UPTAKE AND TRANSLOCATION OF SULPHANILAMIDE AND GRISEOFULVIN BY THE RICE PLANT *

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BOTH sulphanilamide and griseofulvin are known to be absorbed by the roots of several plants and translocated to the shoots.[2,3,5,8] No reports, however, exist on the uptake of sulphanilamide by the rice plant; that of griseofulvin has been observed,[9] but no quantitative assay was made. During the course of a study of their role in the systemic control of brown spot (*Cochliobolus miyabeanus* Ito et Kuribayashi, Drechsler) of rice, the amount of the two compounds taken up by the rice plants was investigated. The results are reported in the present paper.

Rice seed was supplied by the Paddy Specialist,** Agricultural College and Research Institute, Coimbatore, and griseofulvin and *Botrytis allii* culture by Prof. P. W. Brian** of the University of Glasgow. A May and Baker's preparation of sulphanilamide was procured locally. The two chemicals were recrystallised from solutions in acetone and ethanol, respectively, before use.

Plants were grown in liquid culture.[7] At 10 days' age, sulphanilamide at 100 μg./ml. and griseofulvin at 25 μg./ml. were added to the nutrient solution. The free sulphanilamide content of the plants was estimated 3 days after treatment by the methods of Crowdy and Pramer[4] and Crowdy and Rudd Jones,[5] partly modified after Snell and Snell.[6] Free griseofulvin was estimated 4 days after treatment by the bioassay method using *B. allii*.[1]

Fresh plant tissue was used in the studies. Samples consisted of a minimum of 0·5 g. roots and 1 g. shoots. Extracts were made using a hand pestle and mortar. Standards were prepared in untreated plant extracts. Duplicates were run in each case.

Uptake of Sulphanilamide.—The free sulphanilamide content was estimated in the roots and shoots of rice varieties Co_{13} and Adt_{10} separately. The residual sulphanilamide in the nutrient solution was also estimated. The plant tissue was quickly washed, wiped between folds of filter-paper, and killed by dipping in ether for 30 sec. Extracts were filtered and dried over sulphuric acid *in vacuo*. The residue was taken up in 6 ml. water and pH adjusted to 7. To 3 ml. aliquots were added 2 ml. of 15% trichloroacetic acid and the mixture centrifuged for 20 min. at 3000 r.p.m. To 2 ml. of the supernatant was added 0·5 ml. of 0·1% sodium nitrite solution and the tube shaken. This was followed by the addition of 2 ml. of 4% ammonium sulphamate and 1 ml. of 0·1% N-(1-naphthyl) ethylenediaminedihydrochloride. The resulting pink colour was read in a Hilger's Uvispek spectrophotometer at 545 mμ and the quantity of sulphanilamide computed from a standard graph. The amount of sulphanilamide remaining in the nutrient solution was likewise estimated by taking 0·5 ml. aliquots directly. The results are given in Table I.

TABLE I

Free sulphanilamide content of rice plants treated through roots and the balance in the nutrient solution

Variety	Sulphanilamide content (μg./plant) Shoot	Root	Residual sulphanilamide in nutrient solution (μg./ml.)
1. Co_{13}	3·2	1·1	50·25
2. Adt_{10}	2.0	0·8	51·25

It will be seen that 3–4 μg. of free sulphanilamide was detected per plant, while almost half of the quantity supplied was left over in the nutrient solution. The quantity present in the shoots was about 3 times that in the roots.

Uptake of Griseofulvin.—Preliminary experiments showed little difference in the uptake of griseofulvin by the rice varieties Co_{13} and Adt_{10}. Detailed studies were, therefore, undertaken with the variety Adt_{10} alone. Only shoots were analysed. The tissue was killed and extracted as mentioned earlier. The extract was made up to 5 ml. and aliquots used for bioassay. The results are presented in Figs. 1-7.

It will be seen from Figs. 2 and 3 that the abnormalities produced in the germination of the conidia of *B. allii* in the treated plant extract was identical with their germination in the untreated plant extract to which 25 μg./ml. griseofulvin had been added. But a comparison of the dilution end point, 1/16 or 1/32, with the standards (Figs. 4–7) suggested a probable concentration of 6·4 μg./ml. in the extract, thus working to about 2 μg. free griseofulvin per plant.

