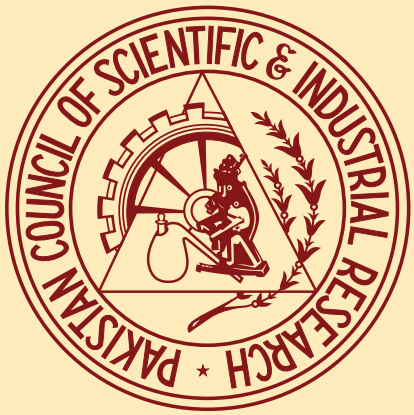
ISSN 2221-6421 (Print), ISSN 2223-2567 (Online)
Coden: PJSIC6 55(2) 59-116 (2012)

Pakistan Journal of Scientific and Industrial Research

Series B: Biological Sciences

Vol. 55, No.2, July-August, 2012



(for on-line access please visit web-site http://www.pjsir.org)

Published by
Scientific Information Centre
Pakistan Council of Scientific and Industrial Research
Karachi, Pakistan

Pakistan Journal of Scientific and Industrial Research
Series B: Biological Sciences

Pakistan Journal of Scientific and Industrial Research started in 1958, has been bifurcated in 2011 into:

Series A: Physical Sciences [**ISSN 2221-6413 (Print); ISSN 2223-2559 (online)**] (appearing as issues of January-February, May-June and September-October) and
Series B: Biological Sciences [**ISSN 2221-6421 (Print); ISSN 2223-2567 (online)**] (appearing as issues of March-April, July-August and November-December).

Each Series will appear three times in a year.

This Journal is indexed/abstracted in Biological Abstracts and Biological Abstracts Reports, Chemical Abstracts, Geo Abstracts, CAB International, BioSciences Information Service, Zoological Record, BIOSIS, NISC, NSDP, Current Contents, CCAB, Rapra Polymer Database, Reviews and Meetings and their CD-ROM counterparts etc.

Subscription rates (including handling and Air Mail postage): ***Local:*** Rs. 2000 per volume, single issue Rs. 350; ***Foreign:*** US$ 400 per volume, single issue US$ 70.

Electronic format of this journal is available with: Bell & Howell Information and Learning, 300, North Zeeb Road, P.O. 1346, Ann Arbor, Michigan 48106, U.S.A; Fax.No.313-677-0108; http://www.umi.com.

Photocopies of back issues can be obtained through submission of complete reference to the Executive Editor against the payment of Rs. 25 per page per copy (by Registered Mail) and Rs. 115 per copy (by Courier Service), within Pakistan; US$ 10 per page per copy (by Registered Mail) and US$25 per page per copy (by Courier Service), for all other countries.

Printed and Published by: PCSIR Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.

Editorial Address

Executive Editor
Pakistan Journal of Scientific and Industrial Research, PCSIR Scientific Information Centre,
PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan
Tel: 92-21-34651739-40, 34651741-43; Fax: 92-21-34651738; Web: http://www.pjsir.org, E-mail: info@pjsir.org

Pakistan Journal of Scientific and Industrial Research
Series B: Biological Sciences
Vol. 55, No. 2, July-August, 2012

Contents

Short Communication

Pak. j. sci. ind. res. Ser. B: biol. sci. 2012 **55** (2) 59-64

Effect of Wheat Residue Incorporation Along with N Starter Dose on Rice Yield and Soil Health Under Saline Sodic Soil

Syed Ishtiaq Hyder*, Muhammad Arshadullah and Arshad Ali
Land Resources Research Institute, National Agricultural Research Centre,
Park Road, Islamabad-45500, Pakistan

(received March 29, 2011; revised November 11, 2011; accepted November 28, 2011)

