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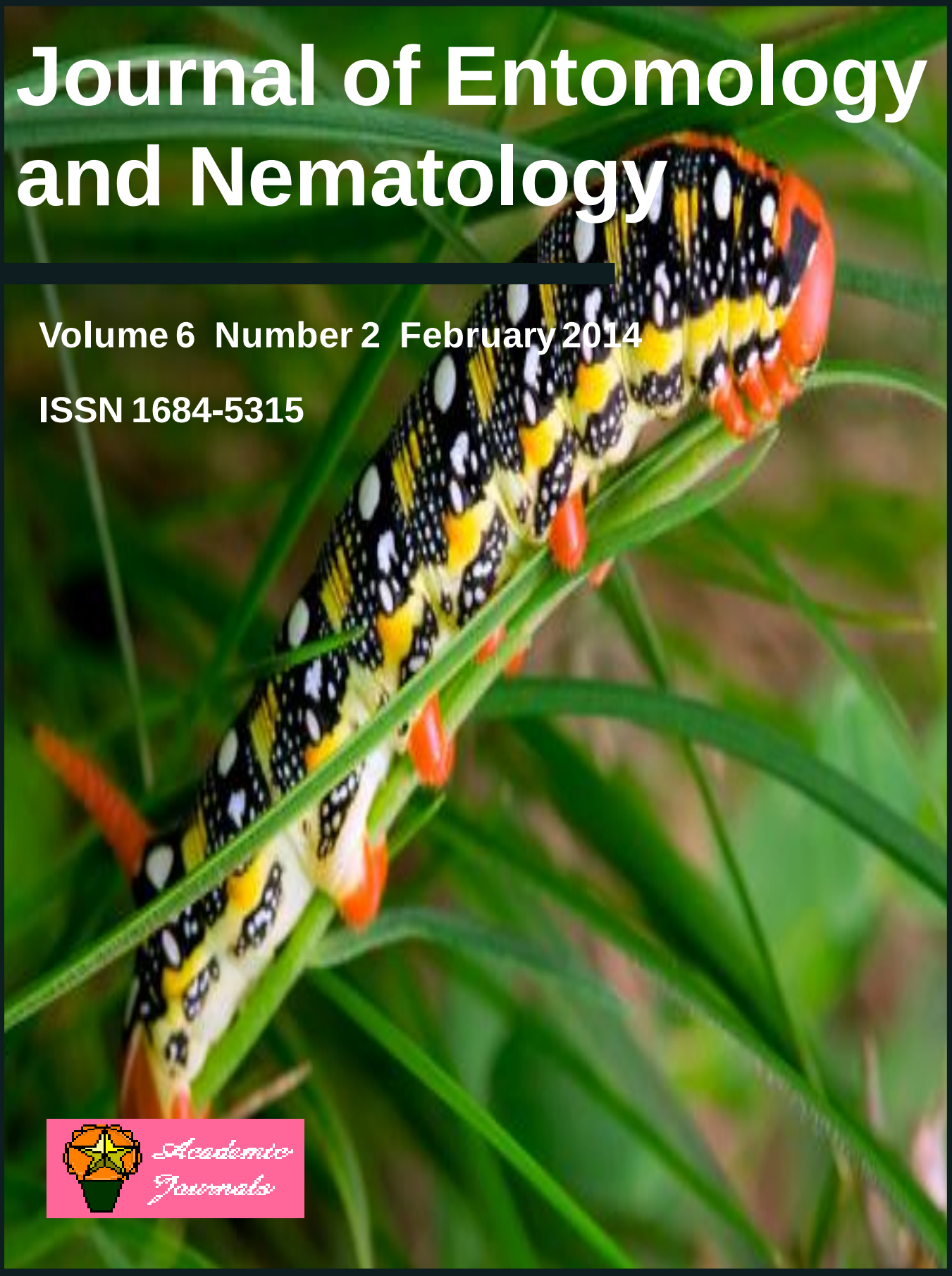

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Journal of Entomology and Nematology

Full Length Research Paper

Aleytrap: An instrumentation to handle adult whitefly, *Bemisia tabaci*, Gennadius

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Whiteflies are fast flying, minute insects in the suborder Homoptera of the Hemiptera; family Aleyrodidae. They are reported to transmit viral diseases in various, economically important agricultural crops. Based on their small size (average wing spans of about 3 mm), whiteflies are difficult to count on plants or capture through insect nets and other tools. Therefore, we developed a feasible and effective method to capture adults and estimate the population size using a new device named "aleytrap". The device took less time to count whiteflies and was found significantly superior over other conventional methods when used in tomato (*Lycopersicon esculentum* Mill.), chili (*Capsicum anuum* L.), brinjal (*Solanum melongena* L.), okra (*Abelmuscus esculentus* L. Moench), cotton (*Gossypium hirsutum* L.), black gram (*Vigna mungo* L., Hepper) and green gram (*Vigna radiata* L., Wilczek).

Key words: Aleytrap, sampling, whitefly, adults, leaf turn method, capture.

INTRODUCTION

Observations on an insect pest in scientific studies under natural conditions involve counting and catching of the individuals on their respective niches. This effort is greatly affected by the flight capacity and behavior as well as size of the concerned insect pest. Counting or catching can be done very easily on weak flying and large size insects as compared to minute and fast flying insects viz., whiteflies and hoppers. Whiteflies and its biotypes are polyphagous pests of great significance in agriculture worldwide (Kontsedalov et al., 2012). It belongs to the family Aleyrodidae from the suborder Homoptera of the order Hemiptera, having 1,556 extant species in 161 genera (Martin and Mound, 2007) and associated with 160 host plant species from 42 families of 113 plant genera of field and fruit crops, ornamentals and forest trees including weeds (Parveen et al., 2010). Hardly exceeding 1.0 mm in length, the adults are of snow-white color which is attributed to the secretion of wax on its body and wings. Adult as well as immature stages inhabit and feed on the lower surface of leaves reducing plant vigor by depletion of plant sap (Bethke et al., 1991). Foliage becomes contaminated with excreted honeydew on which black sooty mould grows thereby reducing the photosynthetic area and lowering the aesthetic appearance of ornamentals. Adults of a small number of species, most notably *Bemisia tabaci* (Gennadius), are important as vectors of many viral diseases than as direct pests and the severe infestation of such viral diseases may cause total yield loss (Gupta and Pathak, 2009). In order to overcome the whitefly menace, an excessive use of pesticides has been done (Roditakis et al., 2005) which led to the development of resistance (Prabhakar et al., 1992). This escalation of problems has prompted many researchers to become involved in management studies of whiteflies and the viruses they are capable of transmitting.

The small size of whiteflies and attraction towards yellow color, natural tendency of upward (Rangaraju

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Table 1. Literature on whitefly counting methods.

Reference	Host	Method of observation
Dharne and Kabre, (2009)	Chili	TMB (leaf turn method)
Zabel et al. (2001)	Tomato	NDM
Gencsoylu (2009)	Cotton	TMB (leaf turn method)
Ali et al. (2004)	Brinjal	TMB (leaf turn method)
Manzano et al. (2003)	Snap bean	Cotyledonary leaf (leaf turn method)
Akhtar et al. (2004)	Cotton	TMB (leaf turn method)
Sanchez-Pena et al. (2006)	Brinjal	NDM
Gupta and Pathak (2009)	Black Gram	NDM
Byrne (2010)	Poinsettia	Leaf turn method
Pasian et al. (2000)	Chrysanthemum and Gerbera	Entire plant counting
Castle et al. (2009)	Melon vine	Fifth terminal leaf (leaf turn method)
Lee et al. (2002)	Tomato	NDM
Sequeira and Naranjo (2008)	Cotton	Abaxial side of single leaf (leaf turn method)
Alicai (1999)	Sweet potato	NDM
Leite et al. (2003)	Brinjal	TMB (leaf turn method)
Nombela et al. (2001)	Tomato	Counting on all leaves of each plant
Mallah et al. (2001)	Cotton	TMB (leaf turn method)
Muniz et al. (2002)	Tomato and Pepper	TMB (leaf turn method)
Muqit et al. (2008)	Tomato	NDM
Rafiq et al. (2008)	Araceae, Asteraceae, Brassicaceae, Cucurbitaceae, Chinopodiaceae, Malvaceae, Meliaceae, Papilionaceae and Solanaceae	NDM
Leite et al. (2005)	Okra	TMB (leaf turn method)
Naranjo et al. (2003)	Cotton	NDM

*TMB- Top, middle and bottom, **NDM- No defined method.

and Chenulu 1980) and towards light orientation (positive photo-taxis) (Holmer et al., 1998; Ahmad et al., 2010) have however made the counting a hectic and trouble-some task. The whitefly adults are active fast fliers, gets away with a slight disturbance and that may be one of the reasons for not mentioning the capture and handling method of adult whiteflies in research papers (Gupta and Pathak, 2009) by the concerned authors. Rangaraju and Chenulu (1980) initiated the efforts to overcome this problem, describing an effective method to count adult whiteflies on crops under field conditions by covering the sample plant with a bell jar of a height according to the respective crops, however, the time consumption is too much in this method.

Apart from the bell jar and leaf turn method, the yellow sticky traps (Lloyd, 1921), muffin fan traps (Byrne et al., 1996) and CC-Trap consisting of transparent disposable cup (Chu and Henneberry, 1998) have also been developed but none of these can be used in counting per plant population in scientific experiments.

Some attempts made by different authors to count adult whiteflies by employing various methods on different host plants are listed in Table 1.

As the table shows, in most of the cases, counting adults was usually based on the leaf turn method involving random selection of a number of leaves (Zanic et al, 2008) or upper, middle and lower leaves (Shirale and Bidgire, 2009). Considering the fragility of whitefly, the leaf turn method could not be considered as an accurate method to count the per plant population of whitefly adults. Therefore, it was felt germane to develop such technology which could be most efficient, less time consuming and relatively more accurate one. In this endeavor, a new device (prototype) named “Aleytrap” after the family “Aleyrodidae”, has been formulated and described.

Structure of the device

Galvanized tin (2 mm thickness) and a transparent glass (10 mm thickness) were used as materials to fabricate the device. The first lower half of the device is of cube shape, facilitated with a small window (facilitated with a lid to close and open) to provide extra brightness during evening time and cloudy weather (open only when required) (Plate 1) otherwise it will interfere with orientation of whitefly adults towards the light. The remaining upper half (trapezium) with tapering walls holding a transparent glass was erected over the lower half square. It is divided into four equal squares, additionally facilitated with clothed sleeve in the company

