EFFICACY OF FOUR PLANT EXTRACTS AGAINST *RIPTORTUS PEDESTRIS* (HEMIPTERA: ALYDIDAE)

A THESIS

BY

MD. JASHIM UDDIN

Student No. 1805236

Session: 2018-2019

Thesis Semester: July-December, 2019

MASTER OF SCIENCE

IN

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BELOVED PARENTS

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The author

EFFICACY OF FOUR PLANT EXTRACTS AGAINST *RIPTORTUS PEDESTRIS* (HEMIPTERA: ALYDIDAE)

ABSTRACT

The bean bug, *Riptortus pedestris* Fabricius (Hemiptera: Alydidae) is a highlypolyphagous and one of the major pests of leguminous crop and many fruit trees, causing serious economic damage in many crops. Plant extracts have long been suggested as alternatives to synthetic ones for pest management because of their high potency, cheaper, available and eco-friendly. The topical spraying and residual methods are used to measure the toxicity and repellency effect of four plant leaf extracts of neem (*Azadirachta indica*), crown flower (*Calotropis gigantean*), water pepper (*Polygonum hydropiper*) and marigold (*Tagetes patula*) against the *R. pedestris*. The experiment was conducted in laboratory conditions of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur; during the period from March to August 2019. The plant extracts were prepared with two solvent and applied at three doses viz., 500, 600 and 700 mg/L water. The maximum mortality of adults was recorded while marigold (acetone) and crown flower (acetone) sprayed topically at 48 hours after treatment at 700 mg/L water. These were followed by neem (ethanol) and water pepper (ethanol) extracts at 54 and 72 hours after treatment at700 mg/L water. On the other hand, at residual method the maximum mortality of adults was also recorded while marigold (acetone) sprayed at 72 hours after treatment at 700 mg/L. The result of repellency test showed that all the applied doses had repellency and direct toxicity effects but marigold showed the best response and it possess the repellency class V followed by crown flower and neem. Therefore, marigold and crown flower extracts showed highly potential to control of *R. pedestris* and can be use for alternative of synthetic chemical pesticides.

Key words: *R. pedestris*, topical spraying, residual spraying, repellency.

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CHAPTER I

INTRODUCTION

Riptortus pedestris Fabricius (Hemiptera: Alydidae) is a seed-sucking and one of the major pests of many crops in Asian countries (Osakabe and Honda, 2002; Kang *et al.*, 2003; Lee *et al.*, 2004) including Japan, China, Korea, Taiwan, Thailand, Indonesia and India (Kikuhara, 2005). It feeds on wide range of host plants such as soybean (*Glycine max* L.), sesame (*Sesamum indicum* L.), rice (*Oryza sativa* L.), apple (*Malus domestica* Borkh.), sweet persimmon (*Diospyros spp*) (Kim *et al.*, 1992, Son *et al.*, 2000, Wada *et al.*, 2006) and causes serious damage to the leguminous crops and the fruits of fruit trees (Kono, 1989; Chung *et al.*, 1995; Kang *et al.*, 2003). Both nymphs and adults causes damage to crops with their piercing and sucking mouthparts, so that the pods fail to mature and finally decreased both yield and quality (Kang *et al.*, 2003).

R. pedestris is highly mobile and making field-by-field control difficult and inefficient (Park *et al.*, 2016). The time when it colonizes in fields may vary due to a number of factors, such as planting date, variety and age of plant and local weather conditions. It has two or three generations per year (Musolin *et al.*, 2001). This species have five instars and overwinters in the adult stage. There are a few management strategies have been introduced against this notorious pest throughout the world. It has strong flying capacity allowing it to avoid to pesticides treated area, in that case chemical pesticides are often ineffective. In addition, chemical pesticides not only increase production cost, but result in reduction of natural enemy populations, cause pest resurgence and other environmental problems (Shepard *et al.,* 1997, Orr 1988). Cultural control methods such as delayed planting and use of resistant varieties have been suggested for *R. pedestris* management (Wada *et al.,* 2006). Biological control is safe and sound pest management approach especially egg parasitoids have been used successfully for the control of *R. pedestris* in augmentation or introduction programs (Orr, 1988).

Even though the chemical has adverse effects but it is commonly used management tactic against *R. pedestris* which is a major pest of leguminous crops (Kim *et al.,* 1992; Lee *et al.,* 2004; Choi *et al.,* 2005; Wada, 2006). Additionally, of chemical pesticides remains a major strategy in integrated pest management (IPM) systems against the insect due to its various advantages (Endo and Tsurumachi, 2001). Major advantages of chemical pesticides include quick action, easy use, convenience, cost-effectiveness, and reliability. Most Farmers rely on the use of synthetic pesticides for the control of insect pests including the *R. pedestris* (Alim and Lim, 2014). *R. pedestris* is a newly introduced invasive pest in Bangladesh in different legume crops like mungbean (*Vigna radiata* L.), soybean (*Glycine max* L.), sesame (*Sesamum indicum* L.), red clover (*Trifolium pretense* L.) etc. The legume growers of Bangladesh face *R. pedestris* as a new insect problem in their field. So, need to identify the suitable insecticides against this pod sucking insect. No research work has been done and published on this pest in Bangladesh yet but published some research in East Asian countries like Korea, Japan and China. Therefore, to ensure sustainable plant protection and eco-friendly environment for the beneficial insect need to identify the suitable pesticides for the controlling of this bug. Therefore, in this study four botanical pesticides are evaluated to control the *R. pedestris* in laboratory condition.

Objectives

1. To evaluate the direct toxicity effect of four botanical plant extracts on the mortality responses of the adult of *Riptortus pedestris*.

2. To find out the residual effect of the four botanical plant extracts against *R. pedestris*.

3. To determine the repellent activity of the botanical plant extracts on *R. pedestris*.

CHAPTER-II

REVIEW OF LITERATURE

Studies on the management of *R. pedestris* by chemical and botanical pesticides are new in Bangladesh. As *R. pedestris* newly introduced pest in Bangladesh which may cause serious damage for different leguminous crops. There is no literature on this insect in Bangladesh but there are a few literatures found in Asian countries like Korea, Japan, China and India. However, available information on the *R. pedestris* has been taken as review of literature.

2.1 Taxonomic position of *R. pedestris*

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hemiptera

Superfamily: Coreoidea

Family: Alydidae

Genus: *Riptortus*

Species: *Riptortus pedestris*

2.2 Biology and life history of *R. pedestris*

Visalakshi *et al.* (1976) studied the biology of *R. pedestris* on cow pea and described that adults paired 2 to 3 days after emergence, female laid about 115 eggs and oviposition period lasted for 30 days. The eggs hatched in 4 days and average nymphal period is 16 days. Adults lived for a total period of about 45-47 days.

Goel *et al.* (1983) studied effect of temperature on oviposition and hatching percentage of *R. linearis*. Under the laboratory conditions, eggs were laid singly or in rows up to 11 on various substrates. The oviposition period was recorded as 2-4 days. At mean temperature of 27 to 29.5°C the hatching percentage was 83.3 to 100 and with increase of temperature to 32.5°C, the hatching was reduced to 45%.

Kohno (1989) reported three generations of *R. clavatus* on soybean. The egg periods of *R. clavatus* under laboratory conditions were 43.4, 19.0, 9.9 and 6.8 days at 15, 20, 25 and 30°C, respectively. According to Allen *et al.* (1996) *R. dentipes* laid eggs in small batches on weeds or other leguminous plants but rarely on beans. There were five nymphal instars.

2.3 Distribution

R. pedestris is widely distributed in Asian countries including Japan, China, Indonesia, and India (Kikuhara, 2005). *R. pedestris* is a multivoltine insect having 2–3 generations per year in Korea and Japan (Kono, 1989; Shin *et al.*, 2012). Based on ovarian development of *R. pedestris* adults, Huh and Park (2005) reported that three generations per year would be possible in the southeastern region of South Korea. Lim (2013) observed that Hemipteran bugs, which were previously considered as secondary pests, have currently become important pests of numerous crops. Among them, *R. pedestris* is a major species that occurs in Korea, Japan, China, and South Asian countries.

Kim *et al.* (2014) conducted an experiment to evaluated that *R. pedestris* start to emerge earlier in spring when reproduction is not possible due to scarcity never became of nutritious food sources, thus host exploitation pattern is not well known. In order to understand the patterns of seasonal occurrence and how it relates to suitability of host plants in the spring, we evaluated the seeds of barley (*Hordeum vulgare* L.), sesame (*Sesamum indicum* L.), and black locust (*Robinia pseudoacacia* L.) as pre-season food sources for *R. pedestris*. When nymphs fed sesame seeds only, *R. pedestris* never became third instars.

2.4 Host Range

R. pedestris is highly mobile and has a wide host range, making field-by-field control difficult and inefficient (Park *et al.,* 2016). They overwinter as an adult near soybean fields or under leaf litter in mountainous areas (Lee *et al.,* 1997; Moriya, 2005). After overwintering, it undergoes 1-2 generations on wild hosts (e.g. Chinese milk vetch) and then colonizes soybean fields in April (Mizutani *et al.,* 2011). They causes the greatest damage to soybean, as it is the most suitable host for the bug (Lim, 2013). *R. pedestris* can consume leaves, stem, and pods of soybean (Ha *et al.*, 1998; Lee *et al.,* 2004).

