

Allelopathic action of *Rauwolfia tetraphylla* L. root extracts on gram (*Cicer arietinum* L.) seeds

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ABSTRACT

Allelopathy refers to the advantageous or detrimental effects of one plant on another plant by liberating the chemicals from its components through leaching, root exudation, volatilization, residue decomposition and others in natural and agricultural systems. The present study has been made to appraise the allelopathic action of *Rauwolfia tetraphylla* L. touching to the alteration of germination, exaggeration of seedling and biochemical actions in gram seed (*Cicer arietinum* L.). Various concentrations of (12.5, 25, 50, 100, 150, and 200mg ml⁻¹) aqueous extracts were prepared from root of *R. tetraphylla* L. and germination studies were conducted. The medium concentrations (50mg ml⁻¹ and 100mg ml⁻¹) of root extracts stimulated the seed germination, growth and biochemical constituents (total sugar, protein, amino acid and DNA and RNA concentrations) of gram. In higher concentrations a more or less stagnant nature was observed in all the parameters after T₄. A significant variation is found among all treatments considering seedling parameters where, seed treatment is always better than control. In biochemical observations, the view is more or less same where T₄ (100mg ml⁻¹) is indicating highest performance excepting fresh and dry weight. The enhanced action in occurrence of root extract can be supportive for early establishment of seedlings especially in water stress location. Therefore, the said treatment can be considered as an invigoration treatment under seed/crop production programme.

Keywords: Allelopathy, biochemical, gram and *Rauwolfia tetraphylla*

Bengal gram or chickpea or gram (*Cicer arietinum* L.) is one of the significant pulse crops in India. It ranks 5th among grain crops (Smithson *et al.*, 1985), and is imperative due to its high nutritive contribution in Indian diet representing the 100g of seed contains 357 calories, 4.5-15.69% moisture, 14.9-24.6g protein, 0.8-6.4 % fat, 2.1-11.7g fiber, 2-4.8g ash, 140-440mg Ca, 190-382mg P, 5.0-23mg Fe, 0.21-1.1mg thiamine, 0.12-0.33mg riboflavin, and 1.3-2.9mg niacin (Duke, 1981; Huisman and van der Poel, 1994). In 2007-08, India produced 5.75 million tonnes considering the average productivity 1448kg ha⁻¹ (Fresh Portal Pvt. Ltd.). But the efficiency in India is far behind as compared to world productivity. So there is a tremendous scope for enrichment of Gram seed production in our region. In farming of this significant crop, the efficiency is deteriorated due to extreme scarce of quality seed. Seed quality may adversely be affected as a result of early desiccation resulting in high levels of green immature and smaller seed, seed-coat cracking under several wetting and drying cycles or during harvesting and handling processes. To modify the seed quality in terms of seed vigour and viability, several works have been done.

The aim of the present work is to evaluate the effect of an important responsive botanicals for enhancement of seed strength through seedling set up as well as seedling vigour which can reflect its ultimate effect in productivity more specifically on quality seed progress. The root extract of *Rauwolfia*

tetraphylla L., a small much-branched woody shrub, contains the alkaloid rauvoscine in 0.1%. The dry root bark is used for medicinal purpose in small dose. The extract of root has been used as allelopathic treatment on some crops for invigoration of the seedlings (Ghayal *et al.* 2011). Therefore, it is considered as a priming treatment object for invigoration of gram (*Cicer arietinum* L.) seeds.

MATERIALS AND METHODS

The roots of matured plant of *Rauwolfia tetraphylla* were collected from the field of Bidhan Chandra Krishi Viswavidya and the extraction procedure was carried out in RKVY laboratory, Department of Seed Science and Technology. The amount of 5g root was extracted in distilled water to attain 10 ml in ultimate. The extract was centrifuged at 5000 rpm for 15 minutes and the supernatant was collected (500mg ml⁻¹). From this extract the treatments were prepared into 6 different concentrations like 12.5mg ml⁻¹ (T₁), 25mg ml⁻¹ (T₂), 50mg ml⁻¹ (T₃), 100mg ml⁻¹ (T₄), 150mg ml⁻¹ (T₅), and 200mg ml⁻¹ (T₆).

