Influence of Light Intensity on Photosynthesis, Capsule Yield, Essential Oil and Insect Pest Incidence of Small Cardamom (*Elettaria cardamomum* (L.) Maton)

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Abstract: *Elettaria cardamomum* (L.) Maton (Small cardamom) is a widely used high value spice with various medicinal properties. Differences in the availability of solar radiation can cause modifications in the structure and function of small cardamom plants through altered source and sink capacities. The objective of the present study was to evaluate the effects of varying levels of light intensity on growth, physiological characteristics, yield, chemical quality and pest incidence of small cardamom (variety Appangala-1) under full sunlight and two shading conditions (75 % shade, 50 % shade). The experiment was performed during 2016-2018 at the Regional Station, ICAR-Indian Institute of Spices Research, Appangala, Karnataka. The experimental data revealed that the plant samples under 50 % shade produced more physiologically active tillers and higher capsule setting as well as yield per plant than those of other plant samples kept under 75 % shade and full sunlight condition. The highest photosynthetic rate, stomatal conductance, transpiration rate, chlorophyll fluorescence and lowest canopy temperature were observed on plants exposed to 75 % shade. Oleoresin content was higher in plant samples under full sunlight as compared with those under shade condition. But no significant difference was observed in essential oil concentrate ion in capsules. The percentage contribution of 1,8-cineole and α-terpinyl acetate that are considered the major constituents of essential oil are different under different illumination conditions. The concentration of α-terpinyl acetate and 1,8-cineol was highest respectively under full sunlight and 75 % shaded levels. Increased borer damage on shoots (*Conogethes punctiferalis* Guen.) was noticed for 75 % shade level. Significant difference was not noticed on the incidence of thrips and borers irrespective of shade levels. Plants under 50 % shade registered higher capsule yield besides having physiologically more active tillers and essential oil constituents and would be hence optimum for increased yield and growth of small cardamom.

Key words: Small cardamom, light intensity, yield, 1,8-cineole, α-terpinyl acetate.

Introduction

Elettaria cardamomum (L.) Maton (Small cardamom) is a highly priced spice. It is cultivated extensively as an under storey crop in the tropical ever green forests of southern Western Ghats (WG) (Kerala, Karnataka and Tamil Nadu) in the altitude ranging from 700-1500 m above MSL with an average annual rainfall between 1500 and 5000 mm and annual lowest and highest temperature ranging from 10-36°C. India produces 20,650 MT of cardamom annually, ranking it second in the world production (Spices Board, 2017-18). The practice of increased shade removal in cultivation of cardamom in the hills has led to the degra-

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dation of cardamom agroforestry systems in India. The effect of increased temperature caused by high light intensity under degraded/open forests, exhibit a larger impact on capsule yield than on vegetative growth. These effects were evident in an early and increased rate of senescence which reduced the ability of the crop to efficiently fill the seeds in the capsules [1, 2].

Under high irradiance, the photosynthetic apparatus of many plants absorbs excessive light energy, resulting in the inactivation or impairment of the chlorophyll containing reaction centers of chloroplasts, and consequently a reduction in photosynthetic activity [3,4]. Pollination is one of the most sensitive phenological stages to high light extremes across all species, and during this reproductive developmental stage high light extremes would greatly affect the setting of capsules in cardamom. Continuous exposure to high temperatures, as high as 32°C, could lead to complete withering of leaves and young tillers provided no irrigation was given during rainless summer period. A relatively higher air temperature during blossoming, especially if associated with a prolonged dry season (more than two months), may cause reduced pollination and abortion of cardamom flowers [5]. High rainfall, surface air temperature and relative humidity are critical for better fruiting and quality of cardamom [6].

