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Improving andrographolide content in kalmegh by integrated nutrient management

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Abstract
Kalmegh is widely used in Indian system of medicine as a "blood purifier" and recommended for use in cases of leprosy, boils, skin eruptions, and chronic and seasonal fevers. Juice or an infusion of fresh leaves is given to infants to relieve griping, irregular bowel habits, and in case of loss of appetite Kalmegh was very useful. The active principal of Kalmegh, andrographolide, has highly bitter taste and is colorless crystalline in appearance. The andrographolide contents are very sensitive to both seasonal and regional variations. In order to offer better package of practices with higher andrographolide content to the farmers, the present investigation on integrated nutrient management was performed. The experiment was laid out in split plot design with the three replication. Treatments consisted of seven organic fertilizer sources were taken as main plot treatment and seven chemical fertilizer sources as sub plot treatments. The three years pooled data revealed that application of vermicompost @ 7.5 t ha^{-1} recorded significantly higher andrographolide yield per hectare followed by application of vermicompost @ 7.5 t ha^{-1}, FYM @ 15 t ha^{-1} and Castor cake 2.5 t ha $^{-1}$. However, these treatments are at par with each other. And Application of Vermicompost @ 7.5 t ha^{-1} recorded significantly higher herbage yield kg ha^{-1}, Dry foliage yield per hectare.

Keywords: Kalmegh, organic manure, herbage yield, andrographolide

1. Introduction
Andrographis paniculata has been reported as having antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic, adaptogenic, anti-inflammatory, emollient, astringent, diuretic, carminative, anthelmintic, antipyretic, gastric and liver tonic. Due to its blood purifying activity it is recommended for use in cases of leprosy, gonorrhea, scabies, boils, skin eruptions, and chronic and seasonal fevers. Juice or an infusion of fresh leaves is given to infants to relieve griping, irregular bowel habits, and loss of appetite. Leaves and root are also used in general debility, during convalescence after fevers, for dyspepsia associated with gaseous distension, and in advanced stages. Reported significant choleretic effects of Andrographolide in rats and guinea pigs [1]. Protection by andrographolide against acetaminophen-induced reduction in volume and contents of bile was better than that produced by silymarin. Multiple-dose pretreatment with arabinogalactan proteins and andrographolide was protective against ethanol induced hepatotoxicity in mice and was deemed comparable to the efficacy of silymarin. The oral preand post-treatment of adult rats with an extract of *Andrographis paniculata* was found protective against ethanol-induced increase in serum transaminase [2]. It has been demonstrated to have potential antimicrobial and antiphrastic activities. It is also reported that the significant antibacterial activity of an aqueous extract is attributed to the combined effect of Andrographolide and arabinogalactan proteins [3].
The characteristic secondary metabolites encountered in the plant have considerably enhanced its importance in the arena of medicinal plants and medicines. It is specifically rated very high in therapeutic action in curing liver disorders and common cough and cold in humans. A number of diterpenoids and diterpenoid glycosides of similar carbon skeleton have been isolated from and rographis, mainly the bitterest compounds among them are andrographolides, neo-andrographolide, deoxy Andrographolide. The leaves of Andrographis contain the highest amount of Andrographolide (2.39%), the most medicinally active phytochemical in the plant, while the seeds contain the lowest [5]. Andrographolide, a colorless and crystalline bitter tasting molecule contains a "lactone function". Andrographolide was firstly isolated in pure form by Gorterin 1911 [6]. The formation of diterpine lactons is strongly influenced by seasonal and regional variation. The concentration of the active components is found highest just before the plant blooms, making early fall the best time to harvest. In those

parts of Asia where Kalmegh is sold commercially as medicine, a variety of lab level methodologies are used to ensure a standardized level of andrographolides such as thin-layer chromatography, ultraviolet spectrophotometry and liquid chromatography. Solvent extraction method using ethanol as solvent is usually employed for extraction of andrographolides.

Material and Method

The experiment was conducted at Nagarjun Medicinal Plant Garden Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (MS). The experiment was laid out in split plot design with the three replication. Treatments consisted of seven organic fertilizer sources as main plot treatment and seven chemical fertilizer sources as sub plot treatments. The crop was subjected to recommended package of agronomic practices to obtain a healthy crop. The net plot is converted in to quintal per hectare by using hectare factor. The three years pooled data is discussed in the present paper.

Treatment details

It includes seven organic treatments viz. M_1- Control, M_2- FYM 10 t ha^{-1},M_3- FYM 15 t ha^{-1}, M_4- Vermicompost 5 t ha^{-1},M_5- Vermicompost 7.5 t ha^{-1}, M_6- Castor cake 1.5 t ha^{-1},M_7- Castor cake 2.5 t ha^{-1} and seven chemical fertilizer treatments viz. T_1- Control, T_2- 40:10:30 Kg/ha NPK,T_3- 60:20:40 Kg/ha NPK,T_4- 80:30:50 Kg/ha NPK,T_5- 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half of N at 25 DAS), T_6- 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half N in two equal splits at 25 and 40 DAS), T_7- 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half N in three equal splits at 25, 40 & 60 DAS). Yield recorded during the investigation and data analyzed statically.

