications.

## Measurements and statistical analysis

We counted the numbers of leaves (2017), chasmogamous (2017) and cleistogamous (2016–2017) flowers, and fruits (2016–2017) of each plant in the interval of 1 or 2 weeks. The chlorophyll content of the leaves was measured as a Soil Plant Analysis Development (SPAD) value using a portable chlorophyll meter (SPAD-502; Konica Minolta, Inc., Osaka, Japan) at 3th August and 5th November 2016 and 28th August 2017. Three leaves were randomly selected per pot and SPAD values were measured and averaged for each measurement. The SPAD sensor was placed on leaf mesophyll tissue only with the main vein avoided. In addition, leaf area and weight of three randomly chosen leaves were measured to estimate the reproductive strategy and leaf functioning under varying light and fertilizer conditions. We measured coverage of each pot in order to estimate the growth rate of the plant, as the coverage is related to not only the size and density of the plant but also the amount of light that can be intercepted by plants. In the case of coverage, images of each plant were captured and analyzed using ImageJ (National Institutes of Health, Bethesda, MD, USA). At the end of the experiment (31 August, 2017), the aboveground materials were cut off at ground level, and the soil was carefully removed from the belowground materials. After each of these above-ground (AG) and below-ground (BG) components were harvested and oven-dried at 70˚C for at least 48 hours, dry weights of AG and BG biomass of each plant were measured. AG/BG ratio was calculated by dividing the dry weights of AG into the dry weights of BG.

The data were statistically analyzed by two-way ANOVA

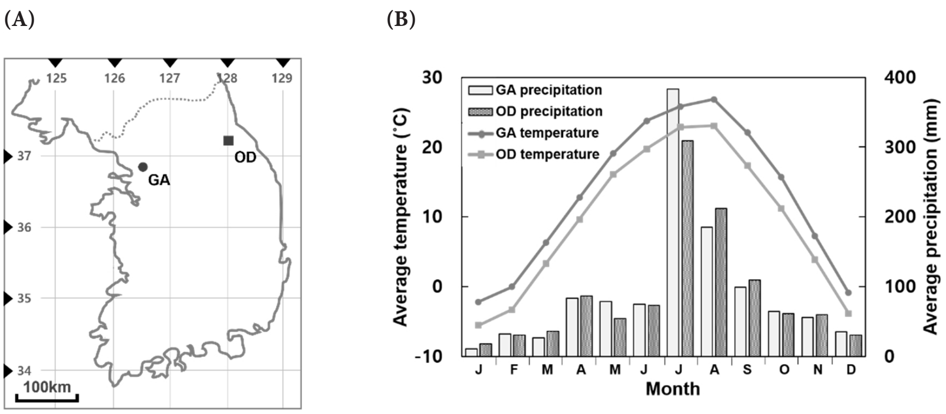


**Fig. 1** Geographical location (**A**) and average temperature (lines) and precipitation (bars) from 2012 to 2016 of two sampling sites (**B**). OD: Mt. Odae; GA: Mt. Gwanak.

using the statistical software environment R ver. 3.3.3, and multiple comparisons were performed using the Duncan test ($p < 0.05$).

## Results

### Growth responses of *Viola collina*

*Viola collina* produced more reproductive organs under shade and fertilized condition than under the other conditions (Fig. 2). The number of chasmogamous flowers was highest (2.88/pot) under shade and fertilized condition, whereas almost no chasmogamous flowers were observed under light conditions (Fig. 2A). The number of cleistogamous flowers showed similar patterns with the number of chasmogamous flowers. The number of cleistogamous flowers was highest (2.76/pot) under shade and fertilized condition, and lowest (1.29/pot) under light and not-fertilized condition (Fig. 2B). The number of total flowers, sum of the number of chasmogamous and cleistogamous flowers, was significantly highest (5.65/pot) under shade and fertilized condition (Fig. 2C). The number of fruits was lowest (17.47/pot) under shade and not-fertilized condition (Fig. 2D).