The ornamental plant, *Caesalpinnia pulcherrima* and the weed, *Cassia occidentalis* were new reports of host plants of *R. pedestris* (Bharathimeena and Sudharma, 2009). The bean bug known as a serious pest of leguminous crops, develops sac-like tissues called crypts at the posterior region of the midgut, the lumen of which is densely colonized by a *Burkholderia symbiont* belonging to the Betaproteobacteria (Kikuchi *et al.,* 2005).

2.5 Oviposition site of *R. pedestris*

Oviposition preference was observed of *R. pedestris* to sites on a plant and within a field, to plants at different developmental stages, and to different leguminous germplasms. The insect laid its eggs mainly on the back surface of leaf in the upper half of plant in both observations from oviposition cage and soybean field. The egg numbers in fields were observed at a statistically-same rate in marginal and inside area, and at a median value of one egg per leaf in oviposited leaves. Full seed stage of soybean hardly affected oviposition preference of the insect (Jung, 2008). The females oviposit not only on host plants but also on non-hosts, which may impose high costs on nymphs in terms of locomotion energy and time searching for host plants (Nakajima *et al.,* 2010).

2.6 Nature of damage

James *et al.* (1982) reported that initial infestation with three leaf footed bug adults per plant during early bloom stages caused an average of 22% pod damage, average rate of seed abortion of 63% and an average rate of seed yield of 54%, whereas late bloom infestations sustained 22% loss in total seed yield in cow pea.

In the forest zone and Guinea savana of Nigeria, yield losses attributed to stink bugs ranged from 25 to 60% (IITA, 1983). Jackai *et al.* (1988) assessed damage caused by stink bugs and reported that seed damage caused by bugs ranged from an average of 32 to 59%. Son *et al.* (2000) reported that the damage caused by stink bugs in determinate types of soybean ranged from 5.0 to 12.5% and indeterminate types, the damage was 36.1- 50.1%. Both adults and nymphs feed on the soybean pods. *R. pedestris* can consume leaves, stem, and pods of soybean (Ha *et al.*, 1998; Lee *et al.,* 2004). Major economic damage occurs when soybean plants are in R3 and R4 stages (i.e. beginning to full pod stage) (Kono, 1989; Jung *et al.*, 2005) which also is the time to apply control measures to reduce *R. pedestris* population densities.

2.7 Seasonal abundance of *R. pedestris*

Singh and Singh (1978) reported the highest incidence of 57 bugs per plant on early red gram variety BS1 in the 39th standard week of October and the population of pest attained its peak (9 bugs/10 plants) by the mid of November.

Lee *et al.* (2004) conducted an experiment on seasonal occurrence of *R. clavatus* in soybean fields. They reported the peak their occurrence was at late of August for late May seeding while at mid of September for mid June seeding.

Bharathimeena *et al.* (2008) conducted an experiment in India, during 2005–2006 to evaluate the seasonal incidence of pod sucking bugs infesting vegetable cowpea and their natural enemies. The nymphal population of the alydid pod bugs, *R. pedestris* F. and *R. linearis* (F.) was significantly higher during the first fortnight of May 2006 and the succeeding three fortnights. Adult population of those peaked significantly during the first and second fortnights of June 2006 respectively, when compared to most other periods of the year.

Mizutani *et al.* (2011) elucidated that the annual life cycle of the *R. pedestris* Fabricius, especially its characteristics on potential host plants before it attacks soybeans. They also investigated the seasonal outbreak of the bean bug in Chinese milk vetch, red clover, and soybean fields from 2004 to 2008. In Chinese milk vetch fields, adults of bug appeared from late April, and their progenies appeared from mid-May to mid-June. Adults and nymphs were found in red clover fields until late August to mid-October. In soybean fields, there were no differences in the immigration and population density of adults and nymphs by year. Following adults, nymphs appeared from late July to early August in soybean fields. Nymphs and adults of the next generation appeared on each host plant, indicating that it can complete its annual life cycle using Chinese milk vetch, red clover and soybean as successive host plants.

Shin *et al.* (2012) investigated that seasonal fluctuation of *R. pedestris* using aggression pheromone traps from April to November in 2010 and 2011. The population density of this pest was high in mid-June, mid-August, and late October in 2010 and in early May, mid-June, mid-September, and early October in 2011 which were distinguished in farmlands and forests, were highest from June to August in the farmland and in September in the forest. Similar numbers of *R. pedestris* were capture in the farmlands and the forest in June-August, September-November, respectively. These results suggest that the insects did not migrate in August because food was plentiful in the forest at 200 m, but they moved to the forest during October due to the scarcity of food and for overwintering. The seasonal fluctuations of this pest in 2011 were affected heavily by the environment, particularly rain precipitation.

2.8 Biological control of *R. pedestris*

Draz *et al.* (2006) reported that green stink bug *Nezara viridula* was affected by an egg parasitoid *Trissolcus basalis*. Percentage of parasitism in egg masses ranged from 33.33% to 77.31%, while among eggs within each egg mass it ranged between 73.21 to 97.59% and sometimes reached 100% parasitism. Czepak *et al.* (1994) observed a polyphagous predator *Nabis punctatus* to prey on egg masses of pentatomid *N. viridula* in the fields of soy bean crop.

Nath and Dutta (1994) reported a predator *O. ratnae* to prey on *N. viridula* in green gram field. Functional response of the reduvid predator *Rhynocoris fuscipes* on the bean bug *R. clavatus* was studied in pigeonpea branch terminals in field cages by Ambrose and Claver (1995) and it was reported that *R. fuscipes* responded positively to changing abundance of prey and exhibited a Holling II functional response. The searching capacity of *R. fuscipes* increased with increasing prey density.

Sarma and Dutta (1997) observed a thomisid spider; *Thomisus* spp. in the field of green gram on pod sucking bug *R. linearis* and a predatory mite, *Tyrophagus putrescentiae* was found cling to various body parts of the coreid. According to Claver and Ambrose (2003), *R. fuscipes* effectively suppressed the infestation of *R. clavatus*. The release of *R. fuscipes* significantly enhanced the yield of healthy pods and was efficient in pest suppression.

According to Proyogo and Sukharsono (2005) a biocontrol agent such as predatory spider, *Oxyopes javanus* controlled the *R. linearis* on soybean crop.

While augmentative release of the parasitoid to control *R. pedestris* can be an alternative control measure, it is not economical (Lim and Mahmoud, 2009). Nakajima *et al.* (2010) indicated that second-instar nymphs might have responded more sensitively to synthetic aggregation pheromone than other instars. It is sometimes difficult to control these bugs with chemical pesticides alone because the bugs are strong that can migrate from field to field (Natsuhara, 1983).

R. pedestris (Heteroptera: Alydidae), formerly known as *R. clavatus*, develops a number of sac-like tissues, called crops or crypts, in the posterior region of the midgut, and these crypts are colonized by dense populations of a *Burkholderia symbiont* (Betaproteobacteria) (Kikuchi *et al.,* 2012). Furthermore, a recent study demonstrated that insecticide-degrading strains of the *Burkholderia symbiont* confer insecticide resistance to the bean bug (Kikuchi *et al.,* 2012).

The seasonal occurrence and within-plant distribution of *R. pedestris* and its two egg parasitoids was conducted to evaluate the effectiveness of parasitoids as a biological control agent in soybean fields. Peak population densities of both eggs and adults were recorded in the R5 stage of soybean, whereas nymphs started in the R6 stage. Seasonal total parasitism by *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) was significantly higher than *Gryon japonicum* (Ashmead) (Hymenoptera: Scelionidae). For the within-plant distribution of *R. pedestris* eggs, 90% were found on leaves and 70% on the under-leaf surface. Egg parasitism by *O. nezarae* was higher on the leaf surface than on pods. Results suggest that *R. pedestris* starts to occur at the R2–R4 stages of field soybeans. Sampling eggs on leaves is an alternative method to measure its populations in the field since most eggs are found on leaves. Since *O. nezarae* was synchronous with the bugs' occurrence, it would be a more efficient biological control agent of *R. pedestris* than *G. japonicum* (Kim and Lim, 2010).

2.9 Chemical control of *R. pedestris*

Chemical pesticides are widely used to reduce bean bug damage in soybeans (Kim *et al.,* 1992; Lee *et al.,* 2004; Choi *et al.,* 2005; Wada *et al.,* 2006). Therefore, pesticides used against this pest not only reduce natural enemies but also antagonists against pests (Shepard *et al.* 1997; Orr, 1988), and also affect their behavior. Behavioral avoidance of pesticides has also been detected in pests (Watson and Barson, 1996; Watson *et al.* 1997). This is particularly serious for insect pests that are physiologically resistant to some pesticides.

Bharathimeena and Sudharma (2009) observed that Imidacloprid 0.005% treated plants recorded significantly lower counts of *R. pedestris* nymphs and the highest yield, the toxic effect being on par with dimethoate 0.05%. Rapid knockdown of adults and nymphs with further persistence in the field was evidently higher for fenvalerate 0.03% and lambda-cyhalothrin 0.002%. The effect of chlorpyrifos 0.03% was on par with imidacloprid 0.005% in 15th day and malathion 0.05% in the 7th day, in suppressing the nymphal and adult populations, respectively after first spraying. Among the botanical pesticides, crude neem oil emulsion 5 ml L^{-1} was on par with 0.03% Neem emulsion 2ml L^{-1} in reducing the nymphal counts of *R. pedestris* and significantly superior in terms of yield when compared with 1% Neem emulsion 2ml L^{-1}.

