EFFECT OF BISHKATALI PLANT (*POLYGONUM HYDROPIPER*) EXTRACT AGAINST RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST)

A THESIS

BY

BHARATI RANI KUNDU

Examination Roll No. 0505005

Registration No. 0505005

Semester: July-December, 2006

MASTER OF SCIENCE (M.S)

IN

ENTOMOLOGY

DEPARTMENT OF ENTOMOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

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Submitted to Department of Entomology
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of

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Approved as to style and content by

Dr. Roushan Ara	**Md. Alamgir Hossain**
Supervisor	**Co-supervisor**

Chairman, Examination Committee

and

Chairman of the Department

DEPARTMENT OF ENTOMOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

NOVEMBER, 2006

DEDICATED

TO MY

BELOVED PARENTS

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The Authoress

EFFECT OF BISHKATALI PLANT (*POLYGONUM HYDROPIPER*) EXTRACT AGAINST RED FLOUR BEETLE (*TRIBOLIUM CASTANEUM*, HERBST)

ABSTRACT

An investigation was carried out in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University, Dinajpur during March-August, 2006, to evaluate the toxicity, repellency and residual effect of Bishkatali plant extracts in two solvent (chloroform and ethyl alcohol) against red flour beetle, *Tribolium castaneum*. Five doses (500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml) of plant extract were used in the experiments. The plant extract in both solvent were moderately toxic to *T. castaneum*. The order of toxicity was- ethyl alcohol extract>chloroform extract after 24 and 72 hours treatment on the insect. Bishkatali plant extract in both solvents showed strong repellent effects against *T. castaneum*. Extracts in chloroform showed better repellency than in ethyl alcohol. The highest repellency 87.3% was observed in chloroform extract at the highest dose of 500mg/ml. While with the same dose (500mg/ml) in ethyl alcohol extract, 80.3% repellency was observed. Bishkatali plant extract in chloroform and ethyl alcohol against *T. castaneum* had remarkable residual effect in progeny reduction. The lowest numbers of F_1 adult progeny (32.7, 25.3 and 27.0) emerged from the wheat flour treated with 500mg/10g chloroform extract when parent released at 7, 12, 17 days after treatment respectively. Whereas with 500mg/10g ethyl alcohol extract, 38.0, 29.7 and 30.3 F_1 adult progeny emerged when parent released at 7, 12, 17 days after treatment respectively. The highest inhibition rate was observed 59.3% and 52.3% in chloroform extract and ethyl alcohol extract, respectively when treated with 500mg/10g dose and the adult released 12 days after treatment. Therefore, *Tribolium castaneum* can be managed effectively with Bishkatali plant extract.

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CHAPTER I
INTRODUCTION

1 INTRODUCTION

Among the stored grain insect pests, Red flour beetle *Tribolium castaneum* Herbst (Coleoptera-Tenebrionidae) is common and most destructive pest. This insect is cosmopolitan in distribution. It is generally found in granaries, mills, ware houses etc. feeding on wheat flour, atta, suji, rice (both husked and unhusked) etc. Neither larva nor adult can generally damage sound grains but they feed on those grains only, which have already been damaged by other pests. This pest has also been reported to attack the germ part (embryo portion) of the grain. It is more serious to flour, atta, suji and similar powders products. Their presence in stored foods directly affects both quantity and quality of the commodity (Burkholder and Faustini, 1991; Mondal, 1992 and 1994). Incase of heavy infestation, flour turns grayish yellow and subsequently becomes mouldy and emits smell (Alam, 1971).

In tropical countries like Bangladesh, the climate and storage conditions are favorable for insect growth and development (Jacobson, 1982; Khan and Mannan, 1991; Talukder and Howse, 1995a). Khan and Mannan (1991) initiated a screening program using stored product insect. Among these *Tribolium castaneum* stands out as an agricultural pest of primary importance in tropics. It was stated that this insect is the most common occurring in situation where grain products are stored (Metcalf and Flint, 1962; Alam, 1971). The world's cereal production is lost to the extent 8% every year due to insect infestation alone in storage. In India, losses caused by insects accounted for 6.5% of stored grains (Raju, 1984). In USA and Canada, 20-26 percent of the stored wheat was infested by insect pest (White *et al.,* 1985). In Bangladesh, the annual grain loss cost as over take 100 crores (Alam, 1971).

At present different kinds of preventive and curative control measures are practiced to protect insect pests. Among them chemical pesticides has been used for a long time, but has serious drawbacks (Sharaby, 1988). Indiscriminate and continuous use of chemical insecticides created several considerable problems in agro-ecosystem such as direct toxicity to beneficial insects, fishes and human (Goodland *et al.,* 1985; Munakata, 1977; Pimentel, 1981), pesticides resistance (Brown, 1968; Georghiou and Taylor, 1997; Waiss *et al.*, 1981) health hazard (Bhaduri *et al.*, 1989) and increased environmental and social costs (Pimentel *et al.*, 1980). Sometimes, resistant pesticides accumulate in higher food chain in both wild life and human and become concentrates by bio-magnification (Metcalf and Luckmann, 1975). The development of cross and multi-resistant strains in many important insect species; resulting from the continuous use of chemical insecticides have been reported from all over the world (Dyte, 1970; Dyte and Hallday, 1985; Irshad and Jilani, 1990; Zettler and Cuperus, 1990; Zettler, 1991).

There is an urgent need for safe but effective and bio-degradable pesticides with no toxic effects on non-target organisms. This has been created a world-wide interest in the development of alternative strategies including the search for new types of insecticides and the re-evaluation and use of age old traditional botanical pest control agents (Heyde *et al.,* 1983). Botanical pesticides are broad-spectrum, safe to apply, unique in action and can be easily processed and used in the pest control. The main advantages of botanicals are that they are easily produced by farmers and are less expensive.

In the rural areas of South Asia, including Bangladesh farmers traditionally mix leaves, barks, seeds, roots or oils of certain plants with stored grains to keep them free from insect attacks. Such techniques have been inherited as part of the

traditional culture (Saxena *et al.,* 1988). The earlier studies (Islam, 1987; Talukder *et al.,* 1990; Talukder and Howse, 1993; 1994a, 1994b and 1995a) established the successful actions of different plant parts and extracts against different major stored product insect pests in Bangladesh. There are about 2000 plant reported to possess pest control properties (Ahmed *et al.,* 1984). Botanicals like Neem, Bishkatali, Marigold, Durba, Pithraj, Ban-kalmi, Nishinda, and Eucalyptus may be grown with minimum maintenance and extracted by indigenous methods.

However, very few scientific research works has been done in Bangladesh to determine the efficacy of our locally available plant materials against stored products pests. Under these circumstances an investigation was undertaken to determine to toxicity, repellency and residual effect of the plant Bishkatali, *Polygonum hydropiper*. In view of the above perspectives extract of the Bishkatali plant was evaluated against Red flour beetle *Tribolium castaneum* with the following objectives-

a. To determine the toxicity of bishkatali plant extract on red flour beetle.

b. To assess the repellency effects of bishkatali plant extract on red flour beetle.

c. To estimate the residual effects of bishkatali plant extract on red flour beetle.

CHAPTER II
REVIEW OF LITERATURE

2 REVIEW OF LITERATURE

Stored grains suffer seriously from the attack of a number of insect pests. Control of insect pest by botanicals has drawn special attention all over the world. The use of botanical as store grain protectant is known since long. There were many reports published on the use of plant materials against stored pests but the information against red flour beetle *T. castaneum* Herbst is scanty. It is mentioned here, that there is very limited research works conducted particularly on the use of Bishkatali plant extract. Relevant information's of the research works in home and abroad on stored product insect pests management by botanical insecticides have been reviewed below:

2.1 Toxicity effect

Abdullah *et al.* (1986) tested the crude extracts of 6 plants such as *Glycine max*, *Chenopodium album*, C. a*mbrosioides, Conyza disocoridis, Artemisia herva-alba* and *Convolvulaus arvensis* against S. *granarius* and *T. castaneum* compared their effects with those of pirimiphos-methyl and malathion. The crude extracts of *C. ambrosioides*, hexane extracts of *C. arvensis* and hexane and ethanol extracts of *Conoyza dioscoridis* were compared. The most active 11 fractions isolated fractions from these crude extracts were studied further. Some of these fractions caused increased mortality, which was comparable to that of the insecticides. *S. ganaris* was generally more sensitive than *T. castaneum* to all fractions. Oviposition and egg hatchability was also greatly reduced by some of the fractions. Several mixtures of the insecticides with the fractions exhibited synergism.

Singh *et al.* (1987) evaluated six extracts of plant-origin against *R. dominica* in the laboratory, extracts of neem *Azadirachta indica, Bassia longifolia* and *Pongamia glabra* were highly toxic. The crude extract of water hyacinth *Eichhornia crassipes* was evaluated for its biological activity against the *T. castaneum, S. oryzae, Callasobruchus maculatus* and *C. cephalonica.*

Trivedi (1987) reported that neem, sesame and linseed oil cake were found effective in controlling *Rhizopertha dominica* and *T. castaneum, w*hen used as a carrier materials with pyrithrin dust. Extracts of *Tagetes erecta* and *Tagetes patula* reduced infestation of diamond black moth, *Plutella xylostella,* when planted around *Brassica campestris, Piper nigrum* and *Capsicum frutescens* were effective when topically applied against stored products insects such as *S. zeamais* and *T. castaneum* (Morallo-Rejesus, 1987). The seed extract of *Gynandrosis gynadra* and rhizome extract of *Cyperus rotendus* were toxic to insects (Chandel *et al.*, 1988).