In general, the duration of climate variables directly affects the occurrence of insect and pest outbreaks and the frequency of these variables are likely to change in the short, medium and long terms [7]. Hence, understanding the infestation of insect pests in cardamom in relation to climate change would be beneficial for the development of the environmental and agricultural sustainability of the cardamom farming society. Therefore, field investigation is necessary to understand the physiology of cardamom yield under varying levels of solar irradiance. The key objectives of the study were:

i. to quantify the influence of light irradiance on growth, photosynthetic characteristics, yield and quality characters of small cardamom, and

ii. to observe the effect of light irradiance on the incidence of major insect and pests outbreaks in small cardamom.

The present investigation would also help establish improved cultivation practices for getting better yield in cardamom growing areas.

Materials and methods

Plant material and experimental details

Appangala-1, a variety of the Malabar type of small cardamom, was used in the present study. Cardamom suckers of the variety were planted on 1st August 2016 under open field in Appangala, Kodagu district, Karnataka (average air temperature of 20.5°C with an average rainfall of 2783 mm during 2016-2018). Plants were subjected to two different shade treatments for 2 years, starting from August, 2016. The treatments comprised of 75 % and 50 % shade levels and full sunlight situation (natural irradiance and no shade was provide). The shade used was green color commercial plastic shading nets (PRATHU AGRO COMPANY, Coimbatore, India). The standard package of practices was followed in all the treatments as per the recommendations of ICAR-Indian Institute of Spices Research. Data of each parameter given in the tables and figures are the averages of seven replications.

Plant growth and yield characters

Plants under different treatments were allowed to grow up to 2 years, after which observations on physiologically active tillers, per cent of capsule setting and capsule yield per plant were recorded.

Environmental conditions and gas exchange measurements

Observations on weather parameters like surface air temperature and relative air humidity near the panicles of the treatment plants were measured using infrared, thermometer and a whirling psychrometer respectively during reproductive stage.

Percent interception of light inside the cardamom canopy was calculated (middle point of a slope) by comparison with a Photometer (Luxor, USA) placed in the open field situation. The measurement was made between mid-day and 2.00 pm as per the method suggested by [8]. Light interception was measured using the following formula:

$LI = (LI_m / LI_o) \times 100$

Where, LI = light interception, LI_m = Mean of light intensity at the middle of the canopy and ground level, LI_o = Light intensity in the open.

Observations on physiological parameters were made from index leaf (4th leaf from the top) of yielding plants (2 year old). Photosynthetic gas exchange parameters were recorded using a portable photosynthetic system (LCpro-SD Advanced Photosynthesis Measurement System, England). The parameters measured were net photosynthetic rate (Pn, µmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}), intercellular CO_2 concentration (Ci, µmol CO_2 mol^{-1}) and transpiration rate (Tr, mmol H_2O m^{-2} s^{-1}). During the experiment period, data were recorded between 9:30 and 11:30 am. The observations were taken when both Pn and g_s were stable. Same leaf was used for chlorophyll (a) fluorescence measurements right after gas exchange measurements. The maximum PS II quantum yield (Fv/Fm) was expressed in terms of chlorophyll fluorescence (Fv / Fm) using a chlorophyll fluorometer (Os-30p) in 10-15 minutes dark adapted leaves between 9:30 and 11:30 am local time according to [9].

Oleoresin estimation

Cured cardamom capsules were dehusked, and seeds were ground into fine powder. 10 g of powder was used for extraction of oleoresin. Oleoresin estimation was carried out using ASTA method [10]. The amount of oleoresin was estimated gravimetrically.

Oleoresin (%) = (Weight of residue (g) / Weight of sample (g)) x 100

Essential oil estimation

Essential oil was extracted using hydro distillation method (ASTA, 1997) on dry whole capsule weight basis [10]. Twenty grams of the whole cured cardamom sample was ground, seeds and husk were transferred to a 1 litre round bottom flask separately to which 500 ml distilled water was added and hydrodistilled for 4 h. The essential oil was carefully collected and dried over anhydrous sodium sulphate, and stored in a sealed glass vial for GC/MS analysis. The percentage of oil was computed as volume by weight basis:

Essential Oil (V/W) (%) = (Amount of Oil collected (ml) / Weight of sample (g)) x 100

