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RESEARCH ARTICLE

Allelopathic Effect of Leaf Extract of *Ochlandra travancorica* on the Germination of *Cicer arietinum*

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ABSTRACT

In the present investigation, aqueous extracts prepared from fresh leaf and dry leaf of *Ochlandra travancorica* showed inhibitory effects on seed germination in *Cicer arietinum* (Bengal gram), in different concentrations. The allelopathic effect of the fresh and dry leaf extracts of *O. travancorica* shows that it decreases the rate of seed germination in *C. arietinum* with an increase in the concentration of aqueous extracts. The maximum percentage of germination was recorded at control condition and the minimum percentage or no growth was recorded in 10%, 20%, 50%, and 100%. The germination rate at a concentration <10% was taken for biochemical and phytochemical analysis. The biochemical components and some active constituents like the secondary metabolites in the shoot, leaf, and cotyledon were also analyzed. The pigment analysis of the germinated leaves was also undertaken. These results indicate that the inhibitory effect on the germination of seeds may be due to allelopathy and the allelochemicals present in the leaves of this bamboo species.

Keywords: Allelopathy, aqueous extact, *Cicer arietinum*, germination inhibition, *Ochlandra travancorica*

INTRODUCTION

Different metabolites are secreted by plants through their root system into the soil, and these metabolites affect the growth of other plants in the neighborhood. Competition is seen between higher plants for moisture, light, and soil nutrients. In this competition, these plants have developed various defense strategies against their neighboring plants; it is known as allelopathy when this defense is chemical in nature. According to Jabeen and Ahmed,[1] the chemicals produced by some plants alter the growth of other plants. Allelochemicals are found in leaves, stems, roots, rhizomes, flowers, fruits, seeds, and pollen. Reduced seed germination and seedling growth are the most common effects of allelopathy. Rice[2] found that allelopathy has a key role in succession, patterning of vegetation, dormancy of seeds, etc. Allelopathic inhibition results from the interaction between flavonoids, alkaloids, terpenoids, steroids, carbohydrates, and amino acids. However, environmental stresses, temperature levels, diseases, and solar radiation can also affect allelopathic inhibition. Allelopathic chemicals remain in the soil and affect neighboring plants.

Whittaker and Feeny[3] stated that most allelochemicals are secondary metabolites. Rizvi *et al*.[4] and Wink *et al*.[5] in their study reported that cell division, production of plant hormones, pollen germination, mineral uptake, pigment synthesis, photosynthesis, respiration, nitrogen fixation, and amino acid synthesis are affected by allelopathy. Tomar *et al*.[6] reported the phytochemical analysis of *Jatropha curcas* in the seedling growth of *Triticum aeativum*. Kruse *et al*.[7] studied the morphological changes in plants due to allelochemicals that include reduced seed germination, coleoptile elongation, radicle development, and retarded growth in shoot and root. Chaves and Escudero[8] conducted a study on the influence of *Cistus ladanifer* on the neighboring plants. They found that the diversity and richness of herbs near Cistus was lower than that of the neighboring plots without this plant. The reason behind it was found as the release of allelochemicals in the form of leaf exudates by Cistus. The allelopathic crops can be used as cover crops, intercrops and green manures because they help in weed infestation,

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kill disease pathogens, improve soil fertility and reduce soil erosion.

There have been several studies on the ecological functions, sustainable uses, and the importance of reed bamboos. However, there has been no systematic study related to allelopathy of reed bamboos such as Ochlandra. The different biologically active compounds can be isolated and can be used in pharmacognosy and also in controlling the growth of several weeds.

MATERIALS AND METHODS

The fresh and dry leaves of *Ochlandra travancorica* were collected from the reserve forest areas of Bonacaud, Thiruvananthapuram, Kerala. 100 g of fresh and dry leaves of *O. travancorica* were put into 500 ml of distilled water and ground fine so that aqueous extracts could be obtained. This solution was the stock solution (100%), and different concentrations such as 10%, 20% and 50% were made from this stock solution by adding distilled water. Apart from this, concentrations from 1% to 9% were also made. Cotton was spread on Petri plates, and sample seeds of *Cicer arietinum* were sown on it. Each Petri plate was labeled. The aqueous extracts of different concentrations were applied to each Petri plates every day and water was given for control plates. These Petri plates were kept at 25°C growth chamber and analyzed for 10 days. The rate of seed germination was noted in all concentrations and the control Petri plates every day.