Dohary *et al.* (1988) reported the neem, rape and coconut oils when used at the rate of 0.5 ml/100 g of gram seeds caused less than 50% adult mortality and high dose (1ml/100g) neem, coconut, sesame and palm oils caused 100 percent adult mortality whereas mustard oil caused 75 percent adult mortality. Mostafa (1988) treated the powder of neem flowers (at 1 and 2 percent) and fruit (at 2, 4, 6 and 8 percent) to *T. castaneum* and the adult mortality was 66 and 61 percent for flower powder and 63, 64, 60 and 69 percent for fruit powder respectively, after 7 days, compared to 22% for untreated rice.

The toxicity due to topical application of acetone extracts of mace and nutmeg, was determined and it was found that extracts were not toxic to adults of *Callasobrachus maculatus*, slightly toxic to *Lasioderma serricorne* and *T. castaneum*, and moderately toxic to *S. oryzae* on surface treated wheat, at 2000

ppm for mace, 2000, 1000 and 500 ppm for nutmeg, similarly treated wheat greatly reduced the number of F_1 progeny of S. *oryzae* (Su, 1989).

Azadirachtin (neem extract) incorporated into wheat flour reduced growth and survival of the *T. castaneum.* Topical application on egg, larvae with 1, 2 and 5µ of neem extracts did not have any adverse effects; application to pupae of less than 6 hour old reduced the emergence of normal adults (Mukherjee and Ramachandran, 1989).

Rani and Jamil (1989) mixed crude extract of water hyacinth, *Eichornia crassiper* with the larval diet of *T. castaneum, C. maculatus, Corcyra cephalonica* and observed higher mortality in the 4th instar larvae.

Mondal and Akhtar (1992) studied the toxicity of caffeine and castor oil on the adults and larvae of *T. castaneum.* The LD_{50} values calculated after 20 days exposure to castor oil were 3659.73, 3692.96 and 1188.99 ppm for males, females and larvae, respectively.

Khalequzzaman and Islam (1992) studied the efficacy of various extracts of the leaves of *Datura metal* against two strains of *T. castaneum.* When applied topically, all extracts were toxic to the pests. Methanol extract was found to be the most toxic.

An information was tabulated by Hussain (1995), the effect of Bishkatali (*Polygonum hydropiper*) leaf powder and its extracts on larvae of *T. castaneum* in laboratory test indicated some toxicity.

Talukder and Howse (1995a) evaluated seed extract of *Aphanamixis ploystachya* for the toxicity to adult of *T. castaneum* and found that all extracts were toxic to beetles but ethanol extract was the most toxic. They also tested ground leaves,

barks and seeds for their residual toxicity to *T. castaneum.* The ground leaves, bark and seeds provided protection for wheat flour by reducing F_1 progeny.

Xie *et al.* (1995) investigated the repellency and toxicity effect of azadirachtin (98% AZA) against three stored product insects, *Cryptolestes ferrugineus*, *S. oryzae* and *T. castaneum.* They confirmed that azadirachtin was largely responsible for both repellent and toxic actions on stored-product pests.

Malek *et al.* (1996) exposed the adults of *T. castaneum* to seed extracts of *Thevetia neriifolia*, *Ricinies communis, Arachis hypogea* and *Carissa carandes* and recorded the adult mortality at 24, 48, 72 and 96 hour after treatment; they found that the extracts of *T. neriifolia* was the most effective causing 50% mortality of the pest.

Prates *et al.* (1998) extracted two secondary metabolites 1, 8-cincole (eucalyptol) form *Eucalyptus sp* and R -(+)- limonene for *Citrus sp.* Essential oils which were bioassay to determine possible fumigant, and ingestion activity against *R. dominica* and *T. castaneum* Both compounds showed insecticidal potency against the two insects.

Liu and Ho (1999) evaluated the toxic activates of *Evodia rutaecarpa* oil against *Sitophilus zeamais* adult and *T. castaneum* larvae and adults. Contact toxicity assayed by tropical application showed that *S. zeamais* adults were significantly more susceptible (LD_{50}= 0.043 µg/mg body wt.) to the essential oil than *T. castaneum* adults (LD_{50} = 0.118 µg/mg body wt.) and *T. castaneum* larvae (LD_{50} = 0.093-0.126 µg/mg body wt).

Ara (2002) studied the efficacy of some plant extract (neem, datura and tabacco) against *T. castaneum.* She found Datura seed extract in ethyl acetate was highly toxic after 72 hours treatment against *T. castaneum.*

2.2 Repellency effect

Jilani and Malik (1973) worked with neem extracts and observed that water and ethanol extracts of leaves and seeds of neem repelled the red flour beetle *T. castaneum,* the khapra beetle *Trogoderma granarium* Everts and lesser grain borer *R. dominica.*

Jilani and Su (1983) reported that turmeric powder repelled the granary weevil, *S. granarius* and *R. dominica* whereas its petroleum ether extract was found to be repellent against the red flour beetle *T. castaneum.*

Acetone extracts of black pepper, *Piper nigrum*, oils of clove; cedar wood oil, *Juniperus virgiana* and karanja, *Pongamia glabra* were tested in India by a choice method to determine their repellent effects on adults of *T. castaneum*, where karanja oil appared more repellency (Sigmony *et al.,* 1984). Seventeen plant extracts were tested in the laboratory at Pakistan for this repellency to *T. castaneum*. Seed extracts of *Intsia bijuga* and neem oil, both had highest repellency.

Saim and Meloan (1986) tested fifteen volatile compounds found in bay leaves, laurus nobilis and crushed bay leaves for their effectiveness as repellents against the adults of *T. castaneum* and reported that when the bay leaves were added to wheat flour, they act as repellent as were in 3 compounds like benzaldehyde, piperidine and geraniol.

Jilani and Saxena (1987) reported that several indigenous plant materials have traditionally been used us stored grain protectant against insect pests in various part of the world. The plants such as neem, turmeric and sweet flag have been found to posses insect repellent properties.

Jilani *et al.* (1988) found that fewer adults of *T. castaneum* settled in rice grain in a food preference chamber treated with 100, 500, 1000 ppm of oil of turmeric, sweet flag and neem. The repellency increased with increasing concentration of the oil.

Qureshi *et al.* (1988) tested 19 plant extracts for their repellent activity against *T. castaneum* and reported that an acetone extract of *Ageratum houstonianum* flowers and alcohol extract of *Valeriana wallichii* rhizomes, at doses ranging from 150-600 mg/cm^2 on filter paper strips, had the strongest repellent activity.

In laboratory experiment, Phillips *et al.* (1993) observed that sesame oil was significantly repellent against *S. oryzae* and *T. castaneum* an external feeding pest of damaged grains and flour. Talukder and Howse (1994a and 1995a) reported that the seed extracts of *Aphanamixis polystachya* had strong repellent effects on red flour beetle and rice weevil. Hussain *et al.* (1995) studied that the extracts of *Polygonum hydropiper* and *Annona squamosa* were repellent effect to adults of *T. castaneum.*

The repellency and toxicity of azadirachtin and 3 neem extracts to three stored product insects, *Cryptolestes ferragineus, S. oryzae* and *T. castaneum* were investigated by Xie *et al.* (1995), when *T. castaneum* was more sensitive to the repellent action of neem than the other 2 species.

Liu and Ho (1999) evaluated repellent activities *Evodia rutaecarpa* oil against *Sitophilus zeamais* adults and *T. castaneum* larvae and adults. In the treated filter paper repellency test, the essential oil was more repellent to *T. castaneum* than to *S. zeamais.*

2.3 Residual effect

Jilani (1986) conducted trials with ethanolic extract of neem seed, hexane extract of sweetflag, *Acorus calamus*, rhizome and thymol, applied *T. castaneum*; *R dominica*; *S. oryzae* and *S. cerealella* in wheat grain and observed significant control of the insect infestation. Trivedi (1987) reported that neem oil, linseeds oil and sesame oil played a little effect on reducing the loss of weight in mung bean seeds. Neem, sesame and linseed oilcakes were found effective in controlling the *R. dominica* and *T. castaneum,* when used as a carrier material with pyrethrin dust.

Bhuiyah and Quinones (1990) treated the corn with leaf powder of bishkatali and found that the leaves powder of bishkatali was effective on oviposition and adult emergence of *S. zeamais.*

Joseph *et al.* (1994) showed that the post embryonic development and adult emergence of *T. castaneum* were adversely affected to varying degrees when larval stages were reared on wheat flour treated separately with either extracts from roots of *Acorus calamus* or *Rauvolfia serpentina,* the fruit cortex of *Sapindus trifoliatus*. There was a marked decline in the reproductive potential, in terms of female fecundity and egg hatchability, when extracts were applied topically or administered orally through diet.

Xu-Hanhong and Zhao-Shanhuan (1994) reported that the cassia oil mixed with wheat or wheat flour at 0.1- 0.2%($^{w}/_{w}$) completely inhibited the reproduction of *S. zeamais*, *R. dominica* and *T. castaneum.*

Talukder and Howse (1994b and 1995b) mixed leaf, bark and seed powders of pithraj with pulses, wheat grains and wheat flour and observed that the treated food effectively reduced the oviposition rate, hatching rate, emergence of F_1 adults of *C. chinensis*, *S. granarius* and *T. castaneum.*

Ho *et al.* (1995) tested clove flower buds, *Syzygium aromaticum*, extracted with n-hexane and the crude extract against *T. castaneum* and the eggs were found to be the stage most susceptible to the clove extracts, with an LC_{50} of 0.01 g/ml. They also found that the F_1 progeny *T. scataneum* was inhibited when the eggs were cultured in rice treated with 2g/m1 extract per 100g of rice. This inhibition was due to newly laid eggs being killed by the extracts.

Rouf *et al.* (1996) investigated the toxicity of the leaf powder of neem, nishinda and bishkatali and their combinations to *C. chinensis* on lentil seed; they observed that 4g of bishkatali leaf powder/50g of lentil seeds was the most effective in reducing oviposition, adult emergence, and damage of seeds by the pest and seed weight loss. The combination of neem and bishkatali leaf powder ranked second followed by neem leaf powder alone. At low doses (1-2g) these 3 plant materials applied either alone or in combination were found to be less effective, germination of lentil seeds was not affected by bishkatali leaf powder. Application of the plant materials at intervals of 15 days up to two month storage did not give better protection of lentil seeds than a single application only.

