user has to click the button 'Search' that will open the options form. Here the user can either plot the data, download the data, save the search, exit the search or go back to edit the search. The software will flash the status and error messages. The form will not be invoked, if no data are selected.

The data visualization and download module enables the user to plot the queried data in line form (Figure 4) as well as by joining each fix. Both the plots show distance covered by the cruises for the queried data in line-km. This evokes the distance facility to measure the distance between any two points on the map. Besides these, the zoom, pan and full extent facilities are available to view data at a convenient resolution.

This module further takes the user to the inventory form, which gives the inventory-level information generated from the queried data. This form gives a link to data download. Before downloading the data, the user needs to enter a password for authentication. The user is guided to a field selection form where he/she can select one, more or all of the fields to download. The user is shown the selected data on a monitor. The user can then press the download button and select a path for saving the download file.

This module allows the administrator to change user password, to process the data or to import new data into the database or allows generating statistics for line, source, ship or country. The module accepts data generated by the 'data import module' and deposits data into the main database. If the variations are beyond the capabilities of the system, then manual intervention and processing may be required.

The GPDAMS has a few limitations like: (1) Its performance depends on the complexity of the query and database size. (2) The accuracy of the line plot is based on the correctness of the line details assigned. (3) The system interface is designed for a 1024×768 monitor resolution. The system checks and adjusts to the required resolution and readjusts to previous setting on exit.

Having the GPDAMS package on a PC, users enjoy access to large volume of multi-disciplinary geophysical data and the means to visualize and extract selected data according to their needs. The software requires a minimum of computing expertise as it is controlled by a system of 'pull-down' menus, backed up by a context-sensitive help system. Efforts are underway to develop a similar system on the Intranet for institutional users and subsequently on Internet for national/international users. It is expected that geophysicists will use GPDAMS for selecting required data. Cruise planners are expected to take advantage of select and display modules for planning purpose. The corporate sector is likely to make use of this information for developmental purpose.

1. Kunte, P. D., Worldwide databases in marine geology: A review. *Mar. Geol.*, 1995, **122**, 263–275.
2. Hittelman, A. M., Groman, R. C., Haworth, R. T., Holcombe, T. L., McHendrie, G. and Smith, S. M., The marine geophysical data exchange format – 'MGD77': KGRD 10. In NOAA, EDIS, Boulder, Colorado, 1977. p. 18.
3. Hittelman, A. M. and Metzger, D. R., Marine geophysics: Database management and supportive graphics. *Comput. Geosci.*, 1983, **9**, 27–33.
4. GEODAS, GEOphysical DAta System (GEODAS) – an online data search and retrieval system (http://www.ngdc.noaa.gov/mgg/gdas/gd_sys.html), 2005.

ACKNOWLEDGEMENTS. We thank Dr S. R. Shetye, Director, National Institute of Oceanography, Goa for his initiation and encouragement. We also thank the Head, Data and Information Division and colleagues at the Data Centre for their cooperation and useful suggestions. We thank all NIO Geophysicists, who have devoted their energy, time and efforts to collect, process, analyse and submit datasets to the Data Centre. This is NIO contribution no. 4116.

Received 4 August 2005; revised accepted 28 February 2006

Studies on the identification of suitable solvents for microbial bioassay

P. Rekha[1], B. Chandra Mouli[1,*], S. G. Vijaya Kumari[1], C. Indumathi[2] and M. S. Mithyantha[1]

[1]Rallis Research Centre, 21 & 22, Phase II, P. B. No. 5813, Peenya Industrial Area, Bangalore 560 058, India
[2]Department of Metallurgy, Indian Institute of Science, Bangalore 560 018, India

