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ORIGINAL ARTICLE

Influence of Homeopathic Treatments on Biochemical parameters of groundnut (*Arachys hypogea* L.) challenged with *Sclerotium rolfsii* Sacc.

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ABSTRACT
Groundnut is an important edible oil crop plant whose quality and yield are greatly affected by various biotic and abiotic stress. The process of mechanisms of recovery from stress are also critical to its productivity, but are currently poorly characterized. Here, we investigated the involvement of groundnut by using different homeopathic drugs to induce resistance against stress which encodes a key enzyme in biosynthesis of chlorophyll. Chlorophyll content in groundnut leaves following seed treatment, foliar application and soil application of homeopathic drugs was recorded using SPAD chlorophyll meter and proline content was recorded using the uv spectrophotometer. In this study the SCMR (SPAD Chlorophyll Meter Reading) and proline content of homeopathy treated plants was recorded to be more in comparison with the untreated control plants. All the homeopathic treatments were found effective in increasing the chlorophyll and proline content in groundnut leaves. Maximum chlorophyll (48.17) and proline (23.14) content was observed in plants treated with the Chelidonium majus in seed treatment method at all the stages of observation.
KEYWORDS: *Homeopathy, Chlorophyll, Groundnut, Proline, Sclerotium rolfsii.*

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INTRODUCTION

Groundnut is called as the king of oilseeds. It is one of the most important food and cash crops of our country. Groundnut is also called as wonder nut and poor men's cashew nut. It is a low priced commodity, but a valuable source of all the nutrients**.** Seeds are a rich source of oil (35–56%), protein (25– 30%), carbohydrates (9.5–19.0%), minerals (P, Ca, Mg and K) and vitamins (E, K and B) [13]. It is cultivated throughout tropical, subtropical and warm temperate regions of the world. The major groundnut producing countries in the world are India, China, Nigeria, Senegal, Sudan, Burma and the United States of America [18]. India occupies the first place, both in regard to the area and the production in the world. In India groundnut is mostly grown in 5 states viz. Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka and Maharashtra, which accounts for 80 per cent of the total area and production of groundnut [22]. In Telangana, groundnut is cultivated in an area of 1.7 lakh ha with an annual production of 3.5 lakh tonnes. The productivity is 2114 kg ha^{-1}. The leading groundnut growing districts in Telangana were Nagarkurnool, Wanaparthi, Mahboobnagar, Gadwal, Mahaboobabad, Vikarabad, Suryapet, Khammam, Bhadradri Kothagudem and Nalgonda [8]. Due to the residual problem and toxicity to the living environment, chemical pesticides are not suitable for crop production. Therefore, products of plant origin have recently gained enormous importance in the quest to develop better alternatives to chemical pesticides [3, 17 and 20-22]. Recently the importance in commonly used medicinal plants has been benefiting from the biological science community. This included isolating and defining secondary plant-generated metabolites, and their use in medicinal preparations as active principles [24]. Plants have limitless ability to synthesize aromatic secondary metabolites, most of which are phenols or their oxygen-substituted derivatives [11]. The so-called secondary metabolites contribute greatly to unique plant odors, tastes and colours. Such

phytochemicals include alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, glycosides, anthraquinones, coumarins, polyphenols, phlobatannins and steroids.

In all organisms, reactive oxygen species (ROS), such as O_2 and hydrogen peroxide (H_2O_2), are formed as by-products of normal, unstressed cellular metabolism. In plants, respiratory and photosynthetic processes responsible for this production take place in several organelles, including mitochondria and chloroplasts [12 and 25]. The photosynthetic electron transport system, a major source of ROS in plants, resides in the thylakoid membranes of chloroplasts [10]. Several forms of biotic and abiotic stress, such as pathogen attack or excess light [16] can damage plant tissues. This in turn may result in the release of chlorophyll from the thylakoid membranes. In such a situation, the chlorophylls need to be degraded quickly to avoid cellular damage by their photodynamic action [23]. Thus, failure in chlorophyll degradation can increase the amount of ROS produced to an extent where the detoxification capacity of the antioxidant systems may be overridden. The toxic molecules formed may result in damage of the organelle and in cell death, or they may act as cellular signals [10 and 25]. It is therefore crucial that the breakdown of chlorophyll is both efficient and tightly regulated [14, 19 and 23].

Hence the present study was conducted to know the effect of homeopathic drug on chlorophyll and proline content of groundnut in comparison with the stem rot pathogen and fungicide treated plants.