CHAPTER III
MATERIALS AND METHODS

3 MATERIALS AND METHODS

The present study on the evaluation of toxicity, repellency and residual action of Bishkatali plant extract against red flour beetle, *Tribolium castaneum* Herbst was conducted in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the period of March 01 to August 31, 2006. The experimental procedures are described below:

3.1 Materials

3.1.1 Bishkatali plant

Bishkatali (whole plant) was used in the form of extracts for the present study.

Bangla name: Bishkatali

English name: Marsh pepper smartweed

Scientific name: *Polygonum hydropiper* L.

Family: Polygonaceae

The Bishkatali plant is mainly distributed in many counties of temperate and sub-tropical Asia, North America, Europe, and many other countries of the world. It grows abundantly in damp soils, low-meadows, cultivated ground, and waste places of Bangladesh. This plant is climbing, 12-30 inches long reproducing by seed (Hooker, 1980). It is a medicinal herb used to control insect pests as repellent and toxic materials.

3.1.2 Red flour beetle

It is one of the most common pests of stored products. Though the red flour beetle has been originated in India, but it is found throughout all tropical countries including Bangladesh, sub-tropical and warm temperate regions of the world.

Scientific name: *Tribolium castaneum* Herbst

Family: Tenebrionidae

Order: Coleoptera

The adult red flour beetle is a small sized, elongated, oblong and hard shelled insect with striated elytra. The length of the body of the adult beetle varies from 3.20-4.00 mm in male and 3.50-4.50 mm in female, average being 3.48 mm and 4.01 mm in male and female respectively. (Haque, 1976). The male possesses a hairy puncture on the ventral surface of the anterior femur but it is absent in the female. The females lay eggs in the commodity through out their adult life.

The larvae and adults feed on wheat flour, rice flour (both husked and unhusked), atta, suji and similar powdered cereal products. This pest has also been reported to attack the germ part of the grain (Alam, 1971). In case of heavy infestation; flour or media turns grayish yellow and subsequently becomes mouldy and emits pungent smell.

Eggs are laid singly by the female beetle among atta, suji, flour etc. Red flour beetle has 4 life stage; a) egg, b) larva, c) pupa and d) adult. The eggs are minute, cylindrical, white and transparent. The surface of freshly laid egg is sticky and therefore, flour or dust particles easily adhere to them. A single female lays on an average 300-400 eggs.

The young grubs (larvae) are yellow-white but it become turns reddish-yellow when mature. The cylindrical grubs are covered with fine hairs. Larva undergoes 6-7 moulting and they become full grown in 20-25 days at 30-35^{0}C and 75% relative humidity (RH). Pupa is naked and pupation occurs in flour. This pupa is a small, elongated, reddish-brown in colour. Adults can live for about six months. *T. castneum* may have five generations in the year in heated premises.

3.2 Collection and rearing of insects

Adult red flour beetles were collected from the mass cultures of the laboratory of Entomology, Hajee Mohammad Danesh Science and Technology University, Dinajpur. Beetles were reared in glass beaker (500ml) with the food medium and kept in an incubator at 30^{0}C ± 0.5^{0}c temperature without light and humidity control. Standard mixture of whole-wheat flour with powdered dry yeast in a ratio of 19:1(Park and Frank, 1948; Park, 1962; Zyromask-Rudzka, 1966) was used as food medium. Rearing media was sterilized at 120^{0}C for six hours in an oven. After sterilization food was not used until at least 15 days to cover its moisture contents. (Khan and Selman,1981; Mondal, 1984).

About 500 beetles were placed in glass beaker and covered with pieces of cloth fastened with rubber bands to prevent the contaminations and escape of insects. A crumpled filter paper was placed inside the beaker for the easy movement of the adults and to avoid the cannibalism of eggs. After 7 days all the adults (parent) are removed from the respective beaker. The first generation emerged within 28-30 days. These newly emerged adults were utilized in the present experiment.

3.3 Collection and processing of plant materials

Bishkatali plant was collected from surrounding villages of HSTU campus and was dried in shade because various compounds may be breakdowns in sunlight.

3.4 Preparation of plant dust/powder

The plant (whole plant) was powdered in a grinding machine. Before grinding the plant was well dried in an oven at 40^0C for 6 hours. The dust was passed through a 60 mesh sieve to obtain fine dust and powdered plant materials were stored in specimen jar under laboratory conditions (Plate 1).

3.5 Extraction techniques

3.5.1 Preparation of chloroform extract

One hundred gram (100g) of plant powder was dissolved in 300ml chloroform. The mixture was stirred for 30 minutes and left to stand for 72 hours and shaking several intervals. The mixture was filtered through a filter paper (Whatman 42) and evaporated the solvent finally hard brownish extract was obtained. The extract was preserved in tightly corked bottles and stored in a refrigerator for experimental use (Plate 2).

3.5.2 Preparation of ethyl alcohol extract

One hundred gram (100g) of plant powder was dissolved in 300ml ethyl alcohol. The mixture was stirred for 30 minutes and left to stand for 72 hours and shaking several intervals. The mixture was filtered through a filter paper (Whatman 42) and evaporated the solvent finally light brownish extract was obtained. The extract was preserved in tightly corked bottles and stored in a refrigerator for experimental use (Plate 2).

Plate 1 A branch of Bishkatali plant (left side) and dust of Bishkatali plant (right side)

Plate 2 Extract preparation from Bishkatali dust (left side) and dose preparation from the extract (right side)

3.6 Insect bioassay

3.6.1 Toxicity test of Bishkatali plant extract

To test the mortality rate or dose response to Bishkatali plant extracts on adult *T. castaneum.* Residual film method (Busvine, 1971) was applied.

Extracts collected from the Bishkatali plant in two solvents namely chloroform and Ethyl alcohol were weighted and dissolved in the respective solvent according to the proportion of the dose concentration of 500mg/ml, 250mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml. Then 1ml liquid of each dose was dropped on petridishes with the help of pipette, covering the whole area of the petridishes internally. The petridishes were then kept in air for drying for a few minutes. When solvents were fully evaporated, 10 adults (3-6 days old) of *T. castaneum* were released in each petridish. Three replications were made for each dose. Control petridishes were treated with the solvent only (Plate 3). The petridishes were then kept in an incubator without food at 25^0 C ± $.5^0$C .

The mortality of insect was recorded at 24, 48 and 72 hours after treatments. The doses were calculated by measuring the dry weight of the crude extract applied in the petridish divided by the surface area of the respective petridish.

The mortality percentage was corrected using Abbott's formula (Abbott, 1925; Busvine, 1971).

$$P_t = \frac{P_o - P_c}{100 - P_c} \times 100$$

Where, P_t = Corrected mortality %

P_o = Observed mortality % and

P_c = Control mortality %

The observed data then subjected to probit analysis according to Finney (1947) and Busvine (1971) using software developed in the Department of Agricultural and Environmental Science, University of Newcastle Upon Tyne, United Kingdom, which adapted the traditional calculations to automatic computation. No provisional graph or tables are required. Heterogeneity is tested by a chi-squared test. If the probability is greater than 5% an automatic correction of heterogeneity is introduced. The program also calculated confidence limits for LD_{50}.

This data is entered into a linear regression program, which first a regression line onto a probit log dose concentration graph. Mortality % and dose concentration can be determined from this graph using the probit transformation table (Busvine, 1980).

3.6.2 Repellency of Bishkatali plant extract

For repellency test Bishkatali was dissolved diluted different quantity of chloroform and ethyl alcohol to get different concentration 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml. The repellency test was conducted according to the method of Talukder and Howse (1994a). Filter papers (Whatman 40, 9cm diameter) were cut in 2 half and one ml of each solution of plant extract was applied to a half filter paper uniformly with a pipette. The treated half discs were then air-dried and attached with the untreated half with a cello-tape. Precautions were taken so that attachment did not interfere with the free movement of insect from one half to another but the distance between the filter paper segments remained sufficient to prevent seepage of test samples from one half of circle to another. Each filter paper was then placed in a petridish (Plate 4). Twenty insects were released at the centre of each filter paper disc and a cover as placed on the petridish. For plant extract and each dose, three replicated were used. Then the insect present on each strip were counted at 2 hours intervals up to the 20 hours.

The data were expressed as percentage repulsion (PR) by the following formula:

$$PR\ (\%) = (Nc - 50) \times 2$$

Where, Nc = the percentage of insects present in the control half .

