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Evaluation of Kalmegh (*Andrographis paniculata*) germplasm for higher biochemical constituents production under agro-climatic conditions of Jharkhand

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Abstract
Keeping in view the importance of variability in biochemical constituents of Kalmegh germplasm, a systematic research trial was undertaken to assess its biochemical contents thus to screen out its most promising cultivar suited for the climatic and edaphic conditions of Jharkhand. 25 Kalmegh germplasm was collected across its natural growing zone and evaluated under uniform edapho-climatic conditions of Jharkhand. Plants of Kalmegh of all germplasm were harvested before flowering stage to evaluate its different biochemical constituents. At before flowering stage, germplasm like $JHAP_1$ (2.39%), $JHAP_3$ (2.21%), OAP_1 (2.00%), $CHAP_1$ (2.36%) and $CHAP_2$ (2.03%) gave maximum andrographolide %. Maximum andrographolide yield was recorded for $JHAP_3$ (35.33 kg/ha) followed by OAP_1, OAP_5, $CHAP_1$ and $MPAP_4$. Maximum neo-andrographolide % was recorded for $JHAP_3$, (2.93%) followed by $JHAP_4$, OAP_1 and OAP_2; however maximum neo-andrographolide yield was recorded for $JHAP_3$ (46.78 kg/ha) followed by $JHAP_4$, OAP_1 and $MPAP_4$. Maximum diterpenoid yield in Kalmegh plants was recorded for $JHAP_3$ (82.11 kg/ha) and OAP_1. So for maximum production of andrographolide, neo-andrographolide and diterpenoid yield from the plants of Kalmegh germplasm harvested before flowering stage; $JHAP_1$, $JHAP_3$, $MPAP_1$, OAP_1 and $CHAP_1$ may be screened out as best germplasm under the climatic and edaphic conditions of Jharkhand.

Keywords: Kalmegh, *Andrographis paniculata*, andrographolide, neo-andrographolide, diterpenoid

Introduction
Genetic variability is the gift of nature and its fruitful utilization calls for systematic collection, evaluation, description and grouping based on economic descriptors. Use of crop genetic resources in crop improvement programme is the ultimate objective of germplasm resource management and improvement in both qualitative and quantitative characters of a crop should be the main aim of any breeding programmes (Simmonds, 1962). The main objective of crop improvement and breeding programme is to screen out the best cultivar of medicinal and aromatic plants germplasm based upon bio-chemical parameters for particular climatic and edaphic conditions. Padua *et al.,* (1999) mentioned that the biochemical constituents of Kalmegh are related with environment, particularly to soil conditions. There are wide differences in the composition of phytochemicals with respect to part used, geography, season, genetic variation and time of harvesting (Pholphana *et al.,* 2004). Sabu (2006) also mentioned that epigenetic variations are mostly induced by the environment in which the plants grow and are also partially affected by developmental events. Therefore, a great deal of information on biochemical parameters is necessary. Considerable variation in biochemical characters has been reported previously in *Andrographis paniculata* (Tongdonae, 2002). The problem of variations is further compounded in medicinal plants which apart from displaying visible variations, synthesize and accumulate an array of plant-specific chemicals.
Medicinal properties in plants are mainly due to the presence of secondary metabolites which plants need in their natural environments under particular conditions of stress and competition (Purwanto *et al*., 2011). Secondary metabolite biosynthesis is controlled by the amount and kinds of enzymes, so its activity is strongly influenced by environmental factors, particularly temperature and humidity. A study of variation in the active principles is often an important element in the investigation of variation in medicinal plants. Considering the diversity and medicinal importance of Kalmegh, understanding of the biochemical constituents variations is important to identify superior genotype. Due to considerably increased demands by national and international pharmaceutical industries of Kalmegh, high production by utilizing improved

high yielding varieties is required.