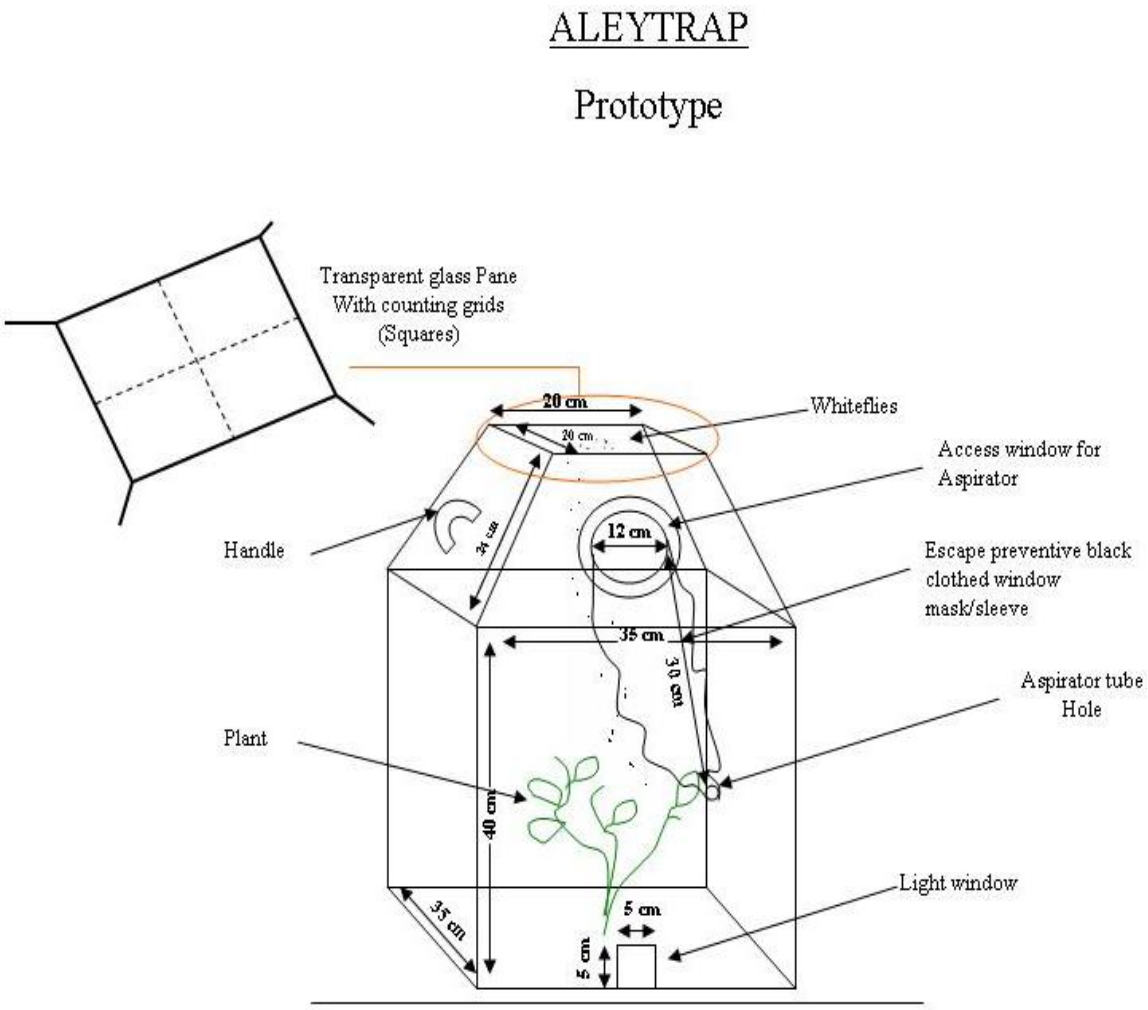

Plate 1. Sketch of Aleytrap.

of a small access hole at its lower end to capture the whitefly adults. Height and width of the device may vary depending upon the canopy of the host plant for population count.

To assure the visibility of adult whiteflies the device was painted in and outside with black color except for the transparent glass (Plate 2). For transportation of the device from the laboratory to the experimental site, a tin handle was provided opposite to the capture sleeve beneath the joint of lower square and upper trapezium. The overall manufacturing cost was estimated at 8-10 $ US, depending upon availability of material in the market and price fluctuation.

Working concept of the device

This device utilizes the phototaxis character (orientation towards light) of the aleyrodid adults (whitefly) (Holmer et al., 1998; Ahmad et al., 2010) to count and capture them. When the device was inverted over the target plant, the adult aleyrodids resting over the plants got oriented towards the source of light and accumulate in clusters underneath the glass pane and hence can easily and clearly be glanced by the device user. For the observation, the user will have to wait (approximately 30 s) for the settlement of all the adult aleyrodids. Sometimes, few adults may remain sitting at the lower internal portion of the device, in this situation, the user is advised to hit the lower portion of device by his/her finger which creates a noise and shake and ultimately will force the adults to settle underneath the glass pane.

The adult population of whiteflies (up to 15) can easily be counted simply by observing the top of the counting desk (glass pane), but in case, the population exceeds over said numbers, the counting desk can be divided into four equal parts and the number observed in that quarter desk can be multiplied by four thus providing a round estimate of whitefly adults present on the host plant. It can be utilized for tomato (*Lycopersicon esculenum* Mill.),

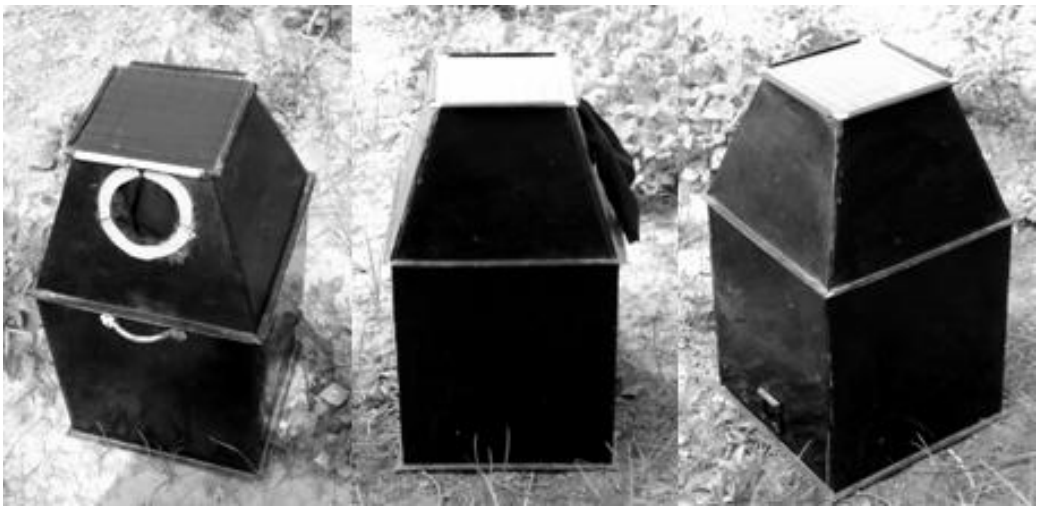

Plate 2. Aleytrap.

Chilli (*Capsicum anuum* L.), brinjal (*Solanum melongena* L.), okra (*Abelmuscus esculentus* L. Moench), cotton (*Gossypium hirsutum* L.,), black gram (*Vigna mungo* L., Hepper) and green gram (*Vigna radiata* L., Wilczek) and many other height-resembling plants.

All the adult insects waiting at the lower side of the counting desk can also be captured simply by inserting the aspirator tube through the hole of access window holding clothed sleeve at lateral side of trapezium. The size and capacity of the aspirator may vary in accordance with the users need.

MATERIALS AND METHODS

Performance of Aleytrap against whitefly on different host plants

To prove the efficacy of this device, seven host plants viz., tomato, chili, brinjal, okra, cotton, black gram and green gram were grown at the experimental fields of Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India. Under field conditions, all the hosts were found to be naturally infested with one or combination of whitefly species, that is, *B. tabaci* and *Trialeurodes vaporariorum* (Westw.). For comparative efficacy of traditional 'leaf turn counting' method and 'aleytrap' device, a control was standardized by using transparent polythene bags (1.0 x 1.0 m). The host plants were covered with these polybags, ensuring the capture of all the adults, anesthetizing them with alpha isomer of allethrin (obtainable from the market under the trade name of "HIT" (Godrej consumer products limited, Mumbai, India) followed by shaking the whole plant inside the poly-bag. This exact amount of aleyrodid adults bagged with poly-bags was taken as control and used to compare the population observed through manual/leaf turn and aleytrap method of counting. Counting of adult aleyrodids in all the methods was made on separate plants of separate plots for each of the tested host. A total of ten counting attempts through each method with ten replications were made on each host plant separately between 10.00-11.00 AM in 2008-09. In aleytrap counting, a population of more than 15 adult aleyrodids was counted by dividing the counting desk (glass pane) into four equal parts (square made with white color paint) and multiplying the population of a square by four. The time spared in counting the whitefly from each of the methods was also recorded using a stop watch and the comparative time consumption was also evaluated.

Statistical analysis

The mean data obtained in each of the counting attempt from the experiments was analyzed using Minitab version 10 and SIGMA PLOT version 10.0 for ANOVA (analysis of variance) and graphical presentation of the findings was made with the help of Microsoft Excel version 2007. The time consumption (in seconds) in counting the adults was also analyzed for analysis of variance (ANOVA). Tukey's HSD test was used to compare the mean of observations of different experiments.

RESULTS

A close parallel relation (*df*-9, 99 and $p<0.05$) between poly-bag capture and aleytrap counting is clearly inferred from the findings on all the host plants whereas leaf turn method of adult counting on all the host plant was found to fall under irregular pattern and show less number of whitefly adults (*df*-9, 99 and $p<0.05$) in most of the attempts (Figure 1). Only two attempts (sixth and ninth) on tomato, the population count through leaf turn method was observed non-significantly at par (*F*-1.61, *p*-0.25, *df*-9, 99 and f-1.39, p-0.30, df-9, 99) with poly-bag and aleytrap counting method. Similar fashion adult population (*df*-9, 99 and $p<0.05$) counted through poly-bag and aleytrap was recorded on chili whereas fifth attempt of leaf turn method of counting on chili exhibited a significant superiority (*F*-5.06, *p*-0.038 and *df*-9, 99) over poly-bag and aleytrap counting attempts. No significant difference was observed among all the counting methods on the eighth (*F*-2.30, p-0.163, df-9, 99) and ninth (*F*-3.06, p-0.103 and df-9, 99) attempt of

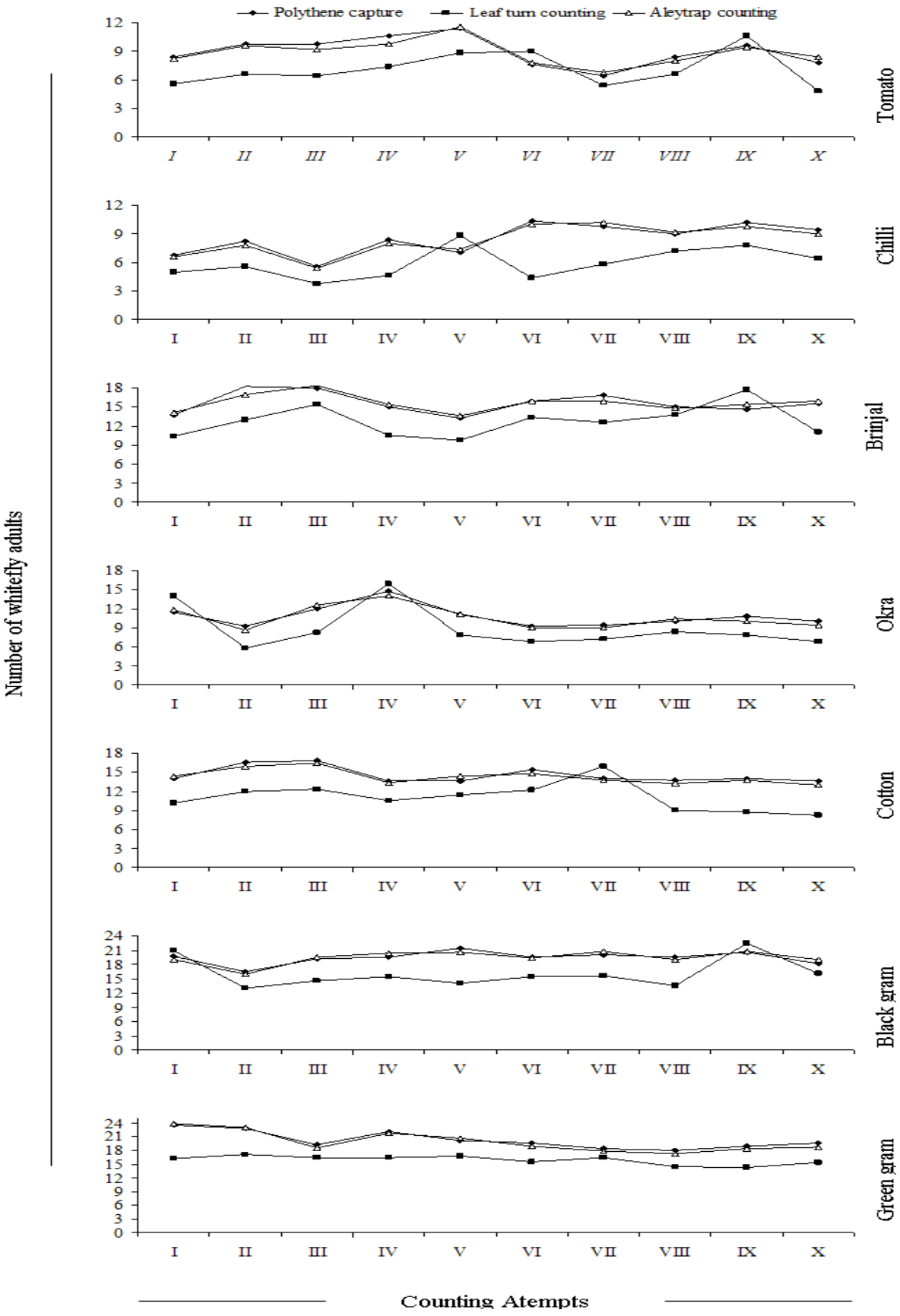

Figure 1. Comparative performance of leaf turn and aleytrap counting method on different host plants.