The susceptibility of the stink bug species *Nezara viridula* (L.), *N. antennata* Scott, *Piezodorus hybneri* (Gmelin), *and R. pedestris* to pesticides was tested, establishing their 50% lethal dose (LD_{50}) values as baseline data. Third instars and adults of the four species were treated by topical application with seven pesticides: fenitrothion, fenthion, etofenprox, silafluofen, dinotefuran, clothianidin, and ethiprole. The weight of the stink bug and weight of the insecticide applied to each bug were used as explanatory variables in the probit regression analysis (Takeuchi and Endo, 2012).

In Korea, 17 pesticides, including diazinon and etofenprox, are registered for the control of hemipteran bugs, and growers apply pesticides two to three times on a regular basis (Lim, 2013). Alim and Lim. (2014) conducted an experiment in the laboratory where acute toxicity of eight pesticides on *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae), a major egg parasitoid of *R. pedestris*. AQmong them, fenitrothion, spinosad, cyfluthrin, etofenprox, and carbosulfan caused 100% mortality of *O. nezarae* within 24 hours by topical application or exposure to residue. Fenitrothion was also highly toxic to the parasitoid when ingested orally.

For soybean pest control in Japan, Lee *et al.* (2004) reported 91% reduction of *R. pedestris* after spraying etofenprox three times, at soybean growing R4, R5, and R6 stages.

2.10 Botanical control of *R. pedestris*

Kwon *et al.* (2011) conducted an experiment while minimizing insecticide use for the environmental friendly agriculture, the control of the *R. pedestris* can be utilized environmental friendly agricultural materials (EFAMs) in the green manure crop fields. The control effects of some EFAMs and most of chemical pesticides to this pest were significantly high. As a result of direct spray of EFAM that contained neem extract, the control effects of six EFAMs were higher than 70% at 120 hours after treatments. Among them, three EFAMs were showed over 90% of control effects.

On the other hand, most of chemical pesticides were showed 100% of control effect against this pest at 48 hours after treatment. As mortality effects of EFAMs were slowly observed until 120h after treatment, we must use selected EFAMs at the beginnings of occurrence and entrance in the field of green manure for effective control of *R. pedestris*.

CHAPTER III

MATERIALS AND METHODS

The experiment on the *Riptortus pedestris* was conducted in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the period from March to August 2019. Details materials and methods were used during the study period are described below.

3.1 Insect rearing

Adults of *R. pedestris* were collected from mungbean infested fields of HSTU campus in May, 2019 and reared in the laboratory of the Department of Entomology (HSTU). The mass culture was maintained in the laboratory according to the methods described by Alim and Lim (2010). The nymphs and adults were kept in separate acryl cages (40L × 40W × 40H cm) with windows in three lateral sides covered with meshed screens for ventilation at ambient temperature 31.62°C ± 0.16, average relative humidity 79.25% ± 1.60 and natural photoperiod 13: 11 (Plate 1). Temperature and relative humidity were measured by the Hydro-thermometer (HTC-1), China. Ascorbic acid dissolved in water and dried soybean seeds were provided to adult as food. The nymphs were transferred into another glass cage with windows in three lateral sides covered with meshed screens for ventilation. Mungbean plants with cotyledonous leaves provided for nymphs as food sources. Four pieces of gauze were placed on upper and bottom corners inside the adult cages as oviposition substrates. Water soaked cotton and mungbean seeds were changed twice a week. The cage were cleaned two times in a week. Eggs were collected from the acryl cages and new gauzes were placed in acryl cages daily and kept in another cage for the use in experiment. Adults were collected for the purpose of experiments.

Plate 1. Rearing cage of *R. pedestris* for mass culture

3.2 Plant materials used for bioassay

Four plant species (Table 1) having insecticidal activities were collected from their natural habitats from different locations of the Hajee Mohammad Danesh Science and Technology University (HSTU), campus Dinajpur. Each plant species was identified and verified according to plant morphology, using live specimens and photographs (Karim and Kabir, 1995) (Plate 2).

Table 1. List of tested plant extracts (fresh leaves)

Sl. No	English name	Scientific name	Family name
1.	Neem	*Azadirachta indica*	Meliacae
2.	Crown flower	*Calotropis gigantean*	Asclepiadaceae
3.	Water pepper	*Polygonum hydropiper*	Polygonaceae
4.	Marigold	*Tagetes patula*	Compositae

3.3 Preparation of plant extracts

The extract of four plant leaves were obtained and isolated by following Abe and Matsuda (2000). For this, fresh leaves of each plant for the purpose of extraction were collected from mature plants (after the fruiting stage). Then the collected leaves were cut into pieces and grinded using a mortar and pester. 10 gm of each category of grinded leaves were separately mixed with 100 ml distilled water at room temperature (31.62ºC). Then the mixture was filtrated through filter paper (Whatman No. 1). 25 ml of each category of filtrated mizture were taken separately in a 600 ml beaker and mixed with each of 100 ml of two different solvents (ethanol and acetone). Then the mixture was shaken for 30 minutes and left stand for separation of layer in separating funnel. After collecting the separating extracts, it was transferred in round bottom flasks and condensed by evaporation of solvent in a rotary evaporator (D 79219, Kika Werke Gmbh and Co. Germany) (Plate 3) at 78 ºC and 56 ºC for ethanol and acetone extracts, respectively. Then the crude extract was transferred quantitatively to a clean and weighted vial and kept in the refrigerator until used for toxicological investigation. For each plant sample 300 gm of fresh leaves were taken to prepare the extract.

Azadirachta indica

Calotropis gigantean

Tagetes patula

Polygonum hydropiper

Plate 2: Fresh leaves used for different botanicals extraction

Plate 3. Rotary evaporator used in the experiment

3.4 Bioassays of plant extracts

All bioassays were conducted in the Entomology laboratory at room temperature in the month of March to August 2019 at 31.62ºC, 79.25 % relative humidity, respectively.

3.4.1 Activity of plant extracts to adults of *R. pedestris* by topical spraying method

Tested four extracts of neem (*Azadirachta indica*), crown flower (*Calotropis gigantean*), water pepper (*Polygonum hydropiper*) and marigold (*Tagetes patula*) were prepared (Plate 4). One adult of *R. pedestris* was placed in a centrifugal tube (50 ml cap.) and sprayed with the 500 mg/L tested solution by a hand sprayer. The individuals were then immediately transferred into a new Petri dish (5.5 D×1.3 H cm) individually and kept at ambient conditions without food sources. Control Petri dishes were treated as same manner with water only. Each test was replicated three times. The treated adults were examined for mortality up to the 72 hours at 6 hours intervals after treatment.

Dead insects were confirmed by showing no responses while probed with small brush when did not show any responses. In the case of four plant extracts with ethanol, *R. pedestris* were examined for mortality at 6 hours after treatment of three concentrations (500 mg/L, 600 mg/L and 700 mg/L), respectively.

Plate 4: Separation of plant extract

3.4.2 Activity of plant extracts to adults of *R. pedestris* by residual method

The bottoms of Petri dishes (5.5 D×1.3 H cm) were sprayed with the concentration of 500 mg/L tested solution by a hand sprayer and the solvent was quickly removed by air drying. After 1 hour drying, thirty adults of *R. pedestris* were introduced into the treated Petri dishes. Control Petri dishes were treated similarly with water. The treated adults were examined for mortality up to the 72 hours at 6 hours interval after treatment.

Adults that did not show any responses when probed with a small brush were considered dead. In the case of four plants with ethanol extracts, *R. pedestris* were examined for mortality at 6 hours after treatment of three concentrations (500 mg/L, 600 mg/L and 700 mg/L), respectively.

3.4.3 Repellency tests

The petiole of two fully expanded leaves was placed in a Petri dishes facing ventral surface upward. One leaf was dipped in the tested plant extract and another was untreated. Thirty adults of *R. pedestris* introduced between the two leaves in the Petridish and the Petridish lid was closed. Number of adults attracted to each leaf was recorded at 6, 12, 18 and 24 hours intervals.

Data were expressed by the formula of Talukder and Howse 1994, as percentage repulsion (PR) as PR (%) = (N_c-50) x 2; Where, N_c= the percentage of insects present in the control half. Positive (+) values express repellency and negative (-) values attractancy. The repellency class was classified as class 0 > 0.01-10.1, class I- 0.1- 20.0, class II- 20.1,-40.0, class III- 40.1-60.0, class IV-60.1 -80.0, and class V -80.1- 100.0% repellency.

3.5 Statistical analyses

Mean values were given with the standard error (SE). Differences in mortalities among treatments were analyzed by univariate comparison test (ANOVA) using SPSS software 20.0 version. The Chi-square and LT_{50} values were obtained by using probit analysis (Robertson *et al.*, 2007). Significant differences among the biopesticides in each assay were recorded when 95% confidence intervals (CI) did not overlap. Values of the tested plant extract and confidence limits were calculated with Log dosage-mortality probit regression equation.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Toxicity of plant extracts against the adults of *R. pedestris* by topical spray methods

Mortalities of R. *pedestris* adults on bean leaves, sprayed with different plant extracts under laboratory conditions showed significant differences (Table 2). Hundred percent adult mortality was recorded while sprayed marigold (acetone) and crown flower (acetone) at 700 mg/L water at 48 hours after treatment. Neem (acetone) also showed 100% mortality at 54 hours after treatment with 700 mg/L water (Table 2). On the other hand, neem (ethanol) and water pepper (acetone) extracts showed 86% and 60% mortality at 42 to 48 hours after treatment with 700 mg/L (figure 3) water (Table 2).

