The Journal of Plant Protection Sciences, **2**(2) : 1-8, 2010

Mass production, formulation, quality control and delivery of *Trichoderma* for plant disease management

B. Ramanujam, R. D. Prasad, S. Sriram and R. Rangeswaran

National Bureau of Agriculturally Important Insects, HA Farm post, P.B.No. 2491, Bellary Road, Bangalore 560024
Directorate of Oil Seeds Research, Rajendranagar, Hyderabad.
E-mail: bonamramanujam58@gmail.com

A B S T R A C T

Trichoderma has gained maximum attention as biocontrol agent due to the fact that it is effective against a large number of soil-borne plant pathogenic fungi, suppressive effects on some root nematodes without adversely affecting beneficial microbes like *Rhizobium* and capable of promoting growth of certain crops. There are two major methods of inoculum production of *Trichoderma* spp. viz., solid state fermentation and liquid state fermentation. In solid fermentation, the fungus is grown on various cereal grains, agricultural wastes and byproducts. The solid state production is highly labour intensive and fit for cottage industry. These products are used mainly for direct soil application in nurseries/main fields to suppress the soil-borne inoculum. In liquid state fermentation, *Trichoderma* is grown in inexpensive media like molasses and yeast medium in deep tanks on a commercial scale. Biomass from the liquid fermentation can be made into different formulations like, dusts, granules, pellets, wettable powders. *Trichoderma* formulations can be applied to the seed either by dry seed treatment or by seed biopriming for control of several soil-borne diseases of some field crops. Similarly, seedlings of horticultural crops and rice are treated by dipping the roots in *Trichoderma* suspensions before planting. Granular or pellets preparations and *Trichoderma* enriched FYM have been used for soil application directly and have provided effective control of diseases both nurseries and field conditions. To ensure that the products of *Trichoderma* do not affect the environment, human beings and other living organisms adversely and to prevent the sale of poor quality products to the farmers, the Central Insecticide Board of Government of India has made registration of microbial pesticides mandatory before commercial production/import/sale. Guidelines and data requirements for registration of microbial pesticides have been provided in the annexure of Insecticide Act. Quality control parameters set by CIB are inadequate for knowing potentiality of a bioagent. Apart from the counts of live propagules in the formulation, bioefficacy also should be taken as a quality parameter to ensure availability of better products to farmers.

Keywords : Trichoderma, mass production, formulation quality control, registration delivery, shelf-life, disease management.

Introduction

Biocontrol technologies have gained momentum in disease control of crop plants in recent times as these technologies not only minimize or replace the usage of harmful chemical pesticides but also found to be cheaper and efficient in certain disease control programmes. Successful use of fungal biocontrol agents like *Trichoderma* spp. for the control of soil borne diseases caused by pathogens like, *Rhizoctonia, Sclerotium, Fusarium, Pythium,* and *Phytophthora* in several crops have been reported (Cook & Baker 1983). *Trichoderma* spp. are under intensive research because of their abundant natural occurrence, biocontrol potential against fungal and nematode diseases as well as host defense inducing ability (Haraman & Kubicek 1998). Bioagents like, *T. viride, T harzianum* and *T.virens* are being successfully used for the control of some of the dreaded diseases like, foot rot of black pepper, root rots of pulses, damping off, collar rots and *Fusarium* wilts of horticultural crops.

Mass Production Technology for Trichoderma spp.

The major aspects of successful biological control technologies include the establishment of product, formulation and delivery system for microorganism that enable them for efficient disease control. The mass production systems should be compatible with industrial and commercial development methods and field application. There are two major methods of inoculum production as (a)Solid state fermentation and (b) Liquid state fermentation.

Solid-state fermentation

Solid fermentation is a very common method for mass production of *Trichoderma*. Various cheap cereal grains like, sorghum, millets, ragi are used as substrates (Jeyarajan 2006). The grains are moistened, sterilized and inoculated with *Trichoderma* and incubated for 10-15 days. *Trichoderma* produces dark green spore coating on the grains. These grains can be powdered finely and used as seed treatment or the grains can be used as it is for enriching FYM for soil application. Other agriculture wastes and byproducts used for mass production of *Trichoderma* are given in Table 1.

