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Impact of Shade Intensities on Rhizome Yield and Quality of *Costus speciosus*: A Viable Option as an Intercrop

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Abstract

The maximum number of stems/plants, stem weight, root length, rhizome spread, fresh rhizome weight and dry rhizome weight were observed under 75% Shade Intensity (SI) followed by 50% natural SI. The light response study revealed that net Photosynthetic Rate (PN) was maximum at 1000 μ mol (photons) m^{-2} s^{-1} and further increase in light intensity decreased the PN. The NPQ increased steadily and this kind of PAR requirement (<750 μ mol) makes the plant suitable for partially shade area with very low light transmission. Diosgenin content in rhizome was low in all shaded plants as compare to 0% SI (open field conditions) in which it was maximum (826 mg g^{-1}). Over all rhizome and diosgenin yield content were maximum under 75% natural SI as an intercrop.

Keywords: *Costus*; Diosgenin; Rhizome yield; Shade intensity

Introduction

Costus specious Koen (*keu*) belongs to the family *Costaceae* is an important ornamental as well as medicinal plant extensively used in indigenous system of medicines for cure of several illnesses. It is commonly grown as ornamental herb in kitchen garden or backyards. Sometimes also grown as pot plant for Indore beautification in India. It is locally known as *keu* and crape ginger in country and erect, perennial, succulent, ornamental herb arising directly from rhizome [1]. The rhizome is tubular, branched having circular nodal scars and few wiry rootlets (yellowish brown in colour) and fibrous. Plants bears large (15-35 and 6-10 cm), thick leaves spirally arranged on stem are elliptical to oblong in shapes. It produces whitish, thick, large flowers and cone like terminal spikes with bright red brackets and yellowish throat of lips. The fruits are globosely trigonous, red capsules type (2 cm) containing black seed with white aril [2]. The rhizome which is the commercial part of plant is used as an alternative source for diosgenin. Rhizome extract exhibit cardiotonic, hydrocholeretic, diuretic depressant activities [3]. It is mostly used to control diabetes and blood sugar levels. Its traditional uses include remedial of pneumonia, rheumatism, dropsy, urinary, gout rheumatism, bronchial asthma and jaundice diseases. Juice of rhizome also applied to head for cooling and relief from head-ache, bruised leaves are applied in fever, decoction of stem is used in fever and dysentery [4]. In general, the diosgenin content in rhizome was reported up to 3.37% on dry weight basis [5].

It is widely distributed in tropical to subtropical parts of the country up to 1200 m above mean sea level. It favours moist tropical evergreen forest but commonly grown along roadside, near water streams and waste land. It is naturally found in plenty from Assam, Meghalaya, Bihar, Orissa, Madhya Pradesh, West Bengal, Western Ghats and Sub Himalayan parts of Uttarakhand, Himachal etc., [1]. It is cultivated well in rich moist clay loam soils in shady area under forest. Best time for cultivation is rainy season which offering high humidity and low temperature type climate in south Indian forests [6, 7]. Tolerance of plant under shade condition sustained the growth due to increase in leaf area, photosynthetic capacity per unit of light and sugar content [8]. The leaves under shade condition made an efficient use of the less intense irradiation reaching up to them like safed musli, rauvolfia, patchouli, turmeric, shatavari and brahmi [9]. Such medicinal plants have been successfully intercropped with fuel wood trees like parkia, chandan (white), Ashoka tree, bael, aonla, sugar apple, Moringa, neem, Indian soapberry, arjun, black myrobalan, in the early years until the tree canopy closes. Therefore, South Asian countries have been a tradition of practicing mixed farming system depending upon the spacing and nature of trees [10]. Systematic efforts to know the exact light requirement in non-traditional area to harvest maximum quantity of *Keu* rhizomes along with good quality are still not standardized. Thus, identification of suitable SI requirements was very much essential to ensure premium produce and its quality for sustainable utilization of natural resources in the era of changing global environment.

Materials and Methods

Experimental site

The research experiment was conducted at the ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat (India) during 2018-2019. The experimental farm is located at an altitude of 45.1 m above mean sea level and at 22˚35'N and 72˚55'E. It has a semi-arid, subtropical type climate with hot dry summers and mild winters.

