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Effect of Tannins on Groundnut (*Arachis hypogaea*)

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Tannins delayed the germination of groundnut seeds. They inhibited the growth of seedlings, and myrobalan tannin was more inhibitory than wattle. Malformations were frequent in the seedlings kept in 4% myrobalan tannin. Tannin treatment reduced the α-naphthylamine oxidizing capacity of roots. Tannins significantly inhibited the shoot and root growth of 42-day-old plants. Chlorophyll and carotenoid contents of tannin treated 25-day-old plants were reduced.

Key Words: Tannin toxicity, Groundnut seed germination, Chlorophyll, Plant growth

Introduction

Tannins are widely distributed in nature and are the fourth most abundant plant constituent after cellulose, hemicellulose and lignin. Tannins occur extensively in higher plants including aquatic angiosperms (Boyd 1970) and in a few lower plants. These are present in leaves, stems, barks, fruits, hairs and sometimes in roots.

Tannin is a major toxic component of tannery effluent (Rao & Mariappan 1972) which finds its way to crop fields, causing stunting, chlorosis and reduction in yield. The scientific basis of these observations has not been carefully investigated. As early as 1967, Floyd and Rice found that gallotannin suppressed the growth of *Digitaria sanguinalis* and *Helianthus annuus*. Tobacco plants treated in tannin developed chlorosis (Einhelling 1971). Till date no analytical study on the effect of tannins on plant growth has been made. In this paper, we present our results on the effect of tannins on groundnut (*Arachis hypogaea*).

Materials and Methods

Groundnut variety TMV 7 obtained from Oil Seeds Research Station, Tindivanam, Tamil Nadu, was used. Wattle and myrobalan tannins were purchased from Tan India Ltd. Madras.

Groundnut seeds of uniform size were selected and washed thoroughly in tap water and surface sterilized with 0.1% mercuric chloride. The seeds were again washed with sterile water for 3 times. Ten seeds were placed in a sterile Petri dish containing filter paper.

Wattle and myrobalan tannin solutions were filter sterilized after adjusting the *p*H to 7. Ten ml each of different tannin concentrations, ranging from 1 to 4% were poured into the Petri dish. After 3 days, the per cent germination of seeds and emergence of radicles were determined.

Surface sterilized seeds were germinated in sterile distilled water. Three-day-old seedlings were placed in a sterile Petri dish containing filter paper. The initial length of the seedlings was measured; five seedlings were placed in each Petri dish and treated with tannin extracts. After 3 days of incubation, final lengths were measured. Ten replicates were maintained for each treatment.

Determination of root activity

Roots from 7-day-old seedlings were washed in water, cut into 1–2 cm segments and pooled. After squeezing the excess water, 1 to 2 g of this sample was weighed and transferred to an Erlenmeyer flask containing 50 ml of 20 ppm α-naphthylamine solution. The flask was shaken for 2–3 hr at 100 strokes/min. Two ml aliquot of α-naphthylamine solution was pipetted out before and after incubation into a graduated test tube and diluted with 10 ml water. Sulphanilic acid, 1 ml (1 g in 100 ml of 30% acetic acid) and 1 ml of 100 ppm sodium nitrite solution were added, and the mixture was made up to 20 ml with water. After 30-60 min, the solution was read at 500 nm in a colorimeter (Oto 1970).

Field soil was collected from groundnut fields and thoroughly mixed to ensure homogeneity. Five kg of soil was taken in each pot measuring 24 cm height and 20 cm diameter. Eight seeds were planted in each pot and upon germination thinned to 5 per pot. The water-holding capacity of the soil was maintained at 50%. Each pot was treated with an aqueous solution of tannin concentrations varying from 1–4% and the pots were kept in the field. Five replicates were maintained for each treatment.

When the plants were 25-days-old, the third leaf from the base of each plant was collected and the chlorophyll (Arnon 1949) and carotenoid content (Goodwin 1955) were estimated. When the plants were 42-days-old, they were gently pulled out and their root and shoot lengths were measured.

The data were analyzed statistically using "t" test (Panse & Sukhatme 1967).