Abstract. A field experiment was conducted to determine the effect of crop residue incorporation @ 5 tons wheat straw/ha along with N starter dose (0, 30 kg N/ha, 60 kg N/ha and 90 kg N/ha) on rice (super basmati) production and soil health under saline sodic soils (ECe=5.32 dS/m, pH=8.52 and SAR=18.38) during 2009. Treatments were arranged using randomized complete block design (RCBD) with three replications. The crop was harvested at maturity, data on tillering, plant height, spike length, number of grains/ spike, 1000-grain weight, straw and paddy yields were recorded. Na, K, Ca and N concentration in grain and straw were estimated using atomic absorption spectroscopy. Tillering, number of grains/spike, 1000-grain weight and paddy yield significantly ($P \leq 0.05$) increased by different levels of doses. Maximum plant height (135.66 cm) and numbers of grains/spike (140.33), spike length (24.66 cm), number of tillers/plant (40.33) and 1000-grain weight (23 g) were recorded at the application of 5 tons wheat straw/ha along with 90 kg N/ha. Grain yield was the maximum (3.32 t/ha) at the application of 5 tons wheat straw /ha along with 90 kg N/ha and 26% more than control treatment. Positive correlations ($r = 0.85$) and ($r = 0.96$) was observed between calcium and potassium contents in grain and grain yield of rice. However, negative correlation (-0.92) between Na contents in grain and paddy yield was found. It indicated presence of significantly higher Ca and K contents in grain receiving wheat straw application combined with N starter dose. Its application helped plants to attain more Ca and K to avoid sodium uptake which has been an added advantage to alleviate salinity/sodicity. Economical analysis showed maximum value cost ratio (5.45:1) with the application of 5 tons wheat straw/ha along with 90 kg N/ha.

Keywords: rice, crop residue incorporation, salt affected soil, N starter dose

Introduction

Soil salinization is one of the major factors that contribute to land degradation and decrease in crop yield (Anjum *et al*., 2005; Yassin, 2005). The negative effects of salinization are intensified by the low levels of soil organic matter (Muhammad *et al.,* 2005). Salinity poses threat to crop production in many areas of the world including Pakistan (Ashraf and Foolad, 2007; Hasegawa *et al*., 2000). It has been estimated that almost 40,000 ha of arable land in Pakistan is being lost due to salinity and the area is rapidly increasing each year (Ashraf *et al*., 2008; Ahmad *et al.,* 2006). Rice-wheat is the largest cropping system in the world. Approximately 85% of the rice-wheat area is in the indo-gangatic plains of South Asia covering Nepal, Bangladesh, India and Pakistan (Timsina and Connor, 2001). Rice is highly valued cash crop that earns substantial foreign exchange, grown on an area of 2.96 million hectare with production of 6.952 million tons (GOP, 2009).

*Author for correspondence; E-mail: hyder292002@yahoo.com

However, the farmers cannot afford chemical fertilizers and pesticides to achieve the potential which is much higher than the national average paddy yield. Hence, there is an urgent need to develop a technology which can fulfill crop production and better utilization of crop residue for soil fertility improvement. As per fertilizer off take data, N: P fertilizer use ratio is 4.67:1, the balance of fertilizer use is in favour of nitrogenous fertilizers (GOP, 2009). Fertilizer off take data showed 5.9% decrease in potassic fertilizer use during 2009 as compared to 2008 which is 25.3 thousand tons. As a result of imbalanced fertilizer use, crop productivity is decreasing. Under present situation the combined use of rice or wheat straw and inorganic fertilizer can, however, increase the yield of rice and wheat in rice-wheat systems (Mahapatra *et al.,*1991) Despite some advantages like killing of deleterious pests and clearing the piles before wheat planting, burning results huge losses of N (up to 80%), P (25%), K (21%) and S (4-60%), air pollution (@ CO_2 13 t/ha) depriving soils of organic matter (SOM). This loss of SOM is one of the

Pak. j. sci. ind. res. Ser. B: biol. sci. 2012 **55** (2) 65-69

Allelopathic Effects of *Eucalyptus camaldulensis* Leaf Leachate on the Growth of Wheat and Green Gram and its Control by Farm Yard Manure

Saima Ibrahim* and Shazia Bibi
Department of Botany, Jinnah University for Women, 5-C Nazimabad, Karachi, Pakistan

(received March 31, 2011; revised May 5, 2012; accepted May 18, 2012)

Abstract. Farm Yard Manure (FYM) significantly reduced the allelopathic effects of *Eucalyptus camaldulensis* leachate. This influence was studied on morphological and physiological aspect of two taxonomically different plants (wheat and green gram). *E. camaldulensis* aqueous leachate applied @ 1% and 5% alone and together with FYM and the results showed that *E. camaldulensis* leaf leachate had inhibitory effects on wheat growth, while promoted shoot and root growth in green gram when supplied in low concentration. The combined effects of litter and FYM reduced the inhibitory effects of leachate and supported the growth of both plants. These results suggested that, if both studied crops have to be cultivated in an agricultural land surrounded by *E. camaldulensis* tree, the possible growth rate could be supported by the application of FYM. But in the absence of this support, the plant growth was significantly arrested due to allelopathic effect of *E. camaldulensis* leaf leachate.

