Article

Elicitors Mediated Response of Growth, Yield and Quality of Kalmegh (*Andrographis paniculata* Wall. ex Nees)

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Abstract: With the objective of studying the influence of elicitors on growth, yield and quality of kalmegh we carried out an investigation for two consecutive years. Nine treatments with three replications were laid out in CRD design. Chitosan, Yeast extract, Jasmonic acid and Salicylic acid were evaluated at different concentrations. CHT 1000 ppm exhibited highest plant height (73.91 cm) and secondary branches (29.07) at the time of harvest. Primary branches and number of leaves per plant were highest in CHT 1000 ppm (26.36; 88.32) which were on par with SA 200 ppm (26.28; 81.51). Plant spread was highest in SA 200 ppm (35.46 cm^2) which was on par with CHT 1000 ppm (35.11 cm^2). CHT and SA sprays were recorded with on par results for yield parameters but highest fresh (42.34 g) and dry (18.30) herbage yield per plant were exhibited with SA 200 ppm. Highest total chlorophyll (4.459 mg g^{-1}) and total andrographolides (3.494%) content were recorded in SA 200 ppm spray. Significant and positive improvement in growth, yield and quality of kalmegh was noticed with Salicylic acid spray @ 200 ppm at 30 and 60 DAS signifying its use in cultivation of kalmegh for high productivity, quality and better returns for farmers.

Keywords: Kalmegh; Elicitors; Growth; Andrograholide; Salicylic acid; Chitosan

Introduction

Since the beginning of human civilization, plants have been one of the most important sources of medicines. Despite the great advancements made in the field of allopathy during the 20th century, plants continue to be one of the primary sources used in both modern and traditional medical systems that are practised by people all over the world. Among these medicinal plants, kalmegh is getting more significance in present day situations owing to its medicinal and curative properties (Farooqi and Sreeramu, 2010). *Andrographis* is one of the most important genera of the family Acanthaceae. Kalmegh plays a significant role in 26 Indian ayurvedic formulas and holds a vital position in the Indian Pharmacopoeia. (Verma *et al.*, 2018). Its antipyretic and antiviral property is immense and thus, it is potential to fight against Covid-19 (Verma *et al.*, 2021).

A wide variety of bioactive substances, including andrographolides and polyphenols, are produced by Kalmegh. One of the pharmacologically significant compounds is andrographolides. A minimum of 26 Ayurvedic remedies used to treat liver problems contain a strongly bitter flavoured andrographolide, a labdane diterpenoid produced from *Andrographis paniculata* (Pandey and Rao, 2018). Andrographolide is important anticancer and immunomodulatory pharmacophore and has potential to be developed as an anticancer chemotherapeutic agent (Mishra *et al.*, 2007). In India, China and other South East Asian nations, kalmegh is used to treat throat infection, fever, colds, and a number of infectious diseases like dysentery, diarrhoea and malaria. The plant also possesses antibacterial, antithrombotic, anti-inflammatory and immunological properties (Verma *et al.*, 2021).

Elicitation is the process of enhancing or inducing the production of metabolites by adding small amounts of elicitors. "Elicitor may be defined as a substance for stress factors which, when applied in small quantity to a living system, induces or improves the biosynthesis of specific compound which do have an important role in the adaptations of plants to a stressful condition" (Radman *et al.*, 2003). In comparison with many applications for enhanced productivity, elicitation is recognised as the most practically possible method for enhancing the synthesis of desirable secondary metabolites from plants without compromising quality (Poornananda and Jameel, 2016).

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For pharmaceutical industries, bulk herb accompanied by high quantity and quality of principle component in the herb is important for ease and worth full extraction. In recent years, studies on use of elicitors, both biotic and abiotic for quality improvement in medicinal plants is increasing but, these studies are confined to *in-vitro* level and only a smaller number of researches were done on *ex-vitro* spray of elicitors. Elicitation is known to increase secondary metabolite. Thus, use of elicitors fulfils both high yield of herb with quality and this in line supports both farmers and buyers for cost effective production and high returns. However, there is scarcity of research to prove yield increment through elicitation. Thus, there is a basic need to study elicitors mediated response of growth, yield and quality in kalmegh. The biosynthesis of bioactive chemicals in medicinal plants and the creation of biomass are both significantly impacted by nutrients and direct or indirect exposure of herbs to foreign molecules (biotic or abiotic) *i.e.*, elicitors. Therefore, studies on efficacy of organic nutrients and elicitors on growth yield and quality of medicinal herbs is required. The emerging global scenario in respect of demand for herbs in various ayurvedic preparations and pharmaceutical industries suggests that kalmegh may become one of the very important crops in the near future. The purpose of the present research work was to acquire insight into the impacts of elicitors [biotic (chitosan and yeast extract) and abiotic (jasmonate and salicylic acid)] on the enhancement of growth, yield, and quality production of kalmegh while keeping in mind the significance of this crop.

Materials and Methods

Elicitation studies for quality production in kalmegh are meagre and only few studies were done in laboratory scale. Exogenous spray of elicitors to enhance the secondary metabolites and also growth and yield in medicinal plants and other crops as reviewed earlier. To extend this application of elicitors in kalmegh crop for quality production and also to check if any variations in growth and yield attribute, the present experiment was done. Extensive reviewing of earlier literatures showed that different concentrations of biotic (0.2 to 2 g L^{-1} or 200 to 2000 ppm) and abiotic elicitors (0.5 to 1 mM or 100 to 200 ppm) were influenced the crop response in positive direction. So, after applying extrapolative and qualitative approach of research the following treatments were fixed and evaluated *viz*., T_1; chitosan @ 500 ppm, T_2; chitosan @ 1000 ppm, T_3; yeast extract @ 500 ppm, T_4; yeast extract @ 1000 ppm, T_5; jasmonic acid @ 100 ppm, T_6; jasmonic acid @ 200 ppm, T_7; salicylic acid @ 100 ppm, T_8; salicylic acid @ 200 ppm, T_9; control (water spray). Elicitors were sprayed at 30 and 60 days after kalmegh was sown. Approximately 100 ml of solution were sprayed on each plant.

Chitosan was dissolved in glacial acetic acid to create a solution (Malekpoor*et al*., 2016), accordingly, 0.50 and 1.00 g of chitosan (500 and 1000 ppm) were combined with distilled water and swirled for 10 minutes. The mixture was then supplemented with 0.50 and 1.00 ml of glacial acetic acid and agitated for another two hours before being diluted to a volume of 1000 ml with distilled water. To obtain 500 and 1000 ppm yeast extract solution, 0.5 and 1 g of yeast extract powder were dissolved in some distilled water, then the volume was increased up to 1000 ml with distilled water. (Maqsood and Abdul, 2017). The different quantity of salicylic acid @ 0.1 and 0.2 g (100 and 200 ppm) were dissolved at first in small quantity of ethanol then distilled water was added for volume made up to one litre (Gorni and Pacheco, 2016; Singh *et al*., 2020).

At the Bidhan Chandra Krishi Viswavidyalaya's herbal garden in Mohanpur, Nadia, West Bengal, India, which is located at 23.5oN latitude and 89oE longitude and has an average elevation of 9.75 m above mean sea level, the investigation was conducted for two consecutive years in 2021 and 2022. The area is classified as subtropical humid. The average annual rainfall is 1500 mm, with summer months having an average temperature range of 25^{o}C to 36.5^{o}C and winter months having an average temperature range of 12^{o}C to 25^{o}C. The experiment's soil was organic Gangetic alluvial soil (Entisol), which had a sandy clay loam texture, good water holding capacity, was well-drained, and had a moderate level of soil fertility. A pot culture experiment with nine treatments and three replications was established using a completely randomised approach. CIM-Megha variety of kalmegh from CIMAP, Lucknow was used for this research.

A total of nine pots per treatment were maintained. 20 cm × 20 cm size plastic containers were used to plant the seeds during January and the crop was harvested during June in both the years. A basal dose of well rotten FYM @ 20 t ha^{-1} and 75:75:50 kg NPK ha^{-1} was incorporated into the soil, the ratio and proportion method wasused in computing the amount of fertilizers and FYM needed in pot experiment (Imakumbili, 2019). Observations on all parameters were recorded in all plants in each replication and the averages were computed. The International Rice Research Institute's Statistical Tool for Agricultural Research (STAR) software was used to pooled analysis on the mean data from two seasons. Each pair of means was compared using Duncan's multiple range test (DMRT) (Duncun, 1955). Utilising the

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Karl-Pearson (1948) formula, correlations were calculated. By comparing correlation coefficients with table values, the significance of correlation coefficients was examined (Fisher and Yates, 1963). The grouping of treatments was done by using UPGMA clustering method as given by Michener and Sokal (1957).

At harvest, the chlorophyll content of leaves from each plant from each replication was randomly selected, and an average was determined as per procedure given by Sadasivam and Manickam (1996). Carotenoid content (mg g^{-1}) was calculated using the formula given by Bajracharya (1996).The extraction and estimation of the andrographolides from the methanol extract of powdered kalmegh sample was done through HPLC at Floriculture and Medicinal Crops Division , ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka.The HPLC studies were completed using a Shimadzu Series LC-10A system (Shimadzu, Kyoto, Japan), which includes a liquid chromatography linked to a UV-VIS detector (10 A) and a binary pump, and the system was controlled by Shimadzu Class VP Workstation software. Gemini, 250 x 4.6 mm, 5 µm C18 (Phenomenex, USA) was the type of column used, and the security guard column was also made of the same material. Shimadzu model SIL-20 A HT autosampler was used to inject the samples. The thermostat was set to 32^0C for the column and guard column. The mobile phase contained phosphate buffer (solvent A) and acetonitrile (solvent B), and the flow rate was 1.5 ml/min. A linear gradient mode was used to operate the equipment. The gradient conditions were 0 to 18 min, 5 to 60 % B, and 18 to 25 min, 47 to 74 % B. At 223 nm the detection was monitored.

Results and Discussion

Plant growth agents are now being used more frequently to improve crop quality and yield. Plants naturally produce some organic molecules during stress conditions, which will help to combat stress by production of secondary metabolites. External application of some substances enhances the production of metabolites and these substances are known as elicitors. Some of the organic substances like chitosan, yeast extract, salicylic acid and jasmonic acid are being used in recent days by farmers in the name of elicitors for increasing growth and yield of crop along with quality. However, application of elicitors for enhancing secondary metabolites is a known factor but combined and full knowledge on effect of elicitors on overall development of plant with respect to growth, yield and quality at a time has rarely been studied. One of the most crucial ways to boost the yield and production of secondary metabolites in plants is by the application of elicitors. Elicitors are the chemical compounds that increase the formation of secondary metabolites in response to stress (Zhao et al., 2005). According to research by Poornananda and Jameel (2016), elicitors increase the bioaccumulation of andrographolide in kalmegh.

