

Influence of abiotic factors on behaviour and adult emergence pattern of coconut white grub, *Leucopholis coneophora* Burmeister (Coleoptera: Scarabaeidae)

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Received: 19 January 2018 / Accepted: 5 April 2018 / Published online: 18 April 2018 © Springer Science+Business Media B.V., part of Springer Nature 2018

Abstract Leucopholis coneophora Burmeister is a subterranean pest associated with coconut based cropping systems in south India. Feeding damage causes yellowing of fronds and yield reduction. To develop appropriate IPM strategy a basic knowledge on insect behaviour is essential. Four years studies indicated that, adult emergence of L. coneophora was commenced with summer shower in April in Kerala. Delay in summer shower delayed the emergence. After a pause in May, the emergence resumed with the setting of south

Key messages

- L. coneophora adult emergence initiated with the summer shower in April, after a pause in May, it resumed with the setting of south west monsoon in June and continued for two weeks in Kerala state, India
- Daily the emergence started at an illuminance of $124.37 \pm 75.5 \ l$ in evening, maximum swarming occurred at $32.6 \pm 15.1 \ l$, female emergence and mating occurred at $12.04 \pm 8.1 \ l$ and remained active till it fall to $1.2 \pm 0.4 \ l$ (between IST 18.15 to 19.20)
- The beetles exhibited a wider operational sex ratio in the initial period (1:10.11) that narrowed down to 1:4.33 in later days.
- Hand picking of beetles is highly significant over light trapping

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west monsoon in June. The beetles did not emerge during dry spells in between the rainy days, when the soil temperature (at 10 cm depth) was ≥34.5 °C. Emergence of the beetles started at an illuminance of 124.37 \pm 75.5 *l* in evening and remained active till $2 \pm 0.4 l$ with a maximum swarming at 32.6 ± 15.1 *l*. Female emergence and mating occurred at 12.04 ± 8.1 *l*. Female based sex pheromone mediated communication is evident. Strong competition among the males for mating with emerging female, which was evident by a wider operational sex ratio in the initial period (1:10.11) that narrowed down to 1:4.33 in later days. The beetles neither congregate on any host plant nor exhibit phototaxis. Number of beetles entrapped in light traps varied from 1.5-16.5% and hand picking is highly significant over light trapping. Hence hand picking of beetles daily in the evening for 2 weeks commencing from the onset of south west monsoon in Kerala, in Indian subcontinent is suggested as a tool in IPM.

Keywords Abiotic factors \cdot Behaviour \cdot Illuminance \cdot *Leucopholis coneophora* \cdot Rainfall \cdot Soil temperature \cdot White grub

Introduction

Coconut (*Cocos nucifera* L.) (F. Arecaceae) is an important palm species cultivated in southern and north eastern states of peninsular India. Coastal states (Kerala, Karnataka and Andhrapradesh) alone contributes 90% of area and production, where it is intermingled with the