Kalmegh (*Andrographis paniculata* Wall. ex Nees.) is an important indigenous medicinal plant, native to India, commonly known as 'King of bitters' (Saraswathy *et al.*, 2004; Chauhan *et al.*, 2009; Gomathinayagam *et al.*, 2009) found throughout tropical and sub-tropical Asia. It is an erect, branched annual herb, extremely bitter in taste, grows to a height of 30-110 cm in moist shady places with glabrous leaves and white flowers with rose-purple spots on the petals (Varaprasad *et al*., 2006). It is a hermaphroditic, self-compatible and habitual inbreeder (Lattoo *et al.*, 2006), low-diverse, endangered and red-listed plant species because of self to often cross-pollinated mating system as well as over exploitation (Valdiani *et al*., 2012a). All parts of this herb are bitter because of presence of diterpene lactone compounds and used extensively as medicine (Prajoubklang, 1998). Consumption of Kalmegh herb is estimated to be 250 tons (Kataky and Handique, 2010) and its demand is increasing day by day (Chauhan *et al.*, 2009). It is one of the 32 prioritized medicinal plants of India with a demand of 2197 tones with annual growth of 3.10% (Anonymous, 2007). The most important secondary metabolite produced from Kalmegh is andrographolide ($C_{20}H_{30}O_5$; molecular weight - 350.45). It is a bitter, colourless and crystalline diterpene lactone found in the aerial parts of the plant. Other major bitter diterpene lactone component is neo-andrographolide ($C_{26}H_{40}O_8$, molecular weight – 480.59) (Fijita *et al.*, 1984; Matsuda *et al.*, 1994; Abeysekera *et al*., 1988).

Andrographolide, the major constituent of Kalmegh having anti-diabetic properties (Verma and Vinayak, 2008), anticancerous properties (Chun *et al*., 2010); anti inflammatory properties (Chandrasekaran *et al.*, 2010); antioxidant and hepatoprotective activity (Kumar *et al*., 2012); anti thrombotic properties (Thisoda *et al.*, 2006); anti herpes properties (Wiart *et al.*, 2005); anti-influenza properties (Chen *et al.*, 2009); analgesic & antipyretic properties (Madav *et al.*, 1995); anti-microbial and antiparasitic properties (Singha *et al.*, 2007), antimalarial properties (Thiyagarajan *et al.*, 2011), antidiarrheal properties (Gupta *et al*., 1993), inhibit pregnancy (Zoha *et al*., 1989), protection against rheumatoid arthritis (Burgos *et al.*, 2009), anti-HIV properties (Calabrese *et al*., 2000), anti-H_1N_1 (Ko *et al.*, 2006), anti-bacterial and anti-viral properties (Reddy *et al*., 2005), cardio protective properties (Ojha *et al*., 2009) protective action against nicotine induced liver, kidney, heart, lung and spleen toxicity (Neogy *et al*., 2008), protection against chronic hepatitis B virus infection (Shukla *et al.*, 1992). Neo-andrographolide having anti-inflammatory properties (Parichatikanond *et al*., 2010); anti-parasitic properties (Misra *et al.*, 1992); hepatoprotective properties (Chander *et al.*, 1995); anti-herpes activities (Wiart *et al.*, 2005) and antioxidant properties (Kamdem *et al.*, 2002).

Materials and Methods

The experimental site was located at Research Farm, Birsa Agricultural University, Kanke, Ranchi, at $23^0$26'30" N latitude and $85^0$18'20" E longitude in Chotanagpur plateau situated in north eastern part of India and at an altitude of 646 m above the mean sea level. The soil of the site is lateritic, developed from granite-gneiss, sandy loam in texture, sedentary in nature and well drained with low water holding capacity and poor consistency. The experimental materials comprised of twenty five germplasm of Kalmegh, for which seeds were collected from its natural habitat. Out of 25 Kalmegh accessions, 4 were collected from Jharkhand ($JHAP_1$, $JHAP_2$, $JHAP_3$, $JHAP_4$), 6 from Odisha (OAP_1, OAP_2, OAP_3, OAP_4, OAP_5, OAP_6), 4 from Chhattisgarh ($CHAP_1$, $CHAP_2$, $CHAP_3$, $CHAP_4$), 4 from Madhya Pradesh ($MPAP_1$, $MPAP_2$, $MPAP_3$, $MPAP_4$), 4 from Karnataka (KAP_1, KAP_2, KAP_3, KAP_4), 2 from NBPGR (IC 111286, IC 471890) and 1 from Gujarat (GAP_1). Collected seed samples were germinated, raised in polytubes and maintained under identical growing conditions and used for analyzing qualitative and quantitative variations.