Growth Parameters

The growth parameters of pooled data *viz.*, height and spread of the plant, primary and secondary branches number per plant changed significantly when elicitors were used compare to control treatment (**Tables 1–5; Figure 1**). Chitosan @ 1000 ppm spray exhibited highest height of plant (73.91 cm) and secondary branches per plant (29.07) at harvest followed by salicylic acid @ 200 ppm spray (71.57 cm and 27.41) whereas, the lowest (56.93 and 23.35 cm) plant height and secondary branches was recorded for control. Plant spread was highest in salicylic acid @ 200 ppm spray (35.46 cm^2) which was *on par* with 1000 ppm chitosan (35.11 cm^2), primary branches number per plant was maximum in chitosan @ 1000 ppm spray (26.36) which was *on par* with 200 ppm salicylic acid (26.28) and control recorded minimum values both the parameters.

Table 1. Details of elicitors used for the experiment.

S.No.	Elicitors used	Specification	Procured from
1	Chitosan	Chitosan (Low MW) extrapure, 90% DA	Sisco Research Laboratories Pvt. Ltd., Kolkata
2	Yeast extract	Brownish yellow powder, Laboratory grade prepared from baker's yeast	
3	Salicylic acid	Salicylic Acid extrapure AR grade, 99.9%	
4	Jasmonic acid	Technical grade jasmonic acid powder 99% pure, for agriculture use	Aquatic Chemicals, Mumbai

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Table 2. Response of plant height to elicitors in kalmegh.

Treatments	Plant height (cm)								
	70 DAS			100 DAS			At harvest		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
T_1: Chitosan spray @ 500 ppm	36.95b^{c}	37.59^{bc}	37.27^{b}	56.84^{b}	57.64^{b}	57.24^{b}	70.66^{b}	70.53^{b}	70.60^{b}
T_2: Chitosan spray @ 1000 ppm	39.91^{a}	40.79^{a}	40.35^{a}	60.60^{a}	61.20^{a}	60.90^{a}	73.97^{a}	73.85^{a}	73.91^{a}
T_3: Yeast extract spray @ 500 ppm	35.55^{c}	35.27^{cd}	35.41^{d}	46.80^{d}	46.60^{d}	46.70^{e}	57.59^{e}	57.51^{ef}	57.55^{ef}
T_4: Yeast extract spray @ 1000 ppm	36.88^{bc}	37.52^{bc}	37.20^{bc}	54.24^{c}	54.54^{c}	54.39^{c}	67.41^{c}	67.75^{c}	67.58^{c}
T_5: Jasmonic acid spray @ 100 ppm	35.54^{c}	35.79^{cd}	35.66^{cd}	46.63^{d}	46.33^{d}	46.48^{e}	58.30^{de}	58.85^{de}	58.57^{e}
T_6: Jasmonic acid spray @ 200 ppm	35.37^{c}	35.09^{d}	35.23^{d}	46.48^{d}	46.28^{d}	46.38^{e}	57.26^{e}	57.18^{ef}	57.22^{f}
T_7: Salicylic acidspray @ 100 ppm	36.52^{c}	37.02^{bcd}	36.77^{bcd}	47.81^{d}	48.01^{d}	47.91^{d}	59.95^{d}	60.25^{d}	60.10^{d}
T_8: Salicylic acid spray @ 200 ppm	39.07^{ab}	39.32^{ab}	39.19^{a}	57.59^{b}	58.09^{b}	57.84^{b}	71.69^{b}	71.44^{b}	71.57^{b}
T_9: Control (Water spray)	35.30^{c}	35.02^{d}	35.16^{d}	46.30^{d}	46.10^{d}	46.20^{e}	56.97^{e}	56.89^{f}	56.93^{f}
S Em±	**0.72**	**0.75**	**0.51**	**0.58**	**0.58**	**0.41**	**0.59**	**0.59**	**0.41**
CD (0.05)	**2.14**	**2.15**	**1.46**	**1.74**	**1.74**	**1.18**	**1.75**	**1.75**	**1.20**
CV (%)	**3.41**	**3.38**	**3.39**	**1.97**	**1.97**	**1.97**	**1.61**	**1.60**	**1.60**

The treatments with same superscript, within each parameter, are not significantly different at $p \leq 0.05$, according to Duncan multiple comparison procedure (DMRT).

Table 3. Response of plant spread to elicitors in kalmegh.

Treatments	Plant spread (cm^2)								
	70 DAS			100 DAS			At harvest		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
T_1: Chitosan spray @ 500 ppm	15.72^{bc}	15.42^{c}	15.57^{c}	23.82^{b}	23.96^{c}	23.89^{c}	33.13^{b}	33.29^{b}	33.21^{b}
T_2: Chitosan spray @ 1000 ppm	17.19^{a}	17.42^{a}	17.31^{a}	26.76^{a}	27.38^{a}	27.07^{a}	35.66^{a}	34.56^{a}	35.11^{a}
T_3: Yeast extract spray @ 500 ppm	13.58^{ef}	13.06^{ef}	13.32^{g}	20.30^{d}	19.68^{f}	19.99^{f}	29.45^{de}	30.59^{c}	30.02^{c}
T_4: Yeast extract spray @ 1000 ppm	15.24^{c}	15.01^{c}	15.13^{d}	22.94^{c}	23.14^{d}	23.04^{d}	32.86^{bc}	32.89^{b}	32.88^{b}
T_5: Jasmonic acid spray @ 100 ppm	14.05^{de}	13.53^{e}	13.79^{f}	20.79^{d}	20.17^{f}	20.48^{f}	29.93^{d}	31.07^{c}	30.50^{c}
T_6: Jasmonic acid spray @ 200 ppm	13.43^{ef}	12.93^{ef}	13.18^{g}	19.16^{e}	18.56^{g}	18.86^{g}	28.39^{ef}	29.49^{d}	28.94^{d}
T_7: Salicylic acidspray @ 100 ppm	14.40^{d}	14.19^{d}	14.29^{e}	22.56^{c}	22.26^{e}	22.41^{e}	31.91^{c}	32.42^{b}	32.16^{b}
T_8: Salicylic acid spray @ 200 ppm	16.23^{b}	16.44^{b}	16.34^{b}	24.39^{b}	24.96^{b}	24.67^{b}	35.85^{a}	35.07^{a}	35.46^{a}
T_9: Control (Water spray)	13.18^{f}	12.68^{f}	12.93^{g}	18.92^{e}	18.32^{g}	18.62^{g}	27.76^{f}	28.86^{d}	28.31^{d}
S Em±	**0.21**	**0.21**	**0.15**	**0.21**	**0.21**	**0.15**	**0.36**	**0.36**	**0.25**
CD (0.05)	**0.64**	**0.64**	**0.43**	**0.64**	**0.64**	**0.43**	**1.06**	**1.06**	**0.72**
CV (%)	**2.52**	**2.57**	**2.55**	**1.68**	**1.69**	**1.69**	**1.97**	**1.94**	**1.95**

The treatments with same superscript, within each parameter, are not significantly different at $p \leq 0.05$, according to Duncan multiple comparison procedure (DMRT).

Table 4. Response of number of primary branches to elicitors in kalmegh.

Treatments	Primary branches (nos.)								
	70 DAS			100 DAS			At harvest		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
T_1: Chitosan spray @ 500 ppm	17.24^{b}	18.76^{b}	18.00^{b}	21.06^{c}	22.26^{b}	21.66^{c}	24.96^{b}	25.58^{a}	25.27^{b}
T_2: Chitosan spray @ 1000 ppm	18.11^{a}	19.81^{a}	18.96^{a}	23.26^{a}	23.33^{a}	23.30^{a}	26.14^{a}	26.58^{a}	26.36^{a}
T_3: Yeast extract spray @ 500 ppm	14.27^{e}	16.04^{e}	15.15^{e}	18.53^{f}	20.14^{e}	19.34^{f}	21.78^{e}	22.53^{cd}	22.16^{d}
T_4: Yeast extract spray @ 1000 ppm	16.20^{c}	18.28^{c}	17.24^{c}	19.91^{d}	21.25^{c}	20.58^{d}	23.69^{c}	24.12^{b}	23.91^{c}
T_5: Jasmonic acid spray @ 100 ppm	14.63^{de}	16.40^{de}	15.51^{de}	18.96e^{f}	20.57^{d}	19.77^{ef}	22.25^{de}	23.00^{bc}	22.63^{d}
T_6: Jasmonic acid spray @ 200 ppm	13.37^{f}	14.57^{f}	13.97^{f}	17.47^{g}	19.82^{e}	18.64^{g}	20.59^{f}	21.54^{d}	21.07^{e}
T_7: Salicylic acidspray @ 100 ppm	14.80^{d}	16.73^{d}	15.76^{d}	19.19^{e}	21.02^{c}	20.11^{e}	23.11^{cd}	22.51^{cd}	22.81^{d}
T_8: Salicylic acid spray @ 200 ppm	17.23^{b}	19.51^{a}	18.37^{a}	22.35^{b}	23.01^{a}	22.68^{b}	26.01^{a}	26.54^{a}	26.28^{a}
T_9: Control (Water spray)	13.02^{f}	14.22^{f}	13.62^{f}	16.50^{h}	18.85^{f}	17.67^{h}	20.35^{f}	21.29^{d}	20.82^{e}
S Em±	**0.16**	**0.16**	**0.11**	**0.17**	**0.14**	**0.11**	**0.31**	**0.40**	**0.25**
CD (0.05)	**0.49**	**0.47**	**0.33**	**0.50**	**0.41**	**0.31**	**0.93**	**1.20**	**0.73**
CV (%)	**1.88**	**1.62**	**1.74**	**1.51**	**1.16**	**1.33**	**2.35**	**2.95**	**2.67**

The treatments with same superscript, within each parameter, are not significantly different at $p \leq 0.05$, according to Duncan multiple comparison procedure (DMRT).

Table 5. Response of number of secondary branches to elicitors in kalmegh.