Keywords: farmyard manure, Allelopathy, *Eucalyptus camaldulensis*, litter, leachate, antagonistic effects

Introduction

Allelopathy has direct or indirect deleterious effect of one plant upon another through the release of chemical inhibitor (phototoxic) and it may be the main reason of failure or poor crop growth in different environmental systems. In Pakistan, Eucalyptus has been the choice tree species in most of the social forestry project in the Asia Pacific regions because of its fast growing nature. From the last few decades, it has registered pronounced deleterious effects on the environment. The Eucalyptus species are considered the most notorious of allelopathic trees causing understory suppression specially in dry climates and water is scarce (rainfall < 400 mm) (May and Ash, 1990). *Eucalyptus* sp. has a high potential of allelochemicals in the form of phenols and essential oils. Vaughan and Ord (1990) reported that most of the phenolic acids released from the eucalyptus plant parts were benzoic and cinnamic acid derivatives. Presence of coumaric, caffeic and gallic acids also identified from *E. globulu*. Similarly gentistic acid, phenolic glycosides and terpenoids were also reported from *E. baxteri.* Iqbal *et al*. (2003) found 16 components in the essential oil of *E. camaldulensis* out of which 5 compounds (alpha pinene, 3-carene, beta-phellandrene, 1-8 cineole and p-cymene) were identified. Ghafar *et al*. (2000) found that these allelochemicals and volatile compounds present in all parts of *E. camaldulensis* have harmful effect on the crops in the ecosystem resulting in the reduction and delaying of germination mortality of seedling and reduction in growth and yield, also reduce the soil pH (Putnum,1984). The release of phenolic compounds adversely affect plant growth through their interference with energy metabolism, cell division, mineral uptake and other biosynthesis processes (Rice, 1984). Different researchers found that the eucalyptus leachate had varying degree of inhibitory and stimulatory effects on germination percentage (Phlomina and Srivasuki, 1996) and plant growth considerably at higher concentration (Jayakumar *et al*., 1990). Therefore, *Eucalyptus*, though a potential industrial crop, is not being recommended as an inter crop in an agroforestry system (Bansal, 1988; Suresh and Rai, 1987), presumably due to the release of allelochemical and phytochemical compounds from the tree (Lisanework and Michelson, 1993). The research evidently pointed out that some crops showed the tolerance towards the inhibitory effects of these allelochemicals. Eucalyptus tree belt have more adverse effect on wheat than legume crop. Similar effects have been reported by Inouye *et al*. (2001), who studied the effects of leaf extracts of *Eucalyptus* spp. on wheat and mung bean in order to support this view.

*Author for correspondence; E-mail: Ibrahim4sept@hotmail.com

Pak. j. sci. ind. res. Ser. B: biol. sci. 2012 **55** (2) 70-81

Decomposition and Nutrients Release Pattern of Leaves, Stems / Vines and Roots of Selected Leguminous and Non-Leguminous Plant Species

Oladele Abdulahi Oguntade[a], Abideen Idowu Adeogun[b]*, Guogiong Tian[c] and Gideon Adeoye[d]

[a]Soil Science Department, University of Agriculture, Abeokuta, Nigeria
[b]Chemistry Department, University of Agriculture, Abeokuta, Nigeria
[c]Environmental Monitoring and Research Division, Research and Development Department, Metropolitan Water Reclamation District of Greater Chicago (MWRD-Chicago), Lue-Hing R&D Complex, 6001 W. Pershing Road, Cicero, IL 60804, USA
[d]Agronomy Department, University of Ibadan, Ibadan, Nigeria

(received November 28, 2011; revised April 12, 2012; accepted May 2, 2012)

Abstract. Decomposition and nutrients release pattern of leaves, stems/vines and roots of leguminous plants (*Pueraria phaseoloides* and *Centrosema brasilianum*) and non-leguminous plants (*Chromolaena odorata* and *Panicum maximum*) were examined for a period of 98 days. The decomposition rate declined in the order *P. maximum* > *C. odorata* > *P. phaseoloides* > *C. brasilianum*. On the 98th day, between 63% and 71% of stems/vines only had decomposed. The % mass of the remaining materials were in the order of *C. odorata* > *P. phaseoloides* > *C. brasilianum* > *P. maximum* and the decomposition rate was in the reverse order. The mass loss and decomposition rates of the parts of the plant species followed the initial N concentrations of the residues in the order of leaves > roots > stems/vines. The initial N contents in plant residues varied from 0.42 to 3.19 g/kg and P from 0.03 to 0.27 g/kg. The pattern of N remaining in the stems/vines of the species at 98 days after placements (DAP) was in the order *P. phaseoloides* (49%) > *C. odorata* (42%) > *C. brasilianum* (41%) > *P. maximum* (36%). The chemical composition of the residues shows that the leaves are richer in N and P than the roots and least in stems/vines in the order *C. odorata* > *C. brasilianum* > *P. phaseoloides* > *P. maximum*. A direct relationship was observed between the initial N contents of the residues and initial polyphenol contents ($r = 0.72$, $p < 0.01$), but no relationships with the P content of the residues. The data reported in this study shows that the decomposition patterns of above and below - ground residues of leguminous and non-leguminous plant species could have positive effect on nutrients requirement of crops.

