put breaks on the decline of the vulture population. However, care should be taken while replacing Diclofenac with other NSAIDs, because the replacement drugs may not be environmentally safe either, and their impacts are unknown. Research into the ecological impact of all major therapeutics, especially the common and widely used ones, should be undertaken urgently to facilitate the policy makers to regulate these compounds with potentially hazardous environmental impacts. The exact physiological mechanism through which Diclofenac acts in three species of only a single genus (*Gyps*), appears mysterious and highly intriguing. The metabolic pathways through which Diclofenac poisoning causes renal complications, gout and consequent mortality in only *Gyps* vultures is yet to be elucidated. As of now, it appears premature to conclude that the Diclofenac residue is the universal causative agent behind the decline of vulture population.


1. Oaks, J. L. *et al.*, *Nature*, 2004, **427**, 630–633.
2. Rae, C. J., *J. Bombay Nat. Hist. Soc.*, 1935, **2**, 18.
3. Bhatnagar, R. K., In *Bird Conservation: Strategies for the Nineties and Beyond* (eds Verghese, A., Sridhar, S. and Chakravarthy, A. K.), Ornithological Society of India, Bangalore, 1993, p. 194.
4. *Threatened Birds of Asia: the BirdLife International Red Data Book*, BirdLife International, Cambridge, UK, 2001.
5. Thiollay, J. M., *J. Raptor Res.*, 1998, **32**, 40–55.
6. Kannan, R., *Newsl. Birdwatchers*, 1993, **33**, 58.
7. John, E., *Outlook*, 1999, **39**, 70.
8. Arun, P. R. and Azeez, P. A., In Proceedings of Indian Environment Congress, Coimbatore, December 2003, 20–21.
9. Singh, B. P., *Curr. Sci.*, 2004, **80**, 484.
10. Borad, C. P., *Sanctuary Asia*, 2001, 84–85.
11. Prakash, V., *J. Bombay Nat. Hist. Soc.*, 1999, **96**, 365–378.
12. Rahmani, A. R., *Newsl. Birdwatchers*, 1998, **38**, 80–81.
13. Rahmani, A. R., *Orient. Bird Club Bull.*, 1998, **28**, 40–41.
14. Satheesan, S. M., *WWF-India Network Newsl.*, 1999.
15. Grubh, B. R., Ph D thesis, University of Bombay, 1974.
16. Grubh, B. R., In Proc. of the 19th Int. Ornithol. Congress, 1986, vol. 2, pp 2763–2767.
17. Grubh, R. B., *J. Bombay Nat. Hist. Soc.*, 1978, **75**, 810–814.
18. Ezra, A., *Bull. Br. Ornithol. Club*, 1918, **38**, 55.
19. Fox, E. B., *J. Bombay Nat. Hist. Soc.*, 1913, **22**, 395–396.
20. Gough, W., *J. Bombay Nat. Hist. Soc.*, 1936, **38**, 624.
21. Grubh, B. R., *J. Bombay Nat. Hist. Soc.*, 1973, **70**, 199–200.
22. Livesey, T. R., *J. Bombay Nat. Hist. Soc.*, 1937, **39**, 398–399.
23. Smith, O. A., *J. Bombay Nat. Hist. Soc.*, 1915, **23**, 579.
24. Bhat, S., *Blackbuck*, 1992, **8**, 85.
25. Gill, E. H., *J. Bombay Nat. Hist. Soc.*, 1921, **27**, 951–952.
26. Jones, A. E., *J. Bombay Nat. Hist. Soc.*, 1916, **24**, 369–370.
27. Kanoje, R., *Newsl. Birdwatchers*, 1996, **36**, 14.
28. Sharma, I. K., *Ostrich*, 1970, **41**, 205–207.
29. Grubh, B. R., *J. Bombay Nat. Hist. Soc.*, 1978, **75**, 1058–1068.
30. Cunningham, A. A. *et al.*, *Animal Conserv.*, 2003, **6**, 189–197.
31. McCarty, J. P., *Conserv. Biol.*, 2001, **15**, 320–331.
32. Daughton, C. G. and Ternes, T. A., *Environ. Health Perspect.*, 1999, **107**, 907–938.
33. Baert, K. and De Backer, P., *Comp. Biochem. Physiol., C*, 2003, 25–33.
34. Tang, W., *Curr. Drug Metab.*, 2003, **4**, 319–329.
35. Masubuchi, Y., Saito, H. and Horie, T., *J. Pharmacol. Exp. Ther.*, 1998, **287**, 208–213.
36. Purcell, P., Henry, D. and Melville, G., *Gut*, 1991, **32**, 1381–1385.
37. Bougie, D., Johnson, S. T., Weitekamp, L. A. and Aster, R. H., *Blood*, 1997, **90**, 407–417.
38. Kim, H. *et al.*, *Anesth. Analg.* (Cleaveland), 1999, **89**, 999–1005.
39. Rossi, E. *et al.*, *Nephron*, 1985, **40**, 491–493.
40. Rothschild, B. M., Tanke, D. and Carpenter, K., *Nature*, 1997, **387**, 357.
41. Schlumberger, H. G., *Lab. Invest.*, 1959, **8**, 1304–1318.
42. Schmidt, R. E. and Hubbard, G. B., In *Atlas of Zoo Animal Pathology*, CRC Press, London, 1987.
43. Siller, W. G., *Lab. Invest.*, 1959, **8**, 1319–1346.
44. Appleby, E. C. and Siller, W. G., *J. Pathol. Bacteria*, 1960, **80**, 427–430.
45. Campion, E. W., Glynn, R. J. and deLabry, L. O., *Am. J. Med.*, 1987, **82**, 421.
46. Prakash, V. *et al.*, *Biol. Conserv.*, 2003, **109**, 381–390.


