Malaysian Journal of Science **31** (1): 8-15 (2012)

BIOASSAY-GUIDED IDENTIFICATION OF CHEMICAL COMPONENTS IN *TYPHONIUM ROXBURGHII* SCHOTT

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Received on 8th August 2011, accepted in revised form 13th February 2012.

ABSTRACT *Typhonium roxburghii* Schott (corm) extracts were screened for insecticidal activity against the storage pest *Tribolium castaneum* (Herbst). Among the different extracts tested, the benzene extract was found to be more effective (LC_{50} 3.042% in 24 h). However, the yellow viscous mass obtained from the petroleum ether fraction of the benzene extract induced absolute mortality at 0.12% concentration (LC_{50} 0.056% in 24 h). It was subjected to GC-MS studies and the chemical constituents were identified as methyl ester of 2-hydroxy benzoic acid, diethyl phthalate and dioctyl phthalate. Cultivation of *T. roxburghii* is possible because it does not prefer natural habitat. It is quick growing and needs no fencing. It can be cultivated in wastelands with fewer expenses. The positive externalities due to cultivation of *T. roxburghii* would be in the form of scenic beauty of the region with its evergreen leaves, virtual absence of mosquitoes even though sewage water be used. Identification of chemical constituents in *T. roxburghii* is a pioneer work and a more extensive exploratory study might reveal more. This can be exploited as an eco-friendly bio-pesticide.

(**Keywords**: *Typhonium roxburghii*, *Tribolium castaneum*, benzene extract, LC_{50} values, chemical constituents.)

INTRODUCTION

There are about 30 species of *Typhonium* [1], 9 species in India and 5 in peninsular India [2]. *Typhonium roxburghii* Schott (Araceae) is distributed in South Asia from India through New Guinea and Sri Lanka; escaped in Africa and Neotropics [3]. In India, it occurs in Southern and Eastern parts [2]. In South India, it occurs in Kerala and Karnataka. In Tamil Nadu, it is cultivated, and occurs as an escape. The species is native in Southern India, Sri Lanka, Malaysia and Indonesia [4]. It grows in plains among grasses in open moist places. It is also grown as an ornamental plant for its evergreen leaves. The corms possess rubifacient properties and they are used in diarrhoea [5]. Corms are reported to be used in Java for eruption on the skin [6] and for dermatitis, in Malaya [7]. Despite this interesting medicinal value, the taxon has not been subjected to systematic evaluation and phytochemical analysis so far. In recent years, extensive survey of the flora has been undertaken to search for potential plant extracts, which could be used in the management of agricultural and household pests. Moreover, investigations on the insecticidal properties of plant extracts have been given an impetus because of imposition of restrictions on the use of chemicals for insect control. In the present study, various extracts obtained from the corm of *T. roxburghii* were subjected to insecticidal activity on the storage pest, *Tribolium castaneum* (Herbst). Red flour beetles attack stored grain products such as flour, cereals, meal, crackers, beans, spices, pasta, cake mix, dried pet food, dried flowers, chocolate, nuts, seeds, and even dried museum specimens. This beetle has chewing mouthparts, but do not bite or sting. It may elicit an allergic response, but is not known to spread disease and does not feed on or damage the structure of a home or furniture [8]. *T. castaneum* is one of the most important pests of stored products in the home and grocery stores. So far, no data were available to determine the insecticidal activity of *T. roxburghii*, and this is the first of this kind in this plant. This bioassay, guided the identification of chemical compounds in the corm of *T. roxburghii*.

MATERIALS AND METHODS

The corms of *T. roxburghii* were collected in the month of January in Palayamkottai, Tirunelveli

District, Tamil Nadu. Authentic specimen is deposited in the Department of Botany, St. John's College, Palayamkottai. The corms were cleaned with water, chopped, dried under shade and ground into powder. 30 g of the powder was extracted successively (8 h / 3 times) with petroleum ether (40°C-60°C), benzene (80°C), chloroform (60°C) and methanol (65°C) using soxhlet apparatus. The extracts were concentrated by distillation and evaporated to dryness under reduced pressure. A pilot study was conducted to know the insecticidal activity of the extracts by filter paper impregnation method [9] using 5 percent concentration of the extracts in their respective solvents against the storage pest *T. castaneum*. The extracts (5% - 5 g of crude extract dissolved in 100 ml of the solvent) which showed the insect mortality rate of 50% or above in 24 h, were further, studied. Among the various treatments administered, the benzene extract of *T. roxburghii* was found to be more effective. Hence, the lethal concentrations of the extract at LC_{50} level against *T. castaneum* was determined by filter paper impregnation method.