GC/MS profiling of essential oil

Qualitative profiling of volatile constituents of cardamom essential oil was done using Shimadzu GC/MS (2010). The separation of compounds was achieved in a Rtx-5 5 % Phenyl and 95 % dimethylpolysiloxane column with the dimension of 30 m x 0.25 mm x 0.25 µm (L x W x ID). The temperature programming of the column was as follows: 65°C for 2 min, then gradient of 3°C min^{-1} to 155°C for 3 minutes. Ion source and interface temperature were set as 200°C and 240°C correspondingly. Other operational parameters were as follows: column over temperature: 65°C; injection temperature: 240°C; helium flow rate: 1.0 mL/min; injection volume: 0.2 µL; injection mode: split (1:50 split ratio); acquisition mode: scan; scan range: 40-650 m/z with a scan speed of 1428.

The mass spectra of the components were compared with the standard mass spectral library of NIST/WILEY and identified by similarity search [11].

Per cent incidence of shoot and capsule borers and thrips

The incidence of thrips (*Sciothrips cardamomi* Ramk.), shoot and capsule borers (*Conogethes punctiferalis* Guen.) was observed under different shade treatments during peak period of infestation. The percent incidence of shoot and capsule borers was recorded in field as well as cured capsules. In field, the number of shoots with bore holes and freshly extruded excreta was recorded in each clump in all treatments. The per cent damage of thrips and borers on capsules was recorded in each treatment. A sample of 100 g capsules was examined for the rasping scar and bore holes in each treatment and expressed in terms of percent damage. The capsule damage includes the damage done by *Conogethes* sp as well as by other borers.

Statistical analysis

Data were subjected to analysis of variance and

Duncan's multiple range tests to differentiate means as described by [12]. Values were considered at a significance level of 95 % ($p < 0.05$). Statistical analyses were performed using WASP-Web Agri Stat Package 2.0 by using RCBD design.

Results and discussion

The data recorded revealed that along with rainfall, surface air temperature and humidity were the most critical for flowering, fruiting and chemical quality of cardamom. The air relative humidity (daily mean) near panicle under full sunlight ranged from 71 to 85 %, 78.6 to 95.4 % under 50 % shade condition and 78.1 to 97.2 % under 75 % shade condition respectively. The temperature was found to be slightly warmer near panicles and the temperature (daily mean) of surface air was found to be in the decreasing order from full sunlight (Min. temp: 18.6°C and Max. temp: 32.5 °C) to 50 % shade (Min. temp: 17.7°C and Max. temp: 24.1°C) and 75 % shade (Min. temp: 15.6 °C and Max. temp: 22.8°C).

Plant growth and yield characters, such as physiologically active tillers, per cent capsule setting, and capsule yield per plant (Table 1), also showed significant variation along with different shade treatments. Among shade levels, 50 % shade recorded maximum number of physiologically active tillers (31.6) and capsule yield per plant (247.7 g) followed by 29.3 physiologically active tillers and 222.1 g of capsule yield per plant in 75 % shade and 22.4 physiologically active tillers and 140.8 g of capsule yield per plant in full sunlight respectively. Full sunlight recorded highest number of senescence tillers (Table 1). The yields of most of the food crops decline sharply at temperatures much above 30°C [13]. Murugan *et al*. reported that cardamom can tolerate surface air temperatures up to 32.2°C and beyond this level both growth and development as well as yield is reduced [14]. In our study, a considerable negative impact of full sunlight on the air temperature and relative humidity has been noticed and the impact was mostly reflected on physiologically active tillers and capsule yield.