It is, therefore, concluded that both sulphanilamide and griseofulvin are absorbed by the roots

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or rice plants and translocated to the shoots within 3–4 days. In treatments with 100 μg./ml. sulphanilamide and 25 μg./ml. griseofulvin, 3–4 μg. of free sulphanilamide and 2 μg. of free griseofulvin could be detected per plant at the end of that period.

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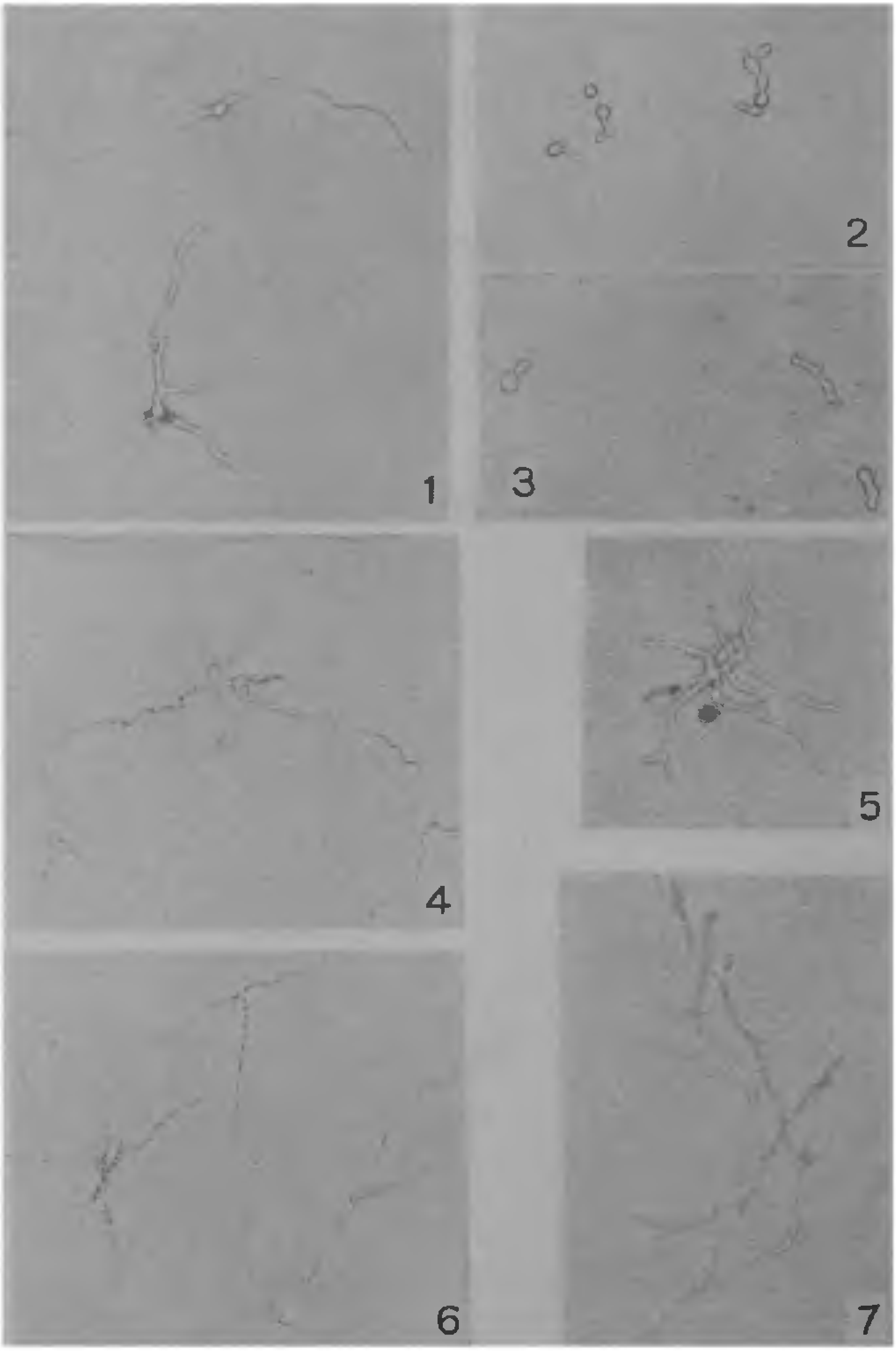