The average number of leaves was significantly highest under shade and fertilized condition (98.65/pot), and lowest under light and not-fertilized condition (63.76/pot) (Fig. 3A). Although the average leaf area of 3 leaves was higher under shade conditions than light conditions, it was not statistically significant difference (Fig. 3B). The average weight of three leaves was highest under light and not-fertilized condition (0.49 g), followed by shade and not-fertilized (0.46 g), shade and fertilized (0.43 g), and light and fertilized (0.34 g) (Fig. 3C). Although the average coverage was higher under shade conditions than light conditions, it was not statistically significant difference (Fig. 3D). Leaf chlorophyll content was higher under shade conditions than light conditions (Fig. 3E).

The average aboveground dry weight showed no significant difference under light and fertilizer treatments ($p = 0.91$) (Fig. 4A). The average belowground dry weight was higher at light conditions than at shade conditions despite the lack of statistical significance ($p = 0.53$) (Fig. 4A). Although the ratio of AG and BG dry weight was higher at shade conditions than at light conditions, it was not statistically significant difference ($p = 0.77$) (Fig. 4B).

### Effects of shade and fertilizer

Both light and fertilizer caused significant differences in reproductive and morphological traits of *V. collina* (Table 1). The number of total flowers and leaves were affected by both shade and fertilizer. On the other hand, the number of chasmogamous flowers, coverage, and chlorophyll showed significant difference under different light conditions only. The number of seeds and reproductive organs, and weight of three leaves per individual were only affected by fertilizer.

## Discussion

*Viola collina* under different light conditions displayed plasticity in reproductive growth, especially in the production of flowers. Unlike many plant species which produce only one type of flower, *V. collina* produce two different

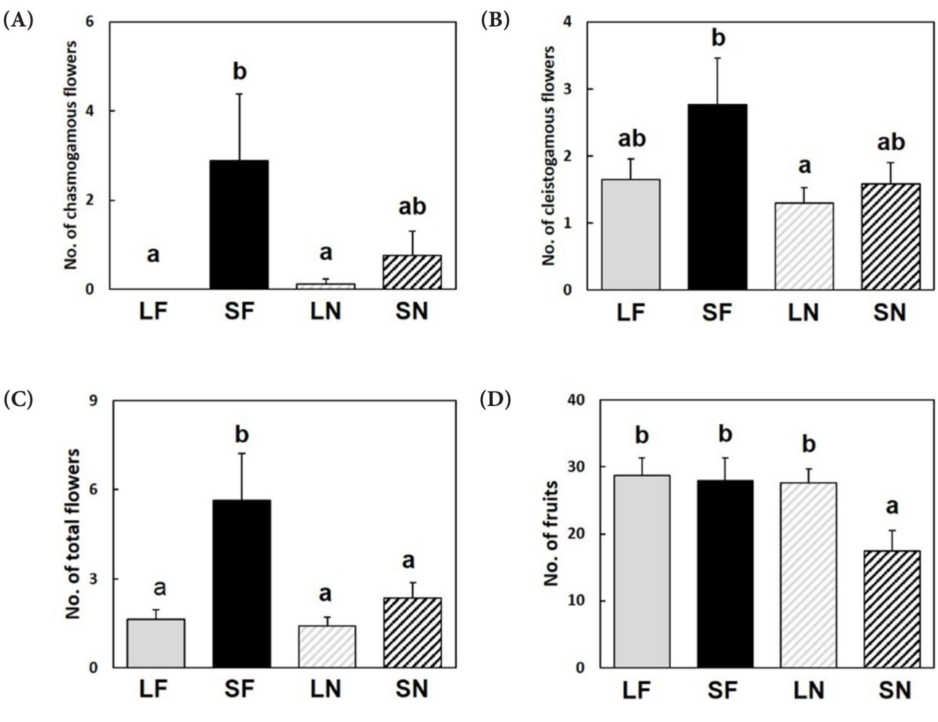


Fig. 2 The average number of chasmogamous flowers (**A**), cleistogamous flowers (**B**), total flowers (**C**), and fruits (**D**) of *V. collina* under different light and soil fertility conditions. Vertical bars indicate standard error. Alphabets indicate significant differences at the 5% level based on Duncan's groups. LF: light and fertilized; SF: shade and fertilized; LN: light and not-fertilized; SN: shade and not-fertilized.