Keywords: nutrients release, plant decomposition, non-leguminous, *Pueraria phaseoloides*, *Centrosema brasilianum*, *Chromolaena odorata*, *Panicum maximum*

Introduction

In agroforestry systems, plant residues enter the soil system as crop residues and tree leaf litter and pruning residues. These plant residues are sources of nutrients and organic matter when they decompose and could contribute to the maintenance of soil fertility. The use of plant residues to improve crop production has become a major focus of soil fertility management in the tropics (Tian, 1998). Thus, bio-availability of nutrients is a function of nutrients supply and nutrients limitation, which in turn depend on differences in the rate of decomposition, mineralization and other processes (Swift *et al*., 1979). Residue management for nutrients recycling involves synchronizing nutrients release with crop demands. This could be achieved by manipulating the nutrients release pattern of the residues through appropriate timing and placement of organic input to meet crop use (Myers *et al*., 1994). For instance, incorporating plant residues into agricultural soils can sustain organic carbon (C) content, improve soil physical properties, enhance biological activities, and increase nutrient availability (Zeng *et al*., 2010; Cayuela *et al*., 2009; Hadas *et al*., 2004).

Residue decomposition rates and nutrient release patterns are controlled by both biotic and abiotic factors, the most important of which is residue quality (Zeng *et al*., 2010; Teklay *et al*., 2007; Mungai and Motavalli, 2006; Silver and Miya, 2001). The initial concentrations of nitrogen (N), phosphorus (P), polyphenol and lignin,

*Author for correspondence; E-mail: abuaisha2k3@yahoo.com

Pak. j. sci. ind. res. Ser. B: biol. sci. 2012 **55** (2) 82-92

Effects of Different Sources of Organic Waste Application on the Growth and Biomass Production of Kenaf (*Hibiscus cannabinus* L.)

Md. Shayeb Shahariar[a]*, Shahrima Tahsin[a], Md. Nasimul Gani[b] and Shah Muhammad Imamul Huq[c]

[a]Soil and Environment Section, Biological Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dr. Qudrat-I-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh

[b]Soil Science Division, Bangladesh Jute Research Institute (BJRI), Manik Mia Avenue, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh

[c]Department of Soil Water and Environment, University of Dhaka, Dhaka-1000, Bangladesh

(received October 6, 2011; revised April 27, 2012; accepted May 24, 2012)

Abstract. The growth and biomass productivity of kenaf (*Hibiscus cannabinus* L.) grown with different sources of organic waste viz. sewage sludge, poultry litter, cow dung and rice straw application were observed in a field experiment. Organic wastes were applied at the rate of 5 t/ha and were compared with recommended dose of fertilizers and control. The plants were harvested at 120 days after sowing (at the flowering stage). Different sources of organic wastes had a significant effect ($P < 0.05$) on the growth indices and biomass productivity of kenaf. Increased plant growth and biomass productivity were in the order of sewage sludge > cow dung>poultry litter > rice straw treatments. Among the four sources of organic wastes, sewage sludge treated plot produced the highest mean biomass of 23.33 t/ha (dry weight basis) which was 14.64% higher than the mean biomass production from control plot.