In our study, maximum capsule setting (50.2 %) was recorded in 50 % shade followed by 75 %

Table 1. The effects of light intensity on growth and yield traits of small cardamom

Treatments	Senescence tillers	Physiologically active tillers per plant	Total tillers	Capsule setting %	100 capsule wt (g)	Number of seed capsule^{-1}	Capsule yield plant^{-1}
Full sunlight	25.6^{a}	22.4^{b}	48.0^{a}	31.2^{b}	16.0^{ns}	18.8^{ns}	140.8^{c}
50 % shade	18.0^{b}	31.6^{a}	49.6^{a}	50.2^{a}	15.7^{ns}	19.5^{ns}	247.7^{a}
75 %shade	12.1^{c}	29.3^{a}	41.4^{b}	47.7^{a}	14.6^{ns}	20.2^{ns}	222.1^{b}
Mean	18.5	27.7	46.3	47.5	15.4	19.5	203.5
CV %	17.7	12.7	10.7	3.94	6.29	14.9	16.2

Values represent mean of seven replications

Same letter within each column indicate no significant differences among the treatments ($P \leq 0.05$)

shade (47.7 %) (Table 1). The data recorded reveals that incidence of heat stress (caused by high light intensity) seriously affected pollination, and significantly suppressed the percentage of capsule setting (31.2 %) under full sunlight, and also the behavioral change of foraging activity of honey bees is mainly influenced by heat stress during day time temperature maxima. A similar observation was recorded by Stanley *et al*. and Murugan *et al*. [14, 15]. In this study, during daytime, the Photosynthetic Photon Flux Density (PPFD) ranged from 950 to 1700, 500 to 900 and 250 to 600 µmols m^{-2} sec^{-1} under full sunlight, 50 % shade and 75 % shade level respectively. Net photosynthetic rate (Pn) varied significantly among shade treatments (Table 2). The Pn value was highest in 75 % shade (8.43) followed by 50 % shade (7.46). Stomatal conductance (g_s) also varied significantly among different shade level treatments. Stomatal conductance (g_s) values of treatment plants grown under full sunlight were lower (0.071). The highest g_s values were observed under 75 % shade (0.182). The optimum light intensity for small cardamom assimilation is between 400 to 900 mmols m^{-2} sec^{-1} [16]. Below and beyond this optimum range, the photosynthetic rate is reduced. In the sub-optimum part, the photosynthetic machinery is limited by the enzymatic reaction rates as the kinetic properties of Rubisco are temperature-dependent. In the supra-optimum part, the oxygenation reaction of Rubisco increases more rapidly than the carboxylation, so that photorespiration becomes more important [17].

The essential oil and oleoresin content showed significant variation among the different light intensity levels (Fig. 1). Among the different shade treatments, full sunlight recorded maximum oleoresin content (4.7 %). The highest essential oil content from the seeds was recorded in 75 % shade (5.37 %) followed by 50 % shade (5.25 %) and full sunlight (5.0 %) respectively though they are not significant (Fig. 1). The highest essential oil content from the whole capsules was recorded in both 75 % shade (7.8 %) and 50 % shade (7.8 %) followed by full sunlight (7.6 %). The results are in agreement with those reported by Manoj *et al* [6].

Qualitative analysis of cardamom essential oil

Table 2. The effects of light intensity on photosynthetic gas exchange of small cardamom

Treatments	Pn (µmol CO_2 m^{-2} s^{-1})	gs (mol H_2O m^{-2} s^{-1})	Tr (mol m^{-2} s^{-1})	Chlorophyll fluorescence (Fv/Fm)	Air temperature ranges near panicle (°C)	Relative humidity near panicle (%)
Full sunlight	5.53^c	0.071^c	1.95^c	0.619^c	18.6-32.5	71.0-85.0
50 % shade	7.46^b	0.106^b	3.27^b	0.728^b	17.7-24.1	78.6-95.4
75 % shade	8.43^a	0.182^a	4.09^a	0.773^a	15.6-22.8	78.1-97.2
CV %	13.21	14.15	13.68	5.29		

Pn = Photosynthetic rate; gs = stotamatal conductance; Tr = Transpiration rate
Values represent mean of seven replications
Same letter within each column indicate no significant differences among the treatments ($P \leq 0.05$)