Solid fermentation results in a product that is generally used as it is for soil application or for enriching organic manures. This technique is suitable for small-scale production in cottage industries or at individual farmer level. The disadvantage of technique is laborious and results in a product which is bulky and prone for contamination. Solid state fermentation technique for commercial/industrial scale production of biocontrol fungi has to worked out in collaboration with industry.

Liquid fermentation

In liquid fermentation system, *Trichoderma* is grown in liquid media in stationary/shaker/ fermentor cultures and formulated and used for field application. Different growth media used for production of *Trichoderma* through liquid fermentation are given in Table 2.

Maximum biomass of *Trichoderma* spp. can be realized in short-time by using appropriate medium in a fermentor with aeration, agitation, temperature, pH and antifoam controls than in shake-flask cultures and will be more suitable for industrial production of *Trichoderma* spp. Studies at erstwhile Project Directorate of Biological Control (PDBC), Bangalore revealed that maximum amount of biomass and viable

Table 1.
Substrates successfully used to produce *Trichoderma* spp. by solid state fermentation

Substrate	Reference
Sorghum grain	Upadhyay and Mukhopadhyay 1986
Wheat bran-saw dust modified medium	Mukhopadhyay *et al*. 1986
Tapioca rind, Tapioca refuse, FYM, press mud	Kousalya Gangadharan & Jeyarajan 1990
FYM, wheat bran, rice bran, peat soil, rice straw	Sangeetha Panicker *et al*. 1993
Groundnut shell medium	Raguchander *et al*. 1993
Spent tea leaf waste and coffee husk	Bhai *et al*. 1994
Wheat bran and biogas manure	Jagadeesh & Geetha 1994
Pigeonpea husk, tapioca waste (after starch extraction) and press mud.	Jayaraj & Ramabadran 1996
Coffee fruit skin, poultry manure and coffee fruit skin composted with cow dung slurry	Sawant & Sawant 1996
Decomposed Coconut Coir pith	Kumar & Marimuthu 1997
Spent malt	Gopalakrishnan *et al.* 2003

propagules of *T. harzianum/T. viride* can be obtained within 96h of fermentation in a fermentor with aeration, agitation, temperature controls (Prasad & Rangeswaran 1998).

Table 2.
Growth media used for production of *Trichoderma* in liquid state fermentation

Growth media	**Reference**
Molasses and brewers yeast	Sankar & Jeyarajan 1996
Potato dextrose broth, V-8 juice and Molasses-yeast medium	Prasad & Rangeshwaran 1998
Molasses soy medium	Prasad & Rangeshwaran 2000
Jaggery-soy medium	Prasad *et al.* 2002

Formulations of Trichoderma spp.

In general, product formed from solid or semi solid-state fermentation do not require sophisticated formulation procedures prior to use. For example, grain or other types of organic matter upon which *Trichoderma* is grown are simply dried, ground and added to the area to be treated. Biomass produced in liquid fermentation either can be separated from medium and concentrated or entire biomass with medium can be incorporated into dusts, granules, pellets, wettable powders or emulsifiable liquids. The carrier material may be inert or a food base or a combination of both. *Trichoderma* spp. can be formulated as pelletts (Papavizas & Lewis 1989), dusts and powders (Nelson & Powelson 1988) and fluid drill gels (Conway 1986). The various types of *Trichoderma* formulations used in biological control of crop diseases are given below.

1. Talc based formulation: In India, talc based formulations of *T. viride* was developed at Tamil Nadu Agricultural University, Coimbatore for seed treatment of pulse crops and rice (Jeyarajan *et al*. 1994). *Trichoderma* is grown in the liquid medium is mixed with talc powder in the ratio of 1:2 and dried to 8% moisture under shade. The talc formulations of *Trichoderma* has shelf life of 3-4 months. It has become quite popular in India for management of several soil-borne diseases of various crops through seed treatment at 4-5g/kg seed. Several private industries produce large quantities of talc formulations in India for supply to the farmers. The annual requirement of *Trichoderma* has been estimated as 5,000 tones to cover 50 per cent area in India (Jeyarajan 2006).

2. Vermiculite-wheat bran formulation (Lewis *et al.* 1991)

Ingredients:

Vermiculite	100g
Wheatbran	33g
Wet fermentor biomass	20g
0.05N HCL	175 ml

Trichoderma is multiplied in molasses-yeast medium for 10 days. Vermiculite and wheat bran are sterilized in an oven at 70 °C for 3 days. Then, 20 gms of fermentor biomass and 0.05N HCl are added, mixed well and dried in shade.