Forty-five days nursery grown old seedlings at 8-10 leaf stage were transplanted in Randomized Block Design with twenty five treatments and three replications at 30 cm × 30 cm spacing level. Ten plants selected randomly from all the treatments were harvested at before flowering stage for the estimation of major active principles, *viz.*, andrographolide and neo-andrographolide using standard HPLC protocol given by Vijaykumar *et al.*, (2007). Samples of selected accessions of *Andrographis paniculata* were dried in a ventilated-electric oven at 55^0C for two days, labeled and numbered. Separated dried plant materials were chopped into small pieces and ground into fine powder and kept in zipped plastic bags. Solvents used for chromatography were methanol (HPLC grade, Merck, Germany) and distilled water. Ten gram each of powdered samples were extracted with 100 ml of methanol for 3 days at room temperature and the process was repeated several times with the same solvent until the solvent portion became colourless. The solvent extracts were concentrated under reduced pressure using a rotary evaporator and concentrated extracts were transferred into conical flasks for removal of residual solvent. Well-dried extracts were placed into small glass containers and after sealing kept in a −20°C freezer for further estimation of active principles in different parts of the plant.

Pure grade andrographolide and neo-andrographolide (Sigma-Aldrich, purity 99.9%) was used as the standard sample. A stock solution of standard andrographolide and neo-andrographolide (1 mg/ml) was prepared in HPLC grade methanol. Quantitative analysis of active principles were done with Perkin Elmer HPLC system equipped with an automatic sampler (96 samples), a series 200 LC pump, a column oven RI 18 column (4.6 mm × 250 mm, 5 µm pore size, used as stationery phase) and a series 200 photodiode array UV detector. Finally, 1 ml from each sample was kept in auto sampler of HPLC apparatus in three replicates before it runs. The separation was carried out using a RI 18 column (4.6 mm × 250 mm, 5 µm pore size) with a injected volume of 10 µl, isocratic mobile phase of methanol: water (6:4; v/v), flow rate of 0.8 ml/min, UV detection at 210 nm, ambient temperature of 36^0C and 15 minutes run time.

Yield of methonolic extract (g) in plants at before flowering stage was calculated by the difference in weight of the flask at before and after distillation stage. Andrographolide and neo-andrographolide % in plants at before flowering stage was calculated by the formula

% of biochemical's in herbage = Methonolic yield factor × % of biochemical's in extract

Andrographolide and neo-andrographolide yield (kg/ha) in plants at before flowering stage was calculated by the formula

Biochemical's yield/ha = Yield of herbage / ha × % of biochemical's in herbage

Diterpenoid lactones yield (kg/ha) in plants at before flowering stage was calculated by adding the value of andrographolide and neo-andrographolide yield. Collected data on bio-chemical parameters was subjected to analysis of variance at 5 and 1% level as per Panse and Sukhatme (1989)

and Box plot analysis for validation of results.

Results and Discussion

Data related to biochemical constituents of different Kalmegh germplasm harvested at before flowering stage is given in Table 1 and 2.

Yield of methonolic extract in Kalmegh plants harvested before flowering

The mean maximum yield of methonolic extract of Kalmegh germplasm was recorded for IC 471890 (2.10 g) and the minimum in $JHAP_2$ (1.54 g) with the mean value of all the 25 germplasm as 1.81 g and different Kalmegh germplasm showed highly significant difference among them. Four germplasm namely KAP_1, KAP_4, IC 471890 and GAP_1 gave higher value of methonolic extract (more than 2.00 g).