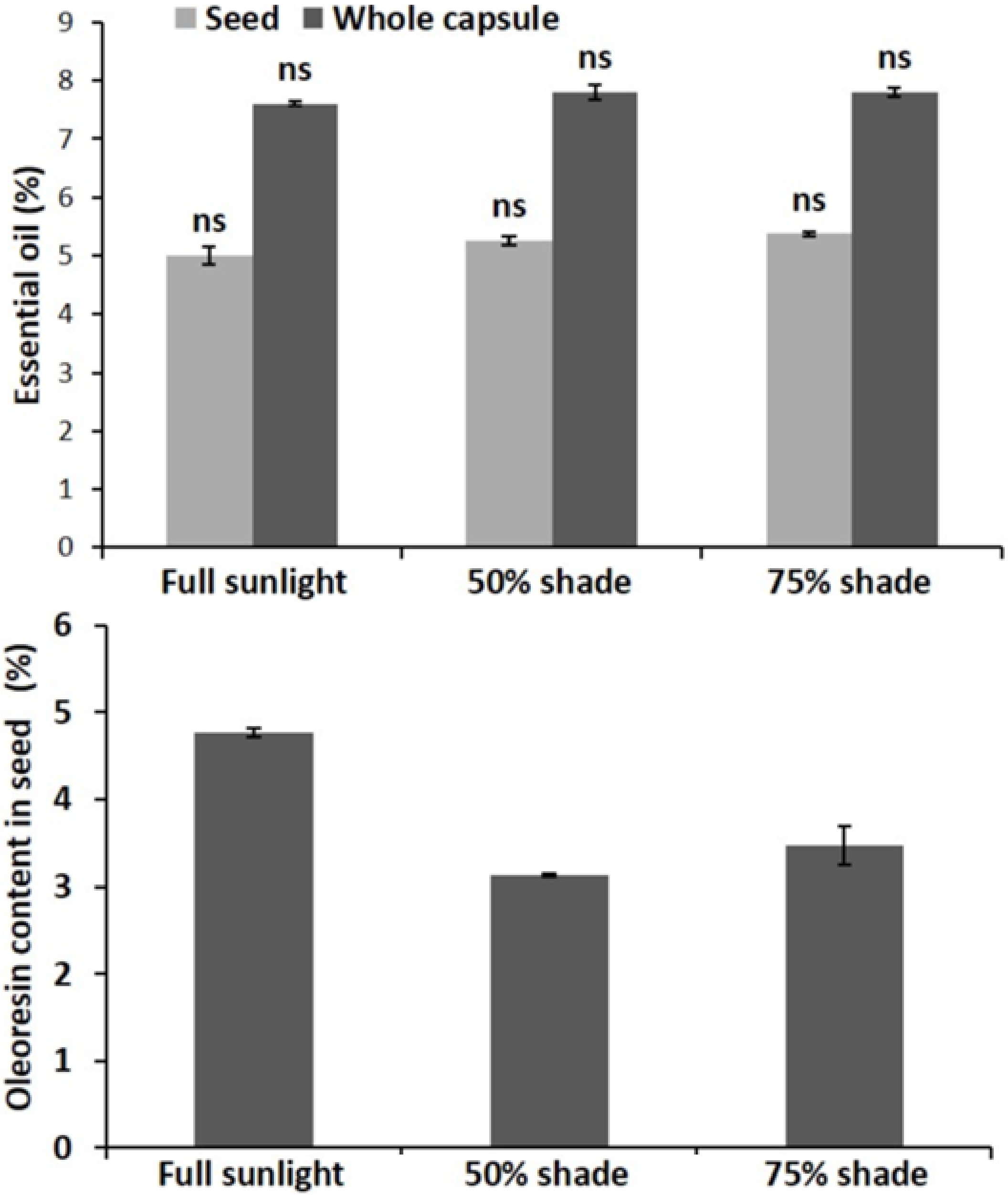

Fig. 1. The effects of light intensity on Essential oil and Oleoresin content in small cardamom capsules