Treatments	**Secondary branches (nos.)**								
	70 DAS			**100 DAS**			**At harvest**		
	2021	**2022**	**Pooled**	**2021**	**2022**	**Pooled**	**2021**	**2022**	**Pooled**
T$_1$: Chitosan spray @ 500 ppm	11.42^{b}	12.86^{ab}	12.14^{b}	22.11^{b}	23.79^{b}	22.95^{bc}	25.43^{b}	28.55^{b}	26.99^{b}
T$_2$: Chitosan spray @ 1000 ppm	12.57^{a}	13.65^{a}	13.11^{a}	24.10^{a}	25.41^{a}	24.76^{a}	27.98^{a}	30.16^{a}	29.07^{a}
T$_3$: Yeast extract spray @ 500 ppm	8.67^{de}	10.21^{e}	9.44^{de}	18.93^{de}	20.93^{de}	19.93^{ef}	22.49^{ef}	26.25^{e}	24.37^{e}
T$_4$: Yeast extract spray @ 1000 ppm	10.87^{b}	12.34^{bc}	11.60^{b}	21.47^{bc}	23.18^{bc}	22.32^{c}	24.58^{c}	27.76^{c}	26.17^{c}
T$_5$: Jasmonic acid spray @ 100 ppm	9.13^{d}	10.67^{de}	9.90^{d}	19.37^{de}	21.37^{de}	20.37^{de}	23.03^{de}	26.79^{de}	24.91^{de}
T$_6$: Jasmonic acid spray @ 200 ppm	8.44^{e}	9.65^{ef}	9.05^{ef}	21.57^{bc}	23.25^{bc}	22.41^{c}	21.90^{fg}	25.39^{f}	23.65^{f}
T$_7$: Salicylic acidspray @ 100 ppm	9.74^{c}	11.41^{cd}	10.57^{c}	20.16^{cd}	22.07^{cd}	21.11^{d}	23.34^{d}	26.92^{d}	25.13^{d}
T$_8$: Salicylic acid spray @ 200 ppm	12.48^{a}	13.91^{a}	13.20^{a}	22.86^{ab}	24.21^{ab}	23.53^{b}	26.02^{b}	28.80^{b}	27.41^{b}
T$_9$: Control (Water spray)	7.86^{f}	9.06^{f}	8.46^{f}	17.89^{e}	20.00^{e}	18.95^{f}	21.61^{g}	25.10^{f}	23.35^{f}
S Em±	**0.19**	**0.36**	**0.20**	**0.48**	**0.49**	**0.34**	**0.23**	**0.19**	**0.15**
CD (0.05)	**0.56**	**1.08**	**0.59**	**1.44**	**1.46**	**0.99**	**0.70**	**0.57**	**0.43**
CV (%)	**3.27**	**5.49**	**4.67**	**4.01**	**3.77**	**3.88**	**1.71**	**1.22**	**1.46**

The treatments with same superscript, within each parameter, are not significantly different at $p \le 0.05$, according to Duncan multiple comparison procedure (DMRT).

Table 6. Response of leaf length, leaf width and leaf area to elicitors in kalmegh.

Treatments	**Leaf parameters at harvest**								
	Leaf length (cm)			**Leaf width (cm)**			**Leaf area (cm^2)**		
	2021	**2022**	**Pooled**	**2021**	**2022**	**Pooled**	**2021**	**2022**	**Pooled**
T$_1$: Chitosan spray @ 500 ppm	5.06^{a}	5.14^{a}	5.10^{a}	1.35^{e}	1.35^{f}	1.35^{de}	6.09^{a}	6.20^{a}	6.15^{c}
T$_2$: Chitosan spray @ 1000 ppm	5.17^{a}	5.48^{a}	5.32^{a}	1.34^{e}	1.30^{g}	1.32^{e}	6.20^{a}	6.42^{a}	6.31^{bc}
T$_3$: Yeast extract spray @ 500 ppm	5.59^{a}	5.40^{a}	5.50^{a}	1.52^{b}	1.53^{b}	1.52^{b}	7.68^{a}	7.44^{a}	7.56^{ab}
T$_4$: Yeast extract spray @ 1000 ppm	5.24^{a}	5.27^{a}	5.25^{a}	1.44^{c}	1.45^{de}	1.44^{c}	6.76^{a}	6.85^{a}	6.80^{ab}
T$_5$: Jasmonic acid spray @ 100 ppm	5.39^{a}	5.22^{a}	5.31^{a}	1.45^{c}	1.48^{cd}	1.46^{c}	7.03^{a}	6.92^{a}	6.98^{ab}
T$_6$: Jasmonic acid spray @ 200 ppm	5.41^{a}	5.33^{a}	5.37^{a}	1.49^{b}	1.51^{bc}	1.50^{b}	7.25^{a}	7.23^{a}	7.24^{ab}
T$_7$: Salicylic acidspray @ 100 ppm	5.23^{a}	5.24^{a}	5.23^{a}	1.43^{c}	1.43^{e}	1.43^{c}	6.71^{a}	6.72^{a}	6.71^{bc}
T$_8$: Salicylic acid spray @ 200 ppm	5.11^{a}	5.18^{a}	5.14^{a}	1.39^{d}	1.36^{f}	1.37^{d}	6.35^{a}	6.31^{a}	6.33^{bc}
T$_9$: Control (Water spray)	5.42^{a}	5.13^{a}	5.28^{a}	1.61^{a}	1.65^{a}	1.63^{a}	7.86^{a}	7.57^{a}	7.71^{a}
S Em±	**0.28**	**0.34**	**0.22**	**0.01**	**0.01**	**0.01**	**0.44**	**0.50**	**0.33**
CD (0.05)	**0.85**	**1.02**	**0.64**	**0.03**	**0.03**	**0.02**	**1.31**	**1.49**	**0.95**
CV (%)	**0.30**	**1.10**	**0.80**	**1.65**	**1.73**	**1.69**	**17.12**	**19.10**	**18.14**

The treatments with same superscript, within each parameter, are not significantly different at $p \le 0.05$, according to Duncan multiple comparison procedure (DMRT).

The findings of the elicitor treatments made it clear that there is a substantial correlation between the plant height and the number of primary branches, which is then correlated with the number of secondary branches and plant spread. Treatments were seen to have an acceptable number of branches and plant spread when they reached their maximum height at maturity. This can be explained by the fact that the taller plants could bear more number of branching nodes and were able to show a high plant spread. Increased availability and uptake of water and vital nutrients through adjusting cell osmotic pressure and reducing the accumulation of harmful free radicals by increasing antioxidant and enzyme activities are likely the causes of chitosan's stimulatory effect on plant growth (Nithin, 2020). Additionally, it might be credited to improved nitrogen transfer to functional leaves, greater photosynthesis, and increased enzymatic nitrogen metabolism activities (nitrate reductase, glutamine synthetase, and protease), all of which facilitated plant growth and development (Mondal *et al*., 2012). Additionally, Chitosan boosted pathways for auxin production via a route tryptophan independent pathway may have increased plant height (Dhakad, 2019). The results obtained from the study are in concurrence with results of Gornik *et al.* (2008), Abdel-Mawgoud *et al.* (2010), Yin *et al.* (2012), Sultana *et al.* (2015), Ahmed *et al.* (2016) and Malekpoor *et al.* (2016).

The longer and more numerous branches that resulted in a tilting outward, which boosted plant spread, may be responsible for the increased plant spread in the chitosan spray treatment. Chitosan increases nitrogen transport in the functioning leaves of kalmegh plants by enhancing the enzyme activities of nitrogen metabolism (nitrate reductase,

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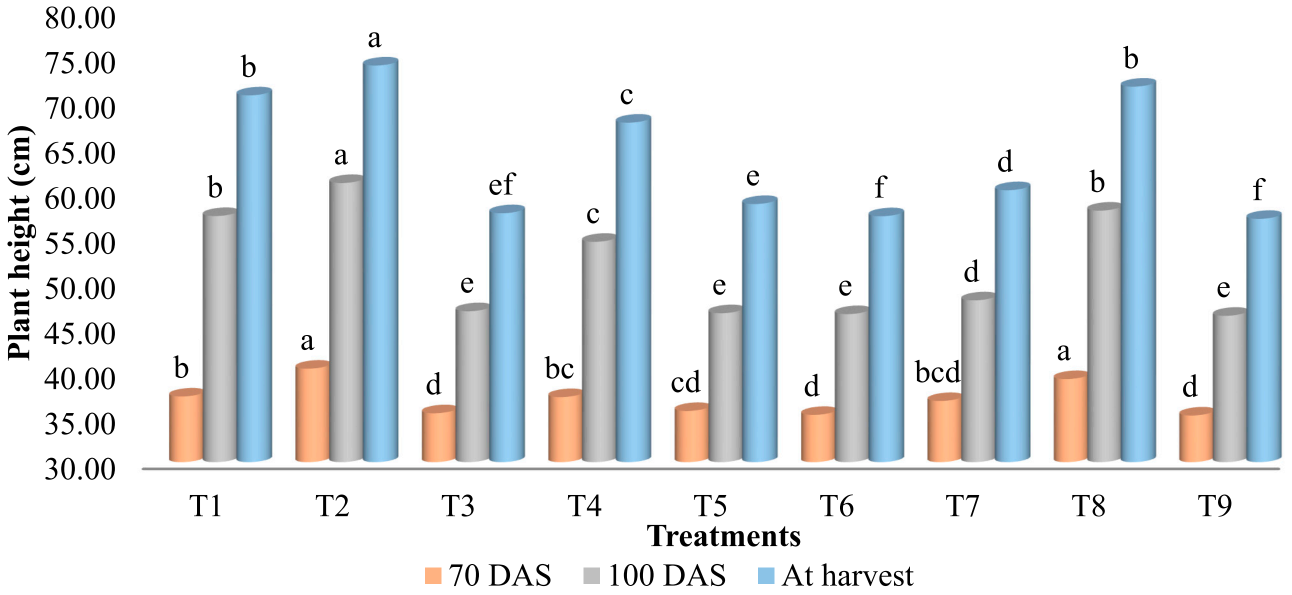
glutamine synthetase, and protease) (Mondal *et al.*,2012). The findings of Gornik*et al*. (2008), Abdel-Mawgoud *et al*. (2010), Pirbalouti *et al*. (2017), and Kra *et al*. (2019) are in agreement with these findings.



T$_1$:	Chitosan spray @ 500 ppm	**T$_2$;**	Chitosan spray @ 1000 ppm
T$_3$:	Yeast Extract spray @ 500 ppm	**T$_4$:**	Yeast Extract spray @ 1000 ppm
T$_5$:	Jasmonic acid spray @ 100 ppm	**T$_6$:**	Jasmonic acid spray @ 200 ppm
T$_7$:	Salicylic acid spray @ 100 ppm	**T$_8$:**	Salicylic acid spray @ 200 ppm
T$_9$:	Control (water spray)		

Figure 1. Elicitors effect on plant height of kalmegh.