MATERIAL AND METHODS

Isolation of the pathogen *Sclerotium rolfsii* (Aneja *et al.,* 2003 and Ali *et al.,* 2006)

Isolation of the pathogen from the stem rot infected groundnut plants was carried out by the tissue segment method under aseptic conditions [2]. Groundnut stem parts containing both the diseased and healthy tissue were cut into small bits with the help of a sterile scalpel. The bits were then surface sterilized by immersing in 1 per cent Sodium hypochlorite for one minute followed by washing with three changes of sterile water and dried by blotting on sterile paper towels. The sterilized bits were transferred to PDA plates under aseptic conditions and incubated at 25 ± 2° C for 3-4 days [1]. The fungal growth emerging from diseased tissues was transferred to PDA plates with sterilized needle under aseptic conditions and pure culture with white coloured mycelium along with light brown coloured sclerotia was obtained.

Treatments

Homeopathic extracts used in the present study *viz*. *Chelidonium majus*, *Colchicum autumnale*, *Natrum muriaticum* and Tebuconazole. Homeopathic solutions were procured from the wholesale homeopathic market in Hyderabad, Telangana.

Groundnut variety Kadiri-6 (K -6) was used for the evaluation of the effect of homeopathic drugs on chlorophyll content of groundnut leaves. 5 seeds/pot were sown in pots (12×09 cm) containing sterilized soil in a glass house. The homeopathic treatments were applied in three methods *viz.* seed treatment, foliar and soil application.

Seed treatment was done prior to sowing. 5 ml of homeopathic drug solution at different concentration was used for seed treatment. Seeds were treated with the suspension of homeopathic solution for 30 minutes and air dried prior to sowing. Foliar application was performed at 30 days old plant stage [6]. For foliar spray the homeopathic solution was sprayed on leaves of the plant. For each plant, 5 ml of homeopathic solution was used for spraying. Soil application was done prior to sowing, 20 ml of solution was mixed in the sterilized soil in pot along with mixing the sclerotia. The experiment was conducted with 10 treatments and 3 replications per each treatment. The following treatments were used *i.e.,* T1- Healthy, T2- only *Sclerotium rolfsii*, T3- *Chelidonium majus*, T4- *Chelidonium majus* along with *Sclerotium rolfsii*; T5- *Colchicum autumnale*; T6- *Colchicum autumnale* along with *Sclerotium rolfsii*; T7- *Natrum muriaticum*; T8- *Natrum muriaticum* along with *Sclerotium rolfsii*; T9- Tebuconazole 500ppm; T10- Tebuconazole 500ppm along with *Sclerotium rolfsii* inoculated plants used in glasshouse studies for determination of chlorophyll and proline content in the host plant following application of homeopathic drugs challenged with the *Sclerotium rolfsii*.

Chlorophyll content

Chlorophyll content in groundnut leaves following seed treatment, foliar application and Soil application of homeopathic drugs was measured after 45, 60 and 75 days of sowing and challenged with *S. rolfsii* according to the method given by Falke *et al.* [9]. Chlorophyll content was measured in younger and older leaves by using SPAD chlorophyll meter to record on each leaflet of the tetra foliate leaf beside the midrib and care was taken to ensure that the SPAD meter sensor fully covered the leaf lamina and the interference from veins and midribs was avoided.

Proline content

0.5 grams of fresh leaf samples were taken and homogenized with 10 ml of 3 % (w/v) sulfosalicylic acid. The extract was filtered through Whatman No. 1 filter paper and the filtrate was used for proline

estimation. An aliquot of 2 ml from each sample was taken in a separate test tube and each test tube was added with 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid and then boiled in a hot water bath for one hour. Then, the test tubes were transferred to an ice water bath for one hour for cooling and the contents of each tube were transferred to a separating funnel. To this, 4 ml of toluene was added, shaken thoroughly and allowed to form two separate layers. The upper toluene layer containing the colour complex due to proline ninhydrin reaction was taken into a separate test tube and colour was read at 520 nm. The proline concentration was determined by using standard curve developed with different concentrations of proline and expressed in µ moles of proline per g fresh weight. [7].

Statistical Analysis

All the data were used for analysis by using one way factor in Randomized Block Design (RBD). All the data were considered as significant at the level of P≤0.05 using XLSTAT software.