3. Pesta granules (Connick *et al.* 1991)

Ingredients :

Wheat flour	100g
Fermentor biomass (FB)	52 ml
Sterile water	sufficient enough to form dough

Fermentor biomass (52 ml) is added to wheat flour (100g) and mixed by gloved hands to form cohesive dough. The dough is kneaded, pressed flat and folded by hand several times. Then one mm thick sheets (pesta) is prepared and air-dried till it breaks crisply. After drying, dough sheet was ground and passed through a 18 mesh (1.0 mm) sieve and granules were collected.

4. Wheat flour- kaolin (Prasad & Rangeswaran 1998)

Ingredients :

Wheat flour	80gm
Kaolin	20 gm
Fermentor biomass	52 ml

52 ml of Fermentor biomass is added to wheat flour (100g) and mixed by gloved hands to form a cohesive dough. The rest of the procedure is as described for pesta granules

5. Alginate prills (Fravel *et al.* 1985)

Ingredients:

Sodium Alginate	25 gm
Wheat flour	50 gm
Fermentor biomass	200 ml

Sodium alginate is dissolved in one portion of distilled water (25g/750 ml) and food base is suspended in another portion (50g/250ml). These preparations are autoclaved and when cool are blended together with biomass. The mixture is added drop wise into $CaCl_2$ solution to form spherical beads, which are air-dried and stored at 5 ^{o}C.

6. Press mud based formulation: Press mud is available as a by product of the sugar factory and this can be used as a substrate for mass multiplication of *Trichoderma*. *Trichoderma* produced and formulated on press mud is sold to farmers as value-added organic manure by a sugar factory in south India. (Jeyarajan 2006).

7. Coffee husk: In Karnataka Sawant & Sawant (1996) developed a *Trichoderma* formulation based on coffee husk which is a waste from coffee curing industry. This product was very effective in managing *Phytophthora* foot rot of black pepper and is widely used in Karnataka and Kerala.

8. Oil-based formulations: They are prepared by mixing the conidia harvested from the solid state/liquid state fermentation with a combination of vegetable/mineral oils in stable emulsion formulation. In such formulations, microbial agents are suspended in a water immiscible solvent such as a petroleum fraction (diesel, mineral oils), and vegetable oils (groundnut etc.) with the aid of a surface-active agent. This can be dispersed in water to form a stable emulsion. Emulsifiable concentrates require a high concentration of an oil soluble emulsifying agent, to give instantaneous formation of a homogenous emulsion on dilution in water. The oils used should not have toxicity to the fungal spores, plants, humans and animals. Such formulations of *Trichoderma, Pseudomonas,* and *Beauveria* are now being used as foliar sprays. Oil-based formulations are supposed to be suitable for foliar sprays under dry weather conditions and to have prolonged shelf life. The spores can survive for longer time in the plant surface even during the dry weather as the spores are covered by oil that protects them from drying. Batta (2005) developed an emulsion formulation of *T. harzianum* for the control of post harvest decay of apple caused by *Botrytis cinerea*. Invert-emulsion formulation of *T. harzianum* with a shelf life of 8 months has been developed using indigenous constituents at the erstwhile Project Directorate of Biological Control (PDBC), Bangalore in India and this his formulation has been and found to be effective against soil-borne diseases of groundnut.

Shelf life of *Trichoderma* formulations

Shelf life of the formulated product of a bio-control agent plays a significant role in successful marketing. In general the antagonists multiplied in an organic food base have longer shelf life than the inert or inorganic food bases. Shelf life of *Trichoderma* in coffee husk was more than 18 months. Talc, peat, lignite and kaolin based formulation of *Trichoderma*, have a shelf life of 3-4 months. The viable propagules of *Trichoderma* in talc formulation was reduced by 50% after 120 days of storage (Sankar & Jeyarajan 1996). Studies on the storage of *T. viride* formulation in poly propylene bags of various colours revealed that the population of *T. viride* was maximum in milky white bags of 100 micron thickness. At PDBC, Bangalore work on increasing shelf life of talc formulations of *Trichoderma* using various ingredients (chitin and glycerol) in production medium and heat shock at the end of log phase of fermentation was carried out which can extend the shelf of talc formulation of *Trichoderma* up to one year. (Sriram *et al*, 2010; Sriram *et al*, 2011)

Delivery of *Trichoderma* for disease management

For successful diseases control, delivery and establishment of *Trichoderma* to the site of action is very important. The most common methods of application of *Trichoderma* are by seed treatment, seedling dip, soil application and wound dressing.

