Volume 14 Number 1, June 2008

Journal of ISSAAS

The International Society for Southeast Asian Agricultural Sciences

CONTENTS

J. ISSAAS Vol. 14, No 1: 33-45 (2008)

SUSTAINABLE WATER MANAGEMENT IN THE RURAL LANDSCAPE OF CIANJUR WATERSHED, CIANJUR DISTRICT, WEST JAVA, INDONESIA

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(Received: August 5, 2005 ; Accepted: June 27, 2007)

ABSTRACT

Water management in the rural landscape of Cianjur Watershed was evaluated during the dry season. Five villages located in the upper stream area (Galudra Dua Hamlet), the middle stream area (Burangkeng and Gasol Satu Hamlet), and the lower stream area (Sayang and Cibakung Hamlet) were studied. A survey method was used in order to find out water quality and quantity, and its management. Water resources from ponds, wells, rivers, paddy fields and springs were analyzed chemically and physically. Water qualities changed significantly, showing a decrease along the Cianjur River and accumulation in the down stream area. Water utilization in the rural landscape indicated optimal improvement of human activity and agricultural production through changes in land use. Disturbance in land use were predicted as related to water quality, and urban land use affected rural areas negatively. Examination of pond characteristics were a best estimate for the entire watershed area. The results on the water cycle in the *pekarangan* was elucidated to find out the management, its availability and its role in the village's ecosystem.

Key words: *pekarangan*, water quality.

INTRODUCTION

The Cianjur watershed is one of the most important watershed areas in Indonesia, especially in West Java Province. It covers 7,250 km^2 area including 6 municipalities and a district in West Java (Whitten *et al.*, 1999) and supports three dams, Saguling, Cirata and Jatiluhur, which are usually used for agricultural needs and other useful activities for rural civilization. The management of the watershed in this area is necessary in order to meet increasing demand for water, like agricultural and household activities. These impacts are now putting pressure on the dams and their catchments areas, and are leading to more sedimentation in the reservoirs and other problems.

The Cianjur watershed is dominated by farmland areas, some of which are found as an agroforestry system. The agroforestry products from this area were marketed mostly in areas outside the watershed, like Jakarta and other urban areas. However, this area must also be conserved because the water stream from this catchment area supplies the water to downstream areas, and severe erosion due to farmland degradation can increase the runoff and lead to flooding in Jakarta and other downstream areas. The agroforestry practiced in Cianjur watershed land use, such as intercropping and multiple cropping, has several beneficial effects. These include using energy optimally, reducing yield-failure risks, and maintaining high biodiversity (Arifin, 2001). On the other hand, land use can indicate optimal energy utilization in the rural area, and it is necessary to figure out the suitable maintenance along the Cianjur watershed.

Water value, as one of main elements in rural landscape of Cianjur watershed, could be seen in its functions and utilization for sustaining life. Water is one of the most important and most attractive element in the farming system of watersheds through quality, quantity, distribution of sedimentation and erosion, and agroforestry system. Water also contributes to the daily life of rural people as a natural feature of sustainability of the rural landscape. Rural landscapes that are managed and developed in a sustainable manner can supply needs of consumption of the owner *(subsistence)* and contribute to food security.

This study sought to analyze qualities and quantities of water elements and sustainability of water management patterns in three different agro-bioclimatic zones in the Cianjur watershed.

STUDY SITES

This research has been conducted as collaboration between Bogor Agricultural University and The University of Tokyo - for the period of 1998-2007. The research was conducted in five villages in the Cianjur watershed that lie between E 106°59'07" - 107°15'00" and between S 6°45'00" - 6°52'30".

Table 1. Classification of five study areas.

Zoning	Study Area	Latitude	Longitude	Altitude
Upper Stream	Galudra	S 6°46'23" - 6°47'15"	E 106°59'07" - 107°03'16"	1300 - 2500 m
Middle Stream	Mangunkerta	S 6°47'44" - 6°48'14"	E 107°03'11" - 107°05'08"	700 - 1000 m
	Gasol	S 6°48'14" - 6°50'17"	E 107°04'14" - 107°03'16"	550 - 700 m
Down Stream	Sayang	S 6°48'06" - 6°50'02"	E 107°08'34" - 108°14'19"	350 - 450 m
	Selajambe	S 6°48'07" - 6°49'18"	E 107°12'17" - 107°14'32"	250 - 300 m

Source: Ground check by GPS (Global Positioning System)

The study sites were categorized by grouping geographical condition of Cianjur watershed into three zones (Table 1). Another factor considered was the location of the urban area, before and after the capital city. These zones have different agro-bioclimatic conditions along gradient slopes of Cianjur River. The three zones in the five villages are positioned in the east slope of Mount Gede. The upper stream of the catchments is a part of Gede Mountain and the down stream is dominated by a riverine plain (Fig. 1).

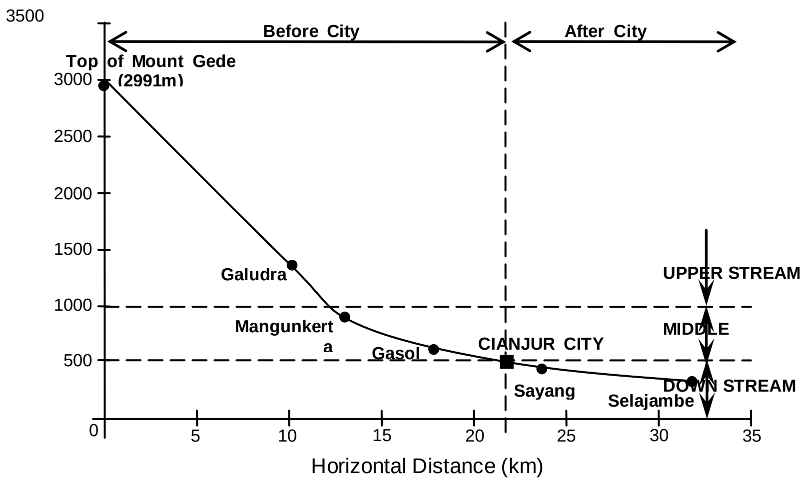

Fig. 1. Research site classification in the Cianjur watershed

The Cianjur watershed area covers 7,250 km^2 and is dominated by agricultural land use, where the types of agricultural land use are paddy field, mixed garden and plantation, especially tea *(Camelia sinensis)* plantations.

MATERIALS AND METHODS

Watershed Landscape Analysis

The land use data was collected using a field survey. Field survey techniques (Veldkamp *et al.*, 2001) in examining land use have a relationship within agroforestry development in rural landscape because at least a small rural landscape has already made some agroforestry practice for needs complementation. On the field surveys, the Integrated Transect Method (ITM) was used to describe land use at the semi-detailed level, using a multidisciplinary approach (Duivenbooden *et al.*, 1996). Spatially explicit ecosystem models could determine their changes (the land use patterns of the study area between 1972 and 1997). Spatially explicit ecosystem models allow the calculation of water and matter dynamics in a landscape that function as a spatial localization of habitat structures and matter input (Seppelt and Voinov, 2002).

Water Sources and Stream Types, Performance and Visualization (Spatial)

The research tools used were rolling meter (50 m), pole meter, leveling staff, altimeter and GPS. Survey methods and measurements using questionnaires in the hamlet area were performed in biophysical and sociological approaches with Proportional Random Sampling. The number of respondent in each hamlet is 60 householders. A total of 300 householders were interviewed with the assumption that a hamlet is a homogenous landscape unit. The mapping data were taken by using previous Indonesia Land Use and Topographical map, sheet 1209-213 Cugenang Area and sheet 1209-214 Cianjur Area with a scale of 1:25,000 (first edition of BAKOSURTANAL, 1990), hydrogeology map of Cianjur District with a scale of 1:100,000 (Direktorat Geologi Tata Lingkungan, 1990) and a semi-detailed land map of Citarum Tengah III Watershed, West Java with a scale of 1:25.000 (Pusat Penelitian Tanah, 1980).

Water Quality and Water Quantity of Rural Landscape Research Sites

Water samples were taken from ponds, paddy fields, wells and water springs around five villages in five hamlets during the dry season. Water samples were taken three times in each location to represent three replications.

The Dissolved Oxygen (DO) concentration was measured directly in the field by using a handy type DO-meter (F-102-5 Shibata, Tokyo). The Chemical Oxygen Demand (COD) concentration was calculated by using a COD: WAK-COD, phosphate (PO_4^{3-}: WAK-PO_4), ammonia (NH_3: WAK-NH_4), nitrite (NO_2^{1-}: WAK-NO_2) and nitrate (NO_3^{1}: WAK-NO_3) by colorimetry using Pack Test Sheets (Kyoritsu, Tokyo). The numbers of *Escherichia coli* and general bacteria were counted by using Test Papers TPA-BG and TPA CG (Kyoritsu, Tokyo), respectively. The process continued by conducting statistical analysis using Duncan Test Method. Water quantity analyses included debit and human needs around the study sites. Water needs were gathered by questionnaires and river water debits were estimated by using a water debit formula.

RESULTS

Land Use Changes in the Cianjur Watershed

According to Landsat Images, the land use in the area was classified into the following three categories: (1) forest, (2) urban area, and (3) agriculture area, including paddy fields, mixed gardens and plantations. Others land use classifications like forest garden and home garden could not be classified in detail using unsupervised classification. Forest gardens (talon) were classified into forest class and home garden, which are always located near settlements to urban areas. The result of this classification shows that forest areas have declined from 1972 to 1997 by about half, which was from 42% in 1972 to 22% in 1997. In addition, the agriculture and urban areas have increased. The agricultural area has increased from 51% to 54% and the urban area has increased from 7% to 24 %. These changes have been faster after the 1980's.

Identifying Stream Types and Water Resources

Biophysical characteristics of water elements could be projected from stream types and existing water sources. Based on its water flow, the Cianjur River is classified as a lotic water body. The field survey of streams was conducted in order to characterize stream attributes, including stream types, using a combination of physical and biological indicators. Watershed slopes distinguish water velocity and higher slopes make water flow higher. Delineation resulting from a contour grid map shows the level of the slope. Upper streams had more than 45% slope levels, and at this level, water dynamics will be close to 3.3528 m/s (Harris and Dines, 1988). It also has an impact on the sedimentation process that impulses erosion and transportation of soil from upper to down stream.

Cianjur Watershed Performance and Visualization

Watershed visualization (spatial) and performance (transect and land use) were produced by relative section and position of land use from Cianjur River. Watershed performance could be indicated by the land use system along the river corridor and depends on a sustainable ecosystem and agroforestry element. The upper stream has much better water performance and visualization than the other because bamboo *talun* (bamboo forest) are within the river position. *Talun* that hemmed in Cianjur River ecologically has many advantages, because the upper streams that have steep slopes are susceptible to erosion and run off.

Water Resource Quality and Quantity

Water quality as an ecological indicator could predict the sustainability of daily human activities of land use practice. Table 2 shows the result of statistical analysis of water quality that decreases along the study site using Duncan Test Method. It is dominantly significant both in the study site and in location, and the quality decreases zone by zone. In addition, the water qualities that were predicted from Gasol and Sayang village (after and before city) decreased quite significantly as an impact of urban landscape.

High concentrations of COD, NO_2, PO_4, NO_3, NH_4 and low concentration of DO were detected in water samples, which were highly contaminated by colon bacillus *(Escherichia coli*) and general bacteria, probably because of cattle in the area. Water quantities became much higher down stream, but the velocity becomes much slower compared to upper stream. Down stream had the highest river debited, 15.36 m/sec, and the upper stream is about 0.24 m/sec. These are in synergy with the Hydrogeology Cianjur District Map (1990) that calculated yearly debit means of upper and down stream water.

Water Resources Utilization

Drinking water sources for meeting the needs of rural society came from ground water and springs. The consumption of drinking water among the study sites was almost relatively similar (Fig. 2). Furthermore, the daily water sources from ponds and wells were utilized in different ratios among the study sites (Table 3).

Table 2. Statistical analysis results of water quality

Study Sites	Sample Location	DO (a)	COD (b)	NO_2 (c)	NO_3 (a)	PO_4 (a)	NH_4 (a)	Temp. (a)	pH (a)	*E. coli* (c)	General Bacteria (a)
Galudra	Well	2.61	8.00	0.01	7.67	0.10	0.20	18.70	7.20	43.33	61.67
	Pond	4.68	7.33	0.04	4.67	0.20	0.10	20.57	8.05	98.33	84.67
	River	4.95	7.00	0.01	3.67	0.20	0.10	21.50	7.91	127.00	101.33
	Paddy Field	3.91	4.67	0.01	1.00	0.10	0.10	23.30	7.67	67.33	85.33
	Spring	3.30	4.00	0.01	4.00	0.10	0.10	19.13	7.80	45.33	45.00
Mangukerta	Well	2.94	6.67	0.01	3.33	0.10	0.17	20.73	7.16	47.67	63.00
	Pond	6.07	6.67	0.03	4.00	0.20	0.23	22.70	8.12	73.33	90.00
	River	6.48	7.67	0.02	2.33	0.20	0.13	23.27	7.89	105.67	109.00
	Paddy Field	8.08	6.00	0.01	1.67	0.20	0.17	24.23	7.37	85.00	96.67
	Spring	4.24	5.33	0.01	2.67	0.10	0.10	22.27	7.03	38.33	53.33
Gasol	Well	4.51	8.00	0.01	2.33	0.10	0.30	19.50	7.02	48.00	65.00
	Pond	8.12	5.00	0.03	3.33	0.20	0.10	20.10	7.52	90.00	93.00
	River	6.60	8.00	0.01	1.67	0.20	0.10	20.80	7.74	105.00	109.00
	Paddy Field	6.14	8.00	0.01	1.67	0.10	0.10	20.50	7.32	98.00	101.00
	Spring	3.89	8.00	0.01	1.00	0.10	0.10	21.40	7.01	45.00	59.00
Sayang	Well	6.21	8.33	0.01	3.33	0.10	0.20	21.30	6.86	51.67	75.00
	Pond	8.23	10.23	0.03	3.67	0.40	0.23	25.32	7.33	80.00	99.00
	River	6.87	8.67	0.01	1.00	0.40	0.13	25.43	7.65	125.33	140.33
	Paddy Field	6.46	10.67	0.01	1.00	0.10	0.17	26.7	7.23	98.33	110.67
	Spring	-	-	-	-	-	-	-	-	-	-
Selajambe	Well	6.50	9.00	0.01	1.00	0.10	0.17	28.30	6.55	52.00	79.33
	Pond	7.82	12.67	0.01	1.00	0.40	0.70	29.10	7.16	81.00	99.00
	River	7.24	9.33	0.02	1.00	0.50	0.20	29.77	7.50	139.33	154.33
	Paddy Field	6.57	11.00	0.02	1.00	0.10	0.30	29.47	7.15	98.67	122.33
	Spring	-	-	-	-	-	-	-	-	-	-

Note:
DO, COD, NO_2*,* NO_3*,* PO_4 *and* NH_4 concentration in ppm unit.
Temperature in °C unit.
Escerichia coli and general bacteria in number of spot unit.

(a). Significantly different, both in the study site and sample location
(b): Significantly different only in the study site, not in the sample location
(c): Significantly different only in the sample location, not in the study site

Table 3. Water sources and disposal of water from daily activities (DA).

Study Site	Water Source for DA (%)		Water Disposal from DA (%)	
	Pond	Well	Small Stream	Septic
Galudra	100.00	0.00	98.33	1.67
Mangunkerta	100.00	0.00	93.33	6.67
Gasol	83.33	16.67	85.00	31.67
Sayang	10.00	90.00	61.67	38.33
Selajambe	8.33	91.67	75.00	25.00

The transfer of utilization of water sources and disposal from upper stream to down stream decreases synergy within the altitude. The disposal of used water to small streams result in decreasing water quality accumulatively. If the upper stream has polluted the stream, automatically the down stream could be polluted, because water resources transport pollutant materials.

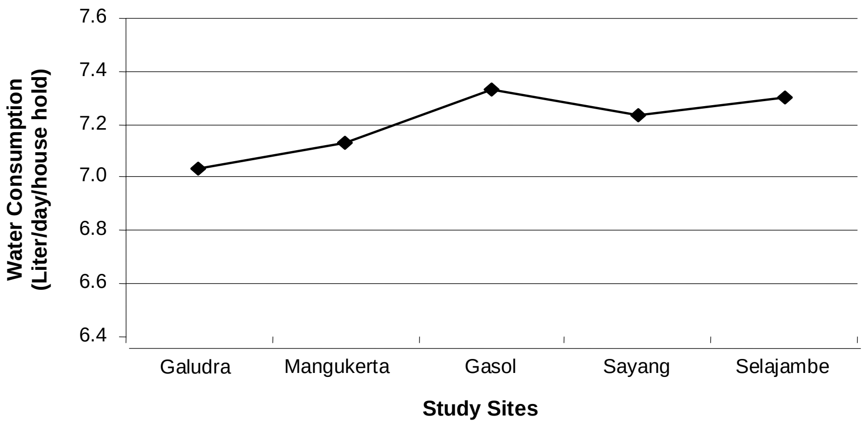

Fig. 2. The average water consumption per household

Water characteristics and functions in rural society are represented by fishpond management, including their number of ownership and treatment. The highest percent ownership was in the middle stream, but the average of area and volume of fishpond belong to down stream (Table 4). Land use, habitat conditions and water quality were significantly different among watersheds.

Table 4. Percent ownership and pond dimensions.

Study Site	Percent Ownership (%)	Area Average (m^2)	Volume Average (m^3)	Shape/Form (%)	
				Regular	Irregular
Galudra	38.33	5.25	7.17	91.30	8.70
Mangunkerta	66.67	7.20	8.48	90.00	10.00
Gasol	91.67	9.85	10.78	56.36	43.64
Sayang	26.67	12.73	13.56	37.50	62.50
Selajambe	21.67	16.69	19.94	23.08	76.92

The most intensive mud cleaning activity of ponds in the middle stream was 26 times/year or twice a week and done together with fish harvesting (Table 5). Almost all of the mud was thrown out to small stream (close to 60 %) and affected the sedimentation process down stream. The water treatment is usually done using $CaCO_3$ (calcium carbonate) with the purpose of recovering the water condition from high acidity and spread manually after fish harvesting. Down stream has the highest number of water treatment of ponds (20 %) but the frequency of treatments was highest at middle stream (4.4 times/year).

Table 5. Mud cleaning frequency and disposal of pond mud

Study Site	Frequency of Mud Cleaning (Times/year)			Location of Mud Disposal (unit)					No. of Ponds
	Average	Max	Min	Home Garden	Small Stream	Paddy Field	Mix Garden	Never	
Galudra	10	24	1	5	18	0	0	0	23
Mangunkerta	12	26	0	9	26	1	3	1	40
Gasol	12	24	4	12	23	15	5	0	55
Sayang	9	16	2	3	3	3	2	0	16
Selajambe	5	12	2	3	3	3	0	0	13
	Total Unit			32	82	22	10	1	147
	Percents (%)			21.77	55.78	14.97	6.80	0.68	100.00

Holistically the water management in rural landscape has positive-correlation with the sustainability of the ecosystem as an agroforestry practice (Fig. 3). In the upper stream, drinking water came mostly from springs; which is different from other areas. The middle stream uses springs and wells as sources of drinking water. However, in down stream, the springs disappeared and were substituted totally by well water. Garbage disposal directly to the river was practiced after middle stream to down stream, and agribusiness practices (e.g. fish, compost, etc.) were observed in downstream areas.

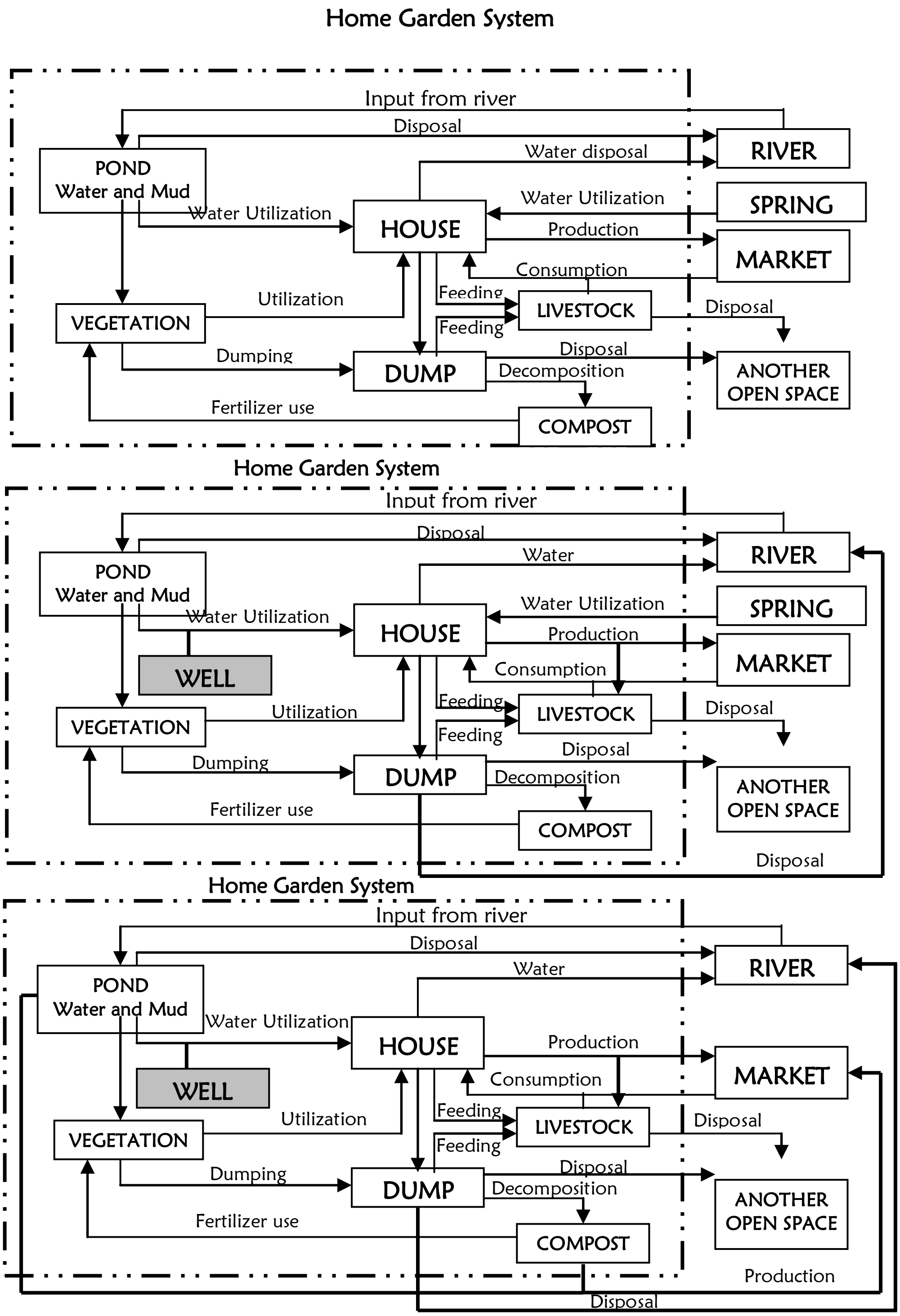

Fig. 3. Water management in the rural landscape watershed:
Upper stream (top); Middle stream (middle); Down stream (bottom)
The bold arrow and shading box indicate the differences

DISCUSSION

Land Use and Cover Change Related to Water Availability

Land use of forest was very important for conservation of natural resource and for keeping all natural function more sustainable. Nevertheless, the needs for agricultural land and settlement areas have led to constant decrease in forest areas. The demand for agricultural area and urban settlements and access to the catchments areas have increased greatly since the 1980's, especially in the urbanized area Cianjur City.

Urbanization process has been faster after 1980s because the government has opened highways from Jakarta to Bogor city. Besides, the landscape along the route from Jakarta to other areas in the eastern part of West Java across this area is beautiful and, therefore, the tourism industry is growing fast in this area, especially through settlements and recreation areas that support this industry and also decreasing process of water quality along Cianjur River.

Land use disturbance has been reflected in the water quality (Stewart *et al.,* 2000) and agricultural land use has escalated landscape sensitivity (Knox, 2001). On the slope of agro-bioclimatic zones, agricultural landscapes are more sensitive to climatic variability than natural landscapes. It is usually caused by tillage and grazing that typically reduces water infiltration and increase magnitudes of surface runoff. However, *talun* has high contribution in producing water availability.