Table 2. Performance of leaf turn and aleytrap counting technique with respect of time consumption (in seconds) on tomato, chili, brinjal, okra, cotton, black gram and green gram.

Host	Whitefly adult	Time consumption	
		Leaf turn method	Aleytrap counting
Tomato	10.375±2.56[b]	171.37±6.52[a]	35.50±3.89[a]
Chilli	7.00±2.00[a]	171.00±4.92[a]	35.00±5.68[a]
Brinjal	16.87±3.13[d]	276.75±6.18[c]	73.37±5.50[b]
Okra	10.37±2.20[b]	224.75±3.84[b]	81.12±4.91[c]
Cotton	13.00±1.85[c]	272.62±7.76[c]	90.62±5.23[d]
Black gram	17.00±2.45[d]	326.12±6.96[d]	102.00±4.27[e]
Green gram	20.12±2.03[e]	326.50±7.11[d]	102.12±5.59[e]
f-value	35.24	819.52	256.56
p-value	0.00	0.00	0.00

*Means followed by the same letters (within a column) showt non-significant difference.

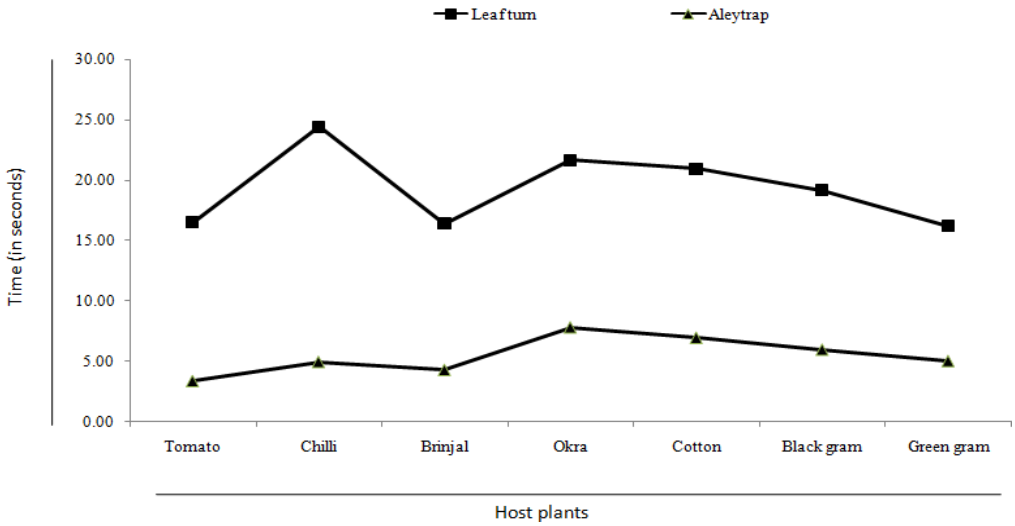

Figure 2. Time consumption throuh leaf turn and aleytrap method on single whitefly adult.

counting through leaf turn method in the case of brinjal, fourth attempt on okra (*F*- 0.75, *p*- 0.502 and *df*- 9, 99), seventh attempt on cotton (*F*- 1.46, *p*- 0.288 and *df*- 9, 99), first and ninth attempt on black gram (*f*-1.09, *p*- 0.382, *df*- 9, 99 and *f*- 0.76, *p*- 0.497, *df*- 9, 99) whereas in these attempts the leaf turn method showed a non-significant superiority over poly-bag and aleytrap counting (Figure 1). The proximity in number of whitefly adults counted through poly-bag and aleytrap counting methods and superiority of aleytrap counting over leaf turn method clearly indicate the effectiveness of aleytrap.

Time consumption

It is inferred from the present findings that aleytrap did consume significant less (*F*-256.56, p-0.00 and df-7, 42) time to count the adults whiteflies than leaf turn method (Table 2). Approximately, a similar duration of time (35.50±3.89 and 35.00±5.68 seconds) was spent to count the whitefly adults through aleytrap on tomato (10.37±2.56 adults/plant) and chili (7.00±2.00 adults) while leaf turn method has utilized relatively much more time on tomato (171.37±6.52 s) and chili (171.00±4.92 seconds) (Table 2). In the case of black gram and green gram, aleytrap has utilized a greater but almost similar time (102.00±4.27 and 102.12±5.59 seconds) to count the adults (17.00±2.45 and 20.12±2.03 adults/plant). When time consumption for a single whitefly adult was analyzed from Table 2, the lowest duration was observed on tomato followed by brinjal and highest on okra (Figure 2).

Justification

The whitefly population prefers lower surface of leave for their rest and feed. Under natural condition, whitefly population is always found to vary from plant to plant thus difficult to count without disturbing the plant. Adults are very agile and sensitive to leave the resting place with a slight disturbance. In the present investigation, poly-bag counting method was considered to compare the efficiency of aleytrap and leaf turn method of whitefly adult counting. The population count on plant basis was found to vary with each other and the performance of poly-bag and aleytrap count was recorded more or less statistically on par as comparison to leaf turn method. The significant variation with respect to aleytrap and poly-bag count, which was recorded rarely, may be attributed to the variation in population of adults on the tagged plants along with the spatial distribution and or migration and immigration from nearby plants. Employing leaf turn method in chili proved to be difficult for adult count on account of small size of leaves. Aleytrap and poly-bag method were found to be convenient and more feasible. Aleytrap nevertheless showed the best performance.

Gusmao et al. (2005) opined that the beating method was significantly superior over the leaf turn method for outdoor tomato crops, but the method is not as cost effective as the device is and in case where one has to assess the residual persistence through bioassay method, it would be inappropriate to beat the leaves and kill the adult whiteflies.

The poly-bag capture was found to be most effective in counting the adult aleyrodid, but to observe the local dynamics and population fluctuation, it cannot be applied as it kills the natural population, indirectly disturbing the natural presence of aleyrodids. Yellow sticky trap (Chu and Henneberry, 1998) and muffin fan trap (Byrne et al., 1996) are also in use but they help only in providing the information on natural occurrence of aleyrodid adults in a particular cultivated area, besides yellow sticky traps which also capture other insects having fondness of yellow color (Chu and Henneberry, 1998). Chu and Henneberry (1998) has developed a new trap (CC Trap) consisting of transparent disposable cup and proved its superiority over yellow sticky trap but these traps cannot count the per plant population of whitefly adults on their respective host plants. Here this device can be considered superior over yellow, muffin fan and CC traps by getting quick information on population count; however it cannot be used to predict the natural occurrence of whitefly as has been observed through yellow sticky, muffin fan and CC traps. The other advantage with this new device is that the sex ratio can be determined by collecting adult through aspirator.

The device is effective in counting adult whiteflies leading to accurate ecological, bio-assay experiments and other studies evolving capture of whitefly adults. On the other hand, it can only be used for low height crops like tomato,chilli, brinjal, black gram and green gram etc. The crops having the height more than one meter *viz*., papaya, pigeon pea and mature cotton cannot be assessed for counting of whitefly adult population using aleytrap. Sometimes, the insects of other groups, having bigger size also get trapped in the device and disturb the cluster settlement of adult whiteflies under the glass pane but they can be removed using clothed sleeve.

In the present findings, the device has been used solely against the adults of the family *Aleyrodidae* family, but possibly it can also be used against other small and fast flying insects having the phototactic character and fondness towards yellow color.

ACKNOWLEDGEMENTS

The authors are very grateful to the Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, India.

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Full Length Research Paper

Effect of different concentrations of *Eriobotrya japonica* extract on control of infection by *Meloidogyne incognita* and *Cephalobus litoralis*

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This study discusses and developed methods for obtaining plant extracts/pure compounds and its usages as a nematicidal agent. Freshly hatched second-stage juveniles of two nematode species, *Meloidogyne incognita* and *Cephalobus litoralis* were used. A bioassay guided isolation of the extract, fractions and pure compounds were done for their nematicidal activity at different concentrations in comparison with *Azadirachta indica,* while distilled water was taken as control. The crude extract showed 90% and ethyl acetate fraction 97% mortality rate after 48 h at 1% concentration against *M. incognita* species and 81 and 50% against *C. litoralis* specie at the same concentration. Among the pure compounds, 4 and 9 showed maximum mortality of 90 and 91% and compounds 8, 3, 6, 2, 5, 7 and 1 showed 89, 88, 88, 82, 80, 80 and 69% mortality, respectively after 48 h in *M. incognita* sp. In *C. litoralis*, compounds 8 and 9 showed 72 and 75%, significant mortality, while 7, 4, 3, 5, 6, 2 and 1 showed 70, 70, 70, 68, 62, 60 and 58% mortality, respectively after 48 h. The plant is of economic importance with nematicidal value.

Key words: *Meloidogyne incognita*, *Cephalobus litoralis*, compound, mortality, crude extract.

INTRODUCTION

Eriobotrya japonica is been used to treat several diseases in East Asia. The leaves of *E. japonica* is widely used in traditional medicine for the treatment of many diseases including cough and asthma. It protects against oxidative stress and cognitive deficits induced by the Aβ peptide. *E. japonica* improves hyperlipidemia and reverses insulin resistance in high-fat-fed mice (Shih, 2010). Agricultural countries study the agricultural productivity which is appropriately protected from pests and diseases caused by insects, nematodes, fungi, viruses and bacteria; (Nasira and Shanina, 2007). Among these, nematodes have been considered universally, as one of the important microscopic organism which play significant role in the agriculture production in different diseases (Alam et al., 1979; Sultana et al., 2010a, b). In the form of plant parasitic nematodes, sometimes, it play very destructive role and causes loss of billions globally (Shurtleff and Averre, 2000).

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Some of the important nematodes species cause severe damage to the economically important crops e.g. *Heterodera avenae, Rotylenchulus reniformis, Pratylenchus* spp., *Hoplolamus* spp., *Xiphinema* spp., *Trichodorus* spp. and root-knot nematodes (Pathan et al., 2008). These nematodes attack almost all parts of plant, including roots, stem, leaves and seeds or fruits and as such damage all variety of crops; some of them are responsible for transmission of soil born viruses which produce deadly diseases in many plants (Shahid et al., 2007).