lives of the local people (Jose 2016). It provides all the requirements of life viz., fuel, food, drink, oil, medicine, fiber, timber, thatch, mats, and domestic utensils. India is the world's third largest coconut producer after Indonesia and Philippines (FAOSTAT 2013). Incidence of pests and diseases is a major constraint in coconut production, among which root eating grub Leucopholis coneophora Burmeister (Scarabaeidae: Coleoptera) is a key pest (Nirula et al. 1952; Shekhar 1958; Abraham and Kurian 1970; Kurian et al. 1974; Abraham and Mohandas 1988a, b and Abraham 1983). Root grubs are major problem in loose sandy soils of Kerala and Karnataka states of Indian subcontinent (Abraham 1983; Veeresh 1983; Kumar 1997). The grubs cause damage to the roots and subterranean parts by feeding. Severe damage on roots causes impaired conduction of water and nutrient which in turn lead to yellowing of fronds, poor production of inflorescence and subsequent yield reduction (Nirula et al. 1952; Rajamani and Nambiar 1970; Abraham and Kurian 1970). Root grub incidence in young seedling cause damage of entire root system and then damage to bole region which gradually lead to total collapse. The pest has annual life cycle with peak adult emergence coinciding with the setting of south west monsoon (Abraham 1983; Abraham 1993 and Prathibha 2015). During monsoon, the rain water percolates down and reaches to aestivating pupae and triggers the adult emergence. The emerging beetles feed, mate and lay the eggs in interspaces. The eggs hatches in about 23.94 days, the larval period ranged 260-270 days, and the pupal period 25.3 and 25.7 days, for males and females, respectively (Abraham and Mohandas 1988b and Mohan and Vidyasagar 1993). The first instar larvae feed on grass roots and soil organic matter. Second instar larvae move towards the root zone area and start to feed on palm roots. Being a polyphagous pest, larval stages feed on a wide array of intercrops viz., sugar cane, sweet potato, tapioca, colocasia, elephant foot yam, Diascoria, banana, fodder grass, cocoa, etc. (Abraham 1993; Bellotti and Schoonhoven 1979; Lal and Pillai 1977; Leefman 1915; Nirula 1958; Veeresh and Viswanath 1983). Presently the grubs are managed by applying soil insecticides belonging to organo phosphorus and neonicotinoid groups which give varying results in farmer's field (Villani et al. 1988; Subaharan et al. 2001; Chenchaiah 2006 and Channakeshavamurthy et al. 2010). To develop an effective IPM package by including all appropriate components, a basic knowledge on field biology and behaviour of the pest is essential. Mass capturing and destruction of adults is one of the components in IPM of white grubs in general. This technique has been successfully evolved and effectively accomplished in the management of grubs of genus *Holotrichia* (Veeresh 1974, 1983, 1984; Yadava et al. 1976). Several ecological and behavioural studies of the Melolonthine root grubs are restricted to the species of the genus *Holotrichia* in India. Scant literature is available on the behaviour of *Leucopholis* spp. The present research attempts to study the behaviour and adult emergence pattern of *L. coneophora* as it would aid to develop strategies for the management of *Leucopholis* grubs.

Material and methods

A four year study was conducted on adult emergence pattern and behaviour of L. coneophora in organically maintained coconut garden of 20,000 m² area at Kasaragod District, Kerala state in India (N12 °31.550' E074 °58.081') during 2011 to 2014. The soil type is coastal sandy and annual rainfall is 3107 mm. Regular observations were taken to study the growth stages present in the field. Different stages of L. coneophora in were extracted from coconut garden at monthly intervals and observed. Four samples (each sampling units of 1 m² area at 15 cm depth) were drawn from interspaces during June-September. Samples were drawn from root zone area (up to 65 cm depth) during September to January, as the second and third instar grubs migrate to root zone area. Observed the biostages of L. coneophora present in the field and recorded. During adult emergence period, adult activities viz., emergence initiation, active swarm, female emergence, mating and feeding behaviour aggregation of beetles in the field were observed between IST 18.15 h to 19.20 h daily in the months of May-June and weekly during the remaining months. The beetles were captured by hand picking and light trapping. They possess sexual dimorphism in antennal and hind tibial characters (Veeresh 1981; Patil and Veeresh 1981). Size of the terminal segments of antennae (lamellae) are comparatively smaller in females than that in males. A pair of spines present at the posterior end of hind tibiae is broad and flattened in female, but are narrow and circular in cross section in males. Based on these morphological characters, males and females were distinguished and the sex ratio was determined. Illuminance in field during initiation of



adult emergence, predatory bird activity, initiation of female emergence, peak swarming of beetles and cessation of emergence were recorded daily between IST 18.15 h to 19.20 h using a digital illuminance meter. Weather data pertaining to daily rainfall and soil temperature at 10 cm depth were obtained from Agro-Meteorological observatory of ICAR- CPCRI. Daily rainfall was measured using graduate cylindrical (standard) rain gauge. The soil temperature was measured using soil thermometer at 10 cm depth and daily mean soil temperature was calculated. A statistical correlation analysis was performed between daily rainfall and number of beetles emerged and between soil temperature and number of beetles emerged separately during each season of study. Duration of emergence period in each season, operational sex ratio and behaviour towards light were observed. Attraction of adults to light was studied by using light traps with incandescent lamp (60 w), and CFL of 11w or 15w. The two light traps were placed in different blocks of the farm @ 1 trap/ha that were switched on between IST 18.00 h to 6.00 h in the next day. Light trap collections were examined daily and the number of males and females collected were recorded. During emergence period the emerging beetles were collected by hand picking, number of beetles captured was compared with the light trap capture. Four years light trap capture data were subjected to analysis of variance (ANOVA). Four years data on adult activity was analyzed by circular statistics using the software 'Oriana'.