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# Development of a novel lyophilization protocol for preservation of mushroom mycelial cultures

The maintenance and production of reliable pure culture spawn with desirable quality is the key operation and the first critical stage in the success of mushroom cultivation. Maintenance of vigour and genetic characteristics of a pure strain in the form of a culture is the main objective of culture preservation. Besides this, strain improvement of cultivated mushrooms demands a well-planned system of maintenance, preservation and availability of genetic diversity[1]. Mushroom culture repositories/gene banks play a vital role in supply of pure and authentic cultures to most of the mushroom spawn-producing units. There are various methods of maintenance and preservation of mushroom cultures and a good culture collection section adopts more than one method to preserve them. Mushrooms might be of academic, medicinal or horticultural importance. Mushroom strains having industrial importance are patented and preserved, although availability of such strains becomes restricted[2]. If no degenerative changes were to take place during preparation or maintenance of mush-

room cultures or spawn, then it would be a relatively simple, routine process. Unfortunately, this is not true. Degeneration of culture spawn refers to the loss of desired traits leading to slow development, poor rate of survival and low productivity[1,3].

Mushrooms are invariably stored as mycelial cultures because spores of heterothallic and secondary homothallic species are produced through a sexual process and have genetic differences, which may not eventually result in fruiting[4]. In the absence of hardy structures like double-walled spores or sclerotia, microbial cultures become susceptible to sudden change in temperature and pressure[5]. Maintenance of mushroom stock cultures under liquid nitrogen has been reported stable with little or no genetic change[6]. Singh *et al.*[7] modified the cryopreservation protocol using mycelium multiplied on wheat grain instead of mycelial disk and experimentally demonstrated genetic stability of mushroom stock cultures in liquid nitrogen.

Cryopreservation under liquid nitrogen gives high survival rates and is universally acceptable. Nevertheless, methods using liquid nitrogen are rather expensive, and regular supply of liquid nitrogen is not always guaranteed. Lyophilization is a good alternative. It employs the preservation of fungi by drying under vacuum from the frozen state by sublimation of ice. Glass ampoules can be stored easily in compact packing without any special requirement. Cultures need not be revived on agar slants prior to dispatch. The product is light, inactive and dry, enabling easy distribution by mail. Fungal cultures containing conidia and spores can be freeze-dried successfully; but despite the early success of the method, many strains of fungi failed to survive, particularly isolates of Mastigomycotina and Basidiomycotina[8]. However, the survival rates of frozen fungal cells increased considerably when cells were cooled[9,10] at the rate of – 1°C/min.

Earlier reports on freeze-drying of fungal hyphae used aqueous solution of culture suspension in Trehalose, skimmed milk, bovine serum albumin, sucrose, glycerol, etc.[11–13]. The survival rate of freeze-dried fungal hyphae of a number of ascomycetes was recorded to be better than two basidiomycetes, *Schizophyllum commune* and *Coronus psychromorbidus*[14]. Deriving inspiration from low rate of survival of filamentous fungi in general, and basidiomycetes in particular, using available freeze-drying protocols, an attempt has been made to improve lyophilization protocols for satisfactory preservation of mushroom mycelial stock cultures.