The different transport of materials emphasizes the fact that the initial point of entry and final point of impact of nutrients can be cumulative downstream and physically separated in both space and time (Edwards and Dennis, 2000). The implications of land use change on nutrient cycling are reviewed by contrasting the properties of nitrogen and phosphorus. In addition, for any particular location selected in the drainage network, the attributes can be quantified for upper stream areas, which make this structure especially useful for identifying point sources of pollution. This is the reason why pollutants were accumulated in downstream area.

Cianjur Stream Type as an Ecological Unit

As a lotic system, Cianjur Streams are particularly conditioned in a flow rate, turbidity, and effects of temperature on the biota of rapids and pools. With a high flow rate over rocks or logs, the surface of a stream is broken, and considerable water turbulence occurs (Forman and Godron, 1986) and there is an increasing sedimentation process. According to Seyhan's categories of watershed (1990) the characteristics of Cianjur Watershed could be influenced by area, shape, slope, vegetation, land use, fishpond and lake number, drainage and pH permeability. Furthermore, Cianjur Watershed can be dominantly categorized into perennial stream type (Hansen, 2001) with the presence of a defined channel. It means a defined channel was entrenched into the landscape or had an active water path that is noticeably scoured, sorted, or settled materials.

Did Water Quality Decrease Stream by Stream?

Landscape elements can be ranked according to their general influence on water quality (Thierfelder, 1998) from the mimicry of an agroforestry system. The highest polluted area is the river. The accumulation of pollutants in the river shows that rural civilization used their resources (e.g. pesticide, fertilizer) in high concentration.

Active and recently extinct volcanoes surrounding the catchments area in the upper Cianjur Watershed were highly contaminated with natural contaminants. The main streams originate from two different sources: (1) a flank spring producing water which is suspected to contain a portion of leaking acid lake water; and (2) artificial sulphur-mud deposits which produce sulphur from crater-lake sediments that were exploited (Sriwana *et al.*, 1998). Those sources considerably impact the water quality along the Cianjur River. Ripl and Hildmann (2000) said that water and matter cycles have strong feedback mechanisms and are closely associated.

Furthermore, pollutant loads at small river watersheds depend on height and aspects of mountain slopes (Yevseev and Krasovskaia, 2001). As a mountainous area, upper streams mostly have clean water to use, and the pollutants are accumulated in down stream area. The upper stream also has the water highest velocity that has advantages and disadvantages. This causes water quality in the upper stream to always cleaned by river flow through transport process but it also results in high density of erosions. Also urban land use had the greatest influence on water quality (Sliva and Williams, 2001); it is in synergy with the statistical results that illustrated water quality decrease significantly after flowing over urban areas.

Rightly or wrongly, it is far simpler to identify each component of the agricultural system separately. Agriculture that uses water sustainability is relatively simple to understand. The concept of sustainability as a whole has been much harder to define (Stevenson and Lee, 2001). Those analyses conclude that the middle stream of Cianjur Watershed has more sustainable indicators as a transition area of rural and urban area identified by water quality.

The most significant contamination of water villages of West Java originates from domestic sewerage. Based on these data for water quality, it is important to set up a restoration system at the most suitable points of water stream with low cost and low energy input. The use of charcoal and plants to remove contamination were suggested (Outridge and Noller, 1991).

Does Pond Size Matter?

Pond characteristics for non-contributing areas are best estimates for the entire basin area (Sophocleous *et al.*, 1999). Utilization of water resources in the Cianjur rural landscape was examined (Tables 3, 4 and 5). The highest pond ownership was found in the middle stream area, because water can be easily accessed from the river to the pond. These are significantly different and show the strong relationships around agro-bioclimatic zone with the area, volume, pond shape and water treatment. Ponds found downstream were more irregular in shape, bigger and were treated more often.

On the other hand, those relationships also explain that accommodation and topography condition did not influence the number of pond ownership, but influenced area, volume and management of pond. The ponds at the upper stream have no fish and few biological activities (e.g. Morin and Hurt, 2001). No major ecological impacts from ponds were expected within the stream and no downstream impact in the watershed were expected as long as the upper stream was not polluted.

The middle stream of Cianjur Watershed had a large number of ponds of small sizes. The middle stream had a higher conservation value and was more sustainable than others. Appropriated by Oertli *et al.* (2002,) a set of ponds of small sizes had more species and had a higher conservation value than a single large pond of the same total area. Also, the mud was disposed mainly to the *pekarangan* (home garden), used as fertilizer in a subsistence agroforestry system and is practiced mostly in the middle stream.

Sustainable Water Management in Rural Landscape

Water management in each zone gradually changes the water cycling system. Upper stream is almost free from house waste disposal, contrary to down stream that pollutes water streams with dump and waste pond water. But the most sustainable cycling system was middle stream where it is supported by a sustainable agroforestry system and has subsistence cycle in home activities and production.

Most farmers households are connected to the potable water system based on gravity. Their concerns focused on water availability, demand, quality and management conflict on the watershed level (Kammerbauer *et al.*, 2001). Anthropogenic activities can act on pristine wetland and change water quantity and water quality (Alvarez-Cobelas *et al.*, 2001). In the Cianjur watershed, the different kinds of management conflicts can be observed as water fluxes in the creek were reduced during the dry season period. Cianjur watershed communities compete for irrigation water for their farming, as clear rules for the assignment of irrigation water were not established effectively. One of the water improvement practices is by using water quality priority scenario that increases the agroforestry system production which is sustainable (Coiner and Polasky., 2001).

One approach to achieve sustainable management of water is ecohydrology (Zalewski, 2000). Degradation of freshwater ecosystems, and those of water resources have two facets: pollution and the disruption of water and nutrient cycles, and pollution can be substantially eliminated by biotechnology. Janauer (2000) dealing management with the optimization of ecotone zones structure in whole watershed usually fights a lack of space for ecohydrologically relevant structure, and a lack of funding, although ecotones and other structural biotic elements provide basis for sustainable landscape management. ??

CONCLUSIONS

Our findings allow the following conclusions:

1. Land use change in Cianjur watershed was under threat, and this may offer the people of the area an unpromising future.

2. Cianjur Watershed performance, according to the land use profile, gradually changes the ecological system (agroforestry system). Cianjur River is a lotic system, high in infiltration and evaporation, categorized by perennial system type.

3. Water qualities were changing, and significantly different. Water quantities were sustainable, supply and demand were in balanced conditions. Urban landscape has influenced rural landscape gradually.

4. Accommodation and topography condition did not influence the number of pond ownership, but influenced area, volume and their management.

5. Water utilization in three zones indicates optimal improvement of human activity and agricultural production. According to material balance cycling system, the middle stream of Cianjur Watershed has more sustainable water management pattern than the others.

ACKNOWLEDGEMENT

This research is collaborative effort between The University of Tokyo, Japan and Bogor Agricultural University, Indonesia under The Core University Program in Applied Bioscience, the Japan Society for the Promotion of Science (JSPS), Japan and Directorate General of Higher Education (DGHE), Indonesia.

We would like express our gratitude to the Landscape Architecture Laboratory, Bogor Agricultural University (IPB), Indonesia and Asian Natural Environmental Science Center, The University of Tokyo, Japan who supported the research budget, tools and materials during the field survey.

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J. ISSAAS Vol. 14, No 1: 46-59 (2008)

SURVEY OF APHID INFESTATION AND VIRAL INFECTION OF POTATOES IN SYRIA

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(Received: February 12, 2008; Accepted: April 16, 2008)

ABSTRACT

The occurrence of aphids and viral diseases on potatoes in Syria was examined. Aphids were trapped in a yellow pan with water at the Tissue Culture Laboratory of the General Organization for Seed Multiplication (GOSM), Al Eeramoun, from June, 2006 to June, 2007. The number of winged aphids trapped increased slightly in autumn and markedly in spring. On autumn-cultivated potato plants in the Aleppo and Hama areas, aphid densities increased from mid-October to early November, just before the harvest, in 2006. On spring-cultivated potatoes, aphid densities decreased from late May, although the densities were higher in mid-April, just after sprouting, in 2007. Virus-infected plants were common among both autumn- and spring-cultivated potatoes in fields not contracted to GOSM, but were few in contracted fields in which virus-free plants grew. Aphid species belonging to 13 genera, including *Myzus persicae*, *Aphis gossypii*, *A. fabae*, *A. craccivora*, *Schizaphis borealis* and *Lipaphis erysimi*, were identified among aphid samples collected from potato plants. In addition, *Rhopalosiphum rufiabdominalis* was found on rhizomes and roots of potato plants in GOSM greenhouses in 2007. The major aphids as potato pests were considered to be *M. persicae* and *A. gossypii*, and *A. fabae* to a lesser extent. To our knowledge, this is the first report of *A. fabae* and *R. rufiabdominalis* infesting potatoes in Syria.

Key words: *Myzus persicae, Aphis gossypii, Aphis fabae,* yellow pan, *Potato Virus Y*

INTRODUCTION

The main agricultural crops in Syria are wheat, barley, olives, cotton, and lentils. Crops such as potatoes, tomatoes, cucumbers, egg plants, green and red peppers, tobacco, cotton, beans, sugar beets and sunflowers are cultivated in limited areas where irrigation systems are set up. Potato (*Solanum tuberosum*) is one of the major crops in the irrigated areas.

In Syria, seed potatoes are imported, propagated and distributed by a national organization, the General Organization for Seed Multiplication, or GOSM (GOSM, 2006). Because a large quantity of seed potatoes is imported annually, a national project to switch from the importation of seed potatoes to domestic production is proceeding in partial cooperation with the Japan International Cooperation Agency (JICA). However, a decline in domestic production as a result of infection with viruses is a serious problem.

GOSM is propagating virus-free potato plants by means of apical culture technology. A system has been constructed for mass production of virus-free seed potatoes in culture rooms, greenhouses, net-houses and open fields. Seed potatoes are produced firstly in net-houses and in the second and third phases in fields, and are called 'Super Elite', 'Elite' and 'Class A', respectively. The 'Class A' seed potatoes are distributed to farmers for commercial production of potatoes.

Potatoes in Syria are seriously infected with *Potato Virus Y* (PVY) (Chikh Ali *et al*., 2006), which is transmitted in the fields by aphids. This situation cannot be solved completely with the use of virus-free seed potatoes. The most important concern is the efficient control of aphids. However, the relationship between the aphids and viral infection has been inadequately investigated and the identity of the aphid species is sufficiently unknown (Katayama, unpublished report of Technical Transfer by JICA Expert, February 2002).

To analyze the population dynamics of aphids and the occurrence of viral infection of potato plants in Syria, we investigated the number of winged aphids trapped in a yellow pan with water, and quantified aphid densities on potato plants and the level of virus-infected plants. In addition, we identified the aphids infesting potato plants.

MATERIALS AND METHODS

Environmental conditions and occurrence of winged aphids

Temperatures were measured at the Tissue Culture Laboratory, GOSM, Al Eeramoun, Syria, at 13:00 from June, 2006 to June, 2007. Weather conditions and grass growth were also observed at the site.

A yellow pan with water (30 cm diameter) with a surface-active agent , from a commercial shampoo, was placed on the ground at the Tissue Culture Laboratory site in June, 2006. The seasonal changes were monitored by counting the number of winged aphids and other insects trapped from June, 2006 to June, 2007.

Occurrence of aphids and viral infection

(1) Autumn-cultivated potatoes in 2006

Commercial farms are classified into those contracted to GOSM and un-contracted fields. Virus-free seed potatoes of the 'Super Elite' and 'Elite' classes were cultivated in contracted fields. Three contracted fields and three un-contracted fields in the Aleppo area in northern Syria and one contracted field and four un-contracted fields in the Hama area in the Syrian midlands, were selected (Table 1). 'Afamia' was the main potato cultivar grown in each field.

The occurrence of the aphids *Myzus persicae* and *Aphis gossypii*, which are the main aphid species in Syria (GOSM, 2005), on potato plants was investigated and samples of the aphids were collected for identification. Numbers of nymphs and adult aphids on 200 randomly selected compound leaves were counted in each field about twice per month. In addition, the number of virus-infected plants, based on the presence of mosaic symptoms on the foliage, was counted for 200 plants.

(2) Spring-cultivated potatoes in 2007

One contracted and four un-contracted fields in the Aleppo area, and one contracted and four un-contracted fields in the Hama area were selected (Table 1). 'Afamia' was the main potato cultivar grown in the fields. The occurrence of aphids and viral disease was investigated, and aphid samples collected, with the same methods as for the autumn-cultivated potatoes.

Table 1. Potato fields, autumn cultivated in 2006 and spring cultivated in 2007, in the Aleppo and Hama areas, Syria.

Areas and abbreviations of field [1)]	Potato cultivar	Area (ha)	Planting date	Former crop
Autumn				
Aleppo				
A1 (G)	Afamia	1.0	Aug. 10	lentil
A2 (G)	Afamia	1.5	Aug. 12	lentil etc.
A3 (G)	Afamia	3.0	Aug. 5	maize
A4	Afamia	2.0	July 25	wheat
A5	Afamia, Merabl, Agria	2.5	July 25	wheat
A6	Afamia, Spunta, Marfona	30.0	July 30	wheat
Hama				
H1 (G)	Afamia	2.0	Aug. 14	wheat
H2	Afamia	1.5	Aug. 4	wheat
H3	Afamia, Spunta	2.0	July 25	wheat
H4	Afamia	1.5	Aug. 5	wheat
H5	Afamia	1.0	Aug. 5	wheat
Spring				
Aleppo				
A1 (G)	Afamia, Loulou	2.0	Feb. 24	lentil
A2	Draga	0.8	Feb. 15	wheat
A3	Afamia, Draga, Fabylla	2.7	Feb. 20	wheat
A4	Marfona	3.0	Feb. 10	wheat
A5	Afamia	12.0	Feb. 22	wheat
Hama				
H1 (G)	Afamia, Loulou	1.0	March 18	barley
H2	Afamia, Atlas, Burren	1.0	March 20	water melon
H3	Afamia	3.0	March 10	water melon
H4	Afamia, Agria	1.5	March 2	barley
H5	Afamia, Agria, Atlas, Burren	6.0	Feb. 25	barley

1) (G) indicates fields contracted to the General Organization for Seed Multiplication (GOSM); the other fields were not contracted to GOSM. Virus-free seed potatoes of the ‘Super Elite’ or ‘Elite’ classes were used in the contracted fields.

Identification of aphids collected on potato plants

Samples of 152 and 376 individual aphids were collected on the potato plants in autumn 2006 and spring 2007, respectively, and were identified from their morphology at Utsunomiya University, Japan. In addition, aphids that infested the underground parts (rhizomes and roots) of the potato plants were collected in the GOSM greenhouses on July 26, 2007, and were also identified.

The aphid samples collected for identification were put into small specimen tubes containing 70% alcohol. In order to examine the structure of the aphids, samples were mounted on microscope slides prepared with Lambers methods. Scientific names of aphids were determined using numerous published keys and references, including Takahashi (1961), Miyazaki (1971), Heie (1980, 1986), Blackman and Eastop (1984), Stroyan (1984), and Torikura (1991).

RESULTS

Environmental conditions and occurrence of winged aphids

(1) Temperature, weather conditions and grass growth

Table 2 shows the temperatures, weather conditions and grass growth in Al Eeramoun, Syria from June, 2006 to June, 2007. The periphery of the study site was wasteland-like desert and few grasses were growing in June, 2006, when the study began. From June until mid-September 2006, fine, dry and hot weather prevailed. During summer, the weather was often very hot and the maximum temperature at 13:00 was above 40°C. In autumn, fine weather continued except in late September, but the temperature decreased slightly and it rained occasionally. During mid-October and early November, rainy and cloudy weather continued and grasses germinated in late October. Shortly afterwards, however, grass growth was inhibited by low temperatures.

During late March until mid-May, 2007, grasses grew densely and blossomed coinciding with the rise in temperature and rainfall. However, the grasses withered in late May following high temperatures and little rainfall.

Table 2. Temperatures, weather conditions and grass growth in Al Eeramoun, Syria from June, 2006 to June, 2007.

Month	Temperature (°C) [1)]			Weather conditions [2)]	Grass growth [2)]
	Mean	Min.	Max.		
June, 2006	36.5	30	41	Fine, dry and hot.	No grasses.
July	38.0	34	42	Fine, dry and hot.	No grasses.
August	38.2	35	42	Fine, dry and hot.	No grasses.
September	32.4	20	41	Sometimes rainy and cool late in the month.	No grasses.
October	24.3	16	32	Fine in the beginning. Rainy and cloudy in the middle and late in the month.	Grasses germinated on one occasion late in the month.
November	15.0	10	18	Fine, dry and cold in the middle and late in the month.	Grass growth occurred, but growth was soon inhibited.
December	10.5	4	16	Fine, dry and cold.	No grasses.
January, 2007	8.2	2	14	Sometimes rainy, cloudy and snowy.	No grasses.
February [3)]	9.9	5	14	Sometimes rainy and cloudy.	No grasses.
March [3)]	17.8	13	20		Grasses grew steadily late in the month.

Month	Temperature (°C) 1)			Weather conditions 2)	Grass growth 2)
	Mean	Min.	Max.		
April	18.7	16	22	Sometimes rainy and cloudy.	Grasses grew densely and blossomed.
May	31.2	25	40	Rainy and cloudy in the beginning and middle. Fine, dry and hot late in the month.	Grasses withered late in the month.
June	39.6	30	46	Fine, dry and hot.	No grasses.

1) The temperatures were measured at 13:00 in the shade at the GOSM Tissue Culture Laboratory, Al Eeramoun, Syria.
2) The weather conditions and grass growth were observed at the same site.
3) Observation was stopped from late February to mid–March, 2007.

(2) Seasonal changes in the number of aphids trapped in the yellow pan

Fig. 1 shows the seasonal changes in the number of winged aphids and other insects trapped by a yellow pan from June 2006 to June 2007 at Al Eeramoun, Syria. From June to September, high-temperature months, a few aphids were trapped. A total of 147 and 58 aphid individuals were trapped in October and November, respectively. However, few aphids were trapped during winter.

In spring, 2007, the number of trapped aphids increased markedly to 1,022 and 765 individuals in April and May, respectively. Few aphids were trapped in June. The seasonal changes in the numbers of other insects trapped in the yellow pan resembled those of aphids (Fig. 1). The insects belonged to a diversity of orders, including Orthoptera, Hemiptera, Neuroptera, Coleoptera, Diptera, Lepidoptera and Hymenoptera.

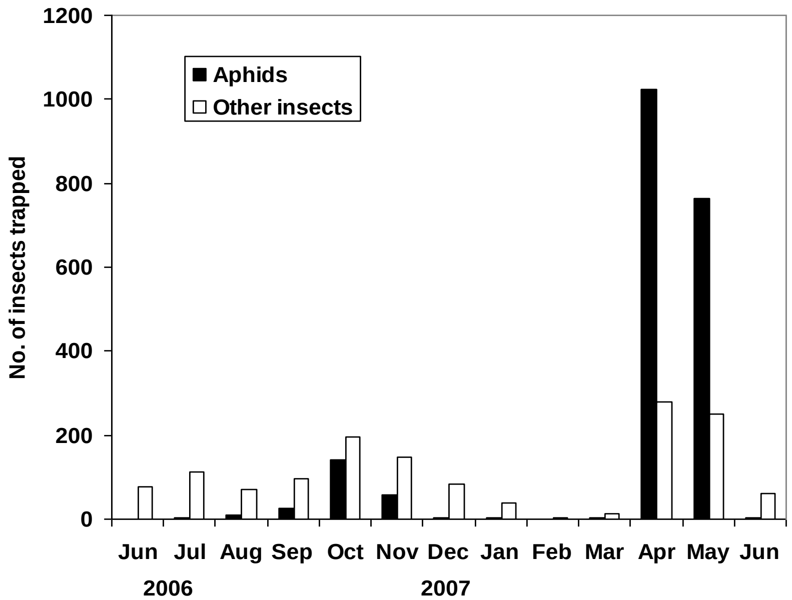

Fig. 1. The number of winged aphids and other insects trapped by a yellow pan from June 2006 to June 2007 at Al Eeramoun, Syria.

2. Occurrence of aphids and viral infection

(1) Autumn-cultivated potatoes in 2006

Table 3 shows the mean number of the aphids *M. persicae* and *A. gossypii* per leaf on potato plants, and mean percentage of plants showing viral mosaic symptoms in autumn-cultivated potato plants, in the Aleppo and Hama areas, Syria in 2006. The densities of winged and non-winged aphids began to increase from the middle of October and increased in early November, 2006, just before the potato harvest, in the contracted and un-contracted fields in both Aleppo and Hama. Large colonies of non-winged aphids were not observed; they formed extremely small colonies or infested plants individually in the same manner as the winged aphids.

The virus-infected plants, which were assumed to be infected with PVY based on the visible symptoms, were already evident on September 3, soon after planting in the contracted and un-contracted fields in Aleppo. The percentage of infected plants increased with time, and the highest percentage of 98% was observed on November 9 in the un-contracted field, H3, in Hama (Table 3). Lower levels of diseased plants were usually observed in the contracted fields.

Table 3. Mean number of the aphids, *Myzus persicae* and *Aphis gossypii* per leaf on potato plants, and mean percentage of plants showing viral mosaic symptoms in autumn-cultivated potato plants, in the Aleppo and Hama areas, Syria in 2006.

Area	Date	Field [1)]	Aphid (No./leaf) [2)]		Virus [3)] (%)
			Myzus persicae	*Aphis gossypii*	
Aleppo	Sep. 3	A1 (G)	0	0	0.5
		A2 (G)	0	0	0
		A3 (G)	0	0	0
		A5	0	0	0.1
		A6	0	0	1.0
	Sep. 20	A1 (G)	0	0	0
		A2 (G)	0	0	0
		A3 (G)	0	0	0.5
	Oct. 4	A3 (G)	0	0	1.0
		A4	0	0	0.5
		A5	0	0	16.0
		A6	0	0	62.5
	Oct. 18	A1 (G)	0.01(W)	0	1.0
		A2 (G)	0	0	1.0
		A3 (G)	0	0	0.5
	Nov. 1	A3 (G)	0.03	0.01	1.0
		A4	0	0	1.5
		A5	0	0.01	51.0
		A6	0.01(W)	0.01, 0.01(W)	76.0
	Nov. 8	A1 (G)	0.04	0	0.5
		A2 (G)	0.06	0	0
		A3 (G)	0.05, 0.01(W)	0	1.0

Area	Date	Field [1)]	Aphid (No./leaf) [2)]		Virus [3)] (%)
			Myzus persicae	*Aphis gossypii*	
Hama	Sep. 18	H1 (G)	0	0	0.5
		H2	0	0	0
		H3	0	0	2.5
		H4	0.02(W)	0	0
		H5	0.01	0	0
	Sep. 27	H1 (G)	0	0	0.5
		H2	0	0	1.5
		H3	0	0	11.0
		H4	0	0	0.5
		H5	0	0	0
	Oct. 12	H1 (G)	0	0	1.5
		H2	0	0	18.0
		H3	0	0	26.5
		H4	0.01(W)	0	4.0
		H5	0.01(W)	0	0.5
	Nov. 2	H1 (G)	0.01	0	1.0
		H2	0.01	0	36.0
		H3	0.06	0.01	93.0
		H4	0.04	0	4.0
		H5	0.01, 0.01(W)	0	1.0
	Nov. 9	H1 (G)	0.05, 0.01(W)	0	0.5
		H2	0.05, 0.01(W)	0	81.5
		H3	0.08	0	98.0
		H4	0.08, 0.01(W)	0	6.0
		H5	0.03	0	0

1) The field codes are as listed in Table 1. (G) indicates fields that were contracted to GOSM.
2) Adults and nymphs of *M. persicae* and *A. gossypii* were counted. The number preceding (W) is the number of winged aphids recorded.
3) The percentage of virus-infected plants showing mosaic symptoms.

(2) Spring-cultivated potatoes in 2007

Table 4 shows the mean number of the aphids *M. persicae* and *A. gossypii* per leaf on potato plants and mean percentage of plants showing virus mosaic symptoms in spring-cultivated potato plants in the Aleppo and Hama areas, Syria in 2007. Winged and non-winged aphids were already present on spring-cultivated potato plants in April, just after sprouting. Higher aphid densities were observed until mid-May, and then lower densities were recorded from late May to harvesting in June or July, in the contracted and un-contracted fields in both Aleppo and Hama. Large colonies of non-winged aphids were not observed.

A large number of virus-infected plants were observed during May and July in the un-contracted fields. The highest percentage of diseased plants (100%) was observed in the un-contracted fields, A2 and H3, at Aleppo and Hama on June 27 and July 4, respectively (Table 4).

