

BIO-FERTILIZERS AND BIO-PESTICIDES RESEARCH AND DEVELOPMENT AT UPLB

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ABSTRACT

With the establishment of the National Institutes of Biotechnology and Applied Microbiology (BIOTECH), University of the Philippines Los Baños (UPLB) researches were done to look for cheaper and locally available but efficient substitutes or supplements to inorganic fertilizer, such as bio-fertilizers and bio-pesticides. Technologies on bio-fertilizers developed by UPLB include BIO-N, the most popular and one of the most effective; Vesicular Arbuscular Mycorrhiza Root Inoculant or VAMRI; Brown Magic; BioGro; Mykovam; NitroPlus; microbial inoculants for the bioconversion of crop residues and agro-industrial by-products into bio-fertilizers; Cocogro or plant growth hormones from coconut water; and BioCon. bio-pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals were also discussed in this paper. Three major types of bio-pesticides were studied: microbial, plant, and biochemical pesticides. Results of experiments and tests showed that these kinds of pesticides may serve as effective and safe alternatives to chemical ones. Bio-fertilizers and bio-pesticides in the Philippines are very promising. Bio-fertilizers are very cheap, easy to use, safe and do not require repeated applications. Several training courses and workshops have already been conducted to disseminate and transfer the bio-fertilizer technologies to target clientele. However, they still lack availability outside UPLB. On the other hand, some bio-pesticides are still unpopular among farmers. The area-wide use of bio-pesticides could be enhanced if village-level mass production and utilization of this technology will be aggressively extended to Filipino farmers by the local government units through organized integrated pest management programs.

Key words: bio-fertilizers, bio-pesticides, BIOTECH-UPLB, bio-fertilizers technologies, microbial pesticides, plant pesticides, biochemical pesticides

INTRODUCTION

For over three decades, the Philippine agricultural sector has depended and relied on inorganic fertilizers and pesticides for food production. Because of the lack of an effective and locally available fertilizer and pesticides technologies, the Philippines resorted to the importation of 85% of the total inorganic fertilizer and more than 90% of pesticide requirements. Large amounts of foreign exchange spent on importation have contributed to the stagnant and limited growth of our economy. It is in this regard that bio-fertilizer and bio-pesticide research in the country was undertaken in the late 70s to come up with more cost-efficient and local

alternatives to imported fertilizers and pesticides.

With the establishment of the National Institutes of Biotechnology and Applied Microbiology (BIOTECH) at the University of the Philippines Los Baños (UPLB), mycorrhizae research grew very rapidly and gained momentum during the last two decades. "Bio-fertilizer Technology Program", formerly "Nitrogen Fixation and Mycorrhizae Program", became one of the major programs of the institute and this program has been mandated to undertake researches in all aspects of mycorrhizal symbiosis, organic composting and nitrogen fixation. The goal was to look for cheaper and locally available but efficient substitutes or supplements to inorganic

fertilizer. Thirty years from its inception, the program has developed several microbial inoculants and bio-fertilizers for agricultural crops, ornamentals and forest trees, which could increase yield/growth of targeted crops, significantly reduce or eliminate chemical fertilizer requirements, and are environmentally sound.

On the other hand, research on the use of plants as bio-pesticides in the Philippines had not gained that momentum as in bio-fertilizers due to inadequate financial support. Most of the bio-pesticide researches in the Philippines have been conducted at the International Rice Research Institute (IRRI) and at UPLB (Departments of Entomology and Plant Pathology). Other research institutions conducted preliminary screening of some crude extracts but results are mostly unpublished. The plant parts being bioassayed are leaves, flowers, stem, vine, bark, fruit peelings, seeds and bulbs. Most of the studies are very preliminary, with crude extracts assayed on a few test organisms.

BIO-FERTILIZERS: TECHNOLOGIES/PRODUCT DESCRIPTION

Title of Technology: BIO-N (inoculants for rice, corn and high value agricultural crops)

Date/Year of development: 1985

Technology description:

BIO-N (Fig. 1) is a microbial-based fertilizer developed by Professor Emeritus Mercedes U. Garcia from nitrogen-fixing bacteria isolated from the roots of “*talahib*” grass (*Sacharrum spontaneum*). It is a product of the ever inquiring mind of Dr. Garcia who had observed the pervasiveness of *talahib* grass even on the hostile soil and environment conditions. She wanted to find out the reason why the



Fig. 1. BIO-N microbial fertilizer

talahib could still grow normally in conditions where other agricultural crops would easily succumb. Dr. Garcia conducted a research study at the National Institute of Molecular Biology and Biotechnology (BIOTECH) to satisfy her curiosity and through years of meticulous work, she was able to isolate a species of nitrogen-fixing bacteria from *talahib* roots called *Azospirillum*. The bacterium has the capability to convert atmospheric nitrogen (N₂) into a form usable by the plants. These bacteria, once associated with roots of rice, corn, sugar cane, and some vegetable plants, can enhance root development, growth, and yield.

It is in powder form and prepared in a 200-gram packet, which could either be used as seed coating or used as a dilute solution for root dipping; or drenching already established young plants. One packet can be used to inoculate 3 kg of corn seeds or 20 kg of rice. Five packets of BIO-N can provide the needs of a one-hectare rice or corn plantation for nitrogenous fertilizer. Fields that have sufficient amounts of other elements respond dramatically to BIO-N. Best responses to inoculation may be obtained when ¼ to ½ of the recommended rate of combined nitrogen (bioorganic and inorganic) for the particular soil is applied. BIO-N can also be used where organic fertilizers are applied basally.

BIO-N enhances root development, growth and yield of the rice and corn and other vegetable crops, maintains the soil natural properties and fertility, and keeps plants healthy and green even during droughts and pest infestation.

BIO-N replaces 30-50% of the total nitrogen requirement of plants. Expenses for nitrogenous fertilizer of PhP 3,200.00 per hectare per cropping cycle are, therefore, cut down to about PhP 300 (Php 60 per pack x 5 packs per ha in lowland rice) per hectare per cropping cycle. The use of BIO-N allows one-time application of nitrogen fertilizer, thereby reducing labor cost to about 50% for the same area per cropping cycle.

Farmers in all regions in the Philippines (Regions 1 to 12) specifically those areas covered by the Department of Agriculture benefit from the use of BIO-N. Trainings on the preparation of the carrier of the BIO-N and on the application of BIO-N are provided to interested clients.

Title of Technology: Vesicular Arbuscular Mycorrhiza Root Inoculant (VAMRI)

(Mycorrhizal inoculant for agricultural and horticultural crops)

Date/Year of Development: 1994

Technology description:

Vesicular Arbuscular Mycorrhiza Root Inoculant or VAMRI (Fig. 2) is a chopped dried corn roots infected with arbuscular mycorrhizal fungus, either *Glomus mosseae* or *Glomus fasciculatum*. These two VAM fungi were chosen among several isolates collected from various localities in the country and 13 isolates purchased from Abbott laboratories. VAMRI serves as bio-fertilizers and bio-control agents of soil-borne diseases of different crops under various conditions. The mycorrhizal fungus has the ability to assist the plant roots in absorbing water and nutrients especially immobile elements such as phosphorus and zinc. They impart a degree of resistance or tolerance against soil-borne pathogens like nematodes, bacteria and fungi. Their interactions, however, vary depending on host cultivar, species of VAM and pathogens, inoculum densities and soil fertility. Thus, VAMRI can substantially reduce or substitute the chemical fertilizer and pesticide requirements of crops. This inoculant can be used for pepper, eggplant, tomato, papaya, banana, pineapple, watermelon, onion, corn, sugarcane, peanut, fruit crops/trees and ornamental plants.

VAMRI can be applied by seed pelleting or coating for direct seeding crops, by mixing with the sowing medium for transplanted crops, and by adding a pinch in potting medium for cuttings or seeds (ornamentals).

VAMRI can replace 50-100% of the plant's chemical fertilizer requirement and other



Fig. 2. VAMRI

amendments like compost depending on soil fertility status. All farmers can use VAMRI on agricultural crops such as vegetables, root crops, sugarcane, onion, fruit crops and ornamentals. VAMRI has been widely used by onion growers in Regions 1, 2 and 3 and sugarcane planters in Bacolod City.

Several trainings, seminars, workshops and on-farm demonstrations were conducted regarding the use of VAMRI. However, most of the trainees were either from academic or research institutions. Hands-on trainings for target clientele were limited due to budget constraints.

Many requests are being received for training on the mass production and handling of mycorrhizal inoculants. Trainors' training on village-level production of inoculants can be done on municipality level and upon requests at BIOTECH. The techniques employed for handling, quality control and maintenance of inoculants are being offered at BIOTECH.

Title of Technology: Brown Magic (inoculant for orchids)

Date/ Year of Development: 1997

Technology description:

Brown Magic (Fig. 3) is a mycorrhizal fungal inoculant that can be utilized as biological fertilizer and bio-control agent of root diseases of orchids. The fungus was chosen from 200 isolates composed of sclerotium or fruiting bodies of fungi and mycelia collected and isolated from orchid roots. Application of Brown Magic increases survival and growth of in- vitro cultured orchid and reduces transplant shock from in-vitro to in-vivo conditions; increases tolerance and resistance of plants against pathogens and diseases; induces early flowering and enhances the production of more suckers and longer spikes. This inoculant is



Fig. 3. Brown Magic

environment-friendly, economical and easy to use since application can be done only once either at transplanting from in vitro to in-vivo conditions or during composting or repotting period.

Brown Magic is being used in Cordillera Administrative region, Region III (Central Luzon) and in Region IV (Calabarzon). The inoculant is available at BIOTECH Sales office at UPLB.