Plant materials

Rhizomes were transplanted at a spacing of 50 × 50 cm in natural shade intensities under 28 years old trees of *Azadarichta indica* (neem) during second week of July, 2018. The natural shade intensity under the tree canopy was measured at about 12.00 hours. The average natural shade intensity just under the tree was nearly 75% (0-4 m adjacent trunk), 50% (4.1-8 m) and 25% (8.1-12 m). The open field was considered as zero shade intensity. Each plot of 405 × 315 cm size was replicated five times. One irrigation in every twenty days interval except rainy season were given. The experiment was layout following the standard horticultural practices. The observations were recorded for plant height, number of stems per plants, stem diameter, number of nodes per plant, number of leaves per stem, leaf length, leaf width, leaf area, rhizome length, rhizome spread, rhizome weight and

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number of feeder roots at harvesting stage. These observations were averaged out to get the mean value of three replication from each treatment after 18 months from the date of planting or second week of December, 2019. Plant height was recorded between the collar region and shoot tip of the main axis. Rhizome were separated from the plant for measuring fresh weight and dried properly after peeling for dry weight until constant moisture content at room temperature.

Photosynthetic light response studies

Chlorophyll fluorescence and photosynthetic performance to different light intensities was studied with portable photosynthesis system, LICOR 6400 XT (LI-COR Inc. Lincon, Nebraska, USA). To measure maximum efficiency of photosynthesis (Fv/Fm), leaves were adapted to dark period of minimum 30 minutes followed by a saturating flash light of 3,000 µmol (photon) $m^{-2}s^{-1}$ to achieve the maximum fluorescence. Subsequently, actual efficiency of photosynthesis (Fv'/Fm') was measured [11]. The net Photosynthetic Rate (PN), Stomatal Conductance (gs) and Transpiration Rate (E) were recorded between 08:00 and 11:00 h. A completely mature leaf was kept in the chamber by ensuring the thermocouple touching it from the underside. Temperature (ambient) was set at giving a stable leaf temperature observation. Based on the light curve studies, the artificial light inside the cuvette was given at 1000 PAR including 10% of blue light. The CO_2 was supplied at the flow rate of 500 m mol s^{-1} to supply 400 ppm CO_2 inside the chamber. Stable reading of flow, CO_2 and H_2O were considered for logging the final reading of gaseous exchange parameters.

Determination of diosgenin content in dry rhizome

Diosgenin is major chemical constituent of *Costus speciosus*, for its determination the root sample dried and ground in cyclone mill (M/s Uday Corporation). The reference standard was purchased for M/s Sigma, India and prepared 100 ppm stock solution in methanol and stored at -20°C in freeze. One gram of dried root powder was extracted by 100 mL methanol three times (3 × 100 mL) by reflux on water bath at temperature 95 ± 5°C. The extracts were combined, filtered and evaporated up to dried by using rotatory evaporator (M/s Hidolph, Germany). The dried extract was hydrolysed by adding 40 mL of 10% HCL on water bath for 30 minutes. The cool down sample solution was partitioned by separating funnel three times with chloroform (3×100 ml). The pooled chloroform extract evaporated on rotary evaporator and residue dissolved in HPLC grade methanol. The sample solution filter through 0.45 µm nylon syringe filter and used for analysis. The chemical analysis was performed on Ultra-Fast Performance Liquid Chromatography (UFLC M/s Shimadzu Corporation, Kyoto, Japan) system. It consists of two L20AD pumps, DGU-20A3 Degasser, SIL-20AC HT auto sampler CTO-10ASvp column oven, CBM-20 a communication bus module and LC Solution software. The analysis performed by using RP-C18 Altima column 100 × 4.6 mm, 3 µm and mobile phase consist of acetonitrile and 0.1% formic acid in water in ratio of 82:18 v/v in isocratic condition. The flow rate was 1 ml/min and total run time 20 min. The peaks were monitor at 190-400 nm using Photodiode Array Detector, and integration and quantification was done at 203 nm. The calibration curve was plotted by loading different concentrations of reference standard against peak area (Figure 1A). The peak of diosgenin in sample was identified based on retention time and quantification by regression equation Y=4.2603e05+0.4745 [12]. The resolved peak chromatogram of reference standard and root extracted sample shown in (Figure 1B).

Statistical analysis

The analysis of variance was done in Randomized Block Design (RBD) for various observations observed during experiment by using statistical software SAS 9.2. DMRT comparisons among the different shade-net intensities. The results were presented at 5% level of significance and Critical Difference (CD) values were calculated to compare the various treatment means.