FIGS. 1–7. The germination of conidia of *Botrytis allii* in aqueous extracts of shoots of rice plants (× 130). Fig. 1. Untreated plant extract, undiluted. Fig. 2. Treated* plant extract, undiluted. Fig. 3. Untreated plant extract + 25 μg./ml griseofulvin. Fig. 4. Treated* plant extract, diluted 16 times. Fig. 5. Untreated plant extract + 0·4 μg./ml griseofulvin. Fig. 6. Treated* plant extract, diluted 32 times. Fig. 7. Untreated plant extract + 0·2 μg./ml. griseofulvin.

*Treated with 25μg./ml. griseofulvin.

Madras, for advice, and the Plant Protection Adviser to the Government of India for permission to undertake the work.

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THE INDIAN SHAD, *HILSA ILISHA* (HAMILTON) IN THE SEA

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THE Indian shad, *Hilsa ilisha* (Ham.), has long been considered as a fluvial anadromous fish with feeding grounds in the sea and spawning grounds along considerable stretches of the lower and middle reaches of big as well as small rivers of India. However, Prashad[1] and Hora[2] cast doubt on the true anadromous habit of this valuable clupeid and considered it as a fluvial fish that does not go down beyond the estuary; since then, although various workers *have* observed either juveniles or adults in inshore waters,[3-9] there has been some reluctance to consider the anadromous habit of hilsa. It is stated that even if hilsa does enter the sea, it is confined to inshore waters or to the "foreshore";[4,8,9] it is however most unlikely that this medium-sized active fish resorts to an intertidal habitat during its extra-fluvial phase. A review of the literature shows that the fluvial anadromous stocks of hilsa *do* enter the sea, but that the available data on the movements in the sea are inadequate to determine the extent of their seaward migration because there are just no observations, negative or positive, beyond the continental shelf.

The recent record by Pillay[10] of maturing, mature and spent hilsa in the sea, 9 to 12 miles off Veraval (in Saurashtra, Gujarat State) on the north-west coast of India adds a new dimension to the known behaviour of this fish; Pillay offers evidence that this particular stock of hilsa probably spawns in the sea. All previous records of mature and spent hilsa are from rivers.

Nikolsky[11] has observed that the reasons why anadromous fishes have not migrated completely to the sea where there are more favourable feeding conditions, but have retained their spawning grounds in freshwater, are

1. if they transferred their spawning grounds also to the sea, they would have to protect their spawn, as otherwise the latter would all be eaten, and
2. since migratory fishes mostly have a considerable hunting area and perform considerable feeding migrations, the adults would starve and die if they were to remain on the spawning ground to protect their spawn through a protracted incubation period. By retaining spawning grounds in the river, the eggs can develop through at least part of the juvenile stage in the river, where they are subject to far less danger than in the sea.

If the Saurashtra stock of hilsa does breed in the sea, how has it solved the problem of spawning in a *milieu* new to the species? Specifically, what is the type of eggs (demersal, semibuoyant or pelagic) and how have the problems of protection of eggs and larvæ and of osmoregulation been solved? The eggs of hilsa spawning in rivers are demersal;[6,12] they rest lightly on the bottom in still water and drift when there is a current;[12,13] the eggs and larvæ are not guarded or protected by the parents. Further, how does the fecundity of the Saurashtra hilsa compare with that of the stocks spawning in rivers? In this connection, it should be noted that in the same region, there is a fluvial anadromous stock spawning in the Narbada River.[12-15] One may expect that if the Saurashtra stock of hilsa does breed in the sea, the fecundity should be at a higher level than that of normal stocks spawning in rivers (where mortality of eggs and larvæ would be much less) if the former stock in its new *milieu* is not to decline. A comparison of the fecundity of mature hilsa from Saurashtra coast and from Narbada River should prove valuable.

The investigations to date on *Hilsa ilisha* thus indicate the possible existence of three ecotypes of this species:

1. Fluvial anadromous stocks that feed and grow in coastal waters and spawn in middle or lower reaches of rivers above