Results and discussion

Field biology and distribution of *L. coneophora* in soil column

The female beetles laid the eggs single in loose, moist sandy soil. Fresh eggs were pearly white and oval in shape, it started swelling prior to hatch. First instar grubs were having brown disproportionally large head. First instar larvae were present scattered in the field during June last week to July in interspaces at 10 cm depth, all the four years of study. It feeds on roots of grasses and soil organic matter present in soil. During August to September the dominant stage present was second instar larvae which were distributed between 10 and 15 cm depth of the soil column. However, a few number of (<10%) first instar larvae were also noticed. The third

instar larvae present in root zone area during September (>7%) to February between 30 and 65 cm depth. Kumar (1997) noticed similar observation on *L. coneophora* in coconut garden at Ullal in Karnataka state. As the soil moisture depletes, the grubs tend to move deeper and deeper layers of soil, by February none of the biostages could be noticed even up to 150 cm depth in unirrigated coconut garden. But third instar grubs were present near emitters in drip irrigated coconut garden at 30–5 0 cm depth. Pupae could not be located even up to 150 cm depth during the course of study.

Influence of rain fall and soil temperature on adult emergence pattern of *L. coneophora* in Kerala

Kerala is situated in the south - western corner of the Indian peninsula and nature has bestowed it with abundant rainfall. The average annual rainfall is about 3000 mm, which is about three times of national average. The monthly distribution of rainfall shows that, it is bimodal with two maxima. The highest rainfall peak for all the districts is associated with southwest monsoon, the four-month period 1st June to 30th September is designated as the SW monsoon season. The period comprising October and November is the phase constituting the withdrawal of the southwest monsoon. Kerala gets 50 cm of rainfall during this season. Precipitation during pre-monsoon is mainly from thundershowers (summer shower) from March–May (Ananthakrishnan and Soman 1989).

During 2011, 2012 and 2013 the early emergence was just initiated in the month of April when soil temperature reached below 30 °C, due to the receipt of summer showers (Table 1). It abruptly stopped with the cessation of summer shower and no emergence was noticed till third week of May. During this period soil temperature was quite high (Average of weekly mean soil tempt at 10 cm depth was 36.28 ± 2.5 °C). It is in accordance with other phytophagous root grub species which need rain water to trigger the emergence (Rai et al. 1969; Yadava

Table 1 Beetle activity in relation to illuminance

Adult activity	Illuminance (<i>l</i>)*
Emergence initiation	124.37 ± 75.5
Peak swarming	32.6 ± 15.0
Mating	12.04 ± 8.1
Cessation	1.2 ± 0.4

^{*}Mean of ten observations



and Saxena 1977; Veeresh 1977a, b; Reddy 1977; Yadava and Sharma 1995; Kumara and Sankar 2009). Abraham (1993) recorded initiation of L. coneophora adult emergence during first half of March in Kerala. After this gap, the adult emergence resumed with the setting of South -West monsoon in the May end and active swarming was noticed from 30/05/11 to 14/06/11 (150th to 166th ordinal day) with a peak emergence on 06/06/11 (157th ordinal day) (Fig. 1) and the capture was 121 beetles. The average temperature of soil at 10 cm depth during this period was 28.92 ± 1.84 °C. During active swarming period, the beetle emergence rate was found to be increasing initially up to the receipt of cumulative rainfall of 200 mm. After that, it was found to be decreasing. However, heavy rainfall during the emergence time on each day decreased beetle activity considerably. Scanty emergence was noticed in the month of August also.