RESULTS

Estimation of chlorophyll content in groundnut leaves following seed treatment, foliar and soil application of homeopathic treatments challenged with *S. rolfsii*

Leaf chlorophyll content (SCMR) was recorded using SPAD chlorophyll meter on the younger and older leaves from the top on the main stem of five randomly selected plants in each treatment. The SCMR was recorded on 45, 60 and 75 DAS following treated with homeopathic treatments and challenged with *S. rolfsii*.

The SCMR of homeopathic treated plants was recorded to be more in comparison with the untreated plants. All the homeopathic treatments were found effective in increasing the chlorophyll content in groundnut leaves. Maximum SCMR was observed in plants treated with the *Chelidonium majus* by seed treatment method in all the three stages of observation.

At 45 DAS the chlorophyll reading in plants treated with seed treatment of potential homeopathic drugs was found to be less when compared with foliar application and micro-injection. Highest chlorophyll reading was observed in foliar application of followed by micro-injection. Lower leaves were observed to have more chlorophyll content followed by upper leaves. Similar trend of SCMR was observed in the groundnut plants at 60 DAS. Maximum chlorophyll content was observed in plants treated with seed treatment of *Chelidonium majus*. Minimum chlorophyll content was recorded in younger leaves and also in soil application treated plants. Maximum chlorophyll content was recorded in seed treatment of homeopathic treatments followed by foliar and soil application. older leaves were recorded to have more chlorophyll content followed by younger leaves.

The results declares that chlorophyll content of homeopathic treated plants was more in comparison with untreated plants and treated controls. Homeopathic treatments have increased the chlorophyll content in all treated plants. It was also observed that, seed treatment method was superior to foliar and soil application in increasing the chlorophyll content. Homeopathic treatment *Chelidonium majus* was found as effective in enhancing the chlorophyll content in treated groundnut leaves.

Estimation of proline content in groundnut leaves following seed treatment, foliar and soil application of homeopathic treatments challenged with *S. rolfsii*

Proline content in the leaf of groundnut was recorded using UV spectrophotometer. The Proline content was recorded after the inoculation of 24, 48 and 72 hours following treated with homeopathic treatments and challenged with *S. rolfsii*.

The proline content of homeopathic treated plants was recorded to be more in comparison with the untreated plants. All the homeopathic treatments were found effective in increasing the proline content in groundnut leaves. Maximum proline content was observed in plants treated with the *Chelidonium majus* by seed treatment method in all the three stages of observation.

After 24 hours of inoculation the proline content in plants treated with seed treatment of potential homeopathic drugs was found to be less when compared with foliar application and soil application. Similar trend of proline content was observed in the groundnut plants after 48 and 72 hours of inoculation. Maximum proline content was observed in plants treated with seed treatment of *Chelidonium majus*. Minimum proline content was recorded in soil application treated plants. Maximum proline content was recorded in seed treatment of homeopathic treatments followed by foliar and soil application.

The results declares that proline content in homeopathic treated plants was more in comparison with untreated plants and treated controls (fungicide). Homeopathic treatments have increased the proline content in all treated plants. It was also observed that, seed treatment method was superior to foliar and soil application in increasing the proline content. Homeopathic treatment *Chelidonium majus* was found as effective in enhancing the proline content in treated groundnut leaves.

Table 1. Chlorophyll content in groundnut leaves treated with different homeopathic treatments.

Treatment	Chlorophyll (SPAD units)																	
	Seed treatment						Foliar application						Soil application					
	DAS																	
	45		60		75		45		60		75		45		60		75	
	Y	O	Y	O	Y	O	Y	O	Y	O	Y	O	Y	O	Y	O	Y	O
T1	20.91	21.14	23.65	24.36	27.28	28.47	20.91	21.14	23.65	24.36	27.28	28.47	20.91	21.14	23.65	24.36	27.28	28.47
T2	19.08	19.23	18.99	19.01	17.31	17.52	19.08	19.23	18.99	19.01	17.31	17.52	19.08	19.23	18.99	19.01	17.31	17.52
T3	35.02	36.17	38.06	38.69	39.97	43.84	34.26	34.68	35.12	36.01	37.78	38.21	32.64	32.98	35.03	35.64	31.82	35.98
T4	37.68	38.76	39.62	39.97	45.47	48.17	36.23	36.74	37.45	38.26	41.20	41.86	34.87	35.73	36.29	36.98	38.46	38.94
T5	31.26	32.61	33.48	34.36	36.79	36.87	30.64	30.92	32.89	33.04	34.84	36.01	31.48	32.35	34.23	34.99	31.66	32.17
T6	35.88	36.04	36.42	36.87	38.41	37.52	34.87	35.22	35.96	36.58	37.97	38.23	33.77	34.71	32.57	33.09	34.38	36.21
T7	34.94	38.94	38.32	39.32	41.98	39.95	32.78	33.06	36.42	37.51	38.71	39.48	30.52	31.38	32.94	33.66	31.71	35.98
T8	36.34	39.12	39.47	39.67	44.86	45.63	33.69	34.22	37.87	38.14	39.33	40.17	31.82	33.06	37.69	38.29	37.22	38.01
T9	21.74	22.13	28.68	29.24	30.59	30.68	26.97	28.30	29.74	30.05	31.87	32.58	25.70	26.33	27.25	28.67	29.63	30.41
T10	22.79	25.67	32.14	32.83	34.75	36.19	31.69	32.08	33.61	33.99	34.18	35.93	29.82	30.82	31.61	32.94	33.05	33.56
C.D (P≤0.05)	5.246		3.282		4.301		4.377		6.140		3.484		3.252		2.923		3.230	
S.E (m)	1.867		1.164		1.520		1.553		2.189		1.233		1.150		1.044		1.620	
S.E (d)	2.630		1.659		2.168		2.207		3.080		1.705		1.636		1.470		1.422	