The toxicity of five organic solvents, viz. *N*,*N*-dimethylformamide (DMF), dimethyl sulphoxide (DMSO), 1,4-dioxan, formamide and *N*-methyl-2-pyrrolidone was evaluated against *Colletotrichum capsici*, *Drechslera oryzae*, *Fusarium oxysporum f.* sp. *cubense*, *Pyricularia oryzae*, *Pythium aphanidermatum* and *Rhizoctonia solani* through poisoned agar bioassay. *N*-methyl-2-pyrrolidone was found to be the most toxic solvent with EC_{50} values of 0.36, 0.70, 0.87, 0.29, 0.83 and 0.36% for the test fungal organisms respectively. DMSO showed the least toxicity with EC_{50} values of 2.91, 3.43, 4.78, 1.46, 3.63 and 1.93% respectively. DMF, formamide and 1,4-dioxan exhibited moderate toxicity. Among the test fungi, *P. oryzae* was the most sensitive with least EC_{50} values and *F. o. cubense* was the least sensitive with high EC_{50} values to all the solvents.

Keywords: Bioassay, EC_{50}, fungi, solvents, toxicity.

USE of organic solvents in pesticide bioassay is often unavoidable, as many of the pesticide technicals have low

*For correspondence. (e-mail: bhogu.mouli@gmail.com)

water solubility and hence must be dissolved in a solvent prior to addition into experimental systems[1]. Organic solvents can cause toxicity on their own[2]. Thus it is generally desirable to use non-toxic solvents in pesticide bioassay, although the actual solvent chosen and the concentration employed are often restricted by problems of pesticide solubility, pipetting accuracy and total test volume[1]. In spite of the frequent use of solvents, no standard method exists which defines the most suitable solvent or the solvent concentration for use in pesticide bioassay studies[3] and little is known about the effects of solvents on biological systems[4].

Though there are allowable limits set for solvent use in aquatic toxicity tests, at 0.05% for acute tests and 0.01% for chronic tests, by the US Environmental Protection Agency (1975)[5], no such guidelines are available for microbial bioassay. In tests involving agar growth media or soil systems, solvent concentrations ranging from 1.0% and above are commonly utilized, due to problems associated with the use of small volumes of test compounds and their solubility and other technical limitations[1].

Hence, the choice of solvent can have a profound effect on the response of an organism to pesticides and its concentration may also be a significant factor in altering toxicity responses. Thus, rather than choosing a solvent and its concentration randomly, a suitable method must be employed that provides adequate data to indicate the interaction that elicits least toxicity on the test organisms[3].

Acetone, methanol, ethanol, tri-ethylene glycol and *N*,*N*-dimethylformamide (DMF) are some solvents suggested for aquatic studies[6]. Acetone is widely used as a solvent of choice in bioassay due to its superior solvent properties rather than its comparative toxicity patterns[7]. Acetone and dimethyl sulphoxide (DMSO) are suitable solvents in toxicity bioassay involving algae because of their low toxicity[2].

In reports on microbial bioassay DMF, ethanol and methanol were the most toxic solvents. Acetone and DMSO were moderately toxic and hexane was the least toxic with *Pythium ultimum*, *Sclerotinia homeocarpa* and *Pestalotia* sp.[1]. Acetone was found to be moderately fungitoxic[8]; it did not affect[9] the growth of the fungi up to 2%. Acetone induced membrane damage in some organisms[4,10]. No significant difference was observed in the toxicity patterns of toluene, acetone, methanol, DMF, and 2-butoxyethanol, when tested on the six wood-decaying fungi[11].

During bioassay studies of new compounds for fungicidal activity against the test organisms using DMF as the solvent, it was found that at high concentrations, DMF arrested the growth of all the test organisms completely and thus was highly toxic. Hence, a study was undertaken to evaluate the most common solvents to identify those which could be used in the bioassay without affecting the inherent fungitoxicity of the test compounds. Five common organic solvents were selected and screened for their toxicity towards the six test organisms.

Six fungal pathogens which are known to cause serious diseases in important agricultural crops, were chosen for bioassay. They were *Colletotrichum capsici* (Syd.) Butler & Bisby, the causal pathogen of fruit rot and anthracnose of chillies; *Drechslera oryzae* (Breda de Haan) Subram & Jain, incitant of brown leaf spot of rice; *Fusarium oxysporum f.* sp. *cubense* (E.F.S) Snyder & Hansen, causative of panama disease of banana; *Pyricularia oryzae* Cav., causative of blast disease of rice; *Pythium aphanidermatum* (Edson.) Fitz., incitant of root and rhizome rot in plantation and vegetable crops, and *Rhizoctonia solani* Kuhn, causative of sheath blight of rice.