The various biochemical constituents analyzed include carbohydrates (Hedge and Hofreiter, 1962), protein (Lowry *et al*., 1951), starch (Hedge and Hofreiter, 1962), reducing sugar (Miller, 1959), amino acid (Moore and Stein,1948), fatty acid (Cox and Peaeson, 1962), total phenolics (Malick and Sigh, 1980), tannin (Robert, 1971), saponin (Fenwick and Oakenfull 1983), alkaloid and flavonoid (spectrophotometer), oxalate (oxalate assay buffer), resin (liquid nitrogen), Vitamin C (Harris and Ray, 1935), Vitamin A (Baker *et. al.,*1980), nutrient dietary fiber and its components (Enzymatic gravimetric method, 1992), and iron (AAS). The moisture content was determined using ISTA method (1996), leaf pigments (spectrophotometer).

RESULTS AND DISCUSSION

The carbohydrate, protein, reducing sugars, amino acids, dietary fibers, phenols, flavonoids, alkaloids, and saponin were analyzed in both shoots and leaves and in cotyledon of the seedlings grown using the different concentrations of fresh and dry leaf extracts of *O. travancorica*. Vitamins A and C, iron, starch, moisture content, tannin, fatty acid, oxalate, resin, and some pigments (chlorophyll a, chlorophyll b, carotenoid, xanthophyll, and pheophytin) were also analyzed in the cotyledons. The analysis was carried out with 10%, 20%, 50%, and 100% concentrations. However, the seeds of *C. arietinum* showed no growth. Hence, further studies were continued with concentrations <10%.

The carbohydrate content of shoot and leaf of bengal gram, on fresh and dry leaf extracts of *O. travancorica*, shows that, as concentration increases from 1% to 9%, the carbohydrate value decreases. The carbohydrate value in cotyledon showed a minimal increase than that present in shoots and leaves. As concentration increases, the carbohydrate value in the cotyledon also decreases. The carbohydrate content in germination of African yam bean and fluted pumpkin was studied by Frank *et al*.[9] and found that the carbohydrate content in both plants is higher than that of other components such as moisture, protein, ash, and total lipid content.

Other biochemical components such as protein, reducing sugar, and amino acid values were also recorded. The concentration is inversely proportional to the values of the components above mentioned. When concentration is increased, their values gradually decreased. It is noted that the protein, amino acids, and reducing sugar in shoot and leaf of germinated seeds with fresh leaf extract are comparatively a little higher than that of dry leaf extract. In cotyledon, the least value is exhibited by amino acids in both fresh and dry leaf extracts. The protein content of the shoot and leaf as well as cotyledon of control was obtained as 6.34 and 3.86, respectively. In the present study, the result obtained as seedlings grown using fresh leaf extract showed more protein content than the dry leaf extract.

Protein content in *Dendrocalamus stockii* and *O. travancorica* was studied by Beena[10] and found that the highest percentage of protein 4.6 was observed at rejuvenated stage. Frank *et al*.[9]

Table 1: Chemical constituents and its variation in shoots of *Cicer arietinum* (fresh leaf extract of *Ochlandra travancorica*)

Component	1%	2%	3%	4%	5%	6%	7%	8%	9%
Carbohydrate	13.61	13.41	12.95	12.35	11.76	10.52	9.86	9.44	9.25
Protein	7.16	6.88	6.48	6.27	5.71	5.48	5.32	5.21	5.15
Reducing sugar	7.26	6.82	6.39	6.06	5.56	5.18	4.79	4.62	4.44
Amino acid	5.41	5.24	4.98	4.81	4.53	4.19	3.95	3.83	3.76
Dietary fiber	8.56	8.26	7.90	7.81	7.72	7.49	7.26	7.17	7.06
Phenols	1.13	1.19	1.26	1.32	1.47	1.55	1.63	1.74	1.85
Flavonoids	0.14	0.22	0.34	0.49	0.63	0.74	0.74	0.92	0.94
Alkaloids	0.08	0.13	0.17	0.21	0.25	0.28	0.31	0.35	0.38
Saponin	0.31	0.36	0.39	0.41	0.43	0.56	0.61	0.64	0.70

