Allelopathic Influence of *Rauwolfia tetraphylla* L. for Enhancing the Seedling Root Vigour of Gram (*Cicer arietinum* L.)

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**ABSTRACT**

**Background:** Advanced genotype may not expose properly due to unhealthy seedling of low-vigour seed which can be upgraded through seed invigoration. The advanced seedling performance primarily depends on steady rhizospheric configuration though roots are least known and least acceptable part in crop study schedule particularly at germination. In existing study, the rhizosphere pattern and seedling performances are evaluated through allelopathic action on seed-vigour. Different seedling parameters with categorized rhizosphere pattern specified the effective role of allelopathy.

**Methods:** The allelopathic action of *Rauwolfia tetraphylla* L. root extracts was assessed on alignment of rhizosphere pattern in gram seedling (*Cicer arietinum* L.). Various aqueous root extracts (200, 150, 100, 50, 25 and 12.5 mg ml⁻¹) were prepared from young roots of *Rauwolfia tetraphylla* L. and the action was observed at 8th and 14th day on seedling especially on root after overnight soaking of seed.

**Result:** Significant variation was found in diverse seedling and root parameters. The roots extract (100 mg ml⁻¹) stimulated total root length, surface area etc. which was also legitimated in shoot length, vigour index of the seedling. In most cases, allelopathic actions on seed enhanced the quality through modification of rhizosphere pattern with seedling performances than control. Therefore, it can be considered for seed invigoration under Gram seed/crop production programme.

**Key words:** Allelopathy, *Cicer arietinum*, *Rauwolfia tetraphylla*, Root extracts, Rhizosphere.

**INTRODUCTION**

The enrichment in crop productivity as well as seed production system depends on several factors like genotype, cultivation method and environmental fluctuation in addition to quality seed. But in cultivation, the exploitation of the superior genotypes is not done properly due to establishment of unhealthy seedling developed from low-grade seed. The plant growth determining seedling primarily depends on steady rhizospheric configuration liable parameters like total root length, surface area, root types etc. The creation of good root system can initiate healthier seedling accommodating the good plant which can confirmed its presence in yield (Mohammadi et al., 2011).

It seems fully reasonable that some of the best-yielding crops are competent to beat others due to their superior efficiency in securing a better and steady supply of water and nutrients. On the other hand, the failure of a crop to prosper in a particular soil may be due to lack of adaptation of its root system to the environment compelled upon it. But the roots are least known, least understood and least acceptable part of the plant in crop study schedule particularly at the stage of seedling or initial germination. In existing study, the linking in between rhizosphere pattern and seedling performances properly exposed the allelopathic effect on seed.

Root length, diameter and distribution are important characteristics to be considered at describing and comparing root systems. Critical root length and variable root-diameter can be achieved through computerised analysis. Using WinRHIZO, a root analysis software package, we performed sensitive scanning protocols for rapid assessment of root characteristics like length and surface area, diameter and tips, root branching patterns, etc. Root length and surface area are important indicators for a potential to uptake the water and nutrients as well as most important input parameters for rhizosphere modelling (Kaushik and Chakraborti, 2019). The aim of the present work is to evaluate the allelopathic effect of phytochemicals related to rhizospheric pattern that may reflect its ultimate effect on productivity and quality of the produce. The advantageous or destructive properties of one plant on unlike plant through discharge of chemicals from any constituent in the manner of leaching, root exudation, volatilization, breakdown of the remaining and other processes under both usual and agricultural systems is referred as Allelopathy. The allelopathic treatment may inhibit (Terzi, 2008; Scognamiglio et al., 2013) or promote (Kocaçalýskan and Terzi, 2001;
Wang et al., 2018) the seedling parameters of a few crops (Inderjit et al., 2005a; b) in which the study on root pattern is very meagre. Therefore, root extract of Rauwolfia tetraphylla L. is used to identify the outcome on root model of gram (Cicer arietinum L.) seedlings in present investigation.