using GC/MS was shown in Fig. 2 & Table 3. The results showed that 1,8-cineole and α-terpinyl acetate as the major constituents of the cardamom oil. The quality of cardamom oil is determined by the proportion of these two compounds. The higher percentage of 1,8-cineole gives camphorous flavor and the elevated levels of α-terpinyl acetate gives mild and sweet aroma to its oil. Usually, larger differences are shown in the concentration of 1,8-cineole in the oils of cv. *Malabar* and *Mysore* [6, 18]. Our results of GC/MS analysis revealed that 75 % shade increased the 1,8-cineole content when compared with full sunlight and 50 % shade condition with subsequent reduction in α-terpenyl acetate content. The reason for the difference in the concentration could be explained by the possible loss of monoterpenes like 1,8-cineole in full sunlight because they have low boiling point. Likewise, α-terpinyl acetate being higher order terpenoid compound with

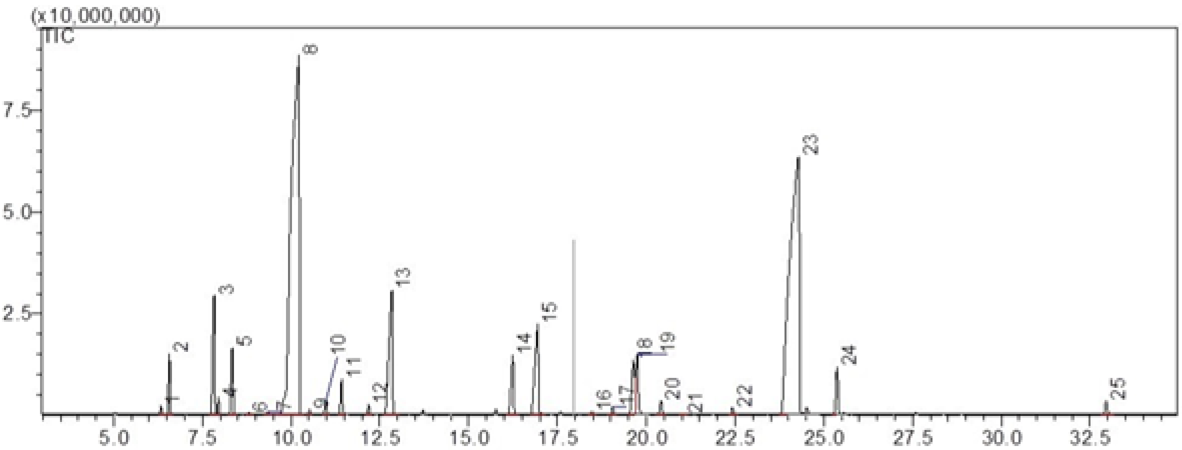

Fig. 2. GC-MS Chromatogram of cardamom essential oil

Table 3. Relative content of major components of cardamom essential oil as analyzed by GC-MS

No.	Compounds	Retention time (min)	Peak area (%) Full sunlight	50 % shade	75 % shade
1	α-Thujene	6.33	0.19	0.19	0.19
2	α-Pinene	6.56	1.27	1.25	1.23
3	Sabinene	7.82	3.19	3.16	3.08
4	β-Pinene	7.94	0.38	0.39	0.33
5	β-Myrcene	8.33	1.78	1.77	1.72
6	Octanal	8.79	0.09	0.10	0.07
7	α-Terpinene	9.32	0.15	0.16	0.16
8	1,8-Cineol	10.17	38.70	39.34	41.81
9	β-Ocimene	10.50	0.12	0.12	0.11
10	γ-Terpinene	10.96	0.33	0.33	0.32
11	*trans*-Sabinene hydrate	11.40	0.94	0.92	0.89
12	α-Terpinolene	12.17	0.27	0.34	0.25
13	Linalool	12.80	5.81	6.01	5.48
14	Terpinen-4-ol	16.22	2.02	2.08	1.84
15	α-Terpineol	16.92	4.13	4.65	3.75
16	Geraniol	18.46	0.11	0.12	0.10
17	Citral	19.03	0.24	0.25	0.18
18	Linayl acetate	19.60	2.03	1.76	1.58
19	β-Geraniol	19.71	2.22	2.50	2.19
20	Geranial	20.38	0.41	0.41	0.30
21	Borneol acetate	21.03	0.04	0.04	0.04
22	Ocimenyl acetate	22.41	0.25	0.24	0.22
23	Terpinyl acetate	24.22	33.22	31.87	32.29
24	Neryl acetate	25.35	1.60	1.51	1.38
25	Nerolidol B	32.94	0.53	0.53	0.49

