SEEDLING BLIGHT OF *HYOSCYAMUS MUTICUS* BY *PYTHIUM BUTLERI* IN INDIA

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HYOSCYAMUS MUTICUS L., commonly known as Egyptian henbane, is one of the most important medicinal plants because of the presence of tropane alkaloids mainly hyoscyamine, hyoscine and atropine[1]. Because of their sedative, antispasmodic and anticholinergic activities, these alkaloids are used in the treatment of human diseases including asthma, whooping cough, intestinal disorders, colic pain and motion sickness[2]. Since November 1984, a severe seedling blight of *H. muticus* has been observed at the experimental plantations of this Institute. The disease caused extensive damage to the seedlings of the crop.

Initial symptoms of the disease appear as yellowish, water-soaked lesions on the hypocotyl region which tend to spread downwards to the root and upwards to the shoot. The infected stem, root and leaves become pulpy and dark brown resulting in drooping and death of the whole plant. Carefully uprooted seedlings showed rotting of primary root system. The pith and stem cortical tissues became water-soaked, typical of soft-rot diseases.

Isolations from the infected plants made on potato-dextrose agar medium, consistently yielded colonies of *Pythium butleri* Subramaniam (CMI No. 305665). The isolate produced copious aerial, white, cottony mycelium on PDA. Pathogenicity tests were carried out on potted seedlings of *H. muticus* raised in sterilized soil, by drenching the soil with mycelial suspension. Inoculated and uninoculated seedlings were incubated in humid chamber of 24 hr. Initial disease symptoms appeared after 24 hr, and within 4 days all the inoculated seedlings collapsed and died (figure 1). The symptoms thus produced were similar to those observed in nature. The same fungus was reisolated from the inoculated plants. The seedlings of *H. niger* also produced similar symptoms on inoculation with the same fungus.

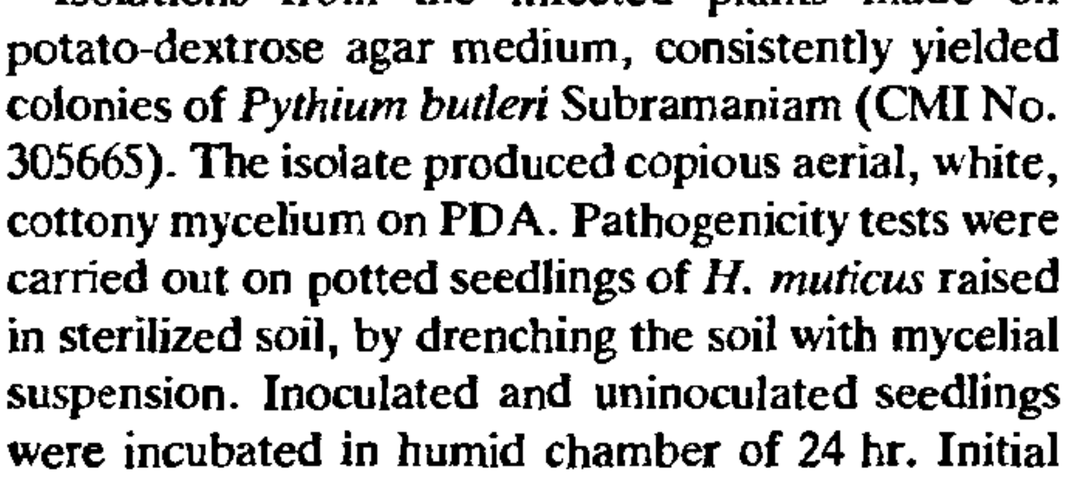

Figure 1. Seedlings of *Hyoscyamus muticus* showing damage caused by *Pythium butleri*.

Review of the literature showed that on *H. muticus*, *Alternaria alternata* causing leaf spot[3] and *Peronospora tabacina* causing blue mould[4] are the only fungal diseases reported so far. *P. butleri* causing seedling blight of *H. muticus* is being reported for the first time.

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GLYCINE ON *IN VITRO* BIOSYNTHESIS OF NIMBIN AND β-SITOSTEROL IN TISSUES OF *AZADIRACHTA INDICA*

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CULTURED dedifferentiated tissues from cotyledons, redifferentiated tissues and normal cotyledons of *Azadirachta indica* A. Juss. (Meliaceae) contain nimbin, glycine (along with some other amino acids) and β-sitosterol. In the latter two tissues, nimbin and glycine occur in larger quantities[1], β-sitosterol being smaller than in callus. This relation provoked the present authors to see whether exogenous glycine helps or enhances nimbin synthesis in callus. Glycine is also a usual component of culture media[2]. We observed an increased biosynthesis of nimbin and a decrease of β-sitosterol by adding glycine in the culture media.

One-month-old callus grown on MS medium[3] supplemented with 1 mg/l naphthalene acetic acid and 0.5 mg/l kinetin was the material. Concentrations of glycine added to the media, were 0, 0.3 mg/l

(the usual dose of MS), and double, four times, six times and eight times of the usual dose. The results were calculated after the third passage (one month each) of callus growth to avoid the influence of the previous nutritional condition. All the cultures grew in a room at 25°C and 400–600 lux light intensity for 10 hr per day. The tissues were extracted after pooling each set together (20 replicates) and drying at 60°C. For extraction, isolation and semi-quantitative estimation of nimbin and β-sitosterol by thin layer chromatography (TLC) and gas liquid chromatography (GLC) the process of Sanyal *et al*[1] was followed.

The growth value rose up to the two-fold dose of glycine (0.6 mg/l) added to the medium. But nimbin continued to increase and β-sitosterol continued to decrease up to the six-fold dose. With an eight-fold dose, nimbin decreased, but β-sitosterol increased considerably (figure 1).