Results

Seed germination

Tannins delayed the seed germination and caused significant inhibition at 3 to 4% concentration. Wattle tannin inhibited the

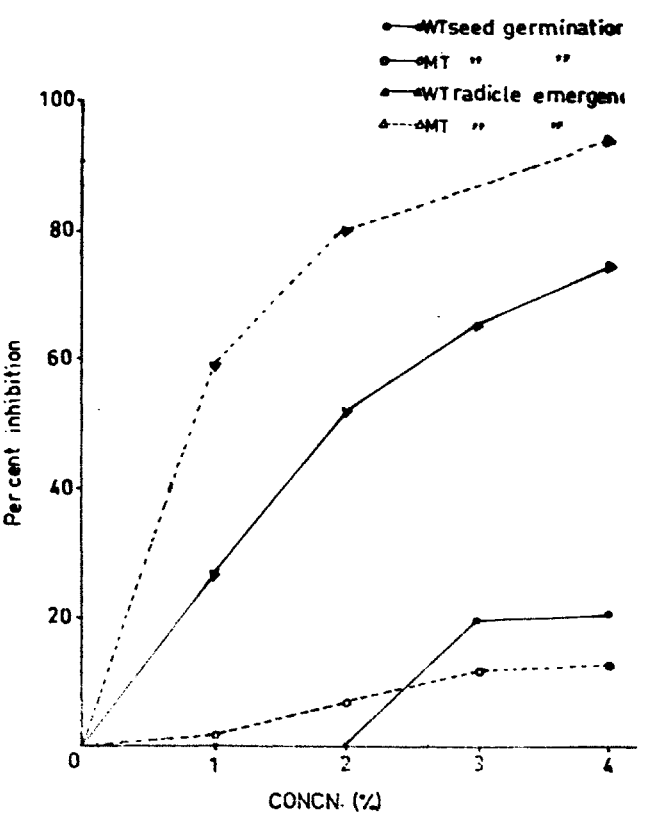


Figure 1 Effect of tannins on groundnut seed germination and radicle emergence

seed germination more than myrobalan tannin did (figure 1). Myrobalan tannin caused more inhibition of radicle emergence than did wattle tannin (figure 2 a,b).

Growth of seedlings

Tannins inhibited the growth of the seedlings. Myrobalan tannin was more inhibitory than wattle tannin. The inhibition was concentration dependent. The radicles became dark and their growth was completely arrested by 4% myrobalan tannin (figure 2d). Secondary root formation was arrested in the tannin treated seedlings. The radicle as well as plumule length was markedly reduced (figure 2c,d). Malformations were frequent in the seedlings kept in high concentrations of myrobalan tannin. The radicle twisted over the cotyledonary leaves (figure 2f). The first leaves developed but the radicles hardly grew. The opening of cotyledonary leaves was also affected by tannins (figure 2f).

Transverse sections of tannin treated roots clearly showed the accumulation of a dark coloured substance in the xylem (figure 2h). The seedlings raised in tannin solutions were washed thoroughly and placed in Petri dishes containing sterile distilled water. After 3 days, the growth of hypocotyl resumed but the radicle did not grow further. The initiation of secondary roots started from the sides of the suppressed radicle (figure 2e, g). Most of the seedlings did not show the growth of the plumule (figure 2g).

Root activity

The α-naphthylamine-oxidizing capacity of tannin-treated and control roots indicated that the former reduced the oxidizing capacity of the roots (figure 3). Myrobalan tannin reduced the root activity more than wattle tannin. The maximum inhibition occurred in myrobalan treated roots at 4%. Wattle tannin reduced the α-naphthylamine oxidizing capacity of the roots; the maximum inhibition was noted in 3% solution. Surprisingly at 4% concentration, the inhibitory effect was less than at 2%.