Lower levels of diseased plants were observed in the contracted fields, as in the autumn-cultivated potatoes.

Table 4. Mean number of the aphids *Myzus persicae* and *Aphis gossypii* per leaf on potato plants and mean percentage of plants showing virus mosaic symptoms in spring-cultivated potato plants in the Aleppo and Hama areas, Syria in 2007.

Area	Date	Field [1)]	Aphid (No./leaf) [2)]		Virus [3)] (%)
			Myzus persicae	*Aphis gossypii*	
Aleppo	April 18	A1 (G)	0.01 (W)	0.01, 0.01 (W)	0
		A2	0	0.01, 0.01 (W)	0
		A3	0	0.01	0
		A4	0.01, 0.01 (W)	0.01 (W)	0
		A5	0.01, 0.01 (W)	0	0
	April 26	A2	0.03, 0.02 (W)	0.02, 0.02 (W)	0
		A4	0.01, 0.01 (W)	0.04, 0.02 (W)	0
		A5	0.03(W)	0.01, 0.14 (W)	0
	May 16	A1 (G)	0.01 (W)	0.03 (W)	0
		A2	0.01 (W)	0.01 (W)	0
		A3	0	0.01 (W)	0
		A4	0	0.01, 0.01 (W)	27.0
		A5	0	0	0
	May 30	A1 (G)	0	0	0
		A2	0	0	29.5
		A3	0	0	2.0
		A4	0	0	91.0
		A5	0	0	10.5
	June 13	A1 (G)	0	0	1.5
		A2	0	0	95.5
		A3	0	0	2.5
		A4	0	0	98.5
		A5	0	0	32.5
	June 27	A1 (G)	0	0	2.0
		A2	0	0	100
		A3	0	0	24.5
Hama	April 11	H1 (G)	0	0.04 (W)	0
		H2	0.01, 0.03 (W)	0.01, 0.01 (W)	0
		H3	0.05 (W)	0.01 (W)	0
		H4	0.01 (W)	0.02 (W)	0
		H5	0.03 (W)	0.01 (W)	0
	April 25	H1 (G)	0.01, 0.04 (W)	0.02, 0.09 (W)	0
		H2	0.08 (W)	0.04 (W)	0
		H3	0.01, 0.12 (W)	0.01, 0.14 (W)	0
		H4	0.05, 0.01 (W)	0.03, 0.06 (W)	0
		H5	0.03, 0.12 (W)	0.02, 0.02 (W)	0
	May 9	H1 (G)	0	0.01 (W)	0
		H2	0.01 (W)	0.01, 0.01 (W)	0
		H3	0	0	0.5
		H4	0	0	0.5
		H5	0	0	0.5

Area	Date	Field [1)]	Aphid (No./leaf) [2)]		Virus [3)] (%)
			Myzus persicae	*Aphis gossypii*	
	May 29	H1 (G)	0	0	0
		H2	0	0.01	1.0
		H3	0.01 (W)	0	89.5
		H4	0	0	7.0
		H5	0	0	6.5
	June 7	H1 (G)	0	0	0.5
		H2	0	0	1.0
		H3	0	0	92.5
		H4	0	0	6.0
		H5	0.01 (W)	0	8.0
	June 20	H1 (G)	0	0	0.5
		H2	0	0	51.0
		H3	0	0	75.0
		H4	0	0	4.5
		H5	0	0	13.0
	July 4	H2	0	0	90.0
		H3	0	0	100
		H4	0	0	5.0
		H5	0	0	5.5

1) The field codes are as listed in Table 1. (G) indicates the fields that were contracted to GOSM.
2) Adults and nymphs of aphids, *M. persicae* and *A. gossypii* were counted. The number preceding (W) is the number of winged aphids recorded.
3) The percentage of virus-infected plants showing mosaic symptoms.

3. Identification of the aphids collected on potato plants

Thirteen aphid species and four groups identified to generic and tribal levels, which collectively belonged to 13 genera, were identified, as shown in Table 5. The four groups were not able to be identified to species level. The major aphid species were *M. persicae* and *A. gossypii,* and to a lesser extent *A. fabae*. Both winged and non-winged individuals of *M. persicae*, *A. gossypii*, *A.fabae*, *A. craccivora*, *L. erysimi* and other unidentified *Aphis* spp. were observed, but only winged individuals were recorded for the other aphid species.

In addition, adult and larval aphids were found on the underground parts (rhizomes and roots) of potato plants in the GOSM greenhouses. These were identified as *Rhopalosiphum rufiabdominalis*.

DISCUSSION

Potatoes are one of the most prosperous crops in Syria. However, the decline in production caused by viral diseases is a serious problem. Potatoes are severely infected with viruses transmitted by potato-infesting aphids in fields, but the relationship between the dynamics of the aphid populations and occurrence of viruses is not sufficiently understood. Moreover, the species of aphids have not been identified precisely (Katayama, unpublished report of Technical Transfer by JICA Expert, February, 2002). To control the aphids efficiently, solutions to these problems are essential.

A few winged aphids were trapped in the yellow pan from June to September (the high-temperature season) in 2006. Little rain was received and few grasses grew in this period, so the development of the host plants was extremely limited. The number of aphids trapped increased somewhat in autumn (October and November), 2006 and more so in spring (April and May), 2007 under moderate temperatures and rainy conditions when grasses germinated at once. These seasonal changes in the numbers of trapped aphids are considered to be caused by the amount of grasses available as host plants. The host plants grew densely under mild seasonal climatic conditions. We also assume that the reproduction and activity of aphids are inhibited by intensely high temperatures.

In Japan, in contrast, the numbers of winged aphids trapped in yellow pans increased in summer in Hokkaido, a cool-climate area (Mizukoshi, 2002), and in spring and autumn in Kanagawa, which experiences a warm climate (Chikaoka *et al.*, 1981). Grasses as host plants grow densely during spring and autumn, therefore the seasonal changes in the numbers of trapped aphids might be determined by the maximum temperatures.

The infestation of potatoes by aphids accompanies the risk of transmitting viruses. Viruses transmitted by the aphids have the greatest impact on potatoes, although direct damage caused by feeding of aphids is also serious (Torikura, 1994). Chikh Ali *et al.* (2006) reported that PVY was the main virus infecting potatoes in Syria. PVY samples in Syria were genetically analyzed in detail (Chikh Ali *et al*., 2007a, b) and a polyclonal antibody was produced (Sankari *et al.*, 2007). The major virus, PVY, is non-persistently transmitted by *M. persicae* or *A. gossypii* (Takanami, 2004). *M. persicae* was the most numerous aphid species among the samples collected on potatoes in Syria, followed by *A. gossypii*. Moreover, *A. fabae* ranked among the major aphid pests of potato, which was examined on potatoes in Turkey by Bostan *et al.* (2006) and can transmit viruses of potatoes (Blackman and Eastop, 1984). In Syria, *A. fabae* is not noted as a potato pest, although *M. persicae* and *A. gossypii* are well known (GOSM, 2005). To our knowledge, this is the first report of the identification of *A. fabae* on potatoes in Syria (GOSM, 2005; Personal communication).

We identified aphids from at least 13 species on potatoes in Syria, but the activities of these aphids as pests on potatoes are unknown except for *M. persicae*, *A. gossypii* and *A. fabae*. Probably numerous individuals among the aphids stayed only temporarily on the potatoes except for *A. craccivora*, *L. erysimi* and other unidentified *Aphis* spp., for which non-winged individuals of each were observed.

In addition, we found adults and nymphs of *R. rufiabdominalis* infesting the underground parts of potato plants. The reproduction of *R. rufiabdominalis* is not known on potatoes in Syria (GOSM, 2005; Personal communication). In Japan, this species is rarely observed on potatoes (Moritsu, 1949), although infestations and reproduction are frequently observed on barley, dry-paddy rice and several other orchard and vegetable crops (Moritsu, 1983). The ability of *R. rufiabdominalis* to transmit viruses of potato is unknown.

In the present study, large colonies of non-winged aphids were not observed on the potato plants, on which they formed extremely small colonies or individuals only. In Japan, however, non-winged aphids usually form large colonies (Fujiie, 1972). It is suggested that the lack of water causes potato plants to become flaccid in the arid climate in Syria and aphids, which are aware of stressed and unhealthy plants, can scarcely reproduce. Viruses are extensively spread as a result of the migratory habit of aphids. It appears that the distribution pattern of aphids in potato fields is random in Syria and that winged aphids randomly fly to potato plants. In Japan, the distribution of aphids is usually concentrated for reproduction (Fujiie, 1972). This phenomenon in Syria should be considered in the future management of aphids in potato fields.

In the autumn-cultivated potato fields, the aphids began to appear in October. Lower percentages of virus-infected plants, which were assumed to be infected by PVY, were observed in September and higher percentages were recorded in October and November. However, in September 11% diseased plants were already found in the un-contracted field, and a lot of diseased plants were present in some un-contracted fields that we incidentally visited in August (data not shown). Early in the growing season (August and September), these viral infections were suspected to be the result of seed potatoes infected with viruses, mostly self-produced ones, for example, and subsequently the viruses were spread by aphids such as *M. persica* and *A. gossypii* late in the growing season (October and November). *A. fabae* was not observed in the season.

In the spring-cultivated potato fields, higher aphid densities were observed in April, just after sprouting, and May. Lower percentages of virus-infected plants were observed in April and higher percentages were recorded from late May in the un-contracted fields. Late in the growing season, from late May to July, the increase in viral infection was assumed to be caused by transmission by aphid species such as *M. persica, A. gossypii* and *A. fabae*.

In the contracted fields, virus-infected plants were seldom observed among both autumn- and spring-cultivated potatoes. Using virus-free seed potatoes of the ‘Super Elite’ or ‘Elite’ classes, eliminated infected plants from the fields, and also spraying with chemical pesticides, through controlling aphid populations, might have reduced the occurrence of diseased plants in the fields.

The broad usage of virus-free seed potatoes and effective management of aphids utilizing several control methods are indispensable to keep virus infection at a low level in Syria. In particular, virus-free seed potatoes should be used, because the disease was suspected to originate from seed potatoes already infected with viruses. However, it is impossible to manage aphids completely with a single method. Needless to say, heavy usage of chemical pesticides should be avoided. The introduction of integrated pest management based on various control methods, predictions of pest emergences and economic injury levels, are mandatory for managing aphids (Fujiie, 1997; Shimanuki *et al.*, 2005).

ACKNOWLEDGEMENT

We gratefully thank Prof. Hidetomo Natsuaki, Utsunomiya University, Japan and Prof. Keiko T. Natsuaki, Tokyo University of Agriculture, Japan for useful comments and advice on the manuscript.

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J. ISSAAS Vol. 14, No 1:60-66 (2008)

SPIDERS IN PADDY FIELDS IN NORTHERN THAILAND

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(Received: October 27, 2007; Accepted: April 14, 2008)

ABSTRACT

A survey of spiders in the paddy fields was carried out from October 2005 to August 2006 at Tambon Mae Faek Mai (approx.19° N, 99° E; 320 meters MSL), Amphoe San Sai, Changwat Chiang Mai, in the northern highland area of Thailand. The spider fauna thus found were *Argiope catenulata* (Doleschall) and *A. inustus* (L. Koch) (Araneidae); *Tetragnatha javana* (Thorell), *T. mandibulata* Walckenaer and, *T. maxillosa* Thorell (Tetragnathidae); *Runcinia acuminata* (Thorell), *Thomisus labefactus* Karsch and *Thomisus* sp. (Thomisidae); *Oxyopes javanus* Thorell and *O. lineatipes* (L. Koch) (Oxyopidae); *Pardosa* (*Lycosa*) *pseudoannulata* (Boesenberg and Strand) (Lycosidae); and *Atypina* (= *Calitrichia*) *formosana* (Oi) (Linyphiidae). All of these were generalist predators of the rice stem borers and rice leaf folder, the dominant lepidopterous pests of rice, and other less important and occasional pests such as rice cutworm, rice caseworm, rice skipper, green rice leafhoppers and rice brown planthopper. The population densities of these insect pests of rice were all below the economic threshold levels and not causing any observable or significant damage during the investigation. These results indicated that the spiders present in the paddy fields served as effective biological control agents.

Key words: generalist predators, biological control agents, rice pests

INTRODUCTION

Natural enemies exhibit close association with the insect pests of rice in the paddy fields. Generally the insect pests of rice do not cause economic damage because of the abundance of the natural enemies of the rice pests including spiders as generalist predators. The importance of the spider fauna in the paddy fields has also long been acknowledged. Okuma *et al*. (1993) prepared an illustrated monograph of the rice field spiders of Bangladesh. Barrion and Litsinger (1994) prepared a chapter on the taxonomy of rice insect pests and their arthropod parasites and predators including spider fauna in South and Southeast Asia. A total of 342 species belonging to 131 genera under 26 families of the rice land spiders of South and Southeast Asia were described and illustrated in Barrion and Litsinger (1995).

The pioneering work on spiders in the paddy fields in Thailand were undertaken by Okuma (1968), Okuma and Wongsiri (1973) and Patarakulpong (1977). Vungsilabutr (no date) prepared an illustrated list of spiders in the paddy fields in Thailand covering 50 species in 36 genera and 14 families highlighting *Pardosa* (*Lycosa) pseudoannulata* and *Tetragnatha* sp. as key natural enemies of the rice brown planthopper (BPH), *Nilaparvata lugens* (Homoptera: Delphacidae), and the rice green leafhopper, *Nephotettix virescecens* (Homoptera: Cicadellidae). Reports concerning several species of natural enemies of rice pests and spiders were also included in Napompeth *et al*. 1983 and Napompeth, 1989). In addition, Yano *et al.* (1997) also made a survey of spiders associated with orchid plants in Thailand. Of the total of 106 specimens of spiders collected, 77 specimens belonging to 12 families were recorded and 29 specimens have yet to be identified. Vungsilabutr (2001) also reported 78 species of diurnal spiders in the citrus plantations of the central plains of Thailand admitting that there might be a lot more of the nocturnal spiders which were not then collected. However, the knowledge about their distribution and abundance in certain microhabitat has not received much attention through the earlier investigations.

This study sought to clarify the spider fauna in association with the insect pests of rice in the paddy fields rotated with vegetables at Tambon Mae Faek Mai, Amphoe San Sai, Changwat Chiang Mai. Rice and vegetables were cultivated under a near "organic" situation where pesticides are hardly used. Since insecticides were not used and only one application of urea was done on vegetables, rice and vegetables cultivated at the site were assumed to be near "organic".

MATERIALS AND METHODS

Site of Investigation

The study site was located at Tambon Mae Faek Mai, Amphoe San Sai, Changwat Chiang Mai in the northern highland area of Thailand. It is served by Highway No. 1001 (Chiang Mai – Phrao) about 20 km north of the city of Chiang Mai. Its geographical position is 18° 57′ - 19° 02′ N latitude and 98° 58′ - 99° 02′ E longitude. The elevation ranges from 320 m on the plain to 947 m on the hilly slope. The temperature averaged from a minimum of 11.6 °C in February to a maximum of 39.3 °C in April, while the average relative humidity (RH) ranged from a low 13% in March to a high 96% from June to December. The overall area is subject to the typical tropical monsoon.

This farming area is cultivated year-round using rice and vegetables in crop rotation and synthetic chemical pesticides are rarely used.

Field Survey, Collection, and Identification of Spiders and Insect Pests

Spiders and their associated pests were collected in the paddy fields for one year from June 2005 to June 2006 to synchronize the collection with the main rice cropping season from June to November and the second cropping season from November to April in Thailand. Field collection was made on a weekly basis during the early morning hours from 08:00-10:00 AM. Since this is strictly a preliminary survey, no attempts were made to collect any quantitative data using any experimental design for statistical analysis.

The spiders were collected by sweep net and/or aspirator and preserved in 70 % ethyl alcohol. For insect pest species on rice they were collected from infested or damaged plant parts. The eggs, immature stages, larvae, pupae or nymphs and adults were collected in the field and transferred to laboratory conditions at the National Biological Control Research Center, Upper Northern Regional Center (NBCRC-UNRC), at Mae Jo University for further laboratory diagnosis and identification, where appropriate. Adults of each species were preserved wet in 70% ethyl alcohol or pinned and dried, as specimens for further identification. Both insect pests and spiders were identified by comparison with the identified specimens deposited at the Natural Enemies Reference Depository (NERD), National Biological Control Research Center (NBCRC) Headquarters at Kasetsart University in Bangkok and other illustrated references such as Okuma (1968), Okuma and Wongsiri (1973), Patarakulpong (1977), Napompeth *et al.* (1983), Barrion and Litsinger (1994), Vungsilabutr (no date), Yano *et al.* (1997), and Vungsilabutr (2001).

All the voucher specimens of spiders and insect pests obtained from this investigation were deposited at the NBCRC-UNRC Insect Collection, Mae Jo University, Chiang Mai, Thailand.

RESULTS

Overview of crop cultivation at the Study Site

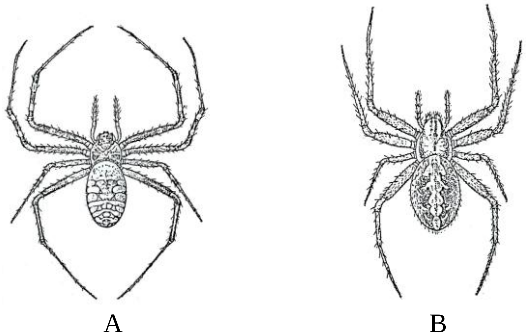
Typical cultivation in Tambon Mae Faek Mai consisted of two crops of rice per annum, the main crop from June to November and the second crop from November to April. Since the area is under irrigation, the rice crop is rotated with some vegetables including cruciferous crops such as cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), Chinese cabbage (*B. chinensis*) and Chinese kale (*B. oleracea* var. *acephala*), cucurbits such as bitter gourd (*Momordica charantia*), angled loofah (*Luffa acutangula*) and cucumber (*Cucumis sativus*), chili pepper (*Capsicum frutescens* and *C. annuum*), eggplant (*Solanum xanthocarpum*), garlic (*Allium sativum*) and yard-long bean (*Vigna unguiculata* subsp. *sesquipedalis*).

Rice varieties cultivated during the main cropping season from June were regular rice Khao Hom Mali 105, sticky rice RD 10, and sticky rice San Pa Tong 1. The rice varieties cultivated as the second crop in November were regular rice Suphan Buri 60 and RD 7, sticky rice RD 10, and sticky rice San Pa Tong 1.

Synthetic chemical pesticides were rarely used in the rice paddies in Tambon Mae Faek Mai for insect pest and weed control. If used, these were synthetic pyrethroids such as permethrin and cypermethrin; organophosphates such as chlorpyrifos, methyl parathion (which has been banned); carbamates such as carbaryl and methomyl; and avermectin and abamectin. Plant extracts also used were rotenone, neem and home-made fermented biological concoctions. Some weeds were controlled by manual weeding and herbicides commonly employed were phosphanate such as glyphosate; bipyridiliums such as paraquat; carbamates such as thiocarbamate; and amides such as alachlor.

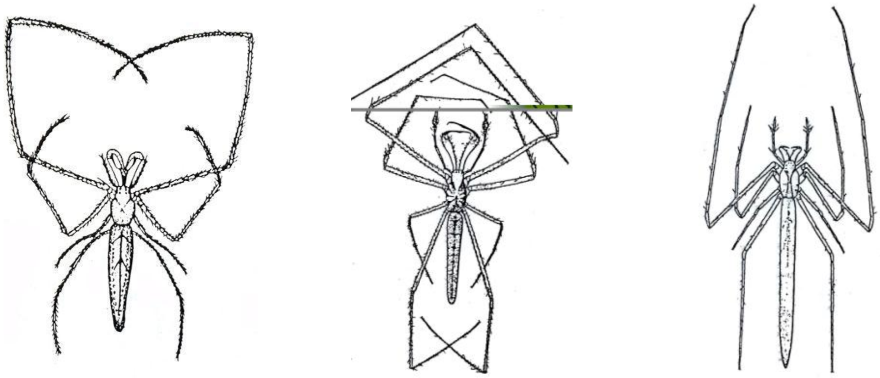
Spiders

In this investigation at least 11 species of spiders were found associated with insect pests of rice in the paddy fields. These were *Argiope catenulata* (Doleschall) and *A. inustus* (L. Koch) (Araneidae) (Fig. 1), *Tetragnatha javana* (Thorell), *T. mandibulata* Walckenaer and, *T. maxillosa* Thorell (Tetragnathidae) (Fig. 2), *Thomisius* sp. and *Runcinia acuminata* (Thorell) (Thomisidae) (Fig. 3), *Oxyopes lineatipes* (L. Koch) and *O. javanus* (Thorell) (Oxyopidae) (Fig. 4), *Pardosa (Lycosa) pseudoannulata* (Boesenberg and Strand) (Lycosidae) (Fig. 5), and *Atypina* (*Calitrichia*) *formosana* (Oi) (Linyphiidae) (Fig. 6).



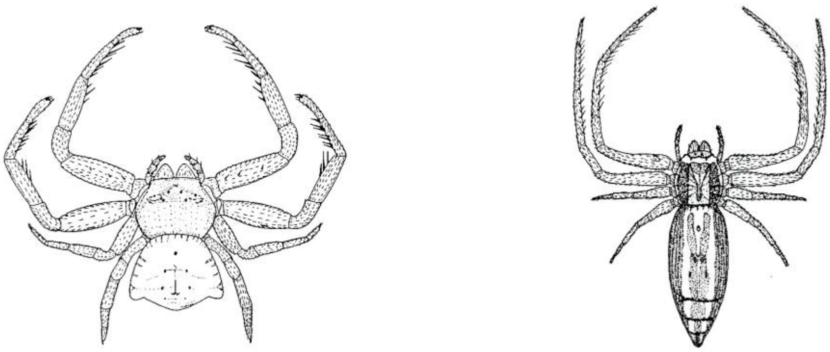
A B

Fig. 1. Family Araneidae: A=*Argiope catenulata* (Doleschall); B=*A. inustus* (L. Koch).



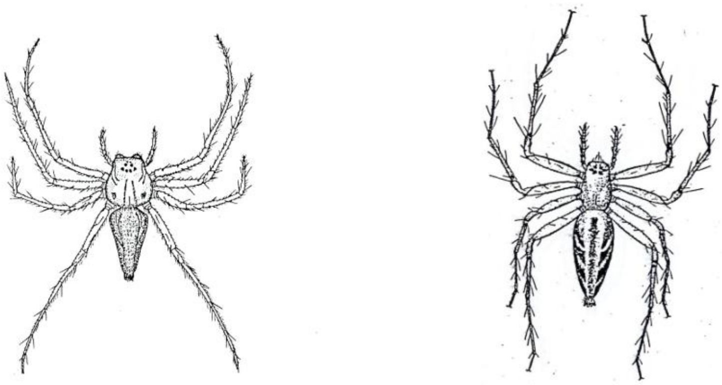
A B C

Fig. 2. Family Tetragnathidae: A = *Tetragnatha maxillosa* Thorell; *B = T. mandibulata* Walckenaer; C = *T. javana* (Thorell).



A B

Fig. 3. Family Thomisidae: A = *Thomisus* sp.; B = *Runcinia acuminata* (Thorell)



A B

Fig. 4. Family Oxyopidae: A =*Oxyopes lineatipe*s (L. Koch); B = *O. javanus* Thorell

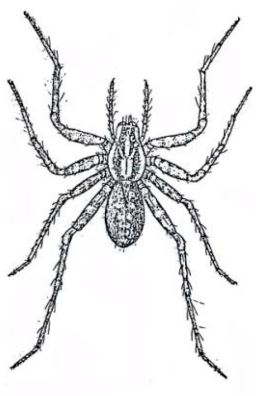


Fig. 5. Family Lycosidae: *Pardosa* (*Lycos*a) *pseudoannulata* (Boesenberg and Strand).

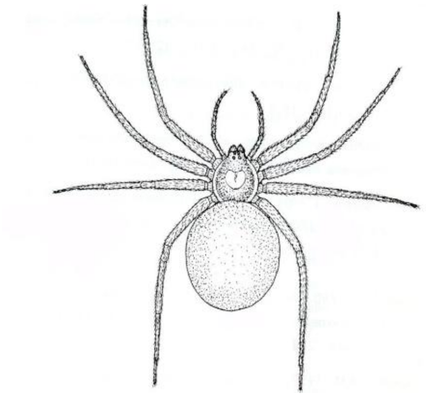


Fig. 6. Family Linyphiidae: *Atypena* (*Calitrachia*) *formosana* (Oi)

Insect pests of rice

For insect pests, the rice stem borer complex was the dominant lepidopterous pests consisting of the yellow rice stem borer (*Scirpophaga incertulas*; Pyralidae), striped rice borer (*Chilo suppressalis*; Pyralidae), and pink stem borer (*Sesamia inferens*; Noctuidae). Other lepidopterous pests which were less important and were found occasionally were rice cutworm (*Mythimna separata*; Noctuidae), rice leaf folder (*Cnaphalocrocis medinalis*; Pyralidae), rice caseworm (*Nymphula depunctalis*; Pyralidae) and rice skipper (*Pelopidas mathias*; Hesperiidae).