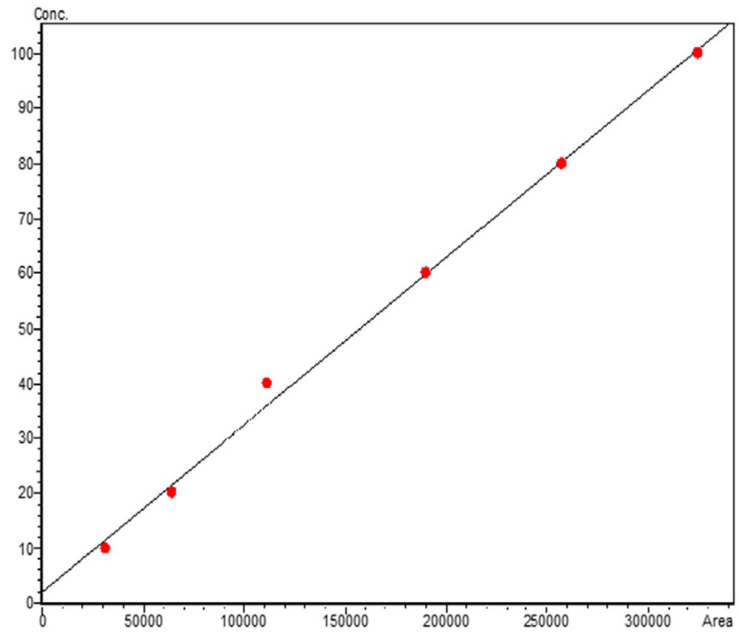

Figure 1: Optimization of chromatographic separation of diosgenin on HPLC A) Specificity and calibration curve of diosgenin.

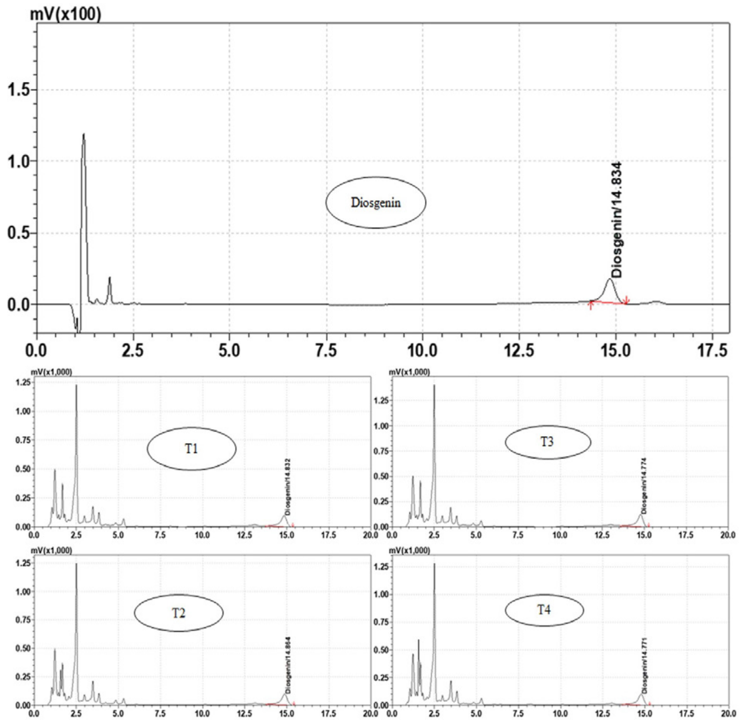

Figure 1B: UHPLC chromatograms of *C. speciosus* under different shade-net intensities.

Table 1: Effect of shade net intensity on rhizome weight and contributing parameters in *Costus speciosus.*

Number of nodes per plant	Number of leaves per tiller	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Rhizome length (cm)	Rhizome spread (cm)	Number of feeder roots	Rhizome weight
15.03c	11.58c	17.70c	7.28c	101.21d	19.90b	31.47b	58.17c	560d
15.73c	14.53 ab	18.36bc	8.88b	136.44c	19.13b	31.88b	60.83b	760c
17.01b	14.76 ab	22.64ab	10.54a	178.91b	19.62b	36.35ab	69.17b	1050b
20.50a	15.79 a	24.32a	10.93a	246.49a	31.12a	37.72a	151.17a	1450a
Means with the same letter (superscript) in the columns do not showing significantly different (P = 0.05) - (Duncan Multiple Range Test)								