Preliminary range finding test was conducted to detect the concentration of the benzene extract causing total mortality. Based on the preliminary test, graded concentrations of the extract 1%, 2%, ... 7% w/v were prepared in benzene. One ml of each solution was spread with the help of the pipette, over a filter paper (Whatmann No.1) of diameter 3 cm, in an embryo cup. The paper was allowed to dry under reduced pressure and after complete evaporation of the solvent; it was placed in a cylindrical plastic container of 3 cm diameter and 4.5 cm height. The solvent without the extract was kept as control. Tiny holes were made in the lid of the container, for the passage of air through it. Laboratory reared adult insects of *T. castaneum* (30 numbers) were released in the container and closed with the holed lid. Mortality counts were undertaken after 24 h, 48 h, 72 h and 96 h and expressed as percent mortality. All the experiments were conducted in triplicate and the data obtained were subjected to log-Probit regression analysis, to calculate the median lethal concentration (LC_{50}) values.

For the identification of the chemical compounds, 300 g of the dry powder was extracted with two litres of benzene (8 h / 3 times / 80°C) using a three litre round bottom flask fitted with a water condenser. Only glass containers were used for storing the powder and the extract. The extract was concentrated after distilling the solvent under reduced pressure. The crude mass obtained was extracted two times with 200 ml of petroleum ether (40-60°C) in a soxhlet apparatus. The petroleum soluble fraction was again partitioned between chloroform and methanol (1:1). The chloroform layer was separated and upon evaporation of the solvent yielded a yellowish viscous mass (TR-PF). It was screened for insecticidal activity by filter paper impregnation method and the (LC_{50}) values were determined. Since the activity was more effective than the crude extract, for the identification of active principles, the TR-PF (0.1 µl) was subjected to systematic GC and MS [Gas Chromatograph – Mass Spectrum] analysis using the SHIMADZU instrument, GC-MS P5000 (Japan). The length and diameter of the column [Carr. Gas Press (*k*Pa) : 24.50] was 20 m and 0.25 mm respectively. The initial temperature was 70ºC and then risen by 10ºC/min. to 300ºC. The temperature 300ºC was maintained for 20 minutes. The results are presented in **Figs. 1 — 4**.

RESULTS AND DISCUSSION

Benzene extract and Petroleum ether fraction of the benzene extract (TR-PF) of *T. roxburghii* corm were toxic to *T. castaneum* (**Tables 1 & 2**). Mortality rate increased with increase in concentration of plant extracts. Total insect mortality was observed within 24 h of exposure at a concentration of 7% in benzene extract. However, the fraction TR - PF (Petroleum ether fraction of the benzene extract of *T. roxburghii* corm) was found to be more effective inducing absolute mortality at 0.12% concentration. Statistical analyses were carried out to determine 24, 48, 72 and 96 h LC_{50} values and the regression equation (**Tables 3 & 4**). Significant results were obtained.

Table 1. Insecticidal activity of the benzene extract of *Typhonium roxburghii* (Corm) against *Tribolium castaneum*

Concentration of the extract (%)	Mortality (%)			
	24 h	48 h	72 h	96h
0	0	0	0	0
1	13.3	23.3	36.7	43.3
2	30.0	36.7	46.7	53.3
3	53.3	56.7	56.7	66.7
4	56.7	66.7	70.0	93.3
5	63.3	73.3	86.7	100
6	76.7	90.0	100	100
7	100	100	100	100

Table 2. Insecticidal activity of the petroleum ether fraction of *Typhonium roxburghii* corm (TR-PF) against *Tribolium castaneum*

Concentration of the extract (%)	Mortality (%)			
	24 h	48 h	72 h	96 h
0	0	0	0	0
0.02	13.3	13.3	43.3	43.3
0.04	23.3	46.7	53.3	53.3
0.06	53.3	56.7	66.7	70.0
0.08	53.3	63.3	70.0	93.3
0.10	86.7	100	100	100
0.12	100	100	100	100

Table 3. *Typhonium roxburghii* (Corm) benzene extract on the LC_{50} parameters of *Tribolium castaneum*