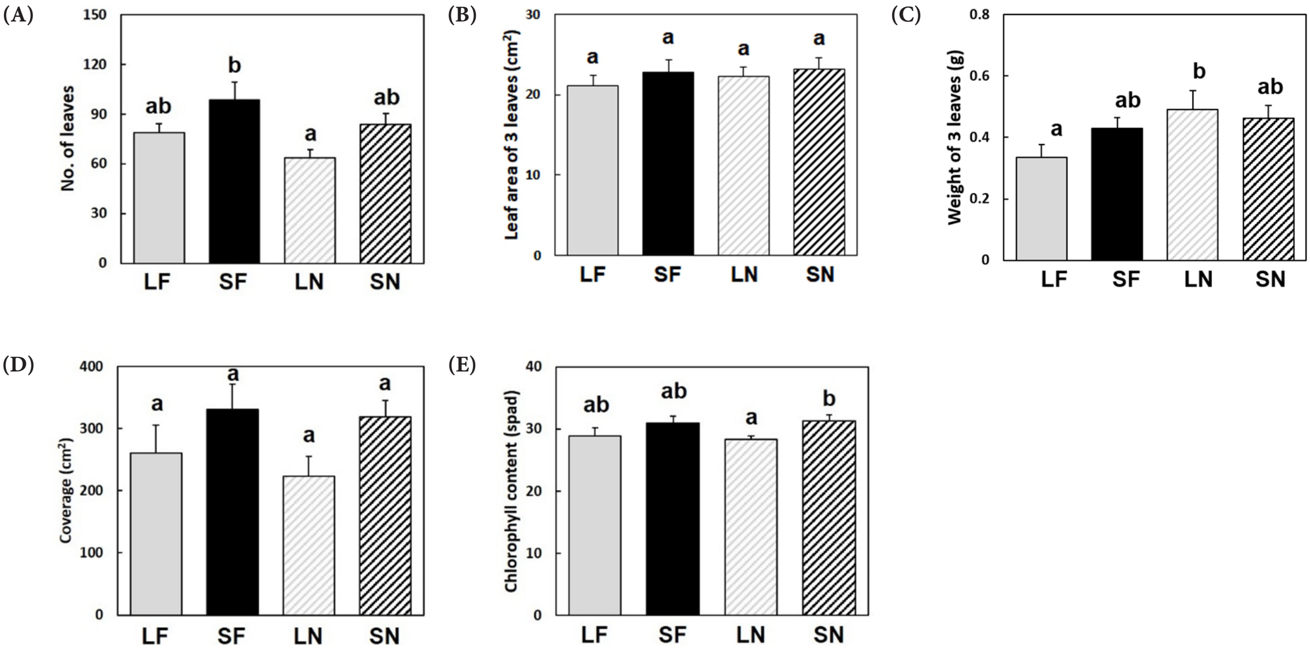


**Fig. 3** The average number of leaves (**A**), leaf area of three leaves (**B**), weight of three leaves (**C**), coverage (**D**), and chlorophyll content (**E**) of *V. collina* under different light and soil fertility conditions. Vertical bars indicate standard error. Alphabets indicate significant differences at the 5% level based on Duncan's groups. LF: light and fertilized; SF: shade and fertilized; LN: light and not-fertilized; SN: shade and not-fertilized.