In single dose response, 700 mg/L water (acetone) with marigold extracts showed significant results (Table 5). All plant extracts showed toxicity to *R. pedestris* in different hours after treatment using topical spraying method. However, marigold (acetone) caused hundred percent mortality at 48 hours after treatment. But crown flower (acetone) and neem (acetone) showed hundred percent mortality at 48 and 54 hours after treatment respectively. On the other hand, dose of 600 mg/L and 500mg /L water with marigold (acetone) were the most toxic and showed 100% mortality at 60 hours after treatment (Figures 1-2). The results of single dose responded mortality is summarized in Tables 3-5.

Table 2: Mortality of different plant extracts against *R. pedestris* by topical spraying at different doses

Plant Extract	Solvent	Dose (mg/L water)	Mortality Percentage							
			18HAT	24HAT	30HAT	36HAT	42HAT	48HAT	54HAT	60HAT
Marigold	Acetone	500	13.33±0.33 c-e	33.33±0.33 bc	63.33±0.33 a	73.33±.33 a	76.67±.33 b	83.33±0.33 b	90.0±0.58 a-b	100.0±0 a
		600	20.00±0.57 bc	33.33±0.66 bc	56.67±0.33 a	76.67±0.33 a	83.0±0.33 ab	86.67±0.33 ab	96.0±0.33 a	100.0±0 a
		700	26.67±0.33 b	43.33±0.33 ab	56.67±0.67 a	80.0±0.58 a	96.67±0.33 a	100.0±0 a	100.0±0 a	100.0±0 a
	Ethanol	500	3.33±0.33 ef	3.33±0.33 gh	13.33±0.33 e-h	13.33±0.33 h-j	26.67±0.33 g-j	30.0±0 i-k	36.67±0.67 i-k	40.0±0 ij
		600	13.33±0.33 c-e	13.33±0.33 d-h	16.67±0.33 d-g	20.0±0 f-i	33.33±0.33 e-j	36.67±0.33 g-j	36.67±0.33i-k	50.0±0 h-j
		700	10.0±0 c -f	23.33±0.33 c-e	26.67±0.33 c-e	33.33±0.33 c-g	46.67±0.33 d-f	53.33±0.33 c-f	60.0±0 d-g	66.67±0.88d-h
Water pepper	Acetone	500	0 g	0 i	3.33±0.33 gh	10.0±0.58 ij	16.67±0.33 j	20.0±0 k	30.0±0.58 k	33.33±0.33 j
		600	0 g	13.33±0.33 d-h	23.33±0.33 c-f	30.0±0.58 c-h	40.0±0.58 d-h	46.67±0.33 d-h	50.0±0.58 e-j	63.33±33 e-h
		700	13.33±0.33 c-e	23.33±0.33 c-e	30.0±0 cd	43.33±0.33 b-d	56.67±0.33 cd	60.0±0 cd	70.0±0.58 cd	73.33±0.33 c-f
	Ethanol	500	6.67±0.33 d-f	16.67±0.33 d-g	20.0±0 c-f	26.67±0.33 d-h	30.0±0 f-j	33.33±0.33 h-k	43.33±0.33 g-k	50.0±0.58 h-j
		600	6.67±0.33 d-f	13.33±0.33 d-h	20.0±0.58 c-f	33.33±0.88 c-g	43.33±0.88 d-g	50.0±1 d-g	63.33±0.88 c-f	70.0±0.58 d-g
		700	13.33±0.33 c-e	20.0±0 d-f	33.33±0.33 bc	43.33±0.33 b-d	46.67±0.33 d-f	50.0±0 d-g	63.33±0.33 c-f	73.33±0.33 c-f
Neem	Acetone	500	6.67±0.33 def	16.67±0.33 d-g	20.0±0 c-f	23.33±0.33 e-i	33.33±0.33 e-j	36.67±0.33 g-j	43.33±0.67 g-k	53.33±0.67 i-k
		600	10.0±0 c -f	16.67±0.33 d-g	23.33±0.33 c-f	33.33±0.33 c-g	33.33±0.33 e-j	43.33±0.33 e-i	53.33±0.33 d-i	56.67±0.33 f-i
		700	13.33±0.33 c-e	16.67±0.33 d-g	26.67±0.67 c-e	33.33±0.33 c-g	43.33±0.33 d-g	56.67±0.33 c-e	66.67±0.33 c-e	80.0±0.58 b-e
	Ethanol	500	10.0±0 c-f	16.67±0.33 d-g	23.33±0.33 c-f	30.0±0.58 c-h	33.33±0.33 e-j	53.33±0.33 c-f	56.67±0.33 d-h	73.33±0.33 c-f
		600	13.33±0.33 c-e	16.67±0.33 d-g	30.0±0 cd	43.33±0.33 b-d	56.67±0.33 cd	66.67±0.33 c	80.0±0 bc	93.33±0.33 ab
		700	16.67±0.33 b-d	26.67±0.33b -d	43.33±0.33 b	53.33±0.33 b	70.0±0.58 bc	86.67±0.33 ab	100.0±0 a	100.0±0 a
Crown flower	Acetone	500	13.33±0.33 c-e	13.33±0.33 d -h	13.33±0.33 e-h	26.67±0.67 d-h	30.0±0.58 f-j	40.0±1.15 f-i	50.0±1.15 e-j	66.67±0.33 d-h
		600	16.67±0.33 b-d	20.0±0.58 d-f	23.33±0.88 c-f	40.0±1 b-e	46.67±1.2 d-f	56.67±0.67 c-e	66.67±0.33 c-e	83.67±0.88 a-d
		700	36.67±0.33 a	46.67±0.88 a	60.0±0.58 a	76.67±0.67 a	96.67±0.33 a	100.0±0 a	100.0±0 a	100.0±0 a
	Ethanol	500	10±0.58 c-f	16.67±0.33 d-g	23.33±0.33 c-f	30.0±0.58 c-h	33.33±0.67 e-j	43.33±0.67 e-i	46.67±0.88 f-k	63.33±0.33 e-h
		600	13.33±0.33 c-e	13.33±0.33 d-h	16.67±0.33 d-g	43.33±0.67 b-d	50.0±0.58 de	50.0±0.58 d-g	66.67±0.33 c-e	73.33±0.33 c-f
		700	16.67±0.33 b-d	20.0±0.58 d-f	26.67±0.88 c-e	46.67±0.88 bc	46.67±0.88 d-f	56.67±0.33 c-e	70.0±0.58 cd	90.0±0 a-c
Control			0 g	0 i	0 i	0 k	0 k	0 l	3.33±0.33 l	3.33±0.33 k

HAT= Hours after treatment

Table 3: Efficacy of different plant extracts against *R. pedestris* by topical application at 500 mg/L

Plant extract	Solvent	n	LT_{50}	95% confidence interval (lower limit, upper limit)	Slope (±) SE	χ^2 df (10)	TI
Marigold	Ethanol	30	28.14 c	19.79-37.06	2.95 ±0.29	41.77	2.27
	Acetone	30	-	-	4.43±0.423	140.26	-
Neem	Ethanol	30	33.00 bc	26.70-39.62	3.78±0.36	28.10	1.94
	Acetone	30	37.18 b	30.65-44.89	3.07±0.32	20.49	1.74
Water pepper	Ethanol	30	34.38 bc	21.72-53.75	2.65±0.28	64.83 (10)	1.86
	Acetone	30	28.43 c	18.16 - 40.07	2.60±0.27	50.22 (10)	2.25
Crown flower	Ethanol	30	13.61 d	8.69-17.94	2.26±0.25	18.48	4.71
	Acetone	30	32.47 bc	22.09- 35.74	2.66±0.28	47.64	1.97
Control		30	64.13 a	60.61- 69.18	8.48±1.23	1.18	1.00

Means followed by different letters significantly different (Robertson *et al.*, 2007), TI = Toxicity index.

Table 4: Efficacy of different plant extracts against *R. pedestris* by topical application at 600 mg/L

Plant extract	Solvent	n	LT_{50}	95% Confidence Interval (lower limit, upper limit)	Slope (±) SE	χ^2 df (10)	TI
Marigold	Ethanol	30	31.61 b	22.16- 42.97	2.82±0.89	44.79	1.88
	Acetone	30	32.90 b	29.28-36.64	3.18±0.32	5.75	1.81
Neem	Ethanol	30	21.90 c	15.10-28.31	2.63±0.27	29.57	2.72
	Acetone	30	-	-	1.81±0.29	111.57	
Water pepper	Ethanol	30	25.38 bc	15.35-36.76	2.47±0.26	42.76	2.34
	Acetone	30	30.85 b	22.17-41.66	1.90±0.24	22.45	1.93
Crown flower	Ethanol	30	13.99 d	9.68-17.86	2.27±0.25	14.55	4.26
	Acetone	30	21.96 c	16.27-27.39	2.412±0.26	18.92	2.71
Control		30	59.69 a	55.02-66.39	5.00±0.66	0.79	1.00

Means followed by different letters significantly different (Robertson *et al.,* 2007); TI = Toxicity index.