In 2012, the setting of South West monsoon was delayed for a week in June and rainfall was less during the initial days (first week of June), and the active swarming period was also delayed accordingly which was during 04/06/12 to 18/06/12 (155th to 169th ordinal day). The peak swarming was noticed on 11/06/12 (162nd ordinal day) the capture was 167 beetles. Average soil temperature during this period was 28.88 ± 2.23 °C (Fig. 2) and the emergence was restricted in June.

But, in 2013, SW monsoon commenced on 21st May (141st ordinal day) itself and by beginning of June cumulative rain fall of 200 mm had been received. The

active swarming and emergence were noticed during 27/05/13 to 13/06/13 (148th to 165th ordinal day). The peak emergence was noticed on 01/06/13 (151nd ordinal day) which is marked by 173 beetle capture. The average soil temperature at 10 cm depth during this period was 28.84 ± 1.64 °C (Fig. 3).

Unlike previous years, in 2014, no beetle emergence was noticed in April, which was devoid of summer shower and the soil temperature was quite high (monthly mean soil temperature at 10 cm depth was $36.6 \pm$ 1.19 °C). Emergence of initial population occurred in the initial rainy days of May and stopped abruptly when the rain restrained i.e., during, 04/05/14 to 12/05/14 (122nd to 132nd ordinal day) (Fig. 4). Weekly mean soil temperature during this period was 31.69 ± 1.64 °C. The emergence was stopped for a while, when the soil temperature attained 34.25 °C on 16/5/14 (134th ordinal day). There was no beetle activity up 18/05/14 (134th to 136th ordinal day) when the average soil temperature was 34.5 ± 0.6 °C. After a gap, emergence resumed on 19th of May (139th ordinal day) along with rainfall and it stopped after 4 days i.e., on 23/05/14 (143rd ordinal day) when soil temperature reached 34.8 °C. There was no beetle activity up to 1st June (152ndordinal day) which was devoid of rain and the weekly mean soil temperature was 34.87 ± 0.5 °C. Beetles emergence resumed on 2nd of June (153rd ordinal day) coinciding with the setting of South - West monsoon and continued up to 18th June (169th ordinal day), when the average

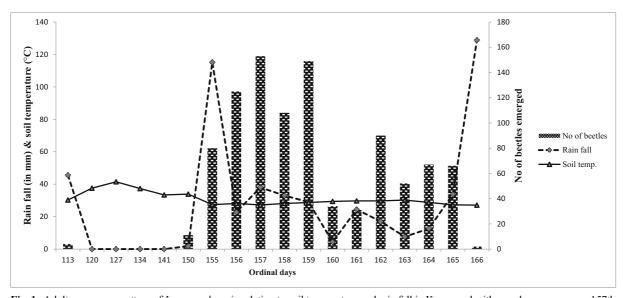


Fig. 1 Adult emergence pattern of *L. coneophora* in relation to soil temperature and rain fall in Kasaragod with a peak emergence on 157th ordinal day in 2011



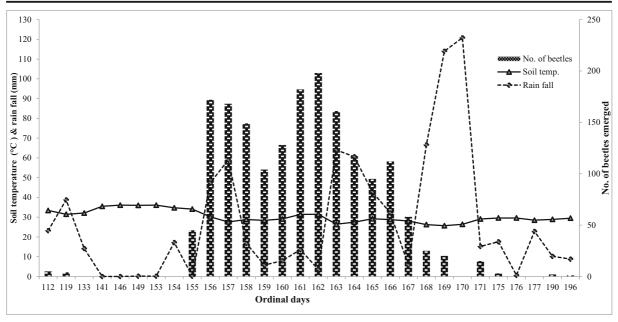


Fig. 2 Adult emergence pattern of *L. coneophora* in relation to soil temperature and rain fall in Kasaragod with a peak emergence on 162th ordinal day in 2012

soil temperature was 29.7 ± 1.4 °C. Total rainfall received during the month of May 2014 was 111.2 mm. Beetle emergence was not noticed in the dry spells between the rainy days. According to Yadava and

Saxena (1977), drought during emergence of *Holotrichia consanguinea* Blanchard during monsoon season caused the death of beetles in the soil itself, which is due to the elevated soil temperature. In the