The realization has prompted increased studies all over the world on nematodes parasites to plants and their control (Amponsah, 2008). Small-scale farmers have limited access to the commercially available nematicidal and pesticidal services owing either to their unavailability or to their high cost. According to different researchers, the plant possesses not only beneficial characteristics but also pesticidal and insecticidal properties (Chitwood, 2003; Javed and Zaki, 2003; Javed et al., 2007). Many modern drugs are derived from plant but there are also an increasing number of herbal products commercially available (Javed et al., 2007, 2008).

MATERIALS AND METHODS

Preparation of plant extracts

The whole plant of *E. japonica* (25 kg) was collected from Swat valley in February. A voucher specimen (KUH # 139(678) was deposited in the Herbarium of Department of Botany, University of Karachi.

Extraction

The whole plant of *E. japonica* (25 kg) was dried in a dryer for three days at 50°C, ground, sieved and socked in 50 L ethanol for one week. The ethanolic extract was concentrated to a gummy material weighed to about 520 g.

Fractions

Crude ethanolic extract was further fractionated into hexane, chloroform, ethyl acetate and methanol.

Preparation of nematode *Cephalobus litoralis* culture

Culture of *C. litoralis* which reproduces parthogenetically was prepared using a single egg. Green peas (*Pisum sativum*) were mashed in small Petri dishes. A single egg was carefully picked under stereoscopic binocular and placed beside pea meal paste (PMP) in a Petri dish.

Nematode eggs hatched within 72 h and after 10 days, large number of nematodes in various stages of life cycle were obtained.

Preparation of nematode root-knot culture

Experiments were performed under laboratory conditions at 28± 2°C. Fresh egg masses collected from stock culture maintained on tomato root tissues were kept in water for egg hatching. The larvae emerged after 48 h from the egg masses incubated at 30°C and were used at test species for larval mortality studies. To determine the nematicidal effect of the various fractions and the pure compounds, freshly hatched second-stage juveniles were taken in tap water. The movements of the nematodes were checked by touching them with the needle.

Preparation of substrate for bioassay

Glass tubes, 15 cm long having a diameter of 8 cm were taken for bioassay. 2, 1 and 0.5 % solution of plant extracts and compounds were prepared in ethanol from stock solution. This solution was passed through Whatman filter paper No. 1 and 3 ml of it was taken in each tube. Four tubes were taken for each treatment whereas another four served as control set.

Inoculation

Nematodes larvae were isolated through modified Baermann funnel technique using Whatman filter paper No. 41 and larvae were counted in a dish with 0.5 cm square at the outer surface to determine their concentration. The required amount of nematode suspension was poured into the tubes each of which contain equal amount of plant extract, fractions and pure compounds 2, 1 and 0.5% had already been added. In other four tubes, distilled water with nematode larvae was taken as control. The experiment was run on benches under room temperature.

Experimental work

Column chromatography was carried out using silica gel of 70-230 mesh and flash chromatography on silica gel 230-400 mesh. Aluminium sheets precoated with silica gel 60 F_{254} (20 x 20 cm, 0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by ceric sulfate as spraying reagent. Optical rotations were measured on a Jasco DIP-360 digital polarimeter. The UV spectra were recorded on a Hitachi UV-3200 spectrometer (λ_{max} in nm). IR spectra were recorded on Shimadzu IR-460 spectrophotometer (v in cm^{-1}). ElMS, HREIMS, FABMS and HRFABMS spectra were recorded on Jeol JMS-HX 110 spectrometer with data system. The ^{1}H NMR spectra were recorded on Bruker AMX-400 MHz instruments using TMS as an internal reference. The chemical shift values are reported in ppm (δ) units and the scalar coupling constants *(J)* are in Hz.

RESULTS AND DISCUSSION

A bioassay guided isolation of the alcoholic extract, hexane, ethyl acetate, chloroform, methanol fractions and pure compounds were done for their nematicidal activity at 0.25, 0.5 and 1% concentrations, respectively, in comparison with *Azadirachta indica.* Structures of pure compounds (1-9) were earlier reported by chemical and spectroscopic methods including one dimensional (^{1}H-NMR, ^{13}C-NMR broad band and DEPT) and two dimensional (COSY-45. NOESY, *J-resolved*, hetero COSY) NMR techniques (Sohail et al., 2008; Kang et al., 2008) Figure 1. The nematicidal activity of the crude ethanolic extract, its fractions (hexane, ethyl acetate, chloroform, methanol) as well as pure compounds (1-9)

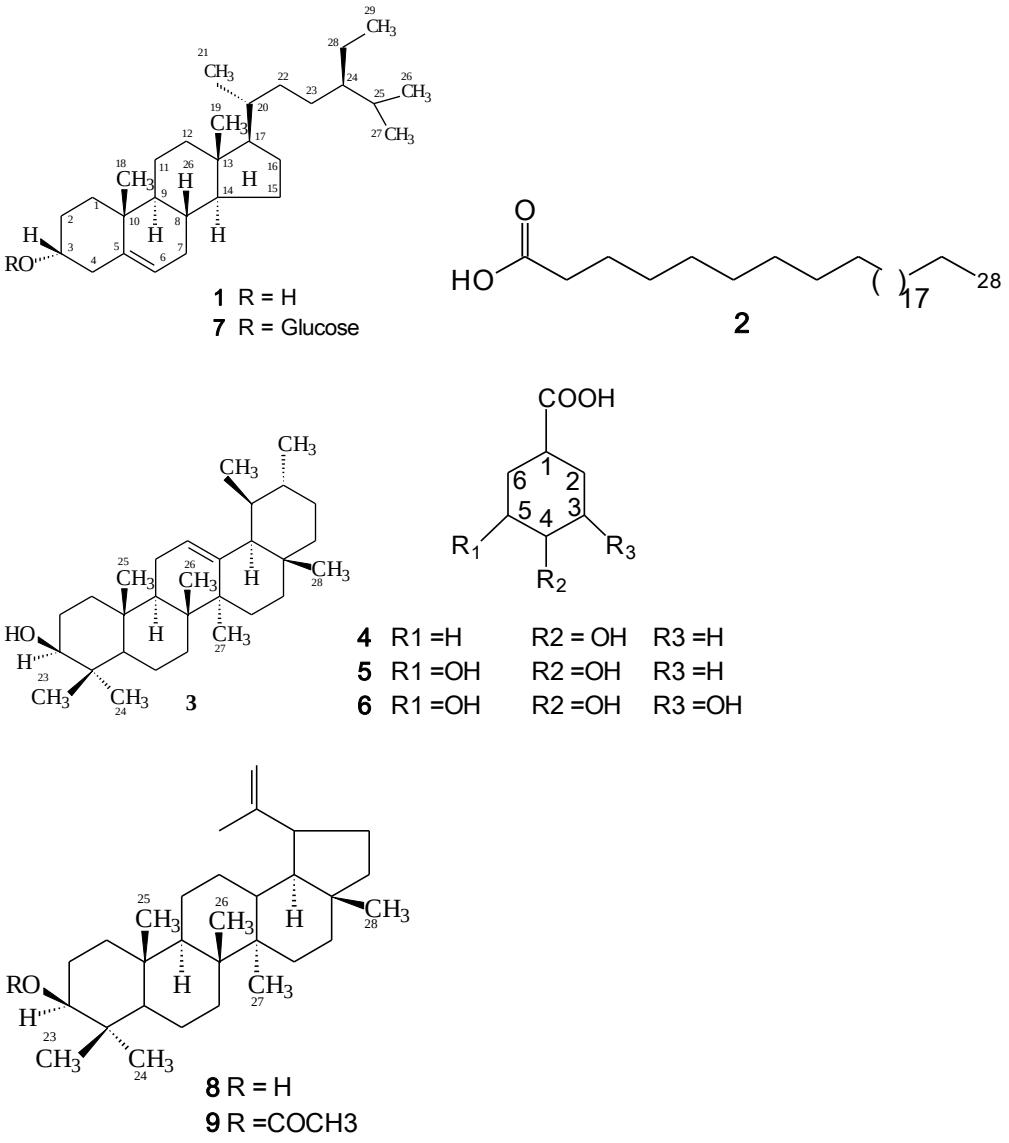

Figure 1. Structures of pure compounds (1 - 9) isolated from *E. japonica.*

were tested against *M. incognita* and *C. litoralis* (Noweer and Hasabo, 2005).

The nematicidal action of *E. japonica* extract, fractions and compounds in *in vitro* investigation against second stage juveniles of both species is shown in Tables 1 to 4. The 1% of crude extract showed 78% mortality and fractions of hexane 18%, ethyl acetate 69%, chloroform 35% and methanol 15% after 24 h, while after 48 h, crude extract showed 90%, fraction of hexane 19%, ethyl acetate 97%, chloroform 45%, methanol 25% at same concentration against *M. incognita* species. Nematicidal activity showed 1, 0.5, 0.25% concentration and control as shown in Table 1.

The pure compounds 1, 2, 3, 4, 5, 6, 7, 8 and 9 showed 57, 73, 71, 81, 70, 75, 72, 74, 76% mortality, respectively after 24 h, while after 48 h, compounds showed 69, 82, 88, 90, 80, 88, 80, 89 and 91% mortality, respectively. Nematicidal activity on 1, 0.5, 0.25% concentration and control is given in Table 2.

The 1% of crude extract, hexane fraction, chloroform fraction, ethyl acetate and methanol soluble fraction showed 77, 30, 29, 50 and 40% mortality against *C. litoralis* respectively after 24 h and 81, 35, 33, 50 and 48% mortality after 48 h, respectively. Nematicidal activity of other concentrations is given in Table 3.

The pure compounds (1-9) were isolated from *E. japonica* and tested for their nematicidal activity on *C. litoralis* larvae. The results of *in vitro* evaluation are shown in Table 4. Compound 9 showed 61%, 8- 54%, 7- 62%, 6- 55%, 5- 58%, 4- 66%, 3- 65%, 2- 50% and 1- 45% mortality after 24 h in 1%concentration while after 48 h, compounds showed 75, 72, 70, 62, 68, 70, 70, 60,

Table 1. The larval mortality of root-knot *M. incognita*.

Fraction	Concentration after 24 h				Concentration after 48 h			
	Percent Mortality				Percent Mortality			
	1%	0.5%	0.25%	Control	1%	0.5%	0.25%	Control
Hexane	18	15	10	1	19	16	10	3
Chloroform	35	29	22	1	45	30	27	4
Ethyl acetate	69	48	30	2	97	72	45	5
Methanol	15	12	11	2	25	20	15	3
Crude	78	50	32	3	90	68	50	5

Table 2. The larval mortality of *M. incognita* (root-knot) nematodes.

Compound	Concentration after 24 h				Concentration after 48 h			
	Percent mortality				Percent mortality			
	1%	0.5%	0.25%	Control	1%	0.5%	0.25%	Control
β-sitosterol	57	44	42	2	69	64	45	3
Octacosanoic acid	73	52	30	1	82	57	62	3
Ursolic acid	71	64	50	4	88	70	68	5
4-hydroxybenzoic acid	81	68	57	2	90	73	62	5
3,4-dihydroxybenzoic acid	70	62	48	1	80	77	59	2
Gallic acid	75	66	50	2	88	71	58	4
β-sitosterol' *3-0-,β-D* glucopyranoside	72	55	47	4	80	73	50	5
Lupeol	89	41	36	2	74	70	57	3
Lupeol acetate	91	61	55	4	76	71	53	2

Table 3. The larval mortality of *Cephalobus litoralis* nematodes.

Fraction	Concentration after 24 h				Concentration after 48 h			
	Percent mortality				Percent mortality			
	1%	0.5%	0.25%	Control	1%	0.5%	0.25%	Control
Hexane	30	17	11	1	33	20	18	2
Chloroform	35	10	7	2	29	18	15	3
Ethyl acetate	55	22	14	2	50	30	22	3
Methanol	49	30	22	3	48	33	20	5
Crude	81	50	28	4	77	56	37	5

68 and 58% mortality in the same concentration. Nematicidal activity of other concentrations is given in Table 4.