Table 5: Efficacy of different plant extracts against *R. pedestris* by topical application at 700 mg/L

Plant extract	Solvent	n	LT_{50}	95% confidence interval (lower limit, upper limit)	Slope (±) SE	χ^2 df (10)	TI
Marigold	Ethanol	30	18.63 c	18.63-24.34	2.55±0.26	28.05	3.39
	Acetone	30	34.42 b	24.51-47.48	2.76±0.29	43.97	1.84
Neem	Ethanol	30	25.39 b	25.39-32.83	2.91±0.28	36.02	2.50
	Acetone	30	38.58 b	34.07-43.80	2.71±0.31	9.50	1.64
Water pepper	Ethanol	30	29.23 b	16.89-43.32	3.38±0.31	90.02	2.16
	Acetone	30	13.00 cd	2.76-21.34	1.38±0.22	29.89	4.87
Crown flower	Ethanol	30	7.22 d	4.89-9.35	2.44±0.32	9.277	8.77
	Acetone	30	22.20 bc	16.61-27.56	2.94±0.28	24.56	2.85
Control		30	63.34 a	58.93-69.91	6.22±0.87	0.65	1.00

Means followed by different letters significantly different (Robertson *et al.*, 2007).

TI = Toxicity index.

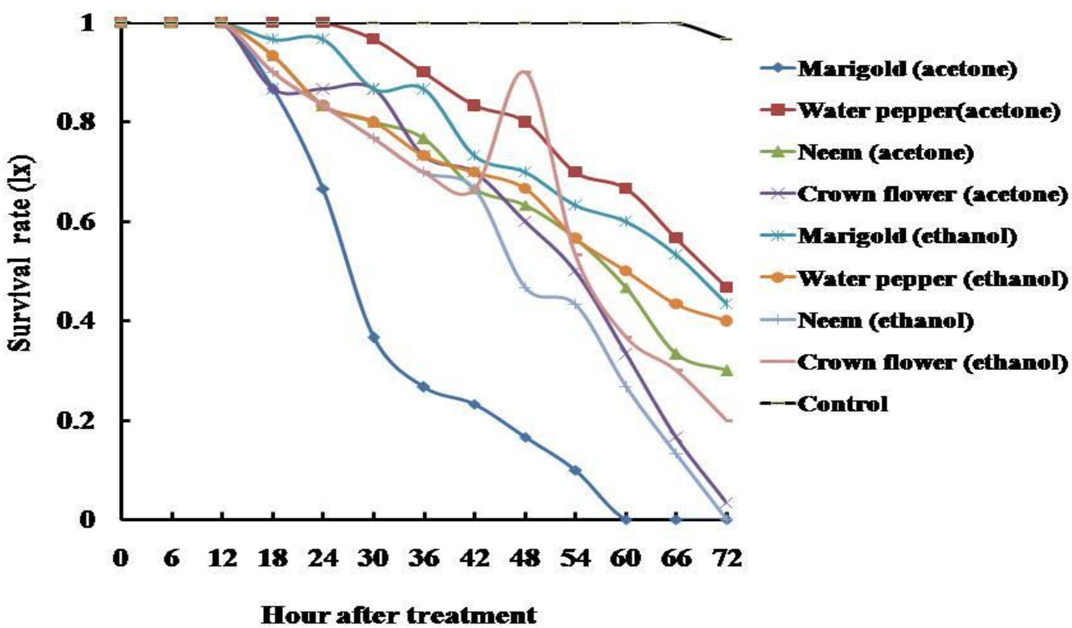

Figure 1. Survivorship of adult *R. pedestris* while exposed to the tested plant extracts by topical application at 500mg/L.

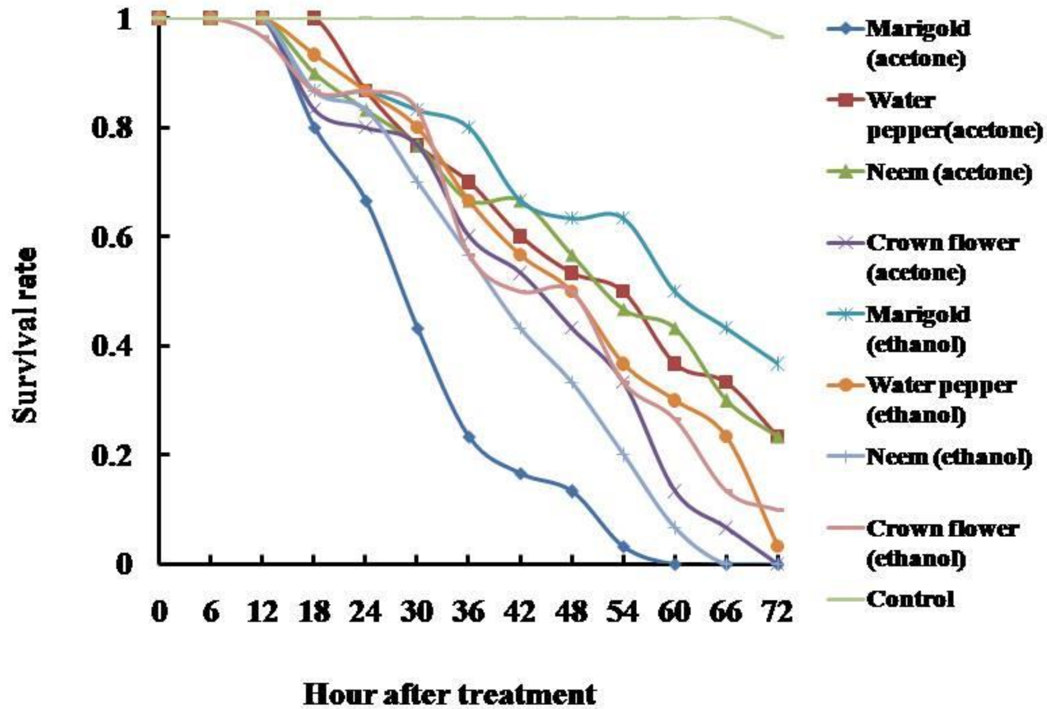

Figure 2. Survivorship of adult *R. pedestris* while exposed to the tested plant extracts by topical application at 600mg/L.

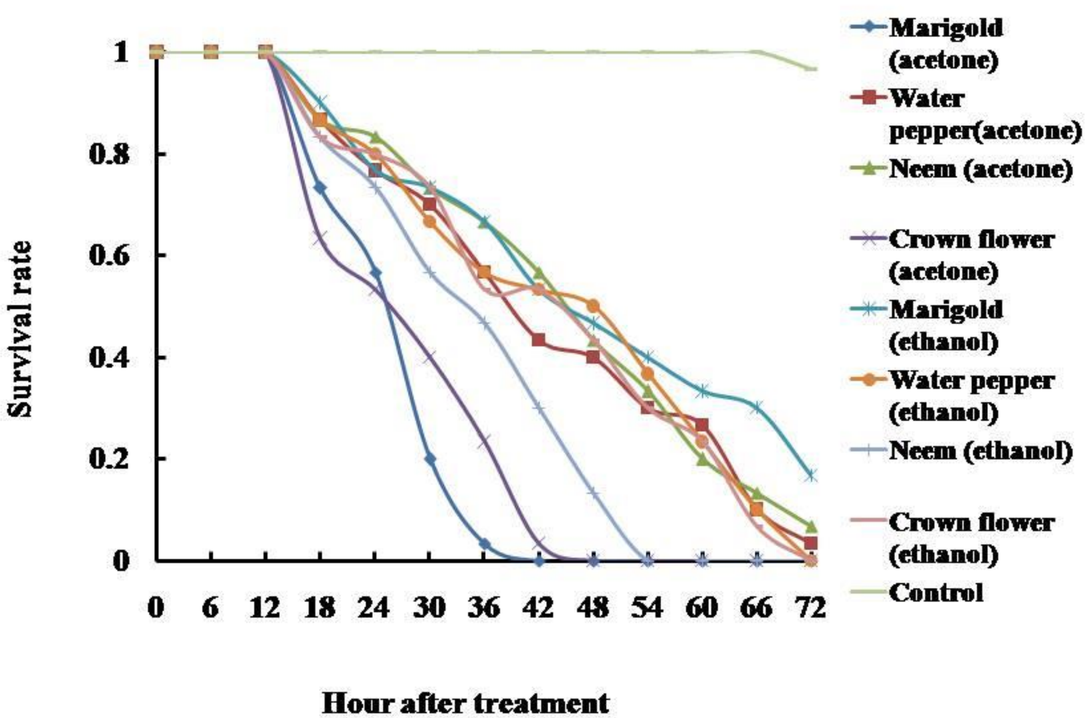

Figure 3. Survivorship of adult *R. pedestris* while exposed to the tested plant extracts by topical application at 700mg/L.

4.2. Toxicity of plant extracts against the adults of *R. pedestris* by residual method

Spraying of tested plant extracts by residual on the adults of *R. pedestris* under the laboratory conditions showed statistically significant (Table 6). Marigold of acetone extract at 700 mg/L water showed the highest mortality (100%) at 72 hours after treatment (Table 6). Marigold of acetone also showed 66.67-90% mortality at 72 hours after treatment at 500 and 600 mg/L water concentrations. On the other hand water pepper of acetone showed 50-60% mortality at 72 hours after treatment at 500 and 600 mg/L water concentrations.

In single dose response 700 mg/L water with marigold of acetone extract showed 100% mortality at 72 hours after treatment (Figure 6) and crown flower of acetone extract, neem (acetone and ethanol) and water pepper (acetone and ethanol) extract caused 50% mortality at 72 hours after treatment.

In single dose 600 mg/L, marigold of acetone extract showed the highest mortality of *R. pedestris* in residual spraying method within 72 hours after treatments (Figure 5). Besides, the most of the plant extracts killed around 70-80% adults within 72 hours after treatment. In single dose 500 mg/L, crown flower of acetone extract showed highest mortality of *R. pedestris* in residual spraying method within 66 and 72 hours after treatments (Figure 4). Moreover, rest of the plant extracts killed 43-70% adults within 72 hours after treatment.