Three different culture media used in the study considering their suitability to the growth of the test fungi were Czapek–Dox agar[12] for *C. capsici*, *D. oryzae* and *R. solani*, Potato dextrose agar[13] for *F. o. cubense* and *P. aphanidermatum* and modified Tanaka[14] medium for *P. oryzae*.

Five organic solvents generally used in bioassay studies of microorganisms, viz. DMF, DMSO, 1,4-dioxan, formamide and *N*-methyl-2-pyrrolidone (MP), were evaluated in the present investigation. All the solvents were of Analytical grade.

The fungitoxicity of the solvents was determined by the poisoned agar technique[15]. The solvent was added to the agar medium and thoroughly mixed by vigorous rotations. The medium was dispensed as 20 ml aliquots into petri dishes and allowed to solidify. The plates were then inoculated with mycelial discs (7 mm diameter) taken from the actively growing peripheral region of fresh fungal stock culture plates, then inverted and incubated at room temperature. Observations on the colony diameter (mm) were recorded at 24 h intervals, until growth in the untreated control reached a diameter of 90 mm. Per cent inhibition values were calculated with reference to the control. Differences in radial growth, resulting from inoculated mycelial discs in control and experimental plates reflected the toxicity of the solvent. EC_{50} values were determined for each solvent, which refer to the effective concentration that causes 50% inhibition of fungal growth.

To determine EC_{50} values for test solvents, each solvent was bioassayed at nine concentrations of 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0% (v/v). All the treatments were replicated three times. Per cent inhibition values were calculated relative to growth in the control (no solvent). EC_{50} values were determined by probit analysis using SPSS analysis package. Analyses of significant differences between solvents ($P = 0.05$) were done with ANOVA using the Stat Pac microcomputer software package.

The results of solvent toxicity data on the test organisms are summarized in Table 1 as per cent inhibition values at various concentrations, along with the EC_{50} values.

C. capsici: All the solvents exhibited moderate toxicity (Table 1), except MP, which incited an EC_{50} value of 0.36%, whereas DMSO was least toxic (2.91%), followed by 1,4-

Table 1. Growth inhibition (%) and EC_{50} (%) at different concentrations of solvent

Solvent	Fungus species, growth inhibition (%)* and EC_{50} (%)																													
	Colletotrichum capsici										*Drechslera oryzae*										*Fusarium oxysporum f.* sp. *cubense*									
	Concentration (%)										Concentration (%)										Concentration (%)									
	0.1	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0	EC_{50}	0.1	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0	EC_{50}	0.1	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0	EC_{50}
DMSO	00^{a}	04^{a}	13^{a}	20^{a}	31^{a}	43^{a}	52^{a}	64^{a}	75^{a}	2.91^{e}	00^{a}	00^{a}	02^{a}	04^{a}	14^{a}	40^{a}	41^{a}	62^{a}	81^{a}	3.43^{e}	01^{a}	02^{a}	08^{a}	11^{a}	18^{a}	18^{a}	27^{a}	38^{a}	51^{a}	4.78^{d}
DMF	01^{ab}	21^{b}	49^{b}	61^{c}	71^{c}	78^{b}	87^{c}	91^{c}	100^{b}	1.39^{c}	02^{a}	15^{b}	25^{b}	49^{c}	57^{c}	69^{c}	85^{c}	100^{d}	100^{b}	1.75^{c}	00^{a}	00^{a}	14^{b}	22^{c}	35^{b}	50^{c}	54^{b}	74^{b}	91^{c}	2.58^{c}
Dioxan	01^{ab}	01^{a}	10^{a}	26^{b}	37^{b}	43^{a}	49^{a}	69^{b}	100^{b}	2.54^{d}	01^{a}	02^{a}	03^{a}	13^{b}	44^{b}	55^{b}	79^{b}	88^{b}	100^{b}	2.22^{d}	00^{a}	00^{a}	13^{ab}	18^{b}	37^{b}	42^{b}	55^{b}	74^{b}	78^{b}	2.69^{c}
Formamide	00^{a}	28^{c}	50^{b}	63^{c}	72^{c}	78^{b}	83^{b}	100^{d}	100^{b}	1.00^{b}	00^{a}	13^{b}	37^{c}	58^{d}	72^{d}	82^{d}	87^{c}	91^{c}	100^{b}	1.26^{b}	16^{c}	44^{c}	67^{c}	69^{d}	79^{c}	83^{d}	88^{c}	91^{c}	100^{d}	0.55^{a}
MP	07^{b}	69^{d}	84^{c}	90^{d}	100^{d}	100^{c}	100^{d}	100^{d}	100^{b}	0.36^{a}	00^{a}	28^{c}	83^{d}	100^{e}	100^{e}	100^{e}	100^{d}	100^{d}	100^{b}	0.70^{a}	08^{b}	38^{b}	64^{c}	83^{e}	90^{d}	91^{e}	100^{d}	100^{d}	100^{d}	0.87^{b}