The plant is of economic importance with nematicidal value. Phytochemicals are used in many drugs, insecticides, pesticides especially for plant diseases. The plants which have these proportions can be used in the manufacture of nematicide (Javed et al., 2006).

It is evident from the above discussion that there is a great likelihood of use of bio-control agents for disease control by nematodes (Akhtar et al., 1991; Javed et al., 2007). Although several potential bio-control agents have been isolated and tested for their efficacy against soil born root pathogens, there is need to discover new potential antagonists or improve strains of already isolated antagonists for better crop production. Possible environmental hazards due to the use of microorganisms as bio-control agents should also be looked into (Jiskani et al., 2005). Development of a simple, cheap and effective method for mass production of bio-control agents is a pre-requisite for the replacement of chemical fungicides by a bio-control agent which also needs

Table 4. The larval mortality of *Cephalobus litoralis* nematodes.

Compound	Concentration after 24 h				Concentration after 48 h			
	Percent mortality				Percent mortality			
	1%	0.5%	0.25%	Control	1%	0.5%	0.25%	Control
β-sitosterol	45	38	25	1	58	43	30	2
Octacosanoic acid	50	30	16	2	60	38	20	4
Ursolic acid	65	45	34	1	70	50	38	2
4-hydroxybenzoic acid	66	53	20	1	70	58	30	2
3,4-dihydroxybenzoic acid	58	34	24	0	68	40	32	1
Gallic acid	55	40	27	1	62	50	34	3
β -sitosterol' *3-0-,β-D* glucopyranoside	62	53	28	0	70	58	37	2
Lupeol	72	19	16	0	54	20	16	1
Lupeol acetate	75	49	37	1	61	56	49	2

investigation (Akhtar et al., 1991; Shakeel et al., 2010).

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Full Length Research Paper

Description of a new species of insect parasitic nematode, *Leidynema* (Schwenck, in Travassos 1929) (Thelastomatidae) from host *Periplaneta americana* of Meerut region - India

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Three different types of insect parasitic nematodes namely - *Leidynema, Thelastoma* and *Hammerschmidtiella,* were collected from hind gut of host *Periplaneta americana*. The new species - *Leidynema meerutensis* sp. nov., is characterized with its females having a typical corpus broader at posterior and an enlarged blind intestinal diverticulum, females with unequally disposed lateral alae with posteriorly directed terminal spine, much enlarged excretory sac/bulb and a short attenuated tail. The male *Leidynema meerutensis* sp. nov. have four pairs of caudal papillae and a truncated tail with a short spine projection.

Key words: New species - *Leidynema meerutensis*, Thelastomatidae, *Periplaneta americana*, unequal lateral alae, Blind Gut diverticulum, four pairs of caudal papillae in males, truncated tail, Meerut region -India.

INTRODUCTION

The animal parasitic nematodes belonging to Order - Oxyurida, constitute two super-families - Oxyuroidea (nematode parasites of vertebrates) and Thelastomatoidea (the nematode parasites of invertebrates essentially arthropods) of order oxyurida. The thelastomatides are essentially the parasites thriving in invertebrate hosts, mostly the arthropods. They are parasitic (or commensal) in the gut of most saprophytic insect and other arthropod hosts. They feed upon host's gut contents like its micro-flora and body fluid (Jex et al., 2005). The super-family Thelastomatidae has been organized into five different families (Adamson and Van Waerebeke, 1992) - Thelastomatidae, Protrelloididae, Hystrignathidae, Travassosinematidae and Pseudonymidae. Family Thelastomatidae is the largest family and have more than 35 genera.

Genus, *Leidynema* was first described by Schwenck (in Travassos, 1929), with *L. appendiculatum* Leidy, 1850 as its type species. At present, eight different species of *Leidynema* have already been described from different regions of world; namely: *L. appendiculatum* Leidy, 1850 (Chitwood, 1932); *L. delatorrei* Chitwood, 1932; *L. periplaneti* Farooqui, 1967; *L. portentosae* Van Waerebeke, 1978; *L. Schwenckei* Farooqui, 1967; *L. socialis* Leidy, 1850 (Adamson et Van Waerebeke,1992); *L. orientalis* Singh and Malti, 2004; and *L. saltense* Achinelly and Camino, 2008. *L.*

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stylopygi (Biswas and Chakravarty, 1963) has been synonymised to *L. appendiculatum* (Kloss, 1966; Farooqui, 1967). *Leidynema meerutensis* sp. nov. is a new species of *Leidynema* from household cockroaches (*Periplaneta americana*) from Meerut region of India (Plate 1-4).

MATERIALS AND METHODS

Different household insects including house hold cockroaches (*P. americana*) were collected from different regions of district Meerut. The collected host insects were anaesthetized and dissected and nematodes were recovered from their hindgut. The collected nematodes were heat killed and fixed in Triethanolamine formalin (TAF) fixative. Nematodes were then dehydrated by slow dehydration method in alcohol glycerin mixture. The fixed nematodes were put into alcohol-glycerin mixture and kept in glass dessicator with anhydrous $CaCl_2$ for 3-5 days to allow slow dehydration. The dehydrated nematodes were mounted on glass slides in anhydrous glycerin with glass wool and sealed. Outline structures were drawn with the help of camera Lucida and photographs were taken using Motic image 2000 DMB 1 microscope. Morphometric measurements were recorded using stage and ocular micrometers. Comparative studies of morphology and morphometric measurements of different specimens of collected of nematode parasites were done using De Mann's formulae and parameters (De Man, 1884). The measurements and parameters are expressed in mean, range and standard deviation from the mean.

For scanning electron microscopy - the heat killed nematodes were transferred in primary fixative - Karnovasky's fixative for overnight and then transferred in 2.5% gluteraldehyde. The fixed nematodes were washed with 0.1 M phosphate buffer (pH - 7.2) at 4°C and gradually dehydrated in different grades of acetone. The dehydrated specimens were dried by critical point drying method using liquid CO_2. Dried specimens were mounted on aluminum stubs and gold coated. Scanning photography was done using LEO435 VP scanning electron microscope and measurements were recorded with LEO-32 annotation programme Tables 1 and 2.

Generic diagnosis

Female

Mouth surrounded with eight large sub-median labiopapillae and a pair of small amphids. Cephalic extremity formed by two annules. Oesophageal corpus divided into anterior narrow and relatively broader posterior portions of nearly equal length, a short distinct isthmus and a valvular spherical end bulb. Intestine have a large blind intestinal diverticulum called cardium. Lateral alae are present and terminate into terminal spine. Excretory pore is present at the posterior to base of oesophagus. Vulva is present at or near mid-body. Vagina anteriorly directed and opens into a common uterus. Ovaries are two in number and directed opposite - didelphic and amphidelphic. Eggs are large, elongate, ellipsoidal, crescent shaped and triangular in cross-section. Tail attenuated to long filliform.

Male

Cephalic extremity is formed by a single expanded annulus. Lateral alae may be present or absent. Oesophagus is simple and without any posterior swellings. Intestine is simple and without diverticulum. Caudal extremity abruptly truncated, with or without short terminal spine (spine like process on its ventral side) or provided with several protrubences. Caudal papillae 3-5 pairs, consisting of one pair of large sub-ventral pre-anal, one to three pairs tiny sub-ventral and one pair sub-lateral post-anal papillae. Spicule is present.

Description

Female

Body cylindrical, tapering at both ends, 2.5 - 3.15 mm in length and with maximum body width of 0.28 - 0.35 mm; head with 2nd annulus much wider and with close-set of 5-6 annuli and then annuli expand abruptly in both, its length and width. First annule with 8 pairs of labial papillae have surrounding mouth. Cuticle is closely annulated throughout the body length. Lateral alae are prominent and each alae terminate into a spine-like projection at the posterior. These lateral alae and its spine-like projections are equally disposed in all species of *Leidynema* described so far (namely: *Leidynema appendiculatum;* however, in the present species - *L. meerutensis* sp. nov., it is much unequally disposed). Oesophagus is 0.37- 0.45 mm long (1/7th) occupying anterior 14-15% of the body length and consists of a 0.29 - 0.31 mm long corpus, 0.020-0.025 mm long distinct isthmus and an end bulb of 0.11 × 0.10 mm dimension. Corpus is demarcated into two distinct regions, the anterior half is narrow and its posterior part is cylindrical and broader. Intestine prominently enlarged at the anterior end and is provided with a posteriorly directed much enlarged characteristic oblong, blind intestinal diverticulum. Nerve ring is at the anterior 1/20th of the body and is located at 0.135 - 0.150 mm from anterior end (NR% - 5.04%). Excretory pore at 0.55 - 0.65 mm from the anterior end occupying anterior 1/5th of the body length (Ex% - 20.74%). The excretory bulb/sac is much enlarged and of the size approximately similar to the oesophageal end-bulb. Ovaries are paired and two in number and are divergent to each other - didelphic, amphidelphic. Vulva is transverse in orientation and slightly anterior to middle of the body at 1.27 - 1.56 mm from anterior end (V% = 48%). Vagina is sclerotized, muscular and anteriorly directed opening into a common uterus. Eggs are elliptical in shape with dimension of 0.125 × 0.050 mm and are laid singly. Tail attenuated and relatively much shorter comprising 1/6th of body length. A pair of phasmid is visible at the anterior of the tail (visible in SEM photomicrograph).

Male

Small body with length 0.80 - 1.15 mm and width 0.06-0.09 mm, curved at the posterior end upon fixation. Cuticle is annulated throughout the body length. Lateral alae present and continue to the tail. Oesophagus 0.18 - 0.20 mm long occupying anterior 1/5th of the body and having a corpus of uniform diameter with length 0.11 - 0.13 mm, a short isthmus 0.02 mm and an end-bulb with dimension 0.03 - 0.04 mm × 0.03 - 0.04 mm. Nerve ring situated at anterior 1/10th at 0.08 - 0.10 mm from the anterior end (NR - 9.53%) and excretory pore occupy anterior 1/3rd of body at 0.27 - 0.35 mm from anterior end (Ex% - 33.12%). Testis is single and reflexed at the tip. Spicule is prominent with its length of 0.033 mm. Caudal extremity in male is abruptly truncated with a short terminal spine like structure, tail 0.015 - 0.017 mm in length from the anus. Caudal papillae four (four pairs) pairs in number and are symmetrically disposed - one pair sub-ventral pre-anal, two pairs ventral post-anal and one pair small sub-dorsal post-anal papillae.

Body dimensions (Mean ± SD in mm)

Holotype female

Total Length = 2.950; Width = 0.310; a (L/W) = 9.516; Oesophagus = 0.430; b (L/E) = 6.860; Tail = 0.480; c (L/T) = 6.146; Nerve ring = 0.150; NR% = 5.08%; Excretory pore = 0.650; Ex% = 22.03%; Vulva = 1.400; V% = 47.46%; Egg = 0.125 mm x 0.040 mm.