Table 6: Efficacy of different doses of plant extracts against *R. pedestris* by residual application

Plant Extract	Solvent	Dose (mg/L Water)	Mortality percentage (Hour after treatment)									
			18 HAT	**24 HAT**	**30 HAT**	**36 HAT**	**42 HAT**	**48 HAT**	**54 HAT**	**60 HAT**	**66 HAT**	**72 HAT**
Marigold	Acetone	500	6.67±0.33 a-c	10.0±0 a-d	13.33±0.33 b-e	16.67±0.33 c-e	23.33±0.33 c-e	26.67±0.33 e	30.0±0 gh	36.67±0.33 d-f	43.33±0.33 g-k	66.67±0.67 b-h
		600	16.67±0.33 a	20.0±0.58 ab	30.0±1 a	36.67±0.33 ab	46.67±0.88 a	60.0±1 a	66.67±0.88 ab	73.33±0.33 ab	86.67±0.88 ab	90.0±0.58 a-b
		700	16.67±0.33 a	16.67±0.33 a-c	23.33±0.88 a-c	33.33±0.33 a-c	46.67±0.88 a	56.67±0.88 ab	66.67±0.33 ab	80.0±1 a	86.67±0.88 ab	100.0±0 a
	Ethanol	500	6.67±0.33 a-c	13.33±0.33 a-c	20.0±0 a-d	26.67±0.33 a-e	30.0±0 a-e	33.33±0.33 c-e	36.67±0.33 e-h	43.33±0.33 c-f	43.33±0.33 g-k	56.67±0.67 e-j
		600	13.33±0.33 ab	23.33±0.33 a	23.33±0.33 a-c	26.67±0.33 a-e	36.67±0.33 a-d	40.0±0.58 a-e	50.0±0.58 a-g	56.67±0.88 a-e	60.0±0.58 c-i	70.0±1.15 b-j
		700	13.33±0.33 ab	16.67±0.33 a-c	20.0±0.58 a-d	20.0±0.58 b-e	36.67±0.33 a-d	50.0±1.15 a-d	56.67±1.45 a-e	63.33±1.45 a-d	63.33±1.45 b-h	76.67±0.88 a-f
water pepper	Acetone	500	6.67±0.33 a-c	10.0±0.58 a-d	16.67±0.67 a-d	16.67±0.68 c-e	23.33±0.33 c-e	30.0±0.58 de	36.67±0.67 e-h	40.0±0.58 c-f	46.67±0.67 f-k	50.0±0.58g-j
		600	3.33±0.33 bc	6.67±0.33 b-d	13.33±0.33 b-e	20.0±0 b-e	30.0±0.58 a-e	36.67±0.88 b-e	43.33±0.88 c-h	50.0±1.15 b-f	53.33±1.2 e-k	60.0±1.54 d-i
		700	6.67±0.33 a-c	13.33±0.33 a-c	16.67±0.33 a-d	16.67±0.33 c-e	23.33±0.33 c-e	33.33±0.33 c-e	36.67±0.33 e-h	46.67±0.67 b-f	50.0±0.58 e-k	50.0±0.58 g-j
	Ethanol	500	6.67±0.33 a-c	13.33±0.33 a-c	13.33±0.33 b-e	16.67±0.33 c-e	23.33±0.33 c-e	33.33±0.33 c-e	43.33±0.33 c-h	53.33±0.33 a-f	60.0±0.58 c-i	66.67±1.2 b-h
		600	16.67±0.33 a	16.67±0.33 a-c	26.67±0.33 ab	36.67±0.33 ab	43.33±0.33 ab	53.33±0.33 a-c	66.67±0.33 ab	73.33±0.67 ab	73.33±0.67 a-e	80.0±0.58 a-e
		700	10.0±0.58 a-c	16.67±0.33 a-c	16.67±0.33 a-d	16.67±0.33 c-e	20.0±0.58 de	30.0±0.58 de	40.0±1.0 d-h	46.67±0.88 b-f	50.0±0.58 e-k	50.0±0.58 g-j
Neem	Acetone	500	6.67±0.33 a-c	13.33±0.33 a-c	13.33±0.33 b-e	23.33±0.88 a-e	26.67±0.67 b-e	33.33±0.88 c-e	40.0±1.0 d-h	46.67±1.2 b-f	50.0±1 e-k	63.33±0.88 c-i
		600	10.0±0.0 a-c	13.33±0.33 a-c	16.67±0.33 a-d	23.33±0.33 a-e	23.33±0.33 c-e	36.67±0.33 b-e	43.33±0.67 c-h	60.0±1.15 a-d	63.33±0.88 b-h	80.0±0.58 a-e
		700	13.33±0.33 bc	16.67±0.33 a-c	16.67±0.33 a-d	23.33±0.33 a-e	33.33±0.33 a-e	56.67±0.33 ab	70.0±0.0 a	80.0±0 a	83.33±0.88 a-c	90.0±0.58 a-b
	Ethanol	500	6.67±0.33 a-c	13.33±0.33 a-c	20.0±0.58 a-d	30.0±0.58 a-d	30.0±0.58 a-e	36.67±0.67 b-e	40±0.58 c-h	43.33±0.33 c-f	50.0±0.58 e-k	60.0±0.58 d-i
		600	10.0±0.58 a-c	16.67±0.33 a-c	20.0±0 a-d	23.33±0.33 a-e	33.33±0.33 a-e	43.33±0.33 a-e	53.33±0.88 a-f	53.33±0.88 a-f	60.0±0.58 c-i	80.0±0.58 a-e
		700	13.33±0.33 ab	16.67±0.33 a-c	23.33±0.33 a-c	33.33±0.33 a-e	46.67±0.88 a	60.0±0.58 a	60.0±0.58 a-d	66.67±0.33 a-c	83.33±0.88 a-c	90.0±0.58 a-b
crown flower	Acetone	500	6.67±0.33 a-c	13.33±0.33 a-c	16.67±0.33 a-d	23.33±0.33 a-e	33.33±0.33 a-e	43.33±0.88 a-e	50.0±0.58 a-g	60.0±0 a-d	70.0±0.58 b-f	70.0±0.58 b-g
		600	16.67±0.33 a	16.67±0.33 a-c	23.33±0.33 a-c	36.67±0.67 ab	46.67±0.33 a	53.33±0.33 a-c	63.33±0.33 a-c	66.67±0.67 a-c	70.0±1 b-f	80.0±0.58 a-e
		700	16.67±0.33 a	20.0±0.58 ab	26.67±0.33 ab	40.0±0.58 a	43.33±0.33 ab	56.67±0.33 ab	66.67±0.33 ab	80.0±0.58 a	83.33±0.88 a-c	96.67±0.33 a
	Ethanol	500	6.67±0.33 a-c	10.0±0 a-d	10.0±0 c-e	13.33±0.33 d-f	16.67±0.33 e	23.33±0.33 e	26.67±0.33 h	30.0±0.58 ef	36.67±0.33 i-k	43.33±0.88 h-i
		600	10.0±0.58 a-c	10.0±0.58 a-d	10.0±0.58 c-e	20.0±1.5 b-e	26.67±0.67 b-e	30.0±1.0 de	33.33±0.88 f-h	43.33±0.33 c-f	50.0±0.58 e-k	53.33±0.88 f-j
		700	16.67±0.33 a	20.0±0 ab	23.33±0.33 a-c	30.0±0.0 a-d	40.0±0.0 a-c	53.33±0.33 a-c	60.0±0.58 a-d	66.67±0.67 a-c	80.0±0.58 a-d	86.67±0.88 a-c
Control			0.0 d	0.0 e	0.0 f	0.0 g	0.0 f	0.0 f	0.0 i	0.0 g	0.0 l	3.33±0.33 k

Table 7: Efficacy of different plant extracts against *R. pedestris* by residual application at 500 mg/L

Plant extract	Solvent	n	LT_{50}	95% confidence interval (lower and upper limit)	Slope (±) SE	χ^2 (df)	TI
Marigold	Acetone	30	33.80 b	29.14-38.45	4.73±0.45	20.00 (10)	1.89
	Ethanol	30	37.19 b	34.41-39.91	5.54±0.54	3.41 (10)	1.72
Neem	Acetone	30	32.78 b	30.09-35.38	5.19±0.48	5.61 (10)	1.95
	Ethanol	30	39.16 b	35.74-42.73	4.12±0.43	3.67 (10)	1.63
Water pepper	Acetone	30	37.72 b	33.52-42.01	5.15±0.49	16.17 (10)	1.70
	Ethanol	30	28.20 bc	25.54-30.72	4.74±0.45	7.75 (10)	2.27
Crown flower	Acetone	30	18.26 d	14.83-21.36	4.11±0.40	16.00 (10)	3.51
	Ethanol	30	25.51 c	22.83-28.02	4.41±0.42	8.66 (10)	2.51
Control		30	64.13 a	60.61-69.18	8.48±1.23	1.18 (10)	1.00

Means followed by different letters significantly different (Robertson *et al.*, 2007)

TI = Toxicity index.