Plant growth

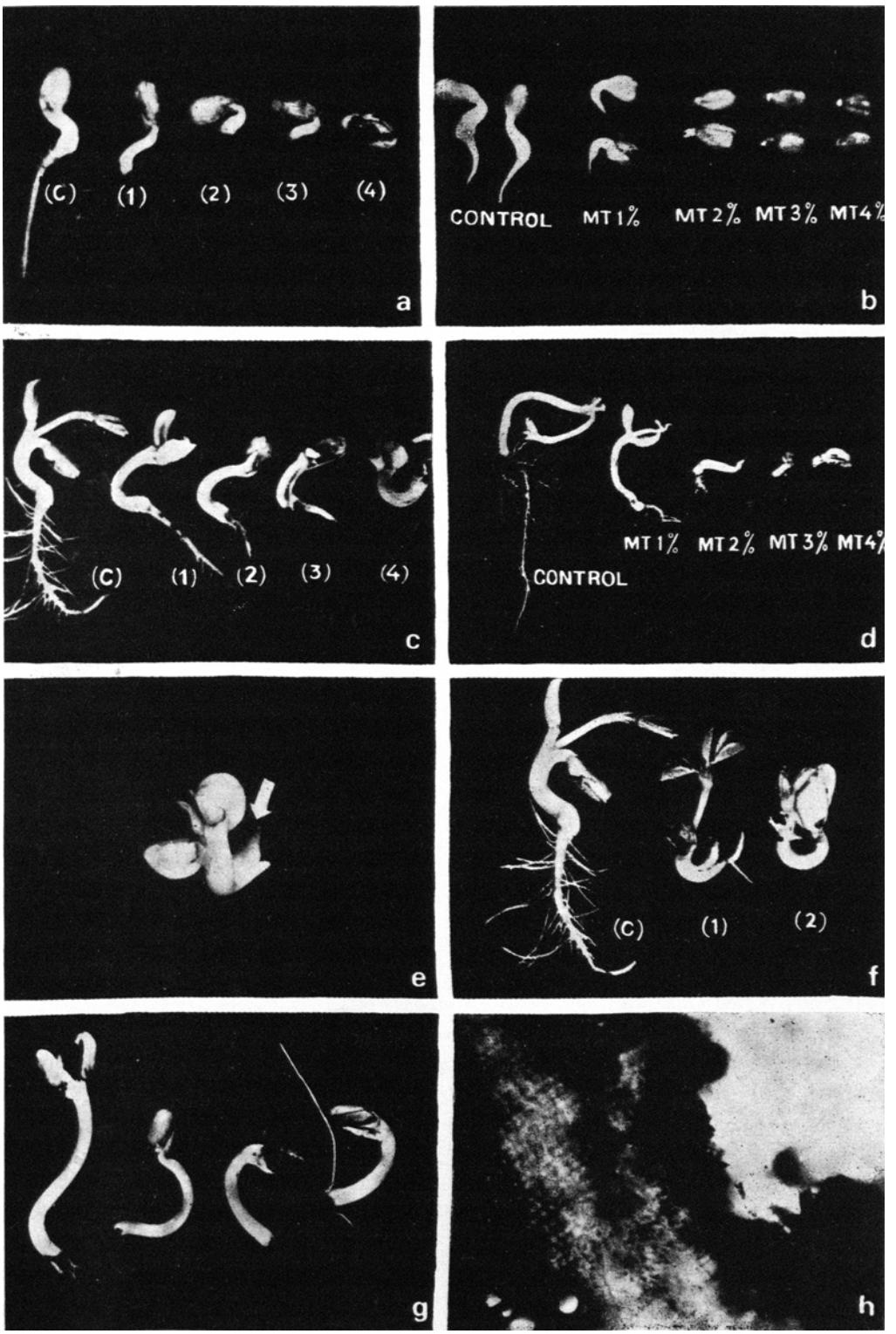
Tannins at 1% significantly inhibited the shoot growth of 42-day-old groundnut plant and the effect increased with concentration. Myrobalan tannin was more toxic than wattle tannin (figure 4). The tap root length was significantly inhibited even by 1% tannin solution (figure 5). The apical meristem was injured and the multi-furcation of the roots occurred only at high concentrations. The length of the roots was inhibited markedly by myrobalan tannin. The effect on root length was pronounced especially in the higher concentrations. The general observations on tannin treated plants were:

(i) Inhibition of tap root growth,
(ii) Secondary roots appeared from damaged root tip or above the root tip, presumably to perform the function of tap root,
(iii) Roots were injured,
(iv) Chlorosis was common,
(v) Plants were stunted.