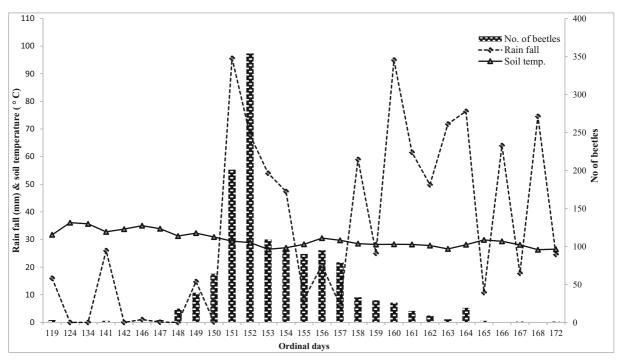


Fig. 3 Adult emergence pattern of *L. coneophora* in relation to soil temperature and rain fall in Kasaragod with a peak emergence on 152nd ordinal day in 2013



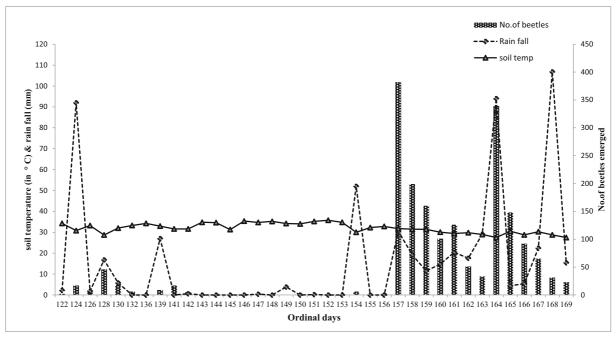


Fig. 4 Adult emergence pattern of *L. coneophora* in relation to soil temperature and rain fall in Kasaragod with a peak emergence on 157th ordinal day in 2014

present study, when the soil temperature came down below critical temperature 34.5 °C due to rain, the emergence occurred and stopped when rain was restrained. Abraham (1993) recorded that, a soil temperature of 37 °C was critical beyond which the emergence would stop. According to Mohan and Vidyasagar (1993) soil temperature is the most important weather parameter which had maximum effect on adult emergence of *L. coneophora* irrespective of the amount of rainfall received. Correlation study between rain fall and number of beetles emerged indicated a weak positive correlation between them during four seasons of study (Pearson correlation coefficient 'r' was 0.129, 0.032,

0.323 .038 respectively during 2011, 2012, 2013 and 2014). Whereas, soil temperature (at 10 cm depth) exhibited a significant negative correlation with daily emergence of beetles (Pearson correlation coefficient 'r' was, -0.625, -0.398, -0.219, -0.448 during 2011, 2012, 2013 and 2014, respectively) (Figs. 5, 6, 7 and 8). Mohan and Vidyasagar (1993) correlated the rainfall data with adult emergence, which indicated the commencement of emergence after 3–5 rainy days irrespective of the amount of rainfall received.

Four years data on adult activity was analyzed by circular statistics using *Oriana*, which indicated that, the activity restricted in second and third quarter of the year

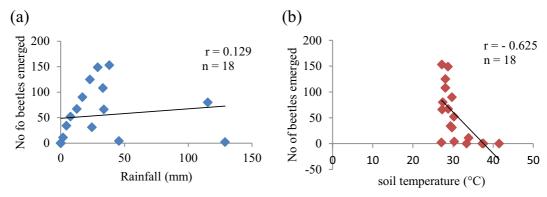


Fig. 5 Correlation of daily adult emergence with (a) daily rain fall and (b) daily mean soil temperature at 10 cm depth in 2011



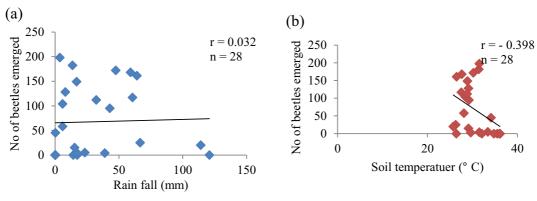


Fig. 6 Correlation of daily adult emergence with (a) daily rain fall and (b) daily mean soil temperature at 10 cm depth in 2012

(the angular degree of 139.4° to 183. 812° at 1% level of significance) with a peak in second quarter (The mean vector (μ) = 161.606° \pm 91.643°); Length of Mean Vector (r) = 0.278 (Fig. 9).