Table 8: Efficacy of different plant extracts against *R. pedestris* by residual application at 600 mg/L

Plant extract	Solvent	n	LT_{50}	95% confidence interval (lower limit, upper limit)	Slope (±) SE	χ^2 (df)	TI
Marigold	Acetone	30	35.62 c	30.74-40.58	4.54±0.44	19.58 (10)	1.67
	Ethanol	30	34.66 c	31.39-37.94	3.95±0.40	3.86 (10)	1.72
Neem	Acetone	30	29.03 d	25.92-31.98	5.25±0.49	11.65 (10)	2.04
	Ethanol	30	62.95 a	58.58-69.41	6.20±0.86	3.13 (10)	0.94
Water pepper	Acetone	30	33.16 c	30.46-35.80	5.12±0.48	9.31 (10)	1.80
	Ethanol	30	44.40 b	40.71-48.51	4.19±0.46	4.86 (10)	1.34
Crown flower	Acetone	30	19.21 e	15.88-22.27	3.40±0.33	11.44 (10)	3.10
	Ethanol	30	20.49 e	17.74-22.96	5.38±0.55	11.73 (10)	2.91
Control		30	59.69 ab	55.02-66.39	5.00±0.66	0.79 (10)	1.00

Means followed by different letters significantly different (Robertson *et al.*, 2007); TI = Toxicity index

Table 9: Efficacy of different plant extracts against *R. pedestris* by residual application at 700 mg/L

Plant extract	Solvent	n	LT_{50}	95% confidence interval (lower limit, upper limit)	Slope (±) SE	χ^2 (df)	TI
Marigold	Acetone	30	25.43 d	23.18-27.56	5.75±0.56	7.04 (10)	2.49
	Ethanol	30	39.06 b	35.08-43.22	4.61±0.46	12.23 (10)	1.62
Neem	Acetone	30	29.78 c	27.26-32.20	5.33±0.50	6.75 (10)	2.12
	Ethanol	30	41.84 b	37.93-46.23	3.60±0.40	6.87 (10)	1.51
Water pepper	Acetone	30	31.80 c	27.82-35.62	7.08±0.66	23.77 (10)	1.99
	Ethanol	30	23.80 d	18.98-28.13	3.66±0.35	19.52 (10)	2.66
Crown flower	Acetone	30	12.12 f	9.86-14.25	4.19±0.44	12.13 (10)	5.22
	Ethanol	30	20.25 e	17.33-22.85	5.59±0.59	13.67 (10)	3.22
Control		30	63.34 a	58.93-69.91	6.22±0.87	0.65 (10)	1.00

Means followed by different letters significantly different (Robertson *et al.*, 2007); TI = Toxicity index

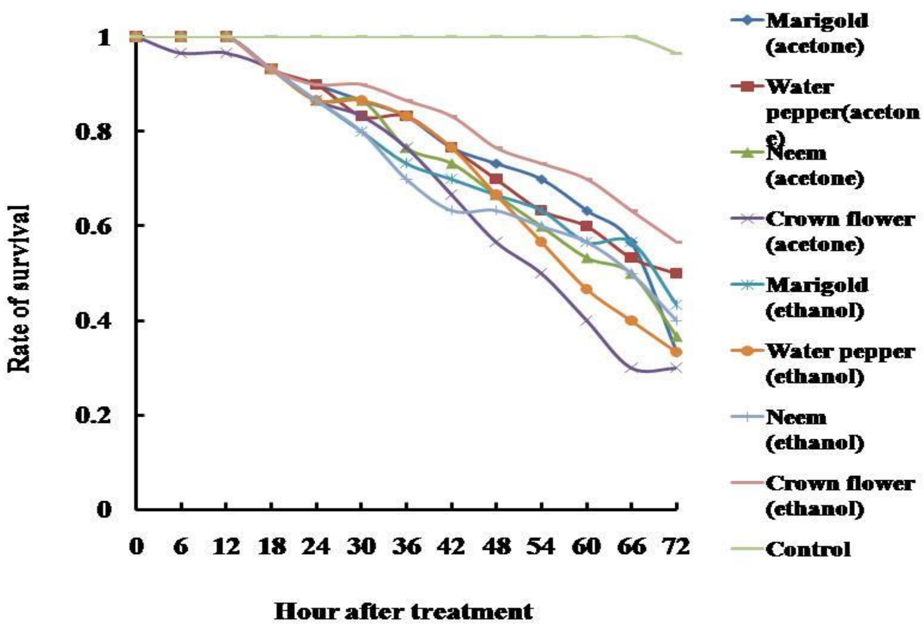

Figure 4. Survivorship of adult *R. pedestris* while exposed to the tested plant extracts by residual application at 500 mg/L

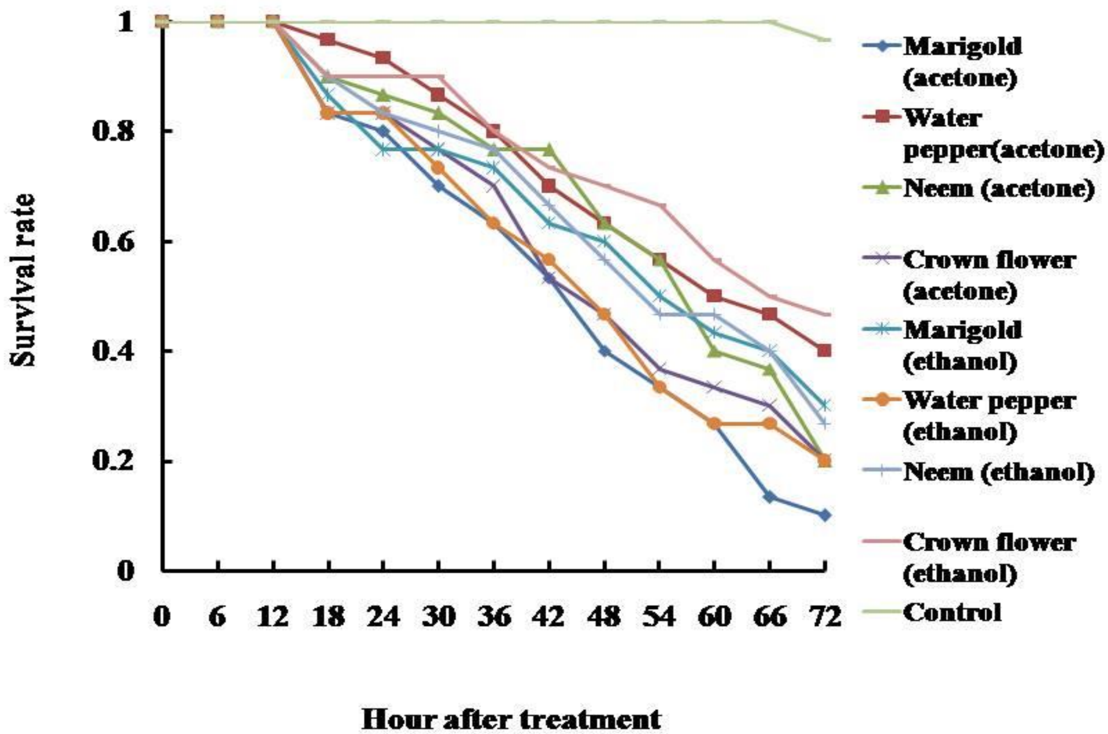

Figure 5. Survivorship of adult *R. pedestris* while exposed to the tested plant extracts by residual application at 600 mg/L

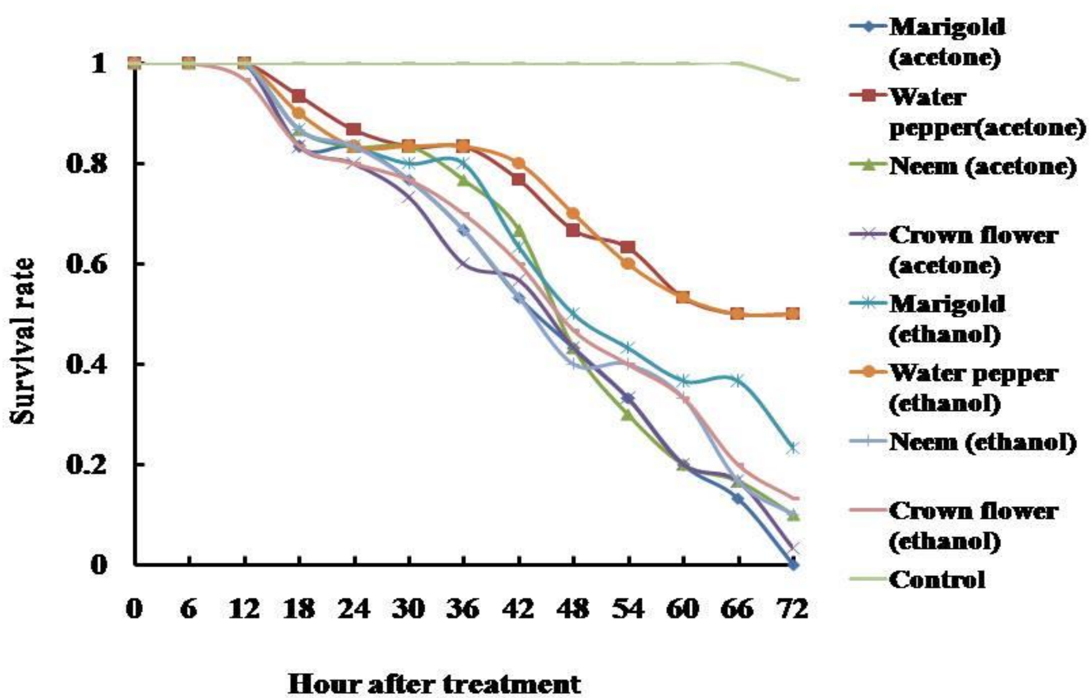

Figure 6. Survivorship of adult *R. pedestris* while exposed to the tested plant extracts by residual application at 700 mg/L.

