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SEED GERMINATION AND INTRODUCTION OF DUBOISIA LEICHHARDTII F. MUELL. AT KARACHI $24^{\circ}\phi$ 59 'N-68° ϕ 56E

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Efforts were made to germinate the seeds of *Duboisia leichhardtii* using different dormancy breaking methods. Excluding gibberellic acid treatments, no other method was found effective. In our experiments different concentrations of gibberellic acid (100, 200, 250 and 300 ppm) responded positively. The best results were found in 200 ppm of gibberellic acid.

Key words: Duboisia leichhardtii, Seed germination and Gibberellic acid.

INTRODUCTION

The few species that comprise the genus Duboisia R.Br. are native to Australia and New Caledonia. The genus Duboisia is represented by three species viz., D. leichhardtii F. Muell., D. myoporoides R. Br. and D. hopwoodii F. Muell. The first two species are rich in hyoscine and hyoscyamine. The leaves of D. leichhardtii contain hyoscine, hyoscyamine, valtropine, butropine and norhyoscyamine [1], whereas the third one i.e. D. hopwoodii contains tobacco alkaloids, nicotine and nornicotine [2,3]. The leaves of Duboisia [4,5,6,7] provide an important and valuable commercial source of drugs as hyoscyamine and hyoscine. The presence of poisonous tropane alkaloids, primarily scopolamine often in concentrations of as much as 4-5 %, have made this plant one of the most sought in the pharmaceutical industry. It also contains other alkaloids such as piturine and duboisine [4,5,6,7]. Duboisia provides specific narcotic effects; the victim of poisoning rapidly falls into a profound state of narcosis. The three species of Dubcisia and a major group of interspecific hybrids have a characteristic alkaloid yields. Accidental poisoning by the plant material is through absorption through the intact mucous membranes of the conjunctivae, nose and upper oropharynx. The corkwood poisoning syndrome is specially that of cerebral, ocular and effects the skin. Cork-eye is the most permanent feature of Duboisia poisoning syndrome [8,9]. More severe symptoms are impaired vision, nausea, headache, dehydration of mucous membrane, loss of balance, depression, cramps and confusion of state [10].

Duboisia is represented by a perennial shrub or small tree. It is less than 5 m tall with thin branches which are peach-like in appearence. The fruit is a berry (Fig. 1) with many seeds, approximately 6-12 seeds. The seeds of Duboisia are difficult to germinate under ordinary and ambient temperature and conditions. They require a special treatment before planting into sterilized seedbed soil to reduce the risk of seedling losses from the damping-off disease.

The seeds were imported from Royal Botanic Garden, Australia, where *Duboisia* plants were growing at 6 km. SW. of Monogorilby, Queensland $26^{\circ} \phi 5'$ S-150° $\phi 58'$ E. Efforts for the introduction and cultivation of *Duboisia* at Karachi has been found to be successful. Harvested seeds germinate poorly and the dormancy of seeds, with a view to practical application, was studied in the laboratory.

MATERIALS AND METHODS

The seeds of *Duboisia* are very tiny (1 mm x 2 mm) and kidney shaped (Fig. 1). Before planting, the seeds were treated with gibberellic acid for 24 hr. at different concentrations of 100, 200, 250 and 300 p.p.m., at a temperature of 40° approximately. The temperature was maintained at a constant level by placing the container with the seeds in gibberellic acid in an electric water bath which had already been brought to 40°.

After treatment the seeds were washed thoroughly with clean water before being dried to ensure the seed coat is absolutely free of chemicals [11]. The promotion of germination by gibberellic acid has also been reported to other hard-seeded species like *Phacelia tanacetifolia* [12,13]. One set of treated seeds with gibberellic acid (100, 200, 250 and 300 p.p.m.) were kept in the dark for a period of 3 weeks and the second set for 6 weeks to compare the difference of germination in each set. In our experiments the percentage of germination is equally encouraging if left for these periods. The seedlings raised from the treated seeds were grown in approximately 60 % shade.

Fifty seeds from each treatment were planted in pots with sterilized soil, and were allowed to germinate in the light at a temperature of about $20-25^{\circ}$. The pH range of the soil was 4.5-5.5. Germination rates were cumulated as observed in 3 weeks and 6 weeks treated seeds separately and the data are incorporated in Fig. 2.

Soon after the germination, nitrogen fertilizer was sprayed at the ratio of 1 teaspoon mixed with 9 litres of water at an interval of seven days. In the early stage *Duboisia* seedlings were susceptible to frost, and so they were kept in a warm place and protected from strong winds.

After 12 weeks the seedlings were about 8''-10'' high; they were then transplanted into the field and filled with sterelized soil. The plants stablished themselves at this stage. They were transplanted at a distance of 10 ft. x 10 ft. and care was taken to ensure that they were planted at the same depth as they were in the nursery Fig. 3 shows a young *Duboisia* tree growing in the field.