Andrographolide percentage in plants of Kalmegh germplasm harvested before flowering The maximum andrographolide % of different Kalmegh germplasm was recorded in $JHAP_1$ (2.39%) and the minimum in KAP_3 (0.93%). The mean value of andrographolide % in different Kalmegh germplasm was calculated as 1.54%. Two germplasm of Jharkhand $JHAP_1$ (2.39%) and $JHAP_3$ (2.21%), one of Odisha, OAP_1 (2.00%) and two of Chhattisgarh like $CHAP_1$ (2.36%) and $CHAP_2$ (2.03%) gave higher percentage of andrographolide, while the germplasm of Karnataka like KAP_3 and KAP_4 gave lower value. Maximum andrographolide % was shown by $JHAP_1$, $JHAP_3$ and $CHAP_1$ (more than 2.00%) however least andrographolide % in plants was observed for KAP_3 and KAP_4 (less than 1.00%). Sharma *et al.,* (1992) reported andrographolide variation between 0.65-0.81% in the plants of Kalmegh in India. Sabu (2006) found moderate variation in andrographolide content from 1.47 to 0.95% in different Kalmegh accessions from Tamilnadu. Patarapanich *et al.,* (2007) also found andrographolide range varied from 2.76 to 4.39%, in Kalmegh germplasm collected from Thailand region.

Pareek *et al.,* (2007) evaluated 30 Kalmegh germplasm with andrographolide content ranged from 1.14% to 2.60%. Lattoo *et al.,* (2008) recorded minimum andrographolide for APJ_{013} (2.67%) and maximum for APJ_{008} and APJ_{046} (5.94%). Sharma *et al.,* (2009) observed andrographolide content in 15 Kalmegh germplasm of Chhattisgarh and adjoining states ranged from 0.69 to 1.85% with a mean value of 1.23%. Anonymous (2009) reported andrographolide content as 1.20%, 1 to 2% and 2.15% for improved varieties of Kalmegh namely AK-1, CIM-Megha and KI_5 respectively. Pandey and Mandal (2010) found andrographolide content from 0.35 to 2.35% in various plant parts of Kalmegh germplasm collected from Madhya Pradesh and Chhatisgarh. They identified AP_4 and AP_5 as promising accessions of Kalmegh having high andrographolide content.

Table 1: Yield of methonolic extract, andrographolide % and its yield in plants of Kalmegh germplasm harvested at before flowering stage

Treatments	Yield (g) of methonolic extract in 10g herbage	Andrographolide (%)	Andrographolide yield (kg/ha)
$JHAP_1$	1.68	2.39	17.05
$JHAP_2$	1.54	1.66	25.56
$JHAP_3$	1.67	2.21	35.33
$JHAP_4$	1.64	1.57	21.19
OAP_1	1.70	2.00	27.49
OAP_2	1.73	1.61	9.87
OAP_3	1.80	1.46	14.20
OAP_4	1.74	1.58	14.41
OAP_5	1.72	1.76	21.55
OAP_6	1.85	1.43	18.93
$CHAP_1$	1.60	2.36	23.27
$CHAP_2$	1.56	2.03	14.01
$CHAP_3$	1.84	1.39	15.94
$CHAP_4$	1.56	1.24	11.43
$MPAP_1$	1.73	1.32	9.23
$MPAP_2$	1.86	1.53	15.13
$MPAP_3$	1.92	1.41	10.01
$MPAP_4$	1.93	1.30	21.12
KAP_1	2.02	1.07	9.61
KAP_2	1.99	1.60	16.97
KAP_3	1.97	0.93	10.05
KAP_4	2.09	1.02	11.45
IC 111286	1.93	1.18	9.28
IC 471890	2.10	1.19	13.05
GAP_1	2.06	1.23	18.58
Grand Mean	1.81	1.54	16.59
$S.E._{(m)}$	0.023	0.029	1.057
$C.D._{5\%}$	0.065	0.083	3.015
$C.D._{1\%}$	0.085	0.107	3.911
$C.V._{(\%)}$	2.178	3.286	11.038

Andrographolide yield from plants of Kalmegh germplasm harvested before flowering stage