Estimation of Andrographolide content

This method is based on the condensation of butenolide ring of andrographolides with picric acid [7]. Andrographolide gives a red- orange colored product with picric acid in alkaline medium and absorbance maxima (λmax) at 481nm. The picric acid and NaOH reagent mixture was made by using ratio of Picric acid: NaOH (80:20). Pure drug (i.e. standard) of Andrographolide was supplied by SPIC pharmaceuticals.

For preparation of linearity curve a stock solution (100µg/mL) of andrographolide was prepared by dissolving 10 mg of drug in 100mL of methanol. From the above solution (0.5 to 5 ml) was transferred into a 10mL volumetric flask, to this flask 5mL of picric acid and NaOH reagent mixture was added. Finally the volume was made up to the mark with methanol. The flask were vortexed and kept aside. The absorbance of the colored chromogen was measured at 481 nm against reagent blank after 20 min. The blank was prepared in same manner without adding a sample. The absorbance values were plotted against the concentration of respective andrographolide to get a linearity curve.

Kalmegh samples were made by drying and crushing aerial parts of *A. paniculata*. 50 mg of fine kalmegh powder was weighed accurately, transferred to a 50 mL volumetric flaskand30mL of methanol was added. The mixture was Ultra sonicated for 1.30 hours for dissolving of andrographolide. Then the sample solution was filtered and diluted further to 50 ml with methanol. To 3mL of this solution, 5mL of picric acid and NaOH reagent mixture was added. Finally the volume was made up to the mark with methanol. The flask was kept aside for 20min. The absorbance of the colored chromogen was measured by spectrophotometer at 481 nm against reagent blank after 20 min. The amount of andrographolide in the sample was calculated from the calibration curve [8].

Result and Discussion

Herbage yield, Andrographolide yield (Kg/ha)

Application of Vermicompost @ 7.5 t ha^{-1} recorded significantly higher herbage yield kg ha^{-1} and dry foliage yield per hectare. Application of vermicompost @ 5 t ha^{-1} was found to be at par with application of vermicompost @ 7.5 t $ha^{-1}$and application of castor cake 2.5 t ha^{-1}. The application of 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half N in three equal splits at 25, 40 & 60 DAS) (T_7) recorded significantly higher herbage yield per hectare. However, Dry Foliage yield was found significantly superior with application of 80:30:50kg ha^{-1}NPK (Half N with full P & K as basal and remaining half N in two equal splits at 25 and 40 DAS) (T6).

Application of vermicompost @ 7.5 t ha^{-1} recorded significantly higher andrographolide yield per hectare followed by application of vermicompost @ 7.5 t ha^{-1}, FYM @ 15 t ha^{-1} and Castor cake 2.5 t ha^{-1}. However, these treatments were at par with each other. Application of 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half N in two equal splits at 25, 40 & 60 DAS) (T_6) recorded significantly higher andrographolide yield per hectare followed by application of 80:30:50 kg per hectare NPK (Half N with full P & K as basal and remaining half N in three equal splits at 25 and 40 DAS) (T_7) and treatments T_5 80:30:50kg per hectare NPK (Half N with full P & K as basal and remaining half N in three equal splits at 25) However, these treatments are at par with each other. The data on pooled mean (Table-1) revealed that interaction effect of manure and fertilizer application produced significantly highest andrographolide yield/ha. The data on pooled mean (Table-2) revealed that the interaction M_5T_6 of Manure application vermicompost 5 t ha^{-1} along with 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half N in two equal splits at 25, 40 & 60 DAS chemical fertilizer application dose and time produced significantly highest Herbage yield than other treatment combinations; however, it was at par with M_5T_6.

Table 1: Andrographolide content (%) and yield (kg/ha) of Kalmegh as influenced different organic and chemical fertilizers treatments

Treatments	Andrographoloide content (%)				Andrographoloide Yield (kg/ha)			
	2015-16	2016-17	2017-18	Pooled Mean	2015-16	2016-17	2017-18	Pooled Mean
Main plot Treatments: Organic manures								
M1- Control	1.75	1.771	1.59	1.70	22.64	38.26	55.14	44.04
M2- FYM 10 t ha-1	1.76	1.824	1.87	1.82	24.91	48.32	64.92	51.32
M3- FYM 15 t ha-1	1.77	1.895	1.86	1.84	26.69	55.46	64.58	55.99
M_4- Vermicompost 5.0 t ha^{-1}	1.78	1.832	1.87	1.83	26.67	52.17	65.09	57.24
M_5- Vermicompost 7.5 t ha^{-1}	1.79	1.897	1.84	1.84	27.66	56.88	63.99	56.67
M_6- Castor cake 1.5 t ha^{-1}	1.76	1.860	1.77	1.80	24.41	47.87	61.43	54.67
M_7- Castor cake 2.5 t ha^{-1}	1.77	1.816	1.84	1.81	25.82	49.72	63.87	55.49