4.3. Repellency effect of plant extracts on adult *R. pedestris*

The repellency rates of different plant extracts on adult *R. pedestris* are presented in Table 10. Neem (ethanol, and acetone), marigold (acetone, ethanol), crown flower (ethanol, acetone) extracted showed highest mean repellency rate (80-84%) and it belongs to the repellency class V followed by water pepper belonged to the class IV.

Table 10: Repellency rates of tested plant extracts against the adults *R. pedestris* at different hours after treatments

Plant Extract	Solvent	Doses (mg/L Water)	Repellency (%) at different HATs				Mean (%)	Repellency classes
			6 HAT	12 HAT	18 HAT	24 HAT		
Marigold	Acetone	500	90.0±0.58 a-d	76.33±0.33 a-d	63.33±0.33 a-f	60.0±1 a-d	72.42	IV
		600	93.33±0.67 a-c	83.33±0.33 a-c	80.0±0.58 a-c	66.67±0.33 a-c	80.83	V
		700	100.0±0 a	90.0±0.58 ab	93.33±0.33 a	76.67±0.33 a	90.00	V
	Ethanol	500	83.33±0.33 a-f	86.67±0.33 a-c	56.67±0.33 b-f	50.0±0.58 b-e	69.17	III
		600	90.0±0.58 a-d	80.0±0 a-d	73.33±0.88 a-d	53.33±0.33 a-e	74.17	IV
		700	86.67±0.88 a-e	80.0±0.58 a-d	46.67±0.88 c-f	53.33±0.88 a-e	66.67	IV
Water pepper	Acetone	500	80.0±0.58 a-g	66.67±0.33 b-e	63.33±0.33 a-f	46.67±0.33 c-e	64.17	II
		600	83.33±0.33 a-f	83.33±0.33 a-c	63.33±0.33 a-f	66.67±0.33 a-c	74.17	III
		700	96.67±0.33 ab	80.0±0.58 a-d	80.0±0.58 abc	56.67±0.33 a-d	78.34	IV
	Ethanol	500	50.0±0.58 hi	33.33±0.88 f	33.33±1.33 f	63.33±0.33 a-c	45.00	II
		600	56.67±0.88 ghi	63.33±0.33 b-e	50.0±0.58 b-f	60.0±1 a-d	57.50	III
		700	80.0±0.58 a-g	66.67±0.88 b-e	63.33±0.33 a-f	56.0±0.88 a-d	66.50	IV
Neem	Acetone	500	96.67±0.33 ab	70.0±0.58 a-e	83.33±0.88 ab	76.67±0.88 a	79.67	IV
		600	93.33±0.67 a-c	80.0±0 a-d	83.33±0.33 ab	73.33±0.67 ab	79.50	IV
		700	96.67±0.33 ab	86.67±0.33 a-c	76.67±0.67 a-d	73.33±0.88 ab	83.34	V
	Ethanol	500	93.33±0.67 a-c	86.67±0.33 a-c	63.33±1.2 a-f	63.33±1.33 a-c	76.67	IV
		600	100.0±0 a	83.33±0.33 a-c	80.0±0.58 a-c	73.33±0.88 ab	84.17	V
		700	100.0±0 a	90.0±0.58 ab	83.33±0.88 ab	76.67±0.33 a	87.50	V
Crown flower	Acetone	500	90.0±0.58 a-d	76.67±0.33 a-d	63.33±0.33 a-f	50.0±0.58 b-e	70.00	III
		600	93.33±0.33 a-c	83.33±0.88 a-c	73.33±0.67 a-d	63.33±0.88 a-c	78.33	IV
		700	100.0±0 a	80.0±0 a-d	76.67±0.88 a-d	60.0±0.58 a-d	80.17	V
	Ethanol	500	93.33±0.67 a-c	76.67±0.33 a-d	73.33±0.33 a-d	66.67±1.2 a-c	77.50	IV
		600	96.67±0.33 ab	83.33±0.88 a-c	70.0±1 a-f	53.33±0.33 a-e	75.83	IV
		700	100.0±0 a	96.0±0.33 a	93.33±0.33 a	60.0±0.58 a-d	87.33	V

4.4. Discussion

Chemicals derived from pyrethrum (*Tanacetum cinerariifolium* Trev.), neem (*Azadirachta indica* Juss) and other plant species are traditionally used as control operations in many crop pests (Saminathan and Jayaraj, 2001). Neem products, tobacco extracts and rosin soap had been found effective against small soft bodied insects (Singh *et al.*, 2005; Lü *et al.*, 2009). Zhang *et al.* (2008) observed that ethanol extracts of *Celosia argentea* L. and *Eupatorium odoratum* L. had high bioactivities against whitefly, *Aleurodicus dispersus* with LC_{50} being 753.40 mg/L and 999.81 mg/L, respectively. Our results indicated that acetone extracted of marigold, crown flower, neem, water pepper at 700 mg/L concentration exhibited significant insecticidal potent against *R. pedestris* adults by topical spraying. Among the four plant extracts, the maximum mortality of adults was recorded for marigold, whether applied by topical spraying or as a residual film (Tables 2-6). Kwon *et al.* (2011) observed that the control effects of neem extracts were higher than 70% at 120 hours after treatments on *R. pedestris* adults. Neem extracts 1.0 and 0.3% were significantly effective against *R. pedestris* adults until the 7 days after first spraying (Bharathimeena, and Sudharma, 2009). Dubey and Sundararaj (2004) reported that fortnightly and three-weekly applications of neem effectively controlled nymphal populations of *A. dispersus*, causing 62.2% mortality even at 21 days after treatment. The different results of toxicity tests might be due to the formulation effect and the response of *R. pedestris* to other toxic components of the marigold.

There are different mechanisms of plant extracts those acted against *R. pedestris* due to various mode of actions. In contrast to action mechanisms of azadirachtin has a diverse biological activity on insects indicating like antifeedant, oviposition deterrent, repellent, insect growth inhibitor, mating disruptor and toxic properties (Xu, 2001). Mixed use of plant extracts with different action mechanisms can enhance the synergism and also delay the development of resistance in *A. dispersus.* In order to reduce the possibility of target site resistance developed by white fly, *B tabaci* after chemical treatments, a mixture of different active compounds is suggested for *B. tabaci* control (Dubey and Sundararaj, 2004). *Myzus persicae* Sulzer developd resistance to pure azadirachtin (the major ingredient of neem insecticide), but not to are fined neem seed extract containing the same absolute amount of azadirachtin together with many other constituent's present (Feng and Zhang, 2007).

Prokash and Rao (1997) conducted an extensive revision of plants containing active secondary substances against insects. They concluded about the information on 866 plant products with insecticidal activity-repellent, antifeedant or regulatory of insect growth used against agricultural pests. Larew and Locke (1990) proposed that the botanical insecticides may involve removal of insects' cuticle wax, physical action, repellency or cell membrane disruption. Sandra (2002) opined that ginger oil has potential as a protectant of tomato seedlings against *Bemisia argentifolli*. The oil of ginger rhizomes *Zingiber officinale* Rosco consisted of α-Zingiberene and Curcumene, which caused increasing of repellency to *B. argentifolli* with increasing ginger oil concentration. Valladares *et al.* (2003) examined the antifeedant activity of an extract of senescent leaves of *Melia azadiracta* on nine insect species, including *A. disperses.* Our results also show the highest repellency rate (95%) on ethanol extracted neem plant (Table 10).

Our data indicated that acetone extract of marigold, ethanol and acetone extract of crown flower, acetone extract of neem generated highest mortality against *R. pedestris.* This may have potential for use in the development of new products for controlling the infestation level of insect pest in legume fields. Therefore, further studies should be carried out to investigate the potential of those plant and their chemical compounds for control of *R. pedestris*.

CHAPTER V

SUMMARY AND CONCLUSION

The bean bug, *Riptortus pedestris* Fabricius (Hemiptera: Alydidae) is a most invasive and one of the major pests of leguminous crops, causing serious economic damage in many crops. The present research work investigated the efficacy of different plant extracts that's commonly used and find out the possibility of different plant extracts for controlling the *R. pedestris.* Plant extracts have long been suggested as alternatives to synthetic ones for pest management because of their high potency, cheaper, available and eco-friendly. The topical spraying and residual contact methods were used to measure the toxicity and repellency effect of four plant leaf extracts against *R. pedestris*. The plant extracts were prepared in two different solvents and applied at doses viz., 500, 600 and 700 mg/L water. The maximum mortality (100%) of adults was recorded by topical spraying for marigold (acetone) at 48 hours after treatment being 700 mg/L. And these were followed by acetone of crown flower, and neem extracts at 48-54 hours after treatment at 700 mg/L. Conversely, at residual film method the maximum mortality (100%) of adults was also recorded on acetone extracted marigold at 72 hours after treatment at 700 mg/L followed by acetone extracts of neem, and crown flower which showed 90-96% mortality. Nonetheless, acetone and ethanol extracted water pepper showed 50% mortality at 72 hours treatment at 700 mg/L water. The result of repellency test showed that all the applied doses had repellency and direct toxicity effects but ethanol extracted marigold and crown flower showed the best response which possess the repellency class V while water pepper showed least repellency. Therefore, considering highest potentiality of acetone extracted marigold may be suggested as alternative of synthetic chemical insecticides to control the *R. pedestris*.

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