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Biochemical studies of thrips infected leaves of groudnut (*Arachis hypogaea L.*): An edible oil seed crop

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Abstract
Groudnut (*Arachis hypogaea* L.) is one of the most consumed legume globally. The Groundnut crop is attacked by several insect pests, mostly thrips (sap suckers). Four genera commonly infest Groundnut namely, *Scirtothrips dorsalis* Distant, *Frankliniella schultzei* Trybom, *Thrips palmi* Karny and *Caliothrips indicus* Bagnall. In the present study, an attempt was made to analyse certain biochemical changes in thrips infected Groundnut leaves collected from Cental Research Institute for Dryland Agriculture (CRIDA), Hyderabad. Thrips belongs to the family of Thysanoptera. In Groundnut crop; thrips feeding starts from seedling emergence to a few weeks following emergence. The severe infestation causes the yield loss and delayed maturity in early stage of crop. Thrips feeding also causes the transmission of viruses such as Tomato spotted wilt virus (TSWV), Groundnut ring spot virus (GRSV) and Groundnut bud necrosis virus (GBNV). In the present study leaf samples of healthy and infected Groundnut plants were collected and processed for biochemical analysis of total chlorophylls, proteins, sugars and starch contents. The results showed significant increase in total leaf proteins and decrease in total chlorophyll, starch and sugar contents in infected leaves compared to healthy leaves. The outcome of the above findings suggested that infestation of thrips on groundnut crops drastically reduce the crop yield through alteration of essential biochemical contents required for plant growth and development.

Keywords: thrips, groundnut, chlorophyll, starch, sugars, protein

Introduction

Groundnut (*Arachis hypogaea* L.) is an important food legume and oil seed crop belongs to the family Leguminosae (Beghnin and Sewadah, 2003) [3]. It is also known as peanut, earthnut, goober, pindar, manila nut etc. Groundnut is a leading oilseed crop in India and an important oilseed crop of tropical and subtropical regions of the world. The seed (kernels) contains up to 50 per cent of a non drying oil, 40-50 per cent fat, 20-50 per cent protein and 10-20 per cent carbohydrate (Mehta J, 2002) [25]. Though India ranks first in area under Groundnut cultivation, the productivity is quite low compared to that of USA, China, Argentina and Indonesia (Anon, 2005) [1]. The reason for low productivity of Groundnut is due to biotic and abiotic stresses during crop growth. Pests and diseases are the major biotic stresses for Groundnut production. Groundnut crop is attacked by about 90 species of insect pests. The sucking insect pest complex comprising thrips, *S. dorsalis* and leaf hoppers, *E. kerri* are the major pests of importance on Groundnut crop specially when raised under summer conditions and bunch varieties are severely infested (David and Ramamurthy, 2011). Groundnut in ranked as 13th most important food crops and 4th most important oilseed crop covering an area of 25.4 M.ha, globally with production of 45.2 Mt and productivity of 1.77 tonnes ha-1(FAOSTAT, 2013). In India it is cultivated in an area of 5.53 M.Ha with annual production of 9.67 MT and productivity of 1750 kg ha_1 which makes India second larger producer after china (FAO, 2013-14).China is the largest producer as well as consumer of Groundnut in the world with 171.50 lakh tonnes in 2017-18 followed by India (91.79 lakh tonnes), United States (32.81 lakh tonnes), Nigeria (24.20 lakh tonnes) and Sudan (16.41 lakh tonnes). Export of Groundnut oil is expected to double in 2019-20 owing to higher demand. The all India rabi crop coverage report, Government of India, as on 30th January 2020, Groundnut was sown in around 4.75 lakh hectares as compared to last year (4.59 lakh ha). Among the states, Telangana stood first in area coverage with 1.16 lakh ha followed by Karnataka (1.07 lakh ha), Tamilnadu (0.99 lakh ha), Andhra Pradesh (0.66 lakh ha), and Odisha (0.70 lakh ha).