Keywords: biomass production, growth, kenaf, organic waste, poultry litter, sewage sludge

Introduction

Kenaf *(Hibiscus cannabinus* L.), a member of the Malvaceae family endemic to Africa (Bassam, 1998) is a warm-season, annual, herbaceous plant that produces high quality cellulose. Although tropical in origin, kenaf cultivars are well adapted to a wide geographical and climatic range (Meints and Smith, 2003). Kenaf is a high yielding plant achieved particular attention as an alternative crop for energy and paper pulp production. It is a good source of low cost natural fibre for a wide range of industrial products, examined for its possible uses in bio-energy sector (Alexopoulou *et al.*, 2004) and has long been used to provide pulp for the paper industry (Taylor and Kugler, 1992). Kenaf has been reported to be 3-5 times more productive per unit area than pulpwood trees, producing pulp with quality equal or superior to that of many wood species (Francois *et al.*, 1992). United States Department of Agriculture (USDA) determined that kenaf is an excellent cellulose fibre source for a wider range of paper products such as newsprint, bond paper, and corrugated liner board. It is also determined that pulping kenaf required less energy and chemicals for processing as compared to standard wood sources (White *et al.*, 1970; Wilson *et al.*, 1965; Nelson *et al.*, 1962; Nieschlag *et al.*, 1960). Recent research and development efforts have further increased the diversity of uses of kenaf by demonstrating its suitability in building materials like particle boards of various densities, thicknesses, with fire and insect resistance, as a substrate mixture adsorbents, textiles, livestock feed and as fibres in new and recycled plastics (injected molded and extruded) (Nielsen, 2004; Webber and Bledsoe, 2002; McMillin *et al.*, 1998; Webber, 1993a; Webber and Bledsoe, 1993). The prospects of kenaf becoming as an alternative crop to obtain large biomass amounts at low production cost (Quaranta *et al.*, 2000). To achieve this, agronomic practices such as soil amendment may be able to improve its productivity, growth and biomass quality (Alexopoulou *et al.*, 2007; Muir, 2002; 2001). Considerable information on kenaf agronomy and biomass productivity has been reported from Australia (Muchow and Carberry, 1993; Carberry *et al.*, 1992; Carberry and Muchow, 1992; Muchow, 1992; Wood *et al.*, 1983; Wood and Muchow,

*Author for correspondence; E-mail: shayeb.shahariar@gmail. com

Pak. j. sci. ind. res. Ser. B: biol. sci. 2012 **55** (2) 93-100

Determination of Lead and Cadmium in Hen Eggs by Graphite France Electrothermal Atomic Absorption Spectrometry and Estimation of the Daily Intake

Ishratullah Siddiqui[a]*, Shaikh Sirajuddin Nizami[b], Farooq Ahmed Khan[a], Durdana Rais Hashmi[a], Uzma Rashid[a], Alia Bano Munshi[a] and Shah Ali-Ul-Qadar[c]

[a]PCSIR Laboratories Complex Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan
[b]Department of Chemistry, University of Karachi, Karachi-75270, Pakistan
[c]Department of Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan

(received June 9, 2011; revised September 9, 2011; accepted September 24, 2011)

Abstract. A total of 54 hen eggs were procured from nine poultry farms of Sindh, Pakistan in different batches to determine lead and cadmium toxicity. The quantitative analysis of lead (Pb) and cadmium (Cd) in egg samples were performed on electrothermal atomic absorption spectrometer (ETAAS), with Zeeman effects background correction. Lead concentrations in hen egg samples ranged from 0.027 to 1.056 µg/g with a mean value of 0.283 µg/g ± 0.86, whereas cadmium concentrations ranged from 0.001 to 0.012 µg/g with a mean value of 0.003 µg/g ± 0.002. Lead concentrations exceeded the normal levels of 0.020 but cadmium was found lower than the normal levels of 0.005 µg/g.

Keywords: hen eggs, lead, cadmium, atomic absorption, spectrometry

Introduction

Fresh eggs are among the most important and common source of animal protein being the nutritious food of daily diet. Eggs are the source of protein, minerals and trace elements and unsaturated fatty acid which are necessary for proper development and functioning of different tissues and organs. In Pakistan consumption of egg per capita significantly increased during the last few years. On the other side, hen eggs contamination by potential toxic metals caused serious health hazards for public health as a source of metal exposure (Fakayode, 2003). Metals can be transferred from parent to offspring and can be sequestered from hen to eggs along the food chain when an animal is fed on metal contaminated feed (Meluzzi *et al.*, 1996), through environmental contamination in the form of atmospheric deposition of metal from industry or vehicular emission (Mochizuki *et al.*, 2002).