Salicylic acid also has a significant impact on the morphology and physiology of the Kalmegh plant. The use of salicylic acid in the current investigation may have increased the photosynthetic activities of the kalmegh plant. These outcomes are consistent with researches of Hashemabadi and Zarchini, Farouk and Osman (2011), Jadhav and Bhamburdekar (2012) and Sharma (2012).Salicylic acid increased the chlorophyll content, which resulted into large quantity of photosynthesis and increased plant growth (Kazemi, 2014)and also due to increased length of internodes more number of internodes which directly increased the height of plant, branches number (Sharma, 2012).Ali and Mahmoud (2013), Rahimi *et al*. (2013) and Mulgir *et al*. (2014) also reported similar results.Increased plant spread by salicylic acid application may be due to higher vegetative growth, chlorophyll content, and branches number as well as root growth, which could result in increased plant spread. The results also support from earlier works of Asgari and Moghadam (2015), Kumar *et al.* (2015), Abd-Elkader (2016), Koppad *et al.* (2017), Nangare (2017) and Sathiyamurthy *et al.* (2017).

Pooled analysis of two years data of single leaf parameters like its length, width and area of leaf showed that control treatment with highest values (4.53 cm, 1.35 cm and 6.04 cm^2) whereas least length and width was recorded by chitosan 1000 ppm spray which was *on par* with 500 ppm chitosan and 200 ppm salicylic acid. In case of leaf area, all the elicitor's treatments recorded *on par* results in both the years.Leaf parameters of plants showed significant difference with elicitor treatment (**Table 7; Figure 2**). Leaves number per plant was highest in chitosan spray @ 1000 ppm (88.32) which was *on par* with chitosan @ 500 ppm (85.59) and salicylic acid @ 200 ppm (81.51). Leaf area of plant and leaf area index recorded maximum value in chitosan @ 1000 ppm (552.59 cm^2 and 1.38) which was *on par* with chitosan @ 500 ppm (521.92 cm^2 and 1.30). The control treatment recorded least values for above three parameters.

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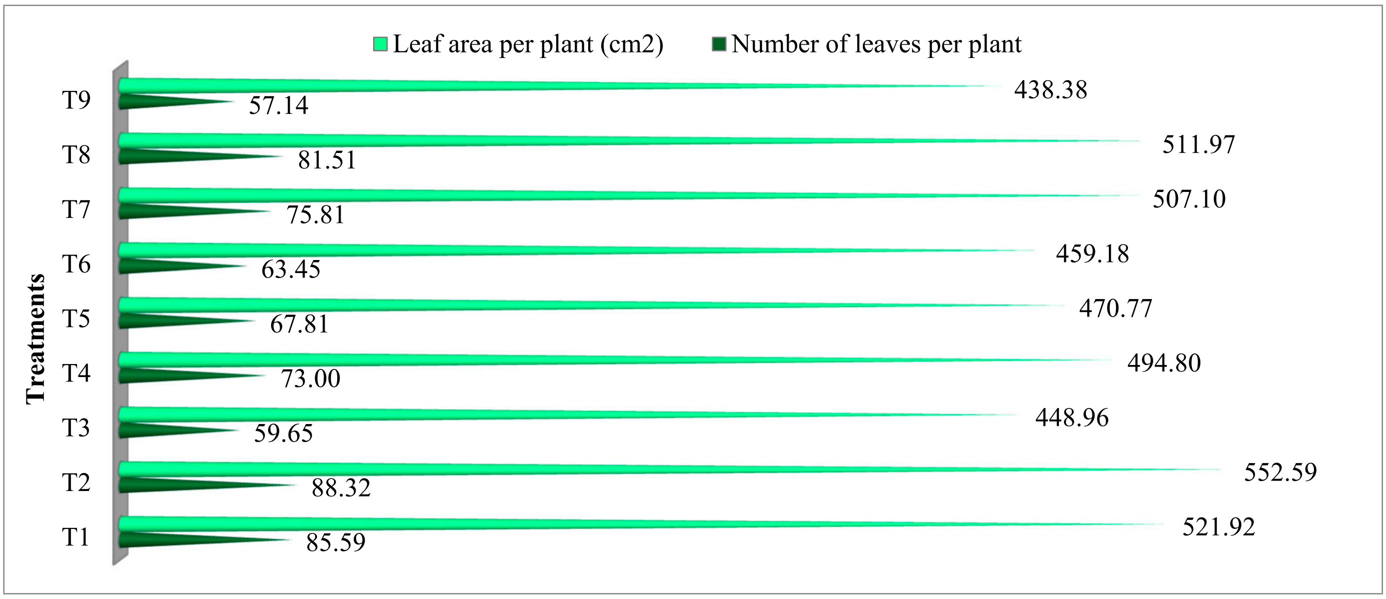
Table 7. Response of number of leaves plant^{-1}, leaf area plant^{-1} and leaf area index to elicitors in kalmegh.

Treatments	Leaf parameters at harvest								
	Number of leaves plant^{-1}			Leaf area plant^{-1}(cm^2)			Leaf area index		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
T_1: Chitosan spray @ 500 ppm	85.59^{a}	85.59^{ab}	85.59^{a}	519.32^{ab}	524.52^{ab}	521.92^{ab}	1.30^{ab}	1.31^{ab}	1.30^{ab}
T_2: Chitosan spray @ 1000 ppm	88.32^{a}	88.32^{a}	88.32^{a}	545.18^{a}	560.00^{a}	552.59^{a}	1.36^{a}	1.40^{a}	1.38^{a}
T_3: Yeast extract spray @ 500 ppm	58.37^{e}	60.92^{de}	59.65^{f}	445.01^{cd}	452.91^{cd}	448.96^{e}	1.11^{cd}	1.13^{cd}	1.12^{e}
T_4: Yeast extract spray @ 1000 ppm	72.75^{bc}	73.25^{bcd}	73.00^{cd}	491.73^{abcd}	497.87^{bcd}	494.80^{bcd}	1.23^{abcd}	1.24^{bcd}	1.24^{bcd}
T_5:Jasmonic acid spray @ 100 ppm	66.96^{cd}	68.66^{cde}	67.81^{de}	469.71^{bcd}	471.83^{bcd}	470.77^{cde}	1.17^{bcd}	1.18^{bcd}	1.18^{cde}
T_6:Jasmonic acid spray @ 200 ppm	63.08^{de}	63.82^{cde}	63.45^{ef}	457.10^{bcd}	461.27^{cd}	459.18^{de}	1.14^{bcd}	1.15^{cd}	1.15^{de}
T_7: Salicylic acidspray @ 100 ppm	75.64^{b}	75.99^{abc}	75.81^{bc}	506.97^{abc}	507.24^{abc}	507.10b^{c}	1.27^{abc}	1.27^{abc}	1.27^{bc}
T_8: Salicylic acid spray @ 200 ppm	80.29^{ab}	82.74^{ab}	81.51^{ab}	508.97^{ab}	514.96^{abc}	511.97^{bc}	1.27^{ab}	1.29^{abc}	1.28^{bc}
T_9: Control (Water spray)	55.87^{e}	58.42^{e}	57.14^{f}	434.88^{d}	441.88^{d}	438.38^{e}	1.09^{d}	1.10^{d}	1.10^{e}
S Em±	**2.61**	**3.91**	**2.35**	**19.04**	**18.90**	**13.42**	**0.50**	**0.05**	**0.03**
CD (0.05)	**7.77**	**11.63**	**6.75**	**56.59**	**56.17**	**38.49**	**1.14**	**0.14**	**0.09**
CV (%)	**6.31**	**9.28**	**7.96**	**6.78**	**6.65**	**6.72**	**6.75**	**6.65**	**6.70**

The treatments with same superscript, within each parameter, are not significantly different at $p \le 0.05$, according to Duncan multiple comparison procedure (DMRT)



T_1:	Chitosan spray @ 500 ppm	**T_2;**	Chitosan spray @ 1000 ppm
T_3:	Yeast Extract spray @ 500 ppm	**T_4:**	Yeast Extract spray @ 1000 ppm
T_5:	Jasmonic acid spray @ 100 ppm	**T_6:**	Jasmonic acid spray @ 200 ppm
T_7:	Salicylic acid spray @ 100 ppm	**T_8:**	Salicylic acid spray @ 200 ppm
T_9:	Control (water spray)		

Figure 2. Elicitors effect on leaf parameters of kalmegh.

In this study, the leaves number was in higher rank in those treatments having prime position in branches number and, in turn, leaves number appeared to have governed the total leaf area of plant. The treatments with more leaves per plant had produced more dry substance simultaneously. This is perhaps due to their higher photo-assimilation capacity

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due to maximum green area of individual plants actively synthesising carbohydrates through photosynthesis. The probable increase in leaves number per plant is owing to increased nutrient absorption. The foliar application of chitosan stimulate molecular signals which served as plant growth promoter that induced higher rate of cell division and cell elongation in sub apical meristems of kalmegh shoots increasing the amount of leaves that each plant produces (Ahmed, 2015). Mondal *et al.* (2012) and Abdel-Mawgoud *et al.* (2010) also obtained the similar results. The likely cause of the increase in leaf area per plant can be linked to an increase in epidermal parenchyma cells and an increase in the number of functional leaves. Furthermore, foliar application of chitosan improved cell osmotic pressure, which improved water availability and nutrient uptake (Mondal *et al.*, 2016) and nitrogen transfer in functional leaves promoted photosynthesis, which in turn improved plant growth and development and increased the leaf area naturally (Mondal *et al.*, 2012). These outcomes are consistent with the work of Gornik *et al.* (2008) and Abdel-Mawgoud *et al.* (2010), Xu and Mou (2018), Thengumpally (2019) and Ashwini (2020).