Chlorophyll and carotenoid contents

Chlorophylls *a* and *b* of tannin treated groundnut plants were significantly reduced

Figure 2 (a) Wattle tannin on seed germination; (b) Myrobalan tannin on seed germination; (c) Wattle tannin on radicle elongation; (d) Myrobalan tannin on radicle elongation; (e) Secondary root emergence from hypocotyl region; (f) Abnormalities developed in tannin treated seedlings; (g) Tannin treated seedlings after washing were placed in distilled water. Emergence of secondary roots; (h) Cross section of tannin treated groundnut root showing the root injury and tannin accumulation in the xylem→


(C) (1) (2) (3) (4)
a
CONTROL MT1% MT2% MT3% MT4%
b
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c
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CONTROL
d
e
(C) (1) (2)
f
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(table 1). Wattle tannin was more effective than myrobalan tannin and the maximum inhibitory effect occurred at 3% concentration. The myrobalan tannin reduced the chlorophyll content of the leaves. The effect increased with increase in concentration with the maximum effect at 4%. Chlorophyll *a* was more affected than chlorophyll *b*.

The carotenoid content of 25-day-old groundnut plant was reduced by tannin treatment. The results (table 1) clearly indicate that wattle tannin was more toxic than myrobalan tannin. Myrobalan caused the maximum effect at 2% and at higher concentrations, the inhibitory effect was less. Wattle tannin caused the maximum effect at 3%. However, 4% caused less effect than the 3% solution.

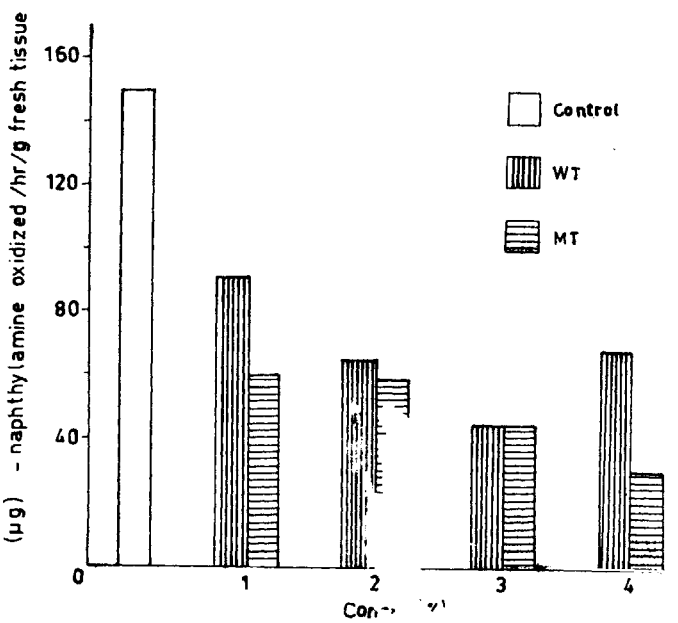

Figure 3 Effect of tannins on roc ty of groundnut

Table 1 *Effect of tannins on chlorophyll and carotenoid content of groundnut leaves*

Treatment	Chlorophyll (µg/g fresh tissue)		Carotenoid (µg/g fresh tissue)
	a	b	
Control	974	944	365
Wattle tannin			
1%	510	616	249
2%	416	478	184
3%	340	469	161
4%	373	513	193
Myrobalan tannin			
1%	632	726	314
2%	448	640	202
3%	449	633	239
4%	385	558	221

Discussion

Tannins were toxic to seed germination, radicle elongation and plant growth. They reduced both chlorophyll and carotenoid contents of the leaves. In general, myrobalan tannin was more toxic than wattle to groundnut plant. Myrobalan tannin is composed of esters of glucose and ellagic acids. The basic monomer of hydrolysable tannin is gallic acid and wattle tannin, flavan 3,4-diol (Haslam 1966). Obviously, chemical structure seems to influence the activity of tannins.

