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Comparative study of seed germination and seed vigour test in *Andrographis paniculata* (Acanthaceae)

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ABSTRACT: Kalmegh *(Andrographis paniculata* Nees.) is an important medicinal plant which is employed in the Indian traditional system of medicine (Folk medicine), Homeopathy, Ayurveda and Unani system of medicine. Its leaves, stem and inflorescence are used as medicine. Its major bitter principles are diterpenoids - andrographolide and neo-andrographolide. It is useful in the treatment of liver disorders, hepatitis, dysentery, febrifuge etc. The herb kalmegh is also used as a liver tonic, in hyperdispsia, wounds, ulcers, chronic fever, malarial and intermittent fevers, inflammations, coughs, bronchitis, skin diseases, leprosy, colic, flatulence, diarrhoea, dysentery, haemorrhoids. This study of germination revealed that germination was essentially complete after 16 d. KNO_3 and GA_3 improved the seed germination percentage. Germination in the presence of $NaHClO_3$ was rapid, taking only 8 d. Being a medicinal plant, KNO_3 and $NaHClO_3$ treatments are economic and easily applied by nursery workers and poor farmers in developing mass planting stocks.

Key words: *Andrographis paniculata,* cancer therapeutic, diterpenoid compound, kalmegh, germination, seed vigour

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INTRODUCTION

Kalmegh *(Andrographis paniculata* Nees.) "King of bitter", a plant belonging to the Acanthaceae family is one of the medicinal plants recommended for cultivation in India, as there is great demand for the plant by the pharmaceutical industries mainly for export. The theurapeutic benefit of this herb has been attributed to adographolide (alkaloid) and its related diterpenoid compound, i.e. deoxandographolide and neonandrographolide (ICH Committee 1996; Bunyaprapatrasara & Chokecharocopons 1997). Pharmaceutical studies suggest anti-inflammatory (Shen *et al.* 2002; Amroyan *et al.* 1999), antipyretic (Madav *et al.* 1995), antiviral (Chang *et al.* 1991), immunostimulaory (Puri *et al.* 1993), potential cancer therapeutic agent (Rajgopal *et al.* 2003), antihyperglycemic (Bu-Chin *et al.* 2003) and antioxidant (Zhang & Tan 2000) properties. According to Ayurveda medicine the plant is bitter, acrid, cooling, laxative, vulnerary, antipyretic, antiperiodic, anti-inflammatory, expectorant, depurative, soporific, anthelmintic, and digestive.

Due to considerably increased demands by national and international pharmaceutical industries of the active principles of this plant, high production by utilizing improved high yielding varieties is required. However, seed germination is a big problem in this crop due to its wild nature. The information available on seed quality and germination of kalmegh *(A. paniculata*) is very meager. Therefore, enhancing seed germination to produce healthy and vigorous seedlings is crucial to meet current requirements. A systematic study to generate information on standardization of a germination test for improved germination is essential. Therefore, studies were undertaken with the objectives to standardize the testing

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procedure for high germination with healthy/vigorous true seed and also to standardize suitable treatments to improve further germination and provide economic opportunities to produce kalmegh crops.

MATERIALS AND METHODS

Seed. Seeds of *A. paniculata* were collected in November, 2009 from Banka District, Bihar state and National gene bank, CIMAP, Lucknow (U.P.). The seeds were dried for a week at room temperature (25±2°C) and stored in screw capped bottles under ambient conditions before experiment during the following April 2010.

Germination test in Petri dish. The seed germination study of kalmegh (*A.paniculata*) was conducted in a completely randomized design with seven treatments replicated thrice on two varieties (CIM-Megha and a locally-collected genetic stock). For germination tests air-dried seeds were disinfected first in 0.04% $HgCl_2$ and washed thoroughly.

Observations. Observations were made on number of days for germination required for the first count; radical length, plumule length, germination initiation and percentage for the two varieties and data were analysed using ANOVA.
Germination percentage = (Total number of seeds germinated/Total number of seeds in all replicates) × 100

Tetrazolium test. The study was conducted in 2009-2010 in a completely Randomized Design with treatments described below and replicated thrice. To ensure that seeds used for the experiment were viable, a seed viability test using the tetrazolium technique (Grabe 1970) was conducted. Three replicates of 50 seeds each were tested using the procedure of Peter (2000). Seeds were imbibed for 24 h in water, cut along the margin without damaging the embryo and soaked in a 0.1% solution of 2,3,5-triphenyl tetrazolium chloride solution for 18 h at 25°C in the dark (Nkomo & Kambizi 2009). The seeds were removed from the TTC solution and washed with distilled water. Seeds were then viewed under a light microscope to observe the stained embryos. Viable seeds appeared bright red in colour. The tetrazolium test showed 80.0± 8.8 and 87.8± 8.4 for the Wild seeds and CIM-Megha respectively.