Next in its dominance to the rice stem borer complex was the leafhopper and planthopper complex consisting of the green rice leafhoppers (*Nephotettix virescens* and *N. nigropictus*; Cicadellidae) and few other planthoppers such as the rice brown planthopper (*Nilaparvata lugens*; Delphacidae). Other occasional, minor or secondary insect pests encountered were rice gall midge (*Orseolia oryzae*; Diptera: Cecidomyiidae), rice hispa (*Dicladispa armigera*; Coleoptera: Chrysomelidae) and rice thrips (*Stenchaetothrips biformis*; Thysanoptera: Thripidae).

Non-insect pests

Non-insect pests found were the rice paddy crabs, *Somaniathelphusa* spp. (Parathelphusidae); the golden apple snail, *Pomacea canaliculata* (Ampullariidae); the rice weaver birds, *Ploceus manyar* and *P. philippinus* (Ploceidae); and rats and mice, *Rattus* spp. and Mus spp. (Muridae).

DISCUSSION

Predatory Natural Biological Control Agents in Rice and Vegetable Fields

Spiders found in this preliminary study played a very important and vital role as generalist predators of several species of insect pests in the paddy fields where agro-pesticides were rarely or less used. They are naturally-occurring biological control agents. As a result of their occurrence and species abundance, the typical damage to rice plants caused by the insect pests during the period of investigation was hardly observed. Yano *et al*. (1997) reported at least 20 identified species as well as 29 unknown species from 107 specimens collected from orchid plants in the wild and from various orchid nurseries in Thailand. While there were only 11 species of spiders in six families found in this study, it could still be concluded that the spider fauna in the paddy fields is relatively rich and diversified.

Among the entire spider species thus found, it was observed by judging from the number of specimens collected in the fields that *Tetragnata* spp. and *Oxyopes* spp. were the most abundant species encountered. The relative densities of these spiders were not investigated in this preliminary study and, therefore, need to be further quantified employing proper and intensive sampling in the fields. The difference in spider fauna diversity and richness at the study site and other sites could be attributed most probably to the higher use of synthetic chemical pesticides in those areas. Cropping systems of rice in rotation with crucifers, cucurbits and other vegetables with low chemical pesticide input also render the study site a more favorable habitat harboring spiders and other parasitic and predatory insect fauna.

The combination of all these factors might have a biotic influence on the low population densities of the insect pests of rice to cause any observable damage to the crop. As a result, no other control measures, especially chemical control, are necessary. In addition, the study site could also serve as a refuge for other beneficial insect species, and increase its diversity eventually to become a more stable habitat within the overall agroecosystem. It is also obvious that the stability of such a habitat is relatively favorable for the adoption of organic rice production systems. This habitat should be maintained and supplemented with an appropriate natural enemy conservation program. It should also be further modified to become a suitable site of organic rice farming eventually.

CONCLUSION

Spiders found in the paddy fields or elsewhere under other agroecosystems are generalists feeders and are by nature not prey-specific. However, collectively their predatory habit is a density-dependent factor which is of primary importance to natural biological control. However, it should be cautioned that spiders could not be easily and cost-effectively reared under captivity. These are also not readily amenable to laboratory mass rearing to obtain large population. As a result, these could not be utilized in the augmentative biological control program. It is, therefore, strongly recommended that a conservation program of spider fauna should be implemented and carried out concurrently with the conservation and augmentative use of other natural enemies in that particular habitat of rice or vegetables cultivation. In addition, any effective microbial control agents such as *Bacillus thuringiensis* and some entomopathogenic fungi could also be integrated, where appropriate as an additional integrated pest management strategy. This approach could then lead to some extent in the reduction of pesticide use and render the production site more environmentally suitable for organic rice cultivation.

ACKNOWLEDGEMENT

The authors would like to express their appreciation to Dr Akimi Fujimoto, Project Leader, Academic Frontier Research Project (AFRP), Tokyo University of Agriculture, Tokyo, for providing partial support to this investigation and Dr Shuji Okajima, AFRP Natural Enemy Team Leader, for guidance and encouragement. Other facilities and support provided by the National Biological Control Research Center (NBCRC) Headquarters, Kasetsart University, Bangkok and NBCRC Upper Northern Regional Center (NBCRC-UNRC), Mae Jo University, Chiang Mai is also herewith acknowledged.

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J. ISSAAS Vol. 14, No 1:67- 79 (2008)

CURRENT VULNERABILITY OF THE RICE PRODUCTION SECTOR TO RAINFALL VARIABILITY AND EXTREMES IN THE PROVINCE OF CAMARINES SUR, PHILIPPINES

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(Received: September 11, 2007; Accepted: April 3, 2008)

ABSTRACT

This paper discusses the results of a study on vulnerability of the rice production sector to rainfall variability in the Province of Camarines Sur. Results of the study show that the province has become more exposed to climate hazards caused by the increase in rainfall variability during the period 1976-2005. It has become more vulnerable to drought, which reduced annual rice production output by nearly 20 percent. The analysis of risk of crop loss associated with drought also shows that this has been increasing over time, costing nearly P1.2 billion during the period 1996-2005. These results suggest that the increase in rainfall variability puts the province at risk of food insufficiency if no mitigating or adaptation measures are instituted.

Key words: risk, drought, climate change

INTRODUCTION

Rice production has always been affected by changes in climatic conditions. Despite advancements in technology such as irrigation and better seed varieties, climate remains to be a key factor determining rice productivity. In the past years, climate change and their impacts on rice productivity, income and over-all food security has become a central focus of government policies and researches particularly in Southeast Asian countries.

Rice is very important to the Philippines and many of its provincial economies. In recent years, rice sufficiency has become synonymous with food sufficiency, with rice accounting for 35 percent of the calorie intake of the population to as high as 60-65 percent for households in the lowest income groups (DA, 2002). In the Bicol Region, the province of Camarines Sur plays a dominant role in rice production. But in the last three decades, the rice production sector of the province has been under increasing pressure to meet the increasing demand for rice by the growing population given that productive resources (land and water) have increasingly become more limited and that farm inputs have become more expensive. Climate hazards brought about by changing climatic conditions are expected to add to this pressure.

The effects of climate on rice production are well established through climate-based, crop-response models. But these approaches fail to predict actual rice productivity because they do not integrate socioeconomic factors that serve to mitigate the effects of changing climatic conditions. The advent of vulnerability studies served to fill this gap. But while there has been a considerable increase in the number of these types of studies dealing with vulnerability to changing climatic conditions during the past several years, most of these studies were undertaken at the global, regional and national scale. These are of limited value to local decision-makers since they require locally relevant information which they can use as bases for designing and prioritizing interventions to address these vulnerabilities. There is therefore a need to understand the effects of climate hazards in different areas so that interventions can be more focused and appropriate.

This paper discusses the current risks posed by rainfall variability and extremes on the sector and the major socioeconomic impacts particularly on the province's rice production output. Such an understanding is particularly important for developing the appropriate strategies for mitigating the effects of these climatic hazards.

CONCEPTUAL FRAMEWORK

The rice production sector of the province of Camarines Sur is considered as a system that is exposed to risks of crop failure due to climate hazards from variability in rainfall. The analysis of risk follows the conceptual framework presented in Fig. 1. In this framework, risk reduction involves enhancing the capacity of stakeholders particularly in the rice farming sector to prepare for, avoid, moderate, and recover from the effects of climate hazards.

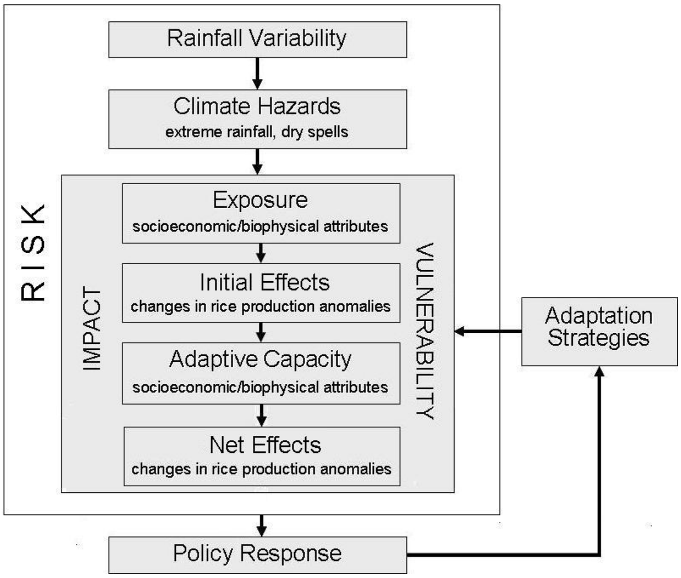


Fig. 1. Climate risk, vulnerability and adaptive capacity framework

Climate Risk

In this paper, risk is viewed as the outcome of climate-related disasters triggered by a physically defined climate hazard (e.g., drought) given the vulnerability of the exposed system. This is compatible with previous definitions of risk being a product of the frequency and consequences of an adverse event (Blaike et al., 1994, cited in Kelly and Adger, 2000; Downing and Patwardhan, 2003; Brooks, 2003; Dolan and Walker, 2004). When applied to the rice production sector, risk is referred to as the loss in rice produce due to the occurrence of a climate hazard such as drought or extreme rainfall events caused by rainfall anomalies.

Formally: $R_i = pH_i \times V_i$ (1)

Where, R_i = risk of crop loss from type of hazard i
pH_i = probability of hazard i
V_i = vulnerability of the sector to hazard i

such that the higher the frequency of a hazard and/or the higher the vulnerability of the sector to the hazard, the greater the risk.

Climate Hazard

Hazard is viewed as a physically defined climatic event that has the potential to cause harm such as extreme rainfall events and/or drought (UNDP, 2003). Climate hazards are typically characterized through hydrological and meteorological variables. In this paper, climate hazards are characterized using the total annual rainfall recorded in the province. A set of criteria was developed to establish the critical values for annual precipitation that would indicate rainfall anomalies or mark the presence of a hazard. Formally,

$$H_i = W_i \qquad W_{lo} \leq W < W_{hi} \qquad (2)$$

Where, H_i = specific type of climate hazard
W_i = actual amount of rainfall
W_{lo} = lower critical value of rainfall for i
W_{hi} = upper critical value of rainfall for i

The probability of a hazard, pH_i in Equation 1, was then associated with the frequency by which the actual amount of rainfall occurs within these critical values over a period of time.

Vulnerability to Climate Hazards

Vulnerability is a composite of the biophysical and socioeconomic attributes of the rice production sector that causes it to become susceptible to and unable to cope with the effects of a climate hazard (Kelly and Adger, 2000; IPCC, 2001, cited in Brooks, 2003). Vulnerability, in this view is systemic and a consequence of the state of development that is often manifested in aspects of human conditions such as undernourishment and poverty (Downing and Patwardhan, 2003). Using this lens, vulnerability is specifically referred to in this paper as the outcomes determined by the interactions between the biophysical and socioeconomic conditions of the rice production sector and rainfall variability measured in terms of anomalies in the amount of rice produced.

RESEARCH METHODOLOGY

Formulation of Risk Criteria

Climate hazards such as drought and extreme rainfall events are known to negatively affect rice productivity at varying degrees, depending on the severity and frequency by which they occur. Thus, a set of criteria was used to mark the occurrence of rainfall anomalies or hazards, and to determine the severity, frequency, level of risk and magnitude of the impacts associated with their occurrence.

The set of criteria used is a modification of the "decile" technique developed by Gibbs and Maher in 1967. The attractiveness of this technique compared to other techniques for measuring rainfall anomalies lies in the ease of calculation using fewer data and assumptions while still providing an accurate statistical measurement of precipitation. The need for a long climatological record to calculate the deciles accurately however is a disadvantage of the decile system (Hayes, 2006).

Using the modified decile method, the annual rainfall data recorded in the province over a 30-year period (1976-2005) obtained from the CSSAC-PAGASA-PCARRD Agromet-Synoptic Station in Pili, Camarines Sur, was divided into fifths of the distribution (Table 1). Each part of the distribution or category was called a "quintile." Ideally, the monthly and annual rainfall data covering a 50-year period at the sub-provincial level is needed to determine the degree of spatial variations in climate hazards across the province. But the choice of data was constrained by the fact that there is only one agromet-synoptic station in the province and was only established in 1975. Thus, with this limited data, it was simply assumed that municipalities were subject to uniform climate hazards. The implication of this assumption of identical climate hazards is that the differences in risk outcomes are assumed to be due solely to variations in vulnerability (i.e., biophysical and socioeconomic conditions) across different municipalities.

Table 1. Categories of annual rainfall

Categories	Description
1st Quintile: Lowest 20 %	Much below normal
2nd Quintile: Next lowest 20 %	Below normal
3rd Quintile: Middle 20 %	Near normal
4th Quintile: Next highest 20 %	Above normal
5th Quintile: Highest 20 %	Much above normal

Characterizing Current Climate Hazards

Using the quintile categories of rainfall, a climate hazard is said to exist when the amount of rainfall occur within the range of the lowest or highest 40% of the rainfall distribution. The probability of a hazard is then measured as the frequency by which it occurs over a period of time. The recorded annual distribution of rainfall over the last 30 years (1976-2005) was further divided into three separate decades to allow for comparison and to determine the trend in the occurrence of climate hazards over the period.

Assessment of Current Vulnerability

It is recognized that the rice production sector is very vulnerable and greatly affected by climate hazards. Vulnerability in this context is measured by the amount of change in the level of rice produced due to the occurrence of a hazard. The initial assessment of the vulnerability of the rice production sector to rainfall variability was made using the 30-year (period 1976-2006) rice production data of the Bureau of Agricultural Statistics (BAS) for Camarines Sur and the corresponding annual rainfall anomalies derived earlier. A comparison of the trend in the anomalies for both annual rice production and annual rainfall was the first step in determining vulnerability.

The anomaly data for annual rice production was determined by calculating the deviation of an observed data from the mean of the data set. Using this method, an observation greater than the mean yields a positive deviation, whereas an observation smaller than the mean yields a negative deviation. The extent of vulnerability of the rice production sector to climate hazards caused by variability in rainfall was then determined by comparing the anomalies for annual rice production and annual rainfall. An increasing negative trend in the anomalies means that the production loss from climate hazards is increasing over time and that the rice production sector is becoming more vulnerable.

The amount of loss in rice production due to the occurrence of a particular climate hazard was estimated by regressing yearly rice production output with the time variable and a dummy variable representing the hazard, and excluding one category, "near normal" rainfall, to serve as basis for comparison.

The following regression equation illustrates the procedure:

$$Y = \alpha + \beta_1 DummyH_1 + \beta_2 DummyH_2 + \beta_3 DummyH_3 + B_4 DummyH_4 + \beta_5 Time + \mu \quad (3)$$

where Y is annual rice production output; α is a constant term; $\beta_1, \ldots, \beta_5$ are regression coefficients; $DummyH_1$ is a hazard dummy representing "much below rainfall", $DummyH_2$ = "below normal" rainfall, $DummyH_3$ = "above normal" rainfall, and $DummyH_4$ = "much above normal" rainfall; *Time* is in years; and μ is an error term. The hazard dummy, say $DummyH_1$, is equal to "1" if the hazard exists, and "0" if otherwise. A negative sign of the regression coefficient, β, suggests that amount of rice produced has declined due to the occurrence of the hazard event. Thus, the value of the coefficient, β, is a good indicator of the decrease in amount of rice produced due to the hazard. It is assumed that the decrease in the amount of rice produced results to a large extent from the occurrence of a climate hazard.

Assessment of Current Climate Risk

Risk is defined in this paper as the product of the probability and consequences of a climate hazard. It is the outcome of the interaction between the climate hazard and the vulnerability of the rice production sector to these hazards. Thus, "risk = probability of a hazard x degree of vulnerability." The degree by which the rice production sector is vulnerable to a particular hazard is based on the amount of loss in annual rice production computed using Equation 3. Thus, the higher the frequency of a hazard and/or degree of vulnerability, the greater is the risk of crop loss. Decadal comparison of the risk posed by climate hazards from rainfall variability was made by modifying Equation 1, such that:

$$\overset{j}{\underset{i=1}{R^T}} = \sum (pHi * Vi)^T \qquad i = 1,\ldots,j \quad (4)$$

Where, T = time period
j = set of climate hazards
i = specific type of climate hazard
pH_i = probability of i^{th} hazard in j at time T
V_i = sector vulnerability to i^{th} hazard in j at time T
$\overset{j}{\underset{i=1}{R^T}}$ = aggregate risk from hazard $i = 1,\ldots,j$ at time T

RESULTS AND DISCUSSION

The province of Camarines Sur is located some 447 kilometers southeast of Manila. It is the largest among the six provinces in the Bicol Region. The province's total land area comprises around

30 percent (548.2 thousand hectares) of the region, over half (262.9 thousand hectares) of which is used for agricultural production (NSO, 2004). In year 2000, the total population of Camarines Sur was 1.55 million, comprising 33 percent of the region's total population. It increased at an average rate of 1.72 percent in 1995-2000. Its total population consisted of 296 thousand families, 67 percent of whom resided in rural areas, where agricultural activities are the main source of income (NSO, 2000).

Rice production is very important to the country and many of its regional economies. In recent years, rice sufficiency has become synonymous with food security, especially among the poor. The rice production sector of Camarines Sur plays a dominant role in the rice economy of the Bicol Region because of its relatively high level of productivity of 3.1 MT per hectare. The province provides 46% of the total rice area as well as 47% of the annual regional production output.

However, similar to other areas of the country, the province's rice production sector has remained underdeveloped. During the past three decades, this sector has been under a lot of pressure to produce enough to meet the increasing demand for rice despite the decline in cultivated area which has shrunk by 12.4 percent during the period 1991-2002. Farming has also become less viable as a business with a reduction in the net profit-cost ratio from 0.15 in 1991 to only 0.06 in 2002 (PhilRice-BAS, 2004). Poverty incidence among rural families has remained high and increased a little from 52.6 percent in 1991 to 57.8 percent in 2000 (PIDS, 2003).

In response, the government launched in 2002 the "Ginintuang Masaganang Ani Rice Program" as one of the core programs of Agriculture and Fisheries Modernization Act with DA as the lead agency and several other government agencies supporting it. The program was expected to benefit the farmers through increase in productivity, income and over-all food security by providing improved seed varieties, access to credit facilities, improved marketing systems and facilities, better irrigation services, and extension and trainings on integrated farming systems. However, what the program was not able to address were concerns related to changing climatic conditions which have reversed some of these gains.

Current Climate Hazards

The discussion in the paper focuses on the assessment of the risks and impacts related to climate hazards due to annual rainfall variability, such as drought and extreme rainfall events, on the rice production sector of Camarines Sur. By and large, these climate hazards are known to adversely affect the productivity of the rice sector in varying degrees, depending on the severity and frequency by which they occur. The set of criteria used to characterize annual rainfall anomalies is presented in Table 2.

Table 2. Categories of annual rainfall, Camarines Sur, 1976-2005

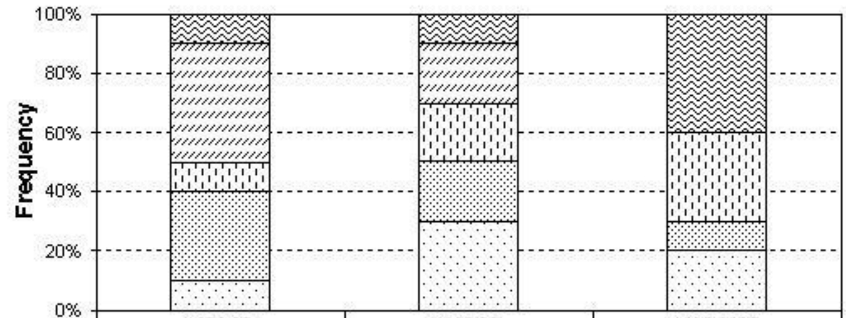
Categories	Amount of Annual Rainfall (mm)	Description
1st Quintile: Lowest 20 %	< 1,690.14	Much below normal
2nd Quintile: Next lowest 20 %	< 2,066.68	Below normal
3rd Quintile: Middle 20 %	< 2,303.34	Near normal
4th Quintile: Next highest 20 %	< 2790.12	Above normal
5th Quintile: Highest 20 %	≥ 2,790.12	Much above normal

As shown in Table 2 for example, the first or lowest quintile includes annual rainfall of less than 1,690 mm and described as "much below normal". Likewise, the second quintile was defined by the amount of annual rainfall (2,066.68 mm) not exceeded by the lowest 40% of the observations. On the other extreme, the 5th quintile includes annual rainfall exceeding 2,790 mm and described as "much above normal". This is the largest amount of annual rainfall (2,790.12 mm) recorded in the province within the last 30-year period (1976-2005).

These categories for annual rainfall were used to determine the presence of climate hazards such as annual rainfalls with levels within the lowest or highest 40 percent of the rainfall distribution. The lowest 40 percent of the rainfall distribution is characterized by dry spells or drought conditions, while the highest 40 percent is often accompanied by the occurrence of tropical cyclones. The probability of a climate hazard was based on the frequency by which these hazards occurred over a period of time. The nature and extent by which the rice production sector was exposed was determined by a decadal comparison of the frequency by which the hazards occurred.

Annual Rainfall Anomalies

The recorded distribution of annual rainfall was categorized based on the procedure described in the previous section and the result of which is shown in Fig. 2 below.



	1976-85	1986-95	1996-2005
Much Above Normal	10%	10%	40%
Above Normal	40%	20%	0%
Near Normal	10%	20%	30%
Below Normal	30%	20%	10%
Much Below Normal	10%	30%	20%

Fig. 2. Decadal comparison of total annual rainfall by quintile classification and frequency, Camarines Sur, 1976-2005

Decadal comparison of total annual rainfall showed that the incidence of "near normal" rainfall has increased over the period, from 10 percent in 1976-1985 to 30 percent in 1996-2005. However, the incidence of "much above normal" rainfall has also increased considerably, from 10 percent in 1976-1985 to 40 percent in 1996-2005. In contrast, the frequency of "above normal" rainfall has considerably decreased, from a high of 40 percent in 1976-1985 to near zero in 1996-2005. On the other hand, while the frequency of "below normal" rainfall has decreased, from 30 percent in 1976-1985 to 10 percent in 1996-2005, the incidence of "much below normal" rainfall has increased from 10 percent in 1976-1985 to 20 percent in 1996-2005. The increase in "much below normal" rainfall in 1986-1995 is consistent with the occurrence of the El Niño phenomenon in the province.

Moreover, similar analysis of total monthly and seasonal rainfall revealed that during the dry season, the province has become increasingly exposed to drought and extreme rainfall conditions. Relatively heavy rainfall activity early in the dry season (November-December) has increased, while relatively low level rainfall activity during the later month of April appeared to extend up to the early months of May to July of the wet season, suggesting a delay in the coming of the monsoon rains. By contrast, the wet season has been characterized by relatively less extreme rainfall conditions, from "near normal" to "above normal" rainfall, with increasing heavy rainfall activity in October.

Overall, the results show that the frequency of extreme weather events in the province has increased over the past three decades, marked by the increase in the occurrence of "much below normal" and "much above normal" annual rainfall. These findings suggest that by and large, rainfall as a resource critical to rice production has increasingly become uncertain and more likely to occur in the extreme, characterized by periods of long dry spells and/or drought conditions as well as heavy rainfall events, particularly during the dry season. As rice production in the province is largely dependent on rainfall, it is reasonable to expect that climate hazards caused by the increase in rainfall variability will have an adverse effect on the rice production sector from shortages in irrigation water and shortened cropping periods.

Current Vulnerability to Climate Hazards

An assessment of the trend in the anomalies in annual rainfall and rice production in the province over 30 years from 1976 to 2005 was undertaken to determine changes in the level of vulnerability of the rice production sector to climate hazards caused by annual rainfall variability.

The deviation of observed rice production data from the mean was computed to obtain the rice production anomaly data. These production anomalies were then grouped into the annual rainfall categories developed in the previous section and plotted over the time period from 1975 to 2005 (Fig. 3). The results show that while there has been a noticeable increase in rice production during the years with "near normal", "below normal" and "much above normal" rainfall, there has been a noticeable decline in rice production during years with "much below normal" rainfall. This means that the production of rice has declined over time due to drought indicating that the rice producing sector of the province has become more vulnerable to the changing rainfall conditions.