MATERIALS AND METHODS
The experiment was conducted on December, 2017 after collection of Rauwolfia tetraphylla roots from field and the extraction was carried out in laboratory, Dept. of Seed Science and Technology, Bidhan Chandra Krishi Viswavidyalaya (BCKV). An amount of 5g extracted root was mixed with distilled water to attain 10 ml in ultimate. The extracted material was centrifuged at 5000 rpm for 15 minutes and the supernatant was collected as base solution.

Six variable treatments were used from the base solution viz. 200 mg ml\(^{-1}\) (T\(_1\)), 150 mg ml\(^{-1}\) (T\(_2\)), 100 mg ml\(^{-1}\) (T\(_3\)), 50 mg ml\(^{-1}\) (T\(_4\)), 25 mg ml\(^{-1}\) (T\(_5\)), 12.5 mg ml\(^{-1}\) (T\(_6\)) in addition to control (T\(_0\), water). Gram seeds of Kabuli type (cv. B-108) were surface sterilised and the seeds were treated for overnight by the above treatments. The treated seeds were evaluated through Glass-plate Method (Chakraborti, 2010) at 8DAS and 14DAS (days after sowing). Moreover, the selective promising outcomes were considered for critical root study at 14DAS through Root Image Analyser, WIN RHIZO (PRO BASIC STD 4800).

Computerised analysis of root length and diameter distribution was done on best-samples consisting of each treatment. The used software, WinRHIZO (Regent Instrument Inc.), worked under Windows and used a skeletonization method for measuring the root parameters (Arsenault et al., 1995; Smit et al., 1994). The program operated with 256 levels greyscale images in TIFF format, which were further converted into threshold (binary) and skeleton images. Threshold images were used by the system for evaluation of root diameter, while root length was measured on so called skeleton images (Regent Instruments, 2000). Measurements involved total root length, average root diameter, surface area, as well as root length measurements as a function of different root diameter classes.

The scanning procedures were done by using one light source below at the flat-bed scanners (Epson Expression/ STD 1600 scanner). The output as greyscale images were analyzed with WinRHIZO using a method of automatic global thresholding.

Completely Randomized Design (CRD) was utilized for analysis of facts associated to these activities. The outcomes were achieved at 1% level of significant by using OPSTAT software. Correlation study was also done using same OPSTAT software.

RESULTS AND DISCUSSION
The values of Table 1 specified the higher concentrations of aqueous root extract of Rauwolfia tetraphylla that amplified the germination percentage of Cicer arietinum seed as seedling emergence though after T\(_4\) it was abruptly reduced. The length of primary root as well as shoot was increased with higher concentrations of aqueous solution (root extract) in both 8DAS and 14DAS (Table 2); though it was non-significant after T\(_4\). Enlarged length of seedling noticeably signified the superior Vigour Index where highest effect came from last three concentrations of root extract, keeping a non-significant deviation among them.

Considering the fresh weight and dry weight of the seedlings (Table 1 and 2), the noted value of the higher concentrations of aqueous extracts of Rauwolfia tetraphylla advanced the seedling growth due to better accumulation of dry matter in healthy seedlings. The accumulation of dry matter was rising significantly with upper concentrations in a continuous way up to the application of treatment T\(_4\) similar to other seedling parameters though an exception was found in 8DAS of fresh wt. indicating a significant upward nature up to treatment T\(_5\) (Table 1). The seedling performance considering parameters was an indication of better plant development where T\(_4\) was continuing its prominence in both extreme exploitation at final count (8DAS) and stable seedling formation in soil (14DAS).

The results of Table 3 specified the maximum whole root length (primary root and others) in medium level concentration (100 mg ml\(^{-1}\)) of aqueous solution (root extract of Rauwolfia tetraphylla) i.e. T\(_4\) and it was gradually decreased with rising of concentration level. The enrichment of total root length signified the additional progress of fresh surface root to initialise the lateral roots that can fully exploit the zone of rhizosphere in specified soil. Consequently, the plant consumed adequate quantity of nutrients and moisture from that soil for evolving optimum growth.