Seed Treatment: Seed coating with *Trichoderma* is one of the easy and effective methods of delivering the antagonist for the management of seed/soil-borne diseases. Seed is coated with dry powder/dusts of *Trichoderma* just before sowing. For commercial purpose, dry powder of antagonist is used at 3 to 10 g per kg seed based on seed size (Mukhopadhyay *et al.* 1992). Propagules of biocontrol agents germinate on the seed surface and colonize roots of germinated seedlings and rhizosphere (Tiwari 1996). *T. harzianum, T. virens* and *T. viride* were found to be effective seed protectants against *Pythium* spp. and *Rhizoctonia solani* (Mukherjee & Mukhopadhyay 1995).

Seed biopriming: Seed biopriming is treating of seeds with *Trichoderma* and incubating under warm and moist conditions until just prior to radical emergence). This technique has potential advantages over simple coating of seeds as it results in rapid and uniform seedling emergence. *Trichoderma* conidia germinate on the seed surface and form a layer around bioprimed seeds. Such seeds tolerate adverse soil conditions better. Biopriming could also reduce the amount of biocontrol agents that is applied to the seed. Seed biopriming was successfully used in tomato, brinjal, soybean and chickpea in Tarai region of Uttaranchal (Mishra *et al.* 2001).

Root treatment: Seedling roots can be treated with spore or cell suspension of antagonists either by drenching the bioagent in nursery beds or by dipping roots in bioagent suspension before transplanting. This method is generally used for the vegetable crops, rice where transplanting is practiced (Singh & Zaidi 2002). There are also reports on the reduction of sheath blight disease of rice by root dip of seedlings before transplantation (Vasudevan *et al.* 2002). Root dipping of tomato seedlings reduces the severity of root knot caused by *Meloidogyne incognita*. Root dipping in antagonist's suspension not only reduces disease severity but also enhances seedling growth in rice, tomato, brinjal, chili and capsicum (Singh & Zaidi 2002).

Soil treatment: There are several reports on the application of biocontrol agents to the soil and other growing media either before or at the time of planting for control of a wide range of soil-borne fungal pathogens (Baby and Manibhushanrao 1996). Such applications are ideally suited for green house and nursery. *Trichoderma* is capable of colonizing farm yard manure (FYM) and therefore application of colonized FYM to the soil is more appropriate and beneficial. This is the most effective method of application of *Trichoderma* particularly for the management of soil-borne diseases.

Aerial spraying / Wound dressing:

Trichoderma has been successful applied to the aerial plant parts for the biocontrol of decay fungi in wounds on shrubs and trees (Papavizas 1985).

Registration and Quality Control

To ensure that the products of microbial BCAs do not affect the environment, human beings and other living organisms adversely and to prevent the sale of poor quality products to the farmers, the Central Insecticide Board (CIB) of the Government of India has made registration of microbial pesticides mandatory before commercial production/import /sale. Guidelines and Data requirements for registration of microbial pesticides (Data on Biological, Physical, Chemical properties and Bio-efficacy to the target pathogen, Effect on non-target organisms, Toxicological reports on laboratory animals, Eco-toxicity, Manufacturing process, Packing and labeling) have been provided in the Annexure of Insecticide Act.

Standards for *Trichoderma* formulations:

1.Colony Forming Units (CFUs) of *Trichoderma* spp. should be a minimum of 2 x 10^6 CFU per ml or gm on selective medium
2.Pathogenic contaminants such as *Salmonella*, *Shigella* or *Vibrio* should not be present. Other microbial contaminants not to exceed 1 x 10^4 count ml/gm
3. Maximum moisture content should not be more than 8% for dry formulation of fungi

Quality control parameters set by CIB are inadequate for knowing potentiality of a bioagent. It is time now for Ministry of Agriculture to identify a Central Agency for quality testing not only in terms of amount of live propagules in the formulation but also their bioefficacy against plant pathogens. Quality of the products in these lines should be periodically checked by the identified agency and that will ensure availability of better products to farmers.