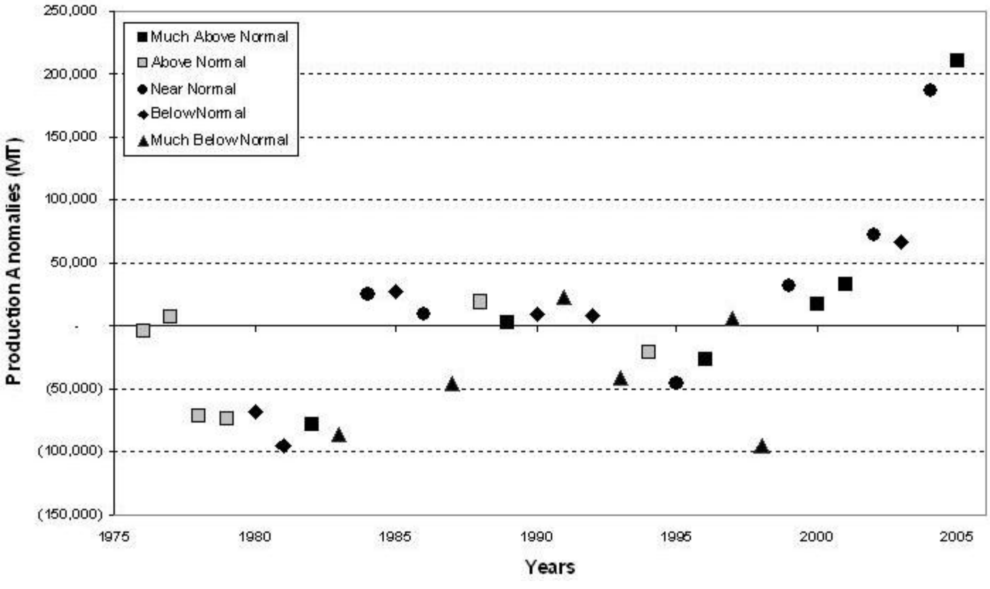


Fig. 3. Time trend of anomalies in annual rainfall and rice production output, Camarines Sur, 1976-2005.

The shifts in the volume of rice production due to the occurrence of annual rainfall anomalies over the period 1976-2005 was estimated using the regression model given by Equation 3. The total annual rice production output taken from the data of BAS was regressed against a time trend and the individual dummy variables representing the five categories of annual rainfall. Prior knowledge suggested that the occurrence of a climate hazard such as drought or extreme rainfall events cause rice production to decrease. A summary of the results of the regression analyses is shown in Table 3 below.

Table 3. Results of the regression model for rice production anomalies, Camarines Sur, 1976-2005.

Predictors	Coefficients	Std. Error	*t*-Value	Sig.
Constant	-10,257,862	2,844,338	-3.606	.001
Time	5,323	1,426	3.734	.001
$DummyH_1$	-68,128	32,398	-2.103	.046
$DummyH_2$	-20,729	33,326	-0.622	.540
$DummyH_3$	-1,456	36,990	-0.039	.969
$DummyH_4$	-23,348	32,020	-0.729	.473

Adjusted RSquare = .395 F = 4.783 Sig. = .004

The results show that the annual rice production is not affected by amount of rainfall which are "below normal" ($DummyH_2$), "above normal" ($DummyH_4$) and "much above normal" ($DummyH_5$). This is indicated by the non-significance of the coefficients. Possibly, this is because the use of total annual rainfall and rice production data is not able to fully capture the timing and nature by which climate hazards occur within the year, as well as its corresponding effects on rice production. The prevalence of "much above normal" rainfall, for instance, may have occurred at a time when farmers have already harvested their rice and so it did not entirely affect the volume of rice produced. In this case, the analysis may be improved by using more detailed data such as the seasonal or monthly distribution of rainfall and rice production output.

On the other hand, the results show that volume of rice produced dropped significantly by around 68,128 MT when the amount of rainfall was much "below normal". This is indicated by the negative and relatively large coefficient (-68,128 MT) of its dummy variable, $DummyH_1$. The decrease in the volume of rice produced may be partly explained by the significant decrease in the total area harvested to rice due to the lack of supply of irrigation water.

The volume of rice lost compared to the "near normal" production as indicated by the coefficient for $DummyH_1$ is estimated at 68,128 MT or 551 kgs./ha during a "much below normal" or drought condition. This value is also indicative of the degree of vulnerability of the province's rice production sector to drought. The crop loss due to drought is estimated at nearly 20 percent of province's average annual rice production output of 361,298 MT. Assuming an average value of P8,740 per MT of unmilled rice, the estimated value of crop loss due to drought is placed at P595.44 million annually.

Moreover, the results suggest that the occurrence of "much below normal" rainfall or drought has an adverse effect on capacity of the province to domestically produce the total rice requirement of its population, that is, to be self-sufficient, for a given period. In 2005, the food demand for rice was 717 MT per day or 258,120 MT per year (NSCB, 2005). This means that, with an average annual rice production of 361,298 MT, the province's production sufficiency ratio was 140 percent, suggesting that it had an excess supply of rice of about 103,178 MT or equivalent to 144 days of supply of rice. But, this also means that, in case of "much below normal" rainfall or drought, the resulting decrease in annual rice production of 68,128 MT will reduce the province's excess supply of rice to only 35,050 MT or equivalent to only 48 days supply of rice. Moreover, the implications of a decrease in Camarines Sur's supply of rice may be expected to extend to other provinces that normally depend on its surplus for their own supply of rice. The "knock-on" effects of the decrease in rice production and income may likewise be felt through the forward and backward linkages of the rice sector with the other sectors of the local economy in terms of reduced demand, income and employment.

The effects of the "much below normal" rainfall occurrence are felt strongly at the farm level. An average rural family in Camarines Sur normally earns an annual income of P72,626, 18.5 percent (P13,436) of which comes from raising crops such as rice in a 2 hectare farm lot (NSO, 2000). Thus, during a time of drought, rural households or 67 percent of the families in the province would lose over 70 percent (P9,634) of their agricultural income. This suggests that, in the event of drought, average rural families will not be unable to produce enough rice to meet their own household requirements. Conceivably, the consequent loss of income can cause rural families to fall back into temporary or transitory poverty. This condition at the farm level can be magnified at the provincial level.

As a whole, the results suggest that the province in general and small farmers in particular are at risk from increased crop loss and food insufficiency due to climate hazards, particularly drought, caused by rainfall variability if no mitigating or adaptation measures are instituted.

Current Climate Risks

The previous findings suggest that compared to other climate hazards, the province is more vulnerable to the occurrence of "much below normal" rainfall or drought. The current risk from drought due to the rainfall variability in 1976-2005 is calculated using Equation 4. The corresponding probability of occurrence of drought and the loss in annual rice production associated with the hazard are obtained from the results of the previous sections. The observations are divided into three decades for comparison to determine whether there are changes in the levels of risk over time. Results of prior analysis show that there has been an increase in the level of risk due to the increase in extreme weather events.

Table 4. Estimated current risk of crop loss due to drought by decade, Camarines Sur, 1976-2005.

Decade	Frequency of Drought	Estimated Crop Loss	
		Volume (metric tons)	Value (billion pesos)
1976-1985	1	68,127	0.595
1986-1995	3	204,381	1.786
1996-2005	2	136,254	1.191

Assumptions: Estimated annual crop loss in volume due to drought is 68,127
Price of unmilled rice is 8,740/MT

The results show that the estimated risk of loss in rice production output due to drought has increased by 100 percent, from P0.595 billion in 1976-85 to P1.191 billion in 1996-2005, brought about by the increase in the frequency of "much below normal" rainfall (Table 4). The amount of risk was highest in 1986-1995, reaching P1.786 billion due to the series of the El Niño events that occurred during the period. Overall, this indicates that the province's rice production sector has increasingly been placed at risk from drought due to rainfall variability, specifically, the occurrence of "much below normal" rainfall. These estimates may be used by concerned agencies as input in developing vulnerability-reducing strategies, particularly in rationalizing the cost of these strategies or interventions.

CONCLUSION

The province is more vulnerable to drought, which is marked by the occurrence of "much below normal" rainfall conditions compared to the other climate hazards from increase in rainfall variability. Over the last three decades, production has been declining due the increasing occurrence of "much below normal" rainfall events. This phenomenon is indicative of the increasing vulnerability of the rice production sector of the province. The province's annual rice production during the period 1976-2005 for example dropped by nearly 20 percent compared to the "near normal" trend because of drought.

Drought conditions, among the different climate hazards mentioned, have the greatest impact on the province's production of rice. With drought or "much below normal" rainfall, the resulting decrease in annual rice production of 68,128 MT will reduce the province's rice surplus to only 35,058 MT which is equivalent to only 48 days supply of rice. Moreover, the implications of a decrease in Camarines Sur's supply of rice may be expected to extend to other provinces that normally depend on its surplus for their own rice supply. Its "knock-on" effects such as reduced demand, income and employment to other industries in the local economy may also be felt through forward and backward linkages of the rice production sector

At the farm level, about 67 percent of rural households in the province loses at least 70 percent (P9,634) of their annual agricultural income due to the "much below normal" rainfall. This suggests that they will not be able to produce enough rice for their own consumption. Similarly, rural families are estimated to lose around P4,380 or 33 percent of their annual farm income in years of "below and/or much above normal" rainfall.

For the rice producing sector of the province, the estimated risk of loss in the volume of rice produced due to the increasing frequency of drought conditions has increased by 100 percent over the last three decades, from P.595 billion for the period 1976-85 to P1.191 billion for the period 1996-2005.

These findings suggest that the changing climatic condition has increased the risks faced by the rice producing sector of the province with a consequent reduction in productivity and farm incomes which threatens the food security especially of small farmers. There is a need therefore to invest in programs which will enhance the capacity especially of small farmers to adapt to these changing climatic conditions through different mitigating measures.

In developing these adaptation programs, it is important to gain a more detailed understanding of key socioeconomic conditions that make local communities vulnerable to the risks posed by current climate hazards, as well as the various adaptation measures that they had actually used to manage these risks. Even if future adaptation strategies would be different, today's adaptation measures will provide valuable inputs for developing those strategies. It is therefore recommended that adaptive capacity studies at the local level should be conducted for this purpose.

The effects of drought compared to typhoons on average rural families are gradual, less dramatic, often invisible but persistent and take them much more time to recover. Often also, the delay in getting vital information on current climate conditions from the National Weather Bureau often results to delays in getting the appropriate assistance from the local and national government units. There is a need then for local government units to be able to monitor on their own the onset of changes in climatic conditions and implement on time appropriate mitigating measures. This becomes especially important with the increase in the likelihood of occurrence of drought and the vulnerability of rural families to this type of hazard. Initially, this task may require enhancing the capacity of local government units to employ drought monitoring tools and develop a planned response that is an integral part of its disaster management program.

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UTILIZATION OF DAIRY CATTLE MANURE-RICE HULL COMPOST USING MICROBIAL INOCULATION

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(Received: September 26, 2007; Accepted: April 10, 2008)

ABSTRACT

The effects of seeding nitrogen-fixing bacterial inoculants into cattle manure-rice hull mixtures in composting were evaluated, particularly in terms of nitrogen gain in the substrate and level of survival of the bacteria. The effects of such compost on wetland rice as test crop was also determined. One hundred-gram weights of dry and screened dairy cattle manure-rice hull mixture (70/30, w/w) were placed in nylon bags and composted for 20 days in actual compost piles of the same materials. Burk medium (without glucose) was added to the substrates. Inoculation treatments of nitrogen-fixing bacteria *Azotobacter* sp. or cellulolytic fungi of *Trichoderma* sp. were imposed on the materials. Carbon loss (66.7 mg C/g vs. control's 59.7) was high and nitrogen gain (14.50 mg N/g vs. control's 7.02) was highest in those treated with nitrogen-fixing bacteria. The survival of the inoculant organisms was highest in those correspondingly treated compost. *Azotobacter* counts in treated compost were 4.22 x 10^6 cfu/g compost, significantly higher than the control with 1.13 x 10^6 cfu/g compost. The inoculated compost produced in this study was tested in wetland rice, and was found to significantly improve the tiller number, panicle number, and grain yield of the test crop at levels similar to those supplied with chemical fertilizers.

Key words: compost, cattle manure-rice hull mixture, nitrogen-fixing bacteria, *Azotobacter* sp., cellulolytic fungi, *Trichoderma* sp.

INTRODUCTION

Composts or organic fertilizers are renewable inputs for crop production. These can be produced from organic wastes whenever needed. The continuing increase in human population coupled with the increase in livestock-raising for food would indicate the magnitude of waste generation which at the same time is directly related to present-day pollution problems. On the other hand, chemical fertilizers are not renewable such that their use must be in regulated amounts. Chemical nitrogen fertilizers make use of depletable fossil fuels while chemical phosphatic fertilizers depend on the continuous mining of rock phosphates.

The use of organic materials in crop production is known to provide nutritional and other benefits (Yoshida, 1995), and may serve to offset the excessive or improper use of chemical fertilizers (Ahmed, 1994). Composts, in particular, can be superior to chemical fertilizers in the cultivation of field plants (Fujio et al, 1986), and when in combination with beneficial microbial inoculants, could further boost sustainability and efficiency. The combined use of organic matter and microbial inoculants had been shown to give nitrogen increases in the order of 6 to 14 mg/g of substrate consumed (Lynch, 1984). Other benefits of microbial inoculation of organic residues such as improvements of soil-stabilizing properties of wheat straw have been noted (Lynch and Wood, 1984). The problem of disposal of cattle manure and rice hull has not been solved yet in many areas of the country. In one province alone**,** such as Batangas in Southern Luzon, dry cattle manure production reaches 647.8 t per year, while rice hull generation amounts to 44.6 t per year (BAS, 1995).

To serve both the ends of sustainable crop production and pollution control, the conversion of organic wastes into organic fertilizers is important. The use of microbial inoculants in composting could provide many benefits not fully known at present. The study sought to determine the effects of seeding nitrogen-fixing bacterial inoculants into cattle manure-rice hull mixtures in composting, particularly in terms of nitrogen gain in the substrate and level of survival of the bacteria. The effects of such compost on wetland rice as test crop was also determined in this study.

MATERIALS AND METHODS

Preparation of Raw Materials

Cattle manure and rice hull were collected in separate lots, air-dried, and oven-dried at 60 °C for several days until constant weight was achieved. The dried materials were ground and sieved through a 2-mm screen. The sieved materials were stored dry in closed polyethylene bags until ready for use.

Nylon-bag Experiment Set-up

The materials were weighed separately, and mixed individually in 100-g lots of cattle manure: rice hull (70/30, w/w), initially in plastic pails to allow for full mixing and wetting overnight with 200 mL Burk medium (without glucose) consisting of the following: K_2HPO_4, 0.8 g/L; KH_2PO_4, 0.2 g/L; $MgSO_4$, 0.20 g/L; NaCl, 0.20 g/L; $CaSO_4{\cdot}2H_2O$, 0.10 g/L; $Fe_2(SO_4)$, 13 mg/L. Each mixed and wetted batch was placed into a nylon net bag (4 cm x 16 cm), tied and closed at one end with a nylon string. The following treatments were used: T1=uninoculated; T2=inoculated with *Trichoderma* sp. SS33 at 1 % (v/w); and T3=inoculated with *Azotobacter* sp. HIBFA4b at 1 % (v/w). One-mL sterile coconut water was added into the uninoculated controls to correspond to the liquid inocula added into the inoculated treatments. The nylon bags containing the samples were embedded at a depth of 0.3 m in a compost pile measuring 2 m x 1m x 0.6 m (L x W x H) of cattle manure-rice hull mixture (70/30, w/w) and allowed to compost for 20 days. Each treatment was replicated 3 times.

In this set-up, the heap (consisted of the same materials) was mainly used as generator of actual composting conditions with the bagged samples as the subject of composting. Comparison was made only among bag samples treated similarly and the equivalent control. All the treatments were subjected to the composting conditions imposed by the heap. The structure of the heap is described in Fig. 1.

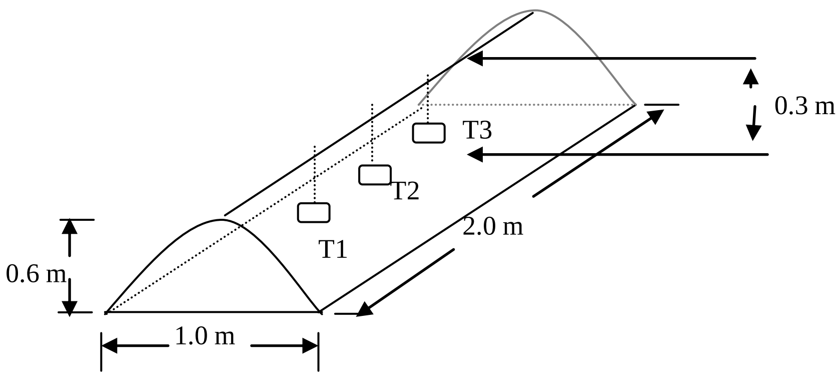


Fig. 1. Schematic diagram of nylon-bag composting experiment.

Microbial Inocula Preparation

The *Trichoderma* sp. SS33 inoculum was prepared by growing seed culture in potato-dextrose broth (PDB) and by dilution technique, separating a clear-cut colony growing in potato dextrose agar (PDA). The colony was grown to abundance in PDA slant for 3 to 4 days. A 1-cm growth was cut from the slant and regrown in 4-mL sterile coconut water in 10-mL sterile tubes. One-mL volumes were taken from the liquid broth for inoculation into the raw materials which were placed in nylon bags. The bacterial inoculants of *Azotobacter* sp. HIBFA 4b were prepared using standard procedures. A loopful of inoculum was taken from a previously purified stock culture and streaked in Burk medium (BM) agar plates. The plates were incubated for 3-4 d at 30 °C and a single colony was picked for re-growing in abundance in 4-mL sterile coconut water in 10-mL tubes with shaking at 30 °C for another 3-4 days. From the cloudy broth of the purified strain, one-mL volumes were taken for inoculation into raw materials that were placed in nylon bags.

Physical and Chemical Analysis

Percent weight loss was determined by deducting the final oven-dry weight of materials from the initial dry weight and multiplying the value by 100. The chemical characteristics of the resulting compost were determined using recommended procedures (PCARRD, 1980). Total N was determined using an automatic nitrogen analyzer. The ignition method was used to determine the total carbon content. The total phosphorus content of the compost was determined using the vanado-molybdate method.

Microbial Count

One-g samples from each treatment was taken after thorough mixing and placed separately in 9-mL sterile distilled water and shaken for 1 hr. Appropriate dilutions were made. Aliquots of 1 mL from 10^{-5} to 10^{-8} dilutions were spread-plated on appropriate growing media (PDA, BM or NA) depending upon the target organisms. Colony-forming units (cfu's) on each plate were counted. The corresponding cfu/g dry weight was calculated. All *Azotobacter*-like colonies were picked out from BM agar plates after a 3 to 4 day growing period. These were tested for morphological and physiological characteristics, and intrinsic antibiotic resistance (IAR) patterns (Tchan, 1984), relative to the inoculum strain. Trichoderma-like fungal isolates from each treatment were evaluated relative to the fungal inoculum strain based on cultural and morphological characteristics (Rolph and Miner, 1998).

Field Testing of Inoculated Compost in Wetland Rice

The use of inoculated compost [i.e. corresponding to T3 of the composting experiment; inoculated with *Azotobacter* sp. HIBFA4b at 1 % (v/w)] was tested in Los Banos, Laguna, during the wet season. The test soil was Maahas clay loam, pH 6.0, 0.35 % total N, and organic matter content of 5.08 %. Wetland rice cv. IR-74 was the test crop. The seedlings were grown in soil bed lined with heavy paper for 10 days, and these were transplanted into plots laid out in randomized complete block design (RCBD) with 4 replications per treatment.

The following treatments were used in the field test: T1 = control (no fertilizer applied); T2 = added with chemical fertilizer at the rate per ha of 90 kg N, 60 kg P, and 60 kg K; T3 = added with chemical fertilizer at the rate per ha of 45 kg N, 30 kg P, and 30 kg K, and inoculated compost (500kg/ha, 30 % moisture); T4 = added with inoculated compost at 500 kg/ha; and T5 = supplied with chemical fertilizer at the rate per ha of 90 kg N, 60 kg P, 60 kg K, and inoculated compost at 500 kg/ha.

Organic fertilizer or inoculated compost used in this experiment came from one stock, and analyzed to have these properties: 1.28 % total N, 0.75 % total P_2O_5, and 1.50 % total K_2O, and 24.59 % total C.

The whole amount of inoculated compost of 500 kg/ha was applied basally and incorporated into the soil during the final leveling of the field. For chemically treated plots, chemical fertilizers were applied in split, 60-60-60 was broadcast basally, and the remaining N at 30-0-0 was topdressed at plant panicle initiation stage. Irrigation water was allowed into the plots one week after transplanting. The grains were harvested when about 90 % of the grains in all plots turned yellow. Plant height and tiller number were taken 30 days after transplanting (30 DAT), and at harvest. Plants, within a 2m x 3m harvest area in each plot, were cut with a sickle and collected. Sample plants were threshed, cleaned and straw weights were recorded. Grains from each plot were weighed and oven-dried at 60°C for 3 days.

RESULTS

Effects of Microbial Inoculation on Total N and C Content and Weight Loss

Inoculation was done where required in all samples prior to composting. Selected properties of raw materials are presented in Table 1. The cattle manure- rice hull mixture had the following initial contents: total N, 1.05 %; C, 30.34 %; and C/N of 29.0. The composting heap was used to provide the actual composting conditions and thus the bagged samples were the ones analyzed. The homogeneity of starting materials was critical for comparison.

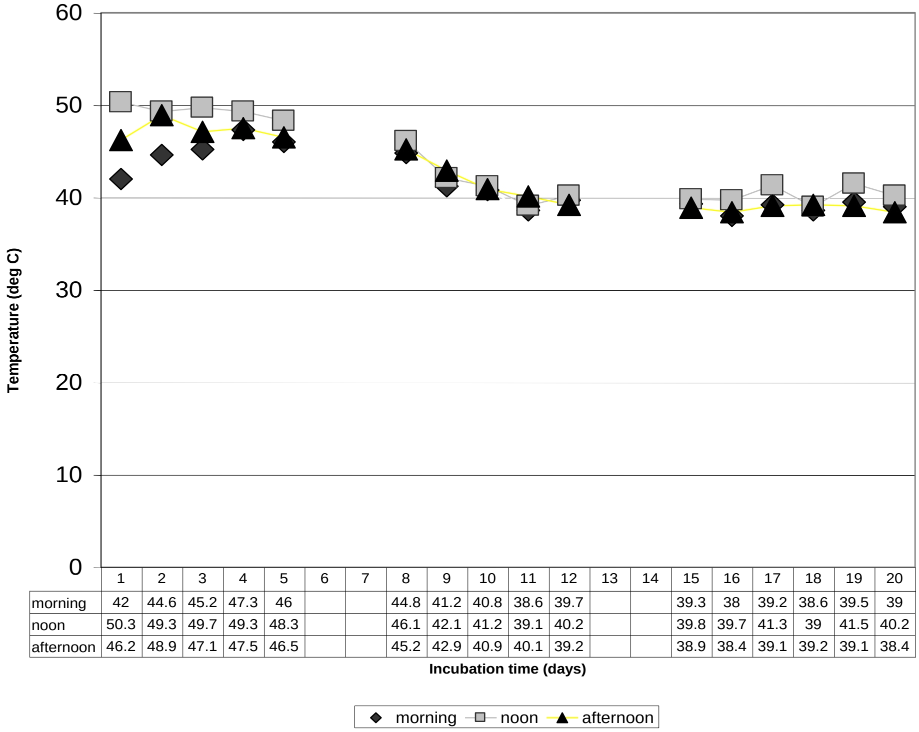
Table 1. Selected properties of raw materials.

Raw Material	N (%)	C (%)	C:N Ratio	P(%)	K(%)
Rice hull	0.25	43.20	172.80	1.00	0.90
Cattle manure	1.04	25.64	24.65	0.60	1.82
Cattle manure-rice hull mixture (70/30, w/w)	1.05	30.34	29.00	0.50	1.33

The compost pile heated up during the composting period. The temperature profile of the heap throughout the 20-day incubation period is shown in Fig. 2. The temperature varied from a mean of 47.2 °C through the first week, then about 41.5 °C in the middle week, and finally about 39.3 °C in the last stages.