Table 2: Chemical constituents and its variation in cotyledon of *Cicer arietinum* (fresh leaf extract of *Ochlandra travancorica*)

Component	1%	2%	3%	4%	5%	6%	7%	8%	9%
Carbohydrate	14.71	13.99	13.31	12.72	11.95	11.49	10.86	10.42	10.23
Protein	7.88	7.59	7.29	6.94	6.37	6.18	5.86	5.74	5.63
Reducing sugar	8.13	7.95	7.06	6.34	6.19	5.95	5.84	5.65	5.36
Amino acid	3.22	2.84	2.30	1.95	1.56	1.38	1.24	1.17	0.16
Dietary fiber	8.53	8.21	7.62	7.34	6.86	6.27	5.73	4.68	3.41
Phenols	0.42	0.53	0.76	1.21	1.34	1.57	1.74	1.82	1.93
Flavonoids	0.16	0.19	0.25	0.29	0.32	0.37	0.45	0.49	0.52
Alkaloids	0.12	0.15	0.19	0.23	0.28	0.32	0.34	0.41	0.45
Saponin	0.14	0.17	0.21	0.25	0.28	0.35	0.37	0.39	0.41

Table 3: Various constituents and its variation in cotyledon of *Cicer arietinum* (fresh leaf extract of *Ochlandra travancorica*)

Component	1%	2%	3%	4%	5%	6%	7%	8%	9%
Vitamin A	1.09	0.86	0.43	0.38	0.29	0.21	0.17	0.13	0.11
Vitamin C	1.82	1.67	1.61	1.56	0.51	0.37	0.19	0.11	0.06
Iron	0.84	0.76	0.68	0.61	0.53	0.42	0.24	0.18	0.13
Starch	6.42	5.86	5.37	4.91	4.56	4.29	3.81	3.66	3.43
Moisture content	89%	83%	79%	74%	66%	61%	57%	53%	49%
Tannin	1.07	0.94	0.84	0.72	0.55	0.35	0.27	0.21	0.16
Fatty acid	2.49	2.52	2.55	2.69	2.72	2.75	2.81	3.02	3.16
Oxalate	0.08	0.13	0.19	0.24	0.34	0.46	0.55	0.68	0.81
Resin	0.31	0.43	0.58	0.78	0.86	0.94	1.06	1.16	1.28

Table 4: Pigment analysis in leaves of *Cicer arietinum* (fresh leaf extract of *Ochlandra travancorica*)

Pigments	1%	2%	3%	4%	5%	6%	7%	8%	9%
Chlorophyll a	10.43	9.51	8.92	8.36	7.85	7.47	6.92	6.38	5.94
Chlorophyll b	7.65	7.27	6.72	6.39	5.84	5.31	4.68	4.42	4.21
Carotenoid	3.29	2.71	2.35	1.42	0.95	0.42	0.37	0.32	0.24
Xanthophyll	2.86	2.73	2.55	2.41	2.26	1.95	1.73	1.57	1.21
Phaeophytin	2.67	2.43	2.37	2.18	1.93	1.72	1.43	1.34	1.25

studied that the protein content is maximum in germinated seeds than in ungerminated seeds in both the plants such as African yam bean and Fluted pumpkin.

