STUDIES ON THE KUDZU BUG, *Megacopta cribraria* (FABRICIUS) (HEMIPTERA: PLATASPIDAE) INFESTING *Lablab purpureus* (L.) (FABACEAE: LABLAB)

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By

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UNDER THE GUIDANCE OF

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CERTIFICATE

This is to certify that this thesis entitled **"STUDIES ON THE KUDZU BUG, *Megacopta cribraria* (FABRICIUS) (HEMIPTERA: PLATASPIDAE) INFESTING *Lablab purpureus* (L.) (FABACEAE: LABLAB)"** is an authentic work carried out by **Ms. SHAMYASREE. M. S** under my supervision and guidance in partial fulfilment of the requirements of the *Degree of Philosophy in Zoology* under the Faculty of Science of the University of Calicut, and that no part thereof has been presented earlier for any other degree, diploma or similar titles.

Malabar Christian College, Calicut.
Date: 30/12/2022

DECLARATION

I do hereby declare that this thesis entitled **"STUDIES ON THE KUDZU BUG, *Megacopta cribraria* (FABRICIUS) (HEMIPTERA: PLATASPIDAE) INFESTING *Lablab purpureus* (L.) (FABACEAE: LABLAB)"** submitted to the University of Calicut in partial fulfilment of the requirements of the *Degree of Philosophy in Zoology*, is a bonafide research work done by me under the supervision of **Dr. Sachin P James**, HOD and Assistant Professor, P.G. & Research Department of Zoology, Malabar Christian College, Calicut, and no part of this thesis presented by me has been thereof used for the award of any other degree, diploma or similar titles.

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CONTENTS

ABSTRACT

Agriculture, with its allied sectors is unquestionably the largest livelihood provider in India, more so in the vast rural areas. It also contributes a significant figure to the Gross Domestic Product (GDP). The Dolichos bean, *Lablab purpureus* (L.) is an important pulse cum vegetable crop belongs to the family Fabaceae, native of Africa and is cultivated throughout the tropics for food. Lablab is a multipurpose legume used as a pulse crop for human consumption, as a fodder crop for livestock, as a rotational and cover crop as well as a pioneer species to improve soil fertility and soil organic matter. Like any other vegetable crop, *Lablab* is also affected by an array of pests mainly arthropods. *Megacopta cribraria* (F.) (Hemiptera: Plataspidae) is one of the pests of *L. purpureus.* It is distributed in Asia, Australia, Georgia and USA. In India it is widely distributed in Madhya Pradesh, Kerala and Karnataka. This insect is voracious feeder on kudzu, but it also feeds on numerous agricultural crops, particularly soya bean, and lablab bean, Pigeon pea *Cajanus indicus* Spreng, *Phaseolus* sps. (Mung beans, kidney beans, lima beans, green beans, etc.) and broad beans (*Viciafaba* L.).

The present study, mainly focused on the life history, ecology and biocontrol agents of *M. cribraria*. Life history studies help the economic entomologist at which stage of the kudzu bug control measure should be adopted. The life table parameters of *M. cribraria* feeding on the host plant, *L. purpureus* var. *Hima* were studied at a temperature of 28 ± 2^0C, RH of 75 ± 5 %, and 14L:10D h photoperiod. Pest populations are influenced by a variety of biotic (parasitoids, predators, microorganisms, etc.) and abiotic factors (temperature, humidity, light, rainfall, etc.) in the field. One of the most important abiotic factor affecting growth, survival and reproduction of insects is temperature. Hence a study was conducted to find out the relationship of five different constant temperatures (15-35^{0}C) on the growth and development of *M. cribraria.* Like temperature, relative humidity also play a key role in the survival, development, fecundity, and population increase in *M. cribraria*. High levels of RH 70 and 80% were associated with higher egg and nymph survival rates, increased adult longevity and female fecundity in *M. cribraria.*

The volatile compounds emitted by host plants that mediate insect–plant interactions. The different parts of host plant were not equally attractive for *M. cribraria.* It was observed that no difference in mean copulation duration among the different sex

ratios of *M. cribraria.* The results may be useful for further research on the effect of prolonged copulation duration on the reproductive biology of *M. cribraria.* VOCs were collected from disturbed and undisturbed adults of *M. cribraria* was analysed by coupled GC-MS revealed that octacosane, decamethyl cyclopentasiloxane, 2-ethylhexanol, tridecane and dodecane, 4,6-dimethyl- were present in the sample from undisturbed *M. cribraria* but these were absent in disturbed *M. cribraria* sample. (E)-2- hexenal and 2-hexene, 4,4,5-trimethyl were only detected from VOCs collected from disturbed *M. cribraria.* Insects were able to responses to various wavelengths of light. *M. cribraria* attracted to white LED light followed by yellow and green LED light. A new species of scelionid wasp, *Paratelenomus anu* (Hymenoptera, Scelionidae) was described based on morphology, scanning electron microscopic studies and DNA barcoding data. The rearing of *P. anu* was done successfully in the laboratory using 20% honey as food source.

In order to assess the feasibility of *P. anu* as a biological control agent for *M. cribraria*, parasitism rate, emergence rate, development time, sex ratio, pre oviposition period, oviposition period, fecundity, number and adult longevity were examined in the laboratory. The parasitism and emergence rate of *P. anu* was 75.86 ± 0.74% and 73.52 ± 0.29%, respectively. Unmated females of *P. anu* only produced males and mated females produced both males and females, with a female biased sex ratio. i.e., in the absence of mating *P. anu* reproduced via arrhenotoky type. Age of host eggs can have substantial effects on parasitism and parasitoid efficacy. *P. anu* preferred to parasitize 12hour old eggs rather than 24hour 48hour and 72hour old eggs. Temperature has a profound effect on the percentage of parasitism, developmental time, emergence rate, sex ratio, oviposition period, fecundity and longevity of *P. anu*. *P. anu* developed on *M. cribraria* eggs at temperatures between 20^0C-30^0C, but performed best at 30^0C. *P. anu* exhibited highest rate of parasitism and percentage of emergence at this temperature.

The success of biological control agent depends upon its field performance. The percentage of parasitism of *P. anu* on *M. cribraria* egg masses was 72.56 ± 1.91% in the release micro plots. The emergence rate of first instar nymphs of host was 86.92 ± 1.86% in the control microplots whereas, 7.18±0.44% in release microplot. The overall reduction in the first instar nymphs in the release plot was 79.74 ± 1.42% than control plot.

CHAPTER 1

INTRODUCTION

Agriculture is the backbone of the Indian economy. On an average about 54.6% of the population is engaged in agriculture and allied activities and it contributes 17.4% to the Gross Domestic Product (GDP). Today, India is a major supplier of several agricultural commodities like tea, coffee, rice, spices, oil meals, fresh fruits, fresh vegetables and its preparations to the international market.

Horticulture is a part of agriculture concerned with intensively cultured plants directly used by peoples for food, for medicinal purpose or for aesthetic gratification (Singh, 2012). Vegetables contribute a major portion of human diet in many parts of the world and play a significant role in nutrition, especially as sources of phyto-nutriceuticals: vitamins (C, A, B1, B6, B9, E), minerals, dietary fiber and phytochemicals (Quebedeaux and Eisa, 1990; Craig and Beck, 1999; Wargovich, 2000; Dias and Ryder, 2011). A high vegetable diet has been associated with the improvement of gastrointestinal health and vision, reduced risk for some forms of cancer, cardiovascular disease, heart disease, stroke, diabetes, anaemia, gastric ulcer, rheumatoid arthritis, and other chronic diseases (Prior and Cao, 2000; Hyson, 2002; Golberg, 2003; Keatinge *et al*., 2010; Mullie and Clarys, 2011). India is the second largest producer of vegetables next only to China in world and popularly known as fruit and vegetable basket of the world.

The diverse agro-climatic conditions and rich diversity in crops and genetic resources enable India to produce a wide range of horticultural crops such as fruits, nuts, vegetables, medicinal and aromatic plants and flowers. Importance of horticulture lies in the fact that it generates much income per hectare of land as compared to other agricultural crops as well as it facilitates employment, nutritional security, poverty alleviation, and earn foreign exchange through export. In India, due to the rapid growth of the population with reduction in land, in order to feed the population, the only strategy should be producing more vegetables from less land, less water with less pesticides and with less detrimental to soil and environment as well. Organic vegetable cultivation is the most sustainable farming systems with recurring benefits not only to long-term soil health but also provides a lasting stability in production by importing better resistance against various biotic and abiotic stresses.

Fabaceae or Leguminosae, commonly known as the legume, pea, or bean family, is the most diverse and economically important family of flowering plants in the world (Beech *et al*., 2017) consist of 770 genera and 19,500 species and considered as the third largest family of angiosperms in species numbers after Asteraceae and Orchidaceae (LPWG, 2017). Plants of this family have economic importance, by having food crops that provide highly nutritious sources of protein and micro nutrients that can benefit health and livelihoods (Graham and Vance, 2003; Yahara *et al*., 2013; Okeke *et al*., 2019). The key feature of this family is the association with soil bacterium of the genus Rhizobium located in root nodules found in many species of Fabaceae, converting atmospheric nitrogen into ammonia, a soluble form used by other plants, aftermath in extremely valuable sports as suppliers of natural fertilizers, in addition to their considerable importance in agriculture, representing their ability to occupy different habitats and diverse life forms (Lewis *et al*., 2005; Sprent *et al*., 2013; Strassburg *et al*., 2017).

Lablab purpureus L. Sweet (2n = 22) belongs to the family Fabaceae, the subfamily *Faboideae*, the tribe Phaseoleae, the subtribe: *Phaseolinae,* the genus *lablab* and *Lablab purpureus* is the only species of the *Lablab* genus (Gowda, 2013). It is commonly known as Country bean, Indian bean, Field bean, Hyacinth bean, Dolichos bean, Egyptian bean, Bonavist bean, Sem bean, etc. (Chandrakant, 2014). Like other legume, Lablab showed greatest variation in its form and growth habit (Piper and Morse, 1915; FAO, 1988a). Most of the species are summer-growing, rampant and vigorously twining herbaceous plants whose wild germplasm is strongly perennial but frequently grown as annual or biennial crop (Piper and Morse, 1915; Duke *et al*., 1983; FAO, 1988a; Deka and Sarkar, 1990; Hall and Naidu, 2004). The trailing, glabrous or pubescent stems can reach 3 to 6 m in length (Duke *et al*., 1983) as well as bushy, semi-erect and prostrate forms also exist (Piper and Morse, 1915; Pengelly and Maass, 2001; Tefera, 2006). The basal stem of may reach up to 4 cm diameter. The nodulated root system consists of a deep taproot from which emerges a lateral root system.

The leaves are alternate and trifoliolate; leaflets are broad ovate-rhomboid in shape, 7.5 to 15 cm long (Schaaffhausen, 1963; Verdcourt, 1979; Duke *et al*., 1983; FAO, 1988b; Maundu *et al*., 1999) acute at the apex, almost smooth above and short haired underneath (FAO, 1988a). Petioles long and slender. Variation in shape of the leaflets is limited whereas its variation in size and colour is high (Piper and Morse, 1915; Pengelly and Maass, 2001). Inflorescences are axillary erect, lax, fascicled and many-flowered

axillary raceme 4– 20 cm long on peduncle 2–40 cm long (Piper and Morse, 1915; Schaaffhausen, 1963; Maundu *et al*., 1999). The flowers may be white to blue or purple in colour depending on the genotype on short pedicels about 1.5 cm long, typically papillonaceous in shape. Lablab pods are very variable in shape and colour; they may be linear, flat or inflated 5–20 cm ×1–5 cm, straight or curved, usually with 3–6 ovoid seeds (Piper and Morse, 1915; Duke *et al*., 1983; FAO, 1988a). The length can vary from 5 to 20 cm, breadth from 1 to 5 cm (FAO, 1988a). Lablab seeds are ovoid, laterally compressed with 0.5–1.2 cm long, 0.3–0.9 cm wide, and 0.2–0.7 cm thick. The beans are variable in colour, depending on variety or cultivar, usually white or cream through to light and dark brown, red to black with a conspicuous linear hilum extending around ⅓ of seed circumference (Piper and Morse, 1915; Schaaffhausen, 1963; FAO, 1988a; Maundu *et al*., 1999; Guretzki and Papenbrock, 2014). some cultivated varieties and wild varieties have mottled seeds (Murphy and Colucci, 1999; Adebisi and Bosch, 2004; Cook *et al*., 2005; FAO, 2014) (Plate. 1).

L. purpureus is believed to be originated in India (Purseglove, 1977; Chowdhury *et al*., 1989) and considered as one of the oldest cultivated crops of the world (Bullivant, 1963). Lablab is widely distributed in Africa, the Indian sub-continent and Southeast Asia (NAS, 1979; Smartt, 1985; Maass *et al*., 2005; Maass, 2006). It is presently widespread throughout the tropics and subtropics (Kimani *et al*., 2012), where it has become naturalized in some areas (Tefera, 2006). The species is extremely diverse (Verdcourt, 1971; Duke *et al*., 1981) and three subspecies are recognised based on difference in the characteristics of pods and seeds (Verdcourt, 1971; Pengelly and Maass, 2001; Maass *et al*., 2005; Tefera, 2006). The subspecies are the wild ssp. *unciantus*, distributed in East Africa which includes the variety *rhomboideus* (Schinz) Verdc. and the two cultivated subspecies, ssp. *purpureus* and ssp. *bengalensis* (Jacq.) Verdc. (Verdcourt, 1970; 1971). In lablab species more than 200 genotypes have been recognized but, most of them remaining unnamed (NAS, 1979). Genotypes can be identified based on differences in size, shape and colours of pods, seeds, flowers and leaves, respectively (Duke *et al*., 1983; Hendrikson and Minson, 1985). India has 227.78 ‘000 hectare area under lablab cultivation with 2,276.95 ‘000 metric tonnes lablab bean production and 10 metric tonnes per hectare productivity (Anonymous, 2018). Within India, it is grown as a field crop in Madhya Pradesh, Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu and Kerala. Outside India, this crop is mainly cultivated in East Africa and Australia.

Lablab is found throughout the tropics and subtropics, ranging from 30° southern to 30° northern latitude and cultivated in arid, semi-arid and humid climates (NAS, 1979; Duke *et al*., 1983; FAO, 1988a; English, 1999; Hill *et al*., 2006). The altitude ranges up to 2500 meters above sea level (masl), but lower elevations are usually preferred (NAS, 1979; FAO, 1988a; Maundu *et al*., 1999; Aganga and Tshwenyane, 2003; Tefera, 2006). Lablab can grow in regions where annual temperatures range between 18 to 30°C and the minimum required temperature for growth is 3°C (Duke *et al*., 1983; FAO, 1988a; Smartt, 1990; Aganga and Tshwenyane, 2003). High temperatures not to affect the development of lablab (Schaaffhausen, 1963; Duke *et al*., 1983; FAO, 1988a; Smartt, 1990; Liu, 1996) but light frosts can damage leaves but will not kill the plant if not occurring for a prolonged time period (Duke *et al*., 1983; FAO, 1988b; Aganga and Tshwenyane, 2003). It is adapted annual rainfall regimes of 200 - 2500 mm (Duke *et al*., 1983; FAO, 1988a; Aganga and Tshwenyane, 2003; Tefera 2006). Lablab can grow in a variety of soils, from sand to clay, in a pH range of 4.5–7.5 (Cook *et al*., 2005). In some areas Lablab is cultivated as a cover crop because its dense green cover reduces soil erosion by wind or rain (Mureithi *et al*., 2003).

Lablab is a multipurpose legume used as a pulse crop for human consumption, as a fodder crop for livestock, as a rotational and cover crop as well as a pioneer species to improve soil fertility and soil organic matter (Karachi, 1987; English, 1999; Pengelly and Lisson, 2003; Hill *et al*., 2006). In addition to this, it is also used as herbal medicine or for ornamental purposes (Maass *et al*., 2010). In tropical region it is cultivated either as a pure crop or mixed with other crops, such as finger millet, groundnut, castor, corn, bajra or sorghum (NAS, 1979; Singh and Mishra, 1988; Hill *et al*., 2006; Njarui and Mureithi, 2010). The crop is mainly grown for its young pods, green and immature seeds for vegetable purpose while the dry seeds are used in various vegetable food preparations. The leaves and flowers may be cooked and eaten like spinach (NAS, 1979; Tefera, 2006).

Lablab has high protein quality (Mortuza and Tzen, 2009) so that it is used in child feeding programmers and food and feed formulations. In addition to protein, adequate concentration of vitamins, carbohydrates and minerals like sodium, potassium, calcium, magnesium, phosphorus and iron are present (Siddhuraju *et al*., 2002). Phytochemical analysis of fresh leaf extracts of lablab contain sugar, alcohols, phenols, steroids, essential oils, alkaloids, tannins, flavonoids, saponins, coumarins, terpenoids, pigments, glycosides

and anthnanoids (Torres and Manalo, 1990; Deoda *et al*., 2012; Balekari, 2013). Phytochemicas study of the raw and aqueous crude extracts of lablab seeds shows that the seeds contained trypsin inhibitor contents, Hemagglutinin content, cyanogenic glycosides, oxalates, phytates, tannins and saponins (Soetan, 2012).

Lablab seeds were used to stimulate stomach, as antidote for poisoning, for menopause and spasms, and for the treatment of cholera, diarrhoea, colic, rheumatism and sunstroke. The juice prepared from the fruit pods was used as astringent, digestive, stomachic, to expel worms and for the treatment of inflamed ears and throats. The flowers were mainly used to treat inflammation of uterus and to increase menstrual flow (Duke and Ayensu, 1985; Shivashankar and Kulkarni, 1998; Kante and Reddy, 2013; Bhogireddy *et al*., 2013) reported that lablab plant was widely used as anti-inflammatory, aphrodisiac, antispasmodic, antidiabetic, febrifuge and for flatulent, bilious, stomachic and phlegmatic disorders.

Like any other vegetable crop, lablab is also affected by an array of pests mainly arthropods. Class Insecta is the largest class of the phylum Arthropoda in the animal kingdom containing up to 75% of the known species of the animals (Hickman *et al*., 2008). Majority of insects are harmless but many of them fed on all kinds of plants including crop plants, forest trees, medicinal plants and weeds. The order Hemiptera is the fifth largest group of insects after Coleoptera, Diptera, Hymenoptera, and Lepidoptera (Schuh and Slater, 1995; Grimaldi and Engel, 2005; Cameron *et al.*, 2006) and recognized as a monophyletic group (Hennig, 1969; Carver *et al*., 1991). They can be recognized by the particular structure of the mouthparts, the mandibles and maxillary laciniae are modified into concentric stylets, the mandibular enclosing the maxillary ones, both forming the food and salivary channels; the multisegmented sheetlike labium is covering the mandibular and maxillary stylets; and the maxillary and labial palpi are absent (Weber, 1930; Hennig, 1969, 1981; Cobben, 1978; Kristensen, 1991) and their feeding habits range from phytophagy to predation, including ectoparasitism and hematophagy. Many of them are considered as important pest species of cultivated crops and some are important vectors of human diseases.

The Plataspidae is a moderately species-rich family within superorder Pentatomoidea comprising about 59 genera and nearly 500 described species. (Davidova-Vilimova and Stys, 1980; Henry, 2009). The members of plataspidea possess a beetle-

like appearance, due to their enormously enlarged scutellum, covering the whole abdomen. *Megacopta cribraria* (Fabricius), is an invasive member of the family Plataspidae originating from Asia and Indian subcontinent (Eger *et al*., 2010; Zhang *et al*., 2012). Numerous common names of *M. cribraria* include bean plataspid (Ahmad and Moizuddin, 1975; Zhang *et al*., 2012), negro bug (Borah and Sarma, 2009a), kudzu bug (Ruberson *et al*., 2013), lablab bug (Sujithra *et al*., 2008) and sting bug (Fukatsu and Hosokawa, 2002; Wu *et al*., 2006; Jenkins and Eaton, 2011).

The appearance of adult bug is small 3.5- 6.0 mm in length, in glabrous and box-like shape and brown to olive green in colour with dark punctuations along the dorsal side. It possesses two-segmented tarsi and second antennal segment one-third or less length of third segment. Head is flat and juga contiguous in front of tylus. The Scutellum is enlarged, width nearly 1.5 times length, truncate or very broadly rounded posteriorly, widest on posterior fourth and base of scutellum with transversely elongate area outlined by distinct impressed line. Adults are morphologically dimorphic externally with respect to the coloration of the sternites, curvature of the terminus, and shape of the genitalia capsule. Female sternites are lighter in colour, but with an area of dark coloration restricted medially, while in males the sternites are usually entirely dark in coloration. Males possess truncated or blunt terminus whereas females have a rounded terminus. The genital segments of the male form a circular cup, but in female it appears as triangular in shape (Eger *et al*., 2010; Zhang *et al*., 2012) (Plate.2).