A narrow window of adult activity that extended up to a maximum period of 3 weeks was noticed in the present study. Abraham (1993) recorded a prolonged adult activity period of 60 days during 1976–1978. Adult emergence initiated during first half of March, continued at low level up to second half of May or till early part of June. Scanty emergence continued up to August and September (Abraham 1993). But, as per present study, there is a huge shift in the emergence pattern of L. coneophora. Climate change could be the major reason for this. A hike in soil temperature (an average increase of 0.22 °C in daily mean soil temperature from March to September) was noticed during 2011–2013 than that in 1977 and 1978. Reports of India Meteorological Department (IMD) reveal a rising level in surface air temperature across west coast between 1961 and 2003 (Attri and Tyagi 2010). Gopakumar (2011) reported a rise of 0.8 °C in maximum and 0.2 °C in minimum temperatures with an increase in average surface air temperature of 0.6 °C. Climate change related factors like rise in temperature, changes in precipitation patterns, milder and shorter winters, rise of sea levels and increased incidence of extreme weather events can directly influence insects by affecting their rate of development, reproduction, distribution, migration, and adaptation. In addition, indirect effects occur through the influence of climate on the insect's host plants, natural enemies and interspecific interactions with other insects (Walther et al. 2002). As insects represent huge numbers of taxa and individuals with their short generation time, high mobility and high reproductive rates, they will respond more quickly to climate changes than long lived organisms (Menendez 2007). As per the concept of thermal constant, a linear relationship between developmental rate and environmental temperature is assumed for plants and poikilothermic animals. Ju et al. (2011) reported reduced life span, adult longevity and oviposition period of lace

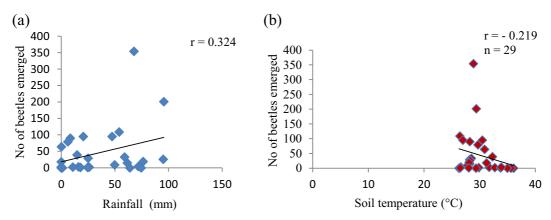


Fig. 7 Correlation of daily adult emergence with (a) daily rain fall and (b) daily mean soil temperature at 10 cm depth in 2013



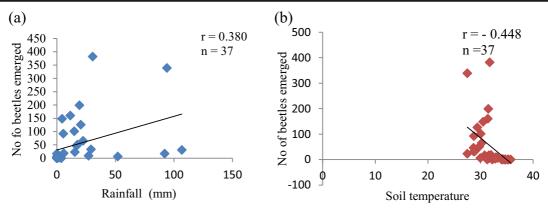


Fig. 8 Correlation of daily adult emergence with (a) daily rain fall and (b) daily mean soil temperature at 10 cm depth in 2014

wing bug at higher temperature regime. Reduction in adult activity window and in oviposition period leads to the building up of chronologically uniform population.

Influence of illuminance on adult activity

The emergence of beetles was initiated on each day when the illuminance fell below $124.37 \pm 75.5\ l$ (Table 1) in the evening (in May–June) and the activity extended till the illuminance reached $1.2 \pm 0.4\ l$ (IST 18.35 h to 19.10 h). During the emergence period birds were found to be predating on beetles and their activity noticed up to $100\ l$ illuminance. Common crow (*Corvus splendens* L.) and jungle crow (*C. macrorhynchos* L.), kingfisher (*Alcedo atthis* L.), brahmini kite (*Haliastur indus* L.) and common egret (*Ardea alba* L.). These birds (except egret) were found roosting on the coconut fronds watching beetle emergence. Whenever the beetles emerged, raptors dived and captured them. Avian

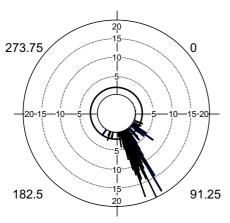
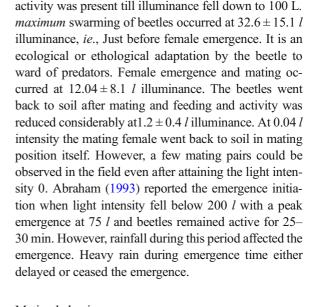