The dietary fiber content in shoot and leaf of fresh and dry leaf extracts of *O. travancorica* showed high values with low concentration and low values in high concentration. The shoot and leaf of fresh leaf extracts possess more dietary fiber content than that of dry leaf extract. The dietary fiber content in cotyledon of dry leaf extracts showed the least value than that of cotyledon of fresh leaf extracts. At high concentration, the value reached minimum in cotyledon of dry leaf extracts. There is much

Table 5: Chemical constituents and its variation in shoots of *Cicer arietinum* (dry leaf extract of *Ochlandra travancorica*)

Component	1%	2%	3%	4%	5%	6%	7%	8%	9%
Carbohydrate	12.32	11.26	10.47	9.87	9.35	8.75	8.13	7.67	7.25
Protein	6.45	5.57	5.28	4. 93	4.72	4.56	4.30	4.06	3.98
Reducing sugar	5.76	5.34	5.05	4.95	4.85	4.67	4.41	4.28	4.09
Amino acid	4.83	3.39	3.65	3.54	3.25	3.21	2.84	2.43	2.17
Dietary fiber	7.53	7.38	7.24	6.92	6.76	6.45	6.21	5.92	5.77
Phenols	1.34	1.46	1.51	1.57	1.68	1.76	1.84	1.87	1.92
Flavonoids	0.13	0.24	0.31	0.43	0.52	0.64	0.75	0.87	0.97
Alkaloids	0.10	0.21	0.27	0.35	0.39	0.43	0.47	0.51	0.58
Saponin	0.23	0.26	0.29	0.32	0.38	0.46	0.51	0.56	0.64

Table 6: Chemical constituents and its variation in cotyledon of *Cicer arietinum* (dry leaf extract of *Ochlandra travancorica*)

Component	1%	2%	3%	4%	5%	6%	7%	8%	9%
Carbohydrate	12.82	12.38	11.76	11.46	11.25	10.94	10.68	10.23	9.86
Protein	6.28	6.02	5.95	5.31	5.04	4.84	4.32	4.15	3.27
Reducing sugar	8.87	8.39	8.16	7.81	7.81	6.97	6.82	6.53	5.59
Amino acid	2.91	2.79	2.62	2.15	1.79	1.61	1.38	1.22	1.12
Dietary fiber	4.27	3.86	3.24	2.95	2.59	2.37	1.62	1.43	1.25
Phenols	0.31	0.52	1.16	1.29	1.42	1.68	1.84	2.10	2.23
Flavonoids	0.15	0.26	0.35	0.58	0.65	0.74	0.78	0.83	0.85
Alkaloids	0.16	0.19	0.24	0.29	0.33	0.37	0.40	0.42	0.46
Saponin	0.15	0.18	0.20	0.23	0.25	0.32	0.34	0.37	0.39

Table 7: Various constituents and its variation in cotyledon of *Cicer arietinum* (dry leaf extract of *Ochlandra travancorica*)

Component	1%	2%	3%	4%	5%	6%	7%	8%	9%
Vitamin A	2.35	2.14	1.75	1.38	1.19	0.94	0.79	0.58	0.36
Vitamin C	2.29	2.16	1.89	1.62	1.25	0.87	0.76	0.63	0.55
Iron	1.57	1.35	1.16	1.06	0.81	0.69	0.46	0.36	0.24
Starch	6.49	6.20	5.96	5.73	5.65	5.42	5.04	4.86	4.35
Moisture content	63%	62%	61%	59%	57%	56%	55%	53 %	51%
Tannin	0.96	1.06	1.07	1.09	1.13	1.16	1.19	1.22	1.26
Fatty acid	3.25	2.81	2.64	1.86	1.33	1.41	1.32	1.28	1.25
Oxalate	0.06	0.16	0.49	0.53	0.77	0.83	1.06	1.14	1.23
Resin	0.62	0.79	0.82	1.11	1.42	1.62	1.84	1.89	1.96

Table 8: Pigment analysis in leaves of *Cicer arietinum* (dry leaf extract of *Ochlandra travancorica*)