The pure mycelial cultures of 11 edible mushroom strains, namely *Agaricus bisporus* (S-11, U-3), *A. bitorquis* (NCB-13), *Pleurotus sajor-caju* (Pl-10 A), *P. ostreatus* (Pl-20), *P. sapidus* (Pl.-40), *P. flabellatus* (Pl.-50) *Auricularia polytricha* (OE-4), *Lentinula edodes* (OE-9), *Morchella esculenta* (ME-1) and *Volvariella volvacea* (OE-12) were procured from the National Mushroom Repository maintained at National Research Centre for Mushroom (NRCM), Solan. All the stock cultures were subcultured on wheat extract agar (WEA) culture medium in test tubes[15].

Each mushroom mycelial culture was multiplied on pearl millet (*Pennisetum typhoides*) grains. The pearl millet grains were thoroughly washed with sufficient water to remove debris, straw particles, undesirable seeds of grasses, etc. Washed grains were soaked in sufficient water for 30 min and then boiled for 20 min. Excess water was drained by sieving through a muslin cloth and dried in shade for 4 h to allow evaporation of any excess water. The boiled grains were mixed with 2% gypsum (calcium sulphate, $CaSO_4$) and 0.5% calcium carbonate ($CaCO_3$) to maintain pH around 7.0. Subsequently, the treated grains were filled in wide-mouth test tubes up to two-thirds of their capacity and plugged with non-absorbent cotton. These grain-containing tubes were autoclaved at 22 lb psi at 126°C for 2 h. After autoclaving, the grain-containing test tubes were allowed to cool overnight to evaporate the moisture on the inner walls of test tubes to avoid contamination. These test tubes were then exposed to ultraviolet light in a laminar flow for 30 min before inoculation. Each mycelial mushroom culture was inoculated in three such test tubes by transferring aseptically growing mycelium from freshly subcultured stocks maintained on WEA. All inoculated test tubes were then incubated at 25°C for 2 weeks, except *V. volvacea* which was incubated at 32°C for 10 days. Mycelial cultures multiplied on pearl millet grain were subjected to lyophilization and also used for pre-lyophilization count.

For lyophilization, glass ampoules were first sterilized in a hot-air oven at 180°C for 2½ h and allowed to cool to ambient temperature. The mycelium that multiplied on pearl millet grains was filled in each sterilized ampoule with the help of sterilized forceps. Each ampoule containing 50–60 grains was plugged with sterilized non-absorbent cotton. The cotton plugs were pushed inside up to the neck of the ampoules. The constrictions were made from above the cotton region in each ampoule and pre-cooled in a deep freezer to – 40°C. When the shelf temperature of the freeze chamber reached –62°C, the ampoules with frozen samples were attached to the lyophilizer (CHRiST, ALPHA 1–2) and vacuum created. The lyophilizer was allowed to run overnight. Next day, when the pressure reached 0.05 mbar, the ampoules were sealed at the point of constriction with the help of a cross-flamed burner connected to oxygen and liquid petroleum gas (LPG). The vacuum was tested with the help of a vacuum-tester. A purple coloured light spark that glows inside the sealed ampoule, verified proper sealing. Six ampoules per strain of edible mushroom were prepared in this way and stored at room temperature.

The survival test of each edible mushroom culture was carried out on malt extract glucose agar (MGA) culture medium (malt extract 10 g; glucose 5 g; agar 20 g; water $l^{-1}$). For each test strain, 21 grains (mycelium containing pearl millet seeds) were placed in three pre-sterilized petri plates containing MGA culture medium. Survival counts were made before lyophilization, and one month and three months after lyophilization. Post-lyophilization survival counts were made by breaking three ampoules and plating seven seeds per ampoule in pre-sterilized petri plates containing MGA. Inoculated petri plates were incubated at 25°C for 10 days for all the strains tested, except *V. volvacea* which was incubated at 32°C in a separate BOD incubator. Per cent survival of different mushroom strains before and after lyophilization was counted on the basis of number of colonies retrieved out of the total number of grains tested.