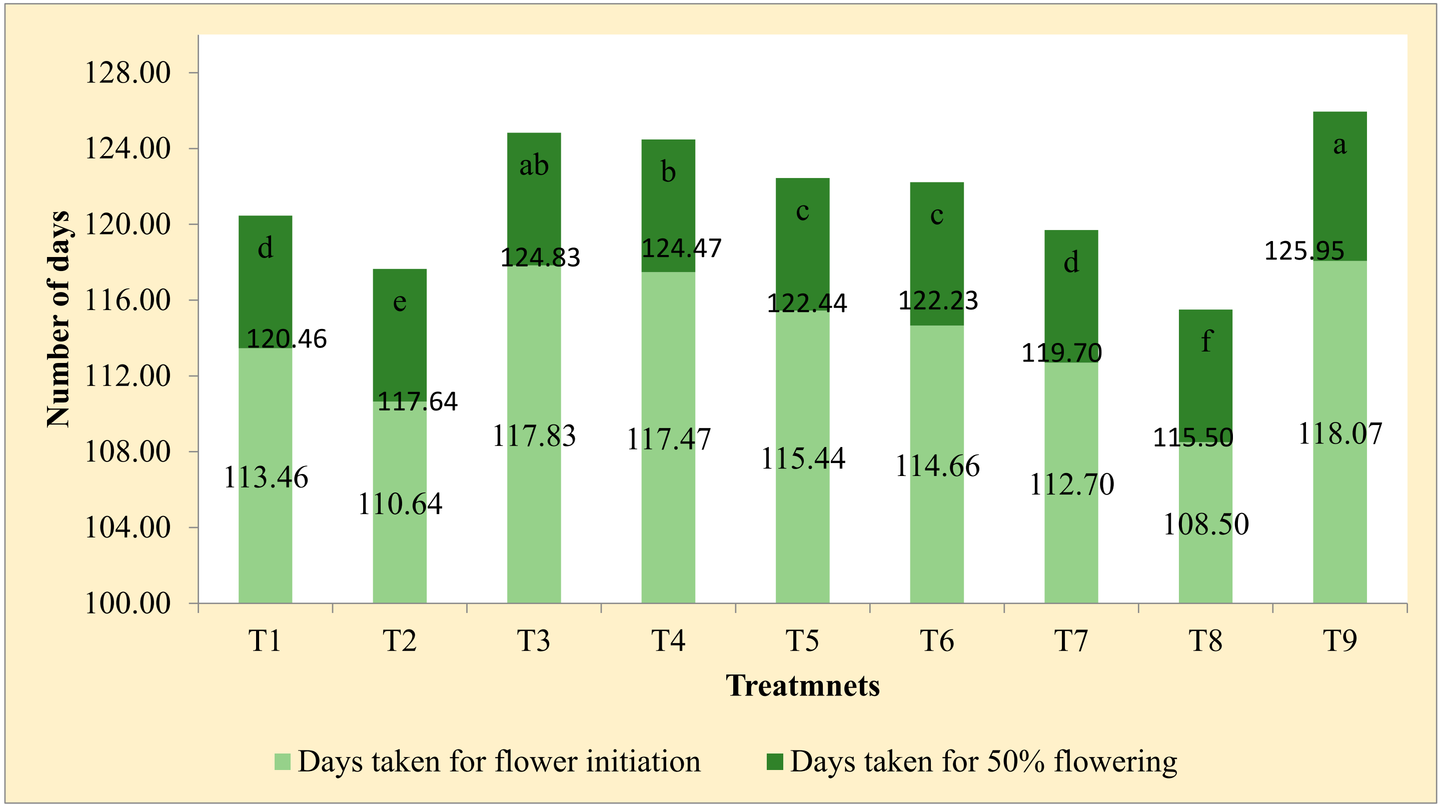
Salicylic acid administration enhanced the number of leaves because the number of leaves positively associated with the number of nodes and primary branches per plant (Bhasker *et al.*, 2020). Andrey *et al.* (2012), Mona *et al.* (2012), Ram *et al.* (2012), Anwar *et al.* (2014), Padmalatha *et al.* (2014), Mahammad (2016) and Manoj (2017) found similar result. The increased number of leaves per plant in Kalmegh as a result of the spraying of salicylic acid @ 200 ppm may be attributable to the peridined division and expansion of the central cell in the leaf axis that would be made possible by the salicylic acid's morphactin-like properties, as a result the number of leaves per plant in Kalmegh might also increase. Meena *et al.* (2016), Koppad *et al.* (2017), Sathiyamurthy *et al.* (2017) and Vitthal (2018) reported similar results.

In pooled analysis, plants sprayed with salicylic acid @ 200 ppm followed by chitosan @ 1000 ppm came for early flowering and harvest (**Table 8; Figure 3**). Maximum days for flower initiation and harvest were taken by control treatment, which was *on par* with yeast extract elicitation. It might be due to salicylic acid promotes flowers, sexual development which results enhancement in flower production (Kumar and Reddy, 2008). It may be also due to induction of early flowering through transition from vegetative to reproductive growth. Maniram *et al.* (2012) and Ram *et al.* (2012) have confirmed this. Salicylic acid's florigenic activity, or more likely its effect on the ratio between flower-promoting and flower-inhibiting components, boosted the synthesis of floral stimuli in an inductive cycle (Padmalatha *et al.*, 2014). Several researchers have reported that salicylic acid causes flower induction in annual crops (Narayanan *et al.*, 2015, Qureshi *et al.*, 2015). Choudhary *et al.* (2016) concluded that salicylic acid induced flowering by acting as floral stimulus in leaves, which may regulate flowering. Salicylic acid serves as an internal growth regulator for flowering and the florigenic response (Pawar *et al.*, 2018).

Table 8. Response of days taken for flower initiation and fifty per cent flowering of kalmegh to elicitors.

Treatments	**Days taken for flower initiation and harvest**					
	Days taken for flower initiation			**Days taken for harvest**		
	2021	**2022**	**Pooled**	**2021**	**2022**	**Pooled**
T1: Chitosan spray @ 500 ppm	113.21[d]	113.71[c]	113.46[c]	120.21[d]	120.71[cd]	120.46[d]
T2: Chitosan spray @ 1000 ppm	110.39[e]	110.89[d]	110.64[d]	117.39[e]	117.89[e]	117.64[e]
T3: Yeast extract spray @ 500 ppm	117.47[a]	118.19[a]	117.83[a]	124.47[b]	125.19[a]	124.83[ab]
T4: Yeast extract spray @ 1000 ppm	117.09[a]	117.86[a]	117.47[a]	124.09[b]	124.86[a]	124.47[b]
T5: Jasmonic acid spray @ 100 ppm	115.40[b]	115.49[b]	115.44[b]	122.40[c]	122.49[b]	122.44[c]
T6: Jasmonic acid spray @ 200 ppm	114.38[c]	114.94[b]	114.66[b]	122.33[c]	122.12b[c]	122.23[c]
T7: Salicylic acidspray @ 100 ppm	112.36[d]	113.04[c]	112.70[c]	119.36[d]	120.04[d]	119.70[d]
T8: Salicylic acid spray @ 200 ppm	108.29[f]	108.71[e]	108.50[e]	115.29[f]	115.71[f]	115.50[f]
T9: Control (Water spray)	117.71[a]	118.43[a]	118.07[a]	125.92[a]	125.97[a]	125.95[a]
S Em±	**0.31**	**0.32**	**0.22**	**0.31**	**0.53**	**0.31**
CD (0.05)	**0.93**	**0.96**	**0.64**	**0.93**	**1.59**	**0.89**
CV (%)	**0.48**	**0.48**	**0.48**	**0.45**	**0.76**	**0.62**

The treatments with same superscript, within each parameter, are not significantly different at $p \leq 0.05$, according to Duncan multiple comparison procedure (DMRT).



T1: Chitosan spray @ 500 ppm
T2; Chitosan spray @ 1000 ppm
T3: Yeast Extract spray @ 500 ppm
T4: Yeast Extract spray @ 1000 ppm
T5: Jasmonic acid spray @ 100 ppm
T6: Jasmonic acid spray @ 200 ppm
T7: Salicylic acid spray @ 100 ppm
T8: Salicylic acid spray @ 200 ppm
T9: Control (water spray)

Figure 3. Elicitors effect on days to flower initiation and fifty per cent flowering of kalmegh.

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From this study, it was noticed that chitosan treated plants starts flowering early as compared to other, which indicates that chitosan stimulates the nutrient absorption and regulates the plant signaling pathways that helped to induce early flowering in kalmegh. While, greater number of days were needed for the first flower bud to appear on water-sprayed plants because of hormonal imbalance that promote vegetative growth only instead of flowering. These outcomes are consistent with those of Farouk and Amany (2012), Salachna and Zawadzinska (2014), Mutka *et al.* (2017), Dhakad (2019) and Nithin (2020). The highest chlorophyll concentration was found in the plants sprayed with salicylic acid and chitosan, which led to greater generation of photosynthates and their accumulation. Additionally, the stimulatory effects of salicylic acid and chitosan on absorption of nutrients may aid in timely nutrient supply with simple uptake throughout overall plant growth and result in early maturity by flowering.

Yield Parameters

The application of elicitors resulted in an increasing trend for the yield parameters, including fresh and dry herbage yield and dry matter content per plant, as compared to the control (**Table 9**). For both the years and the pooled study, chitosan and salicylic acid sprays produced results that were similar in terms of yield characteristics. The yield data for salicylic acid at 200 ppm, chitosan at 1000 ppm, salicylic acid at 100 ppm, and salicylic acid at 500 ppm were reported on par, but significantly better than those for other elicitors treatment and control. Any agricultural plant's production is influenced by the assimilatory surface of the plant system. A reliable source with regard to plant height, LAI, number of branches and leaves is logically capable of increasing the dry matter, and its distribution in various regions is crucial for determining the crop's overall output. In case the treatments are able to perform similarly for holding moisture and dry matter content, they show more or less similar trend in both dry and fresh weight of whole plant. Those treatments having maximum leaf area could exhibit greater values of fresh as well as dry weight of whole plant. Similarly, in the present study, the fresh weight and dry weight values among the treatments are ranked similarly or on par.

Table 9. Response of fresh and dry herbage yield per plant and dry matter content per plant in kalmegh to elicitors.