Pigments	1%	2%	3%	4%	5%	6%	7%	8%	9%
Chlorophyll a	8.51	8.24	7.62	7.49	7.34	7.23	6.84	6.63	6.45
Chlorophyll b	4.92	4.53	4.28	3.95	3.62	3.34	3.06	2.96	2.89
Carotenoid	2.59	2.43	2.10	1.89	1.63	1.34	0.96	0.52	0.23
Xanthophyll	1.96	1.91	1.84	1.79	1.64	1.56	1.41	1.34	1.21
Phaeophytin	1.65	1.46	1.25	1.18	1.12	1.05	0.84	0.63	0.45

difference in dietary fiber content value between cotyledons treated with fresh and dry leaf extracts. Some other allelochemicals such as phenols, flavonoids, alkaloids, and saponin were also tested in shoot and leaf and in cotyledons of Bengal gram. The presence of phenol, flavonoid, alkaloid and saponin were noticed in the shoot and leaf and in cotyledons, grown using both fresh and dry leaf extracts of O.travancorica. All components increased with an increase in concentration and decreased with a decrease in concentration. Among these four components, the least value is shown by alkaloids analyzed in the shoot and leaves treated with fresh leaf extract. Cotyledons contain more amounts of alkaloids than shoot and leaf. Aziz *et al*.[11] studied the alkaloid, saponin,

Table 9: Various constituents and its analysis value in cotyledon of *Cicer arietinum* (using water as control)

Constituents	Control value in cotyledon (mg/g)	Control value in shoot (mg/g)
Carbohydrate	10.67	13.45
Protein	3.86	6.34
Reducing sugar	6.34	5.27
Amino acid	4.55	5.43
Dietary fiber	5.6	7.21
Phenols	0.54	1.11
Flavonoids	0.48	0.65
Alkaloids	0.41	0.56
Saponin	0.36	0.52

Table 10: Various constituents and its variation in cotyledon of *Cicer arietinum* (using water as control)

Constituents	Control value
Vitamin A	0.41
Vitamin C	1.15
Iron	0.98
Starch	7.86
Moisture content	36%
Tannin	0.32
Fatty acid	3.66
Oxalate	0.07
Resin	0.18

Table 11: Pigment analysis in leaves of *Cicer arietinum* (using water as control)

Constituents	Control value(µg/g)
Chlorophyll a	14.16
Chlorophyll b	11.19
Carotenoid	2.67
Xanthophyll	1.76
Phaeophytin	2.79

and flavonoid content in *Bombax ceiba* leaves and seeds. Figures 1-7 are based on 9% concentration of *O. travancorica* leaves on seedlings of *Cicer arietinum.*

Aqueous concentrations of both fresh and dry leaf extracts of *O. travancorica* were used in determining the allelopathic effect on germination of bengal gram. The analysis done with fresh and dry leaves of *O. travancorica* in the germinated shoot and leaves and cotyledons of bengal gram shows that the value of biochemical constituents such as carbohydrate, protein, reducing sugars, amino acids, and dietary fibers decreased as the concentration of the extract was increased. The values of phytochemical constituents such as phenols, flavonoids, alkaloids, and saponins were directly proportional to the concentration of both fresh and dry leaf extracts. As the concentration of the aqueous extract was increased, the amount of secondary metabolites also increased in both the cases. This study has shown that the fresh and dry leaf extracts of *O. travancorica* show more allelopathy. It can be concluded that the