Hours	**Variance**	**Chi-square df=5**	**Regression equation**	**Lower Fiducial Limit**	**Upper Fiducial Limit**	**LC_{50} (%)**
24	0.0042	3.35	y=3.208x + 0.24	2.780	3.303	3.042
48	0.0053	2.89	y=3.107x + 0.67	2.224	2.732	2.478
72	0.0080	5.29	y=3.262x + 0.71	1.784	2.335	2.060
96	0.0080	4.55	y=3.536x + 0.73	1.379	1.846	1.612

Table 4. *Typhonium roxburghii* (Corm) Petroleum ether Fraction (TR-PF) on the LC_{50} parameters of *Tribolium castaneum*

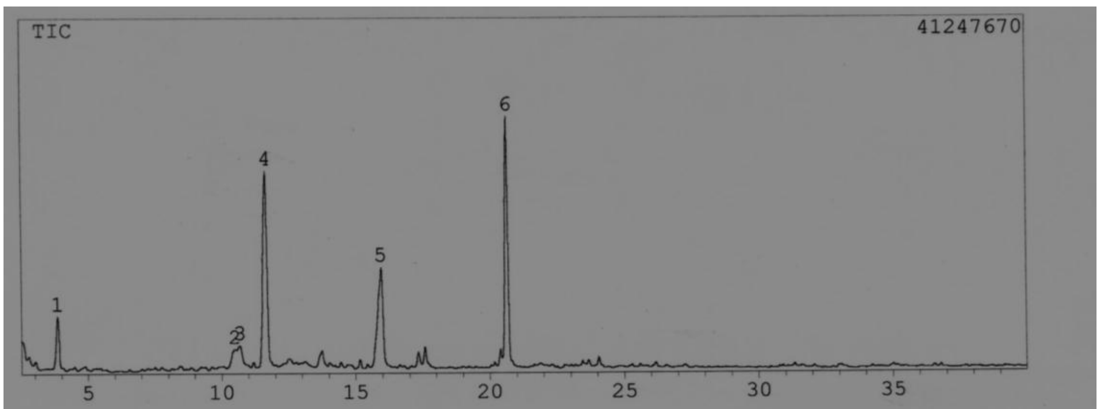
Hours	**Variance**	**Chi-square df=4**	**Regression equation**	**Lower Fiducial Limit**	**Upper Fiducial Limit**	**LC_{50} (%)**
24	0.0032	5.40	y=4.025x – 2.03	0.052	0.060	0.056
48	0.0042	6.44	y=4.387x – 2.38	0.044	0.052	0.048
72	0.0115	6.24	y=3.285x – 0.10	0.031	0.040	0.036
96	0.0085	3.66	y=3.577x – 0.38	0.028	0.036	0.032

Essential oil extracted from the leaves of turmeric (*Curcuma longa*) was investigated for contact and fumigant toxicity and its effect on progeny production in three stored-product beetles, *Rhyzopertha dominica* F. (lesser grain borer), *Sitophilus oryzae* L. (rice weevil), and *Tribolium castaneum* Herbst (red flour beetle) [10]. In *T. castaneum*, the *C. longa* oil reduced oviposition and egg hatching by 72 and 80%, respectively at the concentration of 5.2 mg/cm^2.

At the concentration of 40.5 mg/g food, the oil totally suppressed progeny production of all the three test insects. Seed extracts of custard apple was found to have LD_{50} value 22 mg/cm^2 against *T. castaneum* in film residue method [11]. In the present investigation by filter paper impregnation method one ml of extract / oil was loaded on Whatman filter paper of 3 cm diameter and placed in a vial of 30 ml capacity. 1,8-Cineole isolated from *Artemisia annua* was tested against *T. castaneum* for contact toxicity, fumigant toxicity, and antifeedant activity [12]. The adults of *T. castaneum* were more susceptible than larvae to both contact and fumigant toxicity of 1,8-cineole, and LD_{50} and LC_{50} values of 108.4 µg/mg body weight of adult insect and 1.52 mg/liter air were found, respectively.