The results of pre- and post-lyophilization of 11 edible mushroom mycelial cultures are presented in Table 1. All the 11 mushroom strains were recorded with 100% survival before lyophilization; however a slight variation in survival rate of different edible mushroom strains was not statistically significant. The result exhibited that there was no decline in per cent survival of all the strains after one

**Table 1.** Effect of lyophilization on survival of 11 edible mushroom strains

| Strain | Per cent recovery | | |
|---|---|---|---|
| | Before lyophilization | After one month | After three months |
| *Agaricus bisporus* (S-11) | 100.0 | 95.2 | 95.2 |
| *A. bisporus* (U-3) | 95.2 | 95.2 | 95.2 |
| *A. bitorquis* (NCB-13) | 95.2 | 90.5 | 90.5 |
| *Pleurotus sajor-caju* (Pl 10 A) | 100.0 | 95.2 | 95.2 |
| *Pleurotus ostreatus* (Pl-20) | 100.0 | 100.0 | 100.0 |
| *P. sapidus* (Pl-40) | 100.0 | 100.0 | 100.0 |
| *P. flabellatus* (Pl-50) | 100.0 | 95.2 | 95.2 |
| *Auricularia polytricha* (OE-4) | 95.2 | 90.5 | 90.5 |
| *Lentinula edodes* (OE-9) | 95.2 | 90.5 | 90.5 |
| *Morchella esculenta* (ME-1) | 100.0 | 100.0 | 100.0 |
| *Volvariella volvacea* (OE-12) | 100.0 | 100.0 | 100.0 |
| CD at 5% | 8.5 | 10.5 | 10.5 |

month to three months of storage. *P. sapidus*, *M. esculenta* and *V. volvacea* showed absolute survival before lyophilization and after three months of storage of mycelial cultures in the lyophilized state. *A. bisporus*, *A. bitorquis*, *P. sajor-caju*, and *P. flabellatus* were recorded with a marginal decline in survival counts from before lyophilization to after three months of storage of mycelial cultures in the lyophilized state. Nevertheless, this meagre decline in survival was not statistically significant. *M. esculenta* and *V. volvacea* are low-temperature-sensitive mushroom species[12,16,17]. In the present study, no decline in the survival of any of the 11 mushroom mycelial cultures was due to improvement in the freeze-drying protocols and perfect lyophilization.

Filamentous, non-sporulating fungi which include mushrooms, are considered to be predominantly non-lyophilizable[18,19]. Smith and Onions[8] compiled lyophilization status and stated that only 62 out of 121 species of Basidiomycetes could survive freeze-drying and many mushroom species failed centrifugal freeze-drying at the Common Wealth Mycological Institute. Tan *et al.*[20] freeze-dried fungal hyphae of basidiomycetes *C. psychromorbidus*, *Lepista nuda*, *Perennipora subsida*, *S. commune* and a number of ascomycetes. They reported that programmed slow cooling of hyphae of ascomycetes as well as basidiomycetes survived freeze-drying, although generally better results were obtained with ascomycetes. Morris *et al.*[12] recorded 38% recovery of *V. volvacea* using glycerol at optimum cooling rate of –1°C per min.

The improvement in the preservation protocol was that the preservation as mycelial culture suspension was replaced by multiplication of mushroom mycelium on pearl millet grains. The modified protocol must have given protection to the soft and tender mycelium, concealed inside the grain and thus could have sustained cooling pressure[5,7,10]. The novel protocols developed shall open new vistas to improve available lyophilization technology for long-term conservation of filamentous fungi in general, and mushroom germplasm in particular.


