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RESEARCH PAPER

Seed germination studies in endemic, medicinal plant-*Achyranthes coynei* Santapau

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Abstract: *Achyranthes coynei* (family Amaranthaceae) is a perennial shrub reported to be a rare medicinal and endemic plant species from India. Locally it is known as "*Kempu Uttarani*". Different parts of the plant were used to treat variety of disorders by traditional practitioners. The species is reported to contains triterpenoids which are known for anticancer, antioxidant, antimicrobial properties. The present study was undertaken to evaluate the seed germination under different pre sowing treatments. Total ten pre sowing treatments were used to check the effect on *Achyranthes coynei* seed germination collected from Belagavi and Dharwad districts. The germination parameters were noted along with growth attributes. The seeds of the species show germination of 79.33 per cent in control. The maximum germination percentage (91.00%) and mean daily germination (7.58) were recorded in the seeds treated with 25 ppm gibberellic acid (GA_3). The maximum peak value (14.67) and germination value (106.09) were recorded in the seeds treated with 1 percent Potassium nitrate (KNO_3). Hot water treatment at 100°C for 2 minutes resulted in no germination in *Achyranthes Coynei*. The study suggests that treatments with GA_3 (25 ppm) and 1 per cent KNO_3 are the most effective in improving germination percentage and seedling vigor. Conversely, hot water at 100°C, are detrimental to seed viability. Chilled water treatment and higher concentrations of KNO_3 and GA_3 show less pronounced benefits, highlighting the importance of optimizing treatment conditions for the best germination outcomes.

Key words: *Achyranthes coynei*, Germination value, Gibberellic acid, Pre-sowing treatments, Seed germination

Introduction

Achyranthes coynei (family Amaranthaceae) is a rare and endemic perennial shrub, was reported mainly from Maharashtra, Karnataka and Rajasthan states. The species is locally known as "*Kempu Uttarani*" in Kannada and "*Lal Aghada*" in Marathi. It grows 2 to 4.5 meters tall, has pink petioles, a woody stem, and elliptic leaves that get smaller towards the top. Its flowers are rosy or purplish and arranged in spikes (Pai *et al*., 2011; Satish *et al*., 2015).

Folk healers have been documented the use of *Achyranthes coynei* to treat a variety of human diseases, including fever, cough and piles. Furthermore, the roots are utilized to treat general illness and intestinal worms (Ankad *et al*., 2015; Bhogaonkar and Devarkar, 2002). *Achyranthes coynei* leaves are known to have antibacterial and antioxidant effects (Upadhya *et al.,* 2013). The plant's leaves, stems, roots and inflorescences contain triterpenoids *viz*., betulinic, ursolic and oleanolic acids (Upadhya *et al.,* 2014). These chemicals are known for their anticancer, antioxidant, antibacterial and anti-inflammatory effects (Upadhya, 2014). The literature review revealed a lack of extensive research on pre-sowing treatments for seed germination in *Achyranthes coynei (*Pai and Upadhya 2023*)*. Therefore, this study was undertaken to understand the effects of different treatments on germination.

Material and methods

Seed collection

Mature seeds of *Achyranthes coynei* were collected from Belagavi and Dharwad districts in Karnataka (Plate 1). Using the sinker and floater method (Elhindi *et al*., 2016), 100 seeds from each plant were selected after de-husking and pooling. Seeds that sank were used for germination tests. Ten treatments (T_1 as control) with three replications were applied, as detailed in Table 1. After treatment, 100 seeds were placed on top tissue paper in petri plates and kept in a dark chamber at room temperature. Germination was monitored daily for 7 days, then every alternate day up to 15 days (Plate 2), using a Completely Randomized Design.