Solvent	Fungus species, growth inhibition (%)* and EC_{50} (%)																													
	Pyricularia oryzae										*Pythium aphanidermatum*										*Rhizoctonia solani*									
	Concentration (%)										Concentration (%)										Concentration (%)									
	0.1	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0	EC_{50}	0.1	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0	EC_{50}	0.1	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0	EC_{50}
DMSO	01^{a}	17^{b}	35^{b}	61^{b}	75^{b}	85^{b}	88^{b}	100^{b}	100^{b}	1.46^{c}	00^{a}	00^{a}	09^{a}	15^{a}	22^{a}	24^{a}	41^{a}	57^{a}	72^{a}	3.63^{e}	03^{b}	08^{b}	14^{a}	32^{b}	53^{a}	77^{a}	82^{a}	100^{a}	100^{a}	1.93^{e}
DMF	00^{a}	13^{b}	47^{c}	72^{c}	83^{c}	91^{c}	100^{b}	100^{b}	100^{b}	1.24^{b}	00^{a}	10^{b}	32^{b}	48^{c}	69^{c}	83^{c}	85^{b}	100^{b}	100^{b}	1.64^{c}	00^{a}	20^{c}	58^{b}	78^{c}	91^{c}	100^{b}	100^{b}	100^{a}	100^{a}	1.03^{b}
Dioxan	02^{a}	03^{a}	12^{a}	19^{a}	35^{a}	38^{a}	64^{a}	82^{a}	91^{a}	2.72^{d}	02^{ab}	08^{b}	34^{b}	59^{d}	82^{d}	87^{d}	100^{c}	100^{b}	100^{b}	1.44^{b}	00^{a}	00^{a}	13^{a}	24^{a}	81^{b}	100^{b}	100^{b}	100^{a}	100^{a}	1.60^{d}
Formamide	10^{b}	30^{c}	49^{c}	59^{b}	81^{c}	83^{b}	100^{b}	100^{b}	100^{b}	1.16^{b}	00^{a}	00^{a}	13^{a}	28^{b}	46^{b}	67^{b}	100^{c}	100^{b}	100^{b}	1.97^{d}	00^{a}	00^{a}	51^{b}	78^{c}	90^{c}	100^{b}	100^{b}	100^{a}	100^{a}	1.18^{c}
MP	21^{c}	91^{d}	100^{d}	100^{d}	100^{d}	100^{d}	100^{b}	100^{b}	100^{b}	0.29^{a}	03^{b}	32^{c}	64^{c}	88^{e}	100^{e}	100^{e}	100^{c}	100^{b}	100^{b}	0.83^{a}	09^{c}	80^{d}	100^{c}	100^{d}	100^{d}	100^{b}	100^{b}	100^{a}	100^{a}	0.36^{a}

*Values superscripted by the same letter in each column do not differ significantly at $P = 0.05$.

dioxan (2.54%). The order of toxicity of solvents for *C. capsici* was MP > formamide > DMF > 1,4-dioxan > DMSO.