Positive (+) values expressed repellency and negative (-) values attractancy. Data (PR) were analyzed using analysis of variance (ANOVA) after transformation of arcsin √(percentage) values. The average values were then categorized according to the following scale (McDonald *et al.* 1970)

Class	Repellency rate (%)
0	> 0.01 to 0.1
I	0.1 to 20
II	20.1 to 40
III	40.1 to 60
IV	60.1 to 80
V	80.1 to 100

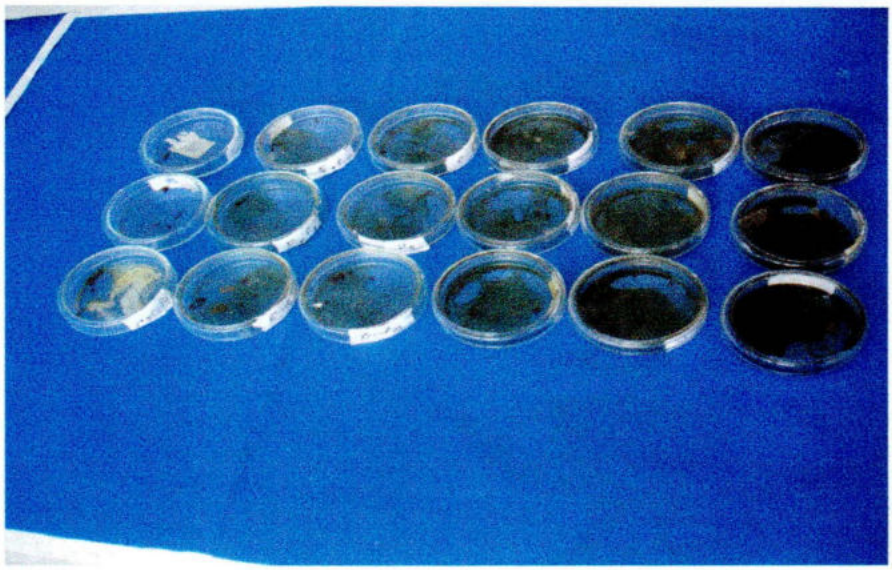

Plate 3 Toxicity test of Bishkatali plant extract (by surface application in petridish)

Plate 4 Repellency test of Bishkatali plant extract (with treated and untreated filter papers)

3.6.3 Residual toxicity of Bishkatali plant extract

Considering time intervals, to determine the residual activity of Bishkatali extract. The test was divided into three sets. Bishkatali plant extracts were weighted and dissolved in respective solvent to obtain 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, and 31.25mg/ml concentration. Ten gram of wheat flour was mixed with each concentration. Then the mixtures were air- dried and put in plastic pots (3.5cm × 4 cm). One pair of newly emerged adult insect was released in each pot and then covers (Plate 5). All the treatments and control were replicated three times. The pots were kept in the incubator at $30\text{-}32^0$ temperatures and 70-75% relative humidity. The adult insects from each pot were removed after 7 days. The same series of work were followed for another 2 sets of adult released at 12 and 17 days after treatment (DAT). The following results were recorded up to the period of 48 days for each set the number of F_1 adult progeny were counted, recorded and the Inhibition rates (IR%), were calculated by the following formula:

$$IR\ (\%) = C_n - T_n/C_n \times 100$$

Where, C_n = number of insects on control pot.

T_n = number of insect on treated pot.

3.6.4 Statistical analysis

Data obtain from repellency and residual effect test were statistically analyzed in accordance with one factor Completely Randomized Design (CRD) and Duncan's Multiple Range Test (Duncan,1951) for comparing treatment mean values.

3.7 Meteorological data record

The present investigation was carried out in the laboratory of Entomology, HSTU, Dinajpur during the period of present research, temperature, relative humidity were rerecorded daily (Appendix table-XV) at twelve hours using the dry and wet bulb thermometer (ZEAL).

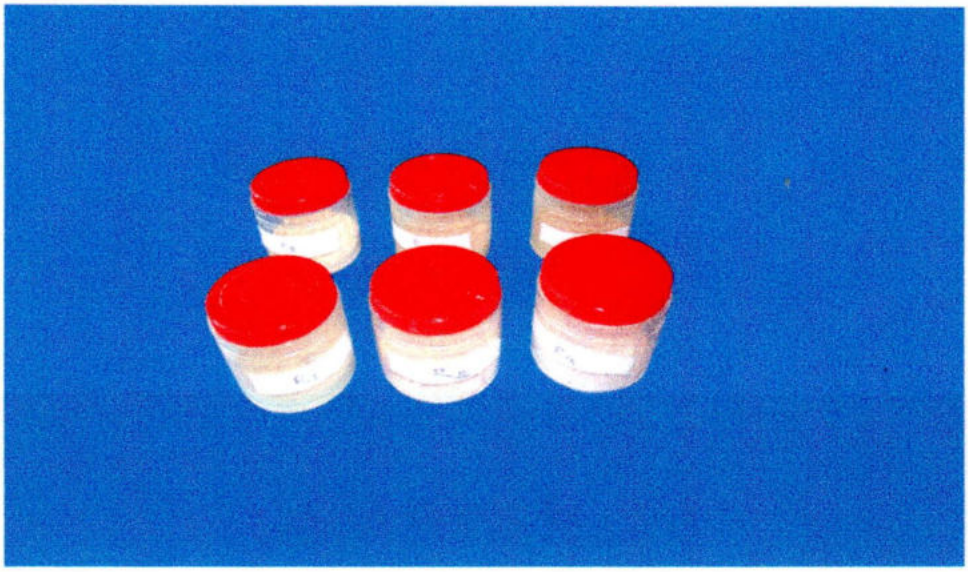

Plate 5 Wheat flour treated with Bishkatali plant extract in plastic pot for residual effect study.

4 RESULTS AND DISCUSSION

The results of the three experiments conducted with Bishkatali plant extract against red flour beetle to determine the toxicity, repellency and residual effect are presented below:

4.1 Toxicity test

The percentages of mortality of *T. castaneum* treated with Bishkatali plant extract in two solvents is shown in Table 1 and Table 2. The LD_{50} values, 95% confidence limits regression equations and χ^2 values were calculated and presented in Table 3 and Table 4. The calculated LD_{50} values were 39.25618, 22.64847, 15.7958 mg/cm^2 in chloroform extract after 24, 48 and 72 hours exposure period respectively and in ethyl alcohol extract the values were 35.3229, 23.42841, 14.85128 mg/cm^2 after 24, 48, and 72 hours of exposure period, respectively. Comparing the LD_{50} values, it was observed that the Bishkatali plant extract in ethyl alcohol showed better than chloroform after 24 hours and 72 hours but chloroform extract gave better result after 48 hours than ethyl alcohol extract.

The mortality of red flour beetles ranged from 10% to 43% in chloroform extract at 24 hours and 7% to 40% in ethyl alcohol extract at 24 hours after treatment. Mortality of beetle ranged from 17% to 50% in chloroform extract at 48 hours and in ethyl alcohol extract 10% to 47% at 48 hours after treatment. Mortality ranged from 17% to 55% and 17% to 57% in chloroform and ethyl alcohol extract after 72 hours respectively.

Table 3 LD_{50}, 95% confidence limits, regression equation and χ^2 value of Bishkatali plant extract in chloroform treated on adult *T. castaneum*.

Solvent used	Duration of Treatment	LD_{50} (mg/cm^2)	95%confidence limit		Regression equation	χ^2 value (df)
			Lower	Upper		
Chloroform	24 hours	39.25618	9.9076	155.54	Y=3.5589+0.9041X	0.9383(3)
	48 hours	22.64847	7.3558	69.73	Y=3.8771+0.8287X	0.8817 (3)
	72 hours	15.7958	6.6617	37.45	Y=3.9287+0.8938X	0.4204 (3)

Table 4 LD_{50}, 95% confidence limits, regression equation and χ^2 value of Bishkatali plant extract in ethyl alcohol treated on adult *T. castaneum*.

Solvent used	Duration of Treatment	LD_{50} (mg/cm^2)	95%confidence limit		Regression equation	χ^2 value (df)
			Lower	Upper		
Ethyl	24 hours	35.3229	9.2474	134.93	Y=3.6373+0.8803X	0.5696 (3)
alcohol	48 hours	23.42841	7.8213	70.18	Y=3.8150+0.8651X	0.7145 (3)
	72 hours	14.85128	6.2758	35.14	Y=3.9875+0.8641X	0.80356 (3)

The probit regression lines of Bishkatali extracts in two different solvents are showed in Figure 1-6. The insect mortality rates showed positively correlation with concentrations, in all cases. The mortality data are also shown in Appendix table I-VI. From the non-significant χ^2 value it was observed that all the equations fitted well with no heterogeneity.

Hussain ,(1995) who investigated Bishkatali (*Polygonum hydropiper*) leaf powder and extract on larvae of *T. castaneum* in laboratory test indicated some toxicity which is similar to the result of the present study. Ferrolion and Padolino (1985) also found that the leaf extract of *Artemisia vulgaris* was highly toxic to *T. castaneum* and *S. zeamis.*

Talukder and Howse (1993) have also noted toxicity effect of Pithraj extract on red flour beetle. Dohary *et al.*(1988) observed toxicity of neem extract on pulse beetle. Sarac and Tunc (1995) argued that oil of *Pimpinella anisum* caused 95% mortality of *T. confusum*. De-pedro and De-pedro (1994) studied the effect of neem bark extract against corn weevil and obtained high mortality of the weevil.

Khanom and Khalequzzaman (2000) investigated the effectiveness of neem seed extracts on larvae and adults of different strains of *T. castaneum*. It was observed that mortality of the adults varied among different strains. The order of toxicity was found as petroleum spirit>methanol> ethyl acetate> acetone extracts in FSS-II and CTC-12 strains while it was found in petroleum spirit> ethyl acetate> methanol> acetone in local and CR-1 strain.

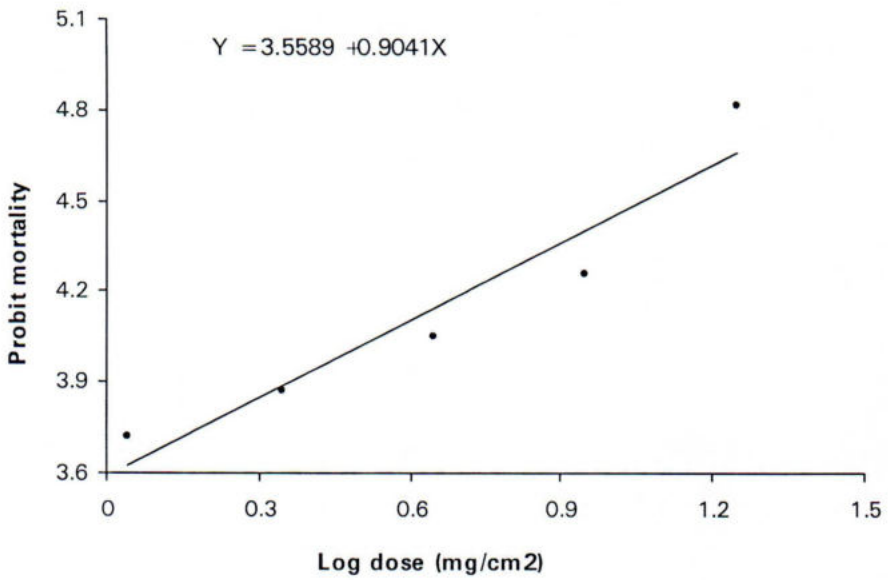

Fig. 1: Regression line of probit mortality on log dose of Bishkatali extract in chloroform after 24 hours on adult *T. castaneum*.