Results and Discussion

Keu was observed under different shade intensities (0, 25, 50 and 75%) for plant growth parameters including rhizome yield and quality parameters (Table 1). The growth parameters like plant height, number of stems, stem diameter, number of nodes per plant, number of leaves, leaf length, leaf width, leaf area, rhizome length, rhizome spread, rhizome weight and number of feeder roots were observed maximum under 75% shade intensity (145.09 cm, 4.75, 2.09 cm, 20.50, 15.79, 24.32 cm, 10.93 cm, 246.49 cm^2, 31.12 cm, 37.72 cm, 1450 g and 151.17, respectively), whereas minimum (66.72 cm, 3.72, 1.32 cm, 15.03, 11.58, 17.70 cm, 7.28 cm, 101.21 cm^2, 19.90 cm, 31.47 cm, 560 g and 58.17 respectively) in 0% shade intensity or open field. Light is an important environmental factor that impacts phenological growth parameters [13]. Similarly, rhizomatous crop like turmeric considered as partial shade-tolerant crop and the total shoot-root biomass was markedly higher in plants raised under 48, 52 and 68% Relative Light Intensity (RLI) as compared to the 100% RLI [14]. Shatavari in arecanut orchard produced fresh root yield (10,666 kg ha^{-1}) and subsidized to maximum kernel equivalent yield (1524 kg ha^{-1}) among sixteen medicinal and aromatic plants as an intercrop [15]. Overall, maximum survival rate, as an intercrop in mulberry orchard was reported in *A. racemosus*, while *Acorus calamus*, *Aloe barbadensis*, *Cyperus scariosus*, *Plumbago zeylanica, Rauvolfia serpentina* also suggested that among these medicinal plants, could be grown as an intercrop [16].

The fresh rhizome yield was highest in 75% SI (55.06 t ha^{-1}) followed by 50% SI (39.71 t ha^{-1}), whereas it was lowest in 0% SI (21.39 t ha^{-1}). Rhizome yield performance was more than double under 75 % shade intensity as compared to 0% SI (Figure 2). It was recorded that maximum weight gain of rhizome taken place with increasing shade intensity. This might be due to medicinal plants prefer to grow in forests under limited shade, organic matter rich moist soils with high Relative Humidity (RH) and trivial temperatures [17].

The light response curve studies performed in *costus* genotypes at a fixed reference of 400 µ mol CO_2 mol^{-1} indicated that plant can initiate photosynthesis with photosynthetic active radiation (PAR) of 250 µ mol (photons) m^{-2} s^{-1} only (Figure 3). Increasing the light intensity, the PN gradually increased till 500 µ mol (photons) m^{-2} s^{-1}. The PN was maximum at 1000 µ mol (photons) m^{-2} s^{-1} and further increase in light intensity decreased the PN. Increasing PAR from 250 to 1000 had resulted in 17% higher PN. The gs become steady after 500 µ mol (photons) m^{-2} s^{-1}. It is interesting to note that the non-

Figure 2: Performance of rhizome yield in *C. speciosus* under different natural shade intensities.

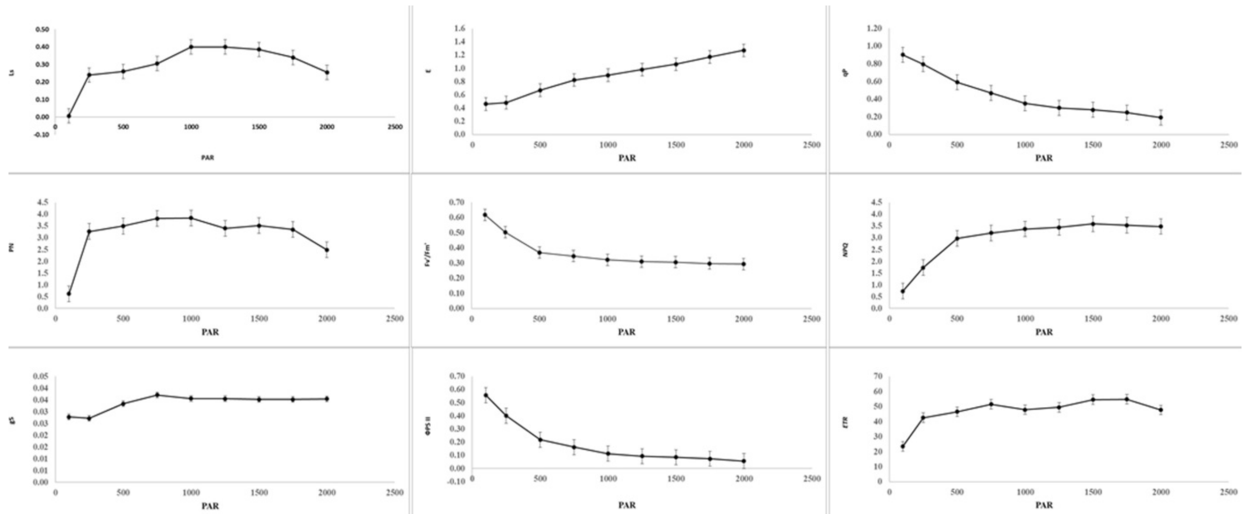

Figure 3: Light response curve performance in *C. speciosus*.