Treatment details

In treatment T_1 (control), seeds were sown without pre-treatment. For T_2, seeds were soaked in cold water for 24 hours (hrs) and for T_3, they were soaked in hot water at 50°C for 2 minutes (min). In T_4, seeds were immersed in boiling water at 100°C for 1 minute, while in T_5, they were soaked in chilled water at 5°C for 24 hours. Treatments T_6, T_7 and T_8 involved soaking seeds in 25 ppm, 50 ppm and 100 ppm gibberellic acid (GA_3) solutions, respectively, for 24 hours. In T_9, seeds were soaked in a 1 percent potassium nitrate (KNO_3) solution, and in T_{10}, in a 2 percent KNO_3 solution, both for 24 hours (Plate 2).

Laboratory studies were carried out at College of Forestry, Sirsi. Germination was monitored daily for 7 days, then every other day up to 15 days. Based on daily germination counts, the following parameters were computed:

Germination percent (GV)

The total number of seeds that germinated normally by the end of the study period was counted and cumulative germination was expressed as a percentage of the total seeds sown. Germination percentage was calculated using the formula prescribed by the International Seed Testing Association (ISTA) (Anon, 2011).

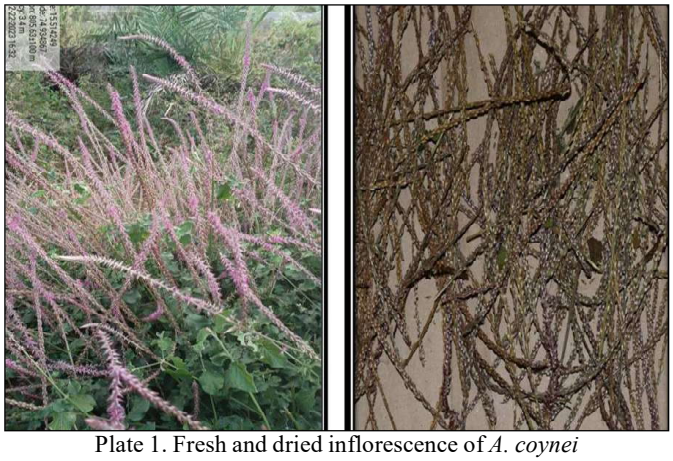

Plate 1. Fresh and dried inflorescence of *A. coynei*

Plate 2. Germination of *A. coynei* seeds

Germination percent = (Number of seeds germinated/Total number of seeds sown)x100

Mean Daily Germination (MDG)

Total germination was expressed as mean daily germination calculated by the cumulative percentage of full seed germination at the end of the test. Mean daily germination was estimated by adopting formula prescribed by ISTA (Anon, 2011).

Mean Daily Germination = Final Germination (percent)/ Total number of days of test

Peak Value (PV)

Peak value refers to maximum mean daily germination reached at any stage of germination period and calculated using the formula given by Czabator (1962)

Peak value = Peak germination (percent) / Days on which maximum germination occurred

Germination value (GV)

The concept of germination value, as defined by Czabator (1962), aims to combine in a single figure an expression of total germination at the end of the test period with an expression of germination energy or speed of germination. It is an index combining speed and completeness of germination. It was calculated using the formula given by Czabator (1962).

Germination value (GV) = PV x MDG

Table 1. Details of pre-sowing seed treatments

Treatment Numbers	Treatment details
T1	Control
T2	Cold water for 24 hrs
T3	Hot water (50°C) for 2 min
T4	Hot water (100°C) for 1 min
T5	Chilled water (5°C) for 24 hrs
T6	25ppm GA_3 for 24 hrs
T7	50ppm GA_3 for 24 hrs
T8	100ppm GA_3 for 24 hrs
T9	1% KNO_3 for 24 hrs
T10	2% KNO_3 for 24 hrs

Shoot length

The shoot length of each seedling was measured from the collar region to the apex of the shoot. The mean shoot length of ten seedlings from each replication was calculated and expressed in centimeters (cm).

Root length

The root length was measured from the collar region to the tip of the tap root. The average root length of ten seedlings was calculated and expressed in centimeters (cm).