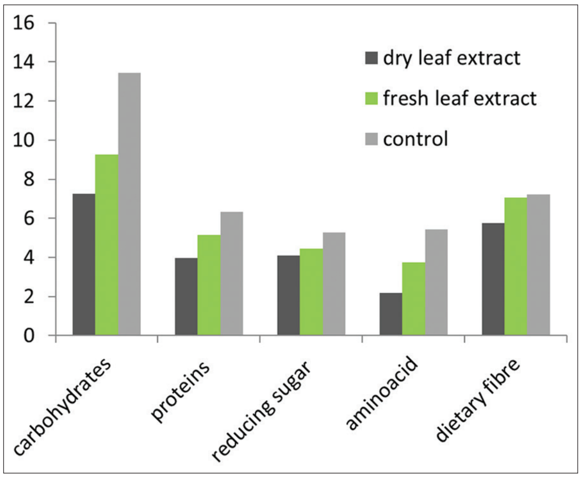

Figure 1: Biochemical constituents in the shoot and leaves of *Cicer arietinum*

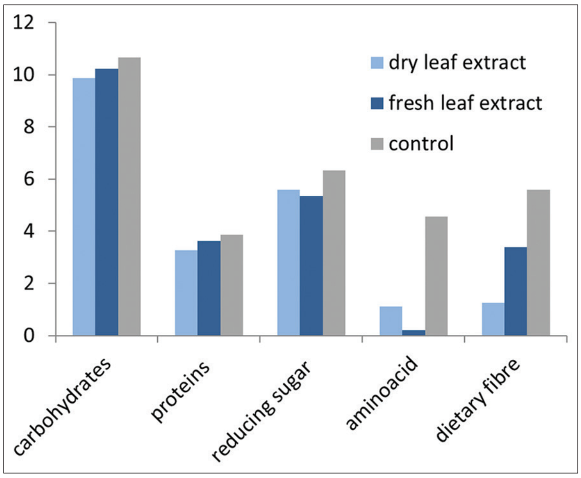

Figure 2: Biochemical constituents in the cotyledons of *Cicer arietinum*

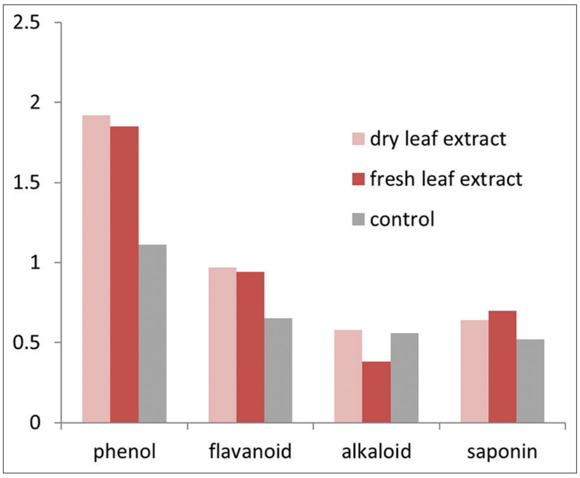

Figure 3: Phytochemical constituents in the shoot and leaves of *Cicer arietinum*

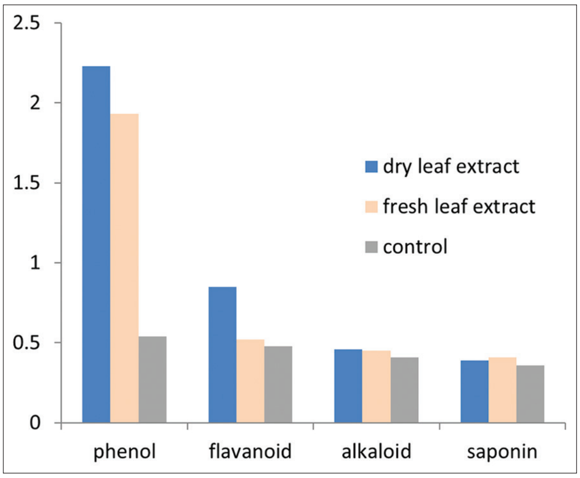

Figure 4: Phytochemical constituents in the cotyledons of *Cicer arietinum*

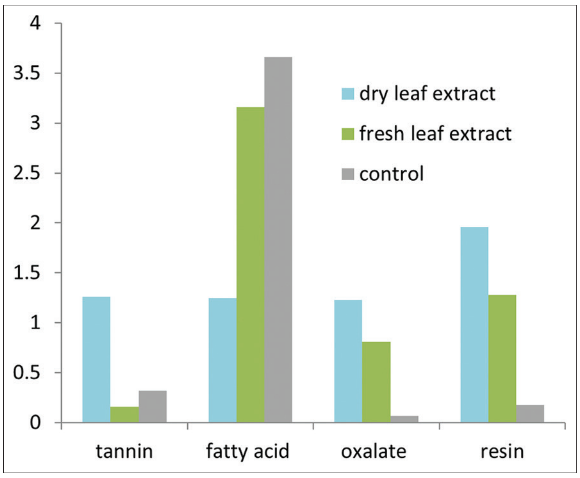

Figure 6: Analysis for tannin, fatty acid, oxalate, and resin in cotyledons of *Cicer arietinum*

