



Original Research Article (Experimental)

## Nutrient analysis of *Kunapa jala* and *Pancha gavya* and their evaluation on germination of *Ashwagandha* and *Kalamegha* seeds: A comparative study



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### ABSTRACT

**Background:** *Vrikshayurveda*, an ancient science of plant life described by *Surapala*, deals with healthy growth and productivity of plants, which has clearly outlined a systematized agricultural practice that insisted the use of *Kunapa jala* (*KJ*) and *Pancha gavya* (*PG*).

**Objective:** An experiment was conducted to validate *KJ* and *PG* by nutrient analysis and their effect on the germination parameters of *Ashwagandha* and *Kalamegha* seeds in comparison with other treatment groups.

**Materials and methods:** *KJ* and *PG* were prepared according to the classical references. The nutrient contents and germination parameters of *KJ* and *PG* were compared with other groups namely control (*Contr*), farmyard manure (*FYM*), humic acid (*HA*) and *NPK*.

**Results:** The pH and EC were 5.793, 2.653 dS/m and 5.584, 2.216 dS/m for *KJ* and *PG* respectively. *KJ* possess highest nutrient contents followed by *PG*. The germination parameters revealed the better activity of *NPK* followed by *KJ*, *PG*, *HA*, *FYM* and *Contr*.

**Conclusion:** *KJ* and *PG* were found to be good in nutrient contents and were found to be effective on studied germination parameters of *Ashwagandha* and *Kalamegha* seeds.

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### 1. Introduction

Cultivation of plants using organic preparations dates back to 1000 AD in India. '*Vrikshayurveda*', is an ancient science of plant life described by *Surapala* and deals with cultivation of various plant species, their healthy growth and productivity. It is an age old agro practice which is of great relevance even today in agriculture and horticulture sectors. It deals with pest and disease management of plants, storage of seeds, sowing, germination, plant propagation, manuring etc [1]. For nourishment of plants, use of a biofertilizer called '*Kunapa jala*' (*KJ*) has been mentioned, references of which can be found in the manuscript of *Vrikshayurveda* written by

*Surapala*, around 1000 AD. The details of *KJ* are also found in *Upavana vinoda*, an anthropological compilation called '*Sharangadhara Paddhati*' written by *Acharya Sharangadhara*, belonging to the 13<sup>th</sup> century. The dictionary meaning of the Sanskrit word *Kunapa* is "smelling like a dead body or stinking". The manure *Kunapambu* or *KJ*, was appropriately named because it involved fermentation of animal remains, such as flesh, marrow etc. with stinking smell [2,3].

*Vrikshayurveda* of *Surapala* mentions varieties of *KJ*. The verse 101 mentions that excreta, bone marrow, brain, flesh and blood of animals are mixed with water and kept as it is for some time to be known as *KJ*. In the subsequent verses it is quoted that bones of horses, dead parrot, fish, horns of sheep and goat, cow dung cake should be boiled in water and later filled along with sufficient quantity of husk in a previously oil smeared pot. Instead of boiling, it can also be roasted in an iron pot and mixed with sesame oil cakes and honey. Good quality black gram and ghee should be added in

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the end. The ingredients mentioned do not have specific measure but the prepared mixture should be kept in a warm place [2]. Besides, Savita et al. referred to *Sharangadhara*, mentioning that almost any animal waste can be used in preparing *KJ* and indicated that the cultivator can choose the animals and their byproducts [4]. Another *Vrikshayurveda* texts *Upavana Vinoda* explains the preparation of *KJ* in the verses 171–174 as, flesh of wild animals should be boiled until properly cooked and transferred to an earthen pot, then tila, honey and ghee should be added followed by hot water. The pot should be kept in warm place for 15 days and the resultant liquid is called *KJ* [5].