Results and discussion

The results pertaining to various germination parameters of *Achyranthes coynei viz*. germination percent, mean daily germination, peak value and germination valueas influenced by various treatments are represented in Table 2.

Germination percent

The highest germination rate (91%) was observed in seeds treated with 25 ppm GA_3 (T6), followed by 1 percent KNO_3 treatment (T_9) (86.67%). Both treatments were similar in

Table 2. Effect of pre-sowing seed treatments on germination parameters of *Achyranthes coynei*

Treatment	Germination Percent	MDG	PV	GV
T_1- Control	79.33 (63.05)	6.67	12.89	85.30
T_2- Normal water soaking for 24 hrs	85.33 (67.55)	7.11	12.33	88.11
T_3- Hot water (50°C) for 2 min	78.33 (62.37)	6.56	13.78	90.13
T_4- Hot water (100°C) for 2 min	0.00 (0.00)	0.00	0.00	0.00
T_5- Chilled water (5°C) for 24 hrs	63.00 (52.55)	5.25	8.55	45.06
T_6- 25 ppm GA_3 for 24 hrs	91.00 (72.61)	7.58	12.89	97.78
T_7- 50 ppm GA_3 for 24 hrs	85.67 (67.77)	7.14	12.33	88.03
T_8- 100 ppm GA_3 for 24 hrs	81.00 (64.16)	6.75	12.45	84.01
T_9- 1% KNO_3 for 24 hrs	86.67 (68.64)	7.22	14.67	106.09
T_{10}-2% KNO_3 for 24 hrs	79.67 (63.24)	6.64	13.00	86.27
S.Em. (±)	1.76	0.15	0.76	6.36
C.D.@5 %	5.23	0.45	2.26	18.91

Note: Values in parenthesis indicates arc sine transformed values

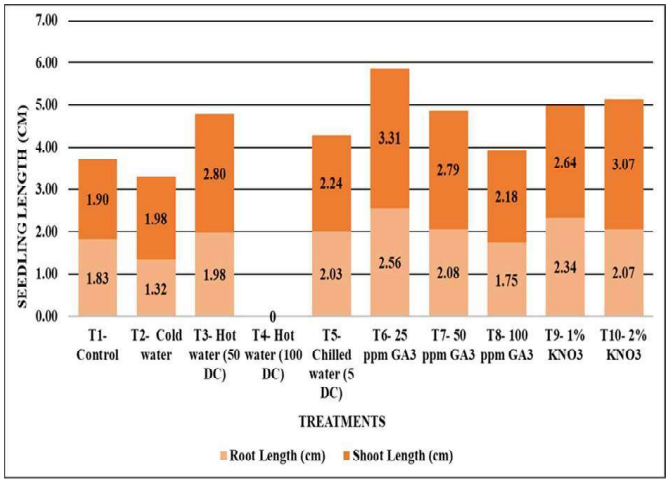

Fig 1. Influence of various pre-sowing treatments on root and shoot length attributes of *Achyranthes coynei*

effectiveness. Soaking seeds in water for 24 hours (85.33%) and in 50 ppm GA*f* (85.67%) also produced better results, while hot water treatment at 100°C (T_4) led to no germination.

Mean Daily Germination (MDG)

The highest mean daily germination was in seeds soaked in 25 ppm GA_3 (7.58), followed by 1 per cent KNO_3 (7.22) and 50 ppm GA_3 (7.14). These treatments were notably more effective than others. No germination was observed for seeds in 100°C hot water. Soaking in 100 ppm GA_3, 2 per cent KNO_3 and 50°C hot water showed values comparable to the control, while chilled water treatment (5.25) had lower germination than the control (6.61) (Table 2).