Title of Technology: BioGroe (plant growth promoter)

Date/Year of Development: 2004

Technology Description:

BioGroe is a solid-based microbial plant growth promoter containing plant growth promoting rhizobacteria (PGPR). PGPR are root-associated bacteria, which influence root growth by producing plant hormones and provide nutrients in soluble form. PGPR can also protect plant surfaces from colonization by pathogenic microbes through direct competitive effects and production of antimicrobial compounds.

BioGroe is easy to use and environment-friendly. The benefits of BioGroe include enhanced root growth and development, increased productivity of crops, and reduced use of toxic or environmentally damaging chemical fertilizers and pesticides. The product can be used in vegetable production, propagation of ornamentals and plantation crops, rice and sugarcane production and in the growth enhancement of fruit and forest trees. BioGroe showed promising results on test crops in Bataan, Laguna, Tarlac and Palawan.

Training on the use of the plant growth promoter and market outlets can be held and established, respectively, in the different regions.

Title of Technology: MYKOVAM (Mycorrhizal inoculant for agricultural crops, fruit trees and forest trees)

Date/Year of Development: 1988

Technology description

Mykovam (Fig. 4) is a soil-based biological fertilizer containing spores, infected roots and propagules of beneficial mycorrhizal fungi called vesicular-arbuscular mycorrhizal fungi (VAM).



Fig. 4. Mykovam

These fungi when inoculated to seedlings will infect the roots, help in the absorption of water and nutrients particularly phosphorus, prevent root infection by pathogens and can increase plant tolerance to drought and heavy metals.

Mykovam is cheap and easy to use. A kilo of Mykovam can fertilize 200-400 plants. The application is once throughout the entire life of a plant. It replaces 60-85% of the plant's chemical fertilizer requirement, thus, farmers may increase their savings and income. Farmers can apply Mykovam on agricultural crops such as vegetables, root crops, fruit trees, forest trees except pines and dipterocarps, and ornamentals except orchids.

Title of Technology: NitroPlus (legume inoculant)

Date/Year of development: 1974 - 1980

Technology Description:

The high cost of chemical nitrogen (N) and the increasing environmental problems gave rise to the need to adapt new ways of supplying N to crops. NitroPlus (Fig. 5) consists of pure effective rhizobia grown in suitable carrier. It



Fig. 5. NitroPlus

comes in powdered form in 100-gram packets which can be used to coat seeds prior to sowing. NitroPlus was developed as a bio-fertilizer for legumes which will replace or supplement chemical N fertilizer.

The need to inoculate legumes has been established. A summary of the results of field inoculation trials in collaboration with various agencies showed that soybean frequently responded to inoculation with an average of 124% yield increase. Mungbean and peanut showed an average yield increase of 29% and 39%, respectively. The average yield of the inoculated treatments was more than one ton in all crops.

Legumes treated with NitroPlus yield as much if not more than legumes fertilized with three bags of ammonium sulfate. At 95% utilization of NitroPlus for soybean, mungbean and peanut production, about \$5 million worth of nitrogen fertilizer per cropping could be saved.

Legume growers will benefit a lot from this low-cost technology. A reduction in production cost will result in steady supply of legumes which in turn will benefit the food industry.

The use of NitroPlus increases crops yield and income. Without nitrogen fertilizer, the use of NitroPlus can increase soybean yield by 124%, mungbean by 29% and peanut by 37%. In a comparative analysis of the economic production of legumes with and without inoculation, NitroPlus in soybean increased net returns per hectare by 251%, from P2,602.40 to P9,198.00; in mungbean by 30%, from P2,806.67 to P3,874.18; and in peanut by 158%, from P3,064.00 to P7,898.75. The potential users are farmers and corporations who are producing legumes, the food industry who are producing legume-based products and the animal producers who use legumes as feeds.

NitroPlus are distributed to private sector, government agencies, cooperatives and non-government organizations. Among the private agencies, San Miguel Corporation, Nestle Philippines and STANFILCO are the biggest Nitro Plus consumers.

Title of Technology: Microbial Inoculants for the Bioconversion of Crop Residues and Agro-Industrial By-Products into Bio-fertilizers

Date/Year of Development: 1991

Technology Description:

This technology provides the microbial inoculants (i.e., Bioquick, Biofix) that promote the bioconversion of crop residues and agro-industrial by-products such as coconut coir dust, mud press, animal manure, and other similar materials, into refined inoculated compost or bioorganic fertilizers (i.e. Biogreen bioorganic fertilizer), which are proven effective for many field crops, horticultural crops, and fishponds.

The formulation of BioQuick, BioFix, and BioGreen (Fig. 6) is part of local innovations on preparation of microbial inoculants for organic matter degradation and soil nutrient enrichment. Data on substrate breakdown and microbial analysis have contributed towards a greater understanding of organic farming and optimum application of organic fertilizers.

Big potential users of these technologies are farmers of field crops such as rice, corn, vegetables; orchardists; fishpond operators; and other plantation owners. Many requests are being received for training at BIOTECH. Several market outlets are open to sell organic fertilizers and microbial inoculants.



Fig. 6. BioQuick, BioFix, and BioGreen



Fig. 7. Cocogro

Title of Technology: Cocogro (plant growth hormones from coconut water)

Technology description:

Cocogro (Fig. 7) is the pooled extract from coconut water which contains approximately 250-300 mg of plant growth hormones/regulators per liter of solution and is not susceptible to microbial action as in fresh coconut water. The product when diluted to 100-500 times with water and applied to seeds, seedlings and cuttings, enhances plant growth and development (root proliferation; stem elongation, flower induction) to 20– 40% more than untreated plants.

Huge volumes of coconut water are being disposed of due to the increase in virgin coconut oil production. A novel approach to get more income and utilize the waste coconut water into usable form is to extract expensive bio-chemicals such as growth hormones thereby reducing the amount of waste water to be disposed in rivers and streams. The extracted growth hormones will enable our country to use the cocogro for agriculture and high tech industries utilizing culture and cell formation. The additional commodities and growth hormones would have been added to the list of coco-based derived chemicals as a source of much needed revenues for the Philippines.

Potential users are tissue culture laboratories, vegetable/seed companies and ornamental plant growers.

Title of Technology: BioCon (a fungus-based wonder)

Technology Description:

BioCon is a product that helps in reducing the expenses on the use of inputs like chemical fertilizers and fungicides. It also mitigates the

effects of chemical fertilizers in the environment. Dr. Virginia Cuevas of the Institute of Biological Sciences, UPLB, screened three *Trichoderma* species (*T. parceramosum*, *T. pseudokoningii*, and a UV-irradiated strain of *T. harzianum*) from a collection that she gathered from different parts of the country. The genus *Trichoderma* is known worldwide as a fungus with high antagonistic properties against fungal pathogens. These properties were established in several laboratory and field experiments conducted in the Philippines and other countries against a wide range of soil-borne and air-borne plant pathogens of various crops. These include *Rhizoctonia*, *Sclerotium*, *Pythium*, *Phytophthora*, *Fusarium*, *Botrytis*, *Sclerotinia*, *Fulvia*, *Macrophomina* and many others. These species were cultured in an organic medium in order to produce BioCon that can be used both as a seed coating and as a soil inoculant.

As a seed coating, BioCon increases shoot and root growth, enhancing the absorption of mineral nutrients. BioCon helps supply the crops with both macro and micronutrients, bringing down the use of chemical fertilizer by as much as 50%. Despite reduction of the use of chemical fertilizer, users are assured that yield will still increase. Farmers must make sure to reduce the use of chemical fertilizer as the fungus will not do its job if chemicals are abundantly present in the soil.

The use of BioCon against damping off pathogens of vegetables was demonstrated in a farmers' field in Laguna province, Philippines. Improved *Trichoderma* pellets introduced into the soil at a rate of 100 g per m² at weeks before seed sowing in beds or direct seeding in the field, a much increase in dosage compared to that used in a previous work, were used. With these innovations, one-time soil introduction of the antagonist minimized activity of *Pythium* spp., *Sclerotium rolfisii* and *Rhizoctonia solani*, thus, damping off disease caused by these pathogens was effectively controlled. Soil population density of *Trichoderma* monitored six weeks after application was highly correlated with the increased percentage seed germination and percentage seedling survival of the test crop, *Brassica chinensis* (pechay).

Performance of BioCon was compared with that of the chemical fungicide mancozeb.

More seeds germinated in *Trichoderma*-treated plots and, consequently, more healthy seedlings were available for transfer to the field by using BioCon than by using chemical fungicide. Growth of *B. chinensis* and *Lycopersicon esculentum* (tomato) seedlings was significantly enhanced in seedbeds treated with *Trichoderma* pellets but not in mancozeb-treated seedbeds and the control. Field application in Benguet, a high-altitude province in northern Philippines, also showed growth enhancement in *Apium graveolens* (celery).

Solanum melongena (eggplant) grown directly in BioCon-treated plots produced more fruits that were bigger than those from the control plots and from plots sprayed with the chemical fungicide mancozeb. A significantly lower number of viable sclerotial bodies of *Rhizoctonia solani* were recovered in BioCon-treated plots compared with the other treatments. A lower incidence of the disease could be expected in the next cropping season. The use of BioCon pellets of *Trichoderma* did not only reduce the incidence of damping off but also enhanced growth and resulted in higher yield of crops (Cuevas *et. al.* 2005).

The use of BioCon is cost-effective. A 250 g bag of BioCon costs only P350, which is equivalent to 2-3 bags of chemical fertilizer (worth about P2,000 – P2,500) and allows the reduction of fertilizer use by 50%. An added value from BioCon is its ability to kill soil-borne pathogens such as damping-off diseases of seedlings, durian die back and corn sheath blight, reducing if not eliminating the use of chemical fungicides. BioCon may be used for any kind of crop including cereals, vegetables, ornamentals, or fruit crops.