The insect pests attacking Groundnut crop, thrips is an important sucking insect pest. Four genera commonly infest Groundnut namely *Scirtothrips dorsalis* Distant, *Frankliniella schultzei* Trybom, *Thrips palmi* Karny and *Caliothrips indicus* Bagnall. Thrips live in young foliage especially between the folded Groundnut leaflets and flowers that inhibit terminal buds and flowers. Both nymphs and adults feed by rasping the surface of rapidly growing leaf tissues and suck the released plant fluid (Chisholm and Lewis, 1984) [7]. Thrips are also known to transmit tomato bud necrosis disease caused by tomato spotted wilt virus in Groundnut and other several crops (Nagaraja *et al*, 2005) [27]. Early season moisture stress associated with thrips injury intensifies the Groundnut yield and quality loss (Funderburk *et al*, 1998).

Thrips adults and immatures primarily cause damage to peanuts by feeding on seedling plants. They feed in the folded leaf lets of the buds of plants causing scarred, deformed leaves which are referred to as possum-earned. Environmental factors can enhance the activity of these secondary pests sometimes resulting in economic loss. As well as causing direct damage to the plant, several of these pests cause indirect damage by transmitting viruses to

peanuts.

Materials and Methods

Study Area and sample Preparation

The Experiment were carried out in the Central Research Institute for Dryland Agriculture (CRIDA). The Groundnut variety of Kadiri-6 (K6) leaf samples were collected from the Research field during *Khariff* -2016& 2017 The healthy and infected leaves were taken and washed with distilled water and kept into the butter paper for drying and kept in to the hot air oven for 3 days at 120-130ºC. The processed leaf samples were grind with the pestle and motor. The grinded leaf samples were used for the estimations.

Estimation of Chlorophyll by Dimethyl Sulphoxide (DMSO) Method

50 milligrams of both healthy and infected samples were taken separately, washed with tap water followed by the distilled water. The samples were macerated in 30 ml of Dimethyl sulphoxide (DMSO). The vials are covered with black cloth and kept in to the dark condition for 24 hrs and color intensity read at 645 nm and 663 nm. Absorbance of the clear extracts was measured using Genesys UV/VIS spectrophotometer (Thermospectronic, Rochester, USA) at 645 and 663 for Chlorophyll a, Chlorophyll b and total chlorophyll, respectively (Porra *et al*, 1989).Concentration of leaf chlorophyll was expressed as mg/g of leaf. The following formulae were used for estimation of total chlorophyll, chlorophyll a and chlorophyll b contents.

Calculation of chlorophyll a, Chlorophyll b, and total chlorophyll is calculated by using the formula.(Sadasivam and Manikam, 2008)

Chlorophyll a (645nm) = (12.7(OD at 663)-2.69 (OD at 645))*v/1000*w

Chlorophyll b (663nm) = (22.9 (OD at645)-4.68 (OD at 663))*v/1000*w

Total chlorophyll = (20.2 (OD at 645)+8.02 (OD at 663))*v/1000*w

Here

V=volume of Dimethyl sulphoxide (DMSO).

W= weight of the sample

Where O.D stands for optical density and d.f for dilution factor. The results were expressed as mg of chlorophyll / g fresh weight.

Estimation of Total Sugars and Starch

The estimation of total sugars was done according to the method of Dubois *et al* (1951) [11] and total starch content by method of McCready *et al*(1950) [24]. Fifty milligrams of healthy and infected leaves were taken, washed thoroughly with tap water followed by distilled water and blotted to dry in between filter paper folds. The leaf samples were cut into bits and macerated with 5 ml of 80%ethanol. The macerates were transferred to centrifuge tubes and centrifuged at 5000 rpm for 15 min. The pellet was washed thrice with 80% ethanol. The supernatants were pooled and made up to known volume with 80% ethanol. The samples were heated in water bath at 85°C until the alcohol was completely lost from the samples. The supernatants were pooled and used for estimation of sugars. The pellet was subsequently used for extraction and estimation of starch.

Estimation of total Sugars

2 ml of healthy and necrosis virus infected pooled supernatants were taken separately into the test tubes. One ml of distilled water and 4 ml of cold anthrone reagent were rapidly added to each tube, shaken well and incubated for 10 min on ice -bath and cooled at room temperature. The blank was prepared by taking 1 ml of distilled water and 4 ml of cold anthrone reagent. The absorbance of the samples was read at 625 nm in a spectrophotometer. Amount of total sugars was estimated by using a standard curve prepared for D-glucose.