Peak value (PV)

The highest peak germination value (14.67) was recorded with 1% KNO_3 treatment (T9), followed by 50°C hot water for 2 minutes (13.78, T3) and 2% KNO_3 (13, T10), showing superior results compared to other treatments. Chilled water treatment had the lowest peak value (8.55), indicating it was less effective, while the values for T2, T6, and T8 were similar to the control (12.89).

Germination value (GV)

The germination value varied dramatically between treatments. The seeds treated with 1 percent KNO_3 for 24 hours showed the highest germination value (106.09), followed by seeds soaked in 25 ppm GA_3 for 24 hours (97.77). The seeds were treated with 50°C hot water for 2 minutes (90.13) and the treatments (cold water and 50 ppm GA_3 for 24 hours) were found to be on par with each other. However, these treatments were significantly superior than the other treatments.

Shoot length (cm)

The various pre-sowing treatments showed a considerable impact on shoot length at 15 days of intervals. Seeds treated with 25 ppm GA_3 for 24 hours resulted in the longest shoots (3.31 cm). This was followed by soaking seeds in 2 percent KNO_3 for 24 hours (3.07 cm) as mentioned in figure 01 and plate 3.

Root length (cm)

Seed treatments had a significant impact on root length. Seeds treated with 25 ppm GA_3 for 24 hours yielded the longest root length (2.56 cm). This was followed by seeds soaked in 1 percent KNO_3 for 24 hours (2.34 cm). The seed that was immersed in cold water (1.32 cm) and 100 ppm GA_3 (1.75 cm) entire day had the smallest root length when compared to the control (1.83 cm) as mentioned in figure 01 and plate 3.

The seed treated in 25 ppm gibberellic acid for 24 hours produced highest seed germination (91%) and also mean daily germination rate (7.58). Tiwari and Kumar (2020) reported that 25 ppm GA_3 was responsible for the higher germination percentage in *Ocimum basilicum*. Bojovic *et al*. (2022), reported

Plate 3. Shoot and Root lengths in different treatments

a maximum germination rate (65%) in *Ocimum basilicum* seeds treated with 50 ppm GA_3 .

Gibberellic acid (GA_3) enhances seed germination by activating enzymes that increase cell wall plasticity, improve water absorption, and stimulate amylase production, which converts starch into sugars, promoting radicle growth (Gillard and Walton, 1973).

In this study, seeds treated with hot water at 100°C showed no germination, suggesting that extreme heat hampers germination by damaging the seed embryo or inhibiting essential enzyme activity (Steckel *et al*., 2004). Similarly, Wambugu and Nyamongo (2010) noted that temperatures above 80°C can harm the seed embryo, preventing germination.

One percent KNO_3 treatment yielded an 86.67 percent germination percentage, which is comparable to 24-hour treatments with 25 ppm and 50 ppm GA_3 , which resulted in 91 percent and 85.67 per cent germination, respectively. Bhandari *et al*. (2020) found that a 5 per cent KNO_3 treatment led to 46 percent, 90 per cent and 94 per cent germination in *Saussurea costus*, *Withania somnifera* and *Ocimum sanctum*, respectively, showing that varied KNO_3 concentrations can enhance germination.

In *Achyranthes coynei* seedlings, the 25 ppm GA_3 treatment resulted in the highest shoot length (3.31 cm) and root length (2.56 cm), indicating GA_3 's effectiveness in promoting early growth through enhanced cell division in the apical meristem (Das *et al*., 2017). Similarly, Kumari *et al*. (2011) observed 100 ppm GA_3 producing the tallest gladiolus seedlings (56.20 cm), as gibberellins promote cell division and elongation (Singh *et al*., 2018).

Conclusion

The study focusses on seed germination in *Achyranthes coynei* concludes that application of 25 ppm GA_3 treatment significantly improves seed germination and helps in quality seedling production in *Achyranthes coynei*. Hence, GA_3 treatment are crucial for optimizing seed dormancy and improving the success rate of seed germination in this species. This study will help in further germination aspects for *Achyranthes coynei* and related species.

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