Y- younger leaves**; O-**older leaves.

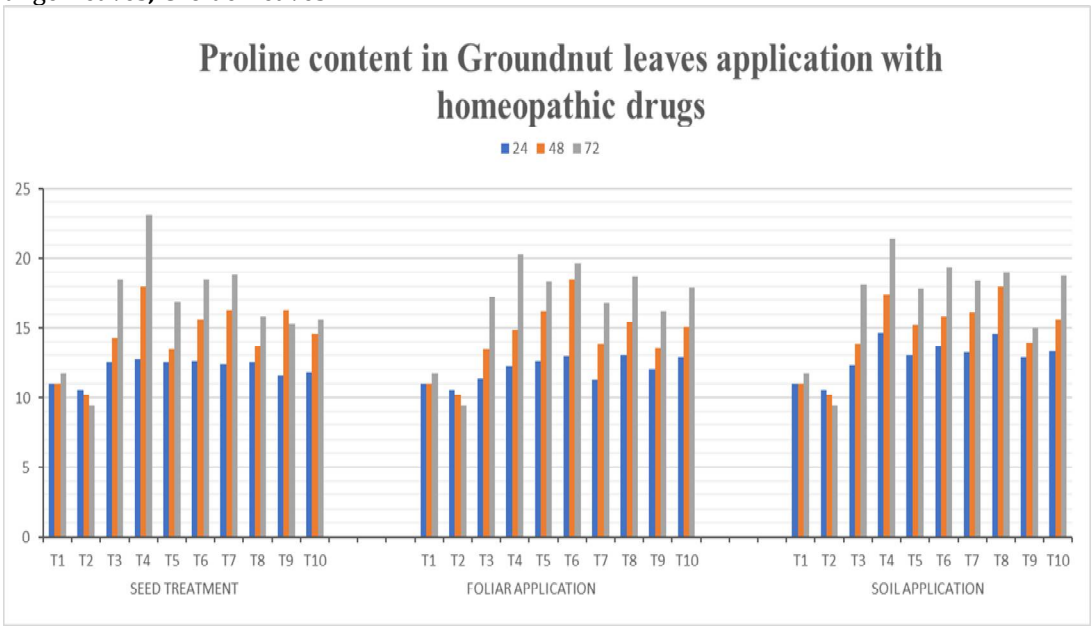

Fig 1: Proline contentin groundnut application with homeopathic drugs

Table 2. Proline content in groundnut leaves treated with different homeopathic treatments.