Fig. 9 Adult emergence pattern of L. coneophora – 2011 to 2014. L. coneophora beetle activity restricted in second and third quarter of the year



Mating behaviour

The males emerged first, located the emerging spots of female by fluttering in soil in inverted position facing antennae down and congregated in the spots were females are about to emerge. This indicates the presence of potential sex pheromone mediated chemical communication among the individuals. There was a strong competition among males for mating during female emergence, which is indicated by a wider operational sex ratio in the initial period (1:10.11) that narrowed down to 1:4.33 in later days. Beetles produced characteristic buzzing sound on aggregation. Similar behaviour was noticed by Abraham (1993) on *L. coneophora*. Males outnumbered the females with an operational sex ratio of 1:5.37. Mating process was typical of other melolonthine beetles, as described by Veeresh (1977a, b) and Yadava



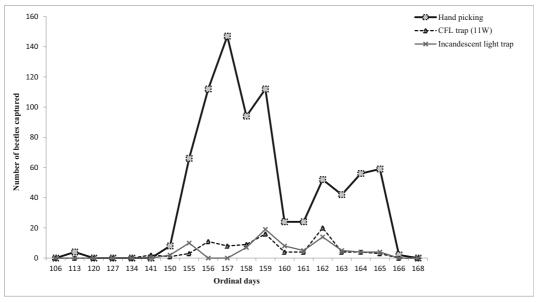


Fig. 10 Comparison of hand picking and light trapping of L. coneophora beetles during emergence period in Kasaragod in 2011

(1981). When females protrude its head, the males pull it out and attempted to mate. Male mounted on female, after establishing the union, it fell upside down on the ground without breaking the union. It remained in mating position for quite a long time (15–20 min). Abraham (1993) noticed that, mating was continued for 7–9 min. Mating pairs were observed in ground as well as on leaf stalks of intercrops as observed by Nirula (1958). Towards the end, the female went back to soil by digging and dragging the male behind. Abraham (1993) observed similar behaviour.

Feeding behaviour

The adult beetles fed on variety of host plants viz., a weed plant Ludwigea (*Ludwigea perennis* L.), cashew (*Anacardium occidentale* L.), mango (*Mangifera indica* L.), okra (*Abelmoschus esculentus* L.), hibiscus (*Hibiscus roasasinensis* L.), ficus (*Ficus* spp.) etc. It did not show congregation on any particular host plant. Many species of root grubs are known for the congregation of their adult stages on a particular host. *Holotrichia* sp.congregates on neem trees immediately after

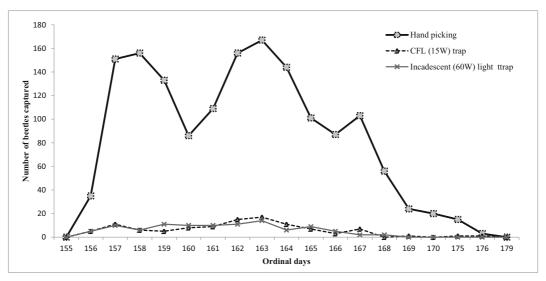


Fig. 11 Comparison of hand picking and light trapping of L. coneophora beetles in Kasaragod in 2012

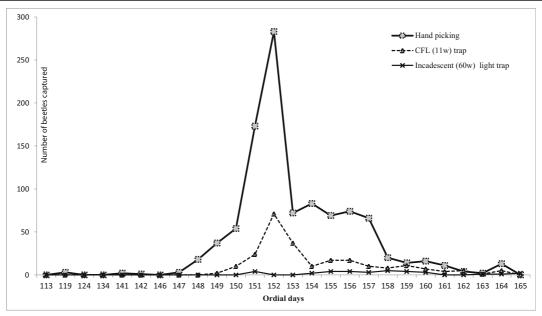


Fig. 12 Comparison of hand picking and light trapping of L. coneophora beetles during emergence period in Kasaragod in 2013

emergence (Gupta 1973; Veeresh 1977a, b; Raodeo and Deshpande 1987).