D. oryzae: The organism was moderately sensitive to the solvents (Table 1) and MP was the most toxic, with an EC_{50} value of 0.70%. DMSO was the least toxic (3.43%) followed by 1,4-dioxan (2.22%), DMF (1.75%) and formamide (1.26%). The order of toxicity of solvents for *D. oryzae* was MP > DMF > formamide > 1,4-dioxan > DMSO.

F. o. cubense: The most toxic solvent to the organism was formamide unlike MP for other organisms and DMSO expressed the least toxicity, with an EC_{50} value of 4.78% (Table 1). The order of toxicity of solvents for *F. o. cubense* was formamide > MP > DMF ≥ 1,4-dioxan > DMSO.

P. oryzae: This test organism was found to be the most sensitive with least EC_{50} values for all the solvents tested (Table 1). MP reflected the highest toxicity with 20% inhibition even at 0.1% concentration and 91% inhibition at 0.5%, whereas 1,4-dioxan was less toxic with an EC_{50} of 2.72%. The order of toxicity of solvents for *P. oryzae* was MP > formamide ≥ DMF > DMSO > 1,4-dioxan.

P. aphanidermatum: All the solvents exhibited better toxicity, except DMSO (Table 1). MP exhibited the highest toxicity with EC_{50} 0.83% followed by 1,4-dioxan (1.44%), DMF (1.64%) and formamide (1.97%), whereas DMSO reflected less toxicity (3.63%). The order of toxicity of solvents for *P. aphanidermatum* was MP > 1,4-dioxan > formamide > DMF > DMSO.

R. solani: This organism was found to be sensitive to all solvents and was also influenced even by slight increase in solvent concentration (Table 1). Growth of the organism was completely inhibited at or above 2.5% of DMF, 1,4-dioxan and formamide, whereas MP at 1% concentration completely arrested the growth. DMSO was the least toxic with an EC_{50} value of 1.93% and MP was the most toxic (0.36%). The order of toxicity of solvents for *R. solani* was MP > DMF > formamide > 1,4-dioxan > DMSO.

In general, MP was found to be the most toxic solvent and DMSO the least toxic solvent. The overall order of toxicity of solvents was MP > formamide ≥ DMF > 1,4-dioxan > DMSO. However, among test fungal species, *P. oryzae* was the most sensitive and *F. o. cubense* was the least sensitive. The overall order of sensitivity was *P. oryzae* ≥ *R. solani* > *C. capsici* > *P. aphanidermatum* ≥ *D. oryzae* > *F. o. cubense*.

In the present study, MP was found to be highly toxic to all the test organisms. This could be due to the antibiotic nature of the solvent, since equisetin, an antibiotic from *Fusarium equiseti* was identified as a derivative of *N*-methyl-2,4-pyrrolidone[16]. Also, Trichosetin, an antimicrobial compound with remarkable activity against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, is a novel tetramic acid (2,4-pyrollidinedione) antibiotic and a homologue of fungal metabolite, equisetin[17].

DMF, formamide and 1,4-dioxan, in general, were observed to show moderate toxicity towards all the test organisms. Furthermore, 1,4-dioxan was found to be the least toxic solvent among the five only towards *P. oryzae*. Hence, 1,4-dioxan would be a solvent of choice for the assay of *P. oryzae*. However, it could not indicate its suitability to other fungal bioassays. DMF was highly toxic to the test organisms, viz. *P. ultimum*, *S. homeocarpa* and *Pestalotia* sp.[1].

The present study indicates that DMSO is the least toxic solvent towards the test fungal organisms and thus could be a suitable solvent in bioassay because of its superior solvent properties, which is consistent with earlier studies[1,2].

The toxic nature of solvents towards test organisms is of primary concern in all *in vitro* pesticide bioassay studies. It has also been reported that the solvent and pesticide can interact additively, synergistically or antagonistically, depending upon the solvent concentration[4,8,18]. For an organism to be altered in its response to the pesticide, a threshold solvent level must be reached such that it becomes more or less sensitive to the pesticide[3]. The present study shows that DMSO could be chosen as a solvent suitable for fungal bioassay because of its low toxicity. The concentration of DMSO that interacts additively with the test compound needs further investigations and in order to choose a solvent concentration that does not affect the inherent pesticide toxicity, interaction studies have to be carried out.