The yellow viscous mass (TR-PF) which was found to be highly toxic to insects, when subjected to GC-MS analysis gave six peaks (**Fig. 1**). The percentages of the six peaks were 6.02%, 3.60%, 3.69%, 31.81%, 22.06% and 32.82% respectively. Comparison of the mass spectra of these compounds to those of the compiled data of Wiley 7n : 1 library attached to the mass spectrometer revealed that compound TR-1 (Peak1) was identical with the compound methyl ester of 2 hydroxy-benzoic acid (**Fig. 2**). Compounds TR-2, TR-3, TR-4 and TR-5 (Peaks 2, 3, 4 and 5) were found to be identical with diethyl phthalate (**Fig. 3**). Compound TR-6 was found to be identical with dioctyl phthalate (**Fig. 4**).

Sample: TR-PF / ANNIE / SJC



**** Peak Report ****

PKNO	R.Time	I.Time - F.Time	Area	Height	A/H(sec)	MK	%Total	Na
1	3.872	3.683 - 4.042	43674968	5941051	7.351		6.02	
2	10.508	10.200 - 10.583	26075936	1986694	13.125		3.60	
3	10.694	10.583 - 10.942	26748111	2319987	11.529	V	3.69	
4	11.669	11.375 - 11.967	230655585	22650635	10.183		31.81	
5	15.983	15.575 - 16.375	159926510	11530871	13.869		22.06	
6	20.667	20.492 - 21.050	237945934	28971670	8.213	V	32.82	
Total			725027043				100.00	

Figure 1. GC-MS chromatogram of the fraction obtained from the Petroleum ether extract of *Typhonium roxburghil* corm (TR-PF) showing six peaks

Methyl ester of 2-hydroxy-benzoic acid

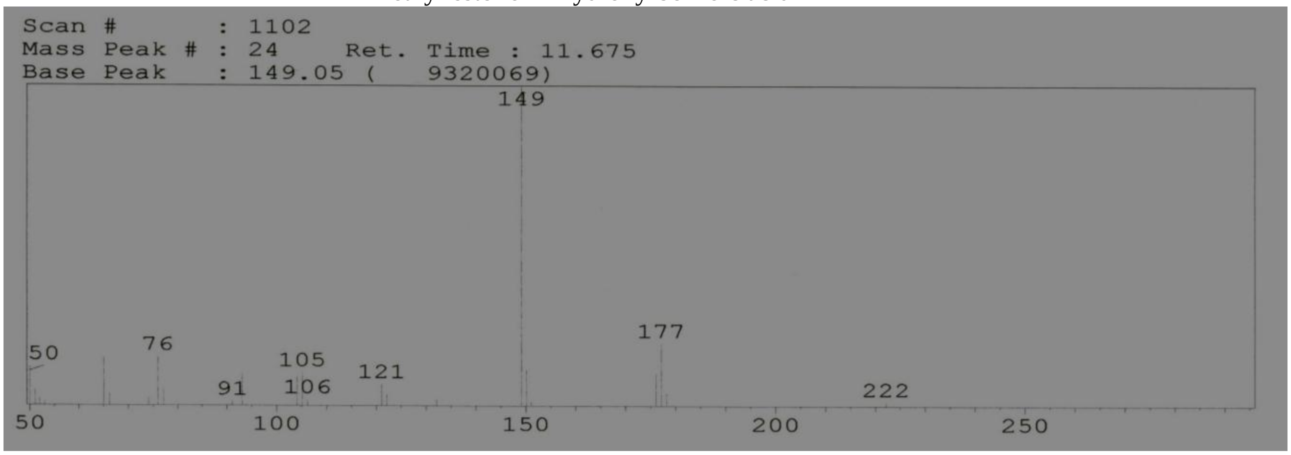


Figure 2. GC – Mass Spectrum of compound 1 (TR - 1) Present in the petroleum ether fraction of *Typhonium roxburghii* corm (TR – PF)