In order to make BioCon available in the market, UPLB entered into a licensing agreement in 2004 with Tribio Technologies, Inc. for the latter to mass-produce and market the inoculant.

BIO-PESTICIDES

Bio-pesticides (also known as biological pesticides) are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. Bio-pesticides fall into three major categories: microbial, plant, and biochemical pesticides.

Microbial Pesticides

Microbial pesticides contain a microorganism (bacterium, fungus, virus, protozoan or alga) as the active ingredient. The most widely known microbial pesticides are varieties of the bacterium *Bacillus thuringiensis* Berliner, or Bt, which can control certain insects in cabbage, corn, potatoes, and other crops. Bt produces a protein that is harmful to specific insect pests. Certain other microbial pesticides act by out-competing pest organisms. Microbial pesticides need to be continuously monitored to ensure they do not become capable of harming non-target organisms, including humans.

Beauveria and *Metarhizium* against invasive pests

Fungi are important microorganisms causing mortality factors often causing drastic reduction in insect population. Gabriel (1968) recorded at least 102 entomopathogenic fungi, some species have been cited by other researchers including foreign scientists and many more await identification. It is quite important to evaluate their pathogenicity to wide array of highly destructive insect pests as a major component of integrated pest management system.

Beauveria bassiana (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sor. are fungi, which cause the disease, known as the white and green muscardine diseases, respectively in insects. When spores of these fungi come in contact with the cuticle of susceptible insects, they germinate and grow directly through the cuticle to the inner body of their host. The fungi proliferate throughout the insect's body, producing toxins and draining the insect of nutrients, eventually killing it. Once the fungi have killed their host, they grow back out through the softer portions of the cuticle, covering the insect with a layer of white/green mold. This downy mold produces millions of new infective spores that are released to the environment. A typical fungus isolate can attack a broad range of insects and various isolates differ in their host range.

M. anisopliae and *B. bassiana* are among the most frequently isolated fungi from insects and the most commonly used for bioassay in the Philippines. These fungi are easily isolated and cultured in ordinary rearing medium and had been evaluated at UPLB

against Asian corn borer, *Ostrinia furnacalis* (Guenee), June beetle, *Leucopholis irrorata* (Chevrolat), migratory locust, *Locusta migratoria manilensis* Meyen, Malayan rice black bug (RBB), *Scotinophara coarctata* (L.) and recently on coconut hispine beetle, *Brontispa longissima* (Gestro).

Several strains of the two fungi were assayed at the National Crop Protection against the larvae of *L. irrorata* and *B. bassiana* was found to cause higher grub mortality compared to grubs treated with *M. anisopliae* (Santiago, 1999). Another strain of *M. anisopliae* was tested against locust hoppers, *Locusta migratoria manilensis* Meyen attacking sugarcane in two farms in Murcia, Negros Occidental in Visayas (Santiago *et al.* 2001). A 95-98% locust mortality was observed with dead hoppers developing fungal growth, which is the cause of death. Most of the hoppers died between the 4th and 7th day after spraying indicating that *M. anisopliae* is a potential bio-control agent against migratory locust.

Fifteen strains of *M. anisopliae* were screened for effectiveness against the Malayan rice black bug (RBB), *Scotinophara coarctata* (L.), which is a highly invasive rice insect pest presently infesting rice in Mindanao and now with reports in Bicol Region (Santiago and Castillo, 2001). Eight strains were originally obtained from naturally infected RBBs found in rice fields while the remaining seven strains were from other insects from the soil. All strains from RBB, except two, were found to be highly effective against the adult RBBs. These strains killed 75 to 95% of the bugs in 12 days. The most effective ones killed 50% of the insects in less than seven days. None of the other strains (from other insects or from soil) killed more than 40% of RBBs in 12 days. These results seem to suggest that only those strains from RBB are capable of causing fatal disease in RBB while those from other sources are less capable. The implication is that for the purpose of developing *M. anisopliae* for RBB control, the strain for eventual commercialization should be selected from those obtained originally from the target insect. Santiago (2001) had both liquid and powder formulations of *M. anisopliae*, which was found effective against nymphs and adults of the invasive Malayan black bug.

Outside UPLB, Braza (1990) was able to isolate a strain of *M. anisopliae* from *L.*

irrorata, which caused 73% grub mortality. However, the fungus has a slower effect (mortality peaks occurred three weeks after fungus infection) compared to insecticides. *M. anisopliae* was also found effective *S. coarctata* (Arida *et al.*, 2006). It can also decimate cultures of both rice plant hoppers, *Nilaparvata lugens* Stal and leafhoppers, *Nephotetix* spp. (Rombach *et al.*, 1994; and Shepard *et al.*, 1987). In a study done at the Bureau of Plant Industry (BPI), an isolate of *M. anisopliae* from rice black bug caused 80% larval infection. *M. anisopliae* is already being mass produced for field evaluation and for actual field application against *Brontispa longissima* (Gestro). The researchers recommended the use of *Metarhizium* and *Beauveria* as an alternative to the use of expensive and hazardous chemical insecticides.

In addition, species of black earwig, *Chelosoichis morio* and *Euborellia annulata*, have been found associated with *B. longissima* and is currently being mass-produced and evaluated at the National Crop Protection Center.

A memorandum of understanding for the technical working group (TWG) was prepared to facilitate sharing of resources especially funds between PCA, DA, BPI and CPC, the partners in the project. Four CPC researchers will be involved in the project 2008-2009 project on the mass production and bio-efficacy trials of several biocon agents against *B. longissima*.

Plant parasitic nematodes particularly the root-knot nematode, *Meloidogyne* sp. has been the bane of many crop plants and ornamentals. *M. incognita* can cause 26.40% yield loss on Cavendish banana inoculated at 1,000 larvae per plant. Although nematode can be controlled by the application of nematicide, the innate problem related to the pollution hazards of chemicals has created an urgent shift for a non-chemical control measure. Biological control offers an alternative approach against plant parasitic nematodes.

The discovery and success of *Paecilomyces lilacinus* as efficient egg parasites of root-knot nematode, search for other potential biological control agents had intensified. Nematophagous fungi such as *M. anisopliae* and *Penicillium oxalicum* are also being developed for use against nematodes. Several studies revealed the potential of these

fungi as biological control agents. In the laboratory percent nematode parasitism was highest on plates grown with *P. oxalicum* (94.3%), followed by *P. lilacinus* (89.4%) and the least *M. anisopliae* (72%) (in tomato, eggplant and okra) (Zorilla and Castillo, 2005). The fungi were mass-produced in sterilized corn grits in flat bottles for three weeks in the laboratory.

Nuclear polyhedrosis virus (NPV) against common cutworm, Spodoptera litura (Fab.)

NPV kills cutworm by attacking the larvae. The larva ingests the virus by feeding on contaminated leaves or plant parts. The virus attacks and rearranges the cell structure of the caterpillar, forming crystals, which are, basically, inanimate and incapable of maintaining life. Infected caterpillars excrete these crystalline structures through their droppings or regurgitated fluid as a sign of stress. When another caterpillar eats any plant part that contains these crystals, the virus will multiply in all the internal organs and tissues of the insect, eventually killing the larvae. Since the digestive tract of the caterpillar is alkaline, it breaks down the crystals releasing the now active virus. However, the virus is safe to humans, including other mammals, birds, fishes and non-target insects because these are acid-based, hence, cannot mass-produce the virus. The virus is more effective than the use of conventional insecticides. Cutworms are hardy, plentiful and healthy. Aside from their nocturnal behavior (active during night time), it is quite difficult to hit the larvae with insecticidal sprays especially in vigorously growing plants.

In order for small farmers to benefit from promising technologies developed in the University, village-level mass production of NPV was initiated by Mr. Mario Navasero of NCPC, UPLB while doing research on the Integrated Pest Management (IPM) system for eggplant in 2000 in Asingan, Pangasinan, (Luzon, Philippines). Farmers through the leadership of Mr. Modesto Gabriel, are members of the Bantug Samahang Nayon Multi-Purpose Cooperative (BSNMPC), who are primarily planting eggplant. Unfortunately, they experienced heavy *S. litura* infestation, which attacked eggplant fruits and flowers. Farmers sprayed insecticides twice a week and even

sprayed a mixture or cocktail of pesticides everyday just to produce a marketable yield. However, due to continuous spraying, still a great proportion of the fruits were damaged by *S. litura*. Majority of the farmers abandoned their fields, while Mr. Gabriel, the chairman of BSNMPC began picking out the *S. litura* by hand but soon stopped this method since it was very tedious especially with the overlapping canopies of 1,300 eggplants in a 1,000 m² farm. Fortunately, Mr. Navasero noticed virus-infected cutworm larvae, which the group formulated and sprayed against the cutworm larvae. One week after the diluted mixture was sprayed on 13-week old eggplants, the destructive cutworms turned sluggish, their greenish brown skin faded to grey brown, and they stopped feeding. After about two to three days, the dreaded cutworms were miraculously being dying before their eyes until their population dropped – without chemical spray. What amazed the farmers more is that they can actually produce the virus – from the dead bodies of infected cutworms.