M. cribraria undergoes hemimetabolous development consists of egg stage, five nymphal stages and adult stage *M. cribraria* laid eggs in two parallel rows and they were most commonly found on the tender leaf sheaths of the growing vine tips but a few also were found on the underside of leaves and older vines of host plant (Ramakrishna Ayyar, 1913; Zhang *et al*., 2012) (Plate.3). The neonates of hatched egg mass aggregate on their own on hatched egg mass and feed on the brown-coloured endosymbiont capsules underneath the egg mass which were deposited by the females (Fukatsu and Hosokawa, 2002; Jenkins *et al*., 2010). These two essential obligate bacterial endosymbionts were the primary γ-proteobacterial, *Candidatus Ishikawaella* capulata and the secondary α-proteobacterium, *Wolbachia* (Hosokawa *et al*., 2005; Hosokawa *et al*., 2007; Jenkins *et al*., 2010), which plays an essential role for proper development, increased fecundity and also support the ecological expansion of *M. cribraria* (Jenkins and Eaton, 2011).

Additionally, Fukatsu and Hosokawa, (2002) and Hosokawa *et al*., (2006) reported that the development period from first instar to adult was significantly longer, and the adult survival rate, body size and pigmentation significantly reduced after the removal of endosymbiont capsules.

General features of each instar from first-to-fifth of kudzu bugs were achieved by measuring body size, observing coloration of the body, noting scalloping of the lateral margin, monitoring development of the wing pads, and counting the number of setae (Ahmad and Moizuddin, 1975). However, Zhang *et al*., (2012) reported that width across the eyes was the most stable parameter for distinguishing instars in addition to overall size. The studies revealed that the developmental duration from egg to adult ranges from 45-58 days and for the completion of the whole life cycle it requires 45-290 days depending on various host plants (Ramakrishna Ayyar, 1913; Ahmad and Moizuddin, 1977; Tayutivutikul and Yano, 1990; Srinivasaperumal *et al*., 1992; Tayutivutikul and Kusigemati, 1992b; Wu *et al*., 1992; Thippeswamy and Rajagopal, 2005b; Del Pozo-Valdivia and Reisig, 2013; Shi *et al*., 2014; Golec *et al*., 2015)

In warmer areas, kudzu bugs may be active around the year (Thippeswamy and Rajagopal, 1998). However, when temperatures decrease, adults start to locate overwintering areas, and enter dormancy during the winter. In spring when temperatures warm, adults will again become active. Golec and Hu, (2015) reported that approximately 15% of females mated before entering winter dormancy and sperm was stored in their spermatheca for up to seven months, oocytes in mated overwintering females proceeded to postblastoderm stage before the onset of spring feeding and mating and all the overwintering males had sperm in their testes, and the ratio of females gradually increased in populations during overwintering. Male and female longevity varies widely depending on the temperature and generation (Wu *et al*., 1992). Zhang and Yu, (2005) stated that first generation adults live 1.5-3 months in a bivoltine population of *M. cribraria* from China, whereas the second generation lives 9-10 months. These differences in adult longevity of subsequent generations arise due to the overwintering dormancy of the second-generation adults and therefore different physiological and ethological profiles for each generation.

The family Plataspidae is preferentially feed on legume species and has become problematic to the production of legume crops throughout the Old World (Yang, 1934;

Hasegawa, 1965; Lal, 1980; Ren, 1984). Adults and nymphs of *M. cribraria* feed on tender stems petioles, leaves, pods, and flowers (Zhang *et al*., 2012; Seiter *et al*., 2013). Damage caused by the feeding activity is seen as purple spots that later coalesce to form large black necrotic regions (Thippeswamy and Rajagopal, 2005a), and extensive feeding may result in defoliation (Chaterjee, 1934). As it is a phloem feeder, *M. cribraria* produces copious honeydew that results in secondary plant issues such as black sooty mold that covers stems and leaves, reduces photosynthesis, and ultimately results in yield loss (Xing *et al*., 2006; Zhang *et al*., 2012). Thippeswamy and Rajagopal, (2005a) studied the life history of *M. cribraria* on field bean, *L. purpureus* var. *lignosus Medikus*, but life table of *M. cribraria* was not studied.

Life-table is the most important basis for qualitative and quantitative study of population ecology which was first applied to insect population by Morris and Miller, (1954). Life tables are used to calculate the vital statistics on pest population dynamics and also give a comprehensive description of the survivorship, development, stage differentiation, fecundity, mortality life expectancy and provides basic data on population growth parameters (Wakisaka *et al*., 1992; Carey, 1993; Southwood, 1995; Harari *et al*., 1997; Medeiros *et al*., 2000; Haseeb *et al*., 2001; Sarfraz *et al*., 2007; Ali and Rizvi, 2008). These tables also describe duration and survival at each life stage in insect life cycle, which allow prediction of the population size and age structure of a pest insect at any time (Carey, 1993; Southwood, 1995). It plays a major role in insect pest management, where developmental stages are discrete and mortality rates may vary widely from one life stage to another (Kakde *et al*., 2014). Thus, by knowing such most vulnerable stages from life table, it may be possible to prepare a time-based application of different control measures for proper management of the pest population.

Traditional female age-specifc life tables (Lewis, 1942; Leslie, 1945; Birch, 1948) are based on only with female populations and ignore the variable developmental rates among individuals and stage differentiation. However, most of the pests are bisexual, and both sexes may cause economical loss. Moreover, developmental rates are also differed between the sexes and among individuals of pest (Istock, 1981). The age-stage, two-sex life table represents an improvement over traditional life table, because it considers males and can describe stage differentiation (Chi and Liu, 1985; Chi, 1988). Furthermore, this type of life table is designed to incorporates survival and fecundity data properly and

takes sex ratio into account, so it is more powerful in the analysis of population development (Chi, 1988). The application of life table includes analysing population stability and structure, estimating extinction probabilities, predicting life history evolution, predicting outbreak in pest species, and examining the dynamics of colonizing or invading species (Vargas *et al*., 1997; Haghani *et al.*, 2006). Life table information may also be useful for predicting population models (Carey, 1993, 2001) and understanding interactions of insect pests and their natural enemies (Omer *et al*., 1996; Chi and Yang, 2003; Yu *et al*., 2005; Chi and Su, 2006).

Environmental factors affect insect population dynamics by influencing development, survival, and fecundity (Logan *et al*., 2006; Broufas *et al*., 2009; Forster *et al*., 2011). Being poikilothermic organisms temperature is one of the most important environmental factors influencing behaviour, distribution, development, survival and reproduction in insects (Huffaker, 1944; Bale *et al*., 2002; Regniere *et al*., 2012). Changes in temperature have impacts on both individual and population level of insects (Nardoni and Belvosky, 2010). Optimal temperature is required for rapid development and reproduction of insects, while temperatures above or below this range can have adverse effects (Zhou *et al*., 2010; Regniere *et al*., 2012) Knowledge on the temperature-dependent population growth potential of insect pests is highly crucial for understanding changes in population dynamics and developing sustainable and environment-friendly pest control strategies (Briere *et al*., 1999; Jarvis and Baker, 2001; Kroschel *et al*., 2013).

Insect phenology and geographical distribution is determined by climate (Wellington, 1957; Hill *et al*., 2011). In addition to commonly considered temperature variables, rainfall and the associated relative humidity can also greatly affect development, fecundity, survival, behaviour (Willmer, 1982; Guarneri *et al*., 2002; Broufas *et al*., 2009) and the population dynamics of insects, but the effect may vary between species or developmental stages (Barlow and Mutchmor, 1963; King, 1972; Mullens and Peterson, 2005; Day, 2006). The RH significantly affects the life table characteristics such as the intrinsic capacity to increase (r_m), net production rate (Ro), the finite rate of increase (γ) and generation time. The combined effect of temperature, rainfall, and the associated ambient humidity have been widely used for pest forecasting and extent of pest infestation (Zhang and Zhang, 2006; Yang *et al*., 2009; Feng *et al*., 2010), to develop strategies for establishing biocontrol agents (Fenoglio and Trumper,

2007), and even to predict long-term shifts of insect populations dynamics and distribution under global climate change (Williams and Liebhold, 2002; Vanhanen *et al*., 2007; Seidl *et al*., 2009).

The ability of phytophagous insects to select a host plant depends on the combination of both extrinsic and intrinsic factors (Bell, 1990). The factors that influence host selection by phytophagous insects includes chemical and physical properties of plants, the insect's internal state, environmental characteristics, interactions like competition, predation etc. (Perez-Contreras, 1999). So, the host plants are not equally attractive for all insects of a species, which may establish a gradient order of host preferences (Singer *et al*., 1992). Host plants emit certain volatile compounds that mediate insect–plant interactions. These compounds act as either attractant or repellent effects on insects which can be used as pest management. The chemical composition and concentration of host plants varies between plant species, and also between different plant parts within the same plant (Schoonhoven *et al*., 2005) that affects food selection by herbivorous insects. Phytophagous insects have the ability to locate host plants both at the individual and population levels. They can cause stunted growth or death of the host plant and significantly influence population dynamics by feeding on flowers, fruits or seeds (Dajoz, 2001).

The mating performance in insects is associated with various factors such as mating or reproductive history (Uhia and Rivera, 2005; Kant *et al*., 2012; Colares *et al*., 2015), age (Kawazu *et al*., 2014; Liu *et al*., 2014; Ekanayake *et al*., 2017), nutritional condition (Aluja *et al*., 2009), body size/mass (Aluja *et al*., 2009; De Luca, 2015; Ekanayake *et al*., 2017), and population density (Shelly *et al*., 2013). The males and females of many insect species form aggregations and mate in them. Two major types of aggregations can be seen in insects (Alexander, 1975). In first one, females aggregate in a particular place like oviposition site or feeding site where males then visit (Fujisaki, 1980, 1981; Mitchell, 1980; McLain, 1984, 1992; Miyatake, 1995) and the other one is often called leks in which males aggregate and appear to attract females (Shelly and Whitter, 1997). In many insect species, prolonged copulation is a common in which genital contact is maintained after insemination has been completed (Thornhill and Alcock, 1983; Alcock, 1994). This phenomenon is explained by postcopulatory mate guarding hypothesis which assumes that males gain fitness through prolonged copulation

by preventing females from remating with other rival males and thereby avoid sperm competition (Parker, 1970; Simmons and Siva-Jothy, 1998).

The true bugs produce a wide variety of chemical compounds that show potential to manage these insects. These bugs, when disturbed produce a pungent odour that consist of a complex mixture of compounds, some of which could be toxic or irritating to predators while others may act as chemical warning signals perceived either by olfaction (highly volatile compounds) or by taste or chemesthesis (Aldrich, 1988; Farine *et al*., 1992; Svadova *et al*., 2013). The Pentatomidae produce these compounds in two different types of glands depending on their life stage (Aldrich, 1988). In the adults the metathoracic scent glands produce these compounds while nymphs produce them in the dorsal abdominal scent glands, these glands can be retained in some Pentatomid adults as functional glands (Aldrich, 1988; Aldrich *et al*., 1995). The common compounds of defensive secretion include short-chain alcohols, aldehydes, oxo-aldehydes, ketones, esters, alkanes, organic acids, monoterpenes and aromatic alcohol/aldehydes (Aldrich, 1988; Moraes *et al*., 2008; Krajicek *et al*., 2016) and the composition of the secretion may depend on the physiological, nutritional and developmental state of the individual and on the season (Gunawardena, 1994). Additionally, the composition of the scent-gland secretion is taxon-specific and sexually dimorphic (Vet, 1999). *M. cribraria* also produce a pungent odour which is used for expelling enemies or for communicating with same species.

Semiochemicals are substances or mixture of substances that act as signals and enable intra- and inter-specific chemical communication (Nordlund and Lewis, 1976; Dicke and Sabelis, 1988; Landolt and Phillips, 1997). They are mainly used for modulating physiological and behavioural activities through the olfactory and taste system (Dicke and Sabelis, 1988; El-Shafe and Faleiro, 2017). The chemicals induce inhibitory, stimulatory or deterrent behaviours in target organisms (El-Sayed, 2015; El-Shafe and Faleiro, 2017). Semiochemicals that mediate intraspecific communication between individuals of the same species are called pheromones; whereas, those that modulate communication between different species are termed allelochemicals (Nordlund and Lewis, 1976; Dicke and Sabelis, 1988; El-Shafe and Faleiro, 2017). The biology of insects depends on pheromones for mating, foraging, aggregation, trailing, dispersion, alarm, territoriality and oviposition behaviour (Navarro-Silva *et al*., 2009;

Stökl and Steiger, 2017; Watentena and Okoye, 2019).

The visual system in insects can detect external light stimulation, which plays an important role in insect foraging, courtship, communication and avoidance of natural enemies (Chapman, 1998). Insects developed phototropic behaviours in response to light sources, which include positive phototropic behaviour toward light sources and negative phototropic behaviour away from light sources (Briscoe and Chittka, 2001). The visual spectrum in insects is mostly concentrated in the wavelength range of 253–700 nm and insects in the same taxonomic group generally have similar responses to light of different wavelengths and preferences for wavelength ranges (Prokopy and Owens, 1983; Briscoe and Chittka, 2001). Recently, LED technology has been used in insect traps which improved trap efficiency by using less energy and producing narrow-spectral bands of light (Nakamoto and Kuba, 2004; Kim and Lee, 2013; Park *et al*. 2015; Xu *et al*., 2017; Sang *et al*., 2018). So, the replacement of traditional light-traps by LED traps will have a significant impact on long-term and area-wide monitoring of pest insects with light-traps and the further use of historical datasets in pest population forecasting.

Biological control is a component of an integrated pest management (IPM) strategy involve the effective means of reducing or mitigating pests and pest effects through the use of natural enemies. It relies on the beneficial action of natural enemies such as predators, parasites, pathogens, and competitors in controlling pests and their damage (Dreistadt, 2007). Biological control for agricultural systems is very old (around 2000 years back) tradition but the modern practices started in the late nineteenth century (DeBach, 1964; Van Lenteren and Godfray, 2005). Biological control differs from other forms of pest control by acting in a density-dependent manner (De Bach and Rosen, 1991). Natural, importation (sometimes called classical biological control), augmentation and conservation are the four different types of biological control (Eilenberg *et al*., 2001; Weller *et al*., 2002; Cock *et al*., 2010).

The survey findings were important in the identification of the key pest populations and the potential natural enemies that could be used for their management. Visual examination of the plants, shaking and beating, sweeping as well as destructive samplings were usually carried out to identify the pests and natural enemies in the field. According to Vacante and Bonsignore, (2017) monitoring is a fundamental step in assessing the dynamics of pests and natural enemy populations in the field, in order to

estimate their presence, possible damage caused by pests, and the population development of natural enemies.

Egg parasitoids show remarkable potential as biological control agents of a wide range of economically important agricultural and forest pests and the most widely produced and released natural enemies in biological control throughout the world (Li, 1994; Van Lenteren, 2000). Parasitoids representing some 15 families of Hymenoptera which include egg parasitoids. They represent the largest group of entomophagous insects. Egg parasitoids belong to Platygastridae (Scelionidae), Mymaridae, and Encyrtidae families were most commonly associated with Heteroptera (Bin, 1994). They usually attack the host before it develops and inflict feeding damage, egg parasitoids show remarkable potential as biological control agent. The host selection process of female parasitoid involves a sequence of phases mediated by physical as well as chemical stimuli from the host, the substrate, and/or associated organisms, eventually leading to successful parasitism (Vinson, 1985; Vet and Dicke, 1992; Godfray, 1994; Vinson, 1998; Hilker and McNeil, 2007).

Phoresy is common phenomenon in the arthropod world (Ferrière, 1926; Clausen, 1976). Phoresy involve the transport of adult parasite or predator females upon the bodies of the host adults and is mostly restricted to egg parasitoids (Clausen, 1976). The great advantage of phoresy among the egg parasites is to reduce the spatial and temporal discontinuity between where hosts mate and where host females oviposit (Clausen, 1976; Vinson, 1998; Fatourous and Huigens, 2012) and facilitates dispersal of the parasitoids along with their hosts. In phoretic species, the host egg age and quality play a critical role in the success of parasitism.

Success in biological control depends on identification of the best host species as well as a good understanding of the ecological requirements of the parasitoid wasp. The effectiveness of egg parasitoids is directly related to its fitness. Survival, fecundity, and development time are the most important parameters that determine the fitness of egg parasitoid wasp. This can be achieved by constructing life tables which evaluate a parasitoid against a host under various climate conditions and host habitats (Birch, 1948; Leslie and Park, 1949; Messenger, 1964; Jervis and Copland, 1996). Furthermore, such demographic data can be very useful for selecting the most effective biocontrol agents, planning mass rearing programs as well as the timing of introduction in inoculative

releases.

The mass-rearing and release of natural enemies are the fundamental basis of augmentative biological control programmes (King, 1993; Elzen and King, 1999; Morales-Ramos and Rojas, 2003). The success of biological control using egg parasitoids depends on the efficiency of mass rearing of the parasitoids. The mass rearing of parasitoids primarily depends on food and it has a significant effect on developmental time, survival, fecundity and longevity of parasitoid species (Hohmann *et al*., 1988; Fuchsberg *et al*., 2007; Özder and Kara, 2010; Tunçbilek *et al*., 2012; Çınar *et al*., 2015). In the field parasitoids can gain carbohydrates in homopteran honeydew, floral and extrafloral nectar (Wäckers *et al*., 2008). Several studies have provided evidence for increased parasitoid abundance and parasitism level when flowering plants are present (Berndt *et al*., 2006; Diaz *et al*., 2012; Masetti *et al*., 2010; Zhu *et al*., 2013). Development of a high-quality artificial diet is more cost-effective method for the mass rearing of parasitoids (Li, 1992; Cônsoli & Parra, 1997, 1999; Grenier, 1997).

The most common mode of reproduction in Hymenoptera is arrhenotoky, characterized by the production of haploid males from unfertilized eggs and fertilized eggs that produce diploid females. Another less common mode of reproduction is thelytoky, that involves the production of diploid females from fertilized and unfertilized eggs (Pintureau *et al*., 1999). In arrhenotokous species, mating is an important factor to consider for the successful use of these species in the field, because unfertilized eggs produce male individuals, which can compromise the maintenance of the parasitoid in the field (Pratissoli *et al*., 2009; Farrokhi *et al*., 2010). On the other hand, field releases of thelytokous populations can be more efficient than arrhenotokous populations (Stouthamer, 1993) because both fertilized and unfertilized eggs will produce females (Hohmann *et al*., 2002).

Egg parasitoids use certain clues to locate hosts as the duration of the egg stage is generally short. Old host eggs have adverse effect on several parasitoid biological parameters, such as percentage parasitism, developmental time (Da Rocha *et al*., 2006), adult emergence (Bruce *et al*., 2009), body size and sex ratio (Ruberson and Kring, 1993). The increase in host egg age can be restrictive because host embryo development depletes the nutrients stored in the egg. Therefore, old eggs were usually considered as low-quality hosts for egg parasitoids (Souza and Spence, 2001; Tunçbilek and Ayvaz, 2003).

Moreover, Strand *et al*., (1986) pointed out that a parasitoid larva cannot capable of digesting the cuticle of its host and therefore cannot consume nutrients from embryos in an advanced stage of development of host eggs. Thus, egg parasitoids usually prefer young or intermediate aged eggs for parasitism because of their better nutritional quality for the development of parasitoid offspring (Reznik and Umarova, 1990; Monje *et al*., 1999; Moreno *et al*., 2009), including members of the Scelionidae (Romeis *et al*., 2000; Da Rocha *et al*., 2006). However, few studies have reports that old eggs do not have any deleterious effect on preference or offspring performance (Pak *et al*., 1986; Jacob *et al*., 2006).

Climatic tolerance of natural enemies is a key factor that determines the establishment and effectiveness of biological control programmes (DeBach, 1965 a,b; Messenger *et al*., 1976 a,b; Hokkanen, 1985). Temperature is an important abiotic factor that strongly determines biological parameters such as the development rate, survival, longevity, parasitism rate, viability, sex ratio, and emergence rate of egg parasitoids (Messenger, 1970; Frazier *et al*., 2006; Moezipour *et al*., 2008; Iranipour *et al*., 2009). Understanding the thermal requirements of egg parasitoids is essential for planning its mass rearing in laboratory and to determine its potential as a biological control agent for a given pest and region.

The parasitism efficiency of natural enemies was evaluated by different techniques, which are classified into direct and indirect techniques. Direct techniques include parasitism rate estimation in the field and laboratory (Prasad, 1989), through parasitism of placed out pray or biological check methods (Jervis, 2005). The most common indirect techniques include observation of pest populations in the presence or absence of natural enemies, such as exclusion of natural enemies or removal of natural enemies (Sule *et al*., 2014). The potential of a parasitoid can be evaluated under laboratory conditions leading to a good idea about the efficacy of the parasitoid. Ode and Heinz, (2002) stated that the efficacy of a parasitoids depends on its ability to locate suitably sized hosts within crop habitats and to kill larvae through host feeding or parasitism. Moreover, the functional response of a parasitoid is a key factor in regulating population dynamics of host-parasite interaction (Pervez and Omkar, 2005), it can be used to verify the efficiency of a parasitoid in managing host populations.

The present study was undertaken with a view to carry out the life history, ecological parameters and natural enemies of *M. cribraria*. This will definitely help in the prediction of emergence of pest population, period of exposure of insecticides, stage of application, exposure of interval etc. Moreover prior to the application of any control measures for any pest, information on its life history, ecological parameter and knowledge about natural enemies are essential. Hence the following objectives are considered for the present study.