It is found that, the daily application of *KJ* has improved the soil fertility and plant growth [6]. The application of *Dhanyagavya* prepared by using cow dung, water and paddy husk helped to eradicate the pest affecting the tea leaves [6]. In *Vrikshayurveda*, several suggestions have been made for enhancing plant growth and for protecting them against diseases. *KJ* is one such prescription, which has been recommended for stimulating growth and development of plants. Administration of *KJ* every 10<sup>th</sup> and 15<sup>th</sup> day, exhibited remarkable enhancement in paddy growth [7]. Besides the classical texts, few modern literatures also suggested to fulfill the nutrient needs to achieve good yield by the application of animal remnants in the form of meat meal, blood meal, fishmeal etc. [8] A study conducted on Langali (*Gloriosa superba* Linn.) revealed, application of modified *KJ* was better in terms of yield in comparison with control and the group cultivated as per the modern agricultural guidelines [9]. Organic farming was a well-developed and systematized agricultural practice during the past and this wisdom was obtained through the *Vedas*, which specify the use of '*Pancha gavya*' (*PG*) in agriculture for the health of soil, plants and humans. It is the blend of five products obtained from cow, namely, dung, urine, milk, curd and ghee. *PG* is used in different forms such as foliar spray, soil application, seed or seedling treatment etc. [10] There have been reports on modified formulations of *PG* and it is found to enhance the biological efficiency of the plants, improved quality of fruits and vegetables [11]. It has also increased the soil fertility [12]. Shailaja et al. reported the potential of *PG* as biofertilizer on *Spinacia oleracea*; there was an increase in biomass, shoot length and root length in *PG* treated plants, and the total viable count and total bacterial count of *Rhizobium*, *Azospirillum* and *Actinomyces* were enhanced in *PG* treated soil [13]. Sarkar et al. proved the efficacy of *KJ* and *PG* individually and in combination in promoting the growth and yield attributes of vegetable crops namely, tomato, chilli and cow pea [14]. Humic acid improves soil fertility and increases availability of nutrient elements by holding surfaces and consequently affecting plant growth and yield [15–17]. The role of 16 essential plant nutrients for plant growth or crop production is well established [18]. Germination studies are essential to predict the growth and development of plants. The successful cultivation of plants depend on the quality and germination behavior of the seeds. Among the stages of plant life cycles, seed germination is one of the most key processes in plant growth and survival [19].

The present study was planned to validate *KJ* and *PG* on *Ashwagandha* and *Kalamegha* as these medicinal plants are mentioned in several Ayurvedic literatures for their wider utility [20]. Besides, both these plants have been identified by the National Medicinal Plant Board (NMPB) of India in the thirty two selected priority medicinal plants, which are in great demand in domestic and international markets [21]. Hence, the present study was aimed to validate the *Vrikshayurveda* practices viz. *KJ* and *PG* by nutrient analysis and evaluated on the germination parameters in comparison with other treatment groups viz control, farmyard manure, humic acid and NPK.

## 2. Materials and methods

### 2.1. Seeds and other raw materials

Mature seeds of *Ashwagandha* (variety: Jawahar *Ashwagandha* 20) were procured from local market. *Kalamegha* seeds were procured from Directorate of Medicinal and Aromatic Plants Research, Anand (GJ), India. Other raw materials required for preparation of *KJ*, *PG*, farmyard manure, commercial brand humic acid and the NPK (in the form of urea, diammonium phosphate and muriate of potash respectively) were procured from local manufacturers.

### 2.2. Preparation of *Kunapa jala* (*KJ*)

*Vrikshayurveda* texts have explained various preparations of *KJ* based on the types of ingredients involved. According to *Sharangadhara* [4] and as explained by Sadhale [2], the *KJ* was prepared with minor modifications. According to availability of ingredients, 1.5 kg each of sheep/goat meat, chicken meat and 1 kg of Indian mackerel fish (*Rastrelliger kanagurta*) were boiled in 16 L of water till properly cooked and transferred to an earthen pot. Each 500 g powders of black gram (*Vigna mungo* L.) and sesame (*Sesamum indicum* L.) were added along with milk (1 L), honey (500 g) and ghee (250 g). The pot was closed with lid and kept in warm place for 30 days with stirring at regular intervals. The content of the pot was filtered on 31<sup>st</sup> day and the resultant filtrate was *KJ*. For application to seeds, 10% of *KJ* was used.

### 2.3. Preparation of *Pancha gavya* (*PG*)

*PG* was prepared referring to the method explained by De et al. [22] with minor modification in quantity of ingredients prepared by Shri Kshetra Revana Siddeshwara Goshala, Hunasevari, Belagavi (KA), India. All ingredients were collected from native Indian breed cow. 20 kg of dung, 10 L each of urine and of tap water were added in an earthen pot and kept for 15 days with stirring every day for an hour in clockwise and anticlockwise direction. On 16<sup>th</sup> day 5 kg of ghee was added, thoroughly mixed by stirring every day and kept for 5 more days. On 21<sup>st</sup> day, 10 L each of milk and curd were added and stirred every day till 30<sup>th</sup> day. On 31<sup>st</sup> day the content of the pot was filtered. For application to seeds, 10% of *PG* was used.