SE (m) ±	0.02	0.010	0.01	0.01	0.64	0.92	50.37	0.889
CD (P=0.05)	NS	0.029	0.04	0.02	1.97	2.84	155.21	2.738
Sub plot Treatments: Chemical fertilizers								
T_1- Control	1.73	1.801	1.92	1.82	20.15	43.49	66.74	48.72
T_2- 40:10:30 Kg/ha NPK	1.74	1.816	1.91	1.82	23.55	45.63	66.22	51.72
T_3- 60:20:40 Kg/ha NPK	1.76	1.830	1.74	1.78	25.02	47.54	60.35	51.03
T_4- 80:30:50 Kg/ha NPK	1.78	1.844	1.57	1.73	26.70	49.52	54.35	50.77
T_5- T_5- 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half of N at 25 DAS)	1.79	1.854	1.76	1.80	27.34	52.05	61.14	56.21
T_6- T_6- 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half N in two equal splits at 25 and 40 DAS)	1.80	1.867	1.90	1.85	27.78	54.54	65.82	58.53
T_7- T_7- 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half N in three equal splits at 25, 40 & 60 DAS)	1.80	1.883	1.85	1.85	28.26	55.90	64.40	58.44
SE (m) ±	0.01	0.009	0.01	0.01	0.52	1.17	47.84	1.283
CD (P=0.05)	0.02	0.025	0.04	0.02	1.45	3.24	132.61	3.557
Interaction (A*B)								
SE (m) ±	0.02	0.02	0.04	0.01	1.39	3.09	126.57	1.283
CD (P=0.05)	0.07	NS	0.10	0.02	3.84	NS	NS	3.557
CV%			3.50	2.51	9.41	10.74	3.50	18.99

Table 2: Herbage yield (kg/ha), Dry foliage yield (kg/ha) of Kalmegh as influenced by different organic and chemical fertilizers treatments

Treatments	**Herbage yield (kg/ha)**				**Dry foliage yield (kg/ha)**			
	2015-16	**2016-17**	**2017-18**	**Pooled Mean**	**2015-16**	**2016-17**	**2017-18**	**Pooled Mean**
Main plot Treatments: Organic manures								
M1- Control	1297	2156	4161	2538	1297	2156	4161	2538
M2- FYM 10 t ha-1	1417	2648	4378	2814	1417	2648	4378	2814
M3- FYM 15 t ha-1	1504	2923	4526	2984	1504	2923	4526	2984
M_4- Vermicompost 5.0 t ha^{-1}	1484	2842	4988	3105	1484	2842	4988	3105
M_5- Vermicompost 7.5 t ha^{-1}	1541	2996	4541	3026	1541	2996	4541	3026
M_6- Castor cake 1.5 t ha^{-1}	1385	2569	4993	2982	1385	2569	4993	2982
M_7- Castor cake 2.5 t ha^{-1}	1456	2734	4984	3058	1456	2734	4984	3058
SE (m) ±	31	48	142	42.517	31	48	142	42.517
CD (P=0.05)	96	149	439	131.020	96	149	439	131.020
Sub plot Treatments: Chemical fertilizers								
T_1- Control	1167	2409	4427	2668	1167	2409	4427	2668
T_2- 40:10:30 Kg/ha NPK	1354	2508	4664	2842	1354	2508	4664	2842
T_3- 60:20:40 Kg/ha NPK	1421	2593	4462	2825	1421	2593	4462	2825
T_4- 80:30:50 Kg/ha NPK	1497	2680	4329	2835	1497	2680	4329	2835
T_5- T_5- 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half of N at 25 DAS)	1528	2801	4876	3069	1528	2801	4876	3069
T_6- T_6- 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half N in two equal splits at 25 and 40 DAS)	1548	2914	4972	3145	1548	2914	4972	3145
T_7- T_7- 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half N in three equal splits at 25, 40 & 60 DAS)	1567	2963	4840	3123	1567	2963	4840	3123
SE (m) ±	28	58.68	143.94	55.206	28	58.68	143.94	55.206
CD (P=0.05)	79	162.65	398.99	153.023	79	162.65	398.99	153.023
Interaction (A*B)								
SE (m) ±	75.20	155.25	380.84	55.206	75.20	155.25	380.84	55.206
CD (P=0.05)	NS	NS	NS	153.023	NS	NS	1056	153.023
CV%	9.04	9.98	14.18	14.96	9.04	9.98	14.18	14.96

Conclusion

Application of vermicompost @ 7.5 t ha^{-1} recorded significantly higher andrographolide yield per hectare followed by application of vermicompost @ 7.5 t ha^{-1}, FYM @ 15 t ha^{-1} and Castor cake 2.5 t ha $^{-1}$. However, these treatments are at par with each other.

Herbage yield was significantly highest with application of(T_6) 80:30:50 Kg/ha NPK (Half N with full P & K as basal and remaining half N in two equal splits at 25, 40 & 60 DAS)as compared to remaining treatments.

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