Estimation of Starch

The pellet which was collected from the above process was solubilized in 5 ml of 52% PCA and boiled at 80°C for 10 min. The solution was filtered through glass wool. The filtrate was measured and made up to 10 ml with PCA. 20 µl of healthy and infected sample extracts were taken separately, added 3 ml of distilled water and 5 ml of anthrone reagent and incubated for 10 min in ice bath. The absorbance of the samples was read at 625 nm in a Spectrophotometer. The amount of starch was calculated by using glucose standard curve.

Estimation of Total Proteins

Total leaf protein content was estimated by the method of Lowry *et al.*1951. 50 milligram of both healthy and sunflower necrosis infected sunflower leaves were taken washed thoroughly and blotted to dry in between filter paper folds. The leaves were cut into bits and homogenized separately in a mortar at 4°C using the grinding buffer (0.1 M Tris HCl, pH 8.3; 0.5 M Sucrose and 0.5%2-mercaptoethanol) at the rate of 2 ml/gm. The homogenate was squeezed through muslin cloth and centrifuged at 10,000 rpm for10 min. The supernatants were collected separately, added equal volume of 20% trichloroacetic acid (TCA) to each sample and kept for 2 hours at 4°C. The TCA precipitate was collected by centrifugation at 10,000 rpm for 10 min. The pellet was washed twice with 5% TCA and thrice with ice cold solvent ether. The final protein pellet was dried under vacuum and solubilized in a minimal known volume of 0.1 N NaOH solutions. Insoluble material was removed by centrifugation at 8000 rpm for 10 min and the soluble protein in the supernatant was estimated according to Lowry *et al.,* 1951. 20 µl of protein obtained from healthy and various infected samples were taken and to each sample added 5 ml of freshly prepared alkaline copper sulphate reagent. The samples were mixed well and the solution was allowed to stand for 10 min at room temperature. After 10 min incubation 0.5 ml of Folin phenol reagent was added to each sample and mixed thoroughly. After 30 min incubation the absorbance of the samples was read at 660 nm by using spectrophotometer. The amount of total leaf protein (mg/g fresh weight) was calculated by using bovine serum albumin (BSA) standard curve.

Result and Discussion

Table 1: Estimation of Chlorophylls:

Sample	Chlorophyll mg/g fresh leaves		
	Total chlorophyll	Chlorophyll a	Chlorophyll b
Healthy	1.7	1.346	0.354
	1.633	1.275	0.358
Infected	1.487	1.154	0.333
	1.429	1.092	0.337

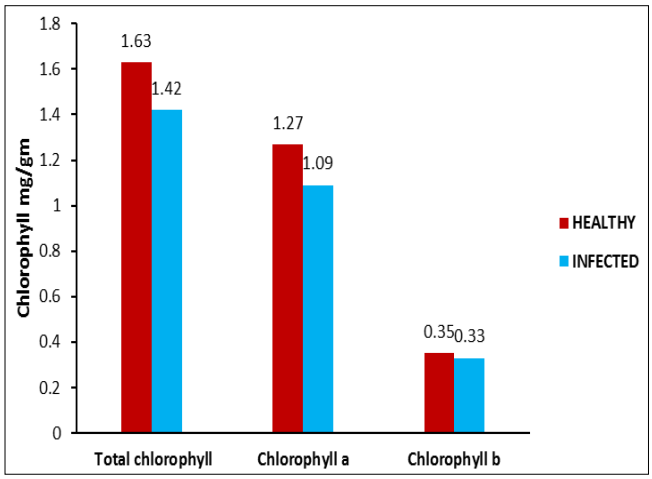

Fig 1

The estimation of Total chlorophyll, Chlorophyll a and Chlrophyll b by using Dimethyl sulphoxide (DMSO) method. In the Healthy Groundnut leaves Total chlorophyll (1.7), (1.63), Chlorophyll a (1.34), (1.27) & Chlorophyll b (0.354), (0.358). In the infected Groundnut leaves the total chlorophyll were (1.487), (1.429), Chlorophyll a (1.154), (1.092) & Chlorophyll b (0.333), (0.337) respectively.