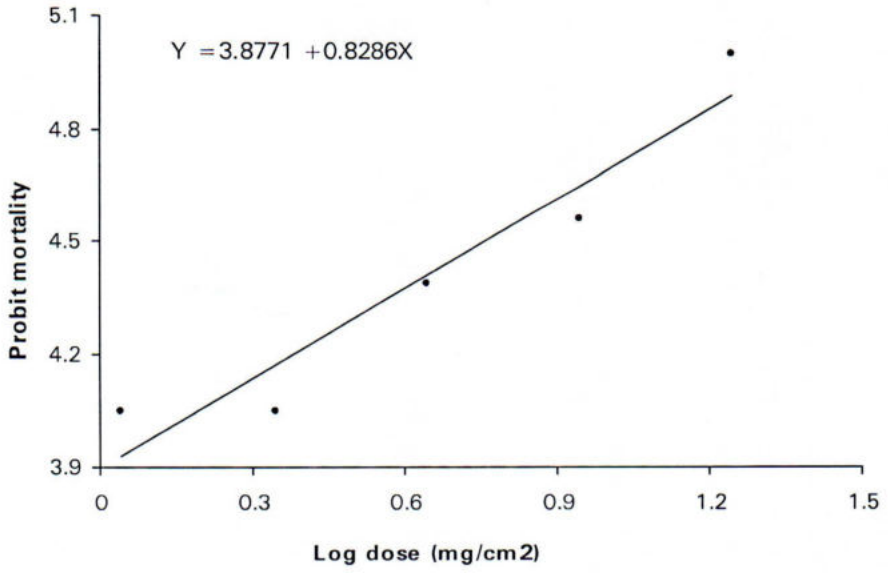

Fig. 2 : Regression line of probit mortality on log dose of Bishkatali extract in chloroform after 48 hours on adult *T. castaneum*.

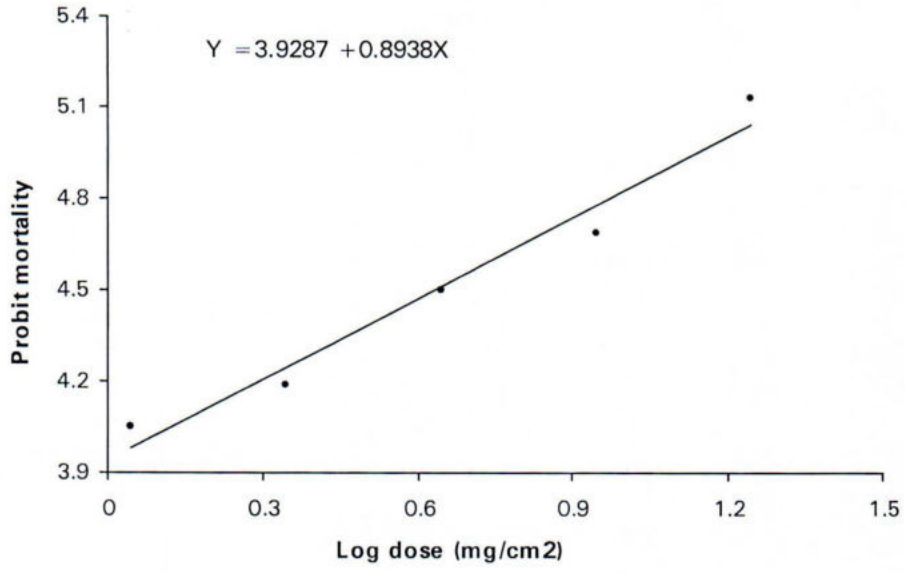

Fig. 3 : Regression line of probit mortality on log dose of Bishkatali extract in chloroform after 72 hours on adult *T. castaneum*.

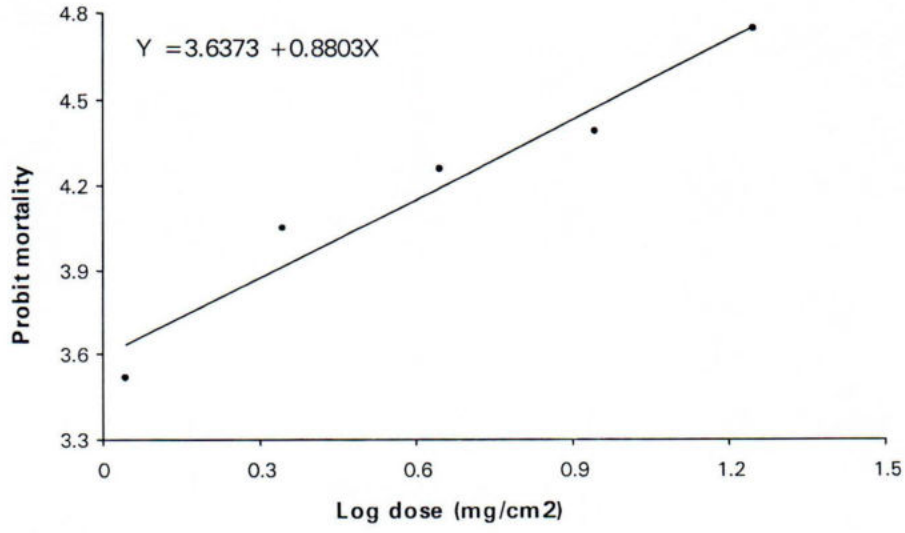

Fig. 4 : Regression line of probit mortality on log dose of Bishkatali extract in ethyl alchol after 24 hours on adult *T. castaneum*.

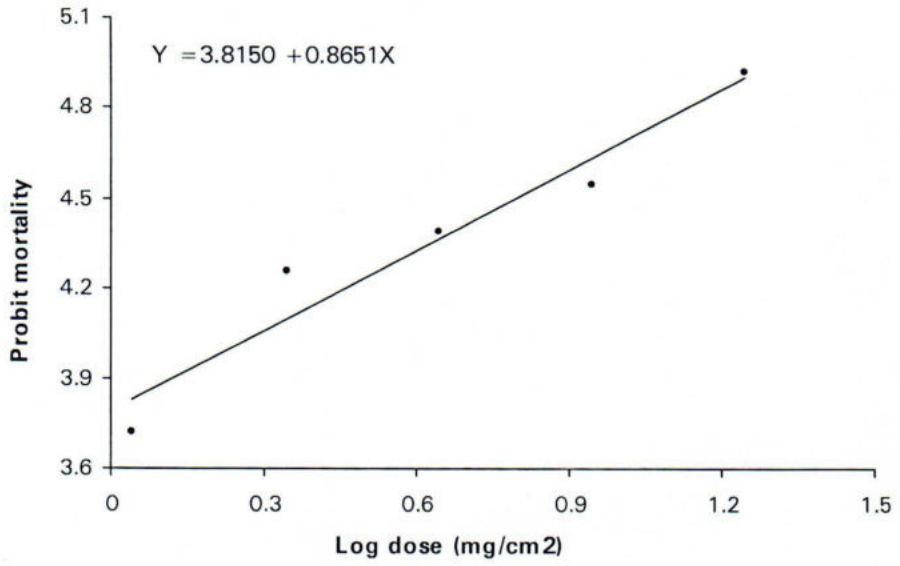

Fig. 5 : Regression line of probit mortality on log dose of Bishkatali extract in ethyl alchol after 48 hours on adult *T. castaneum*.

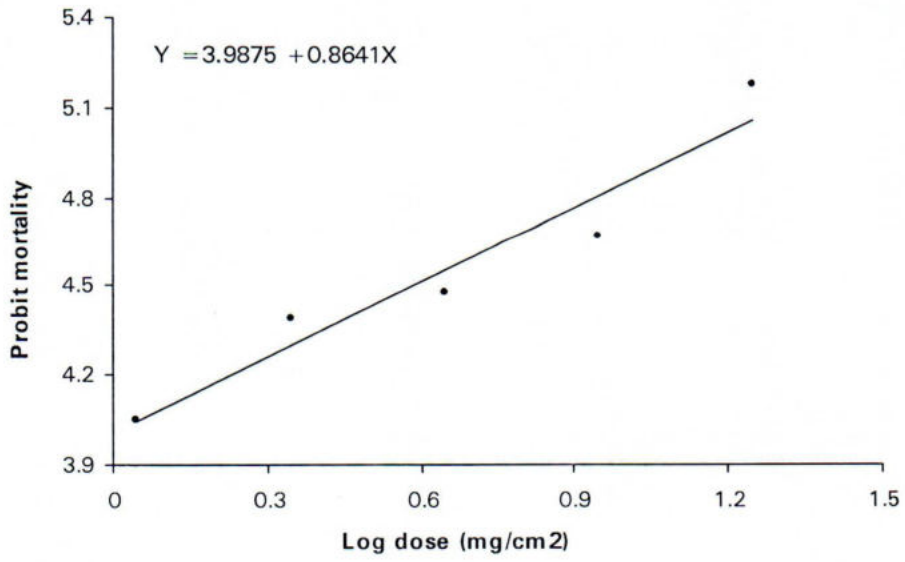

Fig. 6 : Regression line of probit mortality on log dose of Bishkatali extract in ethyl alchol after 72 hours on adult *T. castaneum*.