Diethyl phthalate

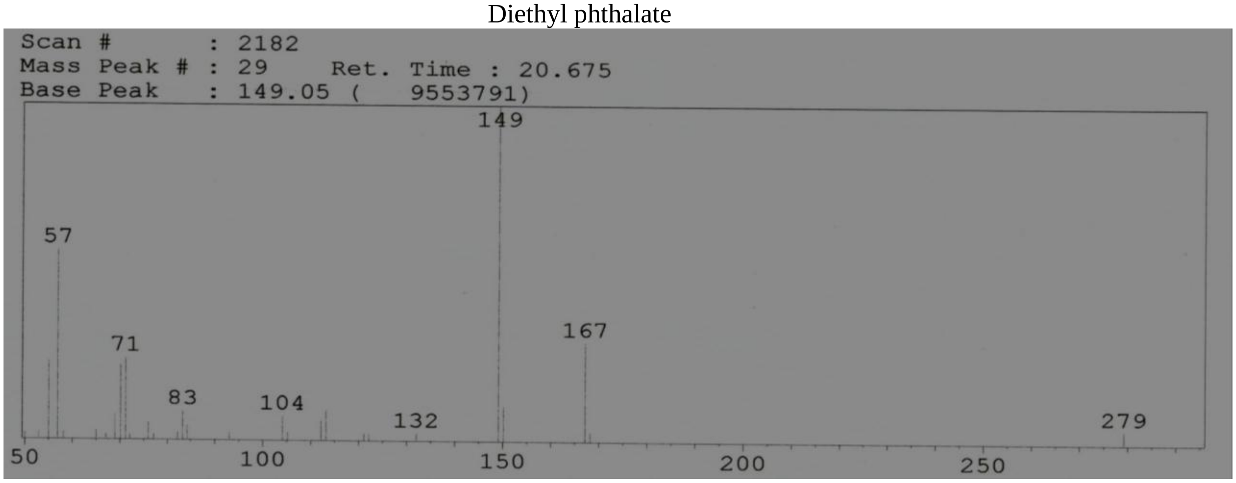


Figure 3. GC– Mass Spectrum of compounds 2-5 (TR – 2-5 of peaks 2, 3, 4 & 5) Present in the petroleum ether fraction of *Typhonium roxburghii* corm(TR–P F)

Dioctyl phthalate

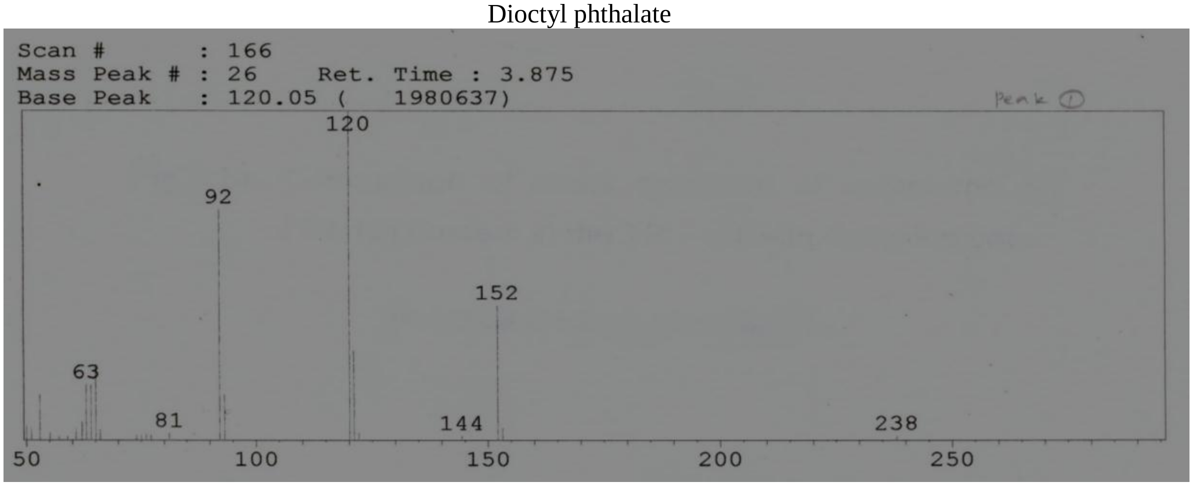
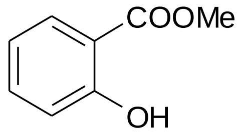
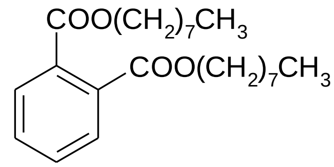


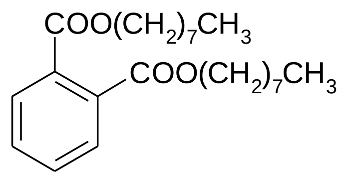
Figure 4. GC – Mass Spectrum of compound 6 (TR - 6) Present in the petroleum ether fraction of *Typhonium roxburghii* corm (TR – PF)



Methyl ester of 2 hydroxy-benzoic acid



Diethyl phthalate



Dioctyl phthalate

Figure 5. Structure of chemical compounds.