The selected properties of cattle manure-rice hull blend after composting are shown in Table 2. Inoculation with the nitrogen-fixing bacteria *Azotobacter* (T3) produced the highest total N in the resulting compost. This N content was significantly higher than that of the control (T1) and that of the *Trichoderma*-inoculated treatment (T2). The total C contents in T3 and T2 were significantly lower than that of the control. The highest loss in dry matter weight (relative to each to each treatment's initial dry weights) was detected in the *Trichoderma*-inoculated samples. Significantly lower weight losses were noted in the control and in the *Azotobacter*-inoculated samples.

Inoculation of cattle manure-rice hull mixture with the nitrogen-fixing bacteria *Azotobacter* significantly enhanced the nitrogen content of the resulting compost as compared to the control and to that inoculated with *Trichoderma* sp. Inoculation with the nitrogen-fixing bacteria showed similar amount of dry matter loss as the control.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
morning	42	44.6	45.2	47.3	46			44.8	41.2	40.8	38.6	39.7			39.3	38	39.2	38.6	39.5	39
noon	50.3	49.3	49.7	49.3	48.3			46.1	42.1	41.2	39.1	40.2			39.8	39.7	41.3	39	41.5	40.2
afternoon	46.2	48.9	47.1	47.5	46.5			45.2	42.9	40.9	40.1	39.2			38.9	38.4	39.1	39.2	39.1	38.4

Fig. 2. Temperature profile of dairy cattle manure-rice hull (70/30, w/w) compost pile.

Table 2. Selected properties of cattle manure-rice hull blend (70/30, w/w) after composting.

Treatment	N (%)	C (%)	C/N	Weight Loss (%)
(T1) Control - uninoculated	1.16 b	24.37 a	21.0 c	15.62 b
(T2) Trichoderma – inoculated	1.04 c	23.31 b	22.0 b	17.35 a
(T3) Azotobacter - inoculated	1.28 a	23.67 b	18.0 d	15.77 b

Note: Raw material at zero time had the following contents, C = 30.34%; N = 1.05%, and initial oven-dry weight of 100 g. Means followed by the same letter within a column are not significantly different at 5% level DMRT.

Effects of Microbial Inoculation on N Gain of Compost

The gain in nitrogen of the control composted material over the uncomposted state was 1.10 mg/g compost. Inoculation with *Azotobacter* doubled that N gain to 2.27 mg/g, a significant increase over the control. No N gain was detected with *Trichoderma* inoculation. N gain efficiency in terms of mg N per g C lost was also significantly highest in the *Azotobacter*-inoculated materials (Table 3); likewise, the highest N gain efficiency in terms of mg N per g substrate consumed. The results showed that inoculation of the cattle manure-rice hull mixture significantly improved the nitrogen gain in compost.

Table 3. Carbon loss and nitrogen gain of cattle manure-rice hull blend (70/30, w/w) after composting.

Treatment	pH	C loss (mg/g)	N gain (mg/g)	N gain (mg N/g C lost)
(T1) Control - uninoculated	7.9 a	59.7 b	1.10 b	18.58 b
(T2) Trichoderma – inoculated	8.1 a	70.3 a	0 c	0 c
(T3) Azotobacter - inoculated	8.4 b	66.7 a	2.27 a	33.83 a

Means followed by the same letter within a column are not significantly different at 5 % level by DMRT

Correlation of C/N with Residual Dry Weight and N Gain

Correlation analyses showed that the resulting C/N of each treatment was due more to N gain and not to weight loss. The correlation of C/N vs. residual dry weight of samples was low and showed negative relationship (r = -0.44, figure not shown), while that of the C/N vs. nitrogen gain was high and also negative (r = -0.98, Fig. 3). The improvement in the nitrogen content appeared to be due mainly to nitrogen fixation and not to material decomposition.

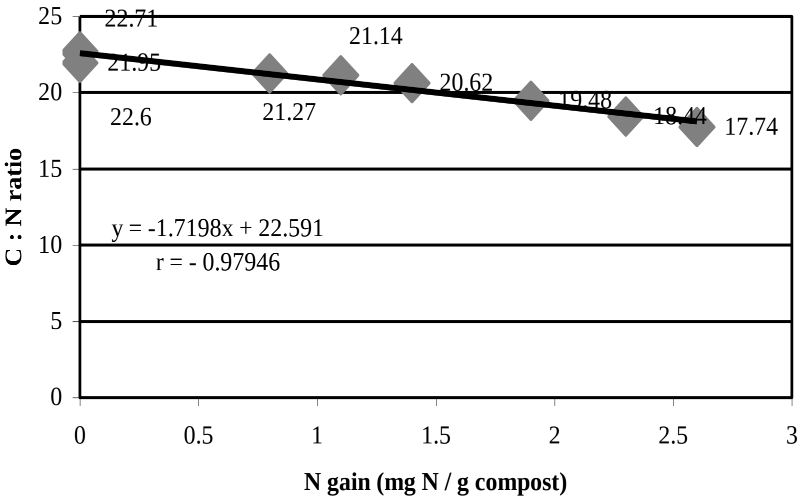


Fig. 3. Regression line and correlation between C:N ratio and N gain in cattle manure-rice hull compost.

Total Bacteria, *Azotobacter* and *Trichoderma* Counts

The load of the inoculant organisms as well as the total bacteria were determined in each treatment after composting. The total bacterial counts are shown in Fig. 4. The highest bacterial count was found in those treated with either *Azotobacter* (35.94 x 10^8 cfu/g compost) or *Trichoderma* (32.92 x 10^8 cfu/g compost). These counts were both significantly higher than those of the control (15.85 x 10^8 cfu/g compost).

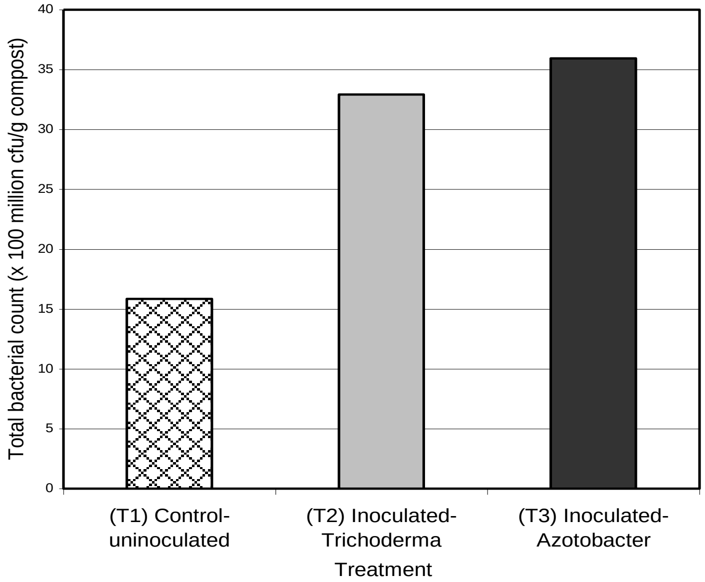


Fig. 4. Total bacterial count in cattle manure-rice hull blend (70/30, w/w) after composting at thermophilic temperatures. (Means followed by the same letter are not significantly different at 5 % level by DMRT).

The *Azotobacter*-inoculated treatment gave the highest *Azotobacter* counts after 30-d composting (4.22 x 10^6 cfu/g compost, Fig. 5). Both the control and the Trichoderma-inoculated treatments showed significantly lower *Azotobacter* counts (1.13 x 10^6 and 1.61 x 10^6 cfu/g compost, respectively).

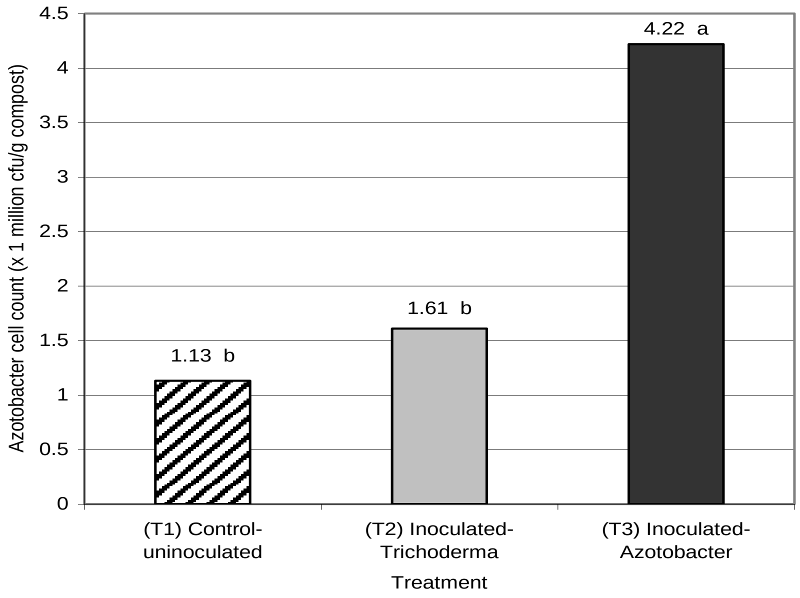


Fig. 5. Count of azotobacters in cattle manure-rice hull blend (70/30, w/w) after composting. Means followed by the same letter are significantly different at 5% level by DMRT.

The highest *Trichoderma* counts (Fig. 6) were found in the *Trichoderma*-inoculated compost. Significantly lower *Trichoderma* counts were found in the control (1.39 x 10^3 cfu/g compost) and in the *Azotobacter*-treated compost (1.38 x 10^3 cfu/g compost).

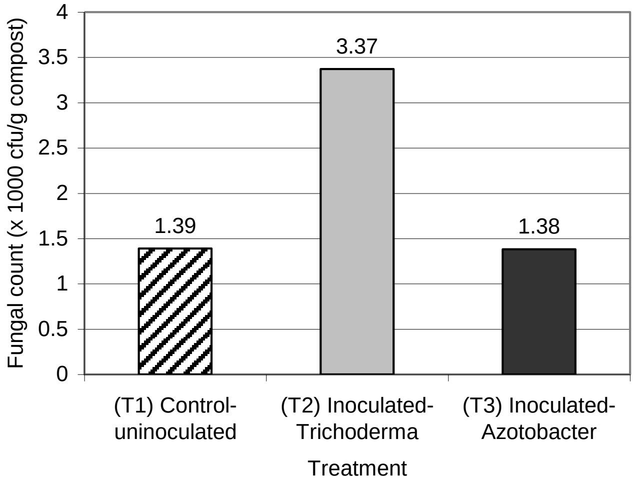


Fig. 6. *Trichoderma* counts in cattle manure-rice hull blend (70/30, w/w) after composting. Means followed by the same letter are significantly different at 5% level by DMRT.

Effects of Inoculated Compost in Field Conditions

At 30 DAT, inoculated compost showed its effects (Table 4). Plants supplied with inoculated compost at 500 kg/ha were taller (53.15 cm/hill) than those of the control (47.75 cm/hill). The combination of chemical fertilizer at N-P-K of 90-60-60 (each nutrient in kg/ha), and inoculated compost at 500 kg/ha, produced the significantly tallest plants at 56.53 cm/hill, which were 18 % taller than the control. At the same early growth stage, the treatment with inoculated compost alone significantly gave the highest tiller number (456.0/m^2) followed by treatment with the combined addition of half chemical fertilizer (45-30-30) and full inoculated compost at 500 kg/ha (446.0/m^2). These two treatments gave 45 % and 42 % increases in tiller count over the control (315.0/m^2). At the same growth stage, tiller count of plants supplied with inoculated compost alone was significantly equivalent to those supplied with full chemical fertilizer (432.0 tillers/m^2).

At harvest, addition of fertilizers, whether chemical or inoculated compost, gave plants significantly taller than the control (Table 5). Inoculated compost application alone at 500 kg/ha gave the highest tiller count at 487.0 tillers/ m^2; significantly higher that those of the control (388.0 tillers/ m^2), and those with full chemical fertilizer (462.0/m^2). The combined application of full chemical fertilizer and inoculated compost gave the highest panicle number at harvest (437.0 panicles/ m^2), followed by those of combined rates of half chemical fertilizer and inoculated compost (358.0/m^2).

Combined application of full chemical fertilizer and inoculated compost gave the highest grain yield of 6.15 t/ha, a 28-percent increase over the control. Half rate of combined application of chemical fertilizer (60-30-30) with full inoculated compost (500 kg of organic fertilizer) gave the second highest grain yield of 6.10 t/ha, an increase of 27 % over the control. Inoculated compost applied alone at 500 kg/ha gave grain yield of 5.92 t/ha, significantly higher than the control by 24 %. The control gave the lowest grain yield of 4.79 t/ha.

Table 4. Effect of inoculated dairy cattle manure-rice hull compost on the average plant height and tiller count of rice cv. IR-74 at 30 DAT [1].

Treatment	Mean plant height (cm/hill)	Tiller number (no./m^2)
T1, Control (no fertilizer)	47.75 b	315.0 b
T2, Chemical fertilizer (90-60-60)	56.40 a	432.0 a
T3, Chemical fertilizer (45-30-30) + Inoculated compost (500 kg/ha)	54.90 a	446.0 a
T4, Inoculated compost (500 kg/ha)	53.15 a	456.0 a
T5, Chemical fertilizer (90-60-60) + Inoculated compost (500 kg/ha)	56.53 a	445.0 a

[1] Values are a mean of 4 replicates; means followed by a common letter are not significantly different at 5 % level by DMRT.

Table 5. Effects of inoculated dairy cattle manure-rice straw compost on the average plant height and tiller count of rice cv. IR-74 at harvest.

Treatment	Mean plant height (cm/hill)	Tiller number (no./m^2)	Panicle number (no. / m^2)	Grain yield (t/ha)
T1, Control (no fertilizer)	59.48 b	388.0 b	195.0 c	4.79 b
T2, Chemical fertilizer (90-60-60)	70.22 a	462.0 a	339.0 a	6.02 a
T3, Chemical fertilizer (45-30-30) + Inoculated compost (500 kg/ha)	71.82 a	476.0 a	358.0 a	6.10 a
T4, Inoculated compost (500 kg/ha)	68.65 a	487.0 a	355.0 a	5.92 a
T5, Chemical fertilizer (90-60-60) + Inoculated compost (500 kg/ha)	68.90 a	462.0 a	437.0 b	6.15 a

[1] Values are a mean of 4 replicates; means followed by a common letter are not significantly different at 5 % level by DMRT.

DISCUSSION

All of the treatments used in this experiment received mineral supplementation in the form of Burk medium minus mannitol or glucose. The minerals were supplied with distilled water as wetting medium. The effects of microbial inoculation, with either the nitrogen-fixing bacteria *Azotobacter* or the cellulolytic fungus *Trichoderma,* on the compost end-product as compared to the uninoculated control, were evaluated in this study.

The raw materials (cattle manure-rice hull mixture) had initial total N content of 1.05 %, C content of 30.34 % and C/N of 29.0 at day 0. The composting process proceeded at a temperature range between 49 to 37 °C. The control and the *Azotobacter*-treated materials showed increases in nitrogen content after composting, giving values of 1.16 and 1.28 %, respectively. Only the *Trichoderma*-treated compost (1.04 % total N) did not show any increase in N after composting. Nitrogen content normally increases with the composting process, due to organic matter degradation and low ammonia losses (Genevini et al., 1997). On the aspect of organic matter degradation, such as in composting, materials of low nitrogen content and easily decomposable carbon offer the potential for dinitrogen fixation (Gibson et al., 1988). The production of fixed nitrogen in organic materials through aerobic nitrogen-fixers and aerobic cellulose decomposers has been shown by some workers (Halsall and Gibson, 1985).

The differences in results were mainly due to the inoculation treatments. All treatments showed high weight losses in the range of 15 to 17 %, indicating high OM degradation. Differences in temperature range, whether high or low, may induce differences in the composition of the microflora inside the composting mass (McKinley and Vestal, 1985; Chino et al., 1983). In spite of the relatively high temperature range, survival of the mesophilic inoculants were detected after composting. Samples inoculated with *Trichoderma* (T2) showed the highest carbon loss (70.3 mg C/g) similar to those inoculated with *Azotobacter* (T3, 66.7 mg/g). Inoculation with either *Trichoderma* or *Azotobacter* consumed the highest carbon during substrate degradation. N gain was highest in materials inoculated with *Azotobacter* (2.27 mg/g), followed by the control (1.10 mg/g). Samples inoculated with *Trichoderma* showed no gain in nitrogen.

In terms of N gain as mg N/g substrate consumed, *Azotobacter* inoculation gave the highest values (14.5 mg/g). The control had significantly lower N gain at 7.02 mg. But the *Trichoderma*-inoculated samples had no gain in nitrogen after composting. The *Azotobacter*-treated samples which showed the highest N gains also exhibited the highest counts of these nitrogen-fixing bacteria, indicating that the greater nitrogen content in the compost is due to these organisms. The control which most likely also accommodated native nitrogen-fixers and cellulolytic fungi had significantly lower N gains.

The highest total bacterial counts were found in those treated with either *Azotobacter* (35.94×10^8 cfu/g compost) or *Trichoderma* (32.92×10^8 cfu/g compost). These counts were equally significantly higher than those of the control (only 15.84×10^8 cfu/g compost). The added inoculants appeared to have encouraged greater growth of microflora which could be due to the positive interactions induced by them.

The N gains obtained by inoculation in the small bagged samples as done in this study were nil. But the magnitude of N gain could be higher under actual large-scale composting conditions, and this is the subject of our subsequent experiments. Factors that could be beneficial to crops other than N gain could be at work in inoculated composts. Other workers have indicated growth-promoting or biocontrol effects that are magnified by inoculated composts (Kostov *et al.*, 1996*;* Hoitink *et al.*, 1993). The changes observed in inoculated compost over uninoculated one as shown in this study could have other beneficial effects on target crops which would need to be elucidated.

Actual composting conditions involve aerobic, anaerobic and microaerophilic sites brought about by the nature of mixed solid substrate degradation such that an array of various types of microbial nitrogen-fixers may be found in the materials. The materials composted in this study were a heterogeneous mass of mixed cattle manure and rice hull with niches of high and low N fractions and sites of varying degrees of oxygen supply. Against this background, both the aerobes and the anaerobes would be active (Lynch and Wood, 1984) subject to the influence of treatment conditions. The field test revealed the beneficial effects of inoculated dairy cattle manure-rice hull compost on the growth and yield of rice. The inoculated compost boosted the tiller and panicle numbers and the grain yield of rice at levels similar to those effected by chemical fertilizers. Organic materials such as manure and rice plant parts contain nutrients and other constituents such as cellulose, hemi-cellulose, lignin, water-soluble sugars, ash, and others (Adachi et al., 1997) which can promote plant growth. Composting conditions as well as bacterial inoculants can provide gains in nutrients and minimization of adverse growth conditions obtaining in the field (Moroyu, 1982; Ponnamperuma, 1984). Other workers (Vien et al., 2006; Yong-Hwan, 2004)) have noted that soil nitrogen could increase under compost application as compared to full rate of chemical NPK fertilizers. It had also been observed that the combination of compost and chemical fertilizers can effect large soil nitrogen pool and more microbial activity. Under these conditions the rice plant could very well benefit from a gradual and stable supply of more available nitrogen coming from the mineralization of soil organic matter and applied compost.

ACKNOWLEDGEMENTS

We would like to acknowledge the funding support of the National Dairy Authority (NDA) and the administrative assistance of the Philippine Council for Agriculture and Resources Research and Development (PCARRD) in the conduct of this study as part of an overall project study on waste management and bioorganic fertilizer production.

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J. ISSAAS Vol. 14, No 1:92-100 (2008)

PROTECTION OF HOT PEPPER AGAINST MULTIPLE INFECTION OF VIRUSES BY UTILIZING ROOT COLONIZING BACTERIA

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(Received: January 21, 2008; Accepted: April 17, 2008)

ABSTRACT

Mixed virus infection is a common phenomena in nature. It results in severe disease symptoms and yield loss. We utilized seven selected root colonizing bacteria (rhizobacteria) isolated from the hot pepper rhizosphere to improve the effectiveness of virus management. The efficacy of those rhizobacteria in inducing plant growth and systemic resistance (ISR) on hot pepper against multiple infection of *Tobacco mosaic virus* (TMV*)*, and *Chili veinal mottle virus* (ChiVMV) was evaluated in greenhouse trials. The rhizobacteria was applied as seed treatment and soil drench. All bacteria treated plants showed better growth character, milder symptom expressions than control and increased peroxidase enzyme activities and ethylene but these depended on the species. It affected slightly the accumulation of TMV, however it suppressed the ChiVMV accumulation. Based on the morphological characters and full length nucleotide sequences analysis of 16S r-RNA, *Bacillus cereus* (I-35) and *Stenotrophomonas sp* (II-10) were the potential isolates as PGPR.

Key words : multiple viral infection, rhizobacteria, *Bacillus cereus*, *Stenotrophomonas* sp, ISR

INTRODUCTION

Hot pepper (*Capsicum annuum*) is one of important vegetables in Indonesia. However, infection by plant pathogens, including plant viruses have become a serious constraint for hot pepper production. The main viral disease infecting hot-pepper are *Chili veinal mottle virus* (ChiVMV), *Pepper veinal mottle virus* (PVMV), *Pepper mottle virus* (PeMoV), *Pepper severe mosaic virus* (PeSMV) and *Cucumber mosaic virus* (CMV). In Indonesia ChiVMV, CMV, TMV and recently Geminivirus are important viruses infecting hot pepper (Duriat, 1996; Sulandari 2004). In nature, multiple infection by pathogens is a natural phenomena which causes damage more severe than a single infection by a pathogen.

Management strategies to control plant viruses in Indonesia are limited to the use of resistant cultivars, culture management and most farmers rely on chemical insecticides to control the insect vectors. To minimize the use of pesticides, which pollute the environment, and to improve the effectiveness of virus disease control, the utilization of beneficial root colonizing bacteria isolated from the plant rhizosphere, referred to as Plant Growth Promoting Rhizobacteria (PGPR), might offer a promising viral disease control. Previously, PGPR reported to be effective in controlling fungi, bacterial and viruses (Maurhofer et al., 1994; De Meyer et al., 1999; Murphy et al., 2000; Murphy et al., 2003). Plants develop an enhanced defensive capacity against a broad spectrum of plant pathogens after colonization of the roots by selected strains of nonpathogenic bio-control bacteria (Pieterse et al., 2000).

In Indonesia, the availability of hot-pepper resistant cultivars against either pest or diseases are limited. Hence, this study sought to find and to evaluate the potential of rhizobacteria eliciting plant growth and induced systemic resistance (ISR) to protect hot pepper against multiple infection of TMV and ChiVMV.

MATERIALS AND METHODS

Rhizobacteria Isolates

Rhizobacteria were isolated from the healthy rhizosphere of hot pepper cultivar in the field at Darmaga Bogor, West Java. The bacteria was isolated and cultured on tryptic soya agar (TSA, Difco, USA). Bacterial isolation was performed as described previously (Lemanceau et al., 1995). Seven rhizobacteria isolates were used: I-2, I-16, I-25, I-30, I-35, II-7, II-10, and evaluated based on their ability to enhance plant growth and their ability to protect hot pepper against multiple virus infection.

Identification of Rhizobacteria

The potential isolate(s) as candidate PGPR was identified by sequencing the 16S r-RNA using a set of primer specific for prokaryotes 16S ribosomal RNA. The forward primer was 68f (5'-CAGGCCTAACACATGCAAGTC-3') and the reverse primer was 1387r (5'-GGGCGGWGTGTACAAGGC-3') as previously described (Marchesi et al., 1998). Further sequencing of the full length of 16S rRNA, additional primers was designed according to the species. The homology and similarity of the nucleotide sequences were analyzed using WU-Blast2 software provided by EMBL-EBI (European Molecular Biology Laboratory - European Bio-Informatics Institute).

Viral Inoculum

TMV was propagated on tobacco plants, while ChiVMV was propagated on hot pepper**.** TMV and ChiVMV were obtained from Laboratory of Virology collection, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University. The viral inoculum was tested serologically before inoculation.

Growth Condition and Rhizobacteria Treatment

The experiments were conducted in greenhouse trials to evaluate the rhizobacterial ability as PGPR to protect hot pepper against mixed infection of viruses. Rhizobacteria treatment (10^9 cfu/ml) and growing conditions were performed as described previously (Damayanti et al., 2007). Plants were grown in the greenhouse with humidity and temperature depending on the natural conditions. Six plants were used for each treatment.