The present result of the investigation is also supported the findings of Islam (1996) who evaluated the toxicity of the leaf, seed, fruit-pericarp, stem-bark and root of the Pithraj plant (*Amoora rohituka*) in four solvent, against different strains of *T. castaneum*. The extractives proved very much promising in controlling the pest by producing high moralities due to contact toxicity.

In the present study, mortality percentage was found directly proportional to the level of concentrations and also with time after treatment. These results are in support with Bhaduri *et al.* (1985), who found that the leaf extract of Bon-kalmi had insecticidal properties against pulse beetle.

4.2 Repellency test

Repellency rate of chloroform extract and ethyl alcohol extract of Bishkatali against *T. castaneum* and are shown in Table 5 and Table 6, respectively.

Bishkatali plant extract in chloroform showed strong repellent effect against red flour beetle. The highest repellency (87.3%) was observed in case of 500mg/ ml extract in chloroform and lowest (63.0%) in case of 31.25 mg/ml extract in chloroform (Table 5). The repellency classes in 500mg/ml, 250mg/ml and 125mg/ml are in class V and in 62.5mg/ml and 31.25mg/ml are in class IV.

Bishkatali plant extract in ethyl alcohol also showed strong repellent effect against red flour beetle. The highest repellency (80.3%) was observed in case of 500mg/ml extract and lowest (52.6%) in case of 31.25 mg/ml extract in ethyl alcohol (Table 6). The repellency classes in 500mg/ml is in class V, 250mg/ml, 125mg/ml, 62.5mg/ml are in class IV and 31.25mg/ml is in class III.

Table 5 Repellency effects of Bishkatali plant extract in chloroform on red flour beetle *T. castaneum* using treated filter paper.

Extract concentration (mg/ml)	Average repellency rate (%)										Mean repellency rate (%)	Repellency class
	2 HAT*	4 HAT	6 HAT	8 HAT	10 HAT	12 HAT	14 HAT	16 HAT	18 HAT	20 HAT		
500	86.7a	86.7a	86.7a	86.7a	90.0a	86.7a	90.0a	86.7a	86.7a	86.7a	87.3	V
250	83.3a	86.7a	83.3ac	80.0ab	86.7ab	83.3a	86.7a	83.3a	86.7a	86.7a	84.6	V
125	76.7ab	76.7ab	76.7ac	76.7bc	80.0bc	80.0ab	83.3ab	83.3a	83.3a	83.3a	80.0	V
62.5	66.7bc	70.0bc	73.3bc	70.0c	73.3cd	70.0bc	76.7b	70.0b	76.7ab	76.7a	72.3	IV
31.25	60.0c	60.0c	66.7c	56.7d	66.7d	60.0c	66.7c	63.3b	66.7b	63.3b	63.0	IV

*HAT=Hours after treatment.

Within column values followed by same letter (s) did not differ significantly at 5% level.

Table 6 Repellency effects of Bishkatali plant extract in ethyl alcohol on red flour beetle *T. castaneum* using treated filter paper.

Extract concentration (mg/ml)	Average repellency rate (%)										Mean repellency rate (%)	Repellency class
	2 HAT*	4 HAT	6 HAT	8 HAT	10 HAT	12 HAT	14 HAT	16 HAT	18 HAT	20 HAT		
500	83.3a	83.3a	80.0a	80.0a	80.0a	76.7a	80.0a	80.0a	76.7a	83.3a	80.3	V
250	73.3ab	73.3b	76.7ab	73.3ab	73.3ab	76.7a	73.3ab	70.0ab	73.3a	73.3ab	73.6	IV
125	73.3ab	66.7bc	66.7ac	66.7bc	66.7bc	70.0ab	66.7bc	66.7ac	66.7ab	63.3bc	67.3	IV
62.5	66.7b	60.0cd	63.3bc	60.0cd	60.0cd	63.3bc	60.0cd	60.0bc	56.7bc	56.7c	60.6	IV
31.25	53.3c	53.3d	53.3c	50.0d	53.3d	53.3c	50.0d	53.3c	53.3c	53.3c	52.6	III

*HAT=Hours after treatment.

Within column values followed by same letter (s) did not differ significantly at 5% level.

The overall results indicated that repellency rate increased proportionally with the increase of concentration of the extract. Replicated data on repellency rate of Bishkatali extract in both solvents at different hours after treatments are shown in Appendix table VII and VIII. Analyses of variance (ANOVA) on repellency of Bishkatali plant extract are given in Appendix table XI-XII. Doses were differed significantly ($P<0.05$) in different hours of treatment (Appendix table XI-XII).

The present result supports the finding of David *et al.* (1988) who showed the repellency activity of *Vitex negundo* against several species of stored product pest. Talukder and Howse (1993) reported strong repellent effect of *Aphanamixis polystachya* on *Tribolium castaneum*. Hussain *et al.* (1995) also reported that the extract of *Polygonum hydropiper* and *Annona squamosa* were repellent effect on adult *T. castaneum*. The present results also agree with the earlier findings. The result of the present investigation is similar to the finding of Nayer *et al.* (1976) who observed that neem treated materials have gustatory repellency.

4.3 Residual effect

Residual effect of Bishkatali plant extract in chloroform and ethyl alcohol extract against *T. castaneum* are presented in Table 7 and Table 8 respectively. The efficacy of Bishkatali plant extract as food protectant against red flour beetle has evaluated by comparing the numbers of F_1 progeny.

The lowest numbers of F_1 adult progeny (32.7, 25.3 and 27.0) emerged from the wheat flour treated with 500mg/10g chloroform extract when parent released at 7, 12, 17 days after treatment respectively (Table 7). But from the wheat flour treated with 500mg/10g ethyl alcohol extract, 38.0, 29.7 and 30.3 F_1 adult progeny emerged when parent released at 7, 12, 17 days after treatment respectively (Table 8).

Table 7 Residual effect of Bishkatali plant extract in chloroform on F_1 progeny of *T. castaneum* at different days after treatment.

Adult remove	Dose	Mean number of F_1 progeny	Inhibition rate (IR %)
After 7days	500	32.7 f	45.7
	250	40.3 e	32.6
	125	45.7 d	24.2
	62.5	48.3 c	19.9
	31.25	53.0 b	12.1
	Control	60.3 a	
After 12 days	500	25.3 f	59.3
	250	33.7 e	45.9
	125	40.3 d	35.3
	62.5	46.0 c	26.1
	31.25	54.3 b	12.8
	Control	62.3 a	
After 17 days	500	27 .0 f	57.3
	250	33.3 e	47.3
	125	41.3 d	37.7
	62.5	48.7 c	23.0
	31.25	55.3 b	12.6
	Control	63.3 a	

Within column values followed by same letter (s) did not differ significantly at 1% level.

Table 8 Residual effect of Bishkatali plant extract in ethyl alcohol on F_1 progeny of *T. castaneum* at different days after treatment.

Adult remove	Dose	Mean number of F_1 progeny	Inhibition rate (IR %)
After 7days	500	38 .0 f	36.9
	250	41.7 e	30.8
	125	46 .0 d	23.7
	62.5	49.3 c	18.2
	31.25	53.0 b	12.1
	Control	60.3 a	
After 12 days	500	29.7 f	52.3
	250	37.7 e	39.4
	125	41 .0 d	34.1
	62.5	47.0 c	24.5
	31.25	54.3 b	12.8
	Control	62.3 a	
After 17 days	500	30.3 f	52.1
	250	37.3 e	41.0
	125	41.0 d	34.7
	62.5	47.3 c	25.2
	31.25	55.0 b	13.1
	Control	63.3 a	

Within column values followed by same letter (s) did not differ significantly at 1% level.

The overall result indicates that the Bishkatali plant extract in both solvent inhibit the population growth of *T. castaneum*. The highest inhibition rate was observed 59.3% and 52.3% in chloroform extract and ethyl alcohol extract, respectively when treated with 500mg/10g dose and the adult released 12 days after treatment.

Replicated data on residual effect of Bishkatali plant extract in both solvents at different days after treatments are shown in Appendix table IX and X. Analyses of variance (ANOVA) on residual effect of Bishkatali plant extract at different days are given in Appendix table XIII-XIV. Doses are significantly ($P<0.01$) differ in different days after treatment (Appendix table XIII-XIV).

The result of present investigation agreed with the finding of Amin *et al.*(2000) who found Akanda, Bishkatali, Neem extract have inhibition activity against lesser grain borer. The present result supports the findings of Pruthi (1937) who reported that neem leaves had strong protective effect on stored grain from insect attack

The over all result indicated that Bishkatali plant extract had residual effect. These result supported the findings of Talukder and Howse (1995a), where the ground leaves, bark and seeds of *Aphanimiuis polystrachya* provided protection of wheat flour by reducing F_1 progeny of *T. castaneum.*

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CHAPTER V
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5 REFERENCES

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APPENDICES

APPENDICES

Appendix table I Doses mortality data of *Tribolium castaneum* adult treated with Bishkatali plant extract in chloroform solvent after 24 hours

Dose	Log dose	Insect used	Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
17.6803	1.2474	30	13	43.33	43	4.82	4.662	4.821	18.03	4.686
8.8401	0.9464	30	7	23.33	23	4.26	4.403	4.270	16.74	4.414
4.420	0.6454	30	5	16.66	17	4.05	4.143	4.056	14.13	4.142
2.210	0.3443	30	4	13.33	13	3.87	3.884	3.873	11.10	3.870
1.105	0.0434	30	3	10.00	10	3.72	3.626	3.730	9.06	3.598
Control	-	30	-	-	-	-	-	-	-	-

LD_{50} =39.25618

Y=3.5589+ 0.9041X

95% confidence limits are 9.9076 to155.54

χ^2 is 0.9383 with 3 degrees of freedom

Appendix table II Doses mortality data of *Tribolium castaneum* adult treated with Bishkatali plant extract in chloroform solvent after 48 hours