OBJECTIVES

1. To study the life history of *M. cribraria* (F.) on *L. purpureus* (L.) var. *Hima*
2. To study the effect of different ecological parameters on the biology and survival of *M. cribraria* (F.) on *L. purpureus* in the laboratory.

    2.1. Effect of different levels of temperature on the life history of *M. cribraria* on *L. purpureus*

    2.2. Effect of different levels of R.H. on the life history of *M. cribraria* on *L. purpureus*
3. To study certain behavioural parameters of *M. cribraria*

    3.1. Food preference, 3.2. Mating, 3.3. Defensive, 3.4. Phototropic and 3.5. Semiochemical response
4. To conduct extensive surveys for identifying natural enemy (predators /parasitoids/ entomopathogens) of *M. cribraria* (F.) in the field
5. To study the life table and develop mass rearing techniques of the most effective biological control agent identified in survey

    5.1. Rearing of biological control agent in the laboratory.

    5.2. To study the effect of mating on parasitism, emergence and sex ratio of biological control

    5.3. To study the effect of host egg age on parasitism, emergence and sex ratio of biological control

    5.4. To study the effect of different levels of temperature on the parasitism and life history of biological control
6. To evaluate the efficiency of biological control agent in the laboratory and in the controlled field conditions [micro plot with potted plants]

CHAPTER 2

REVIEW OF LITERATURE

Lablab is one of the most agro-morphologically diverse (Piper and Morse, 1915; Rivals, 1953; Pengelly and Maass, 2001; Mohan and Aghora, 2006; Islam, 2008) versatile tropical legume species has been documented by archaeo-botanical finds in India prior to 1500 BC (Fuller, 2003) and at Qasr Ibrim in Egyptian Nubia from the 4th century AD (Clapham and Rowley-Conwy, 2007). Lablab is used as pulse, vegetable, forage/green manure, herbal medicine, and even ornamental (Adebisi and Bosch, 2004; NRC, 2006). Additionally, Morris (2009) reviewed its bio-functional properties for use as pharmaceutical or nutraceutical.

Hemipteran insects normally occur as pests on various agriculturally important plants. They pierce tissues of host plants and feed on their juices (Knight, 1941; Mc-Gavin, 1992) lived as entomophagous (Hassanzadeh *et al*., 2009) and many of them are serious pests of plants (Safavi, 1973). Many bug species catch other insects and Acarina and hence beneficial from an agricultural point of view (Linnavuori and Hosseini, 2000; Chandra *et al*., 2011; Chandra and Kushwaha, 2013).

Chandra and Kushwaha, (2013) reported that a total of seven hemipteran pests were found on *L. purpureus*, three of them are from suborder Homopteraviz. *Aphis craccivora* Koch, *Eurybrachys tomentosa* Fabricius and *Leptocentrus taurus* Fabricius, beside these, four of them are belonging to suborder Heteroptera viz *Nezara viridula* L., *Megacopta cribraria* Fabricius, *Riptortus pedestris* Fabricius and *Dolicoris indicus*. Among all of them *Aphis craccivora* Koch and *Megacopta cribraria* showed maximum damage to the lablab plant, as they were found active to the plant throughout the survey. In addition to these *Cheilomenes sexmaculata* Fabricius, an aphidophagous ladybird beetle was also reported from lablab plants of Jabalpur Madhya Pradesh.

Megacopta cribraria was first described from Indian specimens as *Cimex cribraria* by Fabricius, in 1798, later it has been described as *Tetyra cribraria* (Fabricius, 1803), *Thyreocoris cribarius* [sic] (Burmeister, 1835), *Coptosoma cribrarium* (Amyot and Serville, 1843), *Coptosoma xanthochlora* Walker (Walker, 1867); *Coptosoma cribrarium* (Ahmad and Moizuddin, 1975). These names were synonymized as *Megacopta cribraria* by Hsiao and Ren, in 1977. Montandon, (1896) described a closely

related species, *M. punctatissima* (as *Coptosoma punctatissimum*) which exhibits larger body size and darker body colour than

M. cribraria. In the following year, Montandon, (1897) found specimens that were intermediate between *M. cribraria* and *M. punctatissima* but did not formally synonymize the two species. Yang, (1934) revised all 44 Chinese plataspid species and considered *M. punctatissima* to be a variety of *M. cribraria*. However, both names still be used today; especially in Japanese economic literature (Ishihara, 1950; Hasegawa, 1965; Hibino and Ito, 1983; Hirashima, 1989; Imura, 2003; Himuro *et al*., 2006). Hosokawa *et al*., (2007) stated that *M. punctatissima* was a frequent pest of soybean found in mainland Japan, whereas *M. cribraria* was found in the southwestern Japanese islands, rarely causes agricultural problems and considered as harmless to soybean and these two species were capable of interbreeding and their offspring reproduce successfully.

Fukatsu and Hosokawa, (2002) and Hosokawa *et al*., (2006) reported that Females of *M. cribraria* and related species of Plataspidae deposit small brown capsules on the underside of the egg masses and these capsules contain gut symbiotic bacteria (-Proteobacterium *Candidatus Ishikawaella* capsulata). Jenkins *et al*., (2010) found that the 16*S* rRNA gene and the *wsp* gene of the bacterial symbionts of *M. cribraria* were closer to the gene of *Candidatus Ishikawaella* capsulate from *M. punctatissima.* Additionally, the *groEL* chaperone gene was 99% identified from the GenBank sequences of *Candidatus Ishikawaella* capsulate from *M. punctatissima* and *M. cribraria.* Hosokawa *et al*., (2014) strongly suggests that the invading *M. cribraria* populations in the U.S. are derived from a *M. punctatissima* population in the Kyushu region in Japan base on phylogeographical analyses of 8.7 kb mitochondrial DNA sequences of both introduced and East Asian native *Megacopta* populations.

Prior to 2009, *Megacopta* spp. are restricted to the Old World and Oceania where they have been reported from Australia, China, India, Indonesia, Japan, Korea, Macao, Malaysia, Myanmar, New Caledonia, Pakistan, Sri Lanka, Taiwan, Thailand and Vietnam (Montandon, 1896, 1897; Distant, 1902; Kirkaldy, 1910; Matsumura, 1910; Shroff, 1920; Esaki, 1926; Hoffman, 1931, 1935; Yang, 1934; Ishihara, 1937; Esaki and Ishihara, 1951; Ahmad and Moizuddin, 1975; Hsiao and Ren, 1977; Lal, 1980; Ren, 1984; Hirashima, 1989; Easton and Pun, 1997). Froeschner, (1984) concluded that no species of Plataspidae inhabited North America later, Suiter *et al*., (2010) discovered large numbers of *M.*

cribraria associated with kudzu, *Pueraria montana* Lour. (Merr.) variety *lobata* (Willd.), in northeast Georgia were a new United State record and the first known establishment of a species of Plataspidae in the Western Hemisphere in October 2009. Since its first detection, the kudzu bug has spread rapidly throughout13 U.S. states: Alabama, Arkansas, North Carolina, South Carolina, Mississippi, Louisiana, Kentucky, Florida, Tennessee, Virginia, Delaware, Maryland, and Washington D.C. (Gardner *et al*., 2013a).

Eger *et al*., (2010) referenced 20 host legume species and 14 non-leguminous species spanning 14 plant families in its native range. The preferred leguminous (Fabaceae) host plants of *M. cribraria* are *Astragalus sinicus* (L.) (Tayutivutikul and Kusigemati, 1992a), *Cajanus indicus* Spreng (Ramakrishna Ayyar, 1913; Shroff, 1920; Fletcher, 1921; Hoffman, 1932; Borah and Dutta, 1999; Thippeswamy and Rajagopal, 2005b), *Cyanopsis tetragonoloba* (L.) Taub. (as *Cyanopsis psoraloides)* (Ramakrishna Ayyar, 1913; Fletcher, 1921; Hoffmann, 1932), *Glycine max* var. Merill (Ishihara, 1950; Kobayashi, 1981; Kono, 1990; Tayutivutikul and Kusigemati, 1992a; Takagi and Murakami, 1997; Wu and Xu, 2002; Thippeswamy and Rajagopal, 2005b; Xing *et al*., 2006, 2008), *Indigofera* sp. (Ramakrishna Ayyar, 1913), *Lablab purpureus* (L.) (Ramakrishna Ayyar, 1913; Shroff, 1920; Fletcher, 1921; Hoffmann, 1932; Ahmad and Moizuddin, 1975; Thippeswamy and Rajagopal, 1998, 2005a, b; Thejaswi *et al*., 2008), *Lespedeza cyrtobotrya* Miq. (Hibino and Ito, 1983; Tayutivutikul and Kusigemati, 1992a), *Mucuna pruriens* (L.) DC. (Rani and Sridhar, 2004), *Phaseolus lunatus* (L.) (Hoffmann, 1931), *Phaseolus radiatus* (L.) (Shroff, 1920; Easton and Pun, 1997), *Phaseolus* spp. (Hoffmann, 1932), *Phaseolus vulgaris* (L.) (Ishihara, 1950; Easton and Pun, 1997), *Pongamia pinnata* (L.) Pierre (Hoffmann, 1932), *Pueraria montana* (Lour.) Merr. variety *lobata* (Willd.) (Kershaw, 1910; Ishihara, 1950; Hibino and Ito, 1983; Tayutivutikul and Yano, 1990; Tayutivutikul and Kusigemati, 1992a, b; Sun *et al*., 2006; Hosokawa *et al*., 2007), *Sesbania grandiflora* (L.) Pers. (Ramakrishna Ayyar, 1913; Fletcher, 1921; Hoffmann, 1932), *Vigna angularis* (Willd.) Ohwi and Ohashi (Tayutivutikul and Kusigemati, 1992a), *Vicia angustifolia* (L.) (Hibino and Ito, 1983; Easton and Pun, 1997), *Vicia faba* (L.) (Ishihara, 1950), *Vigna mungo* (L.) Hepper (as *Phaseolus mungo* L.) (Fletcher, 1921), and *Wisteria brachybotrys* Sieb.et Zucc. (Tayutivutikul and Kusigemati, 1992a).

Non-leguminous hosts reported for *M. cribraria* are *Citrus* spp. (Tayutivutikul and Kusigemati, 1992a), Compositae (Hoffmann, 1932), *Corchorus capsularis* (L.) (Hoffmann, 1932), *Crossandra infundibuliformis* (L.) Nees (Srinivasaperumal *et al*., 1992), *Deutzia crenata* Siebold and Zucc (Tayutivutikul and Kusigemati, 1992a), *Gossypium hirsutum* (L.) (Srinivasaperumal *et al*., 1992), *Ipomoea batatas* Lam. (Hoffmann, 1932), *Ligustrum sinense* Lour. (Zhang *et al*., 2008), *Morus alba* (L.) (Zhang, 1985), *Oryza sativa* (L.) (Hoffmann, 1932), *Saccharum officinarum* (L.) (Hoffmann, 1932), *Solanum carolinense* (L.) (Imura, 2003), *Solanum tuberosum* (L.) (Hoffmann, 1932) and *Triticum aestivum* (L.) (Tayutivutikul and Kusigemati, 1992a). It is also listed as a pest of Chinese fruit trees including peach (*Amygdalus persica* Linn.), plums (*Prunus* spp.), and jujube (*Ziziphus jujube* Mill.) (Wang *et al*., 1996; Li *et al*., 2001; Wang *et al*., 2004).

M. cribraria have also been investigated as a potential biological control agents for kudzu, an economically important invasive weed, it also poses a threat to soybean and other legume crops (Ishihara, 1950; Tayutivutikul and Yano, 1990; Tayutivutikul and Kusigemati, 1992a; Sun *et al*., 2006). Studies by Zhang *et al*., (2012) demonstrated that *M. cribraria* was found to feed on 10 forest legume species and non-selectively oviposit on 8 species, although adults developed only on soybean and kudzu. The host suitability of kudzu bug is evaluated in 12 plants, including 11 legume species in greenhouse no-choice tests and found that besides kudzu and soybean, *M. cribraria* also can complete development on pigeon pea, followed by black-eye pea (*Vigna sinensis* L.), lima bean (*Phaseolus lunatus* L.) and pinto bean (*Phaseolus vulgaris* L.) (Medal *et al*., 2013). The reproduction host of *M. cribraria* includes mung bean (*Vigna radiate* L. Wilczek) (Golec *et al*., 2015), fava bean (*Vicia faba* L.) (Blount *et al*., 2015), firecracker plant, *Crossandra infundibuliformis* (L.) Nees (Acanthaceae) cotton, *Gossypium hirsutum* (L.) (Malvaceae) and hummingbird tree (*Sesbania grandiflora* Pers.) (Srinivasaperumal *et al*., 1992). Though *M. cribraria* cause economic damage to a wide range of hosts, many crop plants may as bridge hosts to avoid overspecialization on preferred host plants, which are only available at certain seasons (Bernays and Chapman, 1994; Palumbo *et al*., 2016) and essential for population increase before dispersal, as observed in pentatomid pests (Panizzi, 1997).

Ramakrishna Ayyar, (1913) superficially illustrated and described the different immature stages collected from southern India as *C. cribraria*. A broad set of descriptions, illustrations, and accompanying tables of measurements were published by Ahmad and Moizuddin, (1975) from collections made in Karachi, Pakistan as *C. cribrarium* and also by Thippeswamy and Rajagopal, (2005b) from collections made in Bangalore, India as *C. cribraria*. Eger *et al*., (2010) and Zhang *et al*., (2012) collected and described *M. cribraria* Specimens from US.

Like all other Heteroptera, *M. cribraria* undergoes hemimetabolous development and its survival, development and reproduction depend on location, host plants, temperature and other conditions (Ramakrishna Ayyar, 1913; Tayutivutikul and Yano, 1990; Srinivasaperumal *et al*., 1992; Tayutivutikul and Kusigemati, 1992b; Ahmad and Moizuddin, 1977; Thippeswamy and Rajagopal, 2005a, b). Similarly, Observations field tests and greenhouse evaluations were carried out to confirm the life history of *M. cribraria* on soybean and kudzu in the US (Zhang *et al*., 2012; Gardner *et al*., 2013a; Medal *et al*., 2013; Seiter *et al*., 2013; Blount *et al*., 2015; Golec *et al*., 2015). *M. cribraria* population colonized crop fields in April to July and were present until August to October, depending on location and crop (Hibino and Ito, 1983; Tayutivutikul and Yano, 1990; Takagi and Murakami, 1997; Thejaswi *et al*., 2008). In warmer areas, they may be active throughout the year (Thippeswamy and Rajagopal, 1998). The number of generations varies from one to three in a year and *M. cribraria* overwinter as adults (Hibino and Ito, 1983; Tayutivutikul and Kusigemati, 1992b; Wu *et al*., 2006).

Srinivasaperumal *et al*., (1992) studied the host plant preference and life table parameters of *M. cribraria* on *Sesbania grandiflora* Pers., *Crossandra infundibuliformis* (L.) Nees and *Gossypium hirsutum* (L.) and reported the faster development, higher survival rate and higher fecundity were found on *S. grandiflora* as a result its life table characteristics such as intrinsic rate of natural increase (r_m), finite rate of increase (λ), net reproductive rate on this plant were higher than on the other plants.

Many studies were carried to evaluate the effects of temperatures on phytophagous stink bugs in Hemiptera, such as the rice stink bug, *Oeblus pugnax* (F.) (Naresh and Smith, 1983); the squash bug, *Anasa tristis* DeGeer (Fargo and Bonjour, 1988); the Neotropical bug, *Ischnodemus variegates* (Signoret) (Diaz *et al*., 2008); the tarnished plant bug, *Lygus elisus* Van Duzee (Bommireddy *et al*., 2004), and the western

tarnished plant bug, *Lygus hesperus* Knight (Cooper and Spurgeon, 2012). The effect of temperature on development of plataspids was documented first time by Shi *et al*., (2014) and found that temperature had a strong effect on life table parameters through affecting the survival, longevity, and fecundity of *M. cribraria* reared on soybeans. Furthermore, the population growth of *M. cribraria* was restricted at temperatures 17 or 33^0C and the population trend index was highest at 25^0C.

Heavy rainfall will directly result in an increase of environmental humidity. Previous studies demonstrated that an increase in RH after a heavy rainfall promoted directly the increase in population levels of *Adelphocoris* species and that RH may be a key factor associated with the outbreaks of *Adelphocoris* species observed after rainfall in China (Chu and Meng, 1958; Ting, 1964; Cao and Wan, 1983; Wu *et al*., 2002; Lu *et al*., 2008). Relative humidity was a key determinant in the immature survival and development, and adult longevity and fecundity of *Apolygus lucorum* (Meyer-Dur) (Heteroptera: Miridae) (Lu and Wu, 2011) and three *Adelphocoris* species (*Adelphocoris lineolatus* Goeze, *Adelphocoris suturalis* Jakovlev and *Adelphocoris fasciaticollis* Reuter) (Pan *et al*., 2014). Moreover, RH significantly affects the intrinsic capacity for increase (r_m), net production (Ro), and the finite rate of increase (λ) in Hemiptera (Lu and Wu, 2011; Pan *et al*., 2014).

M. cribraria feed on phloem sap of above-ground plant parts, mainly towards the top of the stem of their host plant, or at the base of the petiole (Hibino and Ito, 1983; Tayutivutikul and Yano, 1990; Thippeswamy and Rajagopal, 2005a, b; Kikuchi and Kobayashi, 2010; Seiter *et al*., 2013). Although Thippeswamy and Rajagopal, (2005b) and Wu *et al*., (2006) reported that adults and nymphs feeding on bean pods, but there was no evidence of direct damage to the seed (Seiter *et al*., 2013). Bernays and Chapman, (1994) reported that phytophagous insects use host plants for feeding and developing. The host plants produce specific cues that can be reliable and detectable by monophagous or oligophagous insects (Jermy *et al*., 1988). Among the cues, plants emit volatile organic compounds (VOCs), which have high vapour pressures that enable their evaporation into the surrounding air and play a predominant role in mediating orientation of phytophagous insects (Visser, 1986; Finch and Collier, 2000; Bruce *et al*., 2005; Schoonhoven *et al*., 2005; Fujii *et al*., 2010; Bruce and Pickett, 2011; Beyaert and Hilker, 2014). Certain host plants release constitutive volatiles mainly consist of terpene, ester, ketone, aldehyde and

alcohol (Szendrei and Rodriguez-Saona, 2010) that can attract phytophagous insects, particularly inexperienced insects (Visser, 1986; Dickens, 2000; Brilli *et al*., 2009; Dicke and Baldwin, 2010). However, Bruce *et al*., (2005) reported that the induced volatiles could make the plants adaptive if their effects cause a behavioural change in the interacting herbivore that result in a fitness benefit for the plant. Pan *et al*., (2015) suggested that fragrant volatiles, which were emitted in greater amounts once plants begin to flower, mediate *Apolygus lucorum* (Hemiptera: Miridae) preference to flowering host plants than non-flowering plants.

Hibino and Ito, (1983) and Hibino, (1985) reported that male and female *M. cribraria* aggregate on host plants to mate. Aggregation sizes average around four, but clumps of up to 25 individuals had been observed by Hibino and Ito, (1983). Aggregations consist of mating pairs plus males not in copula (awaiting female arrival) and males play an active role in aggregations through initiation, maintenance, and courtship, whereas females attend aggregations solely to mate (Hibino, 1985). In large mating aggregations, where females select their mates before copulation (Hibino and Itô, 1983; Hibino, 1986; Hosokawa and Suzuki, 1999, 2001). Females usually copulation with larger males (Himuro *et al*., 2006) that aggregate together (Hibino and Ito, 1983), hence female choice is the selective force for male gregariousness. Copulations can last for 24 hours in the laboratory and up to 10 hours in the field, though sperm transfer is regularly completed within 2-4 hours (Hosokawa and Suzuki, 2001). Hosokawa and Suzuki, (2001) and Thippeswamy and Rajagopal, (2005a) reported that mating usually occurs in the early afternoon until early morning the next day, which may relate to predator abundance or optimal time of subsequent oviposition. Prolonged copulation by males may serve to guard the females, which in turn prevent re-mating of females by other males (Parker, 1970; Simmons and Siva-Jothy, 1998), the most common and well-established reproductive behaviour in insects (Alcock, 1994). After copulation, the incubation period of egg laying by *M. cribraria* was approximately 4-7 days (Ahmad and Moizuddin, 1977; Srinivasaperumal *et al*., 1992; Hosokawa and Suzuki, 2001).

Stink bugs, when disturbed, produce large quantities of strong-smelling and irritating defensive chemicals from the metathoracic scent gland (Favaro *et al*., 2011; Favaro and Zarbin, 2013). These volatiles provide as defensive function by discouraging predators (Krall *et al*., 1999; Noge *et al*., 2012). Analysis of composition and function of

chemicals produced by the metathoracic scent glands in Pentatomoidea revealed that each species secretes its own specific blend of components when disturbed, closely related species may secrete identical defensive chemicals or pheromones (Borges *et al*., 1999). The chemical compounds in the alarm pheromones produced by southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (Blum, 1996) and *Piezodorus guildinii* (Westwood) (family Pentatomidae) (Zarbin, 2000) were already reported. The volatiles produced by disturbed shield bug, *Carpocoris fuscispinus* (Boheman) (Hemiptera: Pentatomidae) have been isolated by Durak and Kalender, (2012). Both disturbed and undisturbed *Tessaratoma papillosa* (Drury) (litchi stink bug, family Tessaratomidae) secretions contain tridecane, whereas this stink bug secretes (E)-2-hexenal only when disturbed (Zhao *et al*., 2012). Volatile secretions produced by disturbed species in the family Plataspidae have been characterized by Onnink *et al*., (2017).