### 2.4. Determination of nutrient contents

The pH and electrical conductivity (EC) were determined using pH and EC meter. Determination of macro and micro elements were estimated using atomic absorption techniques and nitrogen content was estimated according to Kjeldahl method [23]. Nutrient contents were estimated for *KJ* and *PG*; same have been compared with organic group (FYM and HA).

### 2.5. Germination parameters

The germination parameters of *Vrikshayurveda* method groups (*KJ* and *PG*) were observed in comparison with organic (FYM and HA), inorganic (NPK) and control groups. Initially both the seeds were washed with 0.1% HgCl<sub>2</sub> for 2 min and rinsed with distilled water for 30 min. In each group, 50 seeds were placed on sterile filter paper disc in petri dishes of 15 cm diameter. 10% of *KJ*, *PG*, FYM, HA, NPK and control treatments were given to respective plates. Germination parameters were determined by the methods given by Srivastava et al. [24] viz. germinability (G %), germination rate index (GRI), emergence index (EI), relative seed germination (RSG), relative root elongation (RRE) and seedling vigor index (SVI). Plates

were observed for 30 days with appropriate irrigation at regular intervals and parameters were recorded.

## 2.6. Statistical analysis

Germination parameters were studied in triplicates; data in the experiments were expressed as mean  $\pm$  SD for three sample replicates ( $n = 3$ ). Means of results of germination parameters obtained were compared against control group for significance using Dunnett multiple comparison test using GraphPad InStat software. Differences were considered significant at  $p < 0.05$ , extremely significant at  $p < 0.01$  and non-significant at  $p > 0.05$  level.

## 3. Results

At the end of 30<sup>th</sup> day of incubation, the resultant liquids *KJ* and *PG* were light brown and greyish black respectively with characteristic pungent odour. *KJ* had pH 5.793, EC 2.653 dS/m while *PG* had pH 5.584, EC 2.216 dS/m. The estimation of nutrient contents for *KJ* and *PG* were estimated and compared with organic groups viz. FYM and HA. Results revealed that *KJ* possessed higher quantities of N, K, S, Ca, Mg, Zn, Fe, Mn and Cu followed by *PG*, HA and FYM. Similarly, *PG* possessed higher quantity of P, which was followed by *KJ*, HA and FYM.

The results of germination parameters of *Ashwagandha* and *Kalamegha* seeds in *KJ* and *PG* along with control, FYM, HA and NPK treatment groups are presented in Figs. 1–9. Fig. 1 shows onset of germination. *Ashwagandha* seeds treated with *KJ* and *PG* germinated on 4<sup>th</sup> day where as *Kalamegha* seeds treated with *KJ* and *PG* germinated on 5<sup>th</sup> and 6<sup>th</sup> day respectively. However, faster germination was observed in NPK treated *Ashwagandha* and *Kalamegha* seeds, i.e on 3<sup>rd</sup> and 4<sup>th</sup> day respectively, whereas germination delayed in both seeds in control group than *Vrikshayurveda* groups. At the end of 30<sup>th</sup> day, root length (Fig. 2) and shoot length (Fig. 3) were recorded. In *Ashwagandha* highest root length was observed in NPK group ( $3.17 \pm 0.13$  cm) followed by *KJ* ( $3.13 \pm 0.17$  cm). Similarly in *Kalamegha*, highest root length was observed in NPK group ( $3.00 \pm 0.14$  cm) followed by *KJ* ( $2.97 \pm 0.17$  cm). Results of shoot length indicated, highest shoot length in *Ashwagandha* was observed in NPK group ( $2.57 \pm 0.97$  cm) followed by *KJ* ( $2.54 \pm 0.95$  cm). Similarly in *Kalamegha* highest shoot length was observed in NPK group ( $3.37 \pm 1.04$  cm) followed by *KJ* ( $3.30 \pm 1.04$  cm).