higher boiling point could withstand the heat of full sunlight as evident by their higher percentage in full sunlight condition.

Apart from these two compounds, linalool and α-terpineol showed variation among the different light exposure or shade treatments. The samples under 50 % shade treatments showed higher linalool content (6.01 %) as compared to full sunlight (5.81 %) and 75 % shade treatments (5.48 %). The same trend was observed with α-terpineol. In general, the content of monoterpene compounds were decreased under full sunlight and the content of terpenoid alcohol compounds like terpinen-4-ol, alpha terpineol, geraniol, citral were increased in 50 % shade condition. The acetylated terpenoid compounds like terpinyl acetate, neryl acetate, ociminyl acteate were present in larger proportion under full sunlight as compared with the different shade conditions. In our study, the data confirmed that the light intensity significantly affected the volatile compounds composition in cardamom capsules. Previous studies involving essential oil of different crop has reported the influence of weather parameters to composition of essential oil. Burbott, suggested that influence of temperature on monoterpene catabolism of peppermint essential oil is due to changes in oxidation-reduction state of co-enzymes of terpenoid biosynthetic pathway which changes the oxidation or reduction state of terpenoids [19]. Their results confirmed that environmental conditions strongly influence the metabolism of monoterpenes in peppermint. Yavaria *et al*. also reported that there is decrease in monoterpene compounds as compared to oxygenated compounds in high temperature condition in essential oil of the crop *Thymus migricus* collected from northwestern Iran [20]. Likewise, Zeljkovi *et al*, reported that the solar exposure has significant effect on essential oil composition and the content of oxygenated compounds was higher in low altitude and high solar exposure area which also showed reduction in monoterpene compounds in essential oil of *Helichrysum italicum* [21]. Thakur *et al*, studied the effect of different shade level on essential oil composition of damask rose. He reported increase in sesquiterpene compounds in open sunlight conditions and decrease of the same with increase in shade level [22]. The results of present study suggested that high light intensity decrease the amount of monoterpene composition of essential oils extracted from small cardamom.

Results of the insect pest incidence under different light intensity showed variations. The borer damage on shoots (*Conogethes* sp.) was significantly different among the different treatments (Fig. 3). The highest incidence of shoot damage was recorded in plants under 75 % shade (10.68 %), followed by 50 % shade (7.70 %) and full sunlight (5.94 %). In contrast, no significant difference of thrips and borers damage was observed among the treatments on cured capsules. The average damage by thrips on capsules ranged between 8.27 and 9.45 %, and the borer damage scaled from 5.0 to 6.68 %.

Conclusion

The data from this experiment reveals that by exposing cardamom plants to 50 % shade could improve small cardamom growth and yield provided water and nutrients are non-limiting factors. Also, the incidence of insect pests like thrips and borers on cardamom capsules was not affected by different shade levels and the damage on shoots by *Conogethes punctiferalis* Guen. was also lower at the 50 % shade level. Further, full sunlight condition increased the sweet aroma as evidenced by increase in α-terpinyl acetate, whereas 75 % shade improved camphorous flavor as it increased the percentage of 1,8-cineole concentration. Therefore, in small cardamom cultivation, 50 % shade level with adoption of standard good package of practices will be beneficial in attaining higher yield and elevated intrinsic chemical quality.