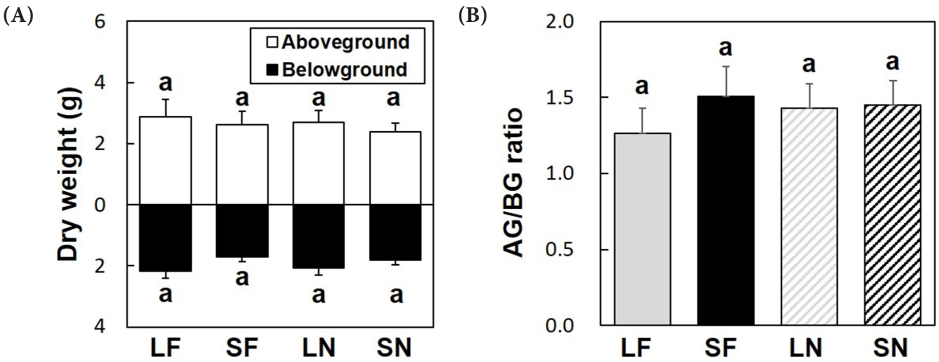


**Fig. 4** The average above-ground (AG) and below-ground (BG) dry weight (**A**) and AG/BG (AG to BG dry weight) ratio (**B**) of *V. collina* under different light and soil fertility conditions. Vertical bars indicate standard error. Alphabets indicate significant differences at the 5% level based on Duncan's groups. LF: light and fertilized; SF: shade and fertilized; LN: light and not-fertilized; SN: shade and not-fertilized.

**Table 1** *F* values from two-way ANOVA results on the effects of light and fertilizer on growth traits

| Growth traits | Light condition | Soil fertility | Light condition × Soil fertility |
|---|---|---|---|
| No. of Ch. flowers | 4.892* | 1.571 | 1.962 |
| No. of Cl. flowers | 2.730 | 3.204 | 0.929 |
| No. of flowers (Ch. + Cl.) | 8.304** | 4.237* | 3.182 |
| No. of fruits | 3.674 | 4.163* | 2.651 |
| No. of leaves | 7.714** | 4.361* | 0.000 |
| Leaf area | 0.860 | 0.357 | 0.077 |
| Leaf weight | 0.483 | 4.140* | 1.743 |
| Coverage | 5.060* | 0.442 | 0.117 |
| Chlorophyll content | 6.036* | 0.194 | 0.621 |
| Aboveground dry weight | 0.481 | 0.256 | 0.006 |
| Belowground dry weight | 2.140 | 0.059 | 0.054 |
| AG/BG ratio | 0.954 | 0.301 | 0.730 |

Degrees of freedom (df) of light, soil fertility, and interaction of two conditions were 1, 1, and 1, respectively.
Ch. flower: Chasmogamous flower; Cl. flower: Cleistogamous flower; AG: aboveground; BG: belowground.
$^{*}p < 0.05$; $^{**}p < 0.01$.

types of flower: chasmogamous and cleistogamous flowers. The number of chasmogamous and cleistogamous flowers increased under shade conditions and chasmogamous flowers were observed almost exclusively in shade conditions. Higher light availability would lead to greater rate of photosynthesis, which results in more resources available for plants to use. Since large and showy chasmogamous flowers require more investment of resources than tiny cleistogamous flowers (Schemske 1978; Waller 1979), conditions of high light availability generally promote production of chasmogamous flowers (Schemske 1978; Yang and Kim 2019; Yang and Kim 2020). Cleistogamous flowers which are less costly to produce would appear under low light availability conditions. However, we found little evidence of a trade-off between allocation to chasmogamous and cleistogamous flowers. In fact, *V. collina* produced relatively more cleistogamous and chasmogamous flowers under shade conditions. Cleistogamous flowers might be more advantageous reproduction strategy in unfavorable environments, such as low light intensity conditions, than chasmogamous flowers (Yang and Kim 2016). However, this could not be applicable to *V. collina*. On the contrary, *V. collina* appears to be a shade tolerant, which suggests that proper shade is necessary for successful reproduction of *V. collina*. It is possible that benefits associated with shade, such as greater air humidity or soil moisture content and less UV radiation stress or heat, outweighed adverse effects of reduction in light availability.

Furthermore, production of *V. collina* flowers including both chasmogamous flowers and cleistogamous flowers was not affected by soil fertility under light conditions, whereas fertilizer facilitated production of flowers under shade conditions. It is likely that plasticity to shade was enhanced by the simultaneous application of soil fertilizer. This is consistent with previous studies that expression of light-dependent plasticity is modified by soil nutrient conditions (Choi et al. 2021; Latham 1992).