Phototactic behaviour of insects has been correlated with characteristics of light and under optimal light exposure times and luminance intensities, LED light sources that emit relatively short wavelengths attract agricultural insect species (Oh *et al*., 2011; Cho and Lee, 2012; Kim *et al*., 2012; Yang *et al*., 2012; Jeon *et al*., 2014; Kim and Lee, 2013; Yang *et al*., 2015 a, b). Based on the phototactic behaviour, green and/or blue LEDs show the highest attraction rate against *Bemisia tabaci* (Kim *et al*., 2012), *Frankliniella occidentalis* (Yang *et al*., 2015), *Liriomyza trifolii* (Kim and Lee, 2013), *Myzus persicae* (Yang *et al*., 2015), *Nilaparvata lugens* and *Sogatella furcifera* (Yang *et al*., 2013), *Plutella xylostella* (Cho and Lee, 2012), *Spodoptera exigua* (Oh *et al*., 2011), *Spodoptera litura* (Yang *et al*., 2012) and *Trialeurodes vaporariorum* (Jeon *et al*., 2014). The relatively long wavelengths of red light and infrared light were repellent for *L. trifolii, S. litura, P. xylostella,* and *T. vaporariorum* (Cho and Lee, 2012; Yang *et al*., 2012; Kim and Lee, 2013; Jeon *et al*., 2014). Matteson and Terry, (1992) reported that both male and female *F. occidentalis* exhibited strong attractiveness to the blue LED traps. Furthermore, Vaishampayan *et al*., (1975) evaluated the ultraviolet, yellow-green region, and red light in attracting *T. vaporari* rapping of *E. postfasciatus* was more efficient using green LED. Hausmann *et al*., (2004) found that the green and blue LEDs were more efficient in attracting and trapping *Anthonomus pomorum* (Coleoptera: Curculionidae) than UV light.

Ahmad and Moizuddin, (1976) reported that Heteropteran predator Reduviids (identified as *Reduviius* [sic] sp.) (Heteroptera: Reduviidae) feeding on adults and fifth-

instar nymphs of *M. cribraria*. The Hymenoptera species reported from eggs of *Megacopta* spp. include, *Ablerus* sp. (Howard) (Aphelinidae) in India (Rajmohan and Narendran, 2001); *Dirphys boswelli* (Girault) (Aphelinidae) in India (Polaszek and Hayat, 1990); *Ooencyrtus nezarae* Ishi (Encyrtidae) in China and Japan (Tayutivutikul and Yano, 1990; Takasu and Hirose, 1991a, b; Hirose *et al*., 1996; Wu *et al*., 2006); *Ooencyrtus* sp. (Encyrtidae) and *Trissolcus* sp. (Scelionidae) in China (Zhang *et al*., 2003); *Paratelenomus saccharalis* (Dodd) (Scelionidae) in China, India and Japan, (Wall, 1928; Watanabe, 1954; Yamagishi, 1990; Hirose *et al*., 1996; Takagi and Murakami, 1997; Rajmohan and Narendran, 2001; Wu *et al*., 2006) ; *Telenomus latisculcus* Crawford (Scelionidae) in India (Mani and Sharma, 1982); and *Telenomus* sp. (Scelionidae) in Pakistan (Ahmad and Moizuddin, 1976). Johnson, (1996) proposed Synonyms for *P. saccharalis* as *Asolcus minor* (Watanabe), *Archiphanurus minor* and *Paratelenomus minor*. Borah and Dutta, (2002) and Borah and Sarma, (2009b) reported the entomopathogenic fungus *Beauveria bassiana* (Balsamo) as a natural biocontrol agent of *M. cribraria* in pigeon pea fields in Assam, India. Borah and Sarma, (2009a) reported the predation of *M. cribraria* by a spider *Oxyopes shweta* (Tikader) (Araneae: Oxyopidae) and a predatory bug *Antilochus coqueberti* (Fabricius) (Pyrrhocoridae).

The field observations and molecular gut-content analysis were carried out by Ruberson *et al*., (2013) and Greenstone *et al*., (2014) reported several existing generalist predators *Euthyrhynchus floridanus* (L.) and *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), *Geocoris uliginosus* (Say) and *G. punctipes* (Say) (Hemiptera: Geocoridae), *Zelus renardii* (Kolenati) (Hemiptera: Reduviidae), *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae), the spiders *Oxyopes salticus* (Hentz) and *Peucetia viridans* (Hentz) (Araneae: Oxyopidae), the entomopathic fungus *Beauveria bassiana* (Balsamo) Vuillemin, and *Phasia robertsonii* (Townsend) (Diptera: Tachinidae) found parasitizing single adult of *M.cribraria*. Additionally, Golec *et al*., (2013) detected the presence of a small parasitic fly *Strongygaster triangulifera* (Loew) (Diptera: Tachinidae) which typically parasitizes only adult insects with a few reports of larval parasitism viewed as abnormal occurrences (Thompson, 1954). *P. saccharalis* emerged from egg masses oviposited by *M. cribraria* were collected from Georgia, Alabama, Mississippi (Gardner

et al., 2013b), and Florida (Medal *et al*., 2015). Ruberson *et al*., (2013) noted that known hosts of *P. saccharalis* were restricted to the family Plataspidae and Gardner *et al*., (2013b) first time the detected presence of *P. saccharalis* in the Western Hemisphere.

The phoretic behaviour had been documented under subfamily Scelioninae include, *Mantibaria mantis* on praying mantids Dodd (Masner, 1976), *Synoditella* Muesebeck, *Sceliocerdo* Muesebeck, and *Scelio* Latreille on grasshoppers (Acrididae) (Brues, 1917; Noble, 1935; Lanham and Evans, 1958; Veenakumari *et al*., 2012), *Sceliocerdo viatrix* Brues, 1917 (Lepidoscelio, in lit.) on *Neorthacris* grasshoppers (Ramachandra rao, 1952; Basavanna, 1953), *Scelio opacus* on three species of grasshoppers (Rees, 1973) and also *Sceliomorpha bisulca* on short-winged locust (Ashmead, 1893), *Thoronella* Masner on *Epiaeschna heros* (Fabr.) (Carlow, 1992), *Mantibaria* Kirby on Mantodea (Kirby, 1900), and *Protelenomus* on coreid bugs (Kohno, 2002).

The life tables of egg parasitoids such as *Fidiobia dominica* Evans on *Diaprepes abbreviatus* L. (Duncan *et al*., 2007), *Psix striaticeps* Dodd on *Canthecona furcellata* Wolff. (Singh *et al.*, 1995), *Telenomus calvus* on *Podisus maculiventris* (Orr *et al*., 1986), *Trichogramma brassicae* Bezdenko on two moths *Anagasta kuehniella* and *Plodia interpunctella* (Iranipour *et al*., 2009), *Trichogrammatoidea lutea* Girault on *Helicoverpa armigera* (Hubner) (Mawela *et al*., 2021) and *Trissolcus* spp. (*Trissolcus brochymenae, Trissolcus teretis* and *Trissolcus urichi*) on *Euschistus heros* eggs (Laumann *et al*., 2008) were studied to evaluate the biology and efficiency of parasitoids.

In commercial augmentative biological control programmes, natural enemies are mass-reared in biofactories for large scale release in the field to obtain immediate control of pests (Van Lenteren, 2012). Most hymenopteran parasitoids require suitable non-host food sources to satisfy their energy needs (Bianchi and Wäckers, 2008), enhancing their life expectancy, realized fecundity and dispersal capacity (Wäckers, 2004; Romeis *et al*., 2005; Wäckers *et al*., 2006, 2007; Bernstein and Jervis, 2008; Géneau *et al*., 2012). Extrafloral nectar, pollen, honey, carbohydrates, and protein have been shown to influence *Trichogramma* sp. life table parameters and parasitisation performance (Ashley and Gonzalez, 1974; Hohmann *et al*., 1988; Baggen and Gurr, 1999; Jervis *et al*., 2004; Lee *et al*., 2004; Shearer and Atanassov, 2004; Zhang *et al*., 2004; Wäckers, 2005; Witting-Bissinger *et al*., 2008).

The success of biological control in Integrated Pest Management depends on the knowledge of the biology and ecology of insect pests and their natural enemies (Cave, 2000). The egg parasitoid mating can be considered before the massive field releases against pest population (Pratissoli *et al*., 2009). The most common reproduction mode in Hymenoptera via parthenogenesis is arrhenotoky type (Jervis *et al*., 2001; Mutitu *et al*., 2013; Pratissoli *et al*., 2014; Queiroz *et al*., 2017). Another less common mode is thelytoky reported in genus Trichogramma (Stouthamer, 1993; Pintureau *et al*., 1999). Queiroz *et al*., (2017) reported the influence of mating on other biological parameters such as the numbers of parasitized eggs, parasitoid emergence (%) and longevity of parental females in *Telenomus remus* (from *Corcyra cephalonica* eggs) that parasitized *Spodoptera frugiperda* eggs. Furthermore, the female longevity of *Trichogramma pretiosum* Riley was significantly influenced by mating (Pratissoli *et al*., 2014).

Previous studies have precisely documented the potential effects of host egg age on key parasitism traits such as parasitism rate, duration and successful offspring developments (Pak *et al*., 1986; Monje *et al*., 1999; Moreau *et al*., 2009; Moreno *et al*., 2009). Egg parasitoids often parasitized young host eggs than older ones (Brand *et al*., 1984; Calvin *et al*., 1997; Pak *et al*., 1986; Moreno *et al*., 2009). Variable results have been reported on the impact of host egg age on % emergence of egg parasitoids (Miura and Kobayashi, 1998; Saour, 2004; Pizzol, 2012; Stinguel *et al*., 2013; Oliveira *et al*., 2014). Similarly, the effect of host egg age on sex ratio was observed in some species of Scelionidae (Da Rocha *et al*., 2006; Bruce *et al*., 2009; Peñaflor *et al*., 2012) and Trichogrammatidae (Calvin *et al*., 1997; Reznik *et al*., 1997; Godin and Boivin, 2000; Takada *et al*., 2000; Tunçbilek and Ayvaz, 2003; Jarjees and Merritt, 2004; Moreno *et al*., 2009; Paraiso *et al*., 2012; Zhang *et al*., 2014; Tabebordbar *et al*., 2020).

Temperature plays a critical role in the life of parasitoids. The relationship between temperature and biology has been studied in a number of scelionid species (Orr *et al*., 1985; Ruberson *et al*., 1995; Olaye *et al*., 1997; Takagi and Murakami, 1997; Torres *et al*., 1997; Canto-Silva *et al*., 2005; Sadoyama, 2007; Austin, 2008; Bueno *et al*., 2008; Iranipour *et al*., 2010; Taguti *et al*., 2019). Olaye *et al*., (1997) studied the impact of 20, 25, 27 and 30^0C on the intrinsic rate of increase (r_m), the generation time (T) and net reproductive rate (R_0) of *Telenomus busseolae* using *Sesamia calamistis* eggs as the host. Moreover, previous studies revealed that temperature play a critical role on life table

parameters of Trichogrammatidae (Pratissoli and Parra, 2000; Pratissoli *et al*., 2004; Kalyebi *et al*., 2006; Bari *et al*., 2015) and Mymaridae (Agboka *et al*., 2004).

Numerous studies were carried out to evaluate the performance of *Trichogramma* wasps under laboratory and field conditions (Sherif *et al*., 2008; Ko *et al*., 2014; Chowdhury *et al*., 2016; Tang *et al*., 2017; Gowda *et al*., 2021; Zouba *et al*. 2022).

So far, no studies on *M. cribraria* natural enemy complex or potential natural enemy, parasitism capacity, life table behaviour, or mass rearing technology have been conducted in the laboratory or in the field. Hence, it is inevitably necessary to undertake a study on *M. cribraria* and its natural enemy complex. This study is mainly focused on these aspects.

CHAPTER 3

MATERIALS AND METHODS

Insect collection, host plant and mass culturing

Stock culture of *M. cribraria* were obtained from eggs, nymphs and adults collected from a *L. purpureus* (L.) field (Malaparamba, Lat.-11.292975, Long.-75.803572) in Kozhikode district, Kerala, India. Field-collected individuals were transferred to transparent plastic jars (25 by 27.5 cm) and reared at room temperature (27 ± 2°C, 80 ± 5% RH and a photoperiod of 14L:10D) in the lab (Plate.4a). The top of the rearing jar was covered with muslin cloth for adequate ventilation. In each jar, *M. cribraria* was reared on fresh tender shoot with 4 -5 leaves of lablab were wrapped with cotton at the cut end and inserted into a small plastic jar (5 by 1 cm) filled with 10% sugar solution to keep them fresh and turgid. The lablab shoots were replaced every day. The lablab shoots and white muslin cloth act as oviposition substrates for *M. cribraria.* Egg masses laid on muslin cloth or plant parts were scrutinized and kept in separate petri dishes until the first instar nymphs emerged. These nymphs were placed in similar containers, that were covered with muslin cloth, and fed in the same way as adults. After completing the immature development adults of *M. cribraria* were collected and managed as described above for the experiments and for colony maintenance.

3.1. Studies on life history of *M. cribraria* on *L. purpureus* var. *Hima*

3.1.1. Developmental Time and Survivorship of *M. cribraria* at room temperature

In order to construct life tables, newly laid eggs on same age were carefully collected from the mass culture and placed inside the petri dishes (8 cm diameter × 1.5 cm high) covered with muslin cloth (Plate.4b). These dishes were incubated at average room temperature of 28 ± 2^0C, with relative humidity 75 ± 5 % and a photoperiod of 14L:10D. The hatching of eggs was recorded daily, and the newly emerged nymphs were collected and placed individually into transparent plastic jars (14.5 by 15.5cm with a muslin cloth covered on the top). Fresh lablab shoot wrapped with cotton at the cut end and soaked in 10% sucrose solution was used as a food source for nymphs. The nymphs were examined daily for ecdysis, based on the presence of cast skin and survival were also recorded. Meanwhile, lablab shoots were replaced daily with fresh ones. After adult

eclosion, the numbers of females and males were recorded. A cohort of 100 eggs was used in the experiments with two replicates. Thus, a total of 200 eggs were used in the life table experiment.

3.1.2. Adult longevity and fecundity of *M. cribraria* at room temperature

With a view to determine the age specific fecundity and adult longevity, newly emerged adults on the same day were paired and each pair placed in a new transparent plastic jar covered with muslin cloth. Fresh lablab tender shoots with leaves served as food source and replaced daily. These jars were maintained at average room temperature (28 ± 2^0C, 75 ± 5% RH and14L:10D). Eggs were collected daily from each plastic jar and the fecundity (the number of eggs produced/female) and survival were recorded daily until the death of each female. Adult pre oviposition period [APOP; the time period between adult female emergence and the onset of oviposition], total pre oviposition period [TPOP; the period counted from egg to first oviposition], oviposition period, total fecundity and longevity of adults were also recorded.

3.1.3. Life table analysis of *M. cribraria* at room temperature

Life table was constructed from age specific survival rate (l_x) and age specific fecundity rate (m_x) of *M. cribraria* using the data from above observations. On each day, the mean number of eggs laid per female per day was derived on the basis of the total number of eggs laid and the total number of females survived on the previous day. In order to arrive at the number of female progeny (m_x), the number of eggs laid per female was divided by Female (F): Male (M) ratio. The fertility life tables were prepared by using the formulae of Birch, (1948), elaborated by Howe, (1953), Watson, (1964) and Atwal and Bains, (1974) viz.,

X = Pivotal age in days

l_x = Survival of female at age 'x'

m_x = Age schedule for female births at age 'x'

Net reproductive rate (Ro)

The values of 'X', 'l_x' and 'm_x' were calculated from the data given in life tables. The sum total of the products 'l_xm_x' is the net reproductive rate (Ro) (Lotka, 1925). The Ro is the rate of multiplication of population in generation measured in terms of females

produced per generation. The number of times a population would multiply per generation was calculated by using following formula:

$$Ro = \sum l_x m_x$$

Mean generation time (Tc)

Mean generation time (Tc) is the average interval separating births of one generation from the next (Carey, 1993). The approximate value of Tc (the mean age of the mother in a cohort at the birth of female offspring) was calculated by using following formula:

$$Tc = \frac{\sum X\, l_x m_x}{Ro}$$

Innate capacity for increase in numbers (r_m)

Total numbers of individuals survived and mean numbers of female offspring birth were recorded at each age interval. i.e. the maximum exponential rate of increase of a population growing within defined physical conditions (Birch, 1948). From these data the arbitrary value of 'r_m (r_c)' was derived by using the following formula:

$$rm = \frac{\log e\, Ro}{Tc}$$

Where,

e = 2.71828

Tc = Mean generation time

The intrinsic rate of increase (rm) was subsequently calculated from the arbitrarily 'r_m' by taking two trial values selected on either side of it differing in the second decimal place and substituting in the equation $\sum e^{7-rmx}. l_x m_x$ (Atwal and Bains, 1974). Thus, the two values of the equation were found which lay immediately above or below 1097. The values of $\sum e^{7-rmx}$. lxmx obtained from the two trials were plotted against their respective arbitrarily 'rm' which give a straight line. The straight line was intersected by a vertical line drawn from the described value at 1097. The two points of intersection gave the accurate 'r_m' value. The precise generation time (T) was calculated by using the following formula:

$$T = \frac{\log e\, Ro}{r_m}$$

The finite rate of natural increase (λ)

The number of females per female per day i.e. finite rate of increase was determined as:

$$\lambda = \text{anti log } e^{rm}$$

From this data the weekly multiplication of the population was calculated.

Weekly multiplication of the population (Wm) was calculated by using the formula:

$$Wm = (e^{rm})^7$$

Doubling time (DT) was the time in days that was required by a population to double in number, (Campbell and Mackauer, 1975) was calculated using the formula:

$$DT = \frac{\log 2}{r_m}$$

3.2. Effect of different levels of temperature on the life history of *M. cribraria*

3.2.1. Developmental times and Survivorship of *M. cribraria* at different temperatures

The relationship between temperature and developmental rate and survivorship of *M. cribraria* were studied under different constant temperatures. Freshly deposited *M. cribraria* eggs (<12 h old) were randomly selected from the mass culture and placed in separate petri dishes (8 cm diameter × 1.5 cm high) covered with muslin cloth. The dishes were then maintained in an environmental chamber having different cabins that can be set at five constant temperatures (15, 20, 25, 30, and 35^0 C), with 83 ± 3% RH and a 14L:10D photoperiod (Plate.5). A total of one hundred eggs were used to initiate every temperature treatment with two replicates. The total number of eggs hatching at each temperature was counted, and the duration of each egg development was also recorded.

Upon hatching, all first instar nymphs were placed individually in a transparent plastic jar, fed with fresh lablab tender shoots every day and maintained in the same cabins in which they had been maintained as eggs. The duration of each instar stage and the number of surviving nymphs were recorded daily. After adult emergence, the sex of progeny was determined to understand if the sex ratio varies with temperature.

3.2.2. Adult longevity and fecundity of *M. cribraria* at different temperatures

The influence of temperature on longevity, fecundity and life table parameters of *M. cribraria* were studied under different constant temperatures. Adults of *M. cribraria* used for the longevity and fecundity experiments were reared from newly emergent nymphs obtained from the five constant temperatures (15, 20, 25, 30 and 35^0C). Upon emergence, the adults were paired and each pair placed in a transparent plastic jar (25 by 27.5 cm), covered with muslin cloth and being assessed at each test temperature. Fresh lablab tender shoots were offered daily as food. The survival and fecundity for females were recorded daily, and all eggs laid were removed from each jar until the death of females. Other parameters including APOP, TPOP, oviposition period, total fecundity and longevity for adults at each temperature were also recorded.

Statistical analysis

For comparisons of developmental time, fecundity, longevity, pre oviposition period and oviposition period for *M. cribraria* among different temperature treatments, a one-way analysis of variance (ANOVA) was performed using SPSS 16.0 software. If significant differences were detected, means were compared using a Student-Newman-Keuls Test with a level of significance at 0.05. Data on development, survival and reproduction of *M. cribraria* at different temperatures were used to calculate life table parameters as described under 3.1.3.

3.3. Effect of different levels of relative humidity on the life history of *M. cribraria*

3.3.1. Developmental times and Survivorship of *M. cribraria* at different RH levels

In the present study, egg and nymph survival and developmental times of *M. cribraria* were studied under different constant relative humidity levels. *M. cribraria* eggs were collected on the same day they were laid and placed in separate petri dishes (8 cm

diameter × 1.5 cm high) covered with muslin cloth. The dishes were held in different cabins in an environmental chamber set at 40, 50, 60, 70, and 80% RH with 30^0C and a photoperiod of 14:10 (L:D). One hundred eggs were examined for each treatment as four replicates. The mean incubation period and percentage hatchability of eggs were recorded daily. At egg hatch, the first instar nymphs were transferred individually to transparent plastic jars and continued at the same RH level. Fresh lablab shoots were provided daily for the nymphs. The duration of each instar stage and the number of surviving instar nymphs were recorded. After emergence, the sex ratio of the adults was recorded for each RH level.