Treatments	**Yield parameters at harvest**								
	Fresh herbage yield plant^{-1} (g)			**Dry herbage yield plant^{-1} (g)**			**Dry matter content plant^{-1} (%)**		
	2021	**2022**	**Pooled**	**2021**	**2022**	**Pooled**	**2021**	**2022**	**Pooled**
T$_1$: Chitosan spray @ 500 ppm	35.35^{b}	39.58^{abc}	37.47^{abc}	13.64^{b}	16.36^{abc}	15.00^{ab}	38.56^{b}	41.13^{ab}	39.84^{ab}
T$_2$: Chitosan spray @ 1000 ppm	38.74^{a}	42.08^{ab}	40.41^{ab}	16.25^{a}	18.59^{ab}	17.42^{a}	41.93^{a}	44.06^{a}	43.00^{a}
T$_3$: Yeast extract spray @ 500 ppm	30.75^{d}	34.84^{bcd}	32.79^{cd}	11.13^{c}	13.60^{bcd}	12.36^{bc}	36.17^{c}	38.83^{bc}	37.50^{b}
T$_4$: Yeast extract spray @ 1000 ppm	33.75^{bc}	38.17^{abc}	35.96^{bcd}	12.29^{c}	14.90^{abc}	13.59^{bc}	36.40^{c}	38.84^{bc}	37.62^{b}
T$_5$: Jasmonic acid spray @ 100 ppm	32.84^{cd}	36.93^{abc}	34.89^{bcd}	11.96^{c}	14.52^{abcd}	13.24^{bc}	36.40^{c}	39.11^{b}	37.75^{b}
T$_6$: Jasmonic acid spray @ 200 ppm	26.91^{e}	32.83^{cd}	29.87^{de}	9.54^{d}	12.52^{cd}	11.03^{cd}	35.44^{c}	37.84^{bc}	36.64^{b}
T$_7$: Salicylic acidspray @ 100 ppm	35.65^{b}	39.68^{abc}	37.66^{abc}	13.76^{b}	16.35^{abc}	15.05^{ab}	38.58^{b}	41.04^{ab}	39.81^{ab}
T$_8$: Salicylic acid spray @ 200 ppm	40.99^{a}	43.68^{a}	42.34^{a}	17.22^{a}	19.37^{a}	18.30^{a}	42.01^{a}	44.25^{a}	43.13^{a}
T$_9$: Control (Water spray)	22.33^{f}	28.25^{d}	25.29^{e}	6.92^{e}	9.76^{d}	8.34^{d}	30.93^{d}	34.11^{c}	32.52^{c}
S Em±	**0.80**	**2.59**	**1.32**	**0.43**	**1.52**	**0.79**	**0.42**	**1.49**	**0.77**
CD (0.05)	**2.38**	**7.48**	**3.79**	**1.28**	**4.52**	**2.26**	**1.26**	**4.44**	**2.2**
CV (%)	**4.21**	**11.68**	**9.20**	**5.98**	**17.44**	**14.02**	**1.96**	**6.50**	**4.93**

The treatments with same superscript, within each parameter, are not significantly different at $p \leq 0.05$, according to Duncan multiple comparison procedure (DMRT)

Yield is a composite trait, governed by polygenes, and associated with several other traits, which contribute in increments. In kalmegh, it is well established that yield is related to improvement in height of plant, leaf area, primary and secondary branches (Jadhav and Bhamburdekar, 2012). Salicylic acid and chitosan were found to considerably improve plant height, spread, number of leaves, branches and leaf area per plant in the current study. Similar to this, during harvest, treatments with salicylic acid and chitosan applications dramatically increased the generation of photosynthates, boosting biomass output and dry matter content accumulation. Salicylic acid and chitosan treatment resulted in an overall increase in growth and yield qualities, which led to better fresh and dried herbage yields (Manoj, 2017).

The plant's ability to regenerate itself is boosted by salicylic acid's inhibition of ethylene synthesis, which may have contributed to the enhanced herbage yield (Pawar *et al.*, 2018). It is generally known that SA promotes cell elongation

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and cell division (Ahmed *et al*., 2013).According to reports, SA can boost other yield in many plant species by improving plant growth factors including height, number of branches, leaves, and leaf area per plant. (Gharib, 2007, Sayyari*et al*., 2013, Mohsen *et al*., 2014, Karimian *et al*., 2015, Pradhan *et al*., 2016, Koppad *et al*., 2017 and Sathiyamurthy*et al*., 2017). Besides, foliar spray of SA and chitosan helped to activate the signalling pathways of growth regulating substances like gibberellins and auxins which might also contributed to increased bio mass of plants by increasing number and size of cells (Supriya, 2015, Gorni and Pacheco, 2016, Youssef *et al*., 2017, Vitthal, 2018, Forouzandeh *et al*., 2019 and Nithin, 2020). The stimulation of physiological processes, improved vegetative growth, active translocation of photoassimilates from source to sink tissues, increased number of leaves and branches per plant, and accumulation of dry matter may all be contributing factors to the increase in yield per plant in the treatment receiving chitosan spray. While, frequent foliar application of water creates moisture stress, which affects the physiological processes of plants, including uptake and translocation of nutrients results in hormonal imbalance, which accounts for reduction of yield per plant in water sprayed treatment. The current findings are consistent with those of Asghari-Zakaria *et al*. (2009), Ghoname*et al*. (2010) and Chookhongkha*et al*. (2013).

The increased plant total dry weight was linked to the maximum vegetative growth characteristics, including a rise in plant height, leaf count, leaf area, and plant spread. This resulted in production and accumulation of a greater amount of photosynthates in plants leading to increased bio mass accumulation, which in turn yields higher dry matter content. Increments in dry matter by chitosan application were in line with Ahmed *et al*. (2016), Bistgani *et al*. (2017), Thengumpally (2019) and Ashwini (2020). Jayalakshmi *et al*. (2010), Bekheta and Iman (2009), Mahammad (2016), Bhasker *et al*. (2020), Mohammadi *et al*. (2020) and Priya (2021) compiled the results on dry matter and their interdependence by salicylic acid.

Quality Parameters

Chlorophyll pigments in leaf varied significantly with elicitor application in kalmegh (**Table 10**). Pooled analysis of two years data showed that highest chlorophyll a, b and total (2.529, 1.909 and 4.459 mg g^{-1}) in salicylic acid 200 ppm spray and the least recorded by control. Carotenoid pigment in leaf was highest in salicylic acid 200 ppm spray (3.674 mg g^{-1}) which was *on par* with 100 ppm SA (3.574 mg g^{-1}) and the control recorded minimum. Maximum chlorophyll content in leaves was found when 200 ppm of salicylic acid was sprayed over the leaves.These outcomes may be explained by regulating the plant water, which carries nutrient components, particularly nitrogen and phosphorus, which increased the overall chlorophyll concentration. (Maity and Bera, 2009) Similar finding was also reported by Bayat*et al*. (2012), Divya*et al*. (2014) and Choudhary *et al*. (2016).

Table 10. Response of chlorophyll a, b and total chlorophyll content in kalmegh to elicitors.

Treatments	Chlorophyll contents (mg g^{-1})								
	Chlorophyll a			Chlorophyll b			Total chlorophyll		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
T_1: Chitosan spray @ 500 ppm	2.004^{e}	2.271^{e}	2.138^{e}	1.490^{e}	1.710^{e}	1.600^{e}	3.510^{e}	3.997^{e}	3.753^{e}
T_2: Chitosan spray @ 1000 ppm	2.051^{d}	2.381^{d}	2.216^{d}	1.542^{d}	1.772^{d}	1.657^{d}	3.611^{d}	4.171^{d}	3.891^{d}
T_3: Yeast extract spray @ 500 ppm	1.725^{h}	1.975^{h}	1.850^{h}	1.275^{h}	1.474^{h}	1.375^{h}	3.013^{h}	3.463^{h}	3.23h^{8}
T_4: Yeast extract spray @ 1000 ppm	1.838^{g}	2.148^{g}	1.993^{g}	1.325^{g}	1.483^{g}	1.404^{g}	3.178^{g}	3.646^{g}	3.412^{g}
T_5: Jasmonic acid spray @ 100 ppm	2.072^{c}	2.407^{c}	2.240^{c}	1.570^{c}	1.805^{c}	1.687^{c}	3.660^{c}	4.230^{c}	3.945^{c}
T_6: Jasmonic acid spray @ 200 ppm	1.950^{f}	2.233^{f}	2.092^{f}	1.438^{f}	1.648^{f}	1.543^{f}	3.403^{f}	3.896^{f}	3.649^{f}
T_7: Salicylic acidspray @ 100 ppm	2.194^{b}	2.554^{b}	2.374^{b}	1.651^{b}	1.911^{b}	1.781^{b}	3.864^{b}	4.484^{b}	4.174^{b}
T_8: Salicylic acid spray @ 200 ppm	2.344^{a}	2.714^{a}	2.529^{a}	1.774^{a}	2.044^{a}	1.909^{a}	4.139^{a}	4.779^{a}	4.459^{a}
T_9: Control (Water spray)	1.604i	1.811^{i}	1.707^{i}	1.171^{i}	1.404^{i}	1.288^{i}	2.779^{i}	3.243^{i}	3.011^{i}
S Em±	**0.001**	**0.002**	**0.001**	**0.001**	**0.001**	**0.001**	**0.005**	**0.002**	**0.003**
CD (0.05)	**0.003**	**0.006**	**0.003**	**0.003**	**0.002**	**0.003**	**0.014**	**0.007**	**0.008**
CV (%)	**0.080**	**0.151**	**0.130**	**0.109**	**0.082**	**0.095**	**0.241**	**0.105**	**0.173**

The treatments with same superscript, within each parameter, are not significantly different at $p \leq 0.05$, according to Duncan multiple comparison procedure (DMRT)

Chlorophyll and carotenoid levels rose after SA applications in plants, which was attributed to the hormone's beneficial effects on nutrient uptake (Kaydan *et al*., 2007). This is because higher levels of Fe, Mg, and Ca can stimulate the biosynthesis of chlorophylls (Kong *et al*., 2014). Additionally, SA's stimulatory influence on the activity of the

Rubisco enzyme and photosynthesis may be the cause of the rise in photosynthetic pigments (Kaur *et al*., 2015). A decrease in stomatal and non-stomatal transpiration, as well as an increase in chlorophyll concentration, can all be attributed to exogenous salicylic acid application on leaf surfaces, which reduces abiotic stressors and improves water usage efficiency. Salicylic acid applied topically to leaves helps to stimulate the enzymes required for the production of chlorophyll in biological processes. These outcomes are in line with those of Mahammad (2016), Pradhan *et al.* (2016), Kirtikumar (2018) and Gothwal (2021).

Quantification of secondary metabolites exhibited varied trends in highest and lowest values of carotene and andrographolides content by different treatments (**Table 11; Figure 4**). Pooled analysis of total andrographolides content in herb showed that salicylic acid 200 ppm spray with highest value (3.494%) this was far more effective than any other treatments. The least value recorded by control (2.263%).The enhanced overall plant growth and metabolism identified in the present investigation may be the cause of the beneficial effect of foliar spray of elicitors on alkaloid synthesis. Through improved plant growth, photosynthesis, and general plant metabolism in the current study, it appears that the elicitor may have enhanced the intrinsic genetic ability of the kalmegh plants to produce an additional yield with improved alkaloid quality via biochemical pathways. Elicitor had the ability to increase metabolic activity, which in turn caused the accumulation of secondary metabolites in kalmegh plants by terpenoid synthesis. According to Rivas-San and Plasencia (2011), metabolic alterations at the chloroplast level (Rubisco enzyme activity, photosystem II efficiency and supply of ATP and NADPH for the carbon reduction cycle) can be attributed to the observed increase in photosynthesis rate in plants sprayed with SA. The stimulatory effect of SA on gas exchange parameters and plant development, however, depends on a number of variables, including the application method, exposure duration, and ontogenetic stage (Pacheco *et al*., 2013).