Virus Inoculation

Plants per treatment were mechanically inoculated with mixed infected plant sap (1:10 w/v) in phosphate buffer (pH 7.0) (Merck, Germany) at 2 weeks post transplanting or 4 weeks after planting (WAP) into pots. The first two leaves on each plant were gently dusted with carborundum, 600 mesh (Nacalai Tesque, Japan) prior to rub-inoculation with sap containing viruses.

Evaluation of Plant Growth Characters and Disease Assessments

The height of each plant was measured from soil line to shoot apex taken at 1 day prior to inoculation (dpi) with viruses and at 4 and 8 weeks post-viral inoculation (wpi). The fresh weight of the above tissues, number of leaves, number of flowers/fruits (taken as single measure) were counted at 6-8 wpi.

Disease Assessments

Disease severity rating was made at 2 wpi using rating scales developed using mock inoculated plants of treatment as a standard by using the following rating scales : 0 = no symptoms, 2 = mild mosaic symptoms on the leaves, 4 = severe mosaic symptoms on the leaves, 6 = mosaic and deformation of leaves, 8 = severe mosaic and severe deformation of leaves and stunted. Disease severity was measured for each plant used in the experiment.

ELISA test. Viral protein accumulation was detected by DAS-ELISA (double antibody sandwich enzyme linked-immunosorbent assay) method at 2 and 4 wpi with TMV, and ChiVMV antiserum. Samples were taken as composite leaf samples per treatment. The procedure was carried out according to manufacture's recommendation (DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany). Viral accumulation was quantitatively measured by using an ELISA reader at 405 nm. The positive sample was considered for the presence of the virus when absorbance value was twice that of healthy control.

Extraction and Quantification of Peroxidase (PO) Enzyme Activities. Peroxidase enzyme activities for 3 minutes was used as a parameter to determine whether ISR occurred or not. Extraction and quantification of peroxidase enzyme activities were done using a spectrophotometric method conducted at 1 wpi according to method described previously (Hammerschmidt et al., 1982; Damayanti et al., 2007) with minor modification. The reaction mixture was incubated at room temperature and the absorbance determined using a spectrophotometer at 420 nm with 30 second intervals for 3 minutes. The enzyme activity was expressed as a change in absorbance ($min^{-1}mg^{-1}$ protein). PO enzyme activity was measured from composite leaf samples of each bacterial treatment.

Quantification of Ethylene Production. The quantification of ethylene production was carried out by gas chromatography (GC) at the Balai Besar Pasca Panen, Cimanggu, Bogor, Indonesia. Leaves samples (2 g/each treatment) of bacteria-treated and control plants were taken at 5 dpi and were measured for ethylene production, expressed as µmol/ g leaf. Ethylene production was measured from composite leaf samples of each bacterial treatment.

Data Analysis

Data was analyzed by analysis of variance (ANOVA) and the treatment means were separated by Duncan's Multiple Range Test (DMRT)($\alpha = 0.05$) using SAS software version 6.12 (SAS Institute, Gary, NC, USA).

RESULTS AND DISCUSSION

Evaluation of rhizobacteria treatment on plant growth characters

Plant height measured at 1 day before viral inoculation (4 WAP) was significantly higher on bacteria treated plants than control. In addition, bacteria treated plants visually exhibited greater vigor, fitness and leaf size than control plants (data not shown). However, I-16 and I-30-treated plants at 8 WAP and at 12 WAP, respectively did not show any difference with control plants (Table 1, Healthy). Similar results were obtained when plants were challenge inoculated by mixed viruses at 4 weeks post inoculation (wpi) (8 WAP) and 8 wpi (12 WAP) (Table 1, Infected). It showed that even if plants were infected severely by the viruses, these were able to grow well, indicating the protective effect of rhizobacteria against viral infection. Further, most bacterial isolates showed the ability to enhance plant growth until 3 months after seeding, suggesting the long term persistence of rhizobacteria in soil. The enhancement of plant health might be due to stimulation of the host plant or mutualistic symbionts and/or effect of hormones which mediate processes of plant cell enlargement, division, and extension in symbiotic as well as non-symbiotic roots (Gardener, 2004).

Table 1. Effect of rhizobacteria treatment on plant height (cm)

Treat-ment	8 WAP*	4 wpi	12 WAP	8 wpi
	Healthy	Infected**	Healthy	Infected**
Control	38.25 ± 3.74 e	25.45 ± 3.56 c	94.17 ± 10.28 b	53.50 ± 18.38 c
I-2	56.25 ± 3.90 c	34.67 ± 2.23 b	107.80 ± 6.99 a	80.48 ± 3.34 ab
I-16	36.17 ± 4.54 e	42.15 ± 2.38 a	115.83 ± 2.64 a	73.83 ± 1.94 b
I-25	61.83 ± 2.73 ab	36.42 ± 4.01 b	111.08 ± 7.23 a	77.17 ± 5.21 ab
I-30	45.83 ± 2.56 d	34.50 ± 4.37 b	93.57 ± 6.80 b	71.32 ± 8.54 b
I-35	64.20 ± 2.54 a	44.10 ± 2.75 a	114.83 ± 8.57 a	87.50 ± 12.88 a
II-7	59.42 ± 5.64 bc	44.00 ± 4.75 a	113.63 ± 6.13 a	83.50 ± 5.60 ab
II-10	62.67 ± 2.88 ab	42.42 ± 4.78 a	111.02 ± 3.17 a	86.20 ± 7.50 a

Number in columns followed by the same letter are not significantly different (α= 0.05) by DMRT
*WAP- week after planting (WAP).
**TMV and ChiVMV were inoculated at 4 WAP. Non-bacterial treated plants were used as control

The bacteria treated plants generally produced more leaves and flowers than control plants (Table 2). Similar results were obtained in the fresh weight of healthy plants. However, the number of flowers of I-16-treated plants decreased similar to control plants. Furthermore, plants treated with I-16, I-30, II-7 and infected with mixed viruses did not show any difference in fresh weight compared to control. Other treatments (I-2, I-25, II-10) showed slight difference while I-35 treated plants were significantly different.

Table 2. Effect of rhizobacteria treatment on plant leaf, flower numbers and fresh weight.

Treat-ment	Leaf Numbers		Flower Numbers		Fresh Weight (g)	
	Healthy	Infected*	Healthy	Infected*	Healthy	Infected*
Control	93.17 ± 29.08 e	35.67 ± 18.04 e	75.83 ± 13.88 b	52.50 ± 22.85d	72.02 ± 16.81 d	58.73 ± 4.75 b
I-2	176.00 ± 16.25 ab	90.00 ± 11.49 bc	191.50 ± 39.93 a	226.33 ± 52.11ab	99.10 ± 7.01 bc	65.61 ± 5.82ab
I-16	140.50 ± 29.51 dc	105.17 ± 20.23 b	202.00 ± 49.39 a	64.00 ± 6.81 d	98.87 ± 4.45 bc	55.47 ± 16.33 b
I-25	156.17 ± 24.77 bc	71.67 ± 16.97 cd	174.83 ± 50.76 a	232.00 ± 56.95 a	97.63 ± 6.86 bc	66.47 ± 14.98 ab
I-30	118.33 ± 9.33de	58.50 ± 16.31 d	185.50 ± 42.40 a	164.17 ± 64.99 c	88.37 ± 9.56 c	48.88 ± 11.12 b
I-35	172.17 ± 38.58 abc	162.50 ± 32.49 a	183.67 ± 34.78 a	137.33 ± 20.49 c	130.37 ± 27.40 a	78.87 ± 26.06 a
II-7	150.17 ± 11.69 bc	107.67 ± 11.69 b	182.17 ± 40.54 a	178.67 ± 21.79bc	90.98 ± 14.01 c	58.90 ± 7.08 b
II-10	199.17 ± 24.56 a	104.33 ± 18.93 b	176.17 ± 39.29 a	221.67 ± 18.93ab	108.76 ± 6.94 b	65.60 ± 6.03 ab

Number in columns followed by the same letter are not significantly different (α = 0.05) by DMRT
* TMV and ChiVMV were inoculated at 4 WAP. Non-bacterial treated plants were used as control

Disease Assessments

All bacteria treated plants inoculated with mixed viruses exhibited phenotype symptoms milder than control plants (Fig. 1). The mildest symptoms were displayed by plants treated with I-16 and I-35. The protective effect afforded by isolates in suppressing the symptoms varied in severity, suggesting that the variety of the bacteria species might lead to different effects on plants after the challenge inoculation of viruses. In addition, the effect of I-16 on disease severity was more prominent than its ability to promote plant growth (Table 1 and Fig. 1).

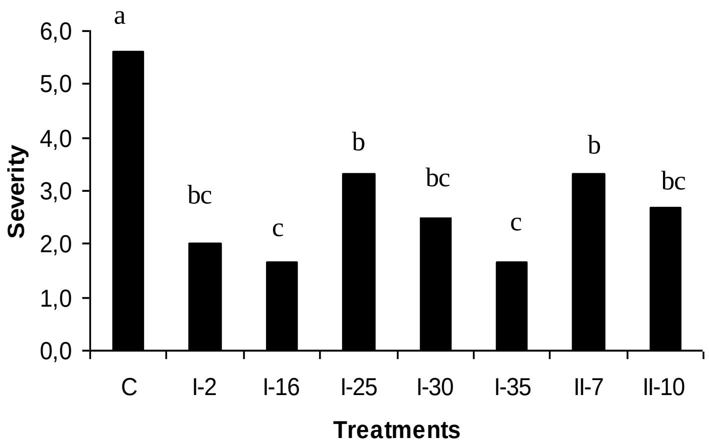

Fig. 1. Disease severity of control plants (C) and bacterial treated plants. inoculated by mix viruses

ELISA test. Based on the ELISA absorbance value, the rhizobacteria treatment affected TMV slightly either at 2 or 4 wpi, while ChiVMV accumulated lower levels of the virus than control plants (Table 3). This suggests that the bacterial treatment was able to suppress the ChiVMV better than TMV.

Table 3. ELISA test of control and inoculated plants infected by virus

Treatment	ELISA Absorbance value*			
	TMV		ChiVMV	
	2 wpi	4 wpi	2 wpi	4 wpi
C**	1.624	1.895	0.326	0.472
I.2	1.889	1.942	0.187	0.386
I.16	2.198	1.765	0.080	0.074
I.25	2.100	1.769	0.221	0.410
I.30	1.810	1.712	0.234	0.360
I.35	2.039	1.751	0.089	0.065
II.7	1.955	1.660	0.195	0.358
II.10	1.925	1.770	0.187	0.341

*Means of ELISA absorbance value obtained from duplex measurement of composite samples per treatment. Positive results of ELISA = twice absorbance of healthy plants Healthy absorbance value of TMV= 0.095, and that of ChiVMV = 0.031
** C = control plants inoculated by mixed viruses

The accumulation of TMV was more prominent than that of ChiVMV, indicating the stability of TMV to compete with other viruses during multiple infection. Alternatively, rhizobacteria treatment did not decrease the TMV accumulation, but it suppressed the severity. Even the rhizobacteria treatment slightly affected TMV, however all rhizobacteria treated plants exhibited milder symptom expression compared with control plants (Fig. 1). The protection afforded to rhizobacteria treated plants appeared to have been a result of the enhanced growth of hot pepper, thereby allowing them to respond to inoculation with viruses.

Peroxidase Enzyme Activities. Generally, all rhizobacteria treated plants had increased PO activity compared with control (healthy), and the PO activities increased to some extent after challenge inoculated with viruses, except for plants treated with I-25, and II-10 (Fig. 2). This suggests that some rhizobacteria treatments might induce plant's systemic resistance through increasing peroxidase enzyme activity (PO dependent) except for I-25 and II-10. It showed that some rhizobacteria might able to enhance plant defense response through elevated PO activity (I-2, I-16, I-30, I-35, II-7) while others might be PO-independent (I-25, I-10) and it might be dependent on the species. The polyphenol oxidase enzyme and peroxidase oxidizes phenolics to quinones and generates hydrogen peroxide (H_2O_2). It well known that H_2O_2 is an antimicrobial compound, which releases highly reactive free radicals and further increases the rate of polymerization of phenolic compounds into lignin-like substances. These substances are then deposited in cell walls and papillae and interfere with the further growth and development of pathogens (Agrios, 2005; Hammond-Kosack and Jones, 1996). Certain PGPR do not induce pathogenesis related (PR) proteins but rather increase accumulation of peroxidase, phenylalanine ammonia lyase, phytoalexins, polyphenol oxidase, and/or chalcone synthase (reviewed by Compant et al., 2005). These experiments also showed that some isolates, except I-25 and II-10, were able to induce peroxidase after challenge with virus infection.

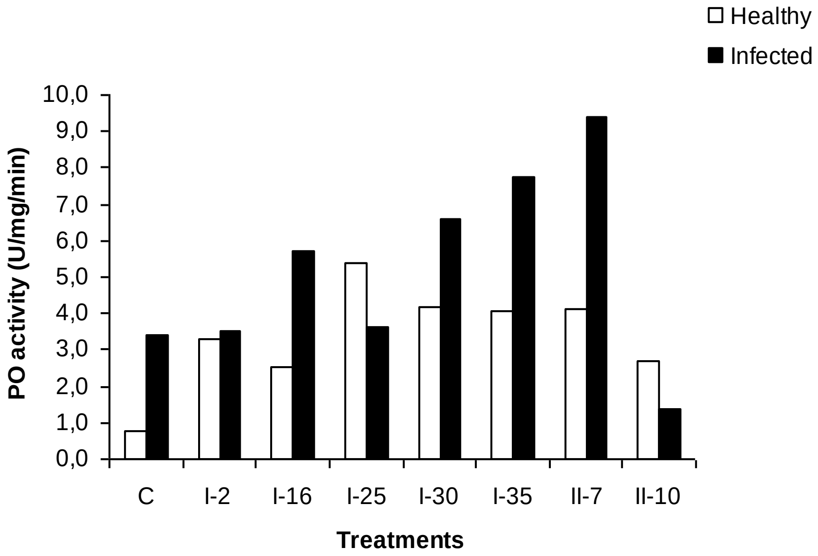

Fig. 2. Peroxidase enzyme activity of healthy and virus infected plants

Ethylene Production. Overall, rhizobacteria treatment did not show increased ethylene production (healthy) (Fig. 3). However, the ethylene production increased when plants were challenge inoculated with viruses, except for I-2 and I-30 treated plants. In this case, induction of ISR either through increased ethylene production or the ACC (1-aminocyclopropane-1carboxylate) converting capacity to ethylene is still unclear and needs further investigation. However, rhizobacteria treatments (I-16, I-25 I-35, II-7, II-10) and challenge inoculation with viruses increased ethylene production compared with healthy plants. A greater production of ethylene in the initial phase of infection might contribute to enhanced resistance against pathogens. In other words, ethylene might act as the primary signal in enhancing the defense capacity or may activate some defense genes. However, even plants treated with I-2 and I-30 did not increase ethylene production, but treated plants showed milder disease severity than control.

Improvement in plant health and productivity by PGPR are mediated by three different ecological mechanisms; (1) antagonism of pests and pathogens, (2) promotion of host nutrition and growth and (3) stimulation of plant host defenses (Gardener, 2004).

The majority of plant growth promoting bacteria that activate ISR appear to do so via an SA-independent pathway involving jasmonate and ethylene signals (reviewed in Compant et al., 2005). It seemed that ISR afforded by isolates might increase the jasmonic acid (JA) production and leads to increased sensitivities to ethylene similar to the case of PGPR WCS417r in *Arabidopsis* (Knoester et al., 1999). ISR is associated with an increase in sensitivity to JA and ethylene rather than an increase in their production, which might lead to the activation of a partial set of defense genes (Hase et al., 2003; Pieterse and van Loon, 1999). Unfortunately, in these experiments, JA production was not measured for further elucidation of the role of JA in ISR on hot pepper plants.

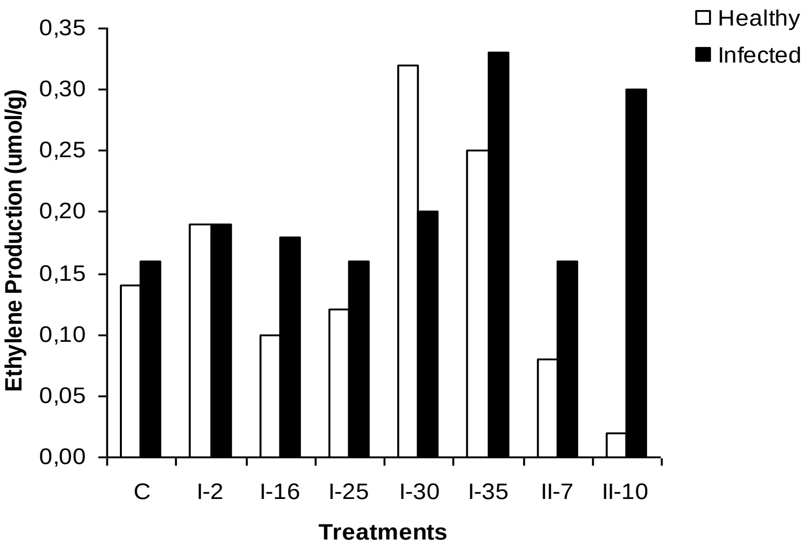

Fig. 3. Ethylene production of healthy and virus infected plants

Identification of Rhizobacteria Species

Isolates I-35 and II-10 have the potential as PGPR as these are able to protect hot pepper plants against multiple virus infection. Previously, I-35 identified as *Bacillus cereus* (DDBJ Accession No.AB288105) was reported to enhance growth and protect hot pepper plants against TMV infection (Damayanti et al., 2007). In most cases, *Bacillus spp* that elicit ISR typically promote plant growth. *B. cereus* has been previously reported to have activities that suppress pests and pathogens or promote plant growth (reviewed in Kloepper et al., 2004). These results also support the previous reports on *B. cereus* (I-35) that consistently exhibited its ability as PGPR and/or enhanced plant systemic resistance event in plants infected by mixed viruses.

Rhizobacteria species were identified by testing the morphological characters of the bacteria (gram type, colony form, cell type) and nucleotide sequences of 16S r-RNA. The II-10 isolate is a gram-negative, whitish colony on TSA, and rod shaped. Based on these characters and 16S r-RNA sequences, the isolate II-10 was identified as *Stenotrophomonas sp* (DDBJ Accession No. AB288107). Few *Stenotrophomonas* genera have been reported as PGPR. However, *Stenotrophomonas maltophilia* was the most frequent species recovered from weeds rhizosphere in Canada and is reportedly able to promote potato growth (Sturz et al., 2001). Our results here extend the role of *Stenotrophomonas* genera as PGPR. These results might be the first evidence of *Stenotrophomonas sp* as inducer of plant systemic resistance against plant viruses in hot pepper.

CONCLUSION

Based on the evaluation of plant growth characters and disease assessments, the potential candidates of root colonizing bacteria as PGPR which could protect hot pepper against multiple infection of viruses are *Bacillus cereus* (I-35) and *Stenotrophomonas* sp (II-10).

ACKNOWLEDGEMENT

This research was financially supported by the Indonesian Toray for Science Foundation (ITSF-2005) in 2006. This was also partially financed by Competition Grant Program B 2006, Department of Plant Protection, Bogor Agricultural University for the author to conduct a part of the work in Japan through the International Linkage Program. I gratefully acknowledge the technical assistance provided by Tetsuro Okuno and Kazuyuki Mise and the DNA sequencing facilities at the Laboratory of Plant Pathology, Graduate School of Agriculture, Kyoto University. I am also grateful to Nisa Rachmania Mubarik, Hendra Pardede and Edi Supardi for excellent work support.

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J. ISSAAS Vol. 14, No 1:101-110 (2008)

TOXICITY OF *CYMBOPOGON CITRATUS* STAPF. (POACEAE) AGAINST THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* L. (LEPIDOPTERA: YPONOMEUTIDAE) LARVAE

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(Received: January 10, 2008; Accepted: May 26, 2008)

ABSTRACT

Cymbopogon citratus Stapf. has been reported to possess antifungal, nematocidal, acaricidal, and insecticidal activities. Active compounds that possess insecticidal activity to the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) larvae have not yet been identified. Therefore, this study was conducted to evaluate the efficacy of the extract of *C. citratus* aerial parts and to elucidate the structure of the active compound causing larval mortality to *P. xylostella*. Bioassay-guided fractionation led to the isolation of the active compound as an essential oil, 3,7-dimethyl-2,6,-octadienal or citral. The LD_{50} value of this compound was 7.7 µg/insect by topical application.

Key words: active compound, botanical insecticide, citral, insecticidal activity

INTRODUCTION

Plant essential oils have been used as material ingredients of many commercial products such as perfumes, analgesic ointments, liniments and food additives. Moreover, they are also known to possess several biological activities to insects such as repellent, ovicidal, larvicidal, antifeedant, and mortality activity. The essential oils of *Litsea elliptica* (Lauraceae) and *Piper aduncum* L. (Piperaceae) are highly toxic to mosquito larvae, *Aedes aegypti* (Diptera: Culicidae). Benzyl benzoate present in oils of *Cinnamon* spp. (Lauraceae) is highly toxic to brine shrimp. Moreover, oils of *L. elliptica*, *Cymbopogon nardus* Rendle (Poaceae) and *C. mollissimum* showed high repellency against female adult *A. aegypti* (Ibrahim *et al*., 1996). Citronella and peppermint inhibit hatchability of *Earias vittella* (F.) (Lepidoptera: Noctuidae) eggs which results in 50% hatchabiity, when eggs are exposed to the volatile oil (Marimuth *et al*., 1997). *C. proximus* significantly inhibits feeding activity of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) at a concentration range of 0.25 - 1.0% (Swidan, 1994).

The herb, lemon grass *C. citratus* Stapf., have leaves of about 1 m. This plant is noted as a source of oleum citronella (Anonymous, 1986); however, *C. citratus* actually yields several essential oils such as linalool (1.1%), neral (25-28%), geral (45-55%), myrcene (8.2-19.2%), geraniol (0.5-0.6%), and citranellol (0.1%), among others. (Weiss, 1997). It is reported to possess antifungal activity against *Alternaria tenuis*, *Botrytis allii*, *Diplodia maydis*, *Fusarium oxysporum*, *Gibberella fujikuroi*, *Ustilago avenae* and *Cladosporium fulvum*, nematocidal activity to *Meloidogyne incognita*, acaricidal activity to *Tetranychus cinnabarinus*, and insecticidal activity to *Aedes aegypti*, *Aphis gossypii* and *Musca domestica* (Grainge and Ahmed, 1988). Therefore, it is important to assay this plant against other economic insect pests in order to develop a botanical insecticide.

MATERIALS AND METHODS

Plant material

The whole plant (mainly stems) of *C. citratus* were obtained from a local market in Bogor, Indonesia. These were air-dried at room temperature for 1-2 weeks before extraction.

Insect

Larvae of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), were obtained from a mass culture. Larvae fed on 6-10 days old radish (*Raphanus sativus* L.) seedlings were kept at 25±1°C under long day 12:12 (L:D) photoperiod regimen in an insect room. Adults, reared in a different cage and fed on 10% honey solution (aqueous), were allowed to mate and oviposit on 6-8 days old radish seedlings. Third instar larvae were used for bioassay.

Extraction

From 5 kg of fresh sample, 395.6 g of air-dried *C. citratus* was collected, cut into small pieces and ground using a mill to make a powder. The powder was poured into a thimble filter (40 mm x 159 mm) and extracted with ether by Soxhlet for 48 hours to obtain 21.0 g of a solid crude extract. The crude extract (20.9 g) was separated into basic, acidic and neutral fractions through a series of solvent-solvent partitioning. The crude extract was dissolved in 300 ml ether and partitioned with 150 ml sodium hydroxide (NaOH), 5% three times. The resulting NaOH fraction (Na-1) and ether fraction (Et-1) were further fractionated.

Aqueous sulfuric acid, 20% was added to the NaOH fraction (Na-1) to attain a pH of 1-2. This was partitioned with ether, 100 ml three times, to get an ether fraction (300 ml, Et-2) and a NaOH fraction (450 ml, Na-2) which was discarded. Meanwhile, the ether fraction (Et-2) was dried with Na_2SO_4 anhydrous (5-8 g) overnight and evaporated under reduced pressure to get an acidic fraction (1.8 g). The ether fraction (Et-1) was partitioned with 5% H_2SO_4 (aqueous), 100 ml three times, to get an ether fraction (Et-3) and H_2SO_4 fraction (300 ml), which was further partitioned with ether. Both fractions were dried with anhydrous Na_2SO_4 overnight. The solvent was evaporated under reduced pressure to get 13.8 g of a neutral fraction (from Et-3 fraction) and 0.8 g of a basic fraction (from H_2SO_4 fraction).