3.3.2. Adult longevity and fecundity of *M. cribraria* at different RH levels

Adult longevity, fecundity, and various life table parameters of *M. cribraria* were worked out under five different RH levels. Upon adult eclosion in the nymphal development studies, the female bugs were paired with an arbitrarily selected male from the same temperature and placed into transparent plastic jars (25 by 27.5 cm). Each of the plastic jars contained fresh lablab tender shoots, which were replaced daily with new ones as food. Observations were made daily to note the pre oviposition period, oviposition period, adult longevity and total lifespan of adults (from egg to adults' death) at each RH level.

Statistical analysis

The effect of relative humidity on survival and development of immature *M. cribraria* and on adult longevity, total lifespan of adults, fecundity, pre oviposition and oviposition days were analysed by one-way analysis of variance (ANOVA) performed by SPSS 20.0 software and separation of means were tested by Student Newman Keuls (S-N-K) test ($P \leq 0.05$). Data on development, survival and reproduction of *M. cribraria* at different RH were used to calculate life table parameters as described under 3.1.3. The effect of relative humidity on egg and nymph survival, preoviposition period, oviposition period, fecundity, adult longevity and life table parameters, such as the intrinsic rate of increase (rm), net reproductive rate (Ro), mean generation time (T), and finite rate of increase (λ) was characterized by linear regression analysis using the model $y= bx + a$, where y is preoviposition period, oviposition period, fecundity, adult longevity or life table parameter, x is RH, and a and b are coefficients obtained from the regression.

3.4. Studies on different behavioural parameters of *M. cribraria*

3.4.1. Food preference

The Y tube olfactometer was used to study the food preference of *M. cribraria.* The olfactometer made up of glass with a main stem 12.5 cm long, each of the two arms 12.5 cm long with 2 cm inner diameter, placed at an angle of 60^0 between them. Teflon tubes were used to connect the two arms of Y tube to a glass cylinder (28.5 cm long and 10.5 cm diameter) in which different parts of host plants could be placed. Before starting the experiment, all equipment was cleaned with acetone. Room air was pumped through activated charcoal filter for purification by using a small aerator which blown air at a rate of 400 ml/min. The filtered air was directed to test glass cylinder (containing single host plant part) and control cylinder and finally opened to each arm of the Y-tube (Plate.6).

Two different tests were conducted to most preferred plant part of host plant lablab which attract *M. cribraria* adults based on the olfactory preference: single-plant part choice test and two-plant part choice test. The different plant parts selected were apices, leaves, inflorescence and green pod. Single-plant part preference test was designed to determine which plant part can attract *M. cribraria* adults. Each plant part was placed separately in one of the odour-source glass cylinders and no plant (i.e. empty chamber) was provided in the other cylinder. Wetted cotton was placed at the distal end of the plant parts to avoid dessecation during the experiment. One adult *M. cribraria* was introduced at the start point in the Y-tube olfactory meter. The direction of movement toward the two arms of the olfactormeter was determined when single *M. cribraria* reached the far end of one of the arms within 10 minutes.

In the two-plant part choice test was designed to determine if one plant part can attract more *M. cribraria* adults than the other and the following combinations of plant parts were compared: apices vs leaves, apices vs inflorescence, apices vs green pod, leaves vs inflorescence, leaves vs green pod, and inflorescence vs green pod. One *M. cribraria* adult was introduced at the start point in the Y-tube olfactometer and its choice to a plant part was recorded.

Tests were repeated 25 times for each sex of *M. cribraria* adult. Same day old insects starved for 24 hours were used for the experiment. After every set of trials, the Y tube was cleaned and washed with acetone and distilled water to remove any potential

odour from previous trails. The arms of the olfactometer were interchanged after every trial to minimise the position effect. Insects did not make a choice within 10 minutes after introduction to the Y-tube were recorded as no response. All bioassays were carried out at 28 ± 2^0C room temperature, 80 ± 5% RH and 14L:10D photoperiod.

Statistical analysis

Data obtained with Y-tube olfactometer assay were analysed with Chi-square tests with a level of significance at = 0.05 using the Microsoft excel software 2010.

3.4.2. Mating

Copulation duration of *M. cribraria* was measured in different sex ratios to determine whether any competition occur between adults that effect the copulation duration (plate.7). The selected sex ratios were one male and one female (1:1), one male and two female (1:2) and two males and one female (2:1). Newly emerged adults were placed into transparent plastic jars (25 by 27.5 cm) covered with muslin cloth at 27 ± 2^0C, 80 ± 5% RH, 14L:10D h photoperiod. Fresh lablab tender stem with leaves was provided as food sources in each jar and were replaced on a daily basis. Copulation duration of the bugs was recorded with a video camera (Sony, AlphaA 58 K). Five replicates were conducted for copulation duration treatments.

Statistical analysis

The copulation duration of the bugs at three different sex ratios were statistically analysed using one-way analysis of variance (ANOVA) performed by SPSS 20.0 software and separation of means were tested by Tukey HSD test ($P \leq 0.05$).

3.4.3. Defensive behaviour

Volatiles emitted from the *M. cribraria* was studied in this experiment. It was noticed that when *M. cribraria* was disturbed it produce a repungent odour. Experiment was conducted to isolate and identify the volatiles emitted by *M. cribraria* in normal condition and in the disturbed conditions.

Volatile compounds extraction

The volatile extraction unit consist of a cylindrical glass jar with 28.5 cm height and 9.5 cm diameter fabricated for this experiment. The lid of the extraction unit was provided with an inlet and outlet apertures and a clip to keep the materials in air tight

condition. Inlet aperture was connected to an aerator and the outlet was connected to an adapter. Air was passed through activated charcoal filter to the chamber using a small aerator where the flow rate was set at 400 ml/min for 6 h. The outlet aperture was loaded with 80 mg of Porapak Q 80-100 mesh medium activated at 200^0C for 24 h in hot air oven (Plate. 8). Twelve *M. cribraria* of each sex (a total of 24 insects) were separately used to collect volatiles emitted from *M. cribraria.* For undisturbed samples, *M. cribraria* were gently removed and placed into a volatile extraction unit using a paint brush. For the disturbed samples, the insects were individually pressed gently without causing any damage between two fingers before being kept into the extraction unit. After each experiment all equipment was cleaned with acetone. Volatiles released by *M. cribraria* were adsorbed by Porapak Q medium for six hours which was later taken for identification. This experiment was replicated six times.

Coupled gas chromatography-Mass spectrometry (GC-MS) analysis of volatiles

Volatiles adsorbed by Porapak Q were eluted with 3.0 ml of HPLC Grade hexane and then concentrated to 25 µl under a gentle stream of inert gas, nitrogen. The concentrated sample was subjected to GC-MS analysis for the identification of volatiles. Analysis was carried out in Agilent Technologies (Model-5975C) system interfaced to a mass spectrometer instruments (MS 7890A). Column used for the elution was DB5-MS fused silica capillary column (30 X 0.25 mm inner thickness X 0.25 µm film thickness, composed of 5% phenyl, 95% dimethyl polysiloxane). Helium (99.999%) was used as the carrier gas. The GC temperature was set at 50^0C/2 minutes, ramped 5^0C/minutes to 250^0C. One microliter of solution was introduced into a splitless injection inlet at 250^0C, and transfer line temperature was 280^0C. Eluted compounds were identified by comparing the obtained spectra with those in the NIST-08 Mass Spectra Library, and the identification was confirmed by comparing the retention times and mass spectra with those obtained from measurements of reference compounds.

3.4.4. Phototropic behaviour

In this study, the behavioural responses of *M. cribraria* when exposed to different coloured LED light stimuli were studied using a Y-tube apparatus. The Y-tube apparatus was made up of glass with a main stem 12.5 cm long, and two branches with an internal diameter of 2 cm, a length of 12.5 cm, and an angle of 60^0 between them. The opening of the main stem was the point of entrance for *M. cribraria*, and LED light was installed at

the end of the test arm and the other as the control arm (no light source). The entire Y tube was covered with black Teflon tape to make it opaque to light (Plate. 9). Tests were conducted in a dark room at a temperature of 29 ± 2^0C and 70 ± 5%RH. The chosen light colours were yellow, white, blue, green and red. The following combinations were tried: (1) yellow vs dark, (2) white vs dark, (3) blue vs dark, (4) green vs dark, (5) red vs dark. One adult *M. cribraria* was introduced at the start point in the Y-tube apparatus. If the insect moves towards the test arm which contained LED light, it was recorded as attractive to that light colour. If the insect moved to the control arm, it was considered that light colour was not attractive to *M. cribraria*.

Based on the result of the above test, the LED light colours that significantly ($P < 0.05$) attracted *M. cribraria* adults were compared: yellow vs white, yellow vs green, and white vs green. Similarly, a single *M. cribraria* was introduced at the base of the Y-tube and the direction of movement toward any of the secondary arm was noticed. Adults did not make a choice within 10 minutes after introduction to the base of the Y-tube were recorded as no response. This test was repeated 25 times with the same day old insects of each sex. After every experiment, the Y tube was cleaned with acetone and distilled water. The arms of the olfactometer were interchanged after every trial to minimise the position effect.

Statistical analysis

Data obtained with Y-tube olfactometer assay were analysed with Chi-square tests with a level of significance at = 0.05 using the Microsoft excel software 2010.

3.5. Surveys to identify natural enemies (predators /parasitoids) of *M. cribraria* in the field

Extensive surveys were conducted at different localities in Calicut, Wayanad and Malappuram districts, Kerala, from June 2015 to 2017, where *Lablab purpureus* plants were grown and the incidence of *M. cribraria* was noticed. *Lablab* plant parts (leaves, apices of stems, inflorescence, tender pods and developed green pods) were examined and plant parts containing eggs and nymphs of *M. cribraria* were collected by hand picking method. In the laboratory, the field collected eggs were kept in small transparent plastic containers (8×11cm) for identifying egg parasitoids if any, and other stages of bugs were reared to identify any entomopathogenic organisms exist. Adult bugs, on which

the parasitoids found as phoretic, were also collected from the field. After the emergence from eggs collected, the parasitic wasps recovered were preserved in ethyl alcohol (100%) for taxonomic study or kept alive in the laboratory by providing honey solution as food source till identification (Plate.10).

3.5.1. Identification of egg parasitoids

Egg parasitoids emerged out from the host eggs were identified and checked out again in the field for further incidence of the same parasitoid. The preserved egg parasitoids were glued to the tip of point cards and examined with Leica M 205A and Zeiss V8 stereo microscopes for taxonomic identification. Extended-focus images were produced with two systems: a Leica DFC 500 camera attached to a Leica M 205 A stereomicroscope with images combined using the Leica Application Suite, and a Macroscopic Solutions Macropod Micro Kit with images combined in Helicon Focus. For further clarification and authentication of the identity of the egg parasitoids a scanning electron microscopy was performed with a Hitachi SU6600 Variable Pressure Field Emission Scanning Electron Microscope (FESEM) and a Hitachi TM3000 Tabletop Microscope and then the parasites were subjected to DNA barcoding.

DNA was extracted from the whole insect using Qiagen DNeasy® 96 Blood and Tissue Kit (Qiagen, Germany). Following DNA extraction, the extracts were subjected to PCR amplification of a 658 bp region near the 5' terminus of the cytochrome c oxidase subunit1 (COI) gene following standard protocol (Hebert *et al*., 2004). The primer pair used was forward primer LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and reverse primer HCO 2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al*., 1994). PCR reactions were carried out in 96-well plates, 50 µL reaction volume containing: 5 µL GeNeiTM Taq buffer, 1 µL GeNeiTM 10mM Dntp mix, 2.5 µL (20 pmol/µL) forward primer, 2.5 µL (20 pmol/µL) reverse primer, 1 µL GeNeiTM Taq DNA polymerase (1 U/µL), 2 µL DNA (50 ng/µL), and 36 µL sterile water. Thermo cycling consisted of an initial denaturation step at 94^0C for 5 minutes, followed by 30 cycles of denaturation at 94^0C for 1 minute, annealing at 55 °C for 1 minute and extension at 72^0C for 1 minute using a C1000™ Thermal Cycler. PCR products were visualized in a 1.5% agarose gel electrophoresis as described by Sambrook and Russell (2001), and sequenced and uploaded to GenBank.

3.6. Life table and development of mass rearing technique of *P. anu*

3.6.1. Rearing of *P. anu* in the laboratory

The rearing of *P. anu* using different concentrations of honey was investigated under laboratory conditions (27 ± 2^0C, 80 ± 5% RH and 14L :10 D photoperiod). A series of concentrations of honey solutions were prepared, i.e. 10%, 20%, 30%, 40% and 50% by adding distilled water. Pure distilled water was used as control. Each honey concentration was placed on the walls of the transparent plastic jars using a thin capillary tube of 1mm diameter. Ten newly emerged adults of the same age were released in to these jars. There were ten replicates for each honey concentration (100 adults). Suitability of the honey concentrations was evaluated by assessing the longevity of the adults.

Statistical analysis

The rearing of *P. anu* using different concentrations of honey was statistically analysed using one-way analysis of variance (ANOVA) performed by IBM SPSS version 22 software and separation of means were done by Student Newman Keuls (S-N-K) test ($P \leq 0.05$).

3.6.2. Life table of *P. anu* on eggs of *M. cribraria* at room temperature

To determine developmental time *P. anu*, newly emerged mated *P. anu* females (<12h old) were individually transferred to small transparent plastic jars (8×11cm) and provided with 20% of honey droplets placed on the walls of the jars as food and closed with muslin cloth (Plate.12). Freshly laid host eggs (<12 h old;) were exposed to each female in the jars. After 24 h, all female parasitoids were removed from the jar and parasitized eggs were incubated at room temperature of 27 ± 2^0C, 80 ± 5% RH and 14 L :10 D photoperiod in the laboratory. The host egg masses were observed twice daily until the emergence of parasitoids. The developmental times from eggs to adults and number of emerged parasitoids were recorded. After emergence of all parasitoids, parasitized host eggs were dissected to examine dead immature parasitoid stages and unemerged adults remaining inside the host eggs. These host eggs that failed to produce adult parasitoids were considered as parasitized host eggs and included in the calculation for rate of parasitism. Therefore, the rate of parasitism was estimated as the number of emerged parasitoids plus clearly parasitized but unhatched eggs over total number of host eggs exposed. Rate of emergence was determined by dividing the number of emerged adults

by the number of visibly parasitized host eggs. The sex ratio of parasitoids was calculated as per the proportion of females on total numbers of adults.

For assessment of fecundity and longevity of *P. anu* adults, newly emerged mated females (<12 h-old) were placed individually into a transparent plastic jar (8×11cm) and fed with 20% of honey droplets. Jars was closed with muslin cloth. Each parasitoid female was supplied daily with a batch of 30 eggs (< 12 h old) of *M. cribraria* until all parasitoids died. After 24 hours, these exposed egg batches were removed and placed in closed petri dishes (8 cm diameter × 1.5 cm high). Longevity of both males and females were recorded daily. Daily records were kept for pre oviposition period, oviposition period, post-oviposition period and total fecundity (total number of eggs produced during an individual's reproductive period) of each *P. anu* female. Those females which were injured during daily handling or those that died because of getting stuck in honey droplets were excluded from the data analysis.

Statistical analysis

All data on number of host eggs parasitized, rate of survival, developmental times from parasitized eggs to adults, sex ratio, pre oviposition period, oviposition period, post-oviposition period and total fecundity and adult longevity were statistically analysed using arithmetic mean and standard error (SE). Data on development, survival and reproduction of *P. anu* on eggs of *M. cribraria* were used to calculate life table parameters as described under 3.1.3.

3.6.3. Effect of mating on parasitism, emergence and sex ratio of *P. anu*

The influence of parasitoid mating on biological parameters of *P. anu* was studied by using newly emerged mated and unmated *P. anu* females (< 12 h old) reared on *M. cribraria* eggs. Each female was individualized in small transparent plastic jar (8×11cm) containing droplets of 20% honey as food and offered thirty eggs (< 12 h old) of *M. cribraria.* The tops of the jars were covered with muslin cloth and maintained at room temperature (27 ± 2^0C, 80 ± 5% RH and 14 L:10 D photoperiod). Six replicates were used for each treatment. After 24 hours, the exposed eggs were removed and placed individually in closed petri dishes until the emergence of adults. The number of parasitized eggs, percentage of parasitoid emergence, sex ratio, and longevity of parental females were recorded.

Statistical analysis

The effect of parasitoid mating system on number of parasitized eggs, percentage of parasitoid emergence, sex ratio, and longevity of parental females were analysed by one-way analysis of variance (ANOVA) performed by IBM SPSS version 22 software.

3.6.4. Effect of host egg age on parasitism, emergence and sex ratio of *P. anu*

The preference of *P. anu* females to discriminate between *M. cribraria* eggs of different ages were evaluated using a no-choice bioassay. *M. cribraria* eggs of 12h, 24h, 48h and 72h of age were selected for parasitisation. Eggs of each age group were placed in a separate transparent plastic jar (8×11cm) 20% of honey droplets placed on the wall of the jar. Newly emerged mated females (<12 h-old) were introduced into the plastic jar containing *M. cribraria* egg mass and maintained at room temperature (27 ± 2^0C, 80 ± 5% RH and 14 L:10 D photoperiod). The plastic jars were sealed with muslin cloth. Eggs were exposed to the parasitoids for 24 h, and then *P. anu* females were removed and the plastic containers kept at room temperature until adult emergence. First instar nymphs of the host bugs that hatched from unparasitized eggs were removed and counted. The experiment was conducted in a completely randomised design with five replicates using thirty host eggs.

After the emergence of parasitoids, number of male and female parasitoids that emerged from parasitized eggs were counted. The remaining parasitized eggs were dissected to examine them for parasitoid larvae and un emerged adults remaining inside the host eggs. These values were used to estimate rate of parasitism and emergence. The percentage of parasitism, total number of emerged parasitoids and sex ratio were determined for the influence of egg age on parasitism.

Statistical analysis

The effect of host egg age on rate of parasitism, rate of emergence, number of host first instar emergence and sex ratio of *P. anu* were analysed by one-way analysis of variance (ANOVA) performed by IBM SPSS version 22 software and separation of means were tested by Duncan Multiple Range Test (DMRT) ($P \leq 0.05$).

3.6.5. Effect of different levels of temperature on the parasitism and life history of *P. anu*

The egg to adult developmental time of *P. anu* was studied under five constant temperatures: 15, 20, 25, 30, and 35^0C. In the beginning of the experiment, newly emerged *P. anu* females (<12 h old, mated and with no previous parasitism experience) were individually placed in transparent plastic jars (8×11cm) with 20% of honey droplets placed on the walls of the containers as food. The egg masses of *M. cribraria* (< 12 h old) were collected from mass culture and exposed to one female parasitoid per treatment. The top of the jars was sealed with muslin cloth. After 24 h exposure, all parasitoids were removed, and parasitised eggs were transferred to transparent plastic jars (8×11cm) and each placed in an environmental chamber with different cabins calibrated to 15, 20, 25, 30 and 35^0C respectively, with relative humidity 83 ± 3% and a photoperiod of 14L:10D. Each treatment was replicated five times. Parasitised egg mass were daily examined until the parasitoids completed their development. The total number of eggs hatching at each temperature was counted, and the duration of each egg development was also recorded. The failures of parasitized eggs to produce adults were dissected to examine the parasitoid larvae and unemerged adults remaining inside the host eggs. These data were included in the calculation for the rate of parasitism and emergence and at each temperature. After emergence of parasitoids, the numbers of females and males were recorded to determine the sex ratio at each temperature.

Adults used for the longevity and fecundity experiments were obtained from parasitized egg masses reared at five different constant temperatures as described above. Newly emerged and mated females were individually isolated in transparent plastic jars (8×11cm) and were fed with 20% of honey droplets. The top of the jar was covered with muslin cloth. Fresh host eggs (<12 h old) were offered daily to females at each temperature. Every 24 h, old eggs were removed and new eggs were provided until death of the female. Exposed egg batches were held under the previously described temperatures until parasitoid emergence. Upon emergence, parasitoids were counted and sexed. Pre oviposition period, oviposition period, fecundity and longevity of adult parasitoids were calculated for each specified temperature regime.

Statistical analysis

For parasitism, emergence, sex ratio, developmental time, fecundity, longevity, pre oviposition period and oviposition period for *P. anu* among temperature treatments, a one-way analysis of variance (ANOVA) were performed using IBM SPSS version 22 software. If significant differences were detected, means were compared using a Tukey's honestly significant difference (HSD) test. The significance level was set at $P \leq 0.05$. Data on development, survival and reproduction of *P. anu* on eggs of *M. cribraria* at different temperatures were used to calculate life table parameters as described under 3.1.3.