photochemical quenching increased steadily with increase in light intensity. This kind of PAR requirement makes the plant suitable for very low light transmission under partially shaded area. The stomatal limitations showed positive relationship with increase in light intensities. It is also worth to note that the ΦPSII was maximum at 100 μ mol (photons) m^{-2} s^{-1} and continuously decreased with higher light intensities. The actual efficiency of photosynthesis was maximum at 100 μ mol (photons) m^{-2} s^{-1} and decreased thereafter. The ETR, E and NPQ increased with increase in light intensities. The *Keu* plant can photosynthetically perform well even below PAR light of 750 μ mol (photons) m^{-2} s^{-1}. *Costus spiralis* and *C. longebracteolatus* plants perform significantly for growth parameters especially pseudo stem length under 50% shading by red and blue nets. This might be due to plant growth and development were repressed under full sun exposure (PAR light >1000 μ mol) along with early leaf necrosis of these species as compared to shade intensities [18].

Dried rhizome (commercial part) was analysed for diosgenin content and average maximum diosgenin content (0.826 mg g^{-1}) was observed under 0% SI followed by 25% and 75% (0.772 mg g^{-1} and 0.764 mg g^{-1}, respectively) SI (Figure 1A and 4). Similarly, the curcumin content was distinctly higher in turmeric plants raised under 59% RLI as compared to the 100% RLI [13]. Although shade-grown leaves are not directly exposed to sunlight, they produce additional chlorophyll to capture diffuse radiation to produce the carbohydrates needed

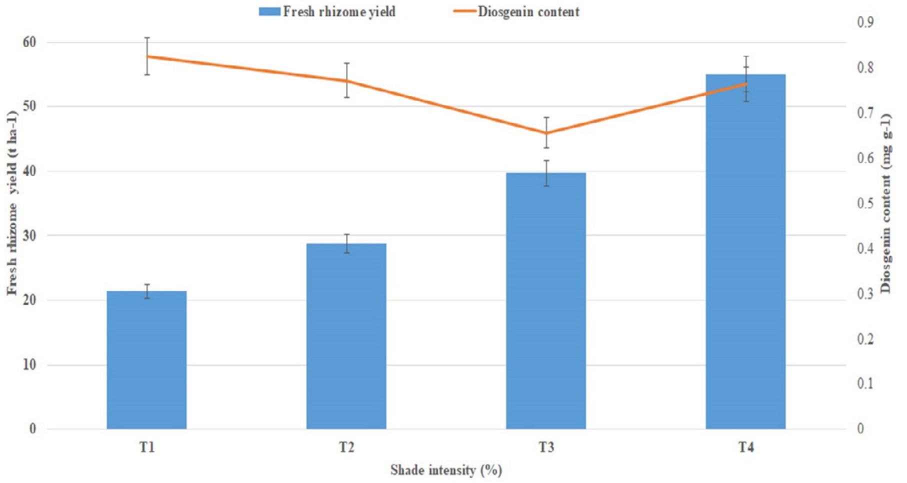

Figure 4: Rhizome yield and diosgenin content in dry rhizome of *C. speciosus* under different natural shade intensities.

Graphical Presentation

for plant growth. While, curcumin (5.42%), oleoresin (10.15%) and essential oil (5.60%) content increased under shaded conditions in turmeric [19]. Shatavarin IV content in Shatavari was enhanced under different shade intensities [9] Graphical Presentation.

Conclusion

In conclusion about double rhizome yield was observed under 75% SI followed by 50% SI. At the same time diosgenin content was not significantly higher in open field condition as compared with shade intensities. For harnessing of rhizome yield and diosgenin content, 75% SI as an intercrop was found most suitable compared with rest SI including open field condition. Over all this crop prefers partial shade condition, therefore, costus is highly suitable as an intercrop under perennial trees like neem for sustainable cultivation.

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