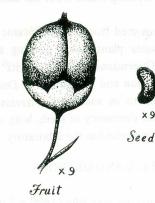


Fig. 1. Duboisia leichhardtii F. Muell.

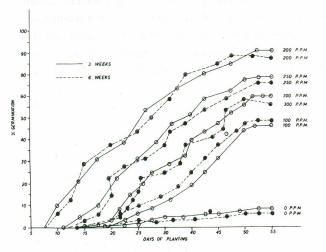


Fig. 2. Effect of gibberellic acid on germination of *Duboisia leichhardtii* seeds.

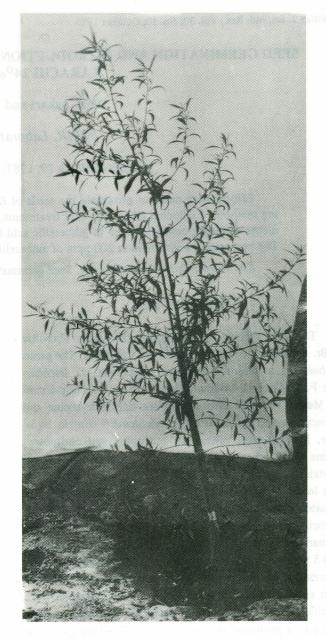


Fig. 3. Showing a young *Duboisia* plant-grown in PCSIR Experimental Farm.

In the other batches of seeds, different treatments such as fire, boiling water, sulphuric acid and light were given to study various effects. A set of *Duboisia* seeds without any treatment was also kept for comparison.

RESULTS AND DISCUSSION

Gibberellic acid is, definitely, by far the best method of breaking the dormancy of *Duboisia* seeds. Other methods such as scarification with zero no. sand-paper, hot water and acid treatments are not effective. They weaken the testa and do not enhance the rate of germination. Nontreated seeds gave very poor results. The dormancy period was greater and germination percentage was very meager. Precisely, gibberellic acid provides the simplest and most reliable means of ensuring almost 90 % germination. As can be observed from Fig. 2, the number of seeds germinated in 200 p.p.m. gave the best results as compared to 300, 250 and 100 p.p.m. Similar observations were made in 6 weeks' dark period treatment but the difference in percentage of germination was very marginal. Keeping in view the observations based on these data, it is clear that the optimum dark period after gibberellic acid treatment lies between 3-6 weeks.

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Prepareton of plant extracts. Parts of the fresh plant were chopped into small phoes. About 100 g of plant material was soaked into 300 ml of 90 % either ethanol, or benzene, or petroleum ether, or water for 2-3 days at rourn femperature, in each case, the solvents used have been mentioned in the table. The crude extracts were obtained from soaked plant material by the percolation method, first after 48 hr and then twice after 24 hr. The proded extracts were concentrated under high vacuum.

Proparation of samples for testing. Plant extracts were not equally soluble in water, and therefore, a solution containing 400 ml of 50 % enhanol and 5g of an emulsifiler Triton^C (Kohm and Hoas, Philadelphia 8a 19105) was used 10 emulsify all and extracts. The above plant extracts were weighed to give 5 % concentration (i.e. 0:05 g of plant extract dissolved in 1 ml solution of ethanol and triton instrure). Aliquots at this concentration were used to test antifungal activity

This argumma Antibagai activity was assed against two common pathogens fung of fruits namely, Appergillan arger and Alternania spo. Both were isolated from infected dirus. Inute. Collines of these fung were maintained on Sabouraud's Dextrose Agar at 4⁰.

Antipingal next. The diffusion plate method was used to task plant extracts (15), O.1 ml of the fungal spore suspenders (grown for 3 days in 10 ml of Sabournad's Destrose Dirator was thoroughly moved with 25 ml of rechted Sabourned Destrose. Again and poured into starikits of perroplates. When the agar was wit, 5 holes of 6 mm diarrecter hore were made on each of the seeded plate. The

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MONDUCTION

For more than 40 years a number of investigation [1.6] have conducted surveys on antimicrobial valuatances, mainly from higher plants. These studies include mostly preliminary data on ornide extendes, filowever, recent studies have also been done on the chamicals responsible for antimicrobial activities [7-13]. It is generally considered that microbial activities [7-13]. It is generally considered that variations in the results by the previous workers mult be partly due to the presence of fortain substances in crute extracts which exert antagonistic effect during testing of the extract that stimulate the growth of microorganisms, and beave interfiere with the effect of inhibitory substances [14].

It is well known that fungal infections are more prevalent in hot and humid areas. This includes plant discusses as well as those of animals and human beings. An such, it was considered worthwhile to take up a programme for screening antifungal substances from indigenous plants, which in final analysis could possibly be used for the control of such discusses. These studies are imply patified because, there exists considerable scope for new funglcides from natural resources.

MATERIALS AND METHODS

Panr margeral acea, All plants wild or sultryated were collected fresh from different places in the district of Karachi Vätiona perts of the plant used during these ito, dies helinde-roots, stems, leavas, fluwers, fruits and secus.