3.7. Field release in microplot and evaluation of control efficiency of *P. anu*

Field performance of *P. anu* was tested by releasing the wasps in microplots containing potted lablab plants. Potted lablab plants of three months old were selected for microplot study which was covered with fine muslin cloth (Plate.12). In these microplots, an equal number of 11 days old gravid females of *M. cribraria* were released. Simultaneously, same number of newly emerged *P. anu* adults were collected from mass culture and released into these micro plots. Controls plots were released with *M. cribraria* only and maintained without the release of *P. anu*. After five days, all *M. cribraria* adults were collected from both test and control plots and taken back to the laboratory. Egg masses were kept in microplot under natural conditions and observed periodically for number of hatched first instar nymphs, emerged wasps and dead eggs from each egg mass. One micro plot was considered as one replication and three replicates were used for both treatments. Egg parasitism and percentage of emergence of *P. anu* were calculated. The percentage of reduction of 1st instar nymphs of *M. cribraria* was calculated by comparing the number of 1st instar nymphs of *M. cribraria* emerged in control plot - number of 1st instar nymphs of *M. cribraria* emerged in release plot.

CHAPTER 4

RESULTS

4.1. The life history of *M. cribraria* on *L. purpureus* var. *Hima*

4.1.1. Developmental Time and Survivorship of *M. cribraria* at room temperature

In the laboratory, the embryonic development time of *M. cribraria* lasted an average of 5.2 ± 0.04 days. The mean duration of the nymphal phase was 40.38 ± 0.40 days with five nymphal instar stages. The first, second, third, fourth, fifth instar stages last for a period of 6.29 ± 0.08, 7.3 ± 0.07, 8.43 ± 0.07, 7.73 ± 0.08 and 10.63 ± 0.09 days respectively (Table1). The total developmental time from egg to adult was 45.58 ± 0.21d at room temperature of 28 ± 2^0 C,75±5% RH and 14L:10D.

Survival percentage of immature stages of *M. cribraria* was given in Table 2. The mean survival percentage of eggs, first instar to second, second to third instar, third to fourth instar and fourth to fifth instar was 90.14 ± 1.03 %, 73.28 ± 2.71%, 53.57 ± 2.36%, 42.43 ± 2.17% and 34.57 ± 1.56%, respectively. The percentage of individuals that survived from egg to adult was 26.57 ± 1.13%.

Table 1. Developmental times of immature stages of *M. cribraria*

Life stage	Duration in days (d)
Incubation period	5.2 ± 0.04
1st instar period	6.29 ± 0.08
2nd instar period	7.3 ± 0.07
3rd instar period	8.43 ± 0.07
4th instar period	7.73 ± 0.08
5th instar period	10.63 ± 0.09
Total developmental time	**45.58 ± 0.21**

Data in the table are represented as Mean ± SE.

Table 2. Survival percentage of immature stages of *M. cribraria*

Life stage	Survival (%)
Egg	90.14 ± 1.03
1st to 2nd instar	73.28 ± 2.71
2nd to 3rd instar	53.57 ± 2.36
3rd to 4th instar	42.43 ± 2.17
4th to 5th instar	34.57 ± 1.56

Data in the table are represented as Mean ± SE.

4.1.2. Adult longevity and fecundity of *M. cribraria* at room temperature

The *M. cribraria* laid eggs 10.72 ± 0.12 days after the completion of pre-oviposition period. The total pre-oviposition period (TPOP) was 56.3 ± 0.24 days, counted from egg to the beginning of oviposition. The mean oviposition period was 26.43 ± 0.15 days. The mean number of eggs produced in female bugs during lifetime (fecundity) was ranged from 180 to 234 eggs /female with an average of 206.49 ± 1.29 eggs /female (Table 3).

The mean adult longevity of female bugs was 45.21 ± 0.26 days, whereas males lived for 32.41 ± 0.38 days at room temperature of 28 ± 2^0C,75 ± 5% RH and 14L:10D. The male total lifespan and female total lifespan of *M. cribraria* reared on lablab were calculated as 77.99 ± 0.47 and 90.79 ± 0.36, days respectively (Table 4).

Table 3. Pre-oviposition period, total pre-oviposition period (TPOP), oviposition period and fecundity of *M. cribraria*

Parameters	Values
preoviposition period (d)	10.72 ± 0.12 d
TPOP (d)	56.3 ± 0.24 d
Oviposition period (d)	26.43 ± 0.15 d
Fecundity /female	206.49 ± 1.29 eggs/female

Data in the table are represented as Mean ± SE; d represent in days.

Table 4. Adult longevity (days) and total lifespan (days) of *M. cribraria*

Parameter	days
Male longevity	32.41 ± 0.38
Female longevity	45.21 ± 0.26
Male total life span	77.99 ± 0.47
Female total life span	90.79 ± 0.36

Data in the table are represented as Mean ± SE.

4.1.3. Life table analysis of *M. cribraria* at room temperature

Age-specific survivorship (l_x) and fecundity (m_x) of *M. cribraria* were shown in Fig.1 based on a comprehensive data in table 5. The first adult female emerged on day 47 and preoviposition period ranged from 47th to 57th days of pivotal age. Females deposited first batch of eggs on 58th day and stopped it after 86th day with l_x values being 1.00 and 0.980, respectively. The numbers of eggs deposited were high in the early days of oviposition period and lower during the final period of oviposition period. The average number of eggs/ female-laid was 206.49 with maximum possible fecundity of 235. The l_x decreased gradually after 84th day of pivotal age due to adult mortality. The last female died on the 97th day. The females could live for a maximum of 51 days as adult. The proportion of female to male observed was 1:0.46.

The population and reproductive parameters of *M. cribraria* were summarized in Table 6. The net reproductive rate (Ro) of *M. cribraria* was 141.428 female offsprings/ female which indicates the rate of multiplication in one generation. The intrinsic rate of increase (r_m) in numbers was 0.075 female per day and the finite rate of increase (λ) was 1.078 female offsprings per female per day with mean generation time (Tc) of 66.024 days. The population would be able to multiply 1.69 times per week. Population doubling time (DT) recorded was 9.242 days.

Table 5. Daily age-specific survival (l_x) and fecundity (m_x) of female *M. cribraria*

Pivotal age in days (X)	Eggs/ female	l_x	F:M	m_x	$l_x m_x$	Xl_xm_x
0-46	Immature stages					
47-57	Pre-oviposition period					
58	12.27	1.000	1.46	8.404	8.404	487.438
59	11.85	1.000	1.46	8.116	8.116	478.869
60	14.39	1.000	1.46	9.856	9.856	591.369
61	12.22	1.000	1.46	8.369	8.369	510.561
62	11.67	1.000	1.46	7.993	7.993	495.575
63	13.86	1.000	1.46	9.493	9.493	598.068
64	17.24	1.000	1.46	11.808	11.808	755.726
65	15.46	1.000	1.46	10.589	10.589	688.287
66	12.93	1.000	1.46	8.856	8.856	584.506
67	13.21	1.000	1.46	9.047	9.047	606.212
68	7.49	1.000	1.46	5.130	5.130	348.849
69	9.35	1.000	1.46	6.404	6.404	441.883
70	12.07	1.000	1.46	8.267	8.267	578.698
71	5.58	1.000	1.46	3.821	3.821	271.356
72	6.01	1.000	1.46	4.116	4.116	296.383
73	6.43	1.000	1.46	4.404	4.404	321.500
74	4.8	1.000	1.46	3.287	3.287	243.287
75	3.35	1.000	1.46	2.294	2.294	172.089
76	4.26	1.000	1.46	2.917	2.917	221.753
77	3.22	1.000	1.46	2.205	2.205	169.821
78	2.27	1.000	1.46	1.554	1.554	121.273
79	1.57	1.000	1.46	1.075	1.075	84.952
80	1.35	1.000	1.46	0.924	0.924	73.972

Continued on next page

Pivotal age in days(x)	Eggs/ female	l_x	F:M	m_x	$l_x m_x$	Xl_xm_x
81	0.93	1.000	1.46	0.636	0.636	51.595
82	1.03	1.000	1.46	0.705	0.705	57.849
83	0.8	1.000	1.46	0.547	0.547	45.479
84	0.49	1.000	1.46	0.335	0.335	28.191
85	0.26	0.990	1.46	0.178	0.176	14.985
86	0.13	0.980	1.46	0.089	0.087	7.504
87	0	0.950	1.46	0.000	0.000	0.000
88	0	0.850	1.46	0.000	0.000	0.000
89	0	0.800	1.46	0.000	0.000	0.000
90	0	0.650	1.46	0.000	0.000	0.000
91	0	0.520	1.46	0.000	0.000	0.000
92	0	0.360	1.46	0.000	0.000	0.000
93	0	0.210	1.46	0.000	0.000	0.000
94	0	0.130	1.46	0.000	0.000	0.000
95	0	0.040	1.46	0.000	0.000	0.000
96	0	0.010	1.46	0.000	0.000	0.000
97	0	0.000	1.46	0.000	0.000	0.000
Total	206.49	33.49	58.4	141.431	141.427	9348.044

Table 6. Life table parameters of *M. cribraria*

Parameters	Values
Net reproductive rate (Ro) (female offspring)	141.428
Mean generation time (Tc) (days)	66.098
Arbitrary value of innate capacity for increase(r_c) (females^{-1} female^{-1} day^{-1})	0.075
Precise value of Intrinsic rate(r_m) (females^{-1} female^{-1} day^{-1})	0.075
Corrected generation time(T) (days)	66.024
Finite rate of increase (λ) (females^{-1} female^{-1} day^{-1})	1.078
Weekly multiplication (Wm) of population	1.690
Doubling time (DT) (days)	9.242

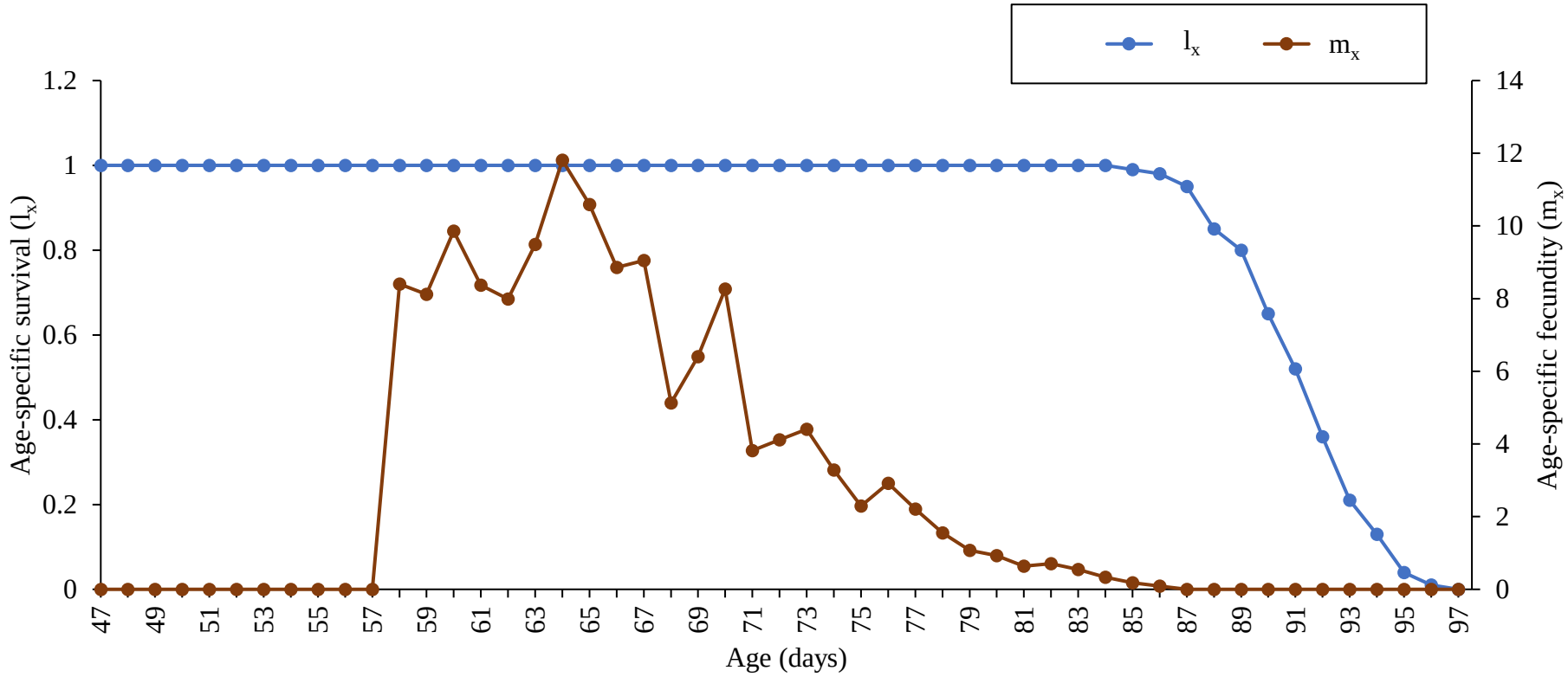

Fig. 1. Age-specific survival (l_x) and age-specific fecundity (m_x) of *M. cribraria*

4.2. Effect of different levels of temperature on the life history of *M. cribraria*

4.2.1. Developmental times and Survivorship of *M. cribraria* at different temperatures

The temperatures within the evaluation range (15–35^{0}C) had a large impact on the development times of *M. cribraria* life stages (Table 7). All eggs which were maintained at 15^{0}C was failed to hatch, hence not shown in the table whereas eggs maintained in 35^{0}C 2nd instar failed to reach 3rd instar stage. The results showed that *M. cribraria* could complete its life cycle at three temperatures 20^{0}C, 25^{0}C and 30^{0}C. Egg development time was significantly impacted by temperature ($F_{3,36}$ =684.419; P<0.05). Mean incubation time was 12.30 ± 0.15, 7.50 ± 0.17, 4.20 ± 0.13 and 3.40 ± 0.16 days for 20, 25, 30 and 35^{0}C respectively. The average developmental time for nymphs was shortened significantly as the temperature increased (1st instar: $F_{3,36}$ =655.304; P<0.05. 2nd instar: $F_{3,36}$ =615.145; P<0.05. 3rd instar: $F_{3,36}$ =2.192; P<0.05. 4th instar: $F_{3,36}$ = 1.573; P<0.05. 5th instar: $F_{3,36}$ =3.152; P<0.05). Total developmental time from egg incubation to adult emergence was decreased significantly with increase in temperature, with the longest period recorded at 20°C (84.90 ± 0.34days) and shortest at 30°C (32.40 ± 0.49days) ($F_{3,36}$ =7.138; P<0.05).

The temperature could significantly affect pre-adult survival rates in *M. cribraria* (Table 8). The eggs of *M. cribraria* had the highest percentage survival at 30^{0}C, followed by 25 and 20^{0}C and the lowest survival occurred at 35^{0}C ($F_{3,16}$ =34.307; P<0.05). Similarly, the proportion of nymphs completing development increased with temperature, except at the highest tested temperature, 35^{0}C (1st to 2nd instar: $F_{3,16}$ =270.597; P<0.05; 2nd to 3rd instar: $F_{3,16}$ =191.917; P<0.05;3rd to 4thinstar: $F_{3,16}$ =333.495; P<0.05; 4th to5thinstar: $F_{3,16}$ =329.996; P<0.05).

4.2.2. Adult longevity and fecundity of *M. cribraria* at different temperatures

The pre oviposition period, oviposition period, fecundity and longevity of *M. cribraria* were significantly influenced by temperature. At 15^{0}C and 35^{0}C *M. cribraria* failed to complete its life cycle. Hence, the result is omitted from the table. The mean pre-

oviposition period (APOP) decreased with an increase in temperature from 48.80 ± 0.25 days at 20^0C to 8.30 ± 0.34 days at 30^0C ($F_{2,27}$=4.260; P<0.05) (Table 9). The length of total pre-oviposition period became progressively shorter from 20^0C to 30^0C ($F_{2,27}$ =7.700; P<0.05). Females reared at 25^0C had the longest oviposition period ($F_{2,27}$ = 2.216; P<0.05), nearly 9.3 times more than that at 20^0 C. The number of eggs laid by *M. cribraria* was increased with an increase in temperature from 20 to 30^0C ($F_{2,27}$ =1.421; P<0.05). The females had the largest fecundity at 30^0C (221.80 ± 3.32 eggs per female) followed by 25^0C (178.70 ± 2.86 eggs per female) and 20^0C (21.30 ± 2.07 eggs per female).

Both female and male longevity were significantly affected among different constant temperatures (male: $F_{2,27}$ =294.478; P<0.05, female: $F_{2,27}$ =916.630; P<0.05) (Table 10). Adults had the shortest longevity at 30^0C and the longevity generally increased with decreasing in temperature. For the same temperature treatment, the adult longevity of females was significantly longer than that of males (20^0C: t = -8.106; df =9; P<0.05; 25^0C: t = -10.647; df =9; P<0.05; 30^0C: -14.478; df =9; P<0.05). The female entire life span showed significant differences at the three temperatures, with the longest (160.30 ± 0.63d) at 20^0C and shortest (70.90 ± 0.65d) at 30^0C ($F_{2,27}$ =3.509; P<0.05). Similarly, the male entire lifespan decreased with the rise in temperatures, with the longest to the shortest longevities at 20^0C > 25^0C > 30^0C respectively ($F_{2,27}$ =1.311; P<0.05).

4.2.3. Life table analysis of *M. cribraria* at different temperatures

Temperature had a significant effect on l_x and m_x values which were presented on Fig. 2, 3 and 4. The l_x value increased as the temperature rose from 20^0C (26.42) to 25^0C (40.64) and then fell at 30^0C (29.22), whereas the m_x increased with an increase in temperature from 20°C (17.36) to 30°C (149.29). The highest value of $\Sigma l_x m_x$ was observed at 30°C followed by 25°C and 20°C. The highest to lowest value of $\Sigma X l_x m_x$ for *M. cribraria* occurred at 25°C (10446.372) > 30°C (8265.049) > 20°C (2429.441), respectively.

Using data on survival rate, fecundity, and sex ratio a life table of *M. cribraria* was constructed under different temperature conditions (Table 11). The highest values in net reproductive rate (R_0) of *M. cribraria* (149.29 female/ Generation) occurred at 30°C

and lowest at 20^0C (17.36029 female/ Generation). Among the three temperature treatments, the total fecundity was maximal at 30°C temperature. The intrinsic rate of increase (r_m) and finite rate of increase (λ) both increased with an increase in temperature from 20 to 30°C. The mean generation time (T) decreased from the maximum at 20°C (142.70 d) to the minimum at 30 °C (55.62 days). The value of doubling time (DT) was lowest (7.70d) at 30°C which means population doubles in size in 7.70 days at this temperature. The Weakly multiplication (Wm) for *M. cribraria* population was the highest at 30°C followed by 25 and 20°C. The results indicated that 30^0C was the most favourable range for *M. cribraria* development, survival and reproduction, where high reproductive potential and shorter generation length were observed.