Germination Experiment. The germination tests were done in 9cm sterile Petri dishes lined with Whatman No.1 filter paper. These were moistened using distilled water (control), gibberellic acid GA_3 (200ppm), potassium nitrate KNO_3 (150mM), sodium hypochlorite $NaHClO_3$ (30min), conc. sulphuric acid H_2SO_4 (120s), sodium chloride NaCl (0.5%) and heat treatment (80°C for 2 min). Each treatment had three replicates of 50 seeds, each in a randomized design under laboratory conditions. Average temperature during germination tests was about 31±2.1°C and relative humidity was 69-71% monitored daily. Germination was recorded after 4 and 16 d. The final germination time was calculated by using the relation:

$MGT=\Sigma (fx)/\Sigma X$

Where,

X=No. of newly germinated seeds on each day

f=No. of days after seeds were set to germinate

RESULTS AND DISCUSSION

Thirty dried seeds of *A. paniculata* were used to determine average fresh weight: 0.077±0.0015 g (wild) and 0.0703±0.0015 g (variety CIM-Megha); length 1.5 mm; width 1mm and thickness 1 mm. Screening of 100 seeds showed that 85% were healthy and 14% shrunk and 1% insect damaged. Significant differences were observed due to the treatments. Among the various treatments, the highest germination (99.2% and 88.3%) in the variety CIM-Megha and wild, respectively was expressed after treatment with GA_3 (200 ppm). The next best treatment was KNO_3 (150 mM) i.e. 97.3% and 85.3% in CIM-Megha and Wild variety, respectively. Treatment with $NaHClO_3$ (30 min) resulted in 88.2% and 81.3% germination in variety CIM-Megha and the Wild collection, respectively (Fig 1). In the Nursery test $NaHClO_3$ (30 min) gave the highest germination percentage i.e. 85.0% and 76.3% in the variety CIM-Megha and Wild stock, respectively. Germination declined to 78.4 and 65.3% in the variety CIM-Megha and Wild collection using KNO_3 (150 mM), while applying GA_3 (200 ppm) resulted in germinations of 86.3% and 68.2%, respectively (Fig 2). Under laboratory conditions, KNO_3 (150 mM) was significantly effective, possibly through oxidized forms of nitrogen causing a shift in respiratory pathway resulting in 5.5cm and 5.1 in variety CIM-Megha and the Wild collection, respectively. GA_3-treated seeds showed the highest plumule length of 5.7 cm and 4.5 cm in Wild and CIM-Megha variety which was closely followed by hot water treatment at 80°C for 2 minutes (5.6 cm and 4.4 cm) in Wild and CIM-Megha, respectively. Plumules at first were spirally coiled, then became straight. In the present study, seeds treated with $NaHClO_3$ turned green, which has not previously been reported in laboratory condition, and in the nursery test full germination was recorded in only 8 d, thus confirming its role as a stimulatory agent.

Krause (1988) reported that soaking seeds for 24-48 h in water at 30°C accelerated the germination of *Catharanthus roseus* (L.) G.Don. seeds. Germination improvement in *Tephrosia purpurea* (Linn.) Pers. was achieved by scarification with sand followed by pre-soaking in hot water at 50°C for five min (Sundraraj *et al.* 1971).

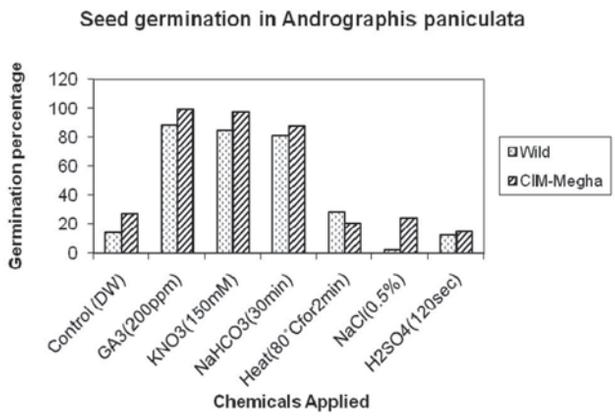

Fig 1. Germination percentage of Wild genetic stocks and CIM-Megha variety of *A. paniculata* given control, heat and chemical treatments in Petri dishes.