In *Ashwagandha*, NPK treatment influenced G% ( $84 \pm 1.63\%$ ), GRI ( $2.80 \pm 0.05$ ), EI ( $4.90 \pm 0.22$ ), RSG ( $126.21 \pm 6.03\%$ ) RRE ( $110.48 \pm 2.94\%$ ) and SVI ( $218.53 \pm 16.08$ ) followed by *KJ* ( $82.67 \pm 0.94\%$ ,  $2.76 \pm 0.03$ ,  $4.86 \pm 0.06$ ,  $124.21 \pm 5.76\%$ ,  $109.60 \pm 9.26\%$  and  $209.40 \pm 16.74$  respectively) and *PG* ( $76.73 \pm 1.78\%$ ,  $2.71 \pm 0.06$ ,  $4.54 \pm 0.11$ ,  $122.07 \pm 2.13\%$ ,  $106.98 \pm 14.58\%$  and  $189.69 \pm 23.59$  respectively) treatment. Results are presented in Fig 4–9.

In *A. paniculata* NPK treatment influenced G% ( $79.49 \pm 0.76\%$ ) as compared to *KJ* ( $78.18 \pm 1.49\%$ ) and *PG* ( $76.09 \pm 1.78\%$ ) groups. GRI was highest in FYM ( $3.56 \pm 0.03$ ) as compared to *KJ* ( $2.87 \pm 0.05$ ) and *PG* ( $2.33 \pm 0.05$ ) groups. EI and SVI were influenced by NPK ( $6.26 \pm 0.15$  and  $267.54 \pm 5.00$  respectively) as compared to *KJ* ( $4.84 \pm 0.08$  and  $258.00 \pm 22.72$  respectively) and *PG* ( $4.10 \pm 0.12$  and  $242.90 \pm 22.34$  respectively) groups. RSG was influenced by FYM ( $155.38 \pm 3.04$  %) as compared to *KJ* ( $125.27 \pm 2.94\%$ ) and *PG* ( $101.93 \pm 1.37\%$ ) treated groups. RRE was observed to be highest in *KJ* ( $124.40 \pm 24.10\%$ ). Results are presented in Fig 4–9.

## 4. Discussion

*Vrikshayurveda* has clearly outlined a systematized agricultural practice that insisted of use of *KJ* and *PG* to enhance the yield and quality of plants. Though, both the practices have been elaborated in *Vrikshayurveda*, there are very few studies conducted to evaluate their efficacy on medicinal plants. Previously, no systematic research was undertaken to develop comprehensive standard operative procedure for the preparation of *KJ* and *PG*, due to the variation in ingredients and their quantity involved in preparation. Though preparation of *KJ* and *PG* seem to be expensive due to type of ingredients used, however these age old preparations are used for application to soil or plants in their diluted forms and hence are cost-effective.

The pH of *PG* is acidic in nature, and was similar to the reports of Gore et al. and Shailaja et al.; the acidic nature might be due to presence of *Lactobacillus* bacteria [25,26]. The results of nutrient content of the present study are in accordance with the results obtained by Jeng et al., where, meat bone meals contain substantial amounts of organic matter and nutritive elements such as N, P and Ca [27]. Similarly, *KJ* derived from animal products containing one or more nutrients like N, P and K are necessary for plant growth (Table 1), which is in accordance with Shaikh et al. and Gupta [28,29]. *PG* contained appropriate amount of nutrients; the results are in compliance with the study made by Geetha et al. [30] and as mentioned by Shubha et al. [31], the nutrient values of *KJ* and *PG* may vary according to the quantity, quality of ingredients used and duration of fermentation [25].

There are no specific references on *KJ* and *PG* as germination enhancers. A study conducted by Khanna et al. on effect of physical and chemical treatments on germination behavior revealed that seeds treated with 150  $\mu\text{g/mL}$  gibberellic acid took 4 days for onset of germination and the germination percentage was 98% [32]. In another study conducted by Afsan et al., germination percentage of GA<sub>3</sub> (500  $\mu\text{g/L}$ ) applied seeds of *Ashwagandha* was  $86 \pm 0.34\%$  [33], wherein present study *KJ* and *PG* treated *Ashwagandha* seeds onset to germinate on 4<sup>th</sup> day and germination percentage of both the groups was  $82.66 \pm 0.94\%$  and  $76.73 \pm 1.77\%$  respectively. Fig. 4 shows, highest germination percentage was achieved in inorganic group ( $84.00 \pm 1.63\%$ ) while that in control group was lowest