Table 7. Duration of immature stages (days) of *M. cribraria* at different temperatures

Temperature	Incubation period (d)	1st instar period (d)	2nd instar period (d)	3rd instar period (d)	4th instar period (d)	5th instar period (d)	Total developmental period (d)
20^0C	12.30±0.15a	12.50±0.17a	11.50±0.17a	15.50±0.17a	13.60±0.16a	19.50±0.17a	84.90±0.35a
25^0C	7.50±0.17b	7.30±0.15b	8.20±0.13b	9.30±0.15b	8.50±0.17b	11.40±0.16b	52.20±0.36b
30^0C	4.20±0.13c	4.50±0.17c	4.30±0.15c	5.40±0.16c	5.50±0.17c	8.50±0.17c	32.40±0.49c
35^0C	3.40±0.16d	3.30±0.15d	3.30±0.15d	0.00	0.00	0.00	0.00

Means (±SE) within a column followed by different letters are significantly different (P<0.05) using Student-Newman-Keuls test; 'd' indicates days

Table 8. Percentage survival of immature stages of *M. cribraria* at different temperatures

Temperature	Egg	1st to 2nd instar	2nd to 3rd instar	3rd to 4th instar	4th to 5th instar
20^0C	83.80±1.56a	68.40±1.75a	48.20±1.24a	33.80±1.59a	24.40±1.29a
25^0C	87.60±1.03ab	71.80±0.86ab	52.60±2.22ab	36.80±0.66a	31.60±0.87b
30^0C	92.40±2.20b	75.00±1.38b	57.40±1.81b	44.20±1.28b	37.40±0.93c
35^0C	71.60±0.93c	30.60±0.81c	9.00±0.71c	0	0

Means (±SE) within a column followed by different letters are significantly different (P<0.05) using Student-Newman-Keuls test

Table 9. Adult pre-oviposition period (APOP), total pre-oviposition period (TPOP), oviposition period and fecundity of *M. cribraria* at different temperatures

Temperature	APOP (d)	TPOP (d)	Oviposition period (d)	Fecundity/female
20^0C	48.80±0.25a	133.70±0.21a	3.20±0.44a	21.30±2.07a
25^0C	19.30±0.37b	71.50±0.64b	29.80±0.20b	178.70±2.86b
30^0C	8.30±0.34c	40.70±0.65c	23.60±0.16c	221.80±3.32c

Means (±SE) within a column followed by different letters are significantly different (P<0.05) using Student-Newman-Keuls test

Table 10. Adult entire lifespan and longevity of *M. cribraria* at different temperatures

Temperature	Male adult longevity (d)	Female adult longevity (d)	Male entire lifespan (d)	Female entire lifespan (d)
20^0C	61.10±1.30a	75.40±0.75a	146.0±1.52a	160.30±0.63a
25^0C	49.70±0.91b	60.90±0.65b	101.90±1.05b	113.10±0.93b
30^0C	27.40±0.67c	38.50±0.37c	59.80±0.89c	70.90±0.65c

Means (±SE) within a column followed by different letters are significantly different (P<0.05) using Student-Newman-Keuls test

Table 11. Life table parameters of *M. cribraria* at different constant temperatures

Temperature	Net Reproductive rate (R_O)	Intrinsic rate of increase (r_m)	Mean generation time (T) (d)	Finite rate of increase (λ)	Weakly multiplication (Wm)	Doubling Time (DT) (d)
20^0C	17.36	0.02	142.71	1.020	1.150	34.657
25^0C	122.85	0.057	84.404	1.059	1.490	12.160
30^0C	149.29	0.09	55.621	1.094	1.877	7.701

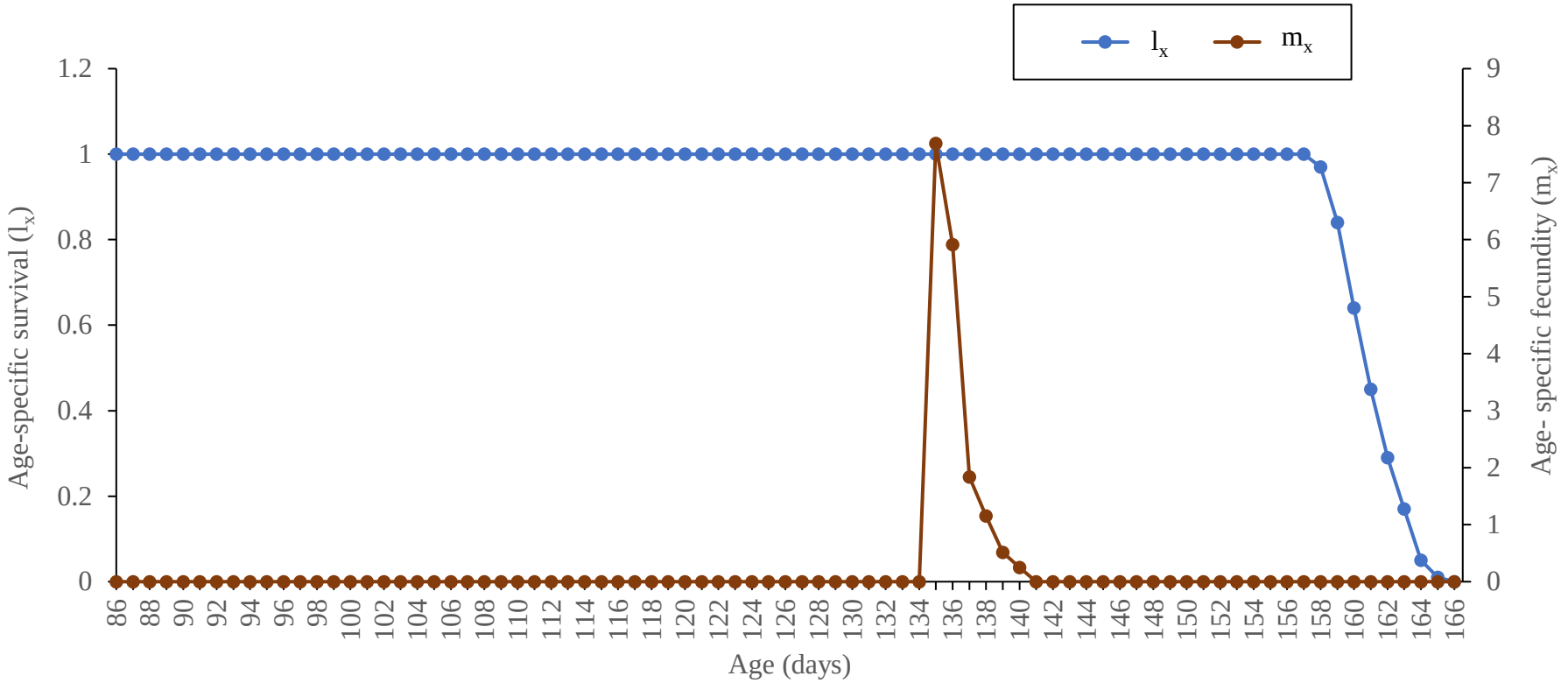

Fig. 2. Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *M. cribraria* reared at 20^0C

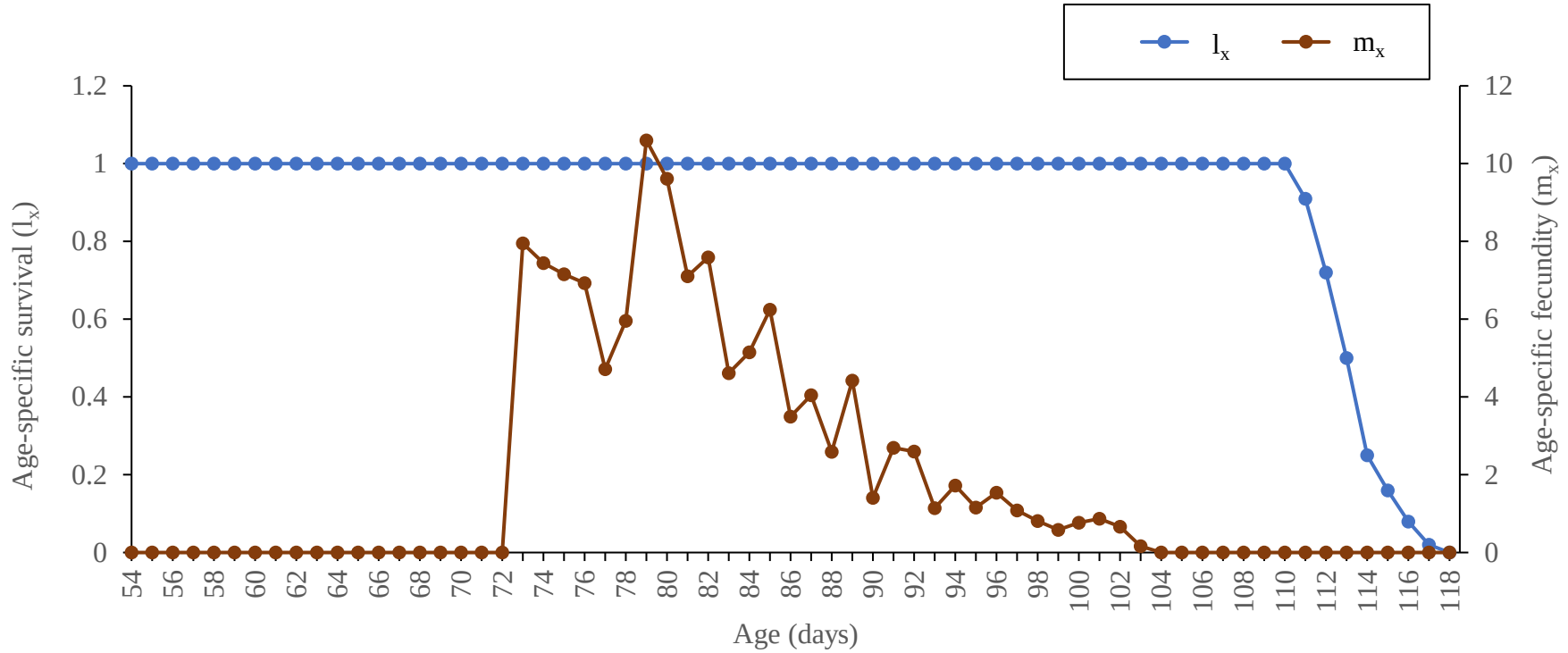

Fig. 3. Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *M. cribraria* reared at 25^0C

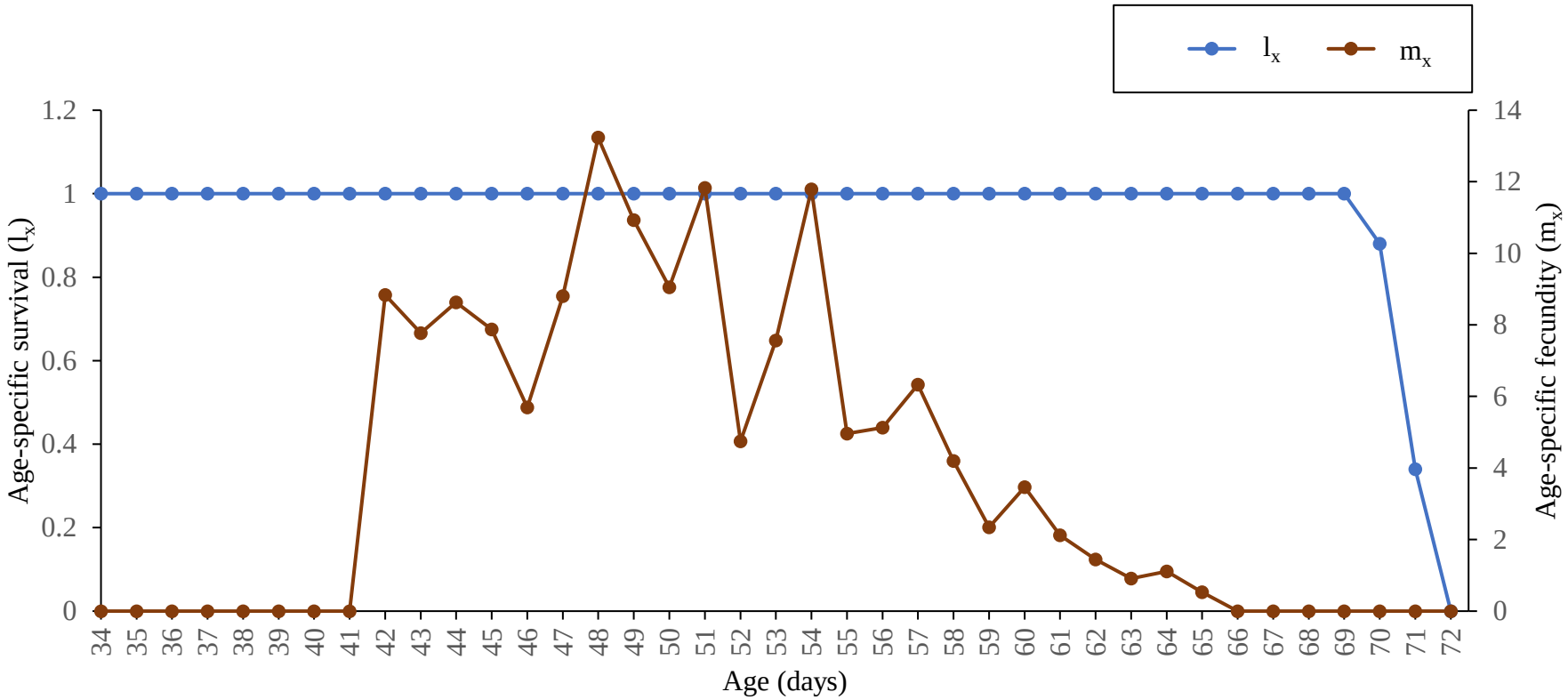

Fig. 4. Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *M. cribraria* reared at 30^0C

4.3. Effect of different levels of relative humidity on the life history of *M. cribraria*

4.3.1. Developmental times and Survivorship of *M. cribraria* at different RH levels

Relative humidity did not greatly affect the developmental duration of eggs and nymphs of *M. cribraria* (egg: $F_{4,25}$=0.488; P=0.745; total nymph: $F_{4,25}$ =2.269; P=0.090) (Table 12). However, the survival percentage of eggs, nymphs and adults were significantly affected by RH (egg: $F_{4,25}$=109.954; P<0.05. 1st to 2nd instar: $F_{4,25}$= 93.126; P<0.05, 2nd to 3rd instar: $F_{4,25}$= 84.555; P<0.05, 3rd to 4th instar: $F_{4,25}$=113.939; P<0.05, 4th to 5th instar: $F_{4,25}$= 108.193; P<0.05). The highest survival rate of eggs was observed at 70% and 80% RH, followed by 60%, 50% and 40%RH respectively. The nymphs of *M. cribraria* had the maximum percentage survival at 80% and 70% RH. The relationships between egg survival rate and relative humidity and between each instar nymph survival rate and relative humidity were good fits to the linear equations, egg: y=0.522x + 49.833 (P<0.05); 1st to 2nd instar: y=0.567x + 30.767 (P<0.05); 2nd to 3rd instar: y=0.448 + 21.467 (P<0.05); 3rd to 4th instar: y=0.525x + 3.833 (P<0.05); 4th to 5th instar: y=0.5383x-5.8333(P<0.05) respectively (Table 12).

4.3.2. Adult longevity and fecundity of *M. cribraria* at different RH levels

Female and male adult longevity differed significantly among relative humidity treatments (male: $F_{4,25}$=146.654; P<0.05, female: $F_{4,25}$=157.458; P<0.05). Females and males had the shortest longevity at 40% RH, and the longevity generally increased with increasing relative humidity (Table 13). However, adult longevities did not exhibit significant differences at 70% and 80% RH. The linear models describing adult longevity (y) as a function of relative humidity (x) were: y=0.490x -2.067 (p = 0.008) for males and y= 0.527x + 1.433(p =0.003) for females.

The number of eggs laid by *M. cribraria* varied substantially among RH levels ($F_{4,25}$=362.323; P<0.05). Females of *M. cribraria* reared from egg hatch at low humidity (40% RH) experienced sharply lower survival and fecundity than females reared at high humidity (70 and 80%). The highest fecundity for *M. cribraria* occurred at 80% RH and these levels were significantly higher than those at 40% RH. There were significant differences in pre oviposition period ($F_{4,25}$= 32.610; P<0.05) and oviposition period ($F_{4,25}$= 166.155; P<0.05) for *M. cribraria* under different RH levels. The mean pre-oviposition period increased with an increase in RH from 5.83 ± 0.30d at 40% RH to 10.0

± 0.36d at 70 and 80% RH. Similar trend was observed in case of oviposition period also (Table13). The oviposition period of females was short at low relative humidity (9.33 ± 0.66d at 40% RH) and long at high relative humidity (24.33 ± 0.21d at 80% RH) respectively.

4.3.3. Life Table analysis of *M. cribraria* at different RH levels

Relative humidity had a significant effect on l_x and m_x values. The net reproductive rates (R_0) in the five RH treatments ranging from the highest to lowest R_0 values were found at 80%,70%,60%,50% and 40% RH ($F_{4,20}$=381.343; P<0.05) (Table 14). Intrinsic rate of increase (r_m) and finite rate of increase (λ) were highest at 80% RH, while the lowest r_m and λ values for *M. cribraria* occurred in individuals reared at 40%RH (r_m: $F_{4,20}$=185.737; P<0.05, λ: $F_{4,20}$=185.262; P<0.05). The mean length of generations (T) increased with increase in RH from 40% RH to 80% RH.

The relationship between RH level and intrinsic rate of increase (r_m), net reproductive rate (Ro), mean generation time (T) and finite rate of increase (λ) were good fit to the linear equations y=0.0003x + 0.0587 (P<0.05), y=2.91x-64.542 (P<0.05), y=0.1871x + 48.398 (P<0.05) and y=0.0003x+1.0603 (P<0.05), respectively (Table14).

4.4. Studies on different behavioural parameters of *M. cribraria*

4.4.1. Food preference

When single plant parts were tested against no plant for preference, 80-90% of *M. cribraria* adults successfully made choices. Within the 10 minute allowed for a response, adults showed a significant preference to plant parts over blank (Table 15). This study also showed that males and females of *M. cribraria* exhibited similar preferences to different plants parts.

From the two-plant choice tests, 90-93% of *M. cribraria* adults used in the experiment successfully made choices. When apices was tested against that of inflorescence, leaves and green pods both male and female showed more response to inflorescence. Order of response was inflorescence > apices > leaves > green pod, respectively. When leaves of *L. purpureus* was tested against that of green pods and inflorescence, it was observed that both males and females were attracted to inflorescence than leaves and least preferred plant part was green pods (Table 16). When inflorescence was compared against green pod, 72.37% of males and 73.57% of females were attracted to inflorescence.

Table 12. Duration and survival rate (± SE) of immature stages (days) of *M. cribraria* at different RH levels

RH	Developmental period (days)		Survival rate (%)				
	Egg	Nymph	Egg	1^{st} to 2^{nd}instar	2^{nd} to 3^{rd} instar	3^{rd} to 4^{th} instar	4^{th} to 5^{th}instar
40%	5.50 ± 0.22a	35.66 ± 0.21a	69.83 ± 1.07a	52.66 ± 1.28a	38.00 ± 0.96a	22.83 ± 1.22a	14.66 ± 0.55a
50%	5.66 ± 0.21a	35.00 ± 0.36a	74.33 ± 0.66b	57.16 ± 0.60b	43.83 ± 0.60b	29.50 ± 0.76b	19.83 ± 0.87b
60%	5.66 ± 0.21a	35.16 ± 0.47a	84.66 ± 0.71c	68.50 ± 0.76c	50.83 ± 0.70c	39.83 ± 0.60c	29.66 ± 0.80c
70%	5.66 ± 0.21a	34.33 ± 0.61a	87.50 ± 0.76d	71.83 ± 0.98d	53.66 ± 0.88d	41.33 ± 0.80cd	33.33 ± 0.98d
80%	5.33 ± 0.21a	33.83 ± 0.60a	89.33 ± 0.80d	73.66 ± 1.05d	55.50 ± 0.76d	43.16 ± 0.54d	34.83 ± 0.94d
Linear model			y=0.522x+49.833	y=0.567x+30.767	y=0.448+21.467	y=0.525x+3.833	y=0.5383x-5.8333
$F_{1,3}$			37.017	36.847	49.939	25.628	41.737
R^2			0.925	0.9247	0 .9433	0.8952	0 .933
P			0.009	0.009	0.006	.015	0.008

Means (±SE) within a column followed by different letters are significantly different (P<0.05).

Table 13. Adult pre-oviposition period, oviposition period, fecundity and adult longevity of *M. cribraria* at different RH levels

RH	pre-oviposition period (d)	Oviposition period (d)	Fecundity/female	Adult longevity (d)	
				Male	Female
40%	5.83 ± 0.30a	9.33 ± 0.66a	46.16 ± 3.98a	15.50 ± 0.76a	21.16 ± 0.70a
50%	7.16 ± 0.30b	14.16 ± 0.30b	104.0 ± 3.39b	23.16 ± 0.70b	27.83 ± 0.47b
60%	8.50 ± 0.22c	18.50 ± 0.56c	172.83 ± 3.79c	29.50 ± 0.76c	34.83 ± 0.47c
70%	10.0 ± 0.36d	23.00 ± 0.51d	209.50 ± 3.37d	33.83 ± 0.60d	39.83 ± 1.07d
80%	10.0 ± 0.36d	24.33 ± 0.21d	218.33 ± 4.62d	34.66 ± 0.42d	41.50 ± 0.42d
Linear model	y=0.112x + 1.6	y=0.3883x-5.433	y= 4.498x -119.733	y=0.490x -2.067	y= 0.527x + 1.433
$F_{1,3}$	51.995	105.823	43.570	39.992	71.464
R^2	0.9454	0.972	0.936	0.930	0.960
P	0.005	0.002	0.007	0.008	0.003

Means (±SE) within a column followed by different letters are significantly different (P<0.05).

Table 14. Life table parameters of *M. cribraria* at different RH levels

RH	Net Reproductive rate (R_O)	Intrinsic rate of increase (r_m)	Mean generation time (T) (d)	Finite rate of increase (λ)
40%	42.03 ± 1.33a	0.068 ± 0.0004a	54.94 ± 0.132a	1.070 ± 0.0004a
50%	81.97 ± 2.49b	0.076 ± 0.0005b	57.65 ± 0.062b	1.079 ± 0.0005b
60%	126.34 ± 2.95c	0.078 ± 0.0003c	61.39 ± 0.075c	1.082 ± 0.0004c
70%	142.86 ± 2.53d	0.080 ± 0.0003d	62.01 ± 0.102d	1.083 ± 0.0003d
80%	157.08 ± 2.49e	0.081 ± 0.0002e	62.11 ± 0.135d	1.084 ± 0.0002e
Linear model	y=2.91x-64.542	y=0.0003x + 0.0587	y=0.187x + 48.398	y=0.0003x + 1.060
$F_{1,3}$	50.686	13.516	17.930	13.632
R^2	0.944	0.818	0.857	0.8196
P	0.006	0.035	0.024	0.034

Means (±SE) within a column followed by different letters are significantly different (P<0.05).