Each of these two phenomena (growth value and synthesis of nimbin) required an optimum dosage of glycine which was higher for the latter. Nimbin accumulation had a reverse relation to β-sitosterol accumulation. It was similar to the redifferentiating tissues where nimbin is high and β-sitosterol low. The natural occurrence[1] of high endogenous glycine and nimbin, and low β-sitosterol in differentiated and cotyledonary tissues, and accumulation of nimbin and decrease of β-sitosterol with increase of exogenous glycine in callus, suggested glycine's role in the biosynthesis of these secondary metabolites in *A. indica*. Redifferentiation of calli appeared to bring about certain metabolic changes, helping nimbin biosynthesis of which increased production of glycine was one.

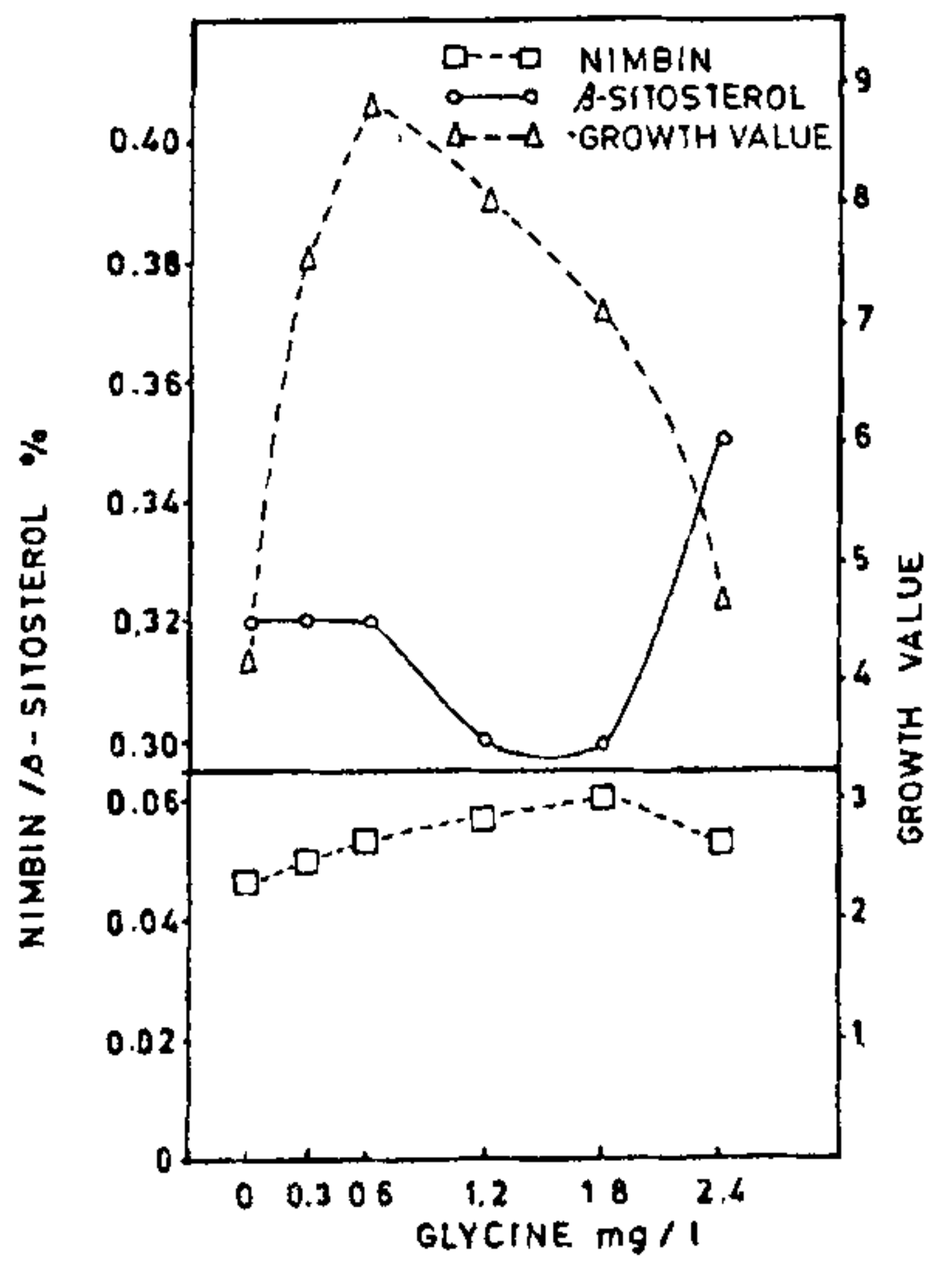

Figure 1. Glycine on nimbin, β-sitosterol and growth value.

A key role of mevalonic acid and squalene as the precursor of all the known plant sterols and triterpenes[4–6] is well established. In that case, following the initial cyclization, many further transformations must occur. The stereochemic requirements for folding of the squalene molecule to conform with cyclization to different types of sterols (like β-sitosterol) and triterpines (like nimbin) are also specific. So, in the changed tissue environment of callus, the stereochemical requirements for folding squalene to nimbin or to β-sitosterol must be different. Glycine probably triggered the biosynthesis of nimbin, blocking the other pathway to β-sitosterol.

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TWO LONG LOST THALLOSE LIVERWORTS RECOLLECTED FROM PINDARI RANGE (WESTERN HIMALAYA)

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DURING a bryo-collection trip to Pindari Glacier (alt. 2900 – 4000 m) in Almora District of Kumaun Himalaya, two extremely rare and interesting thallose liverworts viz. *Sauchia spongiosa* Kash. and *Preissia quadrata* (Scop.) Nees were collected in September 1985. Both are restricted to the high altitude regions of Western Himalaya.