1. Stratton, G. W., The influence of solvent type on solvent–pesticide interactions in bioassay. *Arch. Environ. Contam. Toxicol.*, 1985, **14**, 651–658.
2. Ma, J. and Chen, J., How to accurately assay the algal toxicity of pesticides with low water solubility. *Environ. Pollut.*, 2005, **136**, 267–273.
3. Stratton, G. W., Burrell, R. E. and Corke, C. T., Technique for identifying and minimizing solvent–pesticide interactions in bioassay. *Arch. Environ. Contam. Toxicol.*, 1982, **11**, 437–445.
4. Stratton, G. W., Burrell, R. E., Kurp, L. M. and Corke, C. T., Interactions between the solvent acetone and the pyrethroid insecticide permethrin on activities of the blue-green alga *Anabaena*. *Bull. Environ. Contam. Toxicol.*, 1980, **24**, 562–569.
5. Jay, A. E., Toxic effects of organic solvents on the growth of *Chlorella pyrenoidosa* and *Selenastrum*. *Bull. Environ. Contam. Toxicol.*, 1996, **57**, 191–198.
6. Hughes, J. S. and Vilkas, A. G., Toxicity of *N,N*-dimethylformamide used as a solvent in toxicity tests with green alga, *Selenastrum capricornatum*. *Bull. Environ. Contam. Toxicol.*, 1983, **31**, 98–104.
7. Majewski, H. S., Klaverkamp, J. F. and Scott, D. P., Acute lethality and sub-lethal effects of acetone, ethanol and propylene glycol on the cardiovascular and respiratory systems of rainbow trout (*Salmo gairdneri*). *Water Res.*, 1978, **12**, 217–221.
8. Burrell, R. E. and Corke, C. T., Interactions of the solvent acetone with the fungicides benomyl and captan in fungal assays. *Bull. Environ. Contam. Toxicol.*, 1980, **25**, 554–561.

9. Manten, A., Klopping, H. L. and Van Der Kerk, G. J. M., Investigations on organic fungicides – II – A new method for evaluating antifungal substances in the laboratory. *Antonie van Leeuwenhoek*, 1950, **16**, 282–294.
10. Parasher, C. D., Ozel, M. and Geike, F., Effect of hexachlorobenzene and acetone on algal growth, physiology and ultrastructure. *Chem. Biol. Inter.*, 1978, **20**, 89–95.
11. Bezemer, L. D., Da Costa, E. W. B. and Gay, F. J., Suitability of solvents and dyes for use in laboratory bioassay with fungi and termites. *Int. Biodeterior. Bull.*, 1973, **9**, 101–104.
12. Warcup, J. H., The soil-plate method for isolation of fungi from soil. *Nature*, 1950, **166**, 117–118.
13. Beever, B. E. and Bollard, E. G., The nature of the stimulation of fungal growth by potato extract. *J. Gen. Microbiol.*, 1970, **60**, 273–279.
14. Tanaka, S., Nutrition of *Piricularia oryzae in vitro*. In *The Rice Blast Disease*, Proceedings of a Symposium at The International Rice Research Institute, Oxford & IBH, Calcutta, 1963, pp. 23–25.
15. Nene, Y. L. and Thapliyal, P. N., Evaluation of fungicides. In *Fungicides in Plant Disease Control*, Oxford & IBH, New Delhi, 1993, 3rd edn, pp. 525–542.
16. Vesonder, R. F., Tjarks, L. W., Rohwedder, W. K., Burmeister, H. R. and Laugal, J. A., Equisetin, an antibiotic from *Fusarium equiseti* NRRL 5537, identified as a derivative of N-methyl-2,4-pyrollidone. *J. Antibiot.*, 1979, **32**, 759–761.
17. Marfori, C., Kajiyama, S. I., Fukusaki, E. I. and Koboyashi, A., Trichosetin, a novel tetramic acid antibiotic produced in dual culture of *Trichoderma harzianum* and *Catharanthus roseus* callus. *Z. Naturforsch.*, 2002, **57**, 465–470.
18. Stratton, G. W. and Corke, C. T., Effect of acetone on the toxicity of atrazine towards photosynthesis in *Anabaena*. *J. Environ. Sci. Health*, 1981, **16**, 21–33.