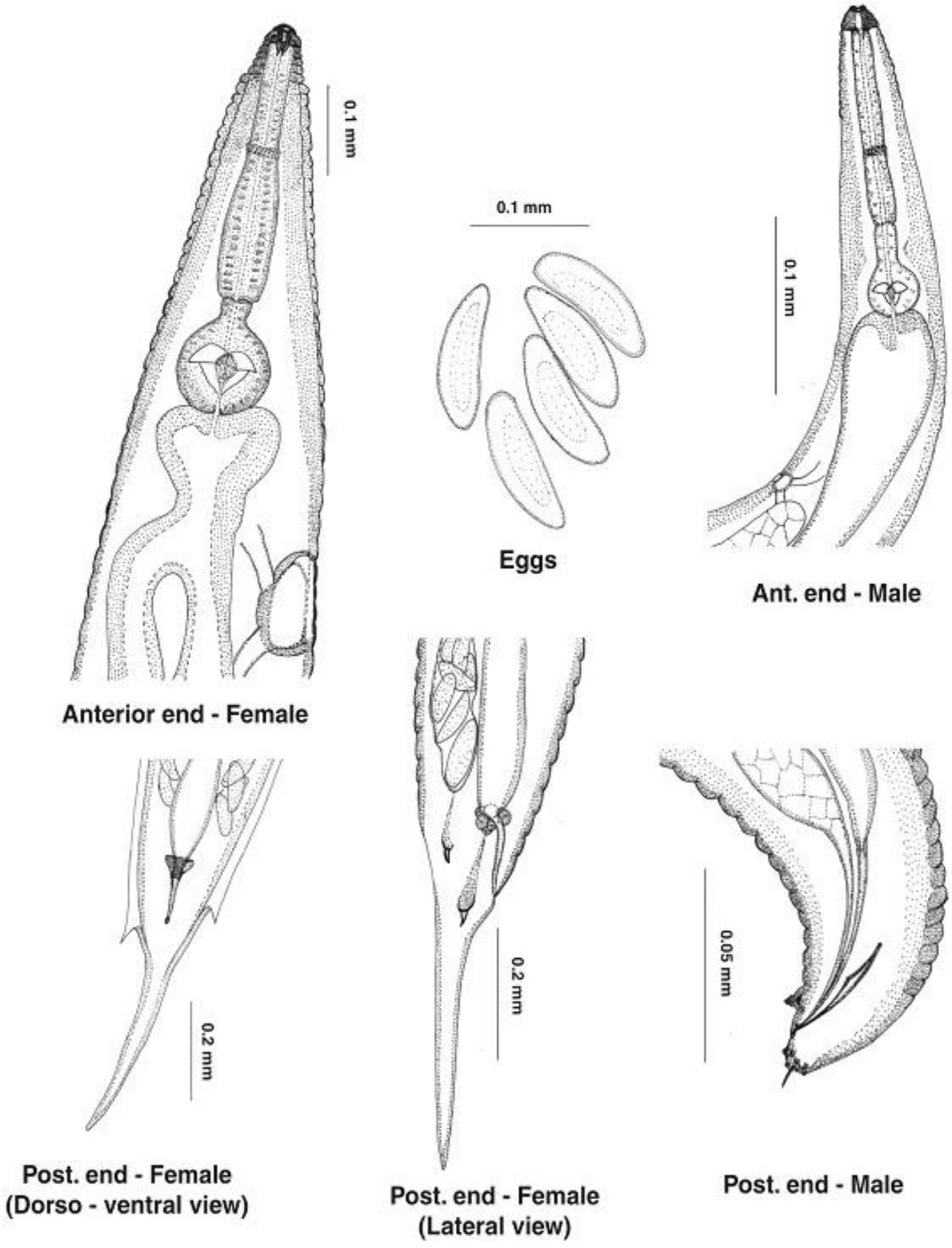
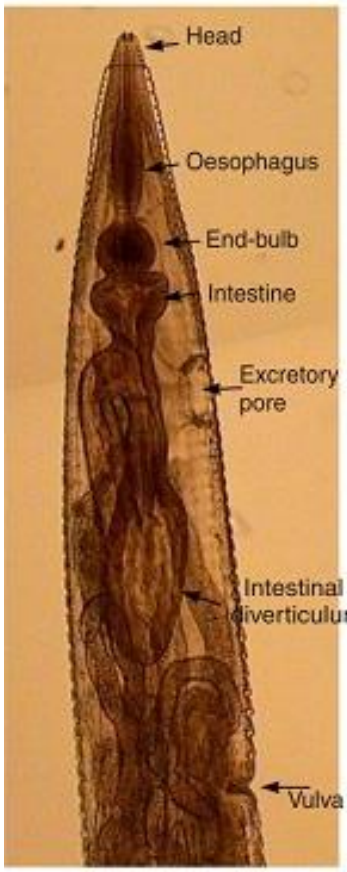
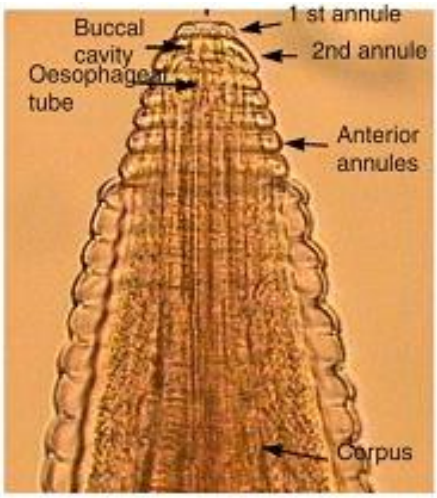


Plate 1. Morphological details of *Leidynema meerutensis* sp.

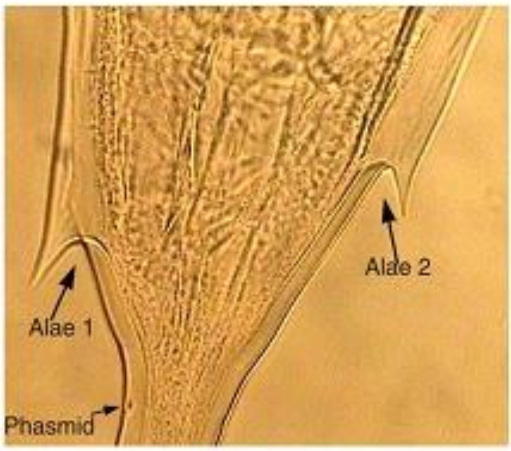


Ant. end - Female

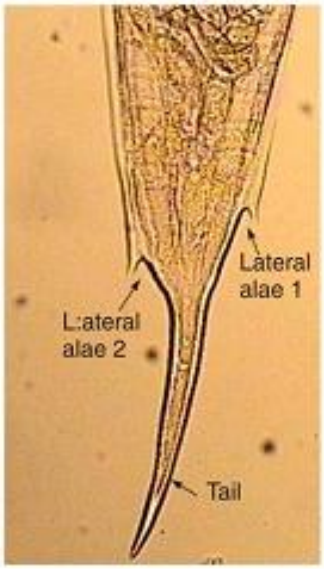
Eggs



Head - Female

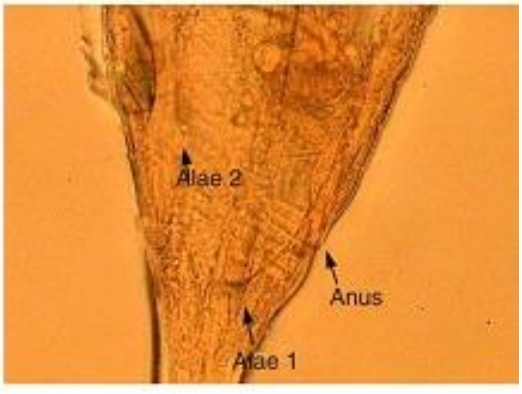


Post. end - Female (Dorso-ventral view)



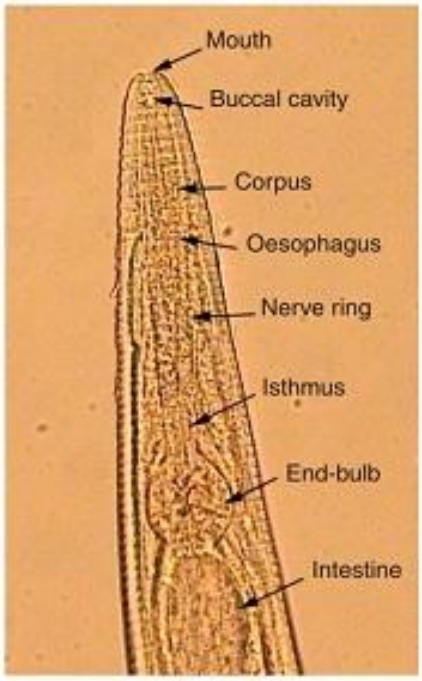
Post. End - Female

Female - W. M.

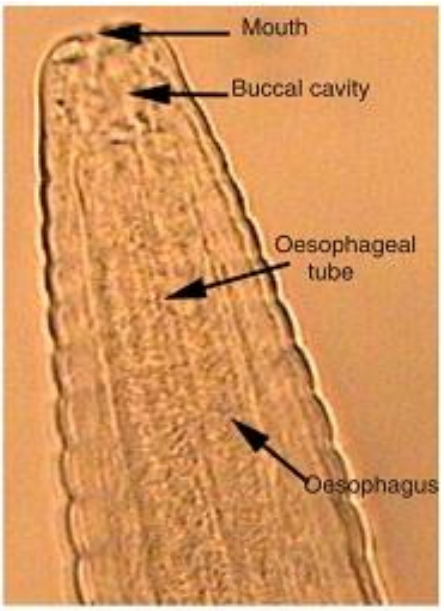


Post. end - Female (Lateral view)

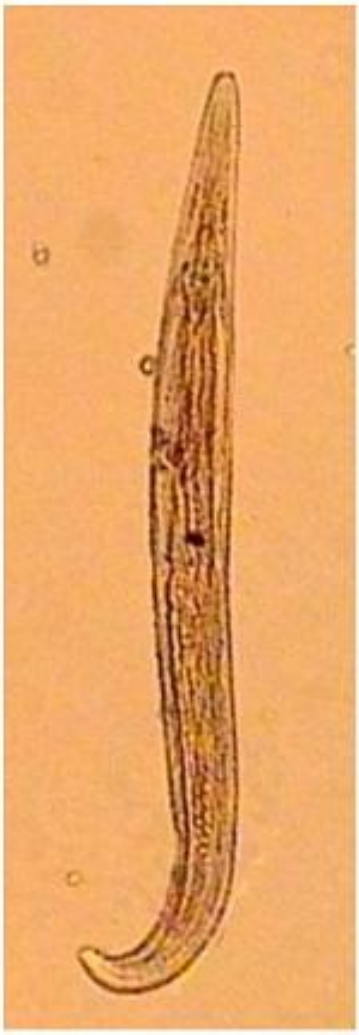
Plate 2. Photomicrographic details of *Leidynema meerutensis* n. sp. (female).



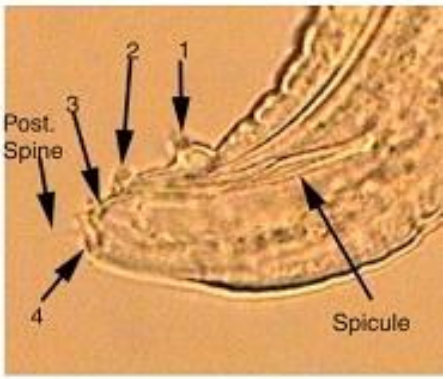
Ant. end - Male



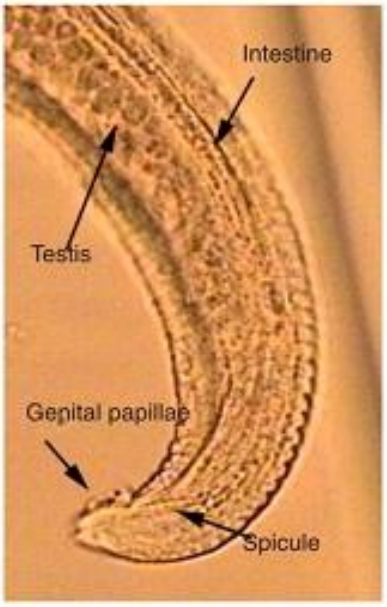
Head - Male



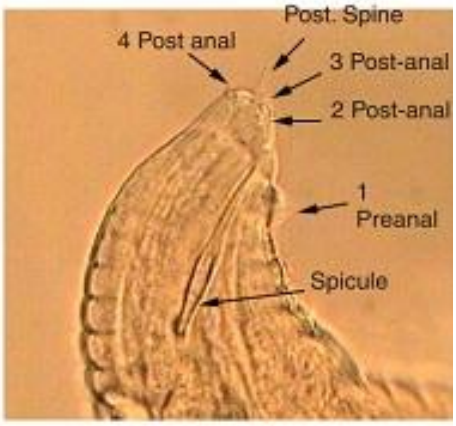
Male - W. M.