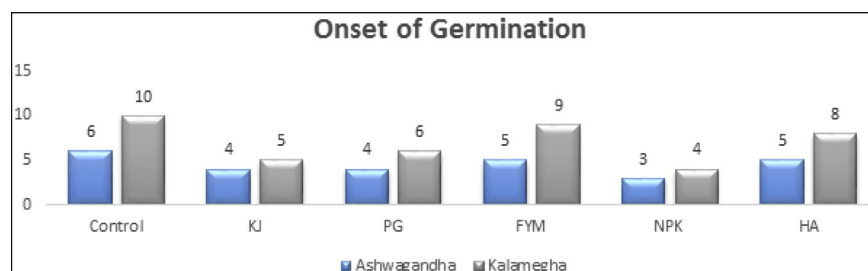


Fig. 1. Onset of germination.

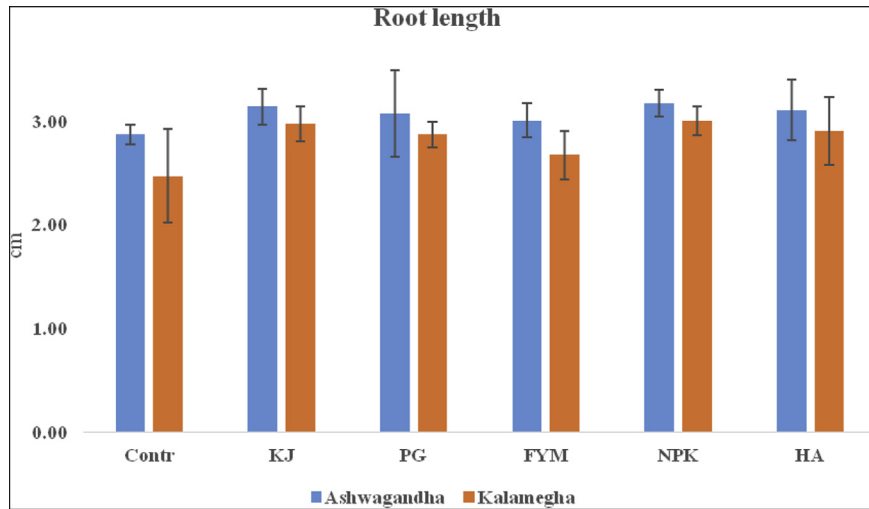


Fig. 2. Root length.

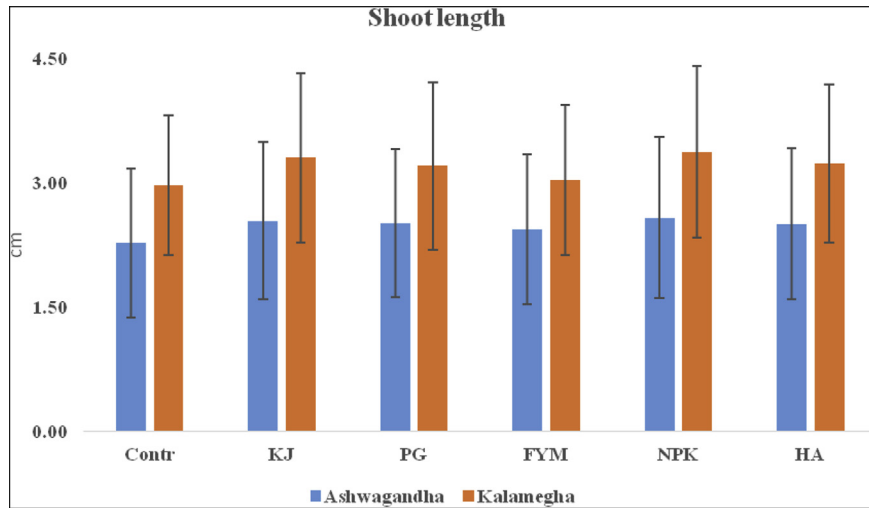


Fig. 3. Shoot length.