ACKNOWLEDGEMENTS. We thank Drs K. S. Rao and K. Sridhara, Department of Synthesis, Rallis Research Centre, Bangalore for providing solvents for the study; Directorate of Rice Research (ICAR), Hyderabad; Indian Institute of Spices Research (ICAR), Calicut; University of Agricultural Sciences, Bangalore and Acharya N. G. Ranga Agricultural University, Hyderabad for providing authentic fungal cultures for evaluation.

Received 16 September 2005; revised accepted 7 February 2006

Studies on the filament of tasar silkworm, *Antheraea mylitta* D (Andhra local ecorace)

G. Shamitha* and A. Purushotham Rao

Department of Zoology, Kakatiya University, Warangal 506 009, India

***Antheraea mylitta* ecorace exclusively found in the northern part of Andhra Pradesh, South India was taken for Scanning Electron Microscope studies of its filament with reference to total indoor and outdoor rearings. Compared to the outdoor-reared cocoons, loose filament structure and wide gaps between filaments were found in indoor cocoons. A comparison was also drawn on the effect of various levels of gamma-irradiation on the silk filament. A dose-dependent decrement in the diameter of the filament was observed.**

Keywords: *Antheraea mylitta*, gamma irradiation, indoor rearing, silk fibre diameter, tasar silk filament.

THE major tropical and commercial tasar varieties of India mainly include Daba tv, Raily, Sukinda, Bagai, Sarihan, Bhandara, etc. Andhra local ecorace is also a tropical tasar variety available only in Andhra Pradesh. It is well known for its superior commercial qualities like hard and compact cocoons, high reelability (69%), high shell ratio (16.85) and low denier (7%)[1,2]. However, it shows heavy mortality of larvae due to predators, parasites[3] and climatic hazards[4].

Outdoor rearing of wild silkworm predisposes larvae to the vagaries of climatic conditions. It makes them more vulnerable not only to pests and diseases, but also to the impacts of temperature, photoperiodism, humidity, etc. In order to overcome these hurdles, several attempts were made to conduct indoor rearing. An artificial diet has been developed for the tropical tasar silkworm containing *Asan* leaf powder, the principal food plant and some success has been achieved[5]. Standardization of chawki-rearing to prevent the early stage larval loss has resulted in 20% increase in the effective rate of rearing[2,6].

In the present investigation, an attempt has been made at Kakatiya University, Warangal at total indoor rearing of the ecorace. Rearing of silkworm was undertaken from brushing stage to cocoon under controlled conditions for three crops. Simultaneously, outdoor rearing was also carried out in the field of *Terminalia arjuna* plantation. The structure of the filament was compared in both outdoor and indoor cocoons.

There is a dearth of appropriate technologies in the post-cocoon sector which has certain drawbacks like lack of uniformity in cocoon structure and silk deposition due to its hardness and this accounts to 50% loss in spinning. The technique of ionizing radiation has been employed to study eggs, larvae and pupae of silkworms to various doses of X-, gamma and UV rays for genetic mutations. Earlier, a study was carried out on the impact of insecticides following irradiation[7]. The effect of UV and gamma irradiation on heartbeat of *Bombyx mori* was investigated[8]. Since the environment has a direct influence on the cocoon surface, sun rays, which are known to possess ultraviolet, X-, gamma rays, etc. at different levels, in the present studies, cocoons are studied by exposing them to gamma rays and observing their effect on the filament.

While studying the cocoon characters, silk fibre diameter was also measured for the outdoor, indoor and irradiated cocoons using a microhardness tester. Earlier, silk thread size measurements were seen using CCD linear line sensor[9].

*For correspondence. (e-mail: shamitha_gang@yahoo.com)