Post. end - Male (Lateral view)



Post end with tail (Male)



Post. end - Male

Plate 3. Photomicrographic details of *Leidynema meerutensis* n. sp. (male).

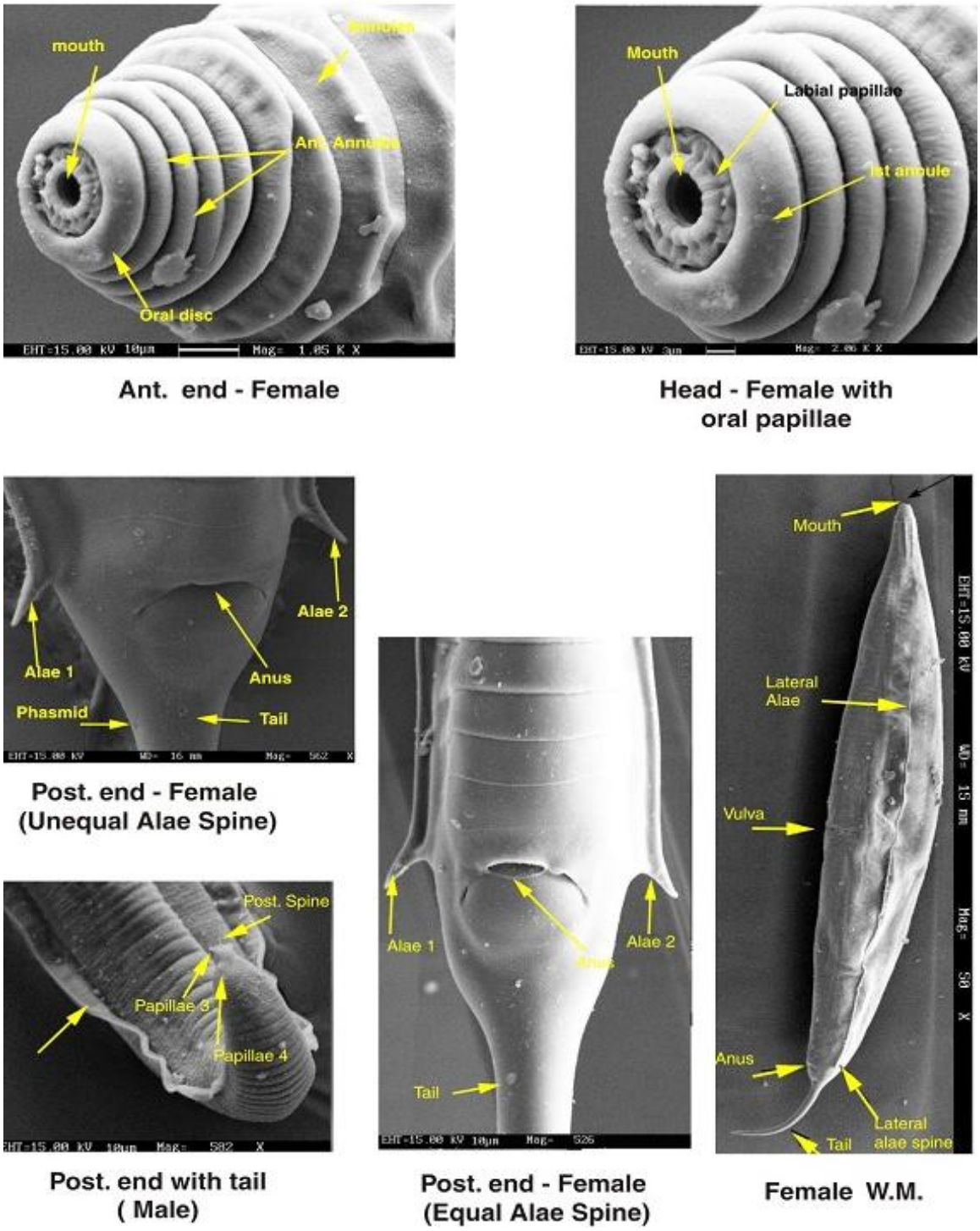


Plate 4. SEM photomicrographic details of *Leidynema meerutensis* n. sp.

Paratype Females (n = 9)

Total Length = 3.00 ± 0.25; Width = 0.31 ± 0.0206; a (L/W) = 9.46 ± 0.353; Oesophagus = 0.426 ± 0.0245; b (L/E) = 7.023 ± 0.305; Tail = 0.513 ± 0.042; c (L/T) = 5.847 ± 0.264; Nerve ring = 0.151 ± 0.008; NR% = 5.04%; Excretory pore = 0.622 ± 0.0363; Ex% = 20.74%; Vulva = 1.44 ± 0.093; V% = 48.00%; Egg = 0.125 mm × 0.035 mm.

Paratype Males (n = 10)

Total Length = 0.918 ± 0.133; Width = 0.074 ± 0.0107; a (L/W) = 12.48 ± 1.376; Oesophagus = 0.192 ± 0.0095; b (L/E) = 4.77 ± 0.579; Tail = 0.0155 ± 0.0008; c (L/T) = 59.016 ± 5.722; Nerve ring = 0.0875 ± 0.0092; NR% = 9.53%; Excretory pore = 0.304 ± 0.028; Ex% = 33.12%; Caudal papillae 4 pairs - 1 pair pre-anal, 2 pairs post-anal sub-

Table 1. Comparative morphometric measurements of different species of *Leidynema* (Female).

Parameter	Described species of *Leidynema*								*Leidynema meerutensis* sp. nov.		
	L. Deltorrei **(Chitwood, 1932)**	***L.Periplaneti*** **(Farooqui, 1967)**	***L.Portentosae*** **(Van Waerebeke, 1978)**	***L.Schwenki*** **(Farooqui, 1967)**	***L.socialis*** **(Adamson et Van Waerebeke,1992)**	***L.Appendiculata*** **(Chitwood, 1932)**	***L.saltense*** **(Achinelly and Camino, 2008)**	***L.orientalis*** **(Singh and Malti, 2004)**	**Range**	**Mean**	**SD**
Length (L)	3.4 - 3.78	1.99 - 2.6	1.85-2.44	1.50-2.80	2.11-4.65	2.168-3.213	3.990	2.60-2.75	2.50-3.15	3.000	0.260
width (W)	0.335-0.450	0.23-0.38	0.130-0.208	0.39-0.41	0.095	0.206-0.284	0.325	0.25-0.28	0.28-0.35	0.317	0.021
a=L/W	8.4 - 10.14	6.84-8.65	11.73-14.23	3.84-6.82	22.21-48.94	10.52-11.31	12.276	9.82-10.40	8.92-9.00	9.465	0.353
Esophagus (E)	0.506 - 0.570	0.37-0.43	0.46-0.598	0.31-0.38	-	0.368-0.446	0.432	0.38-0.40	0.37-0.45	0.427	0.024
b=L/E	6.63 - 6.71	5.37-6.05	4.02-4.08	4.83-7.36	-	5.88-7.20	9.236	6.84-6.87	6.75-7.00	7.024	0.306
Tail (T)	-	0.45-0.54	0.51-0.84	0.34-0.42	1/5 × L	0.491-0.756	-	0.58-0.65	0.43-0.56	0.513	0.042
c=L/T	-	4.42-4.64	2.90-3.62	4.41-6.66	5.000	4.25-4.41	-	4.23-4.48	5.62-5.81	5.847	0.265
Excretory pore	0.840- 0.920	0.59-0.62	0.650	0.46-0.64	-	0.510-0.628	0.460	0.55-0.58	0.55-0.65	0.622	0.036
Ex %	24.33 - 24.70%	23.84 - 29.64%	26.63%	22.8-30.6%	-	19.54-23.52%	11.52%	21.09-21.15%	20.63-22.00%	20.74%	1.03%
Vulva	1.4 -1.63	0.53-1.4	1.10-1.22	1.2-1.38	-	1.020-1.427	1.947	1.5-1.8	1.27-1.56	1.440	0.094
V%	41.17 - 43.12%	53.84%	50.00 - 59.45%	49.28-80.0%	-	44.41-47.04%	48.80%	57.69-65.45%	49.52-50.8%	48.00%	1.55%
Nerve ring	0.000	0.13-0.19	0.208	0.11-0.14	-	0.137-0.156	-	0.12-0.14	0.135-0.150	0.151	0.008
NR%	0.00%	6.53-7.30%	8.52%	5.0-7.33%	-	4.85-6.31%	-	4.61-5.09%	4.76-5.40%	5.04%	0.24%
Egg (lxb)	0.100 - 0.108 × 0.040 - 0.046	0.110 × 0.050	0.095-0.117 × 0.035-0.038	0.122x0.051	0.069-0.038	0.092-0.104 × 0.034-0.036	0.062 × 0.038	0.065-0.068 × 0.028-0.034	0.110-125 × 0.040-0.050	0.11 × 0.04	0.000

ventral, 1 pair post-anal sub-dorsal. Anal Spicule = 0.0348 ± 0.0006 mm.

Taxonomic summery

Type host

Periplaneta americana L. (Orthoptera, Blattidae)

Habitat/site of collection

Hind gut of the host.

Type locality

Meerut region, U. P. (India).

Type species

Leidynema appendiculatum (Leidy, 1850) Chitwood (1932).

Holotype

Leidynema meerutensis sp. nov. deposited at Dept. of Zoology, MCM, C.C.S. University Meerut (U.P. India).

Paratypes

Paratype females labeled as *L. meerutensis* 1-9 and paratype males labeled as *L. meerutensis* 10-19 are deposited at Department of Zoology, MCM, C.C.S. University Meerut (U.P. India).

Etymology

The present species name, *Leidynema meerutensis* is based on type locality of host collection.

Species diagnosis

The new species is characterized by the unequal distribution of lateral alae and terminal spine and short tail in females and 4 pair anal papillae in males. Also, the females have unusually large excretory sac/bulb opening in to excretory pore (not been reported so far). These three features make the species unique and distinct from all other eight species described so far.

RESULTS AND DISCUSSION

Genus *Leidynema* Schwenck, (in Travassos, 1929) with the type species *L. appendiculatum* Leidy 1850 (Chitwood 1932), is characterized by its gut diverticulum. *Leidynema meerutensis* sp. nov. shares the similarities in generic characters with

Table 2. Comparative morphometric measurements of different species *Leidynema* (Male).