The neutral fraction (13.5g) was separated by silica gel column chromatography (Wakogel C-300, glass column: 45 x 3.5 cm i. d.) with *n*-hexane and chloroform as mobile phase. The concentration of chloroform in *n*-hexane increased 5% for each 600 ml added. Each fraction was tested by thin layer chromatography (TLC) (Keiselgel 60 F_{254}, 0.25 mm in thickness, 20 cm x 20 cm) with *n*-hexane and chloroform as development solvents in a glass chamber (25 cm x10 cm x 22 cm). The TLC plates were sprayed with 5% H_2SO_4 (in ethanol) and heated in an oven at 120°C for 2-3 minutes, before viewing under a UV lamp at 254 and 365 nm. The fractions with the same R_f values, represented by spots on TLC, were pooled (Fig. 1).

The active fraction (0.8 g) was further separated by silica gel column chromatography (Wakogel C-300, glass column 35x3 cm i.d) with *n*-hexane and chloroform as eluents with increasing the concentration of chloroform. Resulting active fraction (0.5 g) was further separated by high performance liquid chromatography (Shimadzu LC-8A, column: Nucleosil 50-5, Chemopack

7.5x300 cm, solvent system: *n*-hexane and chloroform (3:1/v:v), flow rate: 2 ml/minute, recorder: C-R4A chromatopac under UV spectrophotometric detector (SPD-6A) at 254 nm). Main peaks were collected by a fraction collector (Shimadzu FCV-100B) connected to the HPLC. Collecting each fraction was based on retention time of peaks. Weight of each fraction was assessed based on the peak area compared to all peak areas recorded by C-R4A chromatopac.

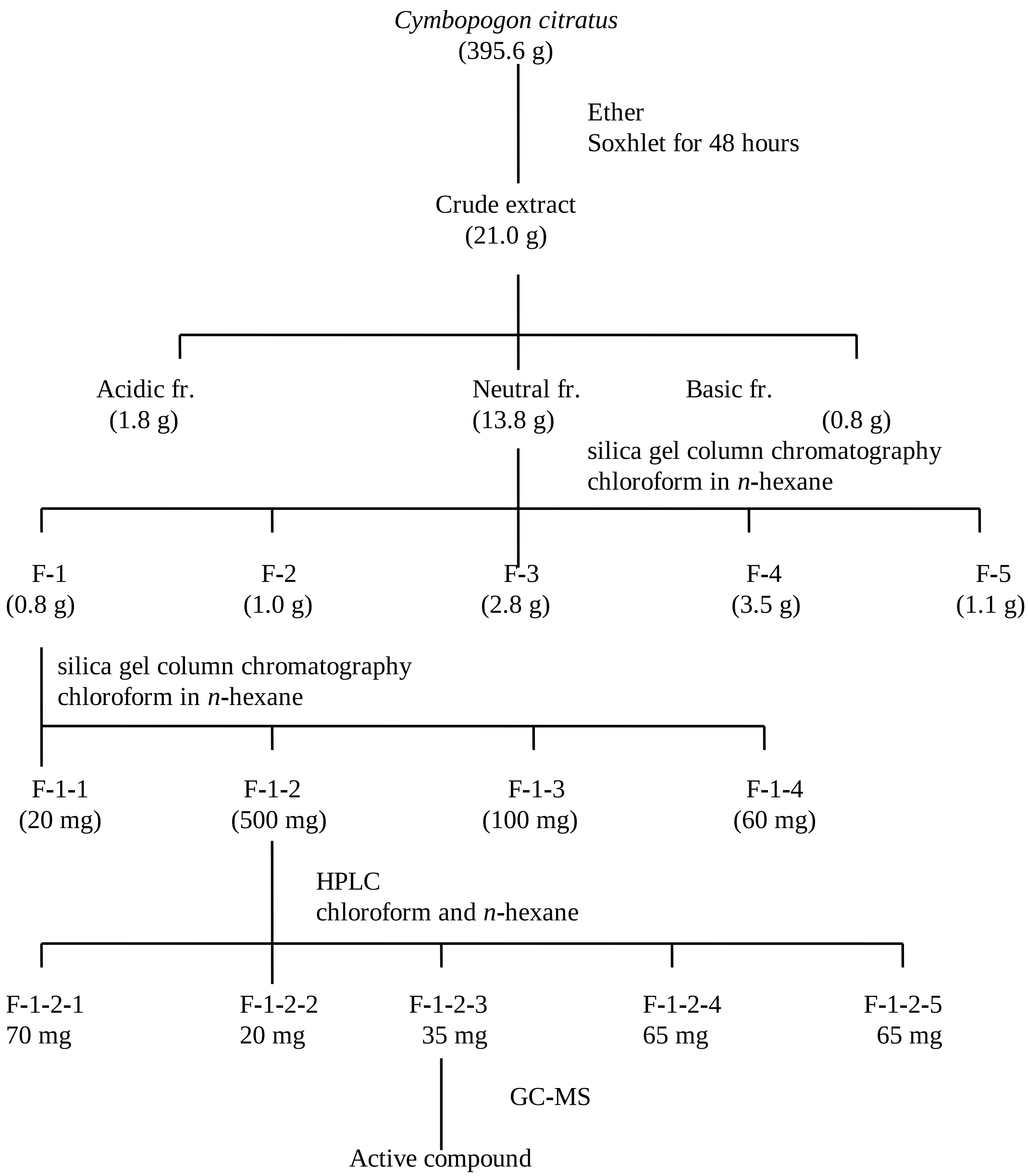

Fig. 1. Isolation scheme for the insecticidal compound from *Cymbopogon citratus*

Analytical instruments

The NMR spectra were recorded on a Jeol α-400 JNM spectrometer in $CDCl_3$ with TMS as an internal standard. Chemical shifts recorded were relative to residual $CDCl_3$ at 77.0 ppm for ^{13}C and TMS at 0.0 ppm for ^{1}H. ^{13}C and ^{1}H spectra were run at 100 and 400 MHz, respectively. Gas chromatography-mass spectrum (GC-MS) was measured by a Shimadzu QP-5000 spectrometer (capillary column DB-1, 0.25 mm i.d. x 30 m, initial GC temperature: 40 ^{O}C for 5 minutes, temperature gradient 40-200^{O}C with 5 ^{O}C /minute increment, He gas as carrier at flow rate of 5 ml/minute).

Bioassay

An appropriate amount of sample was dissolved in acetone to get the desired concentration and this was diluted serially to get lower concentrations. The test solution was applied onto the dorso-thorax of ten larvae (1µl/insect) in a Petri dish, by topical application method using a microsyringe applicator. Insects treated with acetone only served as control. After application, a fresh cabbage leaf (2 cm x 2 cm) was placed into the Petri dish (9 cm in diameter) lined with a distilled water-moistened filter paper (Advantec no.2). Each treatment was replicated 3 times. Insects that did not move when prodded with a fine brush were considered dead. Percent mortality was assessed at 24, 48 and 72 hours after treatment. LD_{50} values were determined by using probit analysis.

RESULTS AND DISCUSSION

Identification of active compound

Fractionation conducted by HPLC resulted in several fractions. Eight main fractions were collected with retention times and fraction weights as follows: 5.18, 7.47, 8.51, 10.12, 12.09, 12.63, 15.95, 17.76 minutes and 70, 20, 35, 65, 65, 36, 40, and 60 mg, respectively (Fig. 2). The fraction with retention time of 8.51 minutes produced mortality activity; while others produced no mortality activity.

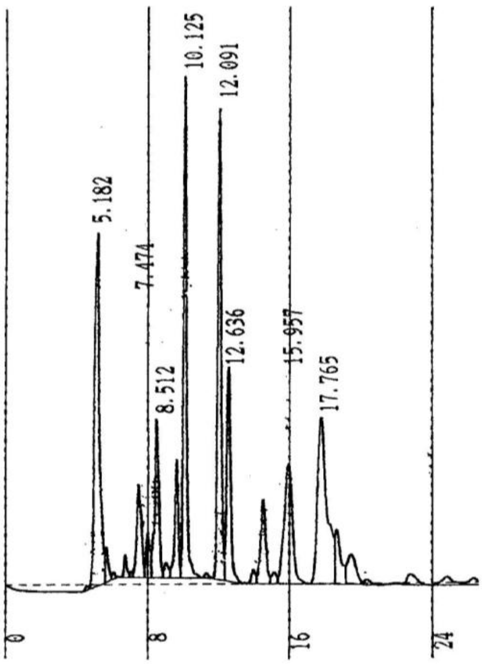

Fig. 2. HPLC chromatogram of active fraction of *C. citratus*

The analysis of the isolated fraction from HPLC by gas chromatography resulted in two peaks with retention times of 25.10 and 26.09 minutes (Fig. 3). Nevertheless, when the mass spectral data of these two peaks were analyzed and mass fragmentation patterns compared, these showed similar fragmentation patterns, at *m/z* 41, 69, 109, and 152. Fragmentation at *m/z* 152 was considered as the parent ion [M^+], so it is suggested that the compounds were isomers with each other (Fig. 4).

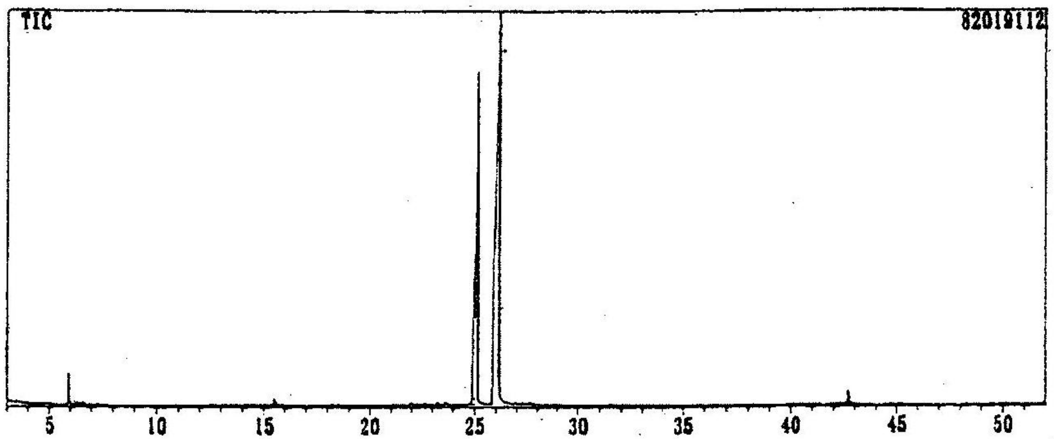

Fig. 3. Gas chromatogram of isolated fraction of *C. citratus*

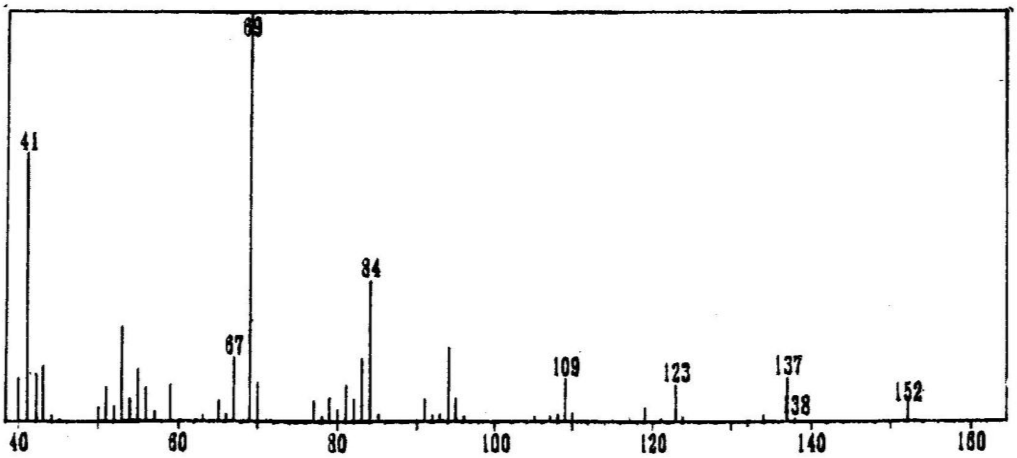

Fig. 4. Mass spectrum of isolated compound from *C. citratus*

The *C. citratus* essential oils contain citral which is racemic, having cis and trans forms (Formacek and Kubecka, 1982). Identification was also supported by ^{13}C-NMR spectrum as shown in Fig. 5. The presence of peaks at 190.8 and 190.3 indicate the presence of aldehyde (CHO) moieties for both neral and geranial compounds (isomer compounds). In addition, peaks found within the range of 122.02-163.45 indicated the presence of double bonds. Meanwhile, the remaining peaks found between 17.20-40.29 ppm indicated methyl moieties.

The position of each carbon atom and mass fragmentation are shown in Fig. 5 and Fig. 6, respectively. The active compound isolated from *C. citratus* was identified as citral, 3,7-dimethyl(-2,6-octadienal), a monoterpenoid compound.

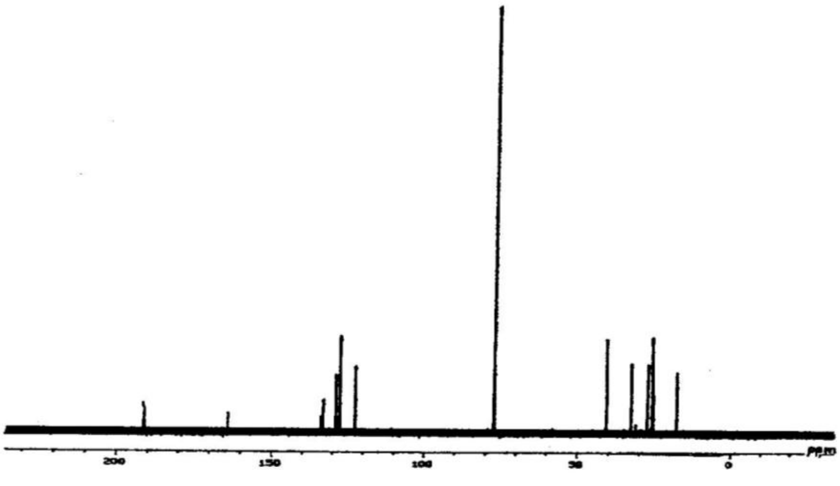

Fig. 5. ^{13}C-NMR spectrum and position of carbons in chemical structure of isolated compounds from *Cymbopogon citraus*

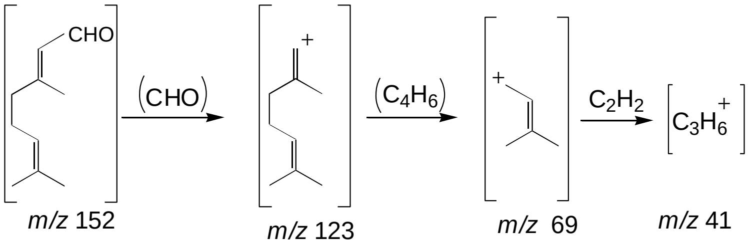

Fig. 6. Mass fragmentations of isolated compound from *C. citratus*

Larval toxicity

The extraction of 395.6 g fresh *C. citratus* aerial parts resulted in 21.0 g of crude extract (5.3%). The dark blue extract caused mortality activity to *P. xylostella* larvae, so fractionation and isolation of the active compound was pursued. Fractionation of the crude extract resulted in acidic, basic and neutral fractions. Bioassay of the fractions showed that the neutral fraction was toxic to *P. xylostella* larvae causing 92% larval mortality at 150 µg/insect within 72 hours of exposure; while acidic and basic fractions at 20 and 8 µg/insect did not cause larval mortality (Fig. 7).

The neutral fraction was separated by column chromatography with *n*-hexane and chloroform as mobile phases. Five fractions were obtained; F-1 eluted with 0-15%, F-2 with 15-25%, F-3 with 30-45, F-4 with 45-70, and F-5 with 70-100%. Bioassay based on equivalent doses at 10, 10, 30, 40, and 15 µg/insect for F-1, F-2, F-3, F-4, and F-5, respectively, all fractions gave no larval mortality activity. Nevertheless, bioassay of all fractions at 50µg/insect resulted in one fraction (F-1) which showed mortality activity. This fraction produced 100% larval mortality within 72 hours exposure; while other fractions did not produce larval mortality activity (Fig. 8).

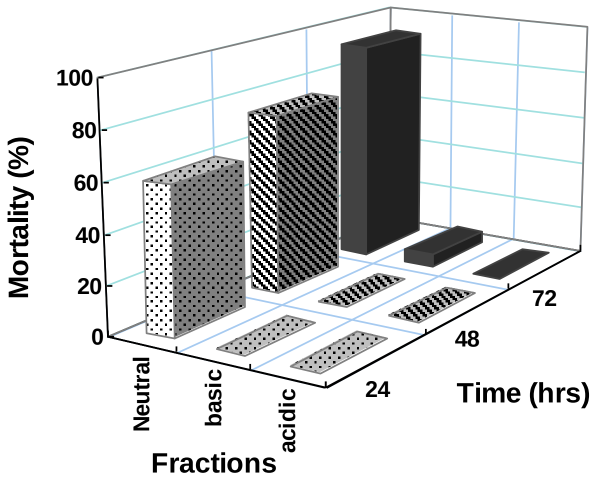

Fig. 7. Larval mortality produced by acidic, basic and neutral fractions of *C. citratus*

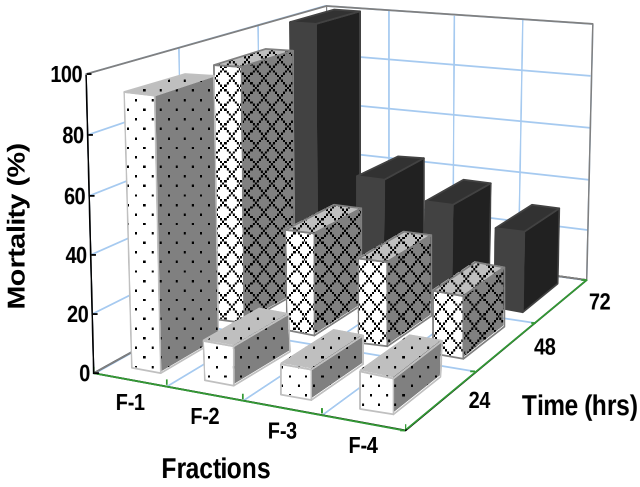

Fig. 8. Larval mortality produced by neutral fractions of *C. citratus* separated by column chromatography

The F-1 fraction was further separated by column chromatography with better elution. The chloroform concentration in *n*-hexane was increased by 1% for each 400 ml eluent added. Four fractions were obtained, as follows: F1-1 eluted with 0-1%, F-1-2 with 1-5%, F-1-3 with 5-15%, and F-1-4 with 15-35%. The bioassays based on equivalent doses at 5, 30, 10, and 5µg/insect for F1-1, F1-2, F-1-3 and F-1-4, respectively showed that F-1-2 yielded the highest larval mortality activity. F-1-2 fraction caused larval mortality by 80% within 72 hours exposure; while other fractions caused no mortality activity (Fig. 9).

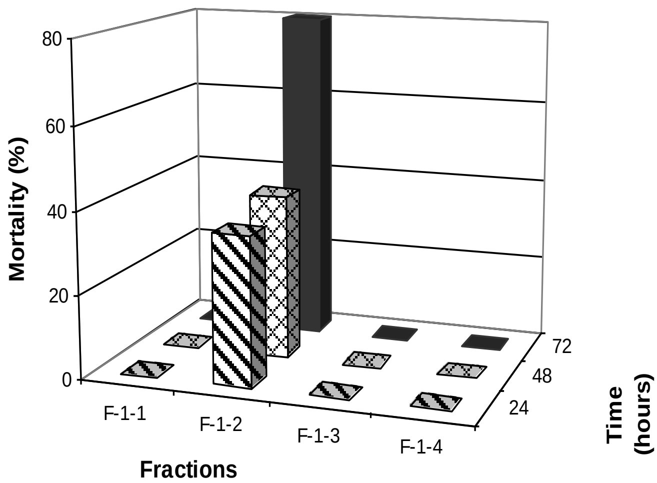

Fig. 9. Larval mortality of F-1 fractions (neutral fraction of *C. citratus*) separated by column chromatography

The separation of the F-1-2 fraction, conducted by HPLC with *n*-hexane and chloroform as mobile phase, resulted in eight main peaks as shown in Fig. 2. Each fraction collected was bioassayed at 20 µg/insect and F-1-2-3 produced the highest larval mortality (75%) within 72 hours exposure. F-1-2-2 and F-1-2-4 gave low mortality activity at 72 hours after treatment; while others were not toxic (Fig. 10). The active compound in F-1-2-3 was identified as citral. This compound is not highly toxic to *P. xylostella* larvae as indicated by its LD_{50} value of 7.7µg/insect.

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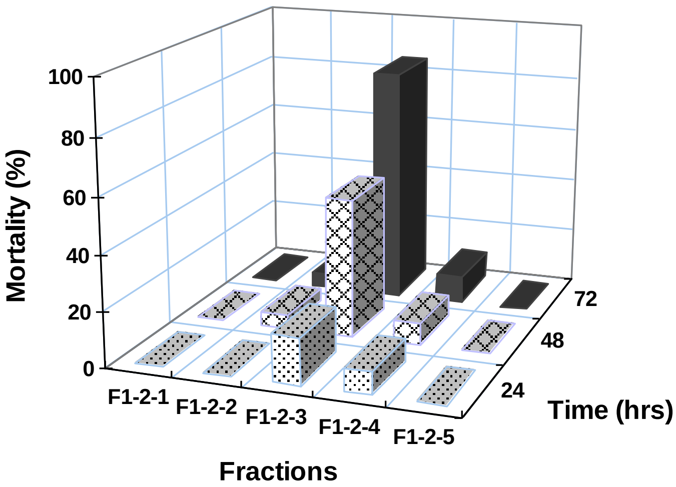

Fig. 10. Larval mortality of *P. xylostella* produced by *C. citratus* HPLC-collected fractions.

The mode of action of citral has not yet been elucidated. By visual observation, however, the onset of symptoms, when larvae were treated topically, were usually rapid and were manifested as agitation, hyperactivity, and quick knock down. The effects suggest that immediate neurotoxicities were induced. The surviving larvae treated with non-active fractions or lower concentrations recovered after several hours because of metabolization or elimination of compounds. Recovering larvae, looked for food sources and ate the food while several larvae treated at high dose struggled through the process. These larvae became weak and motionless because they were still combating the toxic effects, having no energy to move so that they could not reach the food source, eventually dying of starvation.

Several plants are known to contain citral essential oil, including *Zingiber officinale* (Zingiberaceae) and *Magnolia salicifolia* (Magnoliaceae). The pesticidal activity of citral has been reported. Citral in *Z. officinale* is molluscicidal against *Lymnaea acuminata* and *Indoplanorbis exustus* (Singh *et al*., 1997). Acaricidal activity of citral was reported by Ellis and Baxendala (1997) in which citral was toxic to *Acarapis woodi* (Acari: Tarsonemidae). Citral produced ovicidal activity to the house fly, *Musca domestica* (L.) (Diptera: Muscidae), showing 96% inhibition of egg hatchability (Rice and Coats, 1994).

Citral contains an aldehyde functional group and compared to other monoterpenoid compounds, its toxicity depends on its molecular structure because molecular shape, degree of saturation, and types of functional groups influence the insecticidal activity and species-specific insecticide susceptibilities (Lee *et al*., 1997). Alcohol and phenol-containing monoterpenoid compounds were more toxic than monoterpenoid compounds, aldehyde and ketone- moieties against the corn rootworm, *Diabrotica vergifera* (Coleoptera: Chrysomelidae), whereas aldehydes including citral were more toxic than alcohol-containing monoterpenoids by topical and fumigant action against *M. domestica*.

Monoterpenoid compounds are typically rather lipophilic compounds and may interfere with basic metabolic biochemical, physiological and behavioral functions of insects (Brattsten, 1983). Therefore, these chemicals are potentially useful as natural sources for the development of novel insecticides because of their low mammalian toxicity. Certain monoterpenoids are recognized as safe for humans by the Food and Drug Administration of USA and are used commercially as artificial flavorings and perfumes (Coats, 1994).

CONCLUSION

Cymbopogon citratus is toxic to *Plutella xylostella* larvae. The essential oil, citral, 3,7-dimethyl-2,6,-octadienal, was isolated and was responsible for the mortality activity. The LD_{50} value of this compound was 7.7 µg/insect, by topical application.

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