The total surface area of the root was also bigger in T\(_4\) (Table 3) due to similar cause, related to creation of new supplementary roots. The progression in root surface was constructive for exploitation of soil nutrients related to seedling establishment and build up a healthy plant for better yield.

On the other influence, the Table 3 also pointed out the root diameter in an opposite manner. Control showed the highest with a decreasing trend up to T\(_4\) but rose again with increasing the concentrations (T\(_3\) and T\(_5\)). Considering diverse root types, the incidence of fresh root initials may be the cause of these fluctuations. The higher diameter of primary roots in addition to secondary type may enhance the average diameter of roots where number of new root initials (lateral roots) may not the leading in consideration of root diameter. Similarly, the volume of rhizosphere was not dependable on the establishment of new root initials. The new specific root initials at early seedling establishment stage may influence in construction of quality shoot, enhanced seedling dry weight etc. for better plant (Table 1) where the maximum performance value was perceived in T\(_4\) in addition to non-significant trend of extreme higher concentration (T\(_5\)) in some cases.

In categorization of root system, the variable roots on the basis of their thickness were critically observed (Table 3) for estimation of total elongation under growth pattern in an
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Table 1: Influence of various root extracts on seedling performances at 8 DAS.

|            | Control | T₁ | T₂ | T₃ | T₄ | T₅ | T₆ | SEm(±) | LSD (0.01) |
|------------|---------|----|----|----|----|----|----|--------|------------|
| TR value   | 71.28   | 73.81 | 76.12 | 76.12 | 76.25 | 71.19 | 71.19 | 0.561 | 2.36       |
| (Germination %) | (89.18) | (91.7) | (93.69) | (93.73) | (93.77) | (89.1) | (89.1) |        |            |
| Root length (cm) | 4.55 | 4.60 | 5.10 | 5.32 | 5.80 | 5.82 | 5.69 | 0.029 | 0.12       |
| Shoot length (cm) | 4.14 | 4.19 | 4.5 | 5.0 | 5.2 | 5.12 | 5.14 | 0.019 | 0.081      |
| Vigour index | 774.97 | 806.04 | 899.74 | 967.61 | 1031.78 | 974.16 | 965.25 | 1.630 | 4.827      |
| Fresh weight (g) | 0.490 | 0.501 | 0.520 | 0.550 | 0.591 | 0.620 | 0.640 | 0.005 | 0.019      |
| Dry weight (g) | 0.029 | 0.030 | 0.033 | 0.036 | 0.039 | 0.040 | 0.041 | 0.001 | 0.002      |

Table 2: Influence of various root extracts on seedling performances at 14 DAS.

|            | Control | T₁ | T₂ | T₃ | T₄ | T₅ | T₆ | SEm(±) | LSD (0.01) |
|------------|---------|----|----|----|----|----|----|--------|------------|
| TR value   | 71.28   | 73.81 | 76.12 | 76.12 | 76.25 | 71.19 | 71.19 | 0.561 | 2.36       |
| (Germination %) | (89.18) | (91.7) | (93.69) | (93.73) | (93.77) | (89.1) | (89.1) |        |            |
| Root length (cm) | 8.50 | 8.60 | 9.20 | 9.26 | 10.79 | 10.85 | 10.82 | 0.036 | 0.154      |
| Shoot length (cm) | 4.91 | 4.90 | 5.00 | 6.50 | 7.00 | 7.20 | 7.10 | 0.034 | 0.143      |
| Vigour index | 1195.61 | 1237.95 | 1330.71 | 1476.87 | 1668.17 | 1608.55 | 1596.38 | 6.799 | 20.13      |
| Fresh weight (g) | 1.180 | 1.181 | 1.231 | 1.240 | 1.292 | 1.291 | 1.300 | 0.003 | 0.012      |
| Dry weight (g) | 0.056 | 0.057 | 0.058 | 0.062 | 0.068 | 0.067 | 0.069 | 0.001 | 0.002      |