Gram seeds (cv. B-108) were surface sterilised by 0.1% HgCl₂ for 2 minutes and then they were repeatedly washed by distilled water. After that, the seeds were treated for overnight by the said treatments including normal water (C) as control. Then the seeds were evaluated through Glass-Plate method (Chakraborti, 1994) under 3 replicated aseptic condition considering germination percentage in first count, root-shoot length, fresh wt., dry wt. (100°C for

24hrs.), vigour index etc. as seedling parameters at 7th and 14th day. The biochemical characters like total soluble sugar (Gready *et al.*, 1950), soluble protein (Lowry's method), total amino acid content (Moore and Stein, 1948), DNA and RNA (Chowdhury and Chatterjee, 1977), were estimated on 14-days old seedlings (day of final count of gram) which is considered as ultimate day for utilization of food reserves. The statistical calculations were done through Completely Randomized Design (CRD). The outcome was achieved at 5% level of significance by using MS Excel.

RESULTS AND DISCUSSIONS

The results of Table-1, indicated that the higher concentrations of aqueous root extract of *R. tetraphylla* amplified the germination percentage of *C. arietinum* seed at the day of initiation of germination connecting to active participation in germination system though after T₄ it was abruptly reduced. Obviously, the rate was slower in later days though it maintained insignificant value of germination at the end (average) excepting in T₆ and control. The observation on first count at germination can also considered as seed vigour which was very much supportive to seedling establishment in field.

Table-1: Effect of *R. tetraphylla* root extracts on gram seed germination

Treatments	Seed germination (%)			
	3 rd day	4 th day	5 th day	Total
Water	71.40	10.70	7.08	89.18
T ₁	75.40	12.50	3.80	91.70
T ₂	80.56	9.06	4.07	93.69
T ₃	83.60	7.03	3.10	93.73
T ₄	87.30	5.20	1.27	93.77
T ₅	78.70	5.60	5.80	90.10
T ₆	72.20	10.50	6.40	89.10
SEm (±)	0.75	0.30	0.07	0.53
LSD (0.05)	3.16	1.25	0.29	2.22

The representing seedling characters in Table-2 indicated that the length of root as well as shoot was increased with higher concentration of aqueous solution (root extract of *R. tetraphylla*) up to T₄ in both 7 and 14 days; which showed insignificant afterwards. The ratio of root and shoot indicated the rapid growth of root in alliance with root extract at 7th day while this association at 14th day clearly indicated higher growth rate of shoot than control. But longer seedling clearly indicated the superior 'vigour index' where maximum effect came from last three concentrations of root extract, maintaining an insignificant variation at last two stages.

Table 2: Effect of *R. tetraphylla* root extracts on seedling parameters of gram

Treatments	At 7 th day				At 14 th day			
	Root length (cm)	Shoot length (cm)	Root: shoot	Vigour index	Root length (cm)	Shoot length (cm)	Root: shoot	Vigour index
Water	4.55	4.14	1:0.91	774.97	8.5	4.9	1:0.58	1195.91
T ₁	4.59	4.19	1:0.91	805.13	8.6	4.9	1:0.57	1237.95
T ₂	5.10	4.50	1:0.88	900.34	9.2	5.0	1:0.54	1331.82
T ₃	5.30	5.00	1:0.94	966.45	9.25	6.5	1:0.7	1477.82
T ₄	5.80	5.20	1:0.9	1041.50	10.76	7.0	1:0.65	1665.34
T ₅	5.82	5.12	1:0.88	985.70	10.84	7.2	1:0.66	1625.41
T ₆	5.70	5.14	1:0.90	965.85	10.8	7.1	1:0.66	1594.89
SEm (±)	0.07	0.04	--	7.94	0.12	0.07	--	13.64
LSD (0.05)	0.30	0.15	--	33.41	0.49	0.28	--	57.44

The similar trend was also observed in Table-3 indicates that the higher concentration of aqueous extracts of *R. tetraphylla* boost the fresh and dry weights of the sample due to greater accumulation of dry matter in healthy seedlings. But it was contrasting that the accumulation of dry matter was rising significantly with higher concentration in a

continuous way excepting in an insignificant nature in last two cases at 7th day of fresh wt. similar to insignificant nature of last three treatments of other seedling parameters (Table 2).