1. Chang, S. T. and Miles, P. G., In *Edible Mushrooms and their Cultivation*, CBS, Delhi, 1989.
2. Jong, S. C. and Birmingham, J. M., In *Science and Cultivation of Edible Fungi* (ed. Maher), Balkema, Rotterdam, 1991, pp. 831–843.
3. Stadelman, R., *Mushroom J.*, 1986, **158**, 41.
4. Peterson, R. H., *Mycologia*, 1995, **87**, 1–17.
5. Franks, F., In *Biophysics and Biochemistry of Low Temperature on Biological Membranes* (eds Morris, G. J. and Clarkes, A.), Academic Press, London, 1981, pp. 3–19.
6. San Antonio, J. P., *Mushroom Sci.*, 1979, p. 103.
7. Singh, S. K., Upadhyay, R. C., Kamal, S. and Tiwari, M., *Cryoletters*, 2004, **25**, 23–32.
8. Smith, D. and Onions, A. H. S., In *The Preservation and Maintenance of Living Fungi*, Common Wealth Mycological Institute, Kew, UK, 1983, pp. 5–25.
9. Hwang, S. W., Kwolek, W. F. and Haynes, W. C., *Mycologia*, 1976, **68**, 377–387.
10. Grout, B. W. and Morris, G. J., In *Effects of Low Temperature on Biological Systems* (eds Grout B. W. W. and Moris, G. J.), Edward Arnold, Cambridge, UK, 1987, pp. 147–173.
11. Wiemken, A., Trehlose in yeast, stress protectant rather than reserve carbohydrate, *Antonie van Leeuwenhoek Ned, Tijdschr, Hyg*., 1990, **58**, 209–217.
12. Morris, G. J., Smith, D. and Coulson, G. E., *J. Gen. Microbiol.*, 1988, **134**, 2897–2906.
13. Tan, C. S., Ingen, C. W. van., Talsma, H., Miltenburg, J. C. van., Steffensen, C. L., Vlug, I. J. A. and Stelper, J. A., *Cryobiology*, 1995, **32**, 60–67.
14. Tan, C. S., Stalpers, J. A. and van., Ingen, C. W., *Mycologia*, 1991, **83**, 654–657.
15. Singh, S. K., Upadhyay, R. C. and Verma, R. N., *Mushroom Res.*, 2000, **10**, 67–72.
16. Buscot, F., These de Doctoral, Universite de Starbourg, France, 1987, p. 179.
17. Hervey, A., Bistis, G. and Leong, I., *Mycologia*, 1978, **70**, 1269–1273.
18. Simion, F. P. and Brown, E. M., *ATCC Preservation Methods: Freezing and Freeze-Drying*, ATCC Press, Maryland, Rockville, 1991.
19. Smith, D. and Onions, A. H. S., *The Preservation and Maintenance of Living Fungi*, CAB International, Wallingford, UK, 1994.
20. Tan, C. S., Ingen, C. W., van Stalpers, J. A. and van Ingen, C. W., In *Proceedings of First International Seminar on Mushroom Science* (ed. Griensven, L. J. D. Van), Horst, The Netherlands, 1991, pp. 25–30.


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# Protective devices of the carnivorous butterfly, *Spalgis epius* (Westwood) (Lepidoptera: Lycaenidae)

The apefly, *Spalgis epius* is a rare butterfly and an inhabitant of wooded areas. Usually most observers miss it due to its retiring nature, small size and rather drab colour. The species is known to occur in Sikkim, Kolkata, Malda, South India, Burma and Sri Lanka[1–3]. Lycaenids are unique in their larval stage as they eat unrelated food, including flowering plants, fungi, lichens, cycads, ferns, conifers, ant larvae and homopterans[4,5]. The larva of *S. epius* has been recorded as a predator on various species of pseudococcids (mealybugs)[2,6–16] and coccids (scale insects)[8,17,18]. Recently, the larvae of this butterfly were found on different species of croton plants, *Codiaeum* spp. infested with mealybug, *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) at the Jnanabharathi campus, Bangalore University. Though the butterfly is known to be a potential predator of different species of mealybugs[19–23], its activity is rarely noticed these days in the field wherever insecticides are used indiscriminately for the control of various insect pests, particularly homopterans.

Except for being reported as a predator, not much information is available on the biology and behaviour of *S. epius*. Adult of *S. epius* is a small butterfly with dark brown wings above, and grey underside with dark striations. Forewing has a small quadrate spot at the cell end in male (Figure 1 *a*), and larger and somewhat diffuse in female[1,2]. Dorsal side of the thorax is dark and glossy, encircled by tufts of white hair. Dorso-lateral region of abdomen is dark brown, whereas ventral side is covered with layers of white hair. The body length and wingspan of the adult are 9.95–11.50 mm (10.63 ± 0.48) and 18.0–22.5 mm (20.83 ± 1.44) respectively. The butterfly flies rapidly and erratically in the vicinity of bushes infested with mealybugs and it swiftly deposits eggs in the mass of the mealybugs. Occasionally, it has the habit of landing to rest persistently on its preferred perch. Owing to the dull colour, rapid and erratic flight, and swift egg deposition habits of adult *S. epius* females, it is difficult for the predators (birds, lizards, etc.) to attack them in the field.

The larva of the butterfly has a peculiar appearance and it is morphologically different from that of other species of butterflies in general, being short, slug-like and covered with white wax coating (Figure 1 *b*). Legs of the larva are short and hidden. Aitken[6] described the larva and pupa of *S. epius*. The larvae were found feeding voraciously on the egg masses, nymphs and adults of mealybugs. As the young larvae of *S. epius* simulate mealybugs, it is difficult to recognize them amidst the host population. Similarly, the young larvae of *S. lemolea* (Holl.) in Africa[24] and *S. substrigata* (Snell) in Philippines[25] are camouflaged with the mealybug population. The mature larva of *S. epius* measures 14.5–19.0 mm (16.48 ± 1.66) in length and 5.08–6.50 mm (5.72 ± 0.38) in width.