Table 3: Influence of specific root extracts on rhizosphere pattern at 14 DAS.

|            | Control | T₁ | T₂ | T₃ | T₄ | T₅ | T₆ | SEm(±) | LSD (0.01) |
|------------|---------|----|----|----|----|----|----|--------|------------|
| Total root length (cm) | 94.17 | 113.40 | 123.03 | 105.77 | 94.36 | 5.55 | 20.53 |
| Surface area of root (cm²) | 70.30 | 91.47 | 95.08 | 72.80 | 71.83 | 4.88 | 18.12 |
| Rhizosphere volume (cm³) | 5.20 | 6.37 | 3.37 | 5.50 | 5.43 | 0.15 | 0.62  |
| Avg. root diameter (mm) | 3.20 | 2.40 | 2.23 | 2.61 | 2.91 | 0.07 | 0.29  |
| Root length (0-0.5 mm dia.) | 34.43 | 54.96 | 65.47 | 49.99 | 34.22 | 0.32 | 1.35  |
| Root length (0.5-1 mm dia.) | 23.36 | 23.54 | 23.81 | 21.39 | 22.35 | 0.70 | 2.91  |
| Root length (1-1.5 mm dia.) | 11.41 | 9.23 | 9.61 | 10.08 | 9.72 | 1.59 | 7.28  |
| Root length (1.5-4.5 mm dia.) | 18.97 | 14.73 | 13.27 | 16.61 | 19.35 | 0.05 | 0.21  |
| Root length (>4.5 mm dia.) | 9.30 | 7.72 | 7.19 | 7.47 | 9.29 | 0.01 | 0.04  |

exact duration (14DAS). The minimum diameter (0-0.5 mm) containing roots showed maximum elongation in *T₆* though in subsequent types (0.5-1 and 1-1.5 mm), all concentrations showed a non-significant mode including control. But the maximization of root length was shifted to highest concentration and control with the amplification of root diameter i.e. more than 1.5 mm. in diameter (Fig 1).

Pre-sowing treatment of seed with various plant growth regulators was favourable for seedling growth as well as plant establishment (Brijwal and Kumar, 2013) though the physiological role of allelochemicals was not well understood in plants. It may be beneficial or deleterious according to their plant specificity, concentrations, duration etc. (Cheng and Cheng, 2015). The variable concentrations of root extracts motivate the allelopathic action on germinating seeds in modification of physiological or physical parameters that may be favourable to upgrade the seedling vigour. The quick germination of seed and formation of new root initials at surface level (lateral roots) or in other areas linked to primary or secondary root particularly under short duration crop was crucial. The young root initials were competent to reach and exploit local patches of nutrients in the soil (Giehl *et al.*, 2014) through their delicate epidermal layers in contrast to older ones. Greater addition of nutrient enhanced the rate of dry matter accumulation supportive to earlier establishing of vigorous seedlings. The rapid sequence of cell division in advancement of morphological characters, particularly root was very much linked to diverse biochemical markers (Tinus *et al.*, 2000; Yanagida, 2014) like DNA, RNA and protein synthesis etc. (Mandal *et al.*, 2013) that may be inclined in existence of appropriate allelochemicals.

A strong positive correlation was observed for all considerable parameters of seedling excluding germination (Table 4 and 5). In study on rhizosphere pattern (Table 6), the higher positive correlation was reassuring the relationship among root length, surface area with lower diameter containing root length though these were followed negative correlation with root diameter, volume and higher diameter containing root length. In interpretation, the total follow up showed a close association in between rhizosphere and seedling performance in which root length primarily lower diameter containing root shows a vibrant action (Chakraborti and Mandal, 2017).
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Table 4: Correlation study of different seedling characters considering 8 DAS.

|                  | Shoot length | Root length | Fresh weight | Dry weight | Vigour index |
|------------------|--------------|-------------|--------------|------------|--------------|
| TR value (Germination %) | 0.154NS     | 0.999NS     | -0.203NS     | -0.068NS   | 0.337NS      |
| Shoot length     | 0.968**      | 0.902**     | 0.943**      | 0.969**    |              |
| Root length      | 0.920**      | 0.950**     |              | 0.957**    |              |
| Fresh weight     |              | 0.963**     |              |            | 0.814**      |
| Dry weight       |              |             |              |            | 0.879**      |

*Significant; ** Highly significant; NS Non-significant.