On the contrary, vegetative growth, especially morphological traits of leaves, significantly responded to different light and soil fertility conditions (Kim and Kim 2022). The number of leaves were affected by both light and fertilizer, whereas coverage and weight of leaves showed significant difference only under different light and soil fertility conditions, respectively. The number of leaves and coverage increased under shade condition. This can be understood as the result of shade tolerance as the maximization of light capture and photosynthesis in low light intensity conditions (Cooper and Qualls 1967). Leaf chlorophyll content was higher under shade conditions than light conditions. Higher leaf chlorophyll contents under low light intensity condition were widely accepted in previous studies (Cooper and Qualls 1967; Guenni et al. 2018). Higher leaf chlorophyll contents enhance the light use efficiency and thus improve carbon gain leading to the increase in shade tolerance (Givnish 1988; Valladares and Niinemets 2008).

In the case of AG and BG dry weights, the belowground dry weight decreased under shade conditions, and the ratio of AG and BG dry weight under shade conditions was comparatively greater than under light conditions (Fig. 4). This may be attributed to that more biomass was allocated to aboveground for higher carbon assimilation through photosynthesis under low light intensity conditions (Selzer et al. 2013), which has also been observed in other herbaceous species (Martina and von Ende 2012; Park et al. 2019). Although biomass allocation was shifted under different light treatments, it was not statistically significant difference. This is consistent with previous studies that a significantly lower biomass was attained only when light availability was reduced to 10% of full-sunlight intensity (Ryser and Eek 2000; Semchenko et al. 2012).

In the case of AG and BG dry weights, fertilizer treatment has resulted in non-significant change in their biomass (Fig. 4). This may be the result of retainment in phosphorus nutrients to support future growth (Fitter and Setters 1988). Fertilizer experiment in three *Viola* species showed no significant alternation in allocation except in clearly nutrient-deficient condition, and vegetative allocation being less variable (Fitter and Setters 1988). This suggests that fertilizer treatment has increased the reproductive organs' biomass via allocation and for not-fertilized group nutrient wasn't deficient enough to result in growth restriction or reduce in biomass reduction. Furthermore, this is consistent with previous study in Viola blanda where only reproductive biomass was greater in fertilized condition (Griffith 1998).

Fertilizer application could increase the allocation to aboveground organs and reduces allocation to belowground organs due to cheaply obtained nutrients (Bloom et al. 1985). However, this fertilizing could also result in increased leaf turnover, where keeping old leaves could be inefficient (Chabot and Hicks 1982). This could have suppressed the effect of fertilizer application to increase in above ground allocation and higher AG/BG ratio.

On the other hand, there was no significant interaction between light and soil fertility conditions in their effects on reproductive and vegetative growth of *V. collina* (Table 1). Studies have shown that higher light intensity bolsters the effect of fertilizer application (Corre et al. 1983). However, shade treatment did not act as a limiting factor of this species, and this could indicate that V. collina is a shade tolerant species. Thus, no apparent interaction was shown and light and fertilizer showed independent effects on the reproductive and vegetative growth.

## Conclusions

*Viola collina* which appears to be a shade tolerant produced relatively more chasmogamous and cleistogamous flowers under shade conditions, and chasmogamous flowers were observed almost exclusively in shade conditions. Such plasticity in production of flowers was enhanced by the simultaneous application of soil fertilizer only under shade conditions. The effect of fertilizer was minimized due to allocation and retainment of nutrients for future growth. The number of leaves, coverage, and leaf chlorophyll content were higher under shade conditions. In the case of biomass allocation, more biomass was allocated to aboveground under shade conditions. It seems that proper shade and application of soil fertilizer are necessary for the growth of *V. collina*. Our findings could help to predict the reproductive and vegetative growth change of *V. collina* according to climate change. By understanding the basic temporal and spatial niche of *V. collina* will provide essential data for the understanding of *V. collina* habitat characteristics.

### Abbreviations
Not applicable.

### Acknowledgements
Not applicable.

### Authors' contributions
HP did Investigation, data curation, writing - original draft preparation. GS did Investigation, data curation, writing - original draft preparation. JGK did conceptualization, methodology, writing - review & editing.

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### Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate
Not applicable.

### Consent for publication
Not applicable.

### Competing interests
The authors declare that they have no competing interests.

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