The rate of industrialization in urban areas particularly in big cities is increasing with the passage of time. Present level of urbanization in Pakistan is the highest in south Asia; Sindh is the most urbanized province and 60% of urban population of Sindh resides in Karachi which is the largest industrial city of Sindh province. Landhi/Korangi Industrial Zone has a total area of 34.4 km^2 having more then 2000 various types of small and medium sized industrial units of poultry and cattle farms (Mahmood *et al.*, 1998). There are various types of environmental pollution due to indiscriminate discharge of industrial waste which constitute a potential danger to neighboring localities and pose many challenges related to food quality and safety (Abou Donia, 2008) with increasing industrializa-tion, more and more metals are entering into the environment. These metals stay permanently because they can not be degraded in the environment. They enter into the food matrix and from there they ultimately make their passage into animal tissues (Baykov *et al.*, 1996).

Lead, cadmium, mercury and arsenic are among the main toxic metals which accumulate in food chains and have a cumulative effect. There are maximum levels for lead, cadmium and mercury in several foodstuffs such as meat, vegetable or fish nevertheless, no maximum levels exist for these trace elements in eggs (EC, 2006). Lead is ubiquitous in the environment persists indefinitely and can be found at low levels in almost all living organisms (Doganoc, 1996). Cadmium as environmental pollutant is well known to have a

*Author for correspondence; E-mail: pcsirklc_ces@hotmail.com

Pak. j. sci. ind. res. Ser. B: biol. sci. 2012 **55** (2) 101-107

Changes in *Bacillus thuringiensis* Tolerance Levels Due to Hybridization of Bt-Tolerant and Susceptible Silkworm Populations

Hosne Ara Begum[ad]*, Errol Hassan[a], John Dingle[b] and Aisha Alkhayat Alshehi[c]

[a]School of Agronomy and Horticulture, University of Queensland, Gatton, Queensland 4345, Australia
[b]School of Animal Studies, University of Queensland, Gatton, Queensland 4345, Australia
[c]Department of Biology, College of Science, Sultan Qaboos University, Al-Khod 123, Oman
[d]c/o Department of Biology, Sultan Qaboos University, PO Box 36, Al-Khod 123, Muscat, Oman

(received September 12, 2011; revised June 8, 2012; accepted June 20, 2012)

Abstract. Males and females of a Bt-tolerant mulberry silkworm (*Bombyx mori* L.) population were crossed with females and males of a Bt-susceptible population, to produce Bt-tolerant silkworm hybrids, and to determine the expression of the Bt-tolerance pattern in the F1 hybrids. It was observed that when a Bt-tolerant (42% larval mortality) female (BtT ♀) silkworm was crossed with a Bt-susceptible (85% larval mortality) male (BtS ♂), the resultant F1 offspring showed lower levels of Bt-tolerance (87% larval mortality). On the other hand, when a Bt-tolerant male (BtT ♂) was crossed with a Bt-susceptible female (BtS ♀), the F1 hybrid showed higher levels of Bt-tolerance (35% larval mortality) characteristic. The probit statistics showed that both hybrids expressed Bt-tolerance or susceptible levels similar to their male parents. These different patterns of Bt-tolerance in F1 hybrids might be due to the transferring of a Bt-tolerant gene, from the parents to offspring, through the homozygotic male (ZZ) silkworm.

Keywords: *Bacillus thuringiensis*, Bt-tolerance, *Bombyx mori*, insect hybridization, male homozygosity, hybrid performances

Introduction

Different commercial preparations of *Bacillus thuringiensis* (Bt) have been sold and used as biopesticides against crop pests worldwide. Natural Bt is also commonly present on Japanese mulberry plants especially on the leaves (Ohba, 1996). When ingested by silkworm larvae, the parasporal inclusions of Bt are dissolved in larval midgut juice and release protoxins. The Bt Cry1 proteins are specifically toxic to lepidopteran larvae including the *Bombyx mori*. These toxins are solubilized and enzymatically processed in the midgut of susceptible insects. The activated toxins interact with the larval epithelial membrane and induce pore formation in the membrane, which ultimately leads to insect death (McNall and Adang, 2003; Morin *et al.*, 2003). It was reported that the Bt toxins kill insects by binding to specific target sites and disrupting the midgut tissue followed by septicemia (Raymond *et al.*, 2010; Ibiza-Palacios *et al.*, 2008). The Bt affected larvae gradually become brown to black-brown and finally when rotting they turn black (Aruga, 1994).