Table 11. Response of carotenoids and andrographolide contents in kalmegh to elicitors.

Treatments	Quality parameters in kalmegh								
	Carotenoid content (mg g^{-1})			Andrographolide content (%) (A)			Neoandrographolide content (%) (B)		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
T$_1$: Chitosan spray @ 500 ppm	2.806^{c}	3.126^{b}	2.966^{b}	2.477^{d}	2.537^{d}	2.507^{d}	0.364^{c}	0.393^{cd}	0.379^{c}
T$_2$: Chitosan spray @ 1000 ppm	2.858^{c}	3.188^{b}	3.023^{b}	2.646^{c}	2.707^{c}	2.676^{c}	0.338^{c}	0.367^{d}	0.352^{c}
T$_3$: Yeast extract spray @ 500 ppm	2.577^{d}	2.807^{c}	2.692^{c}	2.026^{g}	2.086^{g}	2.056^{g}	0.368^{c}	0.397^{c}	0.382^{c}
T$_4$: Yeast extract spray @ 1000 ppm	2.594^{d}	2.844^{c}	2.719^{c}	2.268^{e}	2.328^{e}	2.298^{e}	0.348^{c}	0.377^{cd}	0.363^{c}
T$_5$:Jasmonic acid spray @ 100 ppm	3.294^{b}	3.629^{a}	3.462^{a}	2.866^{b}	2.926^{b}	2.896^{b}	0.211^{d}	0.240^{e}	0.225^{d}
T$_6$:Jasmonic acid spray @ 200 ppm	2.666^{d}	2.976^{bc}	2.821^{bc}	2.105^{f}	2.165^{f}	2.135^{f}	0.566^{a}	0.595^{a}	0.580^{a}
T$_7$: Salicylic acidspray @ 100 ppm	3.394^{ab}	3.754^{a}	3.574^{a}	2.688^{c}	2.748^{c}	2.718^{c}	0.400^{b}	0.429^{b}	0.414^{b}
T$_8$: Salicylic acid spray @ 200 ppm	3.489^{a}	3.859^{a}	3.674^{a}	2.936^{a}	2.997^{a}	2.966^{a}	0.221^{d}	0.250^{e}	0.236^{d}
T$_9$: Control (Water spray)	2.140^{e}	2.527^{d}	2.334^{d}	1.834^{h}	1.896^{h}	1.865^{h}	0.236^{d}	0.266^{e}	0.251^{d}
S Em±	**0.034**	**0.085**	**0.046**	**0.019**	**0.018**	**0.013**	**0.010**	**0.009**	**0.007**
CD (0.05)	**0.100**	**0.251**	**0.131**	**0.057**	**0.052**	**0.037**	**0.029**	**0.026**	**0.019**
CV (%)	**2.041**	**4.604**	**3.682**	**1.372**	**1.222**	**1.301**	**4.918**	**4.134**	**4.516**

Treatments	Andrographolides content (%)								
	14-Deoxy-11,12-didehydro Andrographolide content(C)			Andrograpanin content (%) (D)			Total Andrographolide content (%) (A+B+C+D)		
	2021	2022	Pooled	2021	2022	Pooled	2021	2022	Pooled
T$_1$: Chitosan spray @ 500 ppm	0.155^{b}	0.182^{b}	0.169^{b}	0.020^{cd}	0.030^{c}	0.025^{c}	3.016^{cd}	3.142^{cd}	3.079^{d}
T$_2$: Chitosan spray @ 1000 ppm	0.105^{c}	0.132^{c}	0.119^{c}	0.015^{cd}	0.024^{cd}	0.019^{cd}	3.104^{bc}	3.230^{bc}	3.167^{cd}
T$_3$: Yeast extract spray @ 500 ppm	0.054^{d}	0.081^{d}	0.068^{d}	0.014^{cd}	0.024^{cd}	0.019^{cd}	2.462^{f}	2.588^{f}	2.525^{g}
T$_4$: Yeast extract spray @ 1000 ppm	0.041^{d}	0.068^{d}	0.055^{d}	0.016^{cd}	0.025^{cd}	0.021^{cd}	2.673^{e}	2.799^{e}	2.736^{f}
T$_5$:Jasmonic acid spray @ 100 ppm	0.105^{c}	0.132^{c}	0.119^{c}	0.016^{cd}	0.025^{cd}	0.020^{cd}	3.197^{bc}	3.323^{b}	3.260^{bc}
T$_6$:Jasmonic acid spray @ 200 ppm	0.166^{d}	0.193^{b}	0.179^{b}	0.031^{b}	0.040^{b}	0.035^{b}	2.867^{d}	2.993^{d}	2.930^{e}
T$_7$: Salicylic acidspray @ 100 ppm	0.119^{c}	0.145^{c}	0.132^{c}	0.021^{c}	0.030^{c}	0.026^{c}	3.227^{b}	3.352^{b}	3.290^{b}
T$_8$: Salicylic acid spray @ 200 ppm	0.233^{a}	0.260^{a}	0.246^{a}	0.041^{a}	0.051^{a}	0.046^{a}	3.432^{a}	3.557^{a}	3.494^{a}
T$_9$: Control (Water spray)	0.120^{c}	0.146^{c}	0.133^{c}	0.010^{d}	0.019^{d}	0.015^{d}	2.199^{g}	2.328^{g}	2.263^{h}
S Em±	**0.006**	**0.006**	**0.004**	**0.003**	**0.003**	**0.002**	**0.038**	**0.035**	**0.026**
CD (0.05)	**0.019**	**0.017**	**0.012**	**0.009**	**0.009**	**0.006**	**0.114**	**0.105**	**0.074**
CV (%)	**9.075**	**6.844**	**7.854**	**26.345**	**16.996**	**20.806**	**2.282**	**2.001**	**2.141**

The treatments with same superscript, within each parameter, are not significantly different at $p \leq 0.05$, according to Duncan multiple comparison procedure (DMRT). Total andrographolide content=andrographolides+neoandrographolides+14-Deoxy-11,12-didehydro Andrographolide+Andrograpanin.

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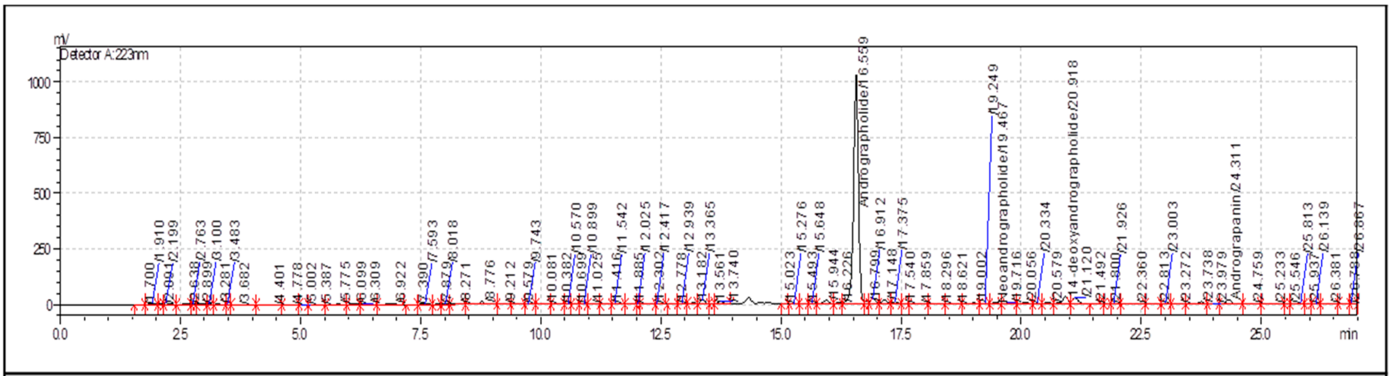
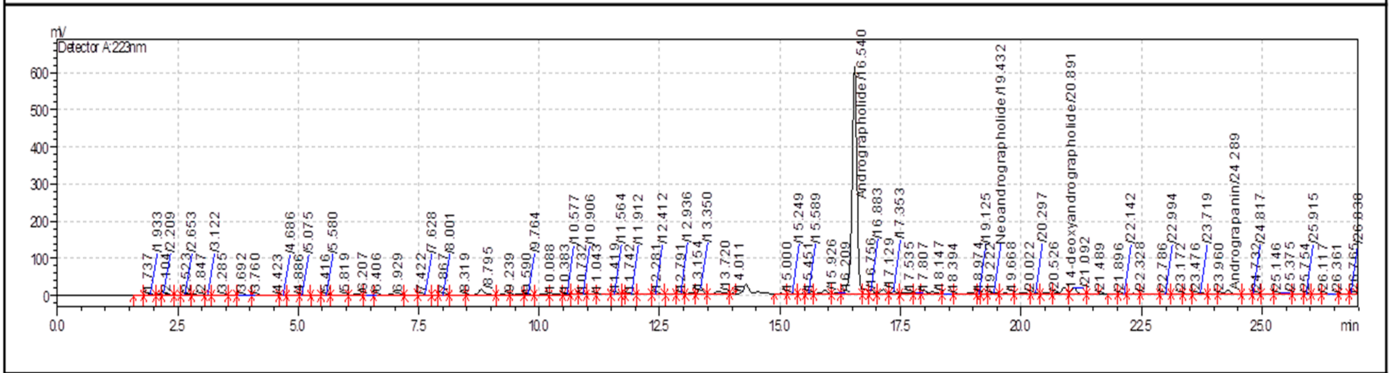


Chromatogram showing highest andrographolides content in T_8; Salicylic acid spray @ 200 ppm



Chromatogram showing lowest andrographolides content in T_9; Control (water spray)

Figure 4. Chromatograms showing highest and lowest andrographolides among elicitor treatments in kalmegh

The andrographolide is a diterpenoid compound. The terpenoid biosynthesis occurs through mevalonate isoprenoid pathway in glands present in the leaves. The improvement in leaf and herbage development had increased the terpenoid yield. In fact, the trichomes in leaves are the major site for biosynthesis of andrographolide alkaloid. Terpenoid biosynthesis is an integration of several steps such as continuous production of precursors, their transport and translocation to active sites for alkaloid biosynthesis. The metabolic pathway for biosynthesis of alkaloid had improved in plants in response to elicitor application especially with salicylic acid.Increased endogenous salicylic acid (SA) levels can activate cell-signaling pathways, which control the expression of genes encoding enzymes connected to the phenylpropanoid pathway (which produces flavonoids) and the mevalonate system (which produces terpinoids).IPP synthase and DMAPP synthase activity, enzymes that diverge from the MVA and MEP pathway to generate terpinoids in plants, are two examples of enzymes whose activity is improved by SA. (Ghasemzadeh and Jaafar 2012, Tounekti *et al.,* 2013).In *Salvia miltiorhiza* and *Michelia chapensis*, it has been demonstrated that SA transcriptionally upregulates the genes involved in isoprenoid production (Cao *et al*., 2011, Yan *et al*., 2015). It has also been demonstrated that SA increases the expression of three prenyl transferases from the primary pathway for the biosynthesis of terpenoids in several species (Shabani *et al.,* 2009 in liquorice and Kai *et al.,* 2010 in *Arabidopsis*). Similar to how SA levels rise during times of drought or salt stress, numerous isoprenoids have also been demonstrated to be upregulated. Despite the fact that the full mechanism of SA in plants is still not fully known, everyone agrees on the crucial role that SA plays in plants (Cappellari *et al*., 2019).