Table 2: Estimation of sugars:

Sample	Concentration	Sugars content mg/g fresh leaves
Healthy	0.1	0.932
	0.2	1.652
Infected	0.1	0.527
	0.2	1.149

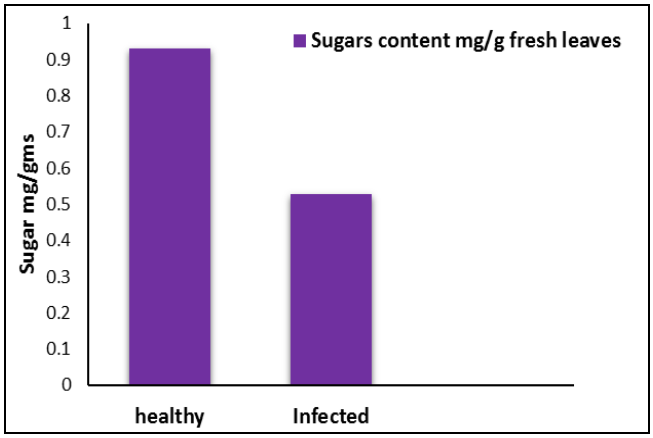

Fig 2

The Estimation of sugars by using two concentrations. The 0.1 concentration in healthy leaves (0.932 mg/g) & infected leaves showed the (0.527mg/g). In the 0.2 concentration the haelthy leave (1.652mg/g) & infected leaves showed the (1.149mg/g) respectively.

Table 3: Estimation of Starch:

Sample	Concentration	Sugars content mg/g fresh leaves
Healthy	0.1	0.8
	0.2	1.34
Infected	0.1	0.63
	0.2	0.78

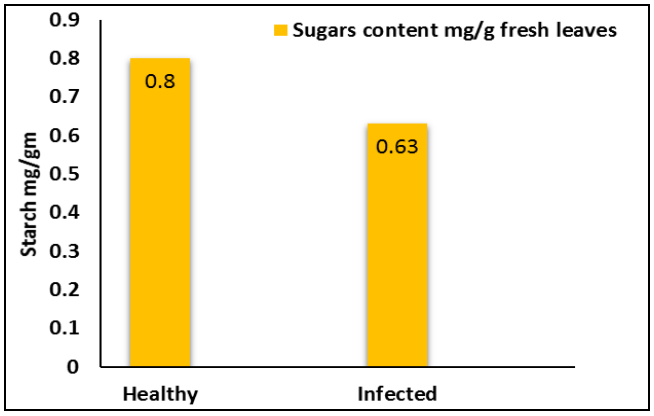

Fig 3

The Estimation of starch in 0.1 & 0.2 concentration in healthy leaves (0.8), (1.34). The infected leaves in two concentrations in 0.1 & 0.2 gives the **(**0.63), (0.78) respectively**.** The starch content was higher in healthy leaves than compare to infected Groundnut leaves. The concentrations increases the starch content in the sample also increased.

Table 4: Estimation of protein

Sample	Concentration(mg/gm)	Protein content mg/g fresh leaves
Healthy	0.1	0.395
	0.2	0.701
Infected	0.1	0.573
	0.2	1.443

The Estimation of protein by Lowry method the two concentrations were taken in the infected leaves 0.1 concentration (0.573) & 0.2 concentration (1.443). The healthy leaves the 0.1 concentration (0.395) & 0.2 concentration (0.701). the results observed that protein content higher in infected leaves than compare to healthy leaves. The concentration increases the protein content in the sample also increased.

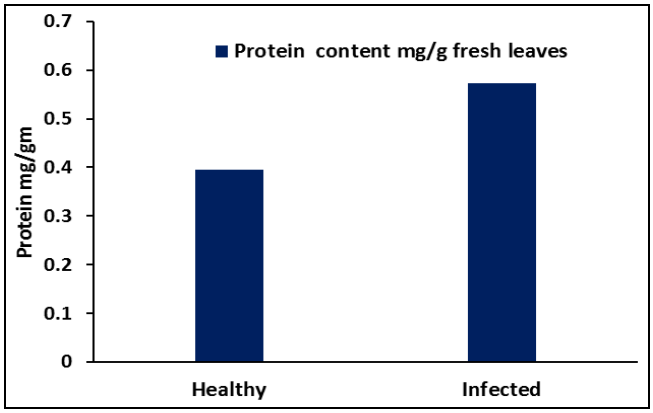

Fig 4

Damage due to pests and diseases interfere with morphological and physiological traits of plants affecting absorption of light energy and thus leading to alteration in the reflectance spectrum (Chaerle and Van Der Straeten, 2000) [6]; Hatfield and Pinter, (1993) [15]. Prabhakar *et al*., (2013) findings on reduction of leaf pigments, particularly leaf chlorophyll, with increased mosaic disease severity. Similar reduction of chlorophyll due to viral diseases have

been reported in mustard (Guo *et al*., 2005) [14], banana (Hooks *et al*., 2008) [16], sugarcane (Grisham *et al*., 2010), tobacco and tomato (Krezhova *et al*., 2009, 2010) [19, 20].