To produce NPV, Mr. Gabriel gathered 10 to 15 moribund and dead larvae that were still firm and even soft-ruptured larvae. Collected larvae were macerated using a stick to squeeze out the liquefied contents. About 30 to 50 ml of water was added and the mixture was stirred for a few minutes and placed in 16L capacity knapsack sprayer. Since NPV is quite sensitive to sunlight, he sprayed in the late afternoon, making sure to wet the surfaces of his eggplant leaves uniformly. One week after spraying, many cutworms died and their population continued to drop even without chemical spray. Infected larvae can be collected, macerated and the NPV suspension can be placed into screw-capped bottles and stored in the freezer of an ordinary refrigerator to prevent spoilage and to prolong the virus infectivity. The container should be labeled as to the number of infected larvae per bottle. Together with his entomologist wife, they trained the other members of the cooperative and the potential trainers, extending the technology to other interested farmers. To ensure that farmers would have enough host plants and cutworms for rearing, they provided mulberry cuttings as food for the cutworm, taught farmers the mass rearing of cutworms on soybean that facilitated advanced mass production of cutworm in the laboratory.

On the other hand, the farmers provided the rearing cages, some facilities and rearing media, and became responsible for their safekeeping in the cooperative. Because of the community's collective efforts, the BSNMPC members are now mass-producing and using NPV as a bio-pesticide in Barangay Bantug. They have also improvised methods to produce more NPV with the least cost, with host plants available in their locality, and with their existing resources.

The training has established the presence of the University in the province and earned the trust of the farmers. Mr. Navasero is collaborating with Drs. Pablito Gonzales and Rivaldo G. Bayot of NCPC on using another NPV against tomato fruit worm, *Helicoverpa armigera* (Hubner). Dr. Pio Javier, also of NCPC, had already extended the technology on the mass rearing and utilization of earwig, *Euborellia annulata* (Fab.) against corn and eggplant insect pests, which the cooperative is already mass-producing against insect pests of corn and vegetables.

***Bacillus thuringiensis* Berliner**

BIOTECH researchers have found out that microorganisms can also control pests and diseases. *B. thuringiensis* or Bt is a bacterium that can produce toxins that control specific insects that affect plants. When ingested by insects, the toxins affect their digestive system leading to starvation and death. They have produced a commercial formulation of Bt called Bactrolep, which is effective against Asiatic corn borer and diamondback moth in crucifers. It is specific by killing only its target pests. Thus, it is safe to human beings and animals.

Plant Pesticides

Plant pesticides are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, scientists can take the gene for the Bt pesticidal protein, and introduce the gene into the plant's own genetic material. Then the plant, instead of the *Bt* bacterium, manufactures the substance that destroys the pest. Both the protein and its genetic material are regulated by EPA but the plant itself is not regulated.

Bt-Corn

The Philippines Department of Agriculture (DA) approved the first Bt pesticidal protein, the Bt corn for propagation and import for direct use in December 2002 after almost six years of trial and safety evaluation. Bt or *Bacillus thuringiensis* is a common soil bacterium that produces its own insecticidal protein. Bt corn is a variety of corn where a specific Bt gene is inserted to produce a protein that protects the corn plant from feeding by Asiatic corn borer (ACB). This makes the corn plant naturally resistant to attack by ACB. The Bt protein controls insects by disrupting the insect's digestive system. Once inside the alkaline gut of the target insect, the Bt protein is activated, and binds to specific receptors. This mid-gut is punctured leaving the insect unable to eat. Within a few days, the insect dies. Since Bt corn is very specific, it would not harm man. The Bt protein will only affect an organism with specific receptor sites in its alkaline gut where the proteins can bind. Human beings and many insects lack these receptors. Besides, the stomach of humans is acidic.

Results of the field trials of Bt corn in the Philippines are very promising. Harvests from Bt corn were found to be about 37% higher than non-Bt counterparts. The computed yield increase between Bt and non-Bt corn ranged from 1.6 to 3.4 tons/ha. This translates to an additional profit of P10,132 per hectare with a reduction in insecticide expenditures of 60%. An incremental net income of P1.34 per kilogram was gained by the Bt-corn users, although the seed cost was twice the ordinary hybrid. After one year of commercialization, the net benefits to farmers in the aggregate amounted to P46.44 million. This was estimated using the area planted to Bt-corn and the reduction in per unit costs (Yarobe 2005).

Increased yield and decreased pesticide cost contribute to a higher net return for Bt corn farmers. It can play an important role in making agriculture, particularly corn, more sustainable and more productive. Since Bt corn no longer requires insecticide application for corn borer, farmers will have more time for other farm management duties. The Philippines imports an average of 300,000 to 500,000 metric tons of corn annually. Increased production of yellow corn can reduce dependency on corn importation.

Another benefit from using Bt corn is

the marked improvements in grain quality through significant reductions in the levels of mycotoxins found in the grain. This delivers a health benefit to the livestock sector that mostly consumes the corn.

The Department of Agriculture has always been vigilant in assuring the entry of safe food for the Filipino consumer. To date, it has no basis to declare Bt corn unsafe. As a policy, the department encourages further studies to provide science-based support to different claims.

Area planted with Bt corn increased from about 120 hectares in 2002 to about 52,000 hectares in 2005 with minimal increase in 2006.

Other Bt crops being worked out in the Philippines include cotton, rice, eggplant, tomato and papaya.

Virus against Papaya Ringspot Disease

The candidate lines of the Papaya Ringspot Virus-Resistant (PRSV-R) papaya are now in their confined trial site. The planting of the first perennial transgenic crop in the Philippines was conducted last February 23, 2007 at the Crop Science Cluster, Institute of Plant Breeding (CSC-IPB), College of Agriculture, UPLB (CA-UPLB). This milestone of a Philippine government-initiated project was carried out after the National Committee on Biosafety of the Philippines (NCBP) formally approved the confined trial through a letter dated February 12, 2007.

Aside from the project leaders and staff, those who attended the planning activity were regulators from the Bureau of Plant Industry-Plant Quarantine Section (BPI-PQS), a representative from the Department of Environment and Natural Resources to the NCBP and a member of the UPLB-Institutional Biosafety Committee (UPLB-IBC). The presence of these respective authorities ensures that the activity is done in accordance to the prescribed regulations of the NCBP.

This confined trial is a research activity, which is a part of the process on the development of the transgenic resistant line. The objective of the confined trial is for disease evaluation screening for resistance to PRSV of the candidate lines. Three candidate T3 PRSV resistant lines were planted plus the control. In total, 135 inoculated T3 seedlings

plus 45 uninoculated and 45 inoculated 'Davao Solo' seedlings were planted. Aside from these, 64 uninoculated 'Davao Solo' seedlings were also transplanted as border plants.

The project is being funded by the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD), Agricultural Biotechnology Support Project (ABSP II), Economic Modernization Through Efficient Reforms and Governance Enhancement (EMERGE) and the International Service for the Acquisition of Agri-biotech Applications (ISAAA) (Lawas and Magdalita, 2007).

Biochemical Pesticides

Biochemical pesticides are naturally-occurring substances that control pests by non-toxic mechanisms. Biochemical pesticides include substances that interfere with growth or mating, such as plant growth regulators, or substances that repel or attract pests, such as pheromones.

The adult mango pulp weevil, *Sternochetus frigidus* (Fab.) has been found feeding on mango flowers with peak of activity observed at 0600-1000 h (de Jesus *et al.* 2003). Weevils are known to imbibe floral secretions including nectar and eat pollen and other floral parts. Nectar contains a mixture of three sugars (glucose, fructose and sucrose) that are digestible to insects (Mitchell 1981).

At full-bloom stage, about 138 volatiles chemicals that are emitted by mango (*Mangifera indica*) flowers were identified by gas chromatography-mass spectrometry (GC-MS). Olfactory attraction of virgin female weevils to 30 single components and blends of selected chemicals emitted by mango flowers were tested in Y-tube bioassays. Acetic acid and decane, in concentrations equivalent to 10 panicles, significantly elicited responses at 66.7% when tested separately. A blend of these components resulted in weaker response from the weevils. Further bioassays led to the identification of a six-component blend that was 70% attractive to the mango pulp weevil. This blend consisted of acetic acid, decane, acetone, linalool, ethyl benzoate and 2-methyl heptenone. Results of the study provide the first evidence for mango pulp weevil attraction to specific compounds present in its mango host.

Plants possess chemical compounds called plant odors and have the ability to release these odors in minute quantities in surrounding air. These emitted odor substances are used by plant feeding insects as cue that could guide them to locate these plants that they perceived as suitable food or suitable sites to lay their eggs or brood. For example, corn plants that are damaged by insects or even those that are mechanically injured emit more different odors than those that are healthy and uninjured and consequently harbored different levels of corn borer populations. This project was conducted to look into the interaction and influence of plant odors present over the headspace of a mixed (herb/vegetable) cropping system on pest infestation level.

Studies along this line had been started at NCPC by Dr. S.M.F. Calumpang. Historically, plants are source of botanical pesticides or bio-pesticides which have been used in many parts of the world especially in the Asian and African countries before 1945. With the advent of synthetic pesticides, which are quite effective, cheap, easy to procure and to apply, the use of bio-pesticides was neglected. However, the unilateral use of pesticides, led to undesirable side effects, such as toxicity to non-target organisms, appearance of pesticide-resistant pests and environmental contamination and more recently the search for organically-grown crops, led to the revival of the search and evaluation of potential bio-pesticides.

The Philippines is endowed with a vast array of plants as an endless source of ecologically sound (biodegradable) and safer pest control agents which can act against pests but spare predators, parasites and other non-target organisms and of low mammalian toxicity. More than 2,000 plant species have been reported or claimed to possess pesticidal activity (Grainge and Ahmed 1988). In the Philippines, plants could also be an abundant source of active compound, which could act as insecticides, fungicides, molluscicides, nematocides, herbicides, etc. These compounds may act as toxicant and/or may influence the growth, development, behavior, reproduction and fitness of the pests in various ways. The utilization of plant-derived insecticides could be the possible solution to the problems associated with synthetic pesticides. Some of these botanicals are also known as insect growth regulator while others act as feeding

and egg laying deterrents, and sterilants.