The maximum andrographolide yield from plants of different Kalmegh germplasm was recorded in $JHAP_3$ (35.33 kg/ha) and the minimum in $MPAP_1$ (9.23 kg/ha). The mean value of different Kalmegh germplasm was calculated as 16.59 kg/ha and different Kalmegh germplasm showed highly significant relation among them. In general, higher andrographolide yield was observed for all Jharkhand germplasm, while lower value was observed in Karnataka germplasm. All the germplasm of Jharkhand ($JHAP_1$ to $JHAP_4$), OAP_1, OAP_5, $CHAP_1$ and $MPAP_4$ gave highest andrographolide yield (more than 20.00

kg/ha), while germplasm like OAP_2, $MPAP_1$, $MAPAP_3$, KAP_1, KAP_3 and IC 111286 gave low andrographolide yield (less than 10.00 kg/ha).

Sabu (2006) selected AP_{36}, the Kalmegh population which yielded highest concentration of andrographolide. Prathanturarug *et al.,* (2007) have selected T_{28A}, T_{31A} and T_{24A} Kalmegh accessions as potentially superior sources for breeding and improvement of cultivars and higher phytomedicine production. Singh *et al.,* (2011) observed maximum andrographolide yield of 67.80 kg/ha for AP_3 Kalmegh germplasm and minimum for AH_{89} (37.80 kg/ha) at pre-flowering stage. Subramanian *et al.,* (2012) reported high level of andrographolide in Kalmegh just before the flowering season (after 90 days) which gradually falls. Valdiani *et al.,* (2012a) found andrographolide percentage ranged from 1.32 to 2.63% in different Kalmegh accessions. They mentioned that andrographolide yield per hectare and andrographolide content per plant tended to increase negligibly with increasing genetic distance. Pholphana *et al.,* (2004) reported wide variation in phytochemicals in Kalmegh with respect to part used, geography, season, genetic variation and time of harvesting. Lattoo *et al.,* (2008) documented that geographical origin can affect both quality and quantity of the active constituents of Kalmegh.

Neo-andrographolide % in plants of Kalmegh germplasm harvested before flowering stage

The maximum mean neo-andrographolide % in plants of Kalmegh germplasm harvested before flowering stage was recorded for $JHAP_3$ (2.93%) and the minimum for $CHAP_2$ (0.83%). The mean of neo-andrographolide % of all the 25 Kalmegh germplasm was calculated as 1.33% with highly significant difference among them. On the average basis, the neo-andrographolide % in plants varied from 1.00 to 1.50%, but lowest neo-andrographolide % was observed in $JHAP_2$ and $CHAP_2$. Bhan *et al.,* (2006) noticed neo-andrographolide concentration ranged from 1.08% (accession 7), 1.81% (accession 6), 2.23 % (accession 1) to 2.81% (accession 3) in Kalmegh. Patarapanich *et al.,* (2007) also found neo-andrographolide varied from 1.42 to 1.89% in Kalmegh germplasm of Thailand region.

Neo-andrographolide yield from plants of Kalmegh germplasm harvested before flowering stage

The maximum mean neo-andrographolide yield from plants of Kalmegh germplasm was recorded in $JHAP_3$ (46.78 kg/ha) and the minimum in $CHAP_2$ (5.72 kg/ha). The mean value of neo-andrographolide yield for all the 25 germplasm was calculated as 14.63 kg/ha with highly significant difference among them. More than 45.00 kg/ha of neo-andrographolide yield was noticed in case of $JHAP_3$, but $CHAP_2$, $MPAP_1$, $MPAP_3$, KAP_1, IC 111286 gave lowest neo-andrographolide yield. Singh *et al.,* (2011) observed maximum neo-andrographolide yield for AP_3 (11.50 kg/ha) and minimum for AH_{89} (6.80 kg/ha). Pholphana *et al.,* (2004) found appreciable variation in the active components of Kalmegh, which was attributed to difference in genotypes, growing environment, time of harvesting, age of leaves at harvest etc.