The physiology of virus infected plant is very important in understanding the involvement of pigments, carbohydrates, proteins, lipids, ascorbic acid and nucleic acid metabolism in tissues infected by a virus. There are several reports in the literature indicating many changes in the physiology and biochemistry of host plants as a consequence of disease. (Matthews, 1970) [23] in virus infected plant, production of chlorophyll may cease (chlorosis, necrosis), cell may either grow and divide rapidly or may grow very slowly and be unable to divide (distorsion, stunting).

The changes in chlorophyll may affect the growth and yield of the plants. Virus induced symptoms involved changes in leaf pigmentation. Reduced chlorophyll content in virus infected plants is attributed to stimulation of normal cell enzymes like chlorophyllase that degrades chlorophyll, and utilization of plastid proteins or their precursors for the synthesis of virus protein os of chlorophyll due to virus have been observed in different virus - host interactions. The decrease in chlorophyll content following virus infection may be accumulation of carbohydrates in the leaves. Watson reported that spraying sugar beet yellows virus infected plants with sucrose increased the carbohydrate content of the sugar beet leaves and enhanced the development of chlorosis. Naidu *et a.l*, (1984) [28] have shown that the chlorophyll 'a/b' ratio was decreased at severe stage of infection mainly due to decreased chlorophyll 'a' levels in peanut green mosaic virus infected peanut leaves. These changes appear to be secondary as faras synthesis is also is considered, they are an important part of the disease process, considering the plant as a whole (Crosbie and Matthews,1974) [8]. The virus infections have a number of indirect effects on host protein metabolism. In relation to the production of symptoms, plant growth by synthesis of significant amounts of a 'foreign protein' and to possible viral controls on expression of the host genome. The present studies amply substantiate the view that "virus infection of plants should be regarded as change in the protein metabolism of the host cells (Bawden and Pierie,1956) [2] Imbalances in total leaf proteins were noticed in the necrotic mosaic infected sunflower leaves. Significant increase in the total leaf proteins was noticed in the mosaic infected sunflower plants than healthy ones. The increased levels of soluble proteins in some virus host plants were earlier reported by several workers in different hosts Mohanty and Sridhar,(1986) [26] (Yadav and Mishra 1997) [32], (Rao *et al*, 1989) [30] (Bhavani *et al* 1998) [4], (Sutha *et al*,1998). The higher protein content in virus infected plants is possibly due to the synthesis of virus coat protein and other virus associated non-structural proteins

Carbon is a major structural element of carbohydrates which is essential for the various physiological activities of plant. The present study showed that the increased amount carbohydrate in plants

Physiological parameters and metabolic activities of the host plant considerably influenced by the attack of the disease. The biochemical changes caused due to morphological variations and the degree of yield loss can be observed by apparent symptoms caused by infected plant (Levy and Marco, 1982). Khalil *et al*., (2014) [18] reported that virus infected tomato plants caused considerable decrease in total of solublesugars: insoluble sugar and carbohydrate contents in stem and plant leaves respectively as compare with control In the present experiment total chlorophyll, chlorophylla & chlorophyll b was reduced in thrips damaged Groundnut leaves than compare to healthy leaves because the damaged leaves having the scarring causes the chlorophyll loss also reduced the staech & sugars in infected leaves shows the Table1,2& 3. Table 4 shows the protein content high in infected leaves than compare to healthy leaves.

Conclusion

The thrips is a sucking pests which causes the premature pods & yield loss in different crops. This pest causes the disease in chlorophyll content which leads to effecting the photosynthesis of different crop plants. The biochemical studies of Thrips infected Groundnut leases shows the decrease tn chlorophyll content, sugars and starch. The biochemical tests protein is higher in infected leaves than compare to healthy Groundnut leaves.

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