Insecticidal plants

Bulk of the studies on plants with insecticidal properties was done by Dr. Belen Morallo-Rejesus, professor emeritus of the Crop Protection Cluster (formerly Department of Entomology). As of 1985, about 100 plants were reported to be insecticidal, with not much significant recent increase in the report. However, these claims are not properly substantiated by entomological bioassay and some studies are not experimentally valid. Some studies used very high dosages, field-collected insects of varying ages and stages, small test samples and no control (Morally-Rejesus, 1985). Still, these claims are quite useful in the preliminary survey of plants with insecticidal activity and could be assayed against insects where they are reported effective.

Out of 46 plants surveyed for insecticidal activity, Morally-Rejesus (1984) reported 34 plants to be insecticidal (supported with at least a crude assay on one or several insects). Twenty-seven plants were found toxic to at least one insect species. Most of the insect species used for bioassay were stored product pests (red flour beetle, corn weevil, lesser grain borer, saw-toothed grain beetle, cowpea bean weevil, rice moth and fig moth), household pests (cockroach, housefly, mosquito), and at least 10 species of agricultural pests.

The three most promising bio-pesticides are the makabuhai (*Tinospora rumphii* Boerl.), which is effective against green leafhopper and brown planthoppers (Leonardo 1983); marigold (*Tagetes erecta* and *T. patula*) against diamondback moth and green peach aphids (Morally-Rejesus and Eroles, 1978; Morally-Rejesus and Decena 1982) and black pepper (*Piper nigrum*) against diamondback moth larvae, adult housefly, cotton stainer, corn weevil and black armyworm (Javier and Morally-Rejesus 1986). The other promising biocides include oregano (*Coleus amboinicus*), sambong (*Blumia balsamifera*), *Ceasalfinia pulcherima* L., wild sunflower (*Tithonia diversifolia*), lantana (*Lantana camara*), malaubi (*Aristolochia tagala*) and timbangan (*A. tagala*) (Morally-Rejesus 1986).

The insecticidal action of water and

organic solvent extracts of 10 plants – makabuhai, “luyang dilaw” (*Curcuma longa* L.), lagundi [*Vitex negundo* (L.)], “lagunding dagat” [*Vitex trifolia* (L.) Van Steem], wild sunflower, derris [*Derris elliptica* (Rosch.) Benth], “kakawate” (*Gliricidia sepium* Jacq.), “hagonoy” [*Chromolaena odorata* (L.) R. M. King & H. Robin], “alagaw-gubat” (*Premna nauseosa* Blco.) and “alagaw” (*Premna odorata* Blco.) were evaluated against brown planthopper (*Nilaparvata lugens* Stal), green leafhopper (*Nephotettix* sp.), diamondback moth (DBM) (*Plutella xylostella* L.) and Asian corn borer [*Ostrinia furnacalis* (Guenee)]. Water extracts of “makabuhai” showed systemic and ovicidal toxicities and growth inhibitory effect (IGR). “Lagundi” and wild sunflower showed contact and ovicidal toxicities and IGR effect. *Derris* sp. and “luyang dilaw” showed contact and ovicidal toxicities (Morallo-Rejesus *et. al.* 1993).

The insecticidal activity of diosgenin isolated from the rhizomes of three species of grape ginger (*Costus speciosus*, *C. lacerus* and *C. globosus*) on DBM was determined. Diosgenin had a low contact and ovicidal activity but a potent insect growth regular and high oviposition deterrent activity. It caused larval and pupal deformities, reduced fecundity and partial sterility. Based on the ED₅₀ (effective dose that produces 50% abnormal test insects), crude diosgenin was more active than purified diosgenin. The DBM parasitoid, *Cotesia plutellae*, was 14x less sensitive to diosgenin compared to DBM suggesting that it is safe to the parasitoid (Pipithsangchan and Morallo-Rejesus 2005).

Fungicidal plants

A total of 169 plants have been surveyed for fungicidal and bactericidal properties (Dumancas, 1976; Pordesimo and Ilag 1976; Lapis and Dumancas 1978; Mendosa 1979; Quebral 1981; Maroon 1983; Agbagala 1984; Franje 1984; and Castro 1986). Of the 98 plants bioassayed for fungicidal activity against three common rice pathogens, 42 plants were inhibitory to *Helminthosporium oryzae*, 22 to *Pyricularia oryzae* and five to *Rhizoctonia solani*. The plants showing fungicidal activity in at least three fungal pathogens were summarized in Table 1. The spraying of

Tagetes erecta extracts effectively controlled *H. oryzae*.

In another study, 23 plants were evaluated on nine common fungal pathogens, where eight were found inhibitory to *Alternaria* sp., 12 to *Cercospora*, seven to *Colletotrichum* sp., four to *Curvularia* sp., seven to *Diplodia*, three to *Fusarium* sp. of okra, four to *Fusarium* sp. of soybean, six to *Helminthosporium* sp. and seven to *Pistotia* sp.

Franje (1984) found that 21 plants showed bactericidal activity against *Xanthomonas phaseoli* var. *sojensis* (Table 2). Two plants, *Allium sativum* and *Pseudocalumna* were found inhibitory to the growth of both *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare (Castro 1986).

Nematicidal plants

The symptoms of plants infected with nematodes are galling and rotting of roots, wilting and yellowing of leaves as well as necrotic lesion in roots and excessive root branching. Root extract of 17 plant species were evaluated against *Meloidogyne incognita* (Li Thi Hoan and Davide 1979). Extracts of 14 plant species suppressed egg hatching and 12 plants reduced nematode infection by 95-100%. This is comparable to the effect of DBCP (Fumazone 75 EC) at 300 ppm. Sarra-Guzman screened solvent extracts of eight plant species for toxicity against *M. incognita* and *Radophalus similis* Caff. The plant species were: *Antiocephalus chinensis* (Lamb) Rich ex Walp, *Desmonium zangelicum* (Linn.) DC, *Artemisia vulgaris* Linn. *Eicornia crassipes* (Marf.) Solm, *Leucaena leucocephala* (Lam) de Wit, *Allium cepa*, *Allium sativum* and *Moringa eleifera* Lam. The extracts showed no definite indication of toxicity in emission bioassay and pathogenecity tests. The purified fractions of *A. cepa*, *A. chinensis* and *E. crassipes* showed high toxicity to the two test nematodes.

Marigold (*Tagetes* spp.) secretes toxic metabolites, alphetathienyl which prevent hatching and larval development of rot-nematodes. Salay or tanglad (Lemon grass, *Cymbogon winterianus*), herbabuena (*Mentha arvensis* L.) and sambong (*Blumea balsamifera* L.) were also not infected by RKNs as evidenced by the root-galling indices, mean number of nematodes per 2g stained roots and

Table 1. List of plants having fungicidal property against at least three fungal pathogens (Lapis and Dumanacas 1978; Franje 1984; and Agbagala 1984).

Botanical Name/ Local/ Common Name	<i>Helminthosporium oryzae</i>	<i>Pyricularia oryzae</i>	<i>Rhizoctonia solani</i>	<i>Collatotrichum Lindemuthianum</i>	<i>Cercospora cruenta</i>
<i>Allium sativum</i> L. Garlic	+	+	+	+	+
<i>Pseudocalymna</i>	+	+	-	+	+
<i>alliaceum</i> (L.) Sandwizt/Garlic vine					
<i>Impatiens balsamina</i> L. Kamantigue	+	+	+	+	+
<i>Euphorbia pucherrima</i>	+	+	+	-	+
Willd./Poinsettia					
<i>Tagetes erecta</i> L. Marigold	+	+	+	-	+
<i>Amaranthus spinosus</i> L. Uray	+	-	-	+	+
<i>Averrhoa bilimbi</i> L. Kamias	+	+	+	-	-
<i>Symphutum officinale</i> Comfrey	+	+	-	-	+
<i>Zingiber officinale</i> Rox Ginger	+	-	-	+	+

mean number of nematodes recovered per 300g soil. These plants could be used as rotation crop or as intercrop in pechay and eggplant fields to effectively reduce the RKN population (Zorilla *et. al.* 2005).

Molluscicidal plants

After its introduction in 1982, about 130,946 hectares lowland rice field had been damaged by the golden apple snail (*Pomacea caniculata*, *P. gigas*, *P. cuprina*) in 1988, making its as the number one pest of transplanted lowland rice in the Philippines. Farmers relied mostly on the use of synthetic molluscicides for its immediate control. However, some of the most popular molluscicides used at that time were the phenyl tin acetate (Brestan) and other organotin, which had several side-effects and were quite expensive. Consequently, research on the use of potential bio-pesticides as an alternative to snail control was initiated.

Seventeen volatile oils were evaluated for

their biotoxicity to golden snails in the concentration range of 1-100 ppm (Maini and Morallo-Rejesus 1992). Oils of sassafras, star anise and oregano showed 100% snail mortality at 10 and 20 ppm against young snails with operculum diameter of 6 mm and mixed population with 6-18 mm operculum diameter, respectively. Oils of lagundi, bitter almond and fennel were observed to be non-toxic to snails at 100 ppm. Isolation of the active constituents of these biologically active oils can be a new source of a molluscicide for golden snail control in the Philippines.

In 1997, Morallo-Rejesus and Punzalan evaluated a total of 119 water extracts and eight volatile oils from 91 plants for molluscicidal activity against the golden snail. Based on the concentrations that caused 80-100% mortality at 48 hours after treatment (HAT), 16 extracts exhibited very high toxicity (200 ppm and below), 16 of high toxicity (>200 -1,000 ppm), 8 of moderate toxicity (>1,000-5,000 ppm) , 40 of low toxicity (>5,000- 10,000

Table 2. List of plant materials showing bactericidal activity against *Xanthomonas phaseoli* var. *sojensis* based on zone of inhibition measured at two days after seeding.