Future Research: The future research should focus on the following aspects for better utilization of *Trichoderma* as a biocontrol agent for crop disease management.

- Suitability of *Trichoderma* for control of foliar/aerial pathogens
- Development of liquid/oil formulations suitable for foliar applications
- Formulations with prolonged shelf life, field persistence and suitable for dry weather conditions
- Scaling up of Solid state production systems with Industry collaboration
- Large scale demonstration of biocontrol technologies in farmers fields
- Fast Track Registration

- Quality control laboratories
- Identification of strains suitable for various soil and environmental conditions (high temperature/ low moisture/saline conditions)

Literature Cited

Baby UI Manibhushanrao K. 1996 Fungal antagonists and VA mycorrhizal fungi for biocontrol of *Rhizoctonia solani*, the rice sheath blight pathogen, pp 1-9. In *Recent Developments in Biocontrol of Plant Pathogens* (Eds Manibhushanrao K Mahadevan A) Today and Tomorrow's Printers and Publishers, New Delhi, 160 pp.

Batta YA. 2005 Postharvest biological control of apple gray mold by *Trichoderma harzianum* Rifai formulated in an invert emulsion. *Crop Protection* **23**(1): 19-26.

Bhai RS Thomas J Naidu R. 1994 Evaluation of carrier media for field application of *Trichoderma* spp. in cardamom growing soils. *Journal of Plantation Crops* **22** (1): 50-52.

Connick WJ Boyette CD McAlpine JR. 1991 Formulation of mycoherbicides using a Pasta like process. *Biological Control* **1**: 281-87.

Conway KE. 1986 Use of fluid drilling gels to deliver biological control agents to soil. *Plant Diseases* **70**: 835-39.

Cook RJ Baker KF. 1983 *The Nature and Practice of Biological control of Plant Pathogens*, APS Books, St. Paul, MN, USA, 539 pp.

Fravel DR Marois JJ Lumsden RD Connick WJ. 1985 Encapsulation of potential biocontrol agents in an alginate-clay matrix. *Phytopathology* **80**: 996.

Gopalakrishnan C Ramanujam B Prasad RD Rao NS Rabindra RJ. 2003 Use of brewer's yeast amended spent malt as substrate for mass production of *Trichoderma*. *Journal of Biological Control* **17**: 167-70.

Harman GE Kubicek CP (Eds) 1998 *Trichoderma* and *Gliocladium*, Vol. 1 & 2 Taylor and Francis, London, 278 pp.

Jagadeesh KS Geeta GS. 1994 Effect of *Trichoderma harzianum* grown on different food bases on the biological control of *Sclerotium rolfsii* Sacc. in groundnut. *Environmental Ecology* **12**: 471-73.

Jayaraj J Ramabadran R. 1996 Evaluation of certain organic substrates and adjuvants for the mass multiplication of *Trichoderma harzianum* Rifai. *Journal of Biological Control* **10**: 129-31.

Jeyarajan R Ramakrishnan G Dinakaran D Sridar R. 1994 Development of products of *Trichoderma viride* and *Bacillus subtilis* for biocontrol of root rot diseases, pp 25-36. In *Biotechnology in India* (Ed Dwivedi B.K.) Bioved Research Society, Allahabad.

Jeyarajan R. 2006 Prospects of indigenous mass production and formulation of Trichoderma, pp 74-80. In *Current Status of Biological Control of Plant diseases using antagonistic organisms in India* (Eds Rabindra RJ Ramanujam B) Project Directorate of Biological Control, Bangalore, 445 pp.

Kousalya Gangadharan Jeyarajan R. 1990 Mass multiplication of *Trichoderma* spp. *Journal of Biological Control* **4**: 70-71.

Kumar A Marimuthu T. 1997 Decomposed Coconut Coirpith - a conducive medium for colonization of *Trichoderma viride*. *Acta Phytopathologica et Entomologica Hungarica* **32**: 51-58.

Lewis JA Papavizas GC Lumsden RD. 1991 A new formulation system for the application of biocontrol fungi to the soil. *Biocontrol Science and Technology* **1** (1): 59-69.

Mishra DS Singh US Dwivedi TS. 2001 Comparative efficacy of normal seed treatment and seed bio-priming with commercial formulations of *Trichoderma* spp. In 53rd *Annual meeting of Indian Phytopathological Society and National symposium on Ecofriendly approaches for plant disease management*, Chennai, India 21-23, 2001.