Parameter	Different species of *Leidynema*								*L.meerutensis* sp. nov.		
	L. Deltorrei **(Chitwood, 1932)**	***L.Periplaneti*** **(Farooqui, 1967)**	***L.Portentosae*** **(Van Waerebeke, 1978)**	***L.Schwenki*** **(Farooqui, 1967)**	***L.socialis*** **(Adamson et Van Waerebeke,1992)**	***L.Appendiculata*** **(Chitwood, 1932)**	***L.orientalis*** **(Singh and Malti, 2004)**	***L.saltense*** **(Achinelly and Camino, 2008)**	**Range**	**mean**	**S.D.**
Length (L)	0.900	0.76	0.81-1.06	0.810	1.27-1.69	0.579-1.119	0.82-0.85	0.656	0.80-1.15	0.918	0.133
width (W)	0.060	0.090	0.06-0.082	0.090	0.064	0.058-0.097	0.09-0.12	0.083	0.06-0.09	0.074	0.011
a=L/W	15.000	8.440	12.92-13.50	9.000	19.84-26.40	9.98-11.53	4.25-9.11	7.900	12.77-13.33	12.482	1.376
Esophagus (E)	0.189	0.120	0.270-0.328	0.150	-	0.157-0.206	0.086-0.11	0.190	0.18-0.21	0.192	0.009
b=L/E	4.760	6.330	3.00-3.23	5.400	-	3.68-5.43	7.72-9.53	3.452	4.44-5.47	4.774	0.579
Corpus	0.145	0.060	0.185-0.229	0.102	-	0.102-0.123	0.05-0.06	-	0.11-0.13	0.121	0.007
Ishthmus	0.014	0.030	0.065-0.071	0.010	-	0.021-0.024	0.005-0.007	-	0.020-0.025	0.021	0.002
End bulb(lxb)	0.030 × 0.028	0.03 × 0.03	0.011-.016 × 0.36-0.44	0.041 × 0.039	-	0.034-0.041-0.029-0.038	0.025-0.027 × 0.024-0.028	-	0.03-00.04 × 0.03-0.04	0.03 × 0.03	0.000
Buccal cavity	-	0.015	-	0.011	-	0.056-0.097	0.012	-	0.010	0.010	0.010
Tail (T)	-	0.020	-	0.020	1/8 of BL	0.009-0.012	0.009-0.012	0.060	0.015-0.017	0.016	0.001
c=L/T	-	38.000	-	40.500	-	64.33-93.25	70.83-91.11	10.933	53.33-67.64	59.016	5.722
Excretory pore	-	0.160	0.432	-	-	0.418	0.11-0.12	0.186	0.28-0.35	0.304	0.028
Ex%	-	21.05%	Aprox 40%	-	-	37.35%	13.41-14.11%	28.35%	30.43-35.00%	33.12 %	2.66 %
Spicules	0.037	0.030	0.042	0.030	0.063	0.0315-0.0328	0.049-0.059	0.054	0.033-0.035	0.035	0.001
Nerve ring	-	0.050	0.082	0.090	-	0.108-0.127	0.062-0.065	-	0.08-0.10	0.088	0.009
NR%	-	6.57%	7.73-10.12%	11.11%	-	11.34-18.65%	7.56-7.64%	-	8.69-10.00%	9.53%	0.59 %
Caudal Papillae	3 pairs	5 pairs	3 pairs	5 pairs	5 pairs	3 pairs	5 pairs	4 pairs	4 pairs	4 pairs	0

other eight known species of *Leidynema* - *L. appendiculatum* Leidy, 1850; *L. delatorrei* Chitwood, 1932; *L. periplaneti* Farooqui, 1967; *L. portentosae* Van Waerebeke, 1978; *L. Schwencki* Farooqui, 1967; *L. socialis* Leidy, 1850 (Adamson et Van Waerebeke, 1992), and *L. saltense* Achinelly and Camino, 2008 in its shape of oesophagus and intestine, vulva near to mid-body in females, and a single spicule, and abruptly truncated caudal extremity with short terminal spine in males.

The present species, *Leidynema meerutensis* sp. nov. is similar to type species *L. appendiculatum* in general body form, but it is different from the type species *L. appendiculatum* as:

(i) The female *Leidynema meerutensis* sp. nov. is relatively longer with more body width (a (L/W) = 9.46 ± 0.353) compared to *L. appendiculatum* (a = 11.146 ± 0.88).

(ii) Tail in *Leidynema meerutensis* sp. nov. is attenuated and shorter (c (L/T) = 5.847 ± 0.264) relative to the filliform and elongated tail in *L. appendiculatum* (c (L/T) = 4.301 ± 0.235).

(iii) Vulva is relatively anterior in position in *L. appendiculatum* (V% = 44%) compared to *Leidynema meerutensis* sp. nov. (V% = 48%).

(iv) Lateral alae in females *L. appendiculatum* and all other species are equally disposed, but in *Leidynema meerutensis* sp. nov.; it is unequally disposed with one side lateral alae ending much shorter to the other.

(v) The male *Leidynema meerutensis* sp. nov. have four (4) pairs of caudal papillae compared to only three (3) pairs of caudal papillae present in *L. appendiculatum.*

The present species *Leidynema meerutensis* sp. nov. is different from all other known species of *Leidynema* in terms of its unequal disposition of lateral alae and number (4 pairs) and disposition of caudal papillae which is three (3) pairs in *L. deltorei, L. pontentosae* and *L.appendiculatum* while five (5) pairs in L. *Schwencki, L. socialis, L. orientalis* and *L. periplaneti* except *L. saltense* which also has four pairs of caudal papillae but differs in its disposition and tail shape. *Leidynema meerutensis* sp. nov. also differs from other species of *Leidynema*, as:

In *L. periplaneti*, the males lack lateral alae. Vulva in females is much posterior to mid-body (V > 60%).

In *L. portentosae,* there are only three pairs of thick papillae in males and it also possesses many ventral cuticular protuberances. The spicule is much longer and thinner. In females, vulva is much posterior (V = 55%).

In *L. Schwencki,* there are two (2) pairs of pre-anal papillae and three (3) pairs of post anal papillae.

L. delatorrei have 3 pairs of papillae and much anterior vulva (V = 41 - 43%) and lateral alae do not end into spine.

L. orientalis have five (5) pairs of caudal papillae and equal lateral alae.

In *L. saltense,* males have four (4) pairs of genital papillae but with different disposition and different tail shape. The females have much shorter oesophagus (b = 9.236) and excretory pore is much anterior (Ex% = 11.52%).

Thus, *L. meerutensis* sp. nov. is a new species of *Leidynema* and characterized as “females with distinct intestinal diverticulum, lateral alae unequally disposed and short tail; and males with four (4) pairs of caudal papillae and with truncated tail with spine”.

Key to different species of *Leidynema*

1. Males with three pairs of papillae and many ventral cuticular protuberances in posterior region. ..... *L. portentosae* Van Waerebeke, 1978
- Males without protuberances in posterior region. 2
2 (1) Caudal papillae 5 pairs in males. Females with equally disposed lateral alae……3
- Caudal papillae 4 pairs in males. Females with equally or unequally disposed lateral alae.....8
3 (2) Caudal papillae 5 pairs in males. Spicule much elongated and > 0.060 mm in length. ..... *L. socialis* Leidy, 1850 (Adamson et Van Waerebeke, 1992).
- Caudal papillae 5 pairs in males. Spicule shorter and < 0.060 mm in length 4
4(3) Lateral alae present only in females. 5
- Lateral alae present in both male and females 6
5 (4) In females lateral alae is pointed at the terminal with spine like projection. ..... *L. Schwencki* Farooqui, 1967.
- In females, lateral alae extends through the length and is not pointed at the terminal spine like projection..... *L. delatorrei* Chitwood, 1932.
6(4) Females with oesophagus longer (1/5th of body), males with 5 pairs of papillae …… ……..…… *L. periplaneti* Farooqui, 1967
- Females with oesophagus shorter (1/7th of body), length, males with 3 or 5 pairs of papillae. ……..……..……7
7(6) Females with vulva much anterior (V% = 44%). Anal papillae 3 pairs in males ……..... *L. appendiculatum* Leidy, 1850
- Females with vulva much posterior (V% > 55%). Anal papillae 5 pairs in males ……..... *L. Orientalis* Singh and Malti, 2004
8(2) Excretory pore much anterior (Ex% = 11.52%) and oesophagus much shorter (b = 9.23), lateral alae equally disposed …… *L. saltense* (Achinelly and Camino, 2008)
- Excretory pore much posterior (Ex% = 20.74%) and oesophagus longer (b = 7.024) lateral alae unequally disposed ……..… *L. meerutensis,* sp. nov.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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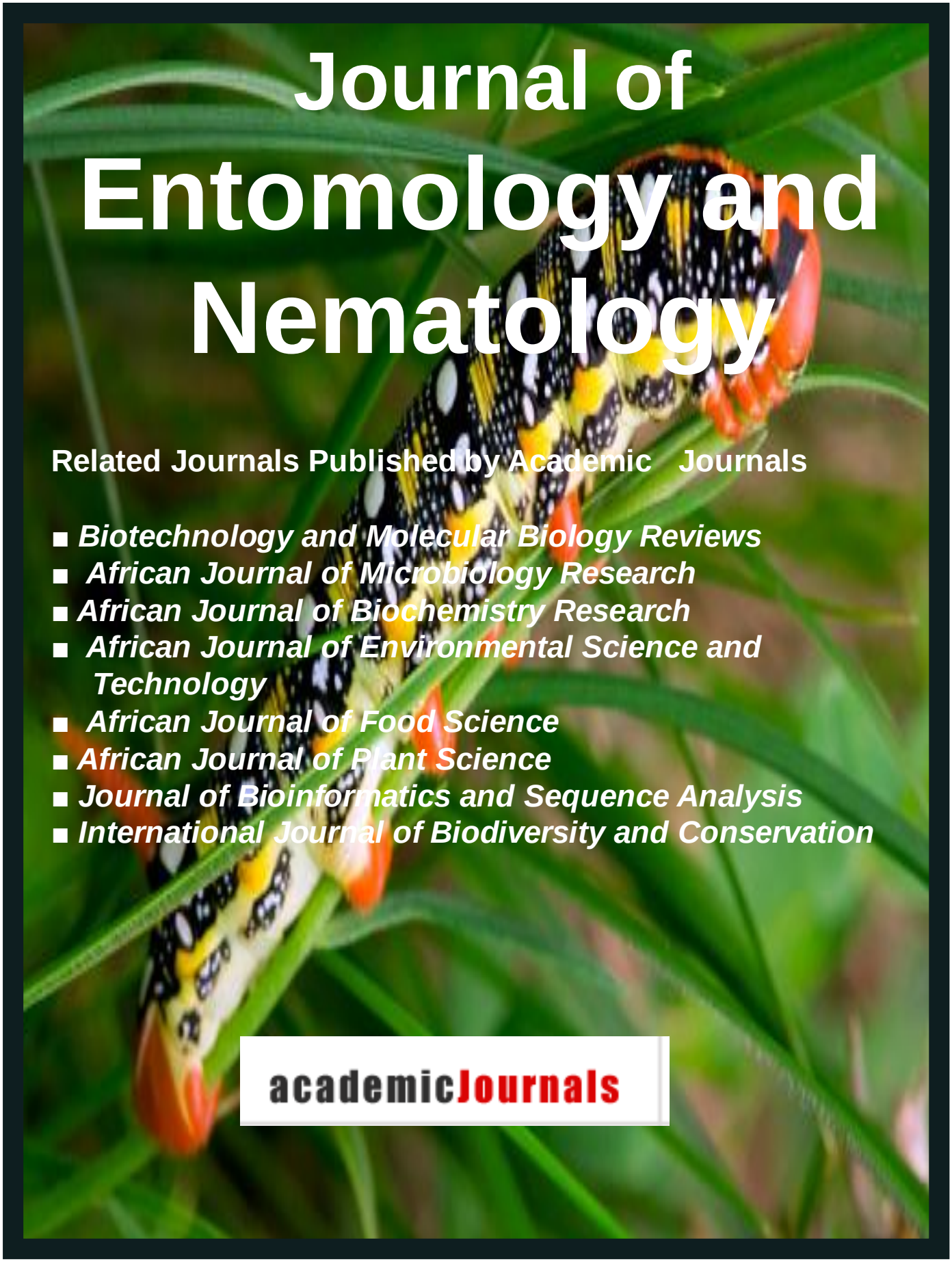

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