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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A B S T R A C T

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 5.739, 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’, 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 Upavina vinoda, an anthropological compilation called ‘Sharangadhara Paddhati’ written by Acharya Sharangadhara, belonging to the 13th 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
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 Sharangadhar, 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 10th and 15th 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 [17]. The germination behavior of the seeds is one of the most key processes in 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 Sharangadhar [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 31st 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 16th day 5 kg of ghee was added, thoroughly mixed by stirring every day and kept for 5 more days. On 21st day, 10 L each of milk and curd were added and stirred every day till 30th day. On 31st 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% HgCl2 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 ± 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 30th 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 4th day where as Kalamegha seeds treated with KJ and PG germinated on 5th and 6th day respectively. However, faster germination was observed in NPK treated Ashwagandha and Kalamegha seeds, i.e on 3rd and 4th day respectively, whereas germination delayed in both seeds in control group than Vrikshayurveda groups. At the end of 30th day, root length (Fig. 2) and shoot length (Fig. 3) were recorded. In Ashwagandha highest root length was observed in NPK group (3.17 ± 0.13 cm) followed by KJ (3.13 ± 0.17 cm). Similarly in Kalamegha, highest root length was observed in NPK group (3.00 ± 0.14 cm) followed by KJ (2.97 ± 0.17 cm). Results of shoot length indicated, highest shoot length in Ashwagandha was observed in NPK group (2.57 ± 0.97 cm) followed by KJ (2.54 ± 0.95 cm). Similarly in Kalamegha highest shoot length was observed in NPK group (3.37 ± 1.04 cm) followed by KJ (3.30 ± 1.04 cm).

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

In A. paniculata NPK treatment influenced G% (79.49 ± 0.76%) as compared to KJ (78.18 ± 1.49%) and PG (76.09 ± 1.78%) groups. GRI was highest in FYM (3.56 ± 0.03) as compared to KJ (2.87 ± 0.05) and PG (2.33 ± 0.05) groups. EI and SMI were influenced by NPK (6.26 ± 0.15 and 267.54 ± 5.00 respectively) as compared to KJ (4.84 ± 0.08 and 258.00 ± 22.72 respectively) and PG (4.10 ± 0.12 and 242.90 ± 22.34 respectively) groups. RSG was influenced by FYM (155.38 ± 3.04 %) as compared to KJ (125.27 ± 2.94%) and PG (101.93 ± 1.37%) treated groups. RRE was observed to be highest in KJ (124.40 ± 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 Shubha 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 μg/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 GA3 (500 μg/L) applied seeds of Ashwagandha was 86 ± 0.34% [33], wherein present study KJ and PG treated Ashwagandha seeds onset to germinate on 4th day and germination percentage of both the groups was 82.66 ± 0.94% and 76.73 ± 1.77% respectively. Fig. 4 shows, highest germination percentage was achieved in inorganic group (84.00 ± 1.63%) while that in control group was lowest.
(72.46 ± 2.71%). In *Kalamegha*, Rawat et al. reported that, seeds treated with GA3 (75 ppm) began to germinate on 3rd 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 GA3 (200 ppm) [35]. Fig. 4 shows, highest germination percentage was achieved in

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**Fig. 2.** Root length.

**Fig. 3.** Shoot length.

**Fig. 4.** Germinability.
NPK group (79.48 ± 0.72%) followed by KJ (78.18 ± 1.48%) and PG (76.08 ± 1.77%). Fig. 5 shows, highest GRI in Ashwagandha was observed in NPK (2.80 ± 0.05) followed by KJ (2.76 ± 0.03), similarly highest GRI in Kalamegha was observed FYM (3.56 ± 0.03) followed by NPK (3.44 ± 0.03) and KJ (2.87 ± 0.05). Fig. 6 shows, highest EI in Ashwagandha was observed in NPK (4.90 ± 0.06) followed by KJ...
(4.86 ± 0.22), similarly highest EI in Kalamegha was observed in NPK (6.26 ± 0.15) followed by FYM (5.72 ± 0.03) and KJ (4.84 ± 0.08). Fig. 7 shows, highest RSG in Ashwagandha was observed in NPK (126.21 ± 6.03%) followed by KJ (124.21 ± 5.76%), similarly highest RSG in Kalamegha was observed in FYM (155.38 ± 3.04%) followed by NPK (150.50 ± 1.82%) and KJ (125.27 ± 2.94%). Fig. 8 shows, highest RRE in Ashwagandha was observed in NPK (110.48 ± 2.94%) followed by KJ (109.60 ± 9.26%), similarly highest RRE in Kalamegha was observed in KJ (124.40 ± 24.10%) followed by NPK (124.38 ± 15.18%). A study conducted by Christian indicates, SVI of fresh seeds of Ashwagandha treated with GA3 (10⁻⁵ M) was 330.0 [36], whereas the present study reports the SVI (Fig. 9) of KJ and PG groups was 209.40 ± 16.74 and 189.68 ± 23.58 respectively.

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.

Table 1
Nutrient analysis.

| Nutrients   | KJ  | PG  | 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)| 3.31 | 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.
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Conflict of interest

None.

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