Dose	Log dose	Insect used	Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
17.6803	1.2474	30	15	50.00	50	5.00	4.892	5.02	18.81	4.910
8.8401	0.9464	30	10	33.33	33	4.56	4.651	4.551	18.03	4.661
4.420	0.6454	30	8	26.66	27	4.39	4.409	4.39	16.74	4.411
2.210	0.3443	30	5	16.66	17	4.05	4.168	4.056	14.13	4.162
1.105	0.0434	30	5	16.66	17	4.05	3.928	4.062	12.15	3.913
Control	-	30	-	-	-	-	-	-	-	-

LD_{50} =22.64847

Y=3.8771+0.8287 X

95% confidence limits are7.3558 to 69.73

χ^2 is 0 .8817 with 3 degrees of freedom

Appendix table III Doses mortality data of *Tribolium castaneum* adult treated with Bishkatali plant extract in chloroform solvent after 72 hours

Dose	Log dose	Insect used	Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
17.6803	1.2474	30	17	56.66	55	5.13	5.044	5.125	19.11	5.043
8.8401	0.9464	30	12	40.00	38	4.69	4.778	4.688	18.48	4.774
4.420	0.6454	30	10	33.33	31	4.50	4.511	4.488	17.43	4.505
2.210	0.3443	30	7	23.33	21	4.19	4.246	4.184	15.09	4.236
1.105	0.0434	30	6	20.00	17	4.05	3.980	4.062	12.15	3.967
Control	-	30	-	-	-	-	-	-	-	-

LD_{50} =15.7958

95% confidence limits are 6.6617 to 37.45

Y=3.9287+0.8938X

χ^2 is 0.4204with 3 degrees of freedom

Appendix table IV Doses mortality data of *Tribolium castaneum* adult treated with Bishkatali plant extract in ethyl alcohol solvent after 24 hours

Dose	Log dose	Insect used	Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
17.6803	1.2474	30	12	40.00	40	4.75	4.754	4.740	18.48	4.735
8.8401	0.9464	30	8	26.66	27	4.39	4.474	4.390	16.74	4.470
4.420	0.6454	30	7	23.33	23	4.26	4.193	4.284	14.13	4.205
2.210	0.3443	30	5	16.66	17	4.05	3.914	4.062	12.15	3.940
1.105	0.0434	30	2	6.66	7	3.52	3.634	3.529	9.059	3.675
Control	-	30	-	-	-	-	-	-	-	-

LD_{50} =35.3229

95% confidence limits are 9.2474 to 134.93

Y=3.6373+0.8803 X

χ^2 is 0 .5696with 3 degrees of freedom

Appendix table V Doses mortality data of *Tribolium castaneum* adult treated with Bishkatali plant extract in ethyl alcohol solvent after 48 hours

Dose	Log dose	Insect used	Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
17.6803	1.2474	30	14	46.66	47	4.92	4.910	4.915	19.02	4.894
8.8401	0.9464	30	10	33.33	33	4.56	4.640	4.551	18.03	4.633
4.420	0.6454	30	8	26.66	27	4.39	4.369	4.394	15.96	4.373
2.210	0.3443	30	7	23.33	23	4.26	4.100	4.283	13.17	4.112
1.105	0.0434	30	3	10.00	10	3.72	3.830	3.720	11.10	3.852
Control	-	30	-	-	-	-	-	-	-	-

LD_{50} =23.42841 95%confidence limits are 7.8213 to70.18

Y=3.81501+ 0.8651X χ^2 is 0.7145 with 3 degrees of freedom

Appendix table VI Doses mortality data of *Tribolium castaneum* adult treated with Bishkatali plant extract in ethyl alcohol solvent after 72 hours

Dose	Log dose	Insect used	Kill	%Kill	Corr %	Emp probit	Expt probit	Work probit	Weight	Final probit
17.6803	1.2474	30	17	56.66	57	5.18	5.062	5.175	19.11	5.065
8.8401	0.9464	30	11	36.66	37	4.67	4.808	4.682	18.81	4.805
4.420	0.6454	30	9	30.00	30	4.48	4.553	4.46	17.43	4.545
2.210	0.3443	30	8	26.66	27	4.39	4.299	4.388	15.09	4.285
1.105	0.0434	30	5	16.66	17	4.05	4.046	4.037	13.17	4.025
Control	-	30	-	-	-	-	-	-	-	-

LD_{50} =14.85128 95% confidence limits are 6.2758to 35.14

Y=3.9875+0.8641 X χ^2 is 0 .80356with 3 degrees of freedom

Appendix table VII Repellency effects of Bishkatali plant extract in chloroform on red flour beetle *T. castaneum* using treated filter paper

Duration of Treatment	Replication	Doses (mg/mi)				
		500	250	125	62.5	31.25
2 HAT	R1	90	80	80	60	50
	R2	80	90	80	70	70
	R3	90	80	70	70	60
4 HAT	R1	80	90	70	60	60
	R2	90	90	80	70	60
	R3	90	80	80	80	60
6 HAT	R1	80	80	80	80	70
	R2	90	90	80	80	70
	R3	90	80	70	60	60
8 HAT	R1	90	80	80	70	60
	R2	90	80	70	70	60
	R3	80	80	80	70	50
10 HAT	R1	90	90	80	70	70
	R2	90	90	80	70	70
	R3	90	80	80	80	60
12 HAT	R1	80	80	80	70	60
	R2	90	90	80	70	70
	R3	90	80	80	70	50
14 HAT	R1	90	90	90	80	70
	R2	90	90	80	80	70
	R3	90	80	80	70	60
16 HAT	R1	90	90	90	80	60
	R2	80	80	80	70	70
	R3	90	80	80	60	60
18 HAT	R1	90	90	80	80	70
	R2	90	90	90	80	70
	R3	80	80	80	70	60
20 HAT	R1	90	90	90	80	70
	R2	90	90	80	80	60
	R3	80	80	80	70	60

Appendix table VIII Repellency effects of Bishkatali plant extract in ethyl alcohol on red flour beetle *T. castaneum* using treated filter paper

Duration of Treatment	Replication	Doses (mg/mi)				
		500	250	125	62.5	31.25
2 HAT	R1	90	80	70	70	50
	R2	80	70	80	70	60
	R3	80	70	70	60	50
4 HAT	R1	90	70	60	60	50
	R2	80	70	70	60	50
	R3	80	80	70	60	60
6 HAT	R1	80	80	60	60	50
	R2	70	70	60	60	60
	R3	90	80	80	70	50
8 HAT	R1	80	70	60	50	50
	R2	80	70	70	60	50
	R3	80	80	70	70	50
10 HAT	R1	80	70	70	60	50
	R2	70	70	60	60	50
	R3	90	80	70	60	60
12 HAT	R1	80	80	80	70	60
	R2	80	70	60	60	50
	R3	70	80	70	60	50
14 HAT	R1	80	80	80	60	50
	R2	80	70	60	60	50
	R3	80	70	60	60	50
16 HAT	R1	80	80	80	70	60
	R2	80	60	60	50	50
	R3	80	70	60	60	50
18 HAT	R1	80	70	70	60	50
	R2	70	70	60	60	60
	R3	80	80	70	50	50
20 HAT	R1	90	80	70	70	60
	R2	80	70	60	50	50
	R3	80	70	60	50	50

Appendix table IX Number of F_1 progeny of *T. castaneum* emergence from Bishkatali plant extract in chloroform at different days after treatment

Adult remove period	Replication	Doses (mg/mi)					
		500	250	125	62.5	31.25	Control
7 days	R1	34	42	46	48	54	62
	R2	32	40	45	49	52	59
	R3	32	39	46	48	53	60
12 days	R1	24	32	40	46	55	65
	R2	24	33	39	45	54	60
	R3	28	36	42	47	54	62
17 days	R1	24	33	40	48	56	67
	R2	27	34	42	49	54	60
	R3	30	33	42	49	56	63

Appendix table X Number of F_1 progeny of *T. castaneum* emergence from Bishkatali plant extract in ethyl alcohol at different days after treatment

Adult remove period	Replication	Doses (mg/mi)					
		500	250	125	62.5	31.25	Control
7 days	R1	39	40	45	49	53	62
	R2	38	42	48	50	53	59
	R3	37	43	45	49	53	60
12 days	R1	30	38	41	48	55	65
	R2	29	37	41	47	54	60
	R3	30	38	41	46	54	62
17 days	R1	31	38	43	48	56	67
	R2	31	37	40	46	53	60
	R3	29	37	41	48	56	63

Appendix table XI ANOVA table on repellency of Bishkatali plant extract in chloroform on red flour beetle

Duration of treatment	Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Prob.
2 hours	Treatment	4	1506.667	376.667	8.071	0.0036
	Error	10	466.667	46.667		
	Total	14	1973.337			
4 hours	Treatment	4	1560.000	390.000	9.750	0.0018
	Error	10	400.000	40.000		
	Total	14	1960.000			
6 hours	Treatment	4	760.000	190.000	3.562	0.0470
	Error	10	533.333	53.333		
	Total	14	1293.333			
8 hours	Treatment	4	1560.000	390.000	19.500	0.0001
	Error	10	200.000	20.000		
	Total	14	1760.000			
10 hours	Treatment	4	1093.333	273.333	13.667	0.0005
	Error	10	200.000	20.000		
	Total	14	1293.333			
12 hours	Treatment	4	1426.667	356.667	10.700	0.0012
	Error	10	333.333	33.333		
	Total	14	1760.000			
14 hours	Treatment	4	1026.667	256.667	9.625	0.0019
	Error	10	266.667	26.667		
	Total	14	1293.333			
16 hours	Treatment	4	1226.667	306.667	6.571	0.0073
	Error	10	466.667	46.667		
	Total	14	1693.333			
18 hours	Treatment	4	866.667	216.667	6.5000	0.0076
	Error	10	333.333	33.333		
	Total	14	1200.00			
20 hours	Treatment	4	1160.000	290.000	8.700	0.0027
	Error	10	33.333	33.333		
	Total	14	1493.333			