The red ant, *Oecophylla smaragdina* (F.) or the black ant, *Crematogaster* sp. attends the mealybugs since they secrete honeydew, as reported earlier[26–29] (Figure 2 *a*). These ants, particularly *O. smaragdina* were found attacking the mature larvae of *S. epius* whenever they encountered them. These larvae are much bigger than the mealybugs and do not secrete honeydew sought by the ants; thus ants interfere with the predatory activity of the larvae. *Crematogaster* sp. was found to be less hostile to *S. epius* caterpillars. However, both the ants did not attack young larvae of *S. epius* that look like mealybugs. Though most of the honey-secreting lycaenids are myrmecophilous (associated with ants), either to protect themselves against larval and pupal enemies, especially parasitoids, or to feed on the larvae of ants[4,8,24,27,29,30–33], a few lycaenid homopterophagous aberrant genera such as *Aslauga* of subfamily Leptininae, and *Spalgis*, *Fenisca* and *Megalopalpus* of subfamily Lycaeninae are amyrmecophilous (not associated with ants). They defend themselves from the homopteran-associated ants by means other than honey secretion and ants derive no benefit from the presence of the lycaenin larvae[8,28,32]. Unlike other lycaenids, the larvae and pupae of these genera lack any sort of organ that yields an ant confection, and ants that visit honey-secreting homopterans are hostile to the caterpillars[8,32]. However, these are protected against ants either by their obscure position among or beneath their host prey, by the thick cuticle on the dorsal body wall, or by their bristly vestiture[8]. The mature larvae of *S. epius* seem to protect themselves against the mealybug-attending ants by maintaining an adequate distance from them and by having hard, thick dorsal cuticle. *S. epius* larva headed for pupation, crawls to the lower surface of the leaf and pupates after firmly attaching on its ventral side to the leaf.

The length, width and height of the pupa are 5.0–6.1 mm (5.6 ± 0.44), 3.5–4.2 mm (3.90 ± 0.19) and 3.2–4.1 mm (3.55 ± 0.32) respectively. The bold pattern on the hard dorsal side of the pupa of *S. epius* resembles the face of a rhesus monkey (Figure 2 *b*). The pupa shows clear spots of eyes, nose, mouth, cheeks and forehead on the dorsal side. *S. epius* pupa is light brown on the dorso-lateral side and whitish-grey on the ventral side. It is known that many edible, unrelated species avoid predation by mimicking the appearance of a dangerous or offensive species. Many lycaenid adults display patterns of colour, structure and behaviour that are consistent with deflecting predator attacks towards a false pattern[34]. There is always a chance of predation of quiescent pupae of *S. epius* by predatory birds or by other natural enemies that visit mealybugs. Though the insect-predatory bird, dull green leaf warbler, *Phylloscopus trochiloides* (Sundevall) seldom visited the mealybug-infested bushes and devoured the crawling mature *S. epius* larvae on the leaves, it

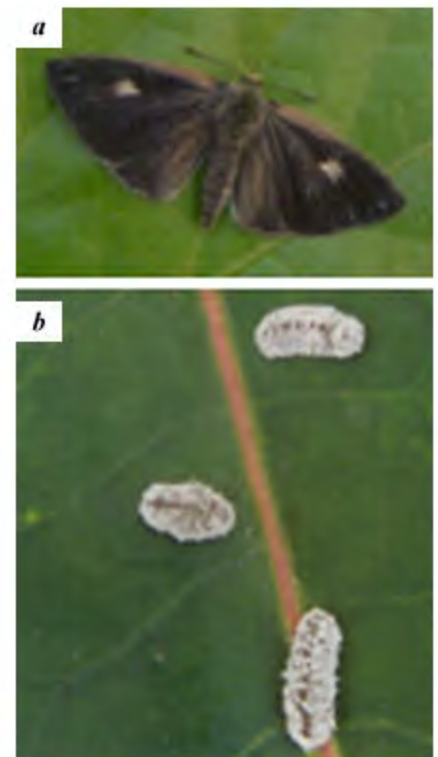


**Figure 1.** ***a***, Male *Spalgis epius* butterfly. ***b***, Fully grown larvae of *S. epius*.