(72.46 ± 2.71%). In *Kalamegha*, Rawat et al. reported that, seeds treated with GA<sub>3</sub> (75 ppm) began to germinate on 3<sup>rd</sup> day with 82% of germination [34] and according to Kumari et al. the highest

germination (99.2% and 88.3%) in the variety CIM-Megha and wild, respectively was expressed after treatment with GA<sub>3</sub> (200 ppm) [35]. Fig. 4 shows, highest germination percentage was achieved in

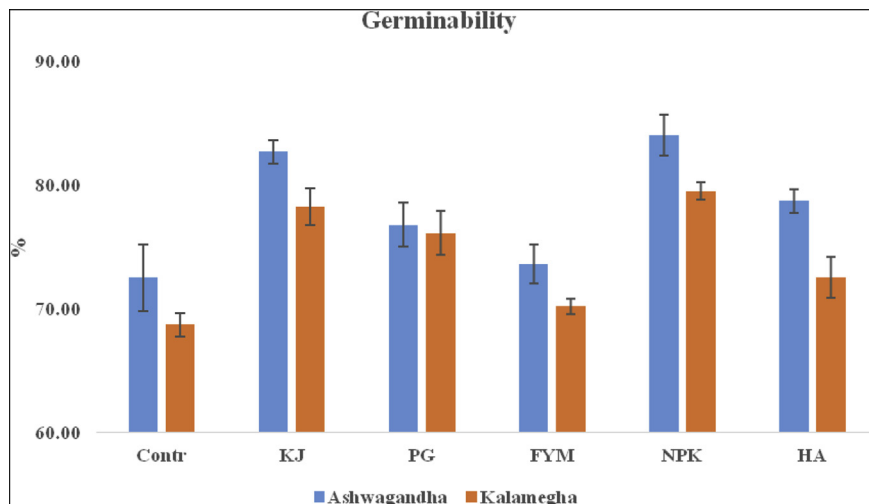


Fig. 4. Germinability.

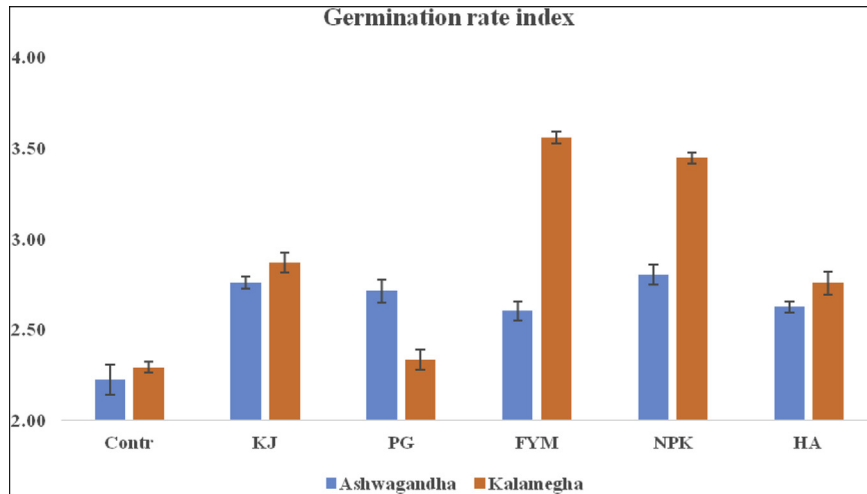


Fig. 5. Germination rate index.

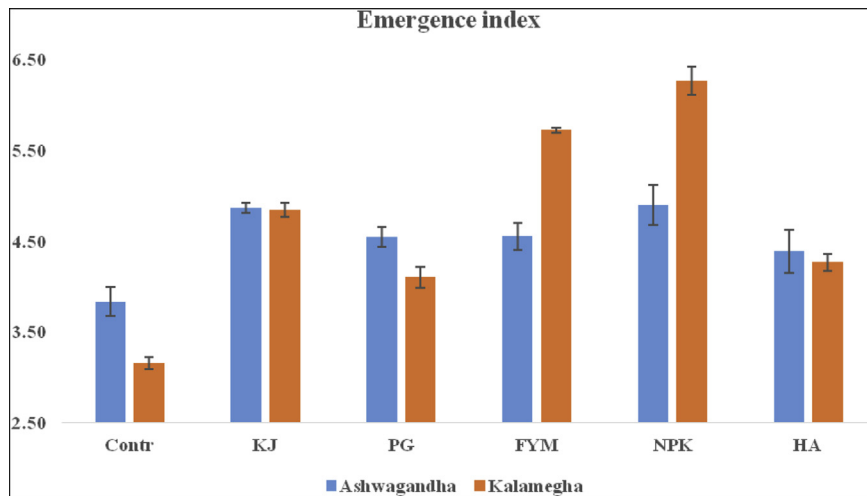


Fig. 6. Emergence index.