*Author for correspondence; E-mail: H.A.Begum@gmail.com

Silkworm (*Bombyx mori* L.) larvae are highly susceptible to *B. thuringiensis* (Bt), causing over 50% mortality of 4th instar larvae at a low concentration of 0.01% of Bt (Pramanik and Somchoudhury, 2001). The silkworm larvae were also found as 2,500-fold more susceptible to the Bt var. *kurstaki* infection than *Spodoptera litura* larvae (Inagaki *et al.*, 1992). *B. thuringiensis* is considered to be the causative agent for flacherie disease of silkworms. Countries with established sericulture industries fear a Bt induced disease out-break and restrict the use of Bt in silkworm/ mulberry growing areas (Van Driesche and Bellows, 1996).

The Bt is widely used in vegetable growing areas of Australia. The establishment of a new sericulture industry in Australia might encounter Bt related disease problems, either from mulberry leaf sources or from the ingress of Bt pesticides from nearby crop areas (Begum *et al.*, 2004). As it is difficult to exclude pathogens from the silkworm rearing environment, Bt might be a major constraint in the silkworm disease management (Balavenkatasubbaiah *et al.*, 1999). Thus, it is important to develop Bt-tolerant silkworm races for the future

Pak. j. sci. ind. res. Ser. B: biol. sci. 2012 **55** (2) 108-113

Modified Protocol for Genomic DNA Extraction from Newly Plucked Feathers of *Lophura leucomelana hamiltoni* (Galliformes) for Genetic Studies and its Endo-restriction Analysis

Saiqa Andleeb[a]*, Sara Shamim[a], Muhammad Naeem Awan[b] and Riaz Aziz Minhas[a]
[a]Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan
[b]Program for Mountain Areas Conservation (PMAC), Regional Office Muzaffarabad, AJK - 13100, Ministry of Environment, Pakistan

(received July 26, 2011; revised February 2, 2012; accepted April 26, 2012)

Abstract. A rapid and accurate protocol was used first time to isolate the high-quality genomic DNA from newly plucked feathers of *Lophura leucomelana*. Two different lysis protocols were used depending on the feather size and it was observed that 55 °C for 3 to 4 days showed better results of feathers lysis as compared with the 37 °C for overnight with gentle shaking. Purification of genomic DNA was also performed with phenol: chloroform: isoamyl alcohol and 100% absolute ethanol precipitation methods. By using this protocol, a significant amount of high-quality genomic DNA was obtained and the purity of DNA was analyzed through endo-restriction analysis. Genomic DNA isolated with this modified method will be used for Southern blotting and also in several polymerase chain reaction systems devoted to sex determination and paternity testing and the evolutionary relationships among the other pheasants.

Keywords: Agarose gel electrophoresis, genomic DNA extraction, *Lophura leucomelana hamiltoni*, endo-restriction analysis

Introduction

The White Kalij (*Lophura leucomelana hamiltoni*), is a pheasant found in forests and thickets, especially in the foothills, from the Indus River to western Thailand (McGowan and Panchen, 1994). *Lophura leucomelana* has nine sub-species and *Lophura leucomelana hamiltoni* is the species found in Pakistan (Johnsgard, 1999; 1986). The speciation pattern in genus *Lophura* is certainly the most complex of any in all pheasant groups, and has been cause of a vast number of species and subspecies being described, many of which have been based on single specimen (Johnsgard, 1986). The pheasants in *Lophura* are sexually dimorphic, with unremarkable and mimetic females, although, males also show cryptic plumages in some species. In previous literature, plumage trait has been used to conclude the evolutionary relationships and taxonomy of the pheasants (Johnsgard, 1986; Delacour, 1977). On the other hand, Kimball *et al.* (1999) demonstrated that most of these phenotypic traits were not diagnostic and might be evolved repeatedly and independently among the pheasants.

In previous literature it has been shown that the DNA was extracted from the mammals, which are often difficult to capture like bears, wolves, primates, elephants (Hausknecht *et al.*, 2007; Schmaltz *et al.*, 2006; McGrew *et al.*, 2004; Eggert *et al.*, 2003; Morin *et al.*, 2001). Similarly, DNA has also been sampled from hair, faeces, urine, fish scales, shed snake skin, and egg shells (Hausknecht *et al.*, 2007; Lucentini *et al.*, 2006; Schmaltz *et al.*, 2006; Eguchi and Eguchi, 2000; Nota and Takenaka, 1999; Goossens *et al.*, 1998; Bricker *et al.*, 1996; Tikel *et al.*, 1996) and can widely be used for sex identification and genetic disease diagnosis, respectively. In the same way, in present research work the genomic DNA extraction was carried out from the newly plucked feathers of White Crested Kalij pheasant instead of blood that is necessary to diminish the stress on the birds because in case of juvenile birds and small parrots the blood vessels are in small size that makes the blood extraction very difficult (Natalia *et al.*, 2001; Hauge, 1997; Alleman, 1996; Davidson *et al.*, 1986).