Table 2: Neo-andrographolide %, neo-andrographolide yield and Diterpenoid lactones yield in plants of Kalmegh germplasm harvested at before flowering stage

Treatments	Neo-andrographolide (%)	Neo-andrographolide yield (kg/ha)	Diterpenoid lactones yield (kg/ha) in plant (before flowering)
$JHAP_1$	1.45	10.36	27.41
$JHAP_2$	0.87	13.35	38.91
$JHAP_3$	2.93	46.78	82.11
$JHAP_4$	1.62	21.82	43.01
OAP_1	1.72	23.56	51.05
OAP_2	1.91	11.73	21.59
OAP_3	1.43	13.89	28.09
OAP_4	1.38	12.61	27.02
OAP_5	1.42	17.35	38.89
OAP_6	1.45	19.16	38.09
$CHAP_1$	1.55	15.25	38.53
$CHAP_2$	0.83	5.72	19.72
$CHAP_3$	1.45	16.67	32.62
$CHAP_4$	1.21	11.12	22.55
$MPAP_1$	1.11	7.73	16.95
$MPAP_2$	1.47	14.55	29.68
$MPAP_3$	1.23	8.73	18.74
$MPAP_4$	1.06	17.26	38.38
KAP_1	0.99	8.85	18.46
KAP_2	1.07	11.29	28.26
KAP_3	0.92	9.94	19.99
KAP_4	1.06	11.91	23.36
IC 111286	1.01	7.97	17.25
IC 471890	1.08	11.84	24.89
GAP_1	1.07	16.20	34.78
Grand Mean	1.33	14.63	31.21
$S.E._{(m)}$	0.022	0.916	1.922
$C.D._{5\%}$	0.062	2.612	5.483
$C.D._{1\%}$	0.081	3.390	7.112
$C.V._{(\%)}$	2.807	10.846	10.667

Bhan *et al.,* (2006) mentioned that deviation in andrographolide and neo-andrographolide concentration was probably arose due to non-uniformity in flowering time and extended phenological phases in different accessions. Further

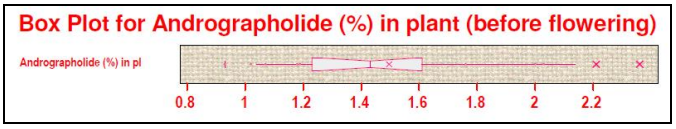
they mentioned that andrographolide and neo-andrographolide is continuously produced during the plant growth due to simultaneous dehydration and glycosylation.

Diterpenoid lactones yield from plants of different Kalmegh germplasm collected before flowering stage

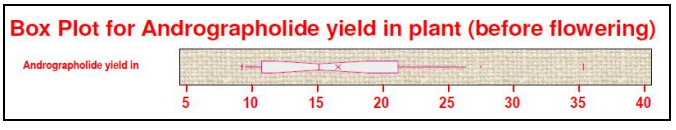
The mean maximum diterpenoid lactones yield in plants of different Kalmegh germplasm was recorded in $JHAP_3$ (82.11 kg/ha) and the minimum in $MPAP_1$ (16.95 kg/ha). The mean of diterpenoid lactones yield from all the 25 Kalmegh germplasm was calculated as 31.21 kg/ha with highly significant difference among them. Germplasm like OAP_1 and $JHAP_4$ also gave higher diterpenoid lactones yield. Minimum yield was recorded in case of IC 111286, KAP_3, KAP_1, $MPAP_1$ and $CHAP_2$. Bhan *et al.,* (2006) recorded andrographolide yield (61.83 kg/ha) in Kalmegh. Ayudhya *et al.,* (1990) found total lactones content in the aerial parts of Kalmegh collected from various provinces of Thailand varied from 3.91-10.29%. As per Department of Medical Sciences, (1995) Kalmegh produce highest yield of total lactones during the blossom period, therefore the suitable time for harvesting is from the beginning of the flowering period to about 50% flowering, or when a plant is about 110-120 days old. Gupta and Pareek (1981) opined that the total leaf area and plant height were significantly correlated with active ingredients. Nemade *et al.,* (2003) recorded that the date of harvesting had a significant influence on yield attributes and andrographolide content.