NPK group ( $79.48 \pm 0.72\%$ ) followed by KJ ( $78.18 \pm 1.48\%$ ) and PG ( $76.08 \pm 1.77\%$ ). Fig. 5 shows, highest GRI in Ashwagandha was observed in NPK ( $2.80 \pm 0.05$ ) followed by KJ ( $2.76 \pm 0.03$ ), similarly

highest GRI in Kalamegha was observed FYM ( $3.56 \pm 0.03$ ) followed by NPK ( $3.44 \pm 0.03$ ) and KJ ( $2.87 \pm 0.05$ ). Fig. 6 shows, highest EI in Ashwagandha was observed in NPK ( $4.90 \pm 0.06$ ) followed by KJ

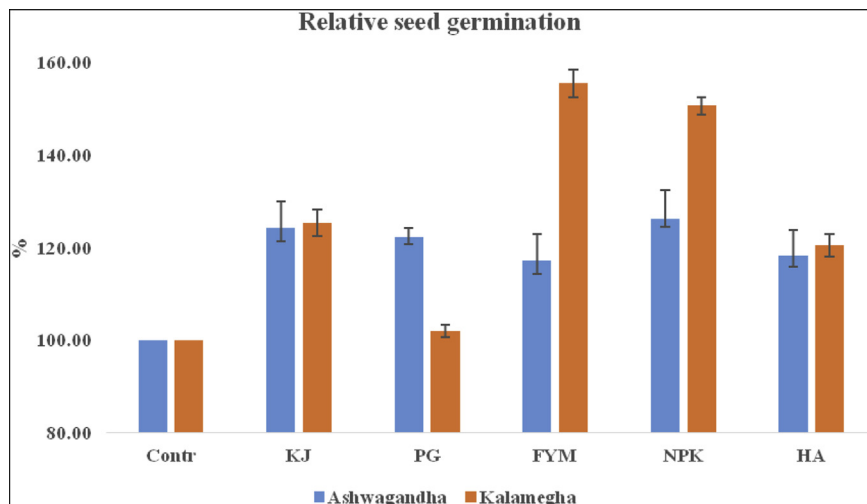


Fig. 7. Relative seed germination.

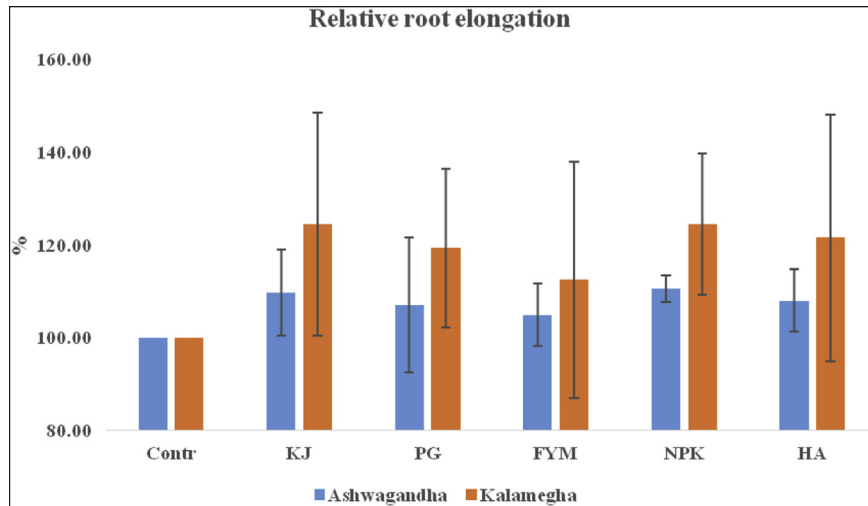


Fig. 8. Relative root elongation.