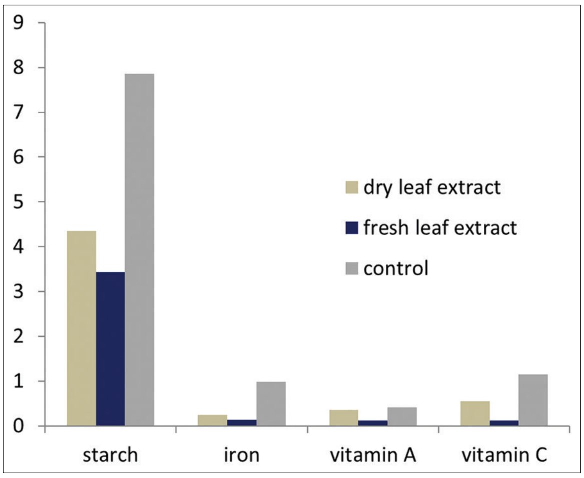

Figure 5: Analysis for starch, iron, Vitamin A, and Vitamin C in cotyledons of *Cicer arietinum*

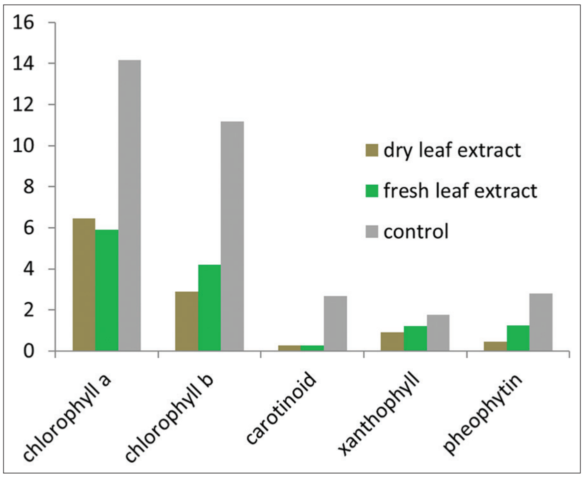

Figure 7: Pigment analysis in the leaves of *Cicer arietinum*

allelochemicals released by this reed bamboo inhibit and affect the growth of other plants as in *C. arietinum*.

The analysis was also done in the cotyledon of the germinated seeds using both fresh and dry leaf extracts of *O. travancorica* for moisture content, Vitamin A, Vitamin C, iron, starch, tannin, fatty acid, oxalate and resin. Moisture content, Vitamin A, Vitamin C, iron, and starch in the cotyledons of Bengal gram was analyzed to be decreasing as the concentration of the aqueous extract of both fresh and dry leaves of bamboo was increased. Oxalate and resin were directly proportional to the concentration of the fresh and dry leaf extracts of bamboo. Tannin in the cotyledon of the bengal gram was observed to be increasing with an increase in the concentration of dry leaf extract, and the analysis of fatty acid with fresh leaf extract was also noted to be increasing with an increase in concentration.

Further pigment analysis was done for the leaves of germinated seeds of *C. arietinum*. Various pigments such as chlorophyll a, chlorophyll b, carotenoid, xanthophyll, and phaeophytin were isolated from seedlings grown both in fresh and dry leaf extracts of bamboo leaves. As the concentration of the leaf extracts was increased, the pigment content in the leaves has gradually decreased, i.e., pigment content is inversely proportional to the concentration of both fresh and dry leaf extracts. Similar studies were carried out by Khaket *et al*.[12] in *Triticum vulgare* after analyzing the effects of senescence leaves of *Populus deltoides* on it.

The present investigation clearly indicates that the aqueous extracts of both fresh and dry leaves of *O. travancorica* are affecting the biochemical as well as phytochemical constituents in the germinating seeds of *C. arietinum*. Both the fresh and dry leaves of *O. travancorica* contain phytochemicals such as phenol, alkaloid, flavonoids, and saponins in considerable quantities. Hence, this reed bamboo is a potential source of useful drugs. It also justifies the therapeutic values of this plant. Therefore, further analysis, isolation, purification, and characterization of the bioactive compounds from the leaves of *O. travncorica* can be recommended.

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