Character association between yield, quality and its component traits in elicitor treatments and UPGMA based grouping of treatments

Correlation analysis among different traits in elicitor treatments (**Table 12**) revealed that fresh herbage yield of plant having highly significant positive association with plant's height (0.80), spread (0.95), primary (0.91) and secondary branches (0.87), leaves number (0.88), leaf area (0.87), leaf area index (0.87), dry herb yield(0.99) and dry

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matter content (0.97) and positive significant association with total chlorophyll (0.81) and total andrographolide contents (0.82). Similar positive association was reported by Jadhav and Bhamburdekar (2012), Sayyari *et al.* (2013), Mohsen *et al.* (2014), Karimian *et al.* (2015), Pradhan *et al.* (2016), Koppad *et al.* (2017), Manoj (2017) and Pawar *et al,* (2018). Plant's dry herb yield exhibited highly significant positive correlation with plant's height (0.82), spread (0.96), primary (0.92) and secondary branches (0.89), leaves number (0.89), leaf area (0.88), leaf area index (0.88), fresh herb yield (0.99), dry matter content (0.97), total chlorophyll (0.81) and total andrographolide content (0.82). Similar results were also reported by Ghoname *et al*. (2010), Chookhongkha *et al.* (2013), Supriya (2015), Gorni and Pacheco (2016), Youssef *et al.* (2017), Vitthal (2018), Forouzandeh *et al*. (2019) and Nithin (2020).Total andrographolides content in kalmegh plant in elicitor treatments showed positive and significant association with plant's leaves number (0.72), leaf area (0.69) and leaf area index (0.69). Positive and highly significant association was found with plant's fresh (0.82) and dry herb yields (0.82), dry matter content (0.82) and total chlorophyll content (0.98). Manoj (2017) recorded the similar positive relation between growth, yield and quality components by elicitor treatment in turmeric and Nithin (2020) in strawberry.

Table 12. Correlation matrix of important parameters of kalmegh (Elicitor treatments).

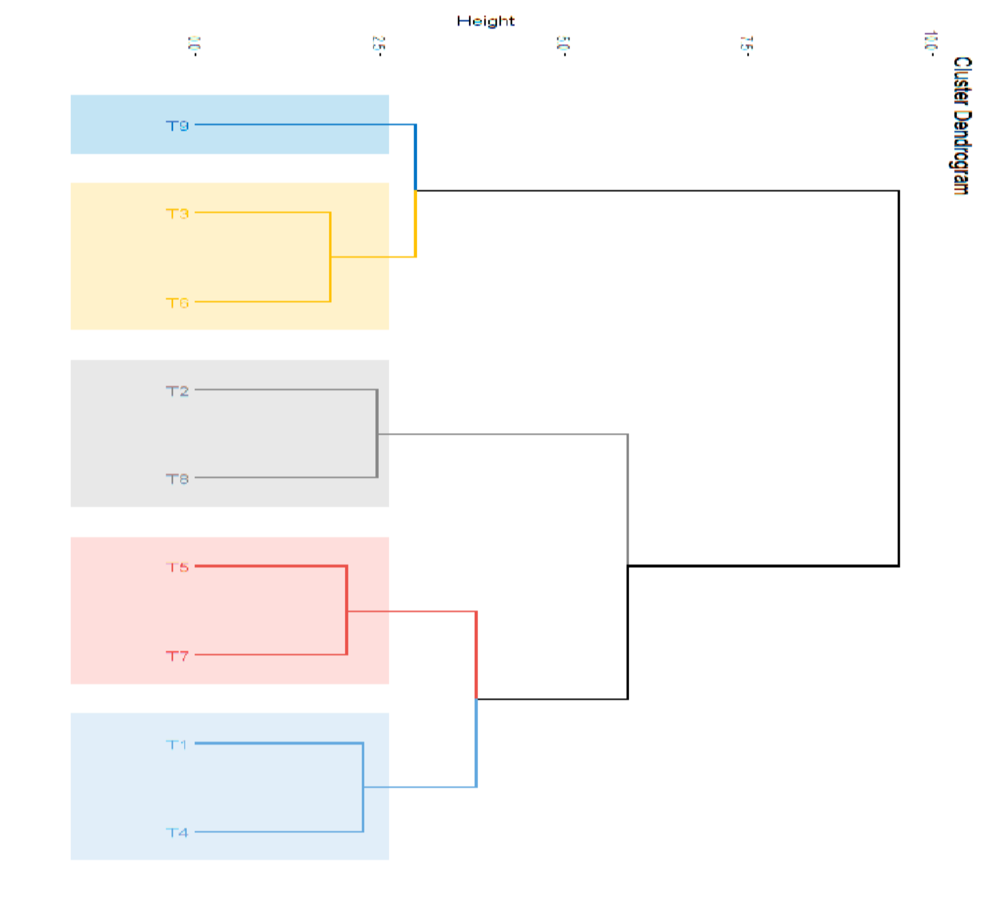
	PH	PS	NPB	NSB	NL	LAP	LAI	FRY	DRY	DMAT	CHLO	ANDR
PH												
PS	0.94**											
NPB	0.97**	0.98**										
NSB	0.96**	0.95**	0.98**									
NL	0.91**	0.92**	0.93**	0.93**								
LAP	0.89**	0.92**	0.90**	0.94**	0.98**							
LAI	0.89**	0.91**	0.90**	0.94**	0.98**	0.99**						
FRY	0.80**	0.95**	0.91**	0.87**	0.88**	0.87**	0.87**					
DRY	0.82**	0.96**	0.92**	0.89**	0.89**	0.88**	0.88**	0.99**				
DMAT	0.79*	0.92**	0.89**	0.87**	0.87**	0.87**	0.87**	0.97**	0.99**			
CHLO	0.47	0.68*	0.61	0.54	0.67*	0.64*	0.65*	0.81**	0.82**	0.81**		
ANDR	0.50	0.68*	0.63	0.59	0.72*	0.69*	0.69*	0.82**	0.82**	0.82**	0.98**	

PH	: Plant height	NL	: Number of leaves	DRY	: Dry herbage yield
PS	: Plant spread	LAP	: Leaf area per plant	DMAT	: Dry matter content
NPB	: Number of primary branches	LAI	: Leaf area index	CHLO	: Total chlorophyll
NSB	: Number of secondary branches	FRY	: Fresh herbage yield	ANDR	: Total andrographolides

*: Significant at 5 % level of significance; **; Significant at 1% level of significance

According to a correlation study of elicitor treatment parameters, plant height, number of primary and secondary branches, leaves number, leaf area per plant, LAI, dry matter content per plant, total chlorophyll content in leaves and total andrographolides content in plants were all positively and significantly correlated with the fresh and dry yield of herb. With the exception of plant height and the branches number, the total andrographolides content in the kalmegh plant was likewise found to be related to the same factors, regardless of level of significance. Therefore, it can be inferred that elicitors achieved improvement in herbage yield and quality of kalmegh by improving above characters in kalmegh production.

Clustering pattern of elicitor treatments based on UPGMA clustering method employing twelve important characters showed five clusters (**Figure 5**). Cluster I consist of chitosan @ 500 ppm and yeast extract @ 1000 ppm spray, cluster II contained chitosan @ 1000 ppm and salicylic acid @ 200 ppm spray. Cluster III with yeast extract @ 500 ppm and jasmonic acid @ 200 ppm spray. Treatments100 ppmjasmonic acid and 100 ppm salicylic acid spray belongs to cluster IV. Control (water spray) treatment falls in cluster V. Treatments belongs to same clusters have relatively same or *on par* effects on yield and quality of kalmegh.



Clustering pattern of elicitor treatments based on UPGMA clustering method		
Clusters	**No. of treatments**	**Treatments**
I	**2**	Chitosan spray @ 500 ppm Yeast Extract spray @ 1000 ppm
II	**2**	Chitosan spray @ 1000 ppm Salicylic acid spray @ 200 ppm
III	**2**	Yeast Extract spray @ 500 ppm Jasmonic acid spray @ 200 ppm
IV	**2**	Jasmonic acid spray @ 100 ppm Salicylic acid spray @ 100 ppm
V	1	Control (water spray)

Where,
T_1: Chitosan spray @ 500 ppm
T_2; Chitosan spray @ 1000 ppm
T_3: Yeast Extract spray @ 500 ppm
T_4: Yeast Extract spray @ 1000 ppm
T_5: Jasmonic acid spray @ 100 ppm
T_6: Jasmonic acid spray @ 200 ppm
T_7: Salicylic acid spray @ 100 ppm
T_8: Salicylic acid spray @ 200 ppm
T_9: Control (water spray)

Figure 5. Grouping of nine elicitor treatments in clusters presented in the form of Dendrogram.

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Significant and positive improvement in growth, yield and quality of kalmegh was noticed with chitosan and salicylic acid spraying. 200 ppm salicylic acid spray at 30 and 60 DAS on kalmegh exhibited best results in quantity and quality production of kalmegh signifying its use in cultivation of kalmegh for high productivity, quality and better returns for farmers and processors. The effect of elicitors as foliar spray on improving the kalmegh quality is being reported for the first time. This novel and useful finding needs further understanding of how, when and where exactly these chemicals elicit accumulation of andrographolides. As concentration and frequency of elicitor application significantly affects various characters under study, still different other concentrations may be tried depending on method of application like seed treatment, seedling dip *etc*. on kalmegh.

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