Appendix table XII ANOVA table on repellency of Bishkatali plant extract in ethyl alcohol on red flour beetle

Duration of treatment	Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Prob.
2 hours	Treatment	4	1466.667	366.667	11.000	0.0011
	Error	10	333.333	33.333		
	Total	14	1800.000			
4 hours	Treatment	4	1626.667	406.667	15.250	0.0003
	Error	10	266.667	26.667		
	Total	14	1893.333			
6 hours	Treatment	4	1373.333	343.333	5.150	0.0163
	Error	10	666.667	66.667		
	Total	14	2040.000			
8 hours	Treatment	4	1626.667	406.667	12.200	0.0007
	Error	10	333.333	33.333		
	Total	14	1960.000			
10 hours	Treatment	4	1733.333	333.333	8.333	0.0032
	Error	10	400.000	40.000		
	Total	14	1733.333			
12 hours	Treatment	4	1173.333	293.333	6.289	0.0085
	Error	10	466.667	46.667		
	Total	14	1640.000			
14 hours	Treatment	4	1626.667	406.667	12.200	0.0007
	Error	10	333.333	33.333		
	Total	14	1960.000			
16 hours	Treatment	4	1226.667	306.667	4.182	0.0303
	Error	10	733.333	73.333		
	Total	14	1960.000			
18 hours	Treatment	4	1240.000	310.000	9.3000	0.0021
	Error	10	333.333	33.333		
	Total	14	1573.333			
20 hours	Treatment	4	1826.667	456.667	8.563	0.0029
	Error	10	533.333	53.333		
	Total	14	2360.000			

Appendix table XIII ANOVA table on residual effect of Bishkatali plant extract in chloroform on F_1 progeny of red flour beetle

Adult remove period	Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Prob.
7 hours	Treatment	5	1400.278	280.056	219.174	0.0000
	Error	12	15.333	1.278		
	Total	17	1415.611			
12 hours	Treatment	5	2744.667	548.933	167.471	0.0000
	Error	12	39.333	3.278		
	Total	17	2784.000			
17 hours	Treatment	5	2789.167	557.833	135.689	0.0000
	Error	12	49.333	4.111		
	Total	17	2838.500			

Appendix table XIV ANOVA table on Residual effect of Bishkatali plant extract in ethyl alcohol on F_1 progeny of red flour beetle

Adult remove period	Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	Prob.
7 hours	Treatment	5	968.944	193.789	129.193	0.0000
	Error	12	18.000	1.5000		
	Total	17	986.944			
12 hours	Treatment	5	2087.333	417.467	300.576	0.0000
	Error	12	16.667	1.389		
	Total	17	2104.000			
17 hours	Treatment	5	2175.778	435.156	126.335	0.0000
	Error	12	41.333	3.444		
	Total	17	2217.111			

Appendix table XV Daily temperature as dry and wet bulb reading and corresponding relative humidity (March-August, 2006)

Month / date		Temperature (^{0}C)		Relative humidity (%)
		Dry bulb	Wet bulb	
March	1	25.5	22.5	77
	2	26.0	23.0	77
	3	27.0	24.5	80
	4	26.5	24.0	80
	5	26.0	22.5	72
	6	26.5	23.0	72
	7	26.5	23.0	69
	8	26.0	22.5	72
	9	26.0	22.5	72
	10	26.0	22.0	70
	11	27.0	23.5	66
	12	26.0	22.5	67
	13	26.5	23.5	71
	14	28.0	23.5	72
	15	28.0	24.0	70
	16	28.5	23.5	62
	17	29.0	25.0	63
	18	29.0	23.5	58
	19	29.5	24.0	64
	20	29.0	23.5	68
	21	30.0	24.0	68
	22	30.0	26.0	68
	23	30.0	26.0	74
	24	30.0	26.0	73
	25	30.0	25.0	71
	26	29.5	25.0	71
	27	30.0	25.5	62
	28	30.0	25.5	70
	29	30.5	27.0	72
	30	30.0	26.5	66
	31	30.0	26.0	73

Appendix table XV..... Continued

Month / date		Temperature (^{0}C)		Relative humidity (%)
		Dry bulb	Wet bulb	
April	1	26.5	23.5	78
	2	26.0	23.0	76
	3	27.0	25.5	79
	4	26.5	24.0	79
	5	26.0	24.5	73
	6	26.5	24.0	71
	7	27.5	23.0	70
	8	26.0	22.5	72
	9	26.0	23.5	73
	10	27.0	22.0	71
	11	27.0	24.5	67
	12	26.0	22.5	67
	13	27.0	23.5	71
	14	28.0	23.5	73
	15	27.0	24.0	70
	16	28.5	23.5	63
	17	28.0	23.0	63
	18	29.0	23.5	59
	19	29.5	24.0	65
	20	29.0	23.5	68
	21	29.0	27.0	69
	22	29.0	30.0	68
	23	29.0	30.0	75
	24	30.0	30.0	74
	25	30.0	30.0	73
	26	30.5	30.0	72
	27	30.0	27.5	63
	28	30.0	27.5	71
	29	29.5	27.0	73
	30	30.0	30.5	67

Appendix table XV ..... Continued

Month / date		Temperature (^{0}C)		Relative humidity (%)
		Dry bulb	Wet bulb	
May	1	30.0	26.5	73
	2	30.5	26.5	72
	3	30.0	26.5	74
	4	30.5	27.0	78
	5	30.5	27.5	78
	6	28.5	25.0	68
	7	30.5	26.0	73
	8	30.5	25.5	77
	9	29.5	27.5	78
	10	29.0	26.5	82
	11	29.0	28.0	72
	12	31.0	29.5	72
	13	33.0	30.0	75
	14	34.0	29.0	73
	15	33.0	30.0	73
	16	33.5	30.5	75
	17	34.0	30.5	75
	18	33.5	29.0	79
	19	32.5	30.5	79
	20	32.0	28.0	79
	21	33.0	29.0	79
	22	33.5	29.0	75
	23	29.5	30.0	72
	24	31.5	28.0	72
	25	30.0	29.0	79
	26	32.5	30.5	79
	27	33.0	30.0	75
	28	33.0	30.0	73
	29	33.0	30.5	79
	30	32.5	30.0	73
	31	32.0	30.5	74

Appendix table XV ..... Continued

Month / date		Temperature (^{0}C)		Relative humidity (%)
		Dry bulb	Wet bulb	
June	1	31.5	30.5	73
	2	33.5	30.0	79
	3	31.0	30.0	79
	4	33.0	29.0	79
	5	31.5	27.5	79
	6	30.5	28.5	79
	7	29.0	26.0	75
	8	29.0	25.0	79
	9	27.0	23.0	79
	10	27.0	23.5	75
	11	27.0	27.0	74
	12	30.0	30.0	73
	13	33.0	31.0	71
	14	31.0	30.5	79
	15	34.0	30.0	79
	16	35.0	30.0	79
	17	34.0	26.0	79
	18	34.0	25.0	79
	19	33.5	27.0	78
	20	31.0	30.0	72
	21	33.0	30.0	70
	22	33.0	30.5	71
	23	31.0	30.0	77
	24	31.5	30.0	73
	25	32.0	29.5	81
	26	32.0	29.5	81
	27	33.0	30.0	81
	28	32.0	30.0	79
	29	32.5	30.0	79
	30	33.0	27.5	79

Appendix table XV ..... Continued

Month / date		Temperature (^{0}C)		Relative humidity (%)
		Dry bulb	Wet bulb	
July	1	33.5	30.0	78
	2	34.0	30.0	81
	3	34.0	31.0	78
	4	34.0	30.0	78
	5	34.5	31.0	78
	6	33.0	30.0	72
	7	32.0	29.0	72
	8	33.0	30.0	72
	9	32.0	30.5	73
	10	32.0	29.0	73
	11	32.0	29.0	79
	12	33.0	30.0	79
	13	33.0	30.5	73
	14	33.0	30.5	73
	15	33.0	30.5	75
	16	33.0	30.0	79
	17	33.0	30.0	79
	18	32.5	30.0	78
	19	32.5	29.5	78
	20	32.0	29.5	78
	21	33.0	29.0	73
	22	33.5	30.0	72
	23	33.5	30.0	73
	24	34.0	30.5	79
	25	33.5	30.5	70
	26	33.5	30.0	73
	27	33.0	30.0	73
	28	32.0	29.0	79
	29	32.0	30.0	73
	30	32.0	30.5	79
	31	31.0	30.5	73

Appendix table XV ..... Continued

Month / date		Temperature (^{0}C)		Relative humidity (%)
		Dry bulb	Wet bulb	
August	1	32.0	30.5	75
	2	32.0	30.0	79
	3	33.5	30.0	79
	4	33.5	30.0	79
	5	32.0	29.0	78
	6	33.5	28.5	78
	7	34.0	29.0	74
	8	34.5	30.5	75
	9	35.0	30.0	73
	10	35.0	30.5	79
	11	34.0	30.0	75
	12	33.5	30.0	71
	13	33.5	30.0	73
	14	31.0	30.0	73
	15	33.0	31.0	79
	16	34.0	30.5	79
	17	34.0	29.5	75
	18	34.0	30.5	73
	19	34.5	30.5	79
	20	33.0	30.0	73
	21	33.0	31.0	73
	22	33.0	31.0	73
	23	34.0	30.5	72
	24	34.0	30.5	75
	25	34.0	29.5	73
	26	34.5	29.0	75
	27	34.0	30.5	79
	28	33.0	30.5	79
	29	33.5	30.5	79
	30	33.0	29.0	78
	31	33.0	29.0	78