Acknowledgements

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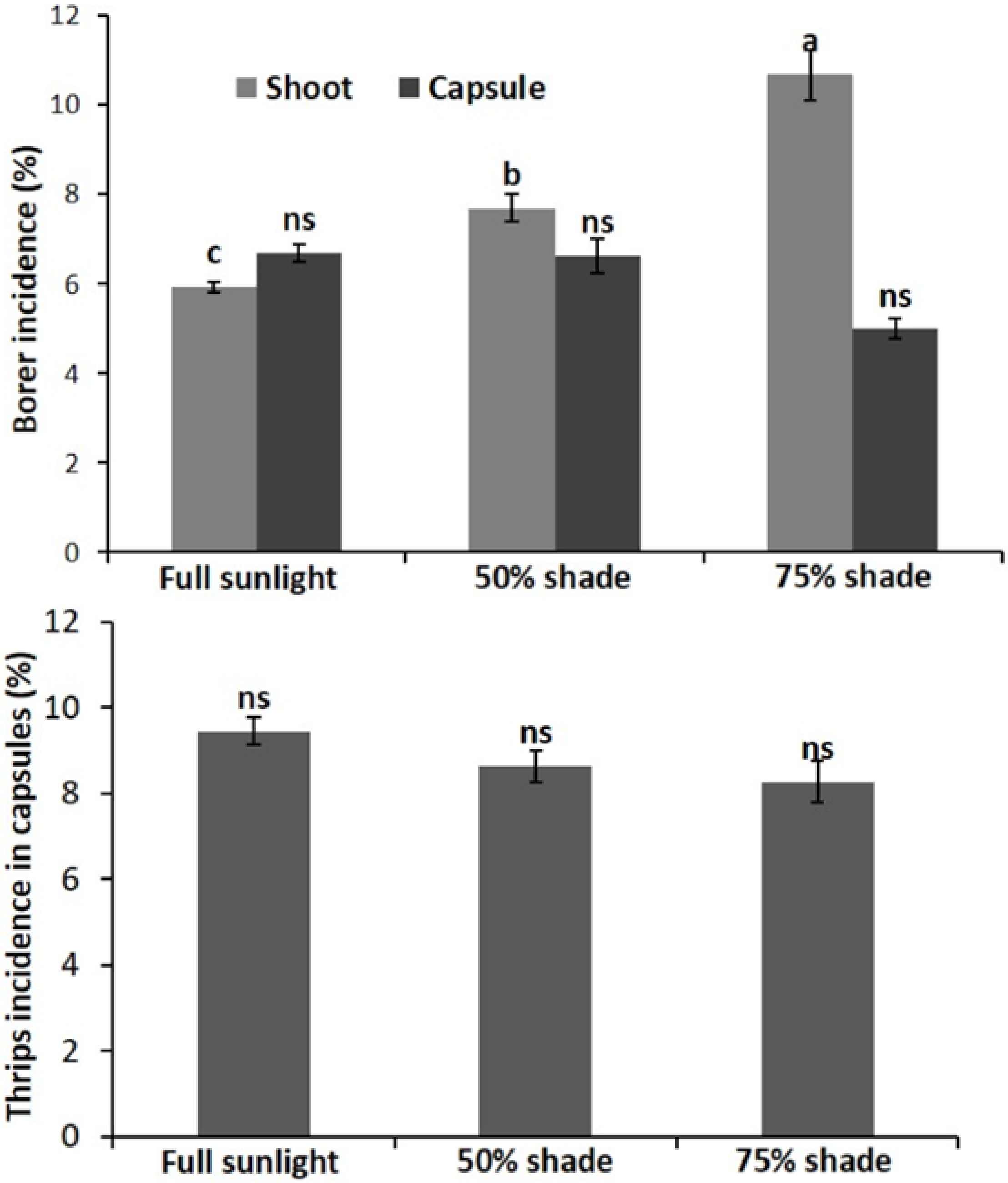

Fig. 3. The effects of light intensity on borer and thrips incidence on shoot and capsules

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