The concentration of tannin profoundly influenced its activity. Generally, the toxic effect increased with rise in concentration. Presumably at high concentrations, the entry of tannins into the cells might have been affected which in turn could have reduced the activity. Presently no suitable technique is available to measure the tannin uptake by plants. Moreover, part of the tannin is

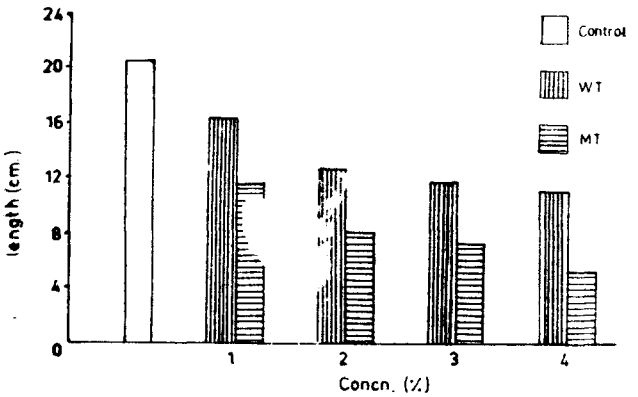

Figure 4 Effect of tannin on shoot length of groundnut

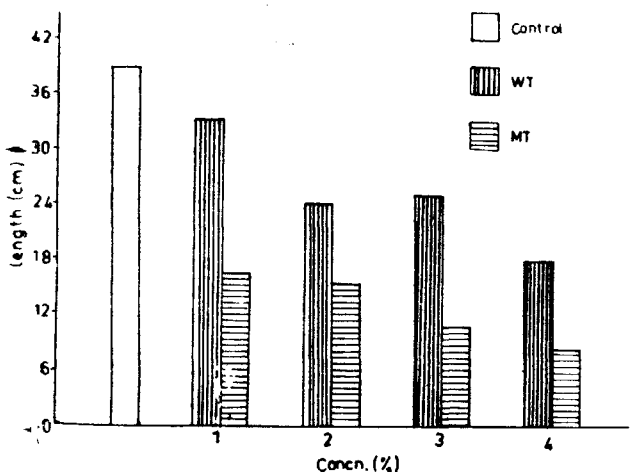

Figure 5 Tannins on the root length of groundnut

rapidly oxidized by the plants making the estimation difficult and unreliable.

Seed germination and radicle elongation were profoundly affected by tannins. The mechanism of inhibition of seed germination by tannin is not known. Perhaps the tannin prevents the water uptake by embryos.

Varga and Koves (1959) identified a hydrolysable tannin as growth and germination inhibitor. Hydrolysable tannins from *Arctostaphylos glandulosa* inhibited seed germination and seedling growth (Chou & Muller 1972). Growth inhibition might involve interference with K^+ and Ca^{++} uptake and photosynthesis (Olmstead & Rice 1970) and interference with gibberellin action (Corcoran et al. 1972). Permanent damage to the apical meristem as shown in this study is the major mechanism of tannin action.

The oxidizing capacity of the roots as measured by α-naphthylamine test is related to the rate of respiration and the tannins strongly inhibited the respiration of groundnut roots. In the absence of published data, it is difficult to postulate the mechanism of action of tannin on plant respiration.

In general, tannin inhibited the growth of groundnut plants. Floyd and Rice (1967) observed that gallotannin inhibited the growth of *Digitaria sanguinalis* and *Helianthus annuus*. Although the mechanism of growth inhibition caused by tannins is not known, Corcoran et al. (1972) implicated that tannins caused stunting by interfering with endogenous gibberellin activity. Experiments are required to verify this hypothesis.

The tannin treated groundnut plants developed chlorosis which confirms the findings of Einhelling (1971) on tobacco. The mechanism of induced chlorosis is not clear. The reduction of available iron in tannin-treated soil has been demonstrated by Sivaswamy and Mahadevan (unpublished). It is assumed that tannin impairs the availability of iron in the soil to the plants which leads to iron chlorosis (Brown 1956). Tannins seem to interfere with chlorophyll synthesis. In fact, Blum and Rice (1969) have reported that gallic acid and tannic acid interfere with porphyrin synthesis in plants. Similarly carotenoid synthesis is also inhibited by tannins but the details of inhibition are not known.

The reduction in shoot and root growth of groundnut seedlings by tannins is caused

by interference with hormonal action (Zinsmeister 1962, Corcoran et al. 1972), photosynthesis (Einhelling 1971), membrane permeability (Muthukumar 1980) and direct injury to the root system (Floyd & Rice 1967). Admittedly the effects of tannins on groundnut plant are complex which culminate in severe abnormalities.

Certainly tannery effluents which are continuously discharged into water streams and fields will cause persistent and deleterious effects on plants and productivity of the soil.

Acknowledgement

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