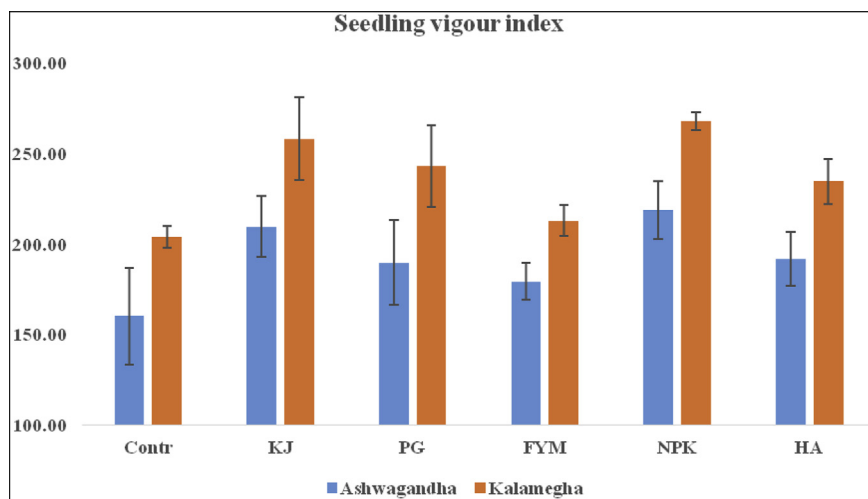


Fig. 9. Seedling vigor index.

( $4.86 \pm 0.22$ ), similarly highest EI in *Kalamegha* was observed in NPK ( $6.26 \pm 0.15$ ) followed by FYM ( $5.72 \pm 0.03$ ) and *KJ* ( $4.84 \pm 0.08$ ). Fig. 7 shows, highest RSG in *Ashwagandha* was observed in NPK ( $126.21 \pm 6.03\%$ ) followed by *KJ* ( $124.21 \pm 5.76\%$ ), similarly highest RSG in *Kalamegha* was observed in FYM ( $155.38 \pm 3.04\%$ ) followed by NPK ( $150.50 \pm 1.82\%$ ) and *KJ* ( $125.27 \pm 2.94\%$ ). Fig. 8 shows, highest RRE in *Ashwagandha* was observed in NPK ( $110.48 \pm 2.94\%$ ) followed by *KJ* ( $109.60 \pm 9.26\%$ ),

similarly highest RRE in *Kalamegha* was observed in *KJ* ( $124.40 \pm 24.10\%$ ) followed by NPK ( $124.38 \pm 15.18\%$ ). A study conducted by Christian indicates, SVI of fresh seeds of *Ashwagandha* treated with  $GA_3$  ( $10^{-5}$  M) was 330.0 [36], whereas the present study reports the SVI (Fig. 9) of *KJ* and *PG* groups was  $209.40 \pm 16.74$  and  $189.68 \pm 23.58$  respectively.

**Table 1**  
Nutrient analysis.

Nutrients	<i>KJ</i>	<i>PG</i>	FYM	HA
Nitrogen (%)	1.822	1.565	0.57	1.12
Phosphate (%)	0.079	0.096	0.039	0.050
Potassium (%)	0.90	0.70	0.25	0.375
Sulfur (ppm)	34.66	25.36	10.29	8.80
Calcium (ppm)	240.00	196.00	48.00	56.00
Magnesium (ppm)	264.00	208.80	73.2	110.80
Zink (ppm)	3.33	2.705	0.945	1.205
Ferrous (ppm)	65.50	52.32	22.42	26.05
Manganese (ppm)	3.18	5.07	1.86	1.96
Copper (ppm)	Traces	Traces	Traces	Traces

*KJ*: *Kunapa jala*; *PG*: *Pancha gavya*; FYM: farmyard manure; HA: humic acid.

## 5. Conclusion

Conclusively, in both the seeds, highest germination was observed in NPK (inorganic) treated group. However, in comparison with organic treated and control groups, *Vrikshayurveda* treatments yielded better results, which can be attributed to their higher nutrient content. To the best of author's knowledge, this is the first comprehensive systematic report on preparation of *KJ* and *PG* and also to comparatively study their effect on germination of *Ashwagandha* and *Kalamegha*. The work also gives a significant statistical implication on the study. Further studies are needed to assess the microbial population responsible for efficacy of *KJ* and *PG*. The efficacy of the *Vrikshayurveda* practices through field studies on these medicinal plants is in progress.

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## Conflict of interest

None.

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