Botanical name	Common Name or Local name
<i>Allium sativum</i> L.	Garlic
<i>Bidens pilosa</i> L.	Burbutak
<i>Plumiera acuminata</i> Alt.	Kalachuchi
<i>Impatiens balsamina</i> L.	Kamantigue
<i>Pseudocalymna alliaceum</i> (Lam)Sandwitz	Garlic vine
<i>Vitex negundo</i> L.	Lagundi
<i>Stachytarpheta jamaicensis</i> Vahl.	Candi-candilaan
<i>Datura metel</i> L.	Talong-punai
<i>Mentha arvensis</i> L.	Herba-buena
<i>Coleus amboinicus</i> Lour.	Oregano
<i>Lantana camara</i> L.	Wild lantana
<i>Andrographis paniculata</i> L.	Sinta
<i>Phyllanthus reticulatus</i> L.	Malinta
<i>Cassia alata</i> L.	Acapulco
<i>Carica papaya</i> L.	Papaya
<i>Imperata cylindrica</i> L.	Cogon
<i>Amaranthus spinosus</i> L.	Uray
<i>Mikania cordata</i> (Bum.) B. L. Robinson	Bicas
<i>Ageratum conyzoides</i> L.	Bulak-manok
<i>Moringa eleifera</i> L.	Malunggay
<i>Allium cepa</i> L.	Onion

ppm) and 58 were inactive (>10,000 ppm). In most cases, the oils were very highly toxic (VHT) at 200 ppm. Based on LC₁₀₀, the order of decreasing toxicity of the volatile oils is: *curcuma longa* > *Blumea balsamifera* > *C. zedoaria* = *Ocimum sanctum* > *cinnamomum mercadoi* > *Zingiber officinale* > *Vitex negundo* > *Azadirachta indica*.

Herbicidal plants

Biological control of weeds generally rely on allelopathy, which is any direct or indirect harmful effect of one plant including microorganisms, on another through the production of chemical compounds that escape into the environment (Rice 1974). Robles (1985) listed 13 major weeds in the Philippines that possess allelopathic properties. The three most studied weeds species include *Rotboellia cochinchinensis*, *Imperata cylindrica* L. (Payawan 1968) and *Pistia stratiotes* L. The water-soluble substances from *R.*

cochinchinensis are found to be inhibitory to *Ipomoea triloba* and *Mimosa pudica* (Payawan 1968; Pamplona, 1973; Mercado and Manuel 1977). The ethyl-soluble substance from the roots of *P. stratiotes* was found to be inhibitory to *R. cochinchinensis* (Mercado and Robles 1982). Advances in the biological control of weeds in the Philippines are quite slow due to the presence of effective and broad spectrum synthetic herbicides.

In broader sense, bio-pesticides may also include living organisms such as insect predators and parasitoids. These biological control agents, which are the major components of pest management, will also be discussed in this paper.

Parasitoids are insects that usually



Fig. 8. Trichogramma adult parasitizing an egg mass.

attack or lay their eggs on eggs or larvae of their host. Parasitoid generally belongs to the order Hymenoptera (wasp) and Diptera (flies). It feeds internally (endoparasitoid) or externally (ectoparasitoid) on other insect's body; it is usually smaller than the host, requires single host to complete its life cycle and always kills the host.

Trichogramma (Fig. 8) is the most widely-used parasitoid against eggs of most lepidopteran (moth) insect pests in the Philippines. It can be considered a breakthrough in the suppression of host insects because the pest is controlled while in the egg stage. *Trichogramma* wasps thrive by laying their eggs inside the eggs of its host. The eggs of *Trichogramma* hatched into larvae, which slowly consume the contents of the host egg. *Trichogramma* could complete its development on eggs of the host in 7-9 days. Examples of *Trichogramma* parasitoids are *T. chilonis*, which parasitizes corn earworm, leaf folder, fruit borer, sugarcane borers, *T. evanescens* against Asian corn borer; *T. japonicum* against rice stem borers and *Trichogrammatoidea cojuancoi* against cacao pod borer.

In sugarcane where the dominant insect pests are the sugarcane borers, the major line of defense employed by growers is with the field releases with *T. chilonis*. Since the parasitoid is very effective against the sugarcane borers, seven *Trichogramma* rearing laboratories were constructed by PHILSURIN, and the parasitoids are given free to growers who are MDDC members (Javier and Gonzales 2000).

Trichogramma can be efficiently mass-produced in the laboratory using eggs of rice moth, *Corcyra cephalonica* Stainton as unnatural host (Gonzales and Javier 2000).

In crucifers, the diamondback moth (DBM), *Plutella xylostella* L. has been the most destructive, which can cause 100% yield loss if effective control is not implemented. To control DBM, farmers commonly use highly toxic and broad spectrum synthetic insecticides. However, the high dosage and continuous application of insecticides do not totally control DBM. The insect develops resistance to practically all insecticides used at an exceptionally very short period, which consequently increased the production cost

and aggravated the hazards to man's health and the environment from the residues of these chemicals.

The IPM to manage DBM involves the field release of parasitoids (*Cotesia plutellae* and *Diadegma semiclausum*) supplemented with the spraying of microbial insecticide (*Bacillus thuringiensis*) based on the economic threshold level (ETL) (Morillo-Rejesus and Sayaboc 1992). The ETL from seedling up to mid-vegetative stage is two larvae per plant and five larvae per plant from pre-heading stage until the crop is ready for harvest. *Cotesia* and *Diadegma* prefer to parasitize the second instar DBM larvae and sustain therein new eggs of *Cotesia/Diadegma*. The DBM larva will eventually die and a new cocoon of *Cotesia* or *Diadegma* will develop and emerge as adult. An adult *Cotesia* or *Diadegma* is capable of parasitizing 450 DBM larvae. In this proportion, the growth of DBM population is easily controlled or destroyed. Thereafter, the population of *Cotesia* or *Diadegma* will increase. They will survive or remain in the field from 12-13 days. Adoption of the technology may reduce the frequent and costly spraying of chemicals from 15-30 times to 4-8 only. Crop yield may increase to 75%, whereas income may increase to 40%. The technology minimizes hazards on man's health from chemical residues on the vegetables and the environment.

Predators are biological control agents that are usually larger than their prey and require several preys to complete their life cycle. One of the common predators, which are commonly used in the field is the earwig, *Euborellia annulata* (Fab.) (Fig. 9) and *Proreus simulans* (Stal). These earwig predators feed on egg masses, young larvae and pupae of lepidoptera, coleopteran diptera and on soft bodied insects like aphids, scale insects, mealybugs, etc. In 2000, Morillo-Rejesus *et. al.* developed the technology on



Fig. 9. *Euborellia annulata*.

the mass rearing of *E. annulata* using dog food as its food in the laboratory. At present, earwigs are being mass reared on fish meal, which is much cheaper than the use of dog food (Javier *et. al.* 2007). The earwigs are released in cornfields at the rate of 10,000 individuals per hectare per release. They are released at the base of the corn plant at about 25 and 32 days after planting.

The field releases of earwigs against ACB increased farmers' income by 25% in green corn and 10% in open-pollinated variety as compared with the application of synthetic insecticides.

Since it is quite easy to mass rear the predator, the mass production technology had already been disseminated to the staff of the Regional Crop Protection Centers and the Department of Agriculture – Regional Field Units (DA-RFU's). There are reports that *E. annulata* is released not only in corn but also in eggplant and legumes, which gave positive results. Preliminary investigations at UPLB revealed that earwigs are also effective in controlling insect pests of eggplant and legumes. However, the plants should be mulched since the predators prefer moist conditions and they generally stay in the soil during daytime and search for their prey during nighttime (Javier and Navasero 2005).

The flower bug, *Orius tantillus* (Motschulsky) is another dominant and effective predator encountered in corn agroecosystem feeding on eggs masses and larvae of ACB, earworm and other lepidopterous pests (Javier and Morallo-Rejesus 2003). In cornfields, the population of this predator could be conserved by maintaining/

planting the weed species, spiny amaranth, *Amaranthus spinosus*, which served as its refuge. Higher population of *Orius* and spiders were monitored in cornfields, where *A. spinosus* are maintained along the borders (Table 3).

O. tantillus has been extensively studied as predator of *Thrips palmi* Karny (Mituda and Calilung 1989; Calilung 1995; Calilung *et al.* 1998; Navasero 1996; and Navasero and Calilung 1997). Although the predator could be mass-reared in the laboratory, rearing is quite difficult and expensive. Therefore, field rearing on *A. spinosus* seemed more practical (Navasero and Rejesus 2000). A good stand of *A. spinosus* can support the development of as many as 150 nymphs and adult *Orius* per plant. An initial plot size of 100m², high density planting of *A. spinosus* at 1 x 0.5 m planting distance is sufficient for a start. At the onset of flowering, adult *Orius* that are initially reared in the laboratory or stocks from inflorescence of volunteer plants near the corn fields are collected and released in 100 m² *A. spinosus*.

Predatory mites

Research on the biological control of mites is focused on the family Phytoseiidae, since they are the most effective and widespread predators of injurious plant-feeding mites. A number of species are now used as biological control agents and several have attained commercial status abroad. However, in the Philippines, phytoseiids or any other group of predatory mites, are still underutilized and little is known about their biology.