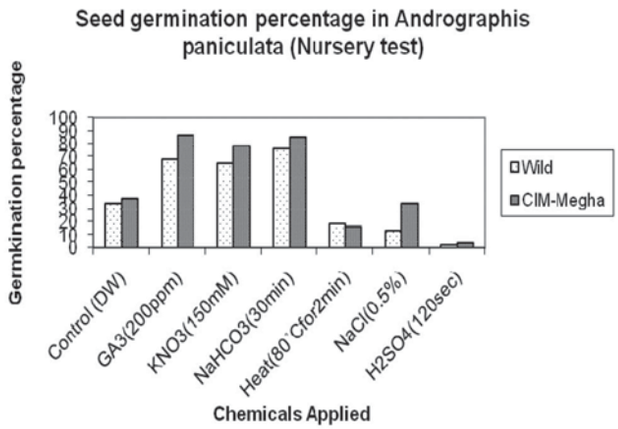

Fig 2. Germination percentage of Wild genetic stocks and CIM-Megha variety of *A. paniculata* given control, heat and chemical treatments in a Net House Nidzo molim te ispravi na y osi u Germination (%) na Fig 1 i 2

According to Yadav *et al.* (2008), scarification treatment not only increased germination with enhanced hypocotyl and radicle growth but also resulted in significant reduction in the total germination time compared with the control. The seeds of most plant species are dormant due to a hard seed coat, which is impermeable to water and gases (Kohli & Kumari 1986). From the work of Thomas (1994) on *Cassia sophera* L., it is obvious that the dormancy of seeds may be exogenous and coat imposed. The seed coat plays a key role in *C. sophera* because it prevents water uptake. *Phragmites karka* Trin. ex Steud. showed 100% germination in NaCl at 15-25°C (Zehra & Khan 2007) whereas in the present germination in NaCl was 24.3% in CIM-Megha and only 2.8% in the wild variety. Potassium nitrate can act as a substitute for light (Copeland 1983) and the germination enhancing effect of KNO_3 was attributed to an increase in cytochrome oxidase activity (ISTA 1976). In the present study, KNO_3 (150mM) gave the highest germination and under the Nursery test in the Net House $NaHClO_3$ also gave a good response. Thus, being a medicinal plant, KNO_3 and $NaHClO_3$ treatment are economic and easily applied by nursery workers and poor farmers to develop mass planting stock compared with costly plant growth regulators and associated technical use in rapid multiplication.

CONCLUSION

These treatments may help in producing germinating seedlings giving them a higher competitive ability and hence reducing chances of their mortality rate throughout year. The study showed that GA_3, KNO_3 and $NaHClO_3$ gave good responses in Laboratory conditions, followed by good results also in a nursery test. Thus, being a valuable medicinal plant, KNO_3 and $NaHClO_3$ treatment would be economic and easily applied by nursery workers and low income earning farmers in developing mass planting stock/planting material. In the study area, generally natural germination of this plant begins towards the rainy season in Northern plains in India only. In this experiment, *Andrographis paniculata* seeds germinated and seedlings were established in summer. This was done to ensure availability of planting material/fresh plants in sufficient quantity for more than one season.

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REZIME

Uporedna studija klijanja semena *Andrographis paniculata* (Acanthaceae)

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Andrographis paniculata Nees. je važna lekovita biljka koja se koristi u indijskoj tradicionalnoj medicini, homeopatiji, te lakalnim medicinama Ajuveda i Unanija. Koriste se listovi, stablo i cvasti ove biljke. Glavni gorki sastojci su diterpenoidi – andrografolidi i neo-andrografolidi. Ova biljka korisna je u tretmanu obolele jetre, hepatitisa, dizenterije i drugih bolesti. Ova studija pokazuje da je klijanje semene ove biljke znatno poboljšano pose 16-o dnevnog tretmana semena sa KNO3 i GA3. Klijanje je ubrzano osmodnevnim tretmanom sa NaHClO3. KNO3 i NaHClO3 tretman se lako rimenjuje u odgajalištima ove ekonomski važne biljke.

Ključne reči: *Andrographis paniculata*, klijanje, semena