The evolutionary relationships and the taxonomy of the subspecies of the silver and Kalij pheasants (*Lophura nycthemera* and *L. leucomelanos*; Phasianidae) are unclear. Delacour (1949) included them in a single superspecies, and pointed out the following systematic problems: (i) their description in a large number of genera before his revision, 49 taxa of silver and Kalij pheasants being split into 28 species belonging to four genera (Del Hoyo *et al.*, 1994); (ii) their large geographical

*Author for correspondence; E-mail: drsaiqa@gmail.com

Pak. j. sci. ind. res. Ser. B: biol. sci. 2012 **55** (2) 114-116

Short Communication

Energy Expended in Processing *Gari* (Cassava Flakes) *Manihot esculenta* Crantz, Using Three Levels of Mechanization

Rahman Akinoso* and Wasiu Olatunde Kasali
Department of Food Technology, Faculty of Technology, University of Ibadan, Ibadan, Nigeria

(received October 1, 2011; revised May 9, 2012; accepted May 16, 2012)

Abstract. The effect of using mechanical equipment for the unit operations involved in *gari* (cassava flakes) processing on individual and total energy demands were studied. The data used to estimate energy demand were the quantity of cassava processed, time taken for each operation, number and gender of labour, method of processing, quantity, and source of energy. Of the seven unit operations for *gari* production, one, three and five operations were mechanized in low, medium and high-level, respectively. Garification (simultaneous cooking and dehydrating operation of *gari*) accounted for 97.38, 97.70 and 47.39% of total energy consumption in low, medium and high levels of mechanization, respectively. Total energy requirements for processing 100 kg of cassava root into *gari* were found to be 771.0 ± 83.71 MJ for low-level mechanized factory, 684.53 ± 26.98 MJ and 73.97 ± 1.84 MJ for medium and high-level mechanized factories, respectively. Energy demand by all unit operations significantly differed ($p<0.05$) between low and high levels of mechanization.

Keywords: cassava, *gari* processing, unit operation, mechanization, energy demand

It is imperative that energy development, management, improvement, utilization and conservation must have predetermined plans and strategies that are capable of driving the economy towards a sure path of sustainable development (Aderemi *et al*., 2009).

Knowledge of energy consumption for each product in a food-processing factory is useful for budgeting, evaluation of energy consumption for a given product, predicting energy requirement in a plant, and for planning plant expansion. Available literature on the estimation of energy input in food processing include sugar-beet production (Mrini *et al*., 2002); cashew nut processing operations (Jekayinfa and Bamgboye, 2006); palm-kernel oil processing operations (Jekayinfa and Bamgboye, 2007); bread making processes (Le-bail *et al*., 2010); sugar production factory (Abubakar *et al*., 2010) and cashew nut processing mills (Atul *et al*., 2010).

Cassava (*Manihot esculenta* Crantz) is a perennial vegetative propagated shrub commonly cultivated within the lowland tropics and *gari* (cassava flakes) is a staple food widely consumed in Nigeria and other West African countries (FIIRO, 2003). Different methods are used for processing cassava in several parts of the world and these methods have resulted in the production of a wide variety of food products (Oladunmoye *et al*., 2010) including *gari*, industrial starch and flour. Labour requirements for traditional processing of cassava into *gari*, *fufu*, *lafun* and other products are huge (Odebode, 2008).

This research examines the effect of using mechanical equipment for the unit operations involved in *gari* (cassava flakes) processing on individual and total energy demands.

Three categories of *gari* producing factories in Ogun State, Nigeria were selected based on the mechanical equipment used for cassava processing. Data for three categories was gathered by direct measurement during normal operation periods (8.00 am to 5.00 pm) of the randomly selected mills production. Four factories were examined for each level of mechanization. The random selection done from the pool of those were up to date in data taking and management practices and are less than 3 years old. Freshly harvested cassava roots (TMS 55752) were used for the production of *gari* and parameters (source of energy, quantity of cassava processed, quantity of fuel consumed, time and number of persons in each unit operation) for evaluating energy inputs were recorded.

At each stage of the unit operations of cassava processing, energy input is required in the form of either thermal energy or manual energy (Samuel *et al*., 2010). The type and magnitude of the energy input is a function of the technology employed and the quantity of cassava

*Author for correspondence; E-mail: akinoso2002@yahoo.com