Bhan *et al.,* (2006) noticed significant variations in chemical characters of different Kalmegh accessions because of their different geographical locations with different genetic makeup. Mean andrographolide concentration across different harvesting dates varied from 4.09% in accession 8 to 3.26% in accession 3. They screened out the last week of October ideal for obtaining maximum andrographolide yield (61.83 kg/ha). Jaleel *et al.,* (2006) found crucial role of growth regulatory compounds in the regulation and coordination of plant growth, morphogenesis, metabolism and role in biosynthesis of secondary metabolites. Singh *et al.,* (2011) observed slightly higher value of total diterpenoid lactones yield at flowering stage (75.70 kg/ha) than at pre-flowering stage (75.50 kg/ha) in Kalmegh germplasm.

Box plot analysis of andrographolide % in Kalmegh plants collected before flowering stage indicated positive skewness; however more number of Kalmegh germplasm showed their presence between 25th to 50th percentile than of 50th to 75th percentile. Box plot analysis of andrographolide yield from Kalmegh plants collected before flowering stage also showed positive skewness but more number of Kalmegh germplasm showed their presence in 50th to 75th percentile than of 25th to 50th percentile.

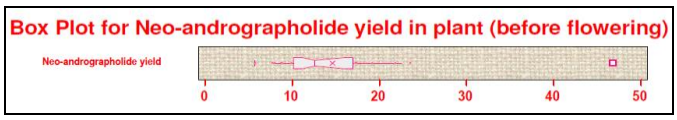


Graph 1: Box plot analysis of andrographolide % in plants of different Kalmegh accessions (before flowering)

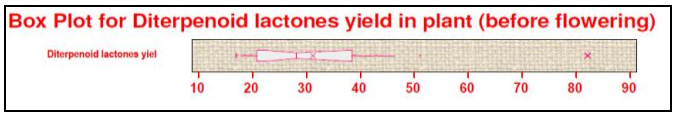


Graph 2: Box plot analysis of andrographolide yield in plants of different Kalmegh accessions (before flowering)

Box plot analysis of neo-andrographolide yield from Kalmegh plants collected before flowering stage indicated positive skewness and more number of Kalmegh germplasm showed their presence in 50th to 75th percentile than of 25th to 50th percentile. Box plot analysis for diterpenoid lactones yield of Kalmegh plants collected before flowering stage also indicated positive skewness and more number of Kalmegh germplasm showed their presence between 50th to 75th percentile than 25th to 50th percentile.



Graph 3: Box plot analysis of neo-andrographolide yield in plants of different Kalmegh germplasm (before flowering)



Graph 4: Box plot analysis of diterpenoid yield in plants of different Kalmegh germplasm (before flowering)

Conclusion

At before flowering stage, mean andrographolide % in plants of Kalmegh germplasm was recorded maximum for $JHAP_1$ (2.39%) followed by $JHAP_3$ (2.21%), OAP_1 (2.00%), $CHAP_1$ (2.36%) and $CHAP_2$ (2.03%). Mean maximum andrographolide yield was recorded for $JHAP_3$ (35.33 kg/ha) which showed highly significant difference with other Kalmegh germplasm. Higher andrographolide yield in plants at before flowering stage was observed for all the germplasm of Jharkhand ($JHAP_1$ to $JHAP_4$), OAP_1, OAP_5, $CHAP_1$ and $MPAP_4$. Maximum mean neo-andrographolide % was recorded for $JHAP_3$ (2.93%) which showed highly significant difference with other Kalmegh germplasm. Maximum mean neo-andrographolide yield was also recorded for $JHAP_3$ (46.78 kg/ha) with highly significant difference from other Kalmegh germplasm. Maximum mean diterpenoid lactones was recorded for $JHAP_3$ (82.11 kg/ha) which showed highly significant difference with other Kalmegh germplasm. So for maximum production of andrographolide, neo-andrographolide and diterpenoid yield from the plants of Kalmegh germplasm collected before flowering stage; $JHAP_1$, $JHAP_3$, $MPAP_1$, OAP_1 and $CHAP_1$ may be screened out as best germplasm under the climatic and edaphic conditions of Jharkhand.

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