Attraction to light trap

Present study indicated that the number of beetles entrapped in light traps were negligible (varied from 1.5–16.5% of total capture per day) when compared to hand picking (Figs. 10, 11, 12 and 13). However, a few numbers of beetles merely fell into light traps during its random movement and not necessarily due to phototaxis. The difference between hand picking and light trap capture was highly significant (*p* value = 0.0001). Among light traps, though the light traps lured with

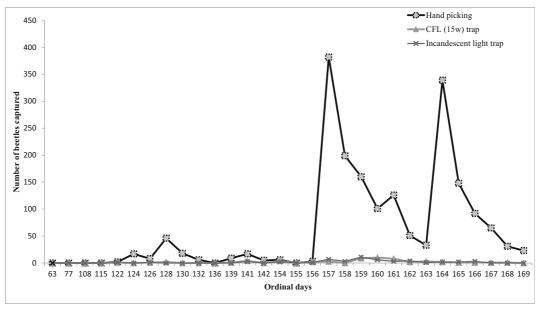


Fig. 13 Comparison of hand picking and light trapping of L. coneophora beetles during emergence period in Kasaragod in 2014



11w CFL captured more number of beetles than incandescent lamp trap, there is no significant difference between captures made by the two light traps. Abraham (1993) and Veeresh et al. (1982) reported that L. burmeisteri and L. lepidophora could not be entrapped by light. In contradiction to this, Jeevan (2014) recorded prolonged and more number (679 adults) of L. coneophora capture by mercury light trap in June. It was followed by U.V. light trap capture during emergence season in 2013. Similarly, Veeresh et al. (1982) reported the attraction of an unidentified species of Leucopholis males to light at Dihatta, West Bengal in India during May 1982. But the information pertaining to source and intensity of light used were not available. However, further studies are needed to take up on behavioural aspects of Leucopholis beetles towards different light sources at different intensities.

Conclusion

Four years studies on adult emergence pattern of L. coneophora beetles indicated that, a combination of rainfall and fall in soil temperature triggered the adult emergence of L. coneophora. During peak swarming period, if the soil temperature (at 10 cm depth) is ≥34.5 °C adult emergence stopped immediately. Heavy rains during evening hours (IST 18.15 to 19.20) considerably reduced the beetle activities and emergence. The emergence pattern apparently varied according to the distribution of rainfall during South West monsoon period. Nevertheless, neither rainfall nor soil temperature exhibit a linear relationship with beetle emergence. The duration of swarming of beetles was noticed only for three weeks during the four seasons of study. During active swarming period, the beetle emergence rate was found to be increasing till the receipt of cumulative rain fall of 200 mm, which was within the first or second week of June in four seasons of study. Seasonal phenology of L. coneophora indicates that it has annual life cycle with adult emergence coinciding with pre monsoon shower (May-June). The success of IPM programmes relies on time and method of execution of each components of IPM. Adult emergence of mating occurred during first two weeks of setting of South West monsoon. By the end of June first instar larvae are present in interspaces and start feed on soil organic matter and grass roots. They are highly susceptible and less amount of insecticide is required to kill them. Hence first round application of insecticide is advisable as blanket application in the month of July second/third week. Rather than targeting third instar grub, it would be more effective and economical to aim first instar stage. The beetles could not capture by light trapping as it does not exhibit photo taxis. Beetle activity noticed between IST 18.15 to 19.20. Hence hand picking and destruction of beetle daily in the evening for two weeks starting form first day of south west monsoon in advisable for the management of *L. coneophora* in coconut based cropping systems of Kerala State in Indian subcontinent.

Acknowledgements Authors are grateful to Director CPCRI and Head Crop protection division, CPCRI and University of Agricultural Sciences Bangalore for providing necessary facilities and fund.

Author's contribution PSP conceived the research, conducted survey and carried out studies on adult emergence pattern and behaviour of *L. coneophora*. ARVK and KS provided guidance and reviewed the research. VV assisted in taking filed observation. All the authors were involved in writing the research paper.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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