Amblyseius longispinosus (Evans) is one of the most common Phytoseiid mites in the Philippines, which has been reported on wide range of fruit crops, field crops and ornamentals (Corpuz-Raros 1989; and Schicha and Corpuz-Raros 1992). The effectiveness of *A. longispinosus* as natural enemy of various plant-feeding mites has been evaluated by a number of researchers worldwide. In the Philippines, it has received some research attention as an important biological control agent against *Tetranychus kanzawai* (Kishida), an important spider mite attacking cassava. Current effort is directed toward developing a mass rearing technique with long-term goal of providing end-users with locally available, effective and cheap predatory mites for the

Table 3. Influence of maintaining *A. spinosus* on the population of *Orius* and spider in corn (Javier and Morallo-Rejesus 2003).

Crop Weed Combination	# Orius	# Spiders
Corn +	134	23
<i>Amaranthus</i>	119	98
Corn alone	70	21

control of various plant feeding mites (de Leon-Facundo and Corpuz-Raros 2005).

OPPORTUNITIES FOR TRAINING OR MARKET OUTLETS OF BIO-FERTILIZERS AND BIO-PESTICIDES

Several training courses and workshops have already been conducted to disseminate and transfer the biofertilizer technologies to target clientele. Training workshops started in 1988. Initially the topics include basic techniques on isolation and identification of potential microbial-based fertilizers. At present, topics include the handling, quality control and on-farm application of the different inoculants. Trainings, lectures and seminars are conducted upon request to the director.

The different products are being sold at BIOTECH Sales office but for market outlets, anybody can enter into a distributor agreement with UPLB.

Among the different bio-fertilizers, BIO-N is so far the most popular, and one of the most effective technologies UPLB has produced, a fertilizer that has outshone all other organic fertilizers in the country and has given multinational companies selling inorganic fertilizers a run for their money.

However, during its infancy in 1985, BIO-N did not get much attention as a technology. It was only through a series of demonstration trials conducted by BIOTECH through Dr. Mercedes U. Garcia (developer of BIO-N) especially in Laguna, Quezon, Rizal and Isabela and by a private individual in Cebu that BIO-N became popular.

To encourage extensive support and patronage of BIO-N by farmers even in marginal and remote areas, radio plugs and TV interview were produced and broadcast in radio and TV stations. The team also intensified information dissemination through national and local newspapers. As a result of the team's hard work, collaboration and linkages were established with different government and non-government organizations.

With BIO-N already a proven technology, the task of persuading farmers of its benefits rests on a team that is overseeing its dissemination. The team got enormous support of a Php11.2 M grant from the Department of Agriculture to implement the "National Program on the Production and

Utilization of BIO-N for Sustainable Production of Agricultural Crops" in 2001. Extension activities were strengthened so that farmers may benefit from BIO-N. Mixing plants for BIO-N were also established in different regions in the Philippines to meet farmers' demand for the product.

Because of the sustained interest and the growing demand for BIO-N, UPLB through BIOTECH has entered into agreements in 2004 with various agencies that would help make the technology easily available in the provinces. UPLB entered into a joint venture with the Technology Livelihood and Resource Center (TLRC) to improve BIO-N production facilities. The project had generated substantial revenues to profitably sustain its business mandate. Due to positive feedback and success stories of farmers of the DA's Ginintuang Masaganang Ani (GMA) corn program, Dr. Artemio Salazar provided funds to the different corn producing regions to finance the establishment of 32 BIO-N mixing plants. A total of ten mixing plants were operating in strategic corn cluster areas. The primary objective is to ensure the steady supply of BIO-N and reduce the production cost incurred by corn farmers. UPLB also entered into an agreement with SUMMA Biotechnologies Corporation allowing the latter to produce, market and distribute BIO-N in Agusan del Norte, Caraga Region and Region 10 within ten years.

In 2005, the team of Dr. Garcia had trained 1,385 model farmers, agricultural technicians and development managers from different regions. After the training, they were given free BIO-N for demonstration trials in their own farms.

A private company is already commercially producing BIO-N as "Vital-N", and aggressively promoting the product. What is important is that thousands of marginal farmers have realized that BIO-N reduces farm costs and it enhances sustainable agricultural practices.

The experiences in developing BIO-N, the strategies and approaches and success in the promotion and acceptance of the technology are worthwhile looking into.

The effectiveness of NPV and Bt isolates had been fully documented by Dr. Leodegario Padua of BIOTECH, UPLB. In fact, several

companies are eyeing to commercialize the product but based on the market demand, commercialization is generally not feasible.

A fungal pathogen found highly effective against parasitic nematode was commercialized by a multinational company. Unfortunately, there were some technical problems concerning intellectual property rights (IPR).

The active principles responsible for the insecticidal activity of at least three botanical pesticides had already been identified but not one had been registered with the Fertilizer and Pesticide Authority (FPA) of the Philippines. The high cost of labor coupled with the regulatory constraints and the problems with formulations and marketing slowed down the advancement of bio-pesticides in the Philippines.

CONCLUSION

There is very bright prospect on the use of bio-fertilizers since it is very cheap, easy to use, safe and do not require repeated applications. The single application of some of the bio-fertilizers can substitute about 30 to 100% of the inorganic fertilizer requirements of the crop. In addition, these bio-fertilizers enhance plant growth rendering them tolerant to pests and some are indeed being used as bio-pesticides. With the exception of Bio-N, the major limitation with majority of these bio-fertilizers is their availability outside UPLB. Registration of these bio-fertilizers with the Fertilizer and Pesticide Authority (FPA) will not be as rigid as the bio-pesticides.

In general, biopesticides such as the *M. anisopliae*, *B. bassiana*, *P. lilacinus* and *Bacillus thuringiensis* and even the use of parasites and predators are still unpopular to our small farmers. On the other hand, plants as biopesticides will face stiff competition with the fast-acting synthetic pesticides. However, since the cost of synthetic pesticides is highly prohibitive nowadays, coupled with its unwanted side-effects, the use of bio-pesticides will be a welcome alternative to the use of chemicals. The area-wide utilization of bio-pesticides could be enhanced if village-level mass production and utilization of this technology will be aggressively extended to

our farmers by the local government units through organized integrated pest management programs.

REFERENCES

- Agbagala, M.L.U. 1984. Assay of fungicidal activity of medicinal plants against some common fungal pathogens of rice (*O. sativa* Linn.). B.S. Thesis, University of the Philippines Los Banos (UPLB), College, Laguna.
- Arida, G.S., A.T. Barrion, A.B. Estoy, R.C. Joshi and H.X. Truong. 2006. Management of the Rice Black Bug. Philrice Rice Technology Bull. 31, 16 p.
- Baroña, M.L.J. 2005. On Focus: The National Crop Protection Center, Tiny wasp bring down Goliaths trampling on cash crops, Horizon, Vol. 7 (1): 4-6, UPLB, College, Laguna.
- Braza, R. D. 1990. Laboratory evaluation of *Metarhizium anisopliae* (Metsch.) Sorokin against *Leucopholis irrorata* (Chev.) (Coleoptera: Scarabaeidae). Philipp. Ent. 8(1): 671-675.
- Calilung, V. J. 1995. Control of the melon thrips, *Thrips palmi* Karny by the anthocorid bug, *Orius tantillus* (Motschulsky). Terminal Report. DOST-PCARRD Funded Research Project, Department of Entomology, UPLB, College, Laguna. 30 p.
- Castro, R.A.Z. 1986. Fungicidal activity of microbial and plant extracts against *Aspergillus flavus* Link and *parasiticus* Speare in stored corn. MS Thesis, UPLB, College, Laguna, Philippines, 145 p.
- Corpuz-Raros, L. A. 1989. Hosts, geographic distribution and predatory mite associations of Philippine phytophagous mites (Acari). Philipp. Agric. 72(3): 303-322.
- De Leon-Facundo, J. B. and L. A. Corpuz-Raros. 2005. Survival, consumption and reproduction of *Amblysius longispinosus* (Evans) (Acari: Phytoseiidae) on various food items and its comparative biology on two species of spider mites. Philipp. Agric. Scientist. 88(1): 72-77.
- Cuevas, V. C., A. M. Sinohin and J. I. Orajay. 2005. Performance of selected Philippine species of *Trichoderma* as biocontrol agents of damping off pathogens and as growth enhancer of vegetables in farmers'