Treatment	µ moles/g of fresh leaf weight								
	Seed Treatment			Foliar Application			Soil Application		
	Time (hours)								
	24	48	72	24	48	72	24	48	72
T1	10.98 ± 2.03	11.01 ± 3.31	11.67 ± 2.50	10.98 ± 2.03	11.01 ± 3.31	11.67 ± 2.50	10.98 ± 2.03	11.01 ± 3.31	11.67 ± 2.50
T2	10.57 ± 0.96	10.21 ± 1.02	9.42 ± 1.35	10.57 ± 0.96	10.21 ± 1.02	9.42 ± 1.35	10.57 ± 0.96	10.21 ± 1.02	9.42 ± 1.35
T3	12.57 ± 1.24	14.28 ± 1.06	18.46 ± 2.13	11.32 ± 0.54	13.48 ± 1.26	17.20 ± 0.51	12.36 ± 1.97	13.89 ± 0.64	18.14 ± 0.67
T4	12.78 ± 0.52	17.99 ± 1.47	23.14 ± 3.48	12.23 ± 1.26	14.87 ± 2.67	20.29 ± 2.36	14.68 ± 2.06	17.38 ± 1.63	21.39 ± 1.25
T5	12.52 ± 3.18	13.48 ± 1.29	16.87 ± 4.27	12.64 ± 1.68	16.18 ± 5.21	18.33 ± 3.14	13.01 ± 1.67	15.22 ± 1.22	17.84 ± 2.03
T6	12.64 ± 1.65	15.62 ± 4.55	18.49 ± 3.16	12.99 ± 1.39	18.47 ± 4.16	19.68 ± 1.69	13.69 ± 2.03	15.87 ± 0.89	19.34 ± 2.58
T7	12.43 ± 1.22	16.28 ± 2.67	18.78 ± 2.02	11.28 ± 1.44	13.89 ± 0.67	16.74 ± 1.22	13.26 ± 2.08	16.15 ± 0.47	18.39 ± 3.49
T8	12.52 ± 2.16	13.66 ± 1.08	15.83 ± 5.23	13.01 ±2.08	15.43 ± 1.85	18.64 ± 0.94	14.55 ± 1.84	17.95 ± 2.41	18.99 ± 3.01
T9	11.58 ± 2.03	16.28 ± 1.99	15.27 ± 2.01	12.02 ± 2.59	13.55 ± 2.41	16.21 ± 0.23	12.86 ± 1.33	13.98 ± 0.88	15.03 ± 2.12
T10	11.74 ± 1.98	14.57 ± 2.07	15.63 ± 3.14	12.89 ± 2.03	15.07 ± 2.06	17.88 ± 2.48	13.31 ± 2.05	15.64 ± 1.45	18.73 ±2.51
C.D (P≤0.05)	**4.015**	**3.169**	**3.006**	**3.514**	**2.051**	**3.128**	**3.129**	**3.114**	**3.028**
S.E (m)	**3.127**	**2.841**	**2.743**	**2.633**	**2.006**	**2.008**	**2.027**	**2.126**	**2.217**
S.E (d)	**3.026**	**2.540**	**2.142**	**2.018**	**1.089**	**1.043**	**2.005**	**2.008**	**1.113**

DISCUSSION

Photosynthesis, pathogen infection and plant defense related signaling molecules or their precursors are generated in the chloroplast and these signals crosstalk and regulate photosynthesis and plant defense. Chloroplast-targeted effectors and phytotoxins produced by elicitors applied, it manipulates chloroplastic functions, especially photosynthesis, to suppress plant defense and promote pathogenicity. Chloroplast plays a central role in the interplay between photosynthesis, pathogen infection, and plant defense. Plant defense is also regulated by photorespiration and light. The roles of photorespiration and photoreceptors in plant defense, reviewed by Ballare and Kangasjarvi *et al*. [5 and 15].

Arunyanar k *et al*. [4] reported that stability in peanut chlorophyll content was related to drought tolerance due to the ability to keep constant biomass production, despite unfavorable conditions. Our findings revealed that chlorophyll content maintained unaltered, and this may be related to a higher root biomass production to increase its exploratory surface in order to improve water uptake. Besides, chlorophyll content may allow plants to deliver sufficient energy to deal with the energy-consuming adaptations to stress. Another possibility is that chlorophyll has a role in control of redox homoeostasis, that is, collaborates in heat dissipation of excess excitation energy within light-collecting chlorophyll and the carotenoid-binding protein complexes of photosystem (PS) II, which are considered major photoprotective mechanisms.

Currently, homeopathic treatments as sustainable approaches to manage pests and disease were more ecologically sustainable than the use of synthetic chemical fungicides. Besides phenolic compounds, there are several other phytochemical mediated plant metabolites like pathogen-related (PR) proteins and defense enzymes had been found to be associated with induction of resistance in the host as many of them are found to be antifungal. The present study is an initiative and it helps in understanding and employing homeopathic treatments which are useful component of integrated management of soil borne pathogens of groundnut. Thus, the proposed work mechanism is novel and as the known control practices are non-selective causing microbial resistance, the natural molecule inhibitors identified through this work are considered as an outcome. This recommends the exploitation of homeopathic treatments on a commercial scale as it is a safe, effective and persistent alternative to chemical pesticides.

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