Table 3: Effects of *R. tetraphylla* root extracts on seedling weight of gram

Treatments	Fresh weight (g)		Dry weight (g)	
	7 th day	14 th day	7 th day	14 th day
Water	0.49	1.18	0.03	0.06
T ₁	0.50	1.18	0.03	0.06
T ₂	0.52	1.23	0.03	0.06
T ₃	0.55	1.24	0.04	0.06
T ₄	0.59	1.26	0.04	0.06
T ₅	0.62	1.29	0.04	0.07
T ₆	0.64	1.32	0.04	0.07
SEm (±)	0.006	0.004	0.001	0.001
LSD (0.05)	0.026	0.015	0.002	0.002

The total soluble sugar content constructed an increasing trend with raising the concentration of root extract of *R. tetraphylla* though last three concentrations 100mg ml⁻¹ (T₄), 150mg ml⁻¹ (T₅), and

Table 4: Effects of *R. tetraphylla* root extracts on activity of bio-molecules (at 14th day) of gram

Treatments	Total soluble sugar (µg mg ⁻¹)	Soluble protein (µg mg ⁻¹)	Amino acid (µg mg ⁻¹)	DNA (µg mg ⁻¹)	RNA (µg mg ⁻¹)
Water	0.23	0.06	0.30	0.10	0.07
T ₁	0.27	0.09	0.45	0.10	0.10
T ₂	0.37	0.12	0.65	0.13	0.12
T ₃	0.51	0.14	0.75	0.14	0.15
T ₄	0.62	0.22	0.89	0.15	0.19
T ₅	0.61	0.20	0.86	0.14	0.15
T ₆	0.59	0.19	0.84	0.13	0.15
SEm (±)	0.02	0.01	0.01	0.01	0.01
LSD (0.05)	0.07	0.03	0.06	0.04	0.03

The different concentration of root extracts stimulates the activity of germinating seeds which can be considered as example of allelopathy. The higher conc. was best though their activity was stagnant or decline after T₄ *i.e.* 100mg ml⁻¹ except fresh and dry weight. The early germination of seed and formation of new surface root initials (lateral roots) from primary root in short duration crop is also vital with the primary root length and dry matter accumulation which may be able to reach and exploit localized patches of nutrients in the soil (Lynch, 1995), to establish the healthy seedlings. After T₄ treatment, the stagnant nature is probably due to formation of more lateral roots. But the rapid sequence of cell division for progress of morphological characters, particularly root is very much linked to various biochemical markers (Tinus *et al.*, 2000) where T₄ was the best. Therefore, T₄ or 100mg ml⁻¹ can be considered as best for getting the most amplified seedlings in field establishment.

200mg ml⁻¹ (T₆) showed insignificant (Table 4). The last three treatments were indicating the synthesis of more carbohydrate in rapid way in contrast to other may be due to the activity of hypogeous cotyledon in *exalbuminous* seed where it adapted for both storage and photosynthesis (Marshall and Kozlowski, 1976). The same trend was also observed in the content of protein and amino acid *i.e.*, the higher inclination was observed with higher concentrations of root extract up to T₄. This may be due to the additional synthesis of the protein material as well as amino acid responsible for cellular activity closely related to the seedling growth at their final day of count (14 days) under last three concentrations of root extract.

In estimation of DNA, the rate was insignificant while in RNA content, the trend was increasing up to T₄. In later stages, the inclination was negative may be due to condensed rate of cell division in formation of growth of root and shoot.

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