- field. Philipp. Agric. Scientist. 88(1): 63-71.
- Dumancas, E. E. 1976. A survey of the higher plants for fungicidal properties against *Helminthosporium oryzae* Bredn de Haas and *Pyricularia oryzae* Cav. MS Thesis, UPLB., College, Laguna. 93 p.
- De Jesus, L. R. A., S. M. F. Calumpang, . R. Medina and K. Ohsawa. 2004. Floral volatiles of *Mangifera indica* L. (c.v. carabao) attractive to *Sternochetus frigidus* (Fab.) Coleoptera: Curculionidae). Philipp. Agric. Scientist. 87(1): 23-35.
- Eser, C.A. M. F. Gilo, K. Natividad and M. L. C. Jarmin. 2005. BIOTECH inks three commercialization MOAs, Horizon, Vol. 7 (4): 2, UPLB, College, Laguna
- Franje, N. S. 1984. Evaluation of medicinal plant extracts as protectants and therapeutants against legume pathogen, P.H. D. Dissertation, UPLB, College, Laguna, Philippines. 192 p.
- Gabriel, B. P. 1968. Entomogenous micro-organisms in the Philippines: new and past records. Philipp. Ent.. 1: 97-130.
- Gonzales, P, G., P. A. Javier, E. P. Cadapan And A. M. Rosales. 2002. *Trichogramma*: kaibigang kulisap laban sa mga pesteng uod ng mais at ng iba pang pananim. National Crop Protection Center, College of Agriculture, UPLB, College, Laguna.
- Grainge, M. and S. Ahmed. 1988. Handbook of plants with pest control activities. John Wiley and Sons. 470 p.
- Jamias, S. B. 2005. On Focus: The National Crop Protection Center, A virus for mass destruction of cutworms, Horizon, Vol. 7 (1): 4-7, UPLB, College, Laguna.
- Javier, P. A. and P. G. Gonzales. 2000. Management of sugarcane borers using *Trichogramma chilonis*. PHILSURIN Leaflet No. 6. Philippine SugarmResearch Institute Foundation, Inc., Makati City, 11 p.
- Javier, P. A. and B. Morallo-Rejesus. 1986. Insecticidal activity of black pepper (*Piper nigrum* L.) extracts. Philipp. Ent. 6(5): 517-525.
- Javier, P. A. and B. Morallo-Rejesus. 2003. Conservation methods for the natural enemies of Asian corn borer. DA BAR Terminal Report. National Crop Protection Center, College of Agriculture, UPLB, College, Laguna. pp. 1-44.
- Javier, P. A., B . Morallo-Rejesus and E.G. Punzalan. 2004. Biocon Notes, Earwig, *Euborellia annulata* (F.), NCPC, UPLB-CA, College, Laguna 3 pp.
- Javier, P. A., B . Morallo-Rejesus and E.G. Punzalan. 2007. Earwig: Pagpaparami at pag-aalpas sa maisan. National Corn RDE Network, UPLB, DA-BAR Publication, UPLB-CA, College, Laguna.
- Lapiz, D. and E.E. Dumancas. 1978. Fungicidal activity of crude plant extracts to *Pyricularia oryzae* Cav. Philipp. Phytopath. 14: 23-27.
- Lapiz, D. and E.E. Dumancas. 1979. Survey of higher plants for fungicidal properties against *Pyreularia oryzae* Cav. Philipp. Phytopath. 15: 23-34.
- Lawas, T. P. and P. Magdalita, April 2007 ABSPH Southeast Asia Newsletter Vol. III No. 2
- Leonardo, R.P.1983. Field evaluation of the insecticidal activity of makabuhai against three major insect pests of rice. B.S. Thesis, UPLB, College, Laguna 51 p.
- Li Thi Hoan and R.G. Davide. 1979. Nematicidal properties of root extracts of seventeen plant species on *Meloidogyne incognita*. Philipp. Agric. 62: 285-295
- Maini, P. N. and B. M. Rejesus. 1992. Toxicity of some volatile oils against golden apple snail, *Pomacea* spp. Philipp. J. Sci. 121(4): 391-397.
- Maroon, C.J.M. 1983. The utilization of plant extracts as fungicides for the control of cercospora leaf spot of mungbean, *Vigna radiata* (L.) Wilzeck. Undergraduate Thesis, UPLB, College, Laguna.
- Mendoza, A. M. 1979. Assay of chemical extracts from higher plants for fungicidal activity against *Helminthosporium oryze*. B.S. Thesis, College of Agriculture, UPLB, College, Laguna. 33 p.
- Mercado, B.L. and J.S. Manuel. 1977. Biology of *Rotboellia exaltata* L.F.: characterization of the inhibitory activity. NRCP Res. Bulletin 32(1): 37-57.
- Mercado, B.L. and R. P. Robles. 1982. Utilization of the aquatic weed, water lettuce (*Pistia stratiotes* L.). NRCP Res. Bulletin. 27(1): 143-152.
- Morallo-Rejesus, B. 1984. Status and prospects of botanical pesticides in the Philippines. Second SEARCA Professorial Chair Lecture. 29 August 1984. UPLB, College, Laguna.

- Morallo-Rejesus, B. 1986. Botanical insecticides against diamondback moth. Proc. First Int'l. Workshop on "Diamondback Moth Management", Tainan, Taiwan, 11-15 March 1985.
- Morallo-Rejesus, B. 1987. Botanical pest control research in the Philippines. Philipp. Ent. 7 (1): 1-30
- Morallo-Rejesus, B. and A. Decena. 1982. The activity, isolation, purification and identification of the insecticidal principles from *Tagetes*. Phil. J. Sci. 7: 31-36.
- Morallo-Rejesus, B. and L. Eroles. 1978. Two insecticidal principles from Marigold (*Tagetes* spp.) roots. Philipp. Ent. 4: 87-98.
- Morallo-Rejesus, B. and E. G. Punzalan. 1997. Molluscicidal action of some Philippine plants on golden snails, *Pomacea* spp. Philipp. Ent. 11(1): 65-79.
- Morallo-Rejesus, B., P. A. Javier and E. G. Punzalan. 2000. Earwig: kaibigang kulisap. National Corn RDE Network, UPLB, DA-BAR Publication, UPLB, College, Laguna.
- Morallo-Rejesus, B. and E. G. Punzalan. 2003. Mass rearing and field augmentation of the earwig, *Euborellia annulata* against ACB. DA BAR Terminal Report. National Crop Protection Center, College of Agriculture, UPLB, College, Laguna. 74p.
- Morallo-Rejesus, B. and A. S. Sayaboc. 1992. Guide to control diamondback moth. Los Banos, Laguna: PCARRD, 1992. 16 p. (PCARRD PRIMER No. 4).
- Navasero, M. V. at M. M. Navasero. 2005. Pangkomunidad na pag-aalaga ng mamumulpol, pagpaparami, pag-iimbak at pagganit ng Bayrus-NPV. UPLB, College, Laguna, 16p.
- Pamplona, P.P. 1973. Dormancy and germination of *Rotboellia exaltata*. M.S. Thesis, UPLB, College, Laguna, Philippines, 65 p.
- Payawan, A. 1968. Effect of aguingay and cogon extracts on the germination and early growth of some plants. Report submitted to the YRAAP (Young Research Apprenticeship Action Program). Society for the advancement of Research. UPLB, College, Laguna.
- Pipithsangchan, S. and B. Morallo-Rejesus. 2005. Insecticidal activity of Diosgenin isolated from three species of grape ginger (*Costus* spp.) on the diamondback moth, *Plutella xylostella* (L.). Philipp. Agric. Scientist. 88(3): 317-327.
- Pordesimo, A. M. and L.L. Ilag. 1976. Toxicity of garlic juice to plant pathogenic organisms. Kalikasan. Philipp. J. Biol. 5:251-258.
- Quebral, F. C. 1981. Assay on the fungicidal properties of some medicinal plants. National Crop Protection Center Ann. Rep. 1981, pp. 21-25.
- Rice, E.L. 1974. Allelopathy. Academic Press, New York.
- Robles, R.P. 1985. Allelopathy and its potential in weed management. Inaugural lecture, Diamond Jubilee Professorial Chair, Department of Agronomy, College of Agriculture, UPLB, College, Laguna.
- Rombach M.C, D. W. Roberts and R. M. Aguda. 1994. Pathogens of rice insects. In: Biology and management of rice insects. Manila (Philippines): International Rice Research Institute. pp 613-655.
- Santiago, D. R. 1999. Fungal biopesticides for management of white grubs in sugarcane. Progress Report. PHILSURIN Project, National Crop Protection Center, University of the Philippines Los Baños, College, Laguna.
- Santiago, D. R. 2000. Primer on the Management of Rice Black Bug. National Crop Protection Center, University of the Philippines Los Baños, College, Laguna, 8p
- Santiago, D. R., A. G. Castillo, R. S. Arapan, M. V. Navasero and J. E. Eusebio. 2001. Efficacy of *Metarhizium anisopliae* (Metsch.) Sor. against the oriental migratory locust, *Locusta migratoria manilensis* Meyen. Philipp. Agric. Scientist. 84 (1): 26-34.
- Santiago, D. R. and B. P. Gabriel. 1998. Selection of *Metarhizium anisopliae* (Metch.) Sorok. isolates for virulence toward four insect pests. Philipp. Agric. 81: 197-202.
- Santiago, D.R. and B. P. Gabriel. 2000. Cuticle-degrading enzyme activity of *Metarhizium anisopliae* (Metsch.) Sorok. isolates pathogenic to Asian corn borer larvae. Philipp. Ent. 14: 15-29.
- Sarra-Guzman, R. 1984. Toxicity screening of various plant extracts against *Meloidogyne incognita* Chintwood and *Radophalus similis* Cobb and characterization of their nematocidal components. Ph.D. dissertation.

- UPLB, College, Laguna.
- Schicha, E. and Corpuz-Raros, L.A. 1992. Phytoseiidae of the Philippines. West Bloomfield, Michigan: Indira Publishing House. 190 p.
- Shepard B.M, A.T. Barrion, J. A.Litsinger. 1987. Helpful insects, spiders, and pathogens. Manila (Philippines): International Rice Research Institute. 127 p.
- Umali, M. M. 2005. On Focus: The National Crop Protection Center, Management of the Asian corn borer, *Horizon*, Vol. 7 (1): 4-6. University of the Philippines Los Baños, College, Laguna.
- Violanta, R.P. and M.M. Umali. 2004. UPLB promotes a winner in BIO-N. *Horizon*, Vol. 6 (10): 5-7, University of the Philippines Los Baños.
- Yarobe Jr., J.M. 2005. After one year of commercial adoption: has the Filipino farmer benefited from Bt corn? *BioLife*, January – March 2005, pp 16-19.
- Zipagan, M. B., C. Gallego and V. Gallego. 2006. Distribution and control of the coconut leaf beetle, *Brontispa longissima* (Gestro) in the Philippines. Proc. 37th Anniv. and Annual scientific conference of the Pest Management Council of the Philippines, Davao City May 2-5, 2006.
- Zorilla, R.A. and A. G. Castillo. 2005. Storage/shelf life of two promising nematophagous fungi. In: Annual Report, National Crop Protection Center, UPLB, College, Laguna, pp. 78-82.
- Zorilla, R.A., D.G. Vargas and A.G. Castillo. 2005. In: NCPC Annual Report, pp.109-111.