Mukherjee PK Mukhopadhyay AN. 1995 *In situ* mycoparasitism of *Gliocladium virens* on *Rhizoctonia solani*. *Indian Phytopathology* **48** (1): 101-02.

Mukhopadhyay AN Brahm Bhat A Patel GJ. 1986 *Trichoderma harzianum*-a potential biocontrol agent for tobacco damping off. *Tobacco Research* **12**: 26-35.

Mukhopadhyay AN Shrestha SM Mukherjee PK. 1992 Biological seed treatment for control of soil borne plant pathogens. *FAO Plant Protection Bulletin* **40**: 21-30.

Nelson ME Powelson ML. 1988 Biological control of grey mold of snap beans by *Trichoderma hamatum*. *Plant Disease* **72:** 727-29.

Papavizas GC Lewis JA. 1989 Effect of *Gliocladium* and *Trichoderma* on damping off and blight of snap bean caused by *Sclerotium rolfsii* in the greenhouse. *Plant Pathology* **38:** 277-86.

Papavizas GC. 1985 *Trichoderma* and *Gliocladium*: Biology and potential for biological control. *Annual Review of Phytopathology* **23**: 23-54.

Prasad RD Rangeshwaran R Anuroop CP Phanikumar PR. 2002 Bioefficacy and shelf life of conidial and chlamydospore formulation of *Trichoderma harzianum*. *Journal of Biological Control* **16**: 145-48.

Prasad RD Rangeshwaran R. 1998 A modified liquid medium for mass production of *Trichoderma* by fermentation process, pp 26. In Abstracts of *National symposium on Eco-friendly approaches in the management of plant diseases* December 22-24, 1998, Shimoga, Karnataka, India.

Prasad RD Rangeshwaran R. 2000 Shelf life and bioefficacy of *Trichoderma harzianum* formulated in various carrier materials. *Plant Disease Research* **15**: 38-42.

Raghuchander T Samiappan R Arjunan G. 1993 Biocontrol of *Macrophomina* root rot of mung bean. *Indian Phytopathology* **46**: 379-382.

Sangeetha P Jeyarajan R Panicker S. 1993 Mass multiplication of biocontrol agent *Trichoderma* spp. *Indian Journal of Mycology and Plant Pathology* **23**: 328-30.

Sankar P Jeyarajan R. 1996 Biological control of sesamum root rot by seed treatment with *Trichoderma spp*. and *Bacillus subtilis*. *Indian Journal of Mycology and Plant Pathology* **26**: 147-53.

Sawant IS Sawant SD. 1996 A simple method for achieving high cfu of *Trichoderma harzianum* on organic wastes for field applications. *Indian Phytopathology* **49**: 185-87.

Singh US Zaidi NW. 2002 Current Status of formulation and delivery of fungal and bacterial antagonists for disease management in India, pp 168-179. In *Microbial Biopesticide Formulations and Application* (Eds Rabindra RJ Hussaini SS Ramanujam B) Project Directorate of Biological Control, Bangalore, 269 pp.

Sriram S Palanna KB Ramanujam B. 2010 Effect of chitin on the shelf life of *Trichoderma harzianum* in talc formulation. *Indian Journal of Agriculture Sciences* **80**: 930-932

Sriram S Roopa KP Savitha MJ. 2011 Extended shelf-life of liquid fermentation derived talc formulations of *Trichoderma harzianum* with the addition of glycerol. *Crop protection* **30:** 1334-1339

Tewari AK. 1996 Biological Control of chickpea wilt-complex using different formulations of *Gliocladium virens* through seed treatment. *Ph D Thesis*, GB Pant University of Agriculture and Technology, Pantnagar, India, 167 pp.

Upadhyay JP Mukhopadhyay AN. 1986 Biological control of *Sclerotium rolfsii* by *Trichoderma harzianum* in Sugarbeet. *Tropical Pest Management* **32**: 215-20.

Vasudevan P Kavitha S Priyadarisini VB Babujee L Gnanamanickamm SS. 2002 Biological control of rice diseases, pp 11-32. In *Biological control of crop diseases.* (Ed Gnanamanickam SS) Marcel Decker, Newyork, 480 pp.