The present study reveals the chemical constituents of TR-PF, which was isolated from the benzene extract of *T. roxburghii* corm and which was ascertained to be toxic to *T. castaneum*. TR-PF was found to contain methyl ester of 2-hydroxy-benzoic acid, diethyl phthalate and dioctyl phthalate. It is evident that any of these phytochemicals or the combined effect of these toxic compounds showed the insecticidal activity. Literature survey reveals that benzoic acids and its esters have been used externally as antiseptic lotions, ointments and mouthwashes [13]. It is more effective as a preservative in foods and pharmaceutical products at low pH [14]. Methyl ester of 2-hydroxy-benzoic acid is the major constituent of "winter green oil" (Wiley 7n:1 library), an essential oil, generally obtained from the leaves of a shrub *Gaultheria procumbens* (Ericaceae) [15]. Diethyl phthalate has lethal toxicity. It affects certain organs at low concentration, specifically the reproductive organs, lungs, kidney and liver [16]. Diethyl phthalate has been reported as an antibacterial agent against *Campylobactor jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica* [17]. Dioctyl phthalate, an antimicrobial compound was also isolated from the methanol extract of the marine brown alga *Sargassum wightti* by purifying through silica gel column and thin layer chromatography [18]. Phthalates are the natural toxic chemicals having germicidal activities [19].

Use of environment friendly and easily biodegradable natural insecticides of plant origin has renewed importance. Such botanicals are least phytotoxic, biodegradable and do not accumulate chemical residues in flora, fauna and soil. Chemical control of insect pests leads to serious problems like environmental pollution, health hazards among people, livestock, and insect resistance to insecticides. Some substances may have delayed toxicity. In less than two decades of the use of the synthetic chemical pesticides in agriculture, the pesticides have been thoroughly distributed that they occur virtually everywhere and now stored in the bodies of majority of human beings regardless of their age. They occur in the mother's milk and probably in the tissues of the unborn child [20]. Insects are affected by wide range of secondary compounds. Phytochemicals have been established to be antifeedants, repellents, growth inhibitors or as insecticides [21]. The trend to utilize botanical pest control agents led to the study of the efficacy of many plant extracts as insecticides. Secondary chemical compounds that occur naturally in plants have the potential for application in environment-friendly management of pests [22].

CONCLUSION

Cultivation of *T. roxburghii* is possible because it does not prefer natural habitat. It is quick growing and needs no fencing. It can be cultivated in wastelands with fewer expenses. The positive externalities due to cultivation of *T. roxburghii* would be in the form of scenic beauty of the region with its evergreen leaves, virtual absence of mosquitoes even though sewage water be used. Identification of chemical constituents in *T. roxburghii* is a pioneer work and a more extensive exploratory study might reveal more. The extract (TR-PF) is easy to obtain and can be exploited commercially as a potent phytochemical biopesticide.

Plate 1. *Typhonium roxburghii* – Potted plant

Plate 2. Plant with inflorescence

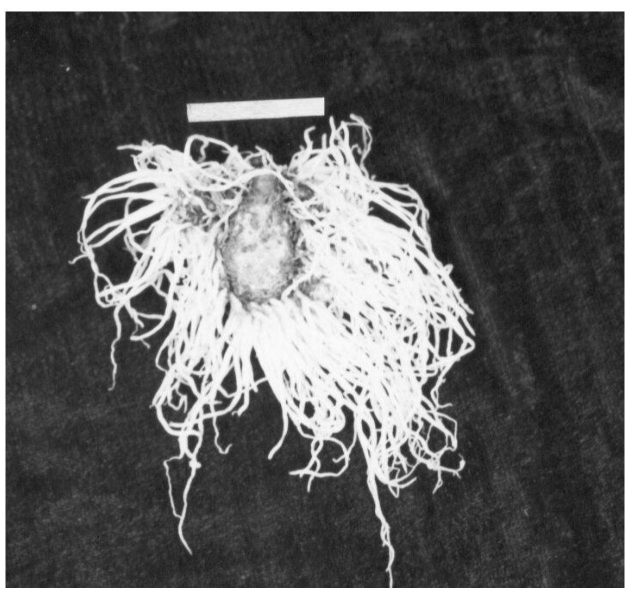


Plate 3. *Typhonium roxburghii* (Corm)

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