Table 5: Correlation study on different seedling characters considering 14 DAS.

|                  | Shoot length | Root length | Fresh weight | Dry weight | Vigour index |
|------------------|--------------|-------------|--------------|------------|--------------|
| Germination % (TR value) | -0.054NS    | -0.086NS    | 0.020NS      | -0.066NS   | -0.013NS     |
| Shoot length     | 0.918**      | 0.924**     | 0.957**      | 0.996**    |              |
| Root length      | 0.974**      | 0.955**     | 0.984**      |            |              |
| Fresh weight     |              | 0.950**     |              | 0.987**    |              |
| Dry weight       |              |             |              |            | 0.919**      |

*Significant; ** Highly significant; NS Non-significant.

Table 6: Correlation study of different root characters considering 14 DAS.

|                  | Shoot length | Root length (0-0.5 mm dia.) | Root length (0.5-1 mm dia.) | Root length (1-1.5 mm dia.) | Root length (1.5-4.5 mm dia.) | Root length (>4.5 mm dia.) |
|------------------|--------------|------------------------------|-----------------------------|-----------------------------|-------------------------------|-----------------------------|
| Surface area of | 0.911*       | -0.832*                      | -0.908**                    | 0.980**                     | 0.376NS                       | -0.980**                    | -0.896*                     |
| rhizosphere      |              |                              |                             |                             |                               |                             |                             |
| volume           |              |                              |                             |                             |                               |                             |                             |
| Avg. root       | -0.887*      | -0.824*                      | -0.880**                    | 0.505**                     | -0.525NS                      | -0.925*                     | -0.714NS                    |
| diameter        |              |                              |                             |                             |                               |                             |                             |
| (0.5-1 mm dia.) | -0.904*      | -0.904*                      | -0.795NS                    | 0.378NS                     | 0.53NS                        | -0.980**                    | 0.561NS                     |
| (1-1.5 mm dia.) |              |                              |                             |                             |                               |                             |                             |
| (1.5-4.5 mm dia.)| -0.904*      | -0.904*                      | -0.795NS                    | 0.378NS                     | 0.53NS                        | -0.980**                    | 0.561NS                     |
| (<4.5 mm dia.)  | -0.904*      | -0.904*                      | -0.795NS                    | 0.378NS                     | 0.53NS                        | -0.980**                    | 0.561NS                     |
| (0-0.5 mm dia.) | -0.832*      | -0.832*                      | -0.824*                     | 0.505**                     | -0.525NS                      | -0.925*                     | -0.714NS                    |
| (0.5-1 mm dia.) | -0.904*      | -0.904*                      | -0.795NS                    | 0.378NS                     | 0.53NS                        | -0.980**                    | 0.561NS                     |
| (1-1.5 mm dia.) | -0.904*      | -0.904*                      | -0.795NS                    | 0.378NS                     | 0.53NS                        | -0.980**                    | 0.561NS                     |
| (1.5-4.5 mm dia.)| -0.904*      | -0.904*                      | -0.795NS                    | 0.378NS                     | 0.53NS                        | -0.980**                    | 0.561NS                     |

* Significant; ** Highly significant; NS Non-significant.
CONCLUSION
To enrich the seedling vigour, a precise observation on root features was essential that was authenticated by current observation in connection to the shoot progression and dry matter accumulation. The allelopathic action of precise root extracts (T₄) in cultivation of Gram crop may enhance the quantity and quality of the produce through modification of rhizosphere pattern as well as seedling performance. Therefore, the specific allelopathic treatment must be included in cultivation manual for crop or seed production as an invigoration treatment.

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