Table 15. Chi-square statistics and associated p-values of *M. cribraria* preferences to single plant part

Plant parts vs blank	Male			Female		
	% Preference to plant parts*	$\chi 2$	P value	% Preference to plant parts*	$\chi 2$	P value
Apices	70.72%	18.84	0.004	74.67%	14.72	0.023
Leaves	68.20%	19.70	0.003	71.82%	22.16	0.001
Inflorescence	72.039%	13.83	0.031	75.33%	13.71	0.033
Green pod	64.04%	20.49	0.002	63.49%	18.96	0.004

*Significant preference with > 50% preference to plant indicates that plant part attracted *M. cribraria.*

Table 16. Chi-square statistics and associated p-values of *M. cribraria* preferences to the combination of two plant parts

Combination of two plant parts tested	Male			Female		
	Preferred plant part (% choice)	$\chi 2$	P value	Preferred plant part (%choice)	$\chi 2$	P value
Apices vs leaves	Apices (65.89%)	16.74	0.010	Apices (61.18%)	25.90	0.000
Apices vs inflorescence	Inflorescence (53.07%)	13.07	0.042	Inflorescence (56.91%)	25.70	0.000
Apices vs green pod	Apices (71.71%)	14.92	0.020	Apices (69.95%)	16.71	0.010
Leaves vs inflorescence	Inflorescence (70.17%)	14.17	0.027	Inflorescence (73.21%)	16.97	0.009
Leaves vs green pod	Leaves (53.83%)	21.62	0.001	Leaves (56.03%)	20.37	0.002
Inflorescence vs green pod	Inflorescence (72.37%)	17.71	0.007	Inflorescence (73.57%)	14.31	0.026

4.4.2. Mating

Mean copulation duration of *M. cribraria* at 1:1, 1:2 and 2:1 sex ratio was 9.80 ± 0.33h, 10.73 ± 0.41h and 11.03 ± 0.79 hour, respectively (Fig.5). There was no significant difference in mean copulation duration among the three sex ratios ($F_{2,12}$=1.39, P > 0.05).

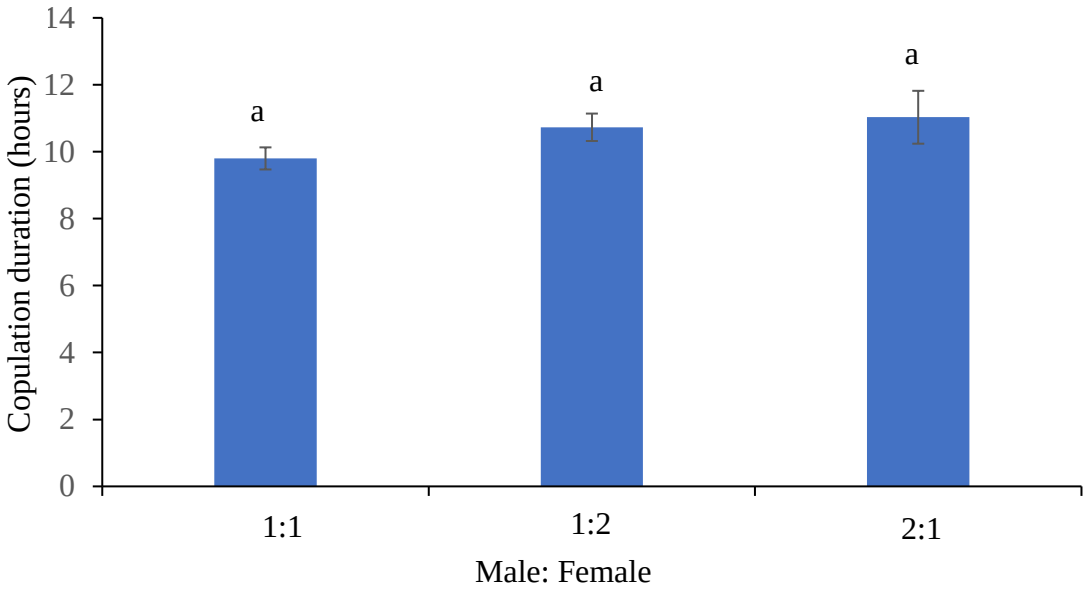

Fig. 5. Copulation duration (h) of *M. cribraria* under different sex ratios (Mean ± SE)

4.4.3. Defensive response

VOCs were collected from disturbed and undisturbed adults of *M. cribraria* was analysed by coupled GC-MS. Numerous VOCs were detected by the GC based on the retention times from 4.782 to 56.397 and the detected compound were identified based on matches in the NIST 08 database (Table 17). Fifty four different compounds were detected in the GC-MS study. Among, the samples 1 and 10 were detected only in disturbed samples whereas 6,12,15,16 and 43 compounds were only present in undisturbed samples. Compound no. 34 is present in both samples at highest concentrations. When comparing the area of chromatogram, in the disturbed samples second highest compound was 1. Most probably this compound can be the potential compound which invoke behavioural response among host insect or predators. Octacosane, decamethyl cyclopentasiloxane, 2-ethylhexanol, tridecane and dodecane, 4,6-dimethyl- were present in the sample from undisturbed *M. cribraria* but these were

absent from disturbed *M. cribraria* sample. (E)-2- hexenal and 2-hexene, 4,4,5-trimethyl were unique to the VOCs collected from disturbed *M. cribraria* eluted with a retention time of 4.782 and 11.182 minutes. These two compounds were further verified using standards and further studies on electro antennogram (EAG) and single cell receptors were referred to know its role as a behaviour modifying chemical.

4.4.4. Phototropic response

When yellow, white, blue, green and red coloured lights were tested against dark, 83-92% of *M. cribraria* adults successfully made choices. *M. cribraria* adults exhibited a significant ($P < 0.05$) preference to yellow, white and green coloured light (Table 18). Blue and red coloured lights were not attractive to both male and female bugs.

From the two-coloured light choice tests, 91-93% of adult bugs successfully made choices in Y tube olfactometer assay. White light colour attracted more bugs than yellow and green light. While comparing yellow and green light, both male and female bugs preferred yellow light. The overall order of attraction of light source tested on *M. cribraria* was white > yellow > green respectively (Table 19).

4.5. Survey of egg parasitoids associated with *M. cribraria* in the field

Results from the surveys were summarised in Table 20. It was noticed that two egg parasitoids were emerged out from field collected egg masses of *M. cribraria*. The emerged egg parasitoids were identified as new parasitoid to the science, *Paratelenomus anu* Rajmohana, Sachin & Talamas, (Hymenoptera, Scelionidae) and already identified one, *Encarsiella boswelli* (Girault) (Aphelinidae) (Polaszek and Hayat, 1990, 1992). No entomopathogenic fungus were detected from the field survey. It was observed that in both field and laboratory conditions, females of *P. anu* were found to be phoretic on their bug hosts, with up to five wasps on a single host bug. *P. anu* was the most abundant species with 73.7 ± 7.3 % of mean parasitism rate, whereas *E. boswelli* showed relatively low level of parasitism of 18.64 ± 2.0 % (Table 20). So, *P. anu* was studied extensively in all possible angles.

Table 17. List of VOCs emitted by both disturbed and undisturbed *M. cribraria* identified and analysed by GC-MS

Compound name	RT	Area	Disturbed *M. cribraria*	Undisturbed *M. cribraria*
1. (E)-2-hexenal	4.782	167553101	✓	-
2. Cyclopentanol, 1-methyl	5.891	3179388	✓	✓
3. Heptane, 2,4-dimethyl-	6.654	4461224	✓	✓
4. 2,4-Dimethyl-1-heptene	7.273	26138539	✓	✓
5. 2-Pentanone, 4-hydroxy-4-methyl	7.397	2981661	✓	✓
6. 2-Ethylhexanol	9.384	4444699	-	✓
7. Pentanal, 2,2-dimethyl	9.999	9858481	✓	✓
8. Oxalic acid, cyclohexyl pentyl ester	11.116	21072897	✓	✓
9. Cyclopropane, 2-bromo-1,1,3-trimethyl	11.129	36099688	✓	✓
10. 2-Hexene, 4,4,5-trimethyl-	11.182	4498416	✓	-
11. Decane	11.778	3062731	✓	✓
12. Decamethyl cyclopentasiloxane	Tridecane	16.952	3560765	-
13. 3-Tetradecene, (Z)-	Tridecane	16.952	3560765	-
14. 1-Octanol, 3,7-dimethyl-/	Tridecane	16.952	3560765	-
15. Tridecane	Tridecane	16.952	3560765	-

Continued next page

Compound name	RT	Area	Disturbed *M. cribraria*	Undisturbed *M. cribraria*
16. Dodecane, 4,6-dimethyl-	16.740	2197282	-	✓
17. Benzene, 1,3-bis(1,1-dimethylethyl)-	17.122	17136901	✓	✓
18. 2-Isopropyl-5-methyl-1-heptanol	18.030	9558603	✓	✓
19. Tetradecane	19.801	2918996	✓	✓
20. Nonadecane	20.999	5224715	✓	✓
21. Phenol, 2,4-bis(1,1-dimethylethyl)-	21.717	15117244	✓	✓
22. Dodecanoic acid, methyl ester	21.840	17905508	✓	✓
23. 1-Hexadecanol, 2-methyl-	21.949	8006750	✓	✓
24. 1-Hexadecanol, 3,7,11,15-tetramethyl	22.111	6130411	✓	✓
25. 3-Eicosene, (E)-	22.266	3714017	✓	✓
26. Methyl tetradecanoate	24.841	14925433	✓	✓
27. Cyclohexane, 1,3,5-trimethyl-2-octadecyl	24.975	5276276	✓	✓
28. Oleic Acid	25.541	3809086	✓	✓
29. Phytol	26.282	40090238	✓	✓
30. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol	26.324	8689430	✓	✓
31. 9,12-Octadecadienoic acid (Z,Z)-	26.868	11293736	✓	✓
32. n-Hexadecanoic acid	28.139	169778405	✓	✓

Continued next page

Compound name	RT	Area	Disturbed *M. cribraria*	Undisturbed *M. cribraria*
33. 3,4 Oxazolidine dicarboxylic acid, 2-(1-methylethyl)-, 3-methyl ester, (2R-cis)-	30.033	4563190	✓	✓
34. 9,12-Octadecadienoic acid (Z,Z)-	30.433	2561356714	✓	✓
35. Octadecanal, 2-bromo-	31.905	2416124	✓	✓
36. Oleic acid, 3-(octadecyloxy) propyl ester	32.970	5313314	✓	✓
37. 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	33.733	31712026	✓	✓
38. 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	33.777	23778717	✓	✓
39. Eicosane	33.993	8193976	✓	✓
40. 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	34.345	8456546	✓	✓
41. Heptacosane	35.929	6569433	✓	✓
42. Hexacosane	34.979	10043221	✓	✓
43. Octacosane	36.848	8832181	-	✓
44. Squalene	36.975	16667007	✓	✓
45. Tetratetracontane	38.701	5663079	✓	✓

Continued next page

Compound name	RT	Area	Disturbed *M. cribraria*	Undisturbed *M. cribraria*
46. Hexadecanoic acid, butyl ester	39.573	364445	✓	✓
47. Stigmasterol	41.190	7001215	✓	✓
48. Cyclopentane carboxylic acid, 2-amino-, cis-	42.683	134267	✓	✓
49. Pentadecanal	44.926	8724777	✓	✓
50. β-Sitosterol	48.062	12931723	✓	✓
51. 1-Docosene	52.860	2395502	✓	✓
52. Oxirane, hexadecyl-	53.505	8027776	✓	✓
53. α-Amyrin	53.897	3957036	✓	✓
54. 7-Hexadecenal, (Z)	56.397	2420391	✓	✓

Table 18. Chi-square statistics and associated p-values of *M. cribraria* preferences to single different coloured light

Light colour vs dark	Male			Female		
	% Preference to light colour*	χ 2	P value	% Preference to light colour*	χ 2	P value
Yellow	71.88%	21.24	0.002	67.70%	20.40	0.002
White	75.52%	20.96	0.001	72.39%	14.97	0.020
Blue	47.39%	1.04	0.983	44.27%	1.59	0.953
Green	63.020%	13.84	0.032	69.27%	15.55	0.016
Red	44.27%	4.73	0.58	41.67%	5.25	0.512

*Significant preference with < 50% preference to light source indicates that light source not attracted by *M. cribraria*.

Table 19. Chi-square statistics and associated p-values of *M. cribraria* preferences to the combination of two light colours

Comparison of two colours	Male			Female		
	Preferred light colour (% choice)	χ 2	P value	Preferred light colour (% choice)	χ 2	P value
Yellow vs white	White (54.69%)	12.91	0.044	White (58.58%)	13.13	0.041
Yellow vs green	Yellow (65.10%)	15.14	0.019	Yellow (68.23%)	16.16	0.013
White vs green	White (69.27%)	14.72	0.022	White (72.39%)	13.35	0.038

Table 20. Summary of the percentage parasitism of *M. cribraria* egg masses by *P. anu* and *E.boswelli* in field during 2015-2021

Location	% Parasitism by	
	P.anu	*E.boswelli*
1) Malaparamba near providence college, Lat. 11.292975, Long. -75.803572	73.58	23.87
2) Calicut University, Lat. 11.131442, Long -75.894595	76.21	0
3) Koyilandi- Nelluli tazham, Lat. 11.472937, Long -75.676152	75.29	18.95
4) Kunnamangalam Markaz, Lat. 11.306922, Long -75.894595	74.95	0
5) Palayattunada Maniyoor, Vatakara Lat. 11.573128, Long-75.61774	73.66	0
6) Nallalam-padam bus stop, Lat. 11.258753N, Long-75.780411	76.79	0
7) Kizhur poovadithara Lat.11.5218472, Long-75.63299	69.75	0
8) Azhiyur-Mahi Lat.11.69931, Long-75.54601	74.45	0
9) Aravind Gosh bus stop -Puthuppanam Lat.11.57621, Long-75.60383	71.9	0
10) Payyoli- near court complex Lat.11.51628, Long-75.62120	64.88	0
11) Poyiloor -near north LP school Lat.11.76724, Long-75.64362	69.33	0
12) Kadampuzha- Vettichira Lat.10.94401, Long-76.04369	78.26	0

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Location	% parasitism by	
	P.anu	*E.boswelli*
13) Thanikkudam -Vellanikkara road, Madakkathara Lat.10.550655, Long-76.265859	75.23	0
14) Thondinmal kinar bus stop, Mukkam Lat.11.34010, Long-75.99502	72.2	16.07
15) Uppumpetti Koyilandy-Edavanna road Lat.11.44694, Long-75.86935	75.26	0
16) Anakkulam-near SARBTM-Govt.college, Muchukunnu Lat.11.49255, Long-75.66950	77.7	0
17) Thenakkuzhi- Kizhakkayil road near Balussery Town Lat.11.44816, Long-75.86037	72.38	0
18) BST ground-near Kallai railway station, Lat.11.23495, Long-75.78899	75.8	14.17
Mean percentage of parasitism	**73.7 ± 7.3**	**18.64 ± 2.0**

4.5.1. Diagnostic features of *P. anu*

Results of the taxonomic identification is described below.

Plate. 13

Material examined: Holotype, female: INDIA: Kerala St., Malapparamba, near Providence College, 9.VI.2015, J. Sachin, ZSI/WGRS/I.R-INV.5069 (deposited in ZSIK). Paratypes: INDIA: 17 females, 2 males, CNC494969–494970 (CNCI); 21987/H3–21999/H3 (ZSIC); ZSI/WGRS/I.R-INV.5070–5073 (ZSIK).

Body length: Female: 0.65–0.71mm. Male: 0.66–0.68mm. Colour. Body black to honey brown; first metasomal tergite slightly xanthic, weakly contrasting with posterior metasomal segments; antenna and legs yellow to brown; wings hyaline; wing venation brown.

Head: Frons mostly smooth with coriaceous sculpture dorsally; central keel attenuated dorsally, not bifurcating around median ocellus; submedian carina absent; orbital carina present; a single row of equidistant setae present along orbital carina; gena dorsally coriaceous, as on vertex, but smooth toward mandibular articulation; occipital carina incomplete medially; crenulae arising from occipital carina short; labrum pentagonal, slightly more than 2× wider than long, apex bidentate medially; antennal clava 4-merous; claval formula A11–A8: 1-2-2-1; A5 in males with tyloid.

Mesosoma: Notauli absent to weakly present posteriorly; mesoscutum with coriaceous sculpture; parapsidal lines present; mesoscutal humeral sulcus and mesoscutal suprahumeral sulcus indicated by cells; transscutellar articulation narrowed medially, wider and crenulate laterally; foveae of posterior mesoscutellar sulcus of uniform size; mesoscutellum abutting mesoscutum medially; disc of mesoscutellum semicircular, with coriaceous sculpture; setal bases on mesoscutellum simple, not pustulate; metascutellum rugulose; mesopleural carina absent; intercoxal space narrow, not completely occluded by postacetabular and mesopleural epicoxal sulci; acetabular field small, finely setose, and coriaceous; episternal fovea present; femoral depression weakly indicated; prespecular sulcus present; metapleural triangle present; metapleural carina present; paracoxal sulcus absent; posterodorsal metapleural sulcus present. Metasoma. T1 longitudinally costate, with two lateral setae; T2 striate, striae absent in lateral and posterior portions of tergite.

Male: Similar to female, except antennae filiform and metasoma with 8 external tergites and 7 external sternites.

Sequence analysis: The CO1 sequence of *Paratelenomus anu* (KT896660.1) was analyzed using the online BLAST tool of NCBI for comparison with other sequences in the GenBank database. The barcoding revealed that *P. anu* showed 85% sequence identity with *P. saccharalis* (KC778442.1) with 520/628 identities, and 7 gaps that accounted for about 1% of the total alignment length. This degree of sequence divergence is congruent with treatment of *P. saccharalis* and *P. anu* as separate species. Results of the DNA barcoding showed that examined specimen is new to science.

4.6. Life table and development of mass rearing technique of *P. anu*

4.6.1. Rearing of *P. anu* in the laboratory

The rearing of *P. anu* was done successfully in the laboratory using honey as food source for the emerged parasitoids. Longevity of *P. anu* significantly varied with different concentrations of honey ($F_{5,54}$= 458.912; P<0.05). The highest longevity 12.27 ± 0.15 days was found when they were fed with 20% honey and the lowest longevity (3.33 ± 0.16 days) was recorded when the parasitoids were fed with distilled water (Fig.6).

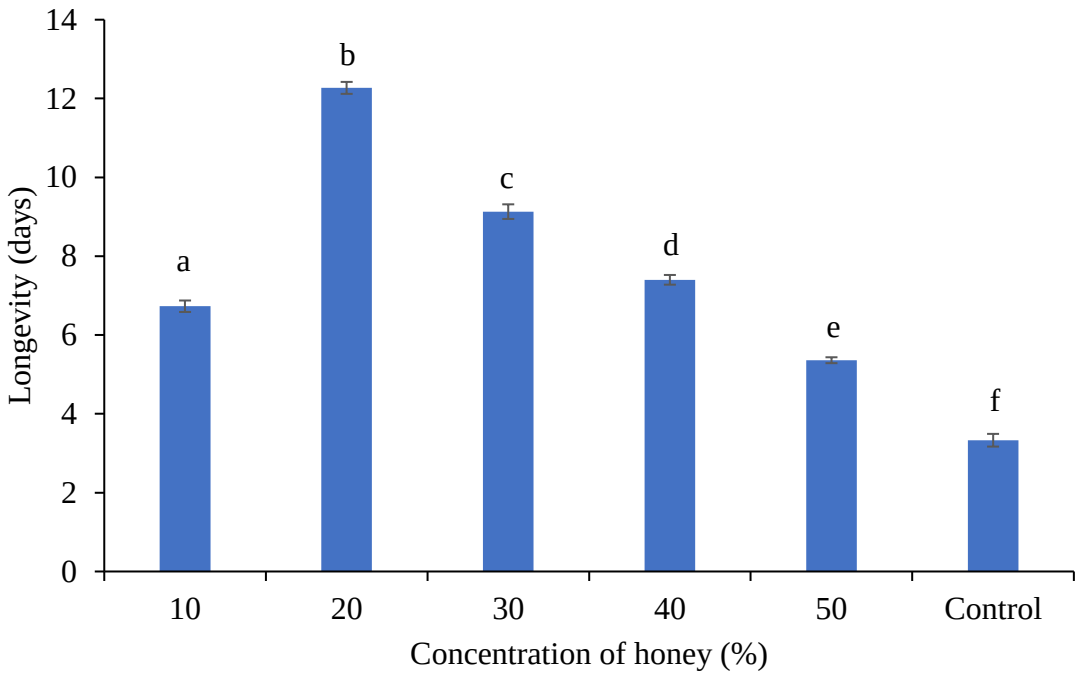

Fig. 6. Adult longevity of *P. anu* fed on different concentrations of honey. [Bars with different letters indicated significant differences between treatments (n = 10; S-N-K test P < 0.05)].

4.6.2. Life table of *P. anu* on eggs of *M. cribraria* at room temperature

In the laboratory, the first instar nymphs of *M. cribraria* emerged at 5.2 ± 0.04d from un parasitized eggs, while the average length of the egg-adult period of *P. anu* was 13.4 ± 0.071 d. The parasitism rate of *P. anu* on *M. cribraria* eggs was 75.86 ± 0.74 % at room temperature 27 ± 2^0C, 80 ± 5% RH and 14 L :10 D photoperiod. Among the parasitized egg masses, the first instar nymph emergence from unparasitized eggs was 7.54 ± 0.41%. The percentage of emergence of parasitoids was 73.52 ± 0.29 % (Table

21). The percent emergence of male and female parasitoids was 10.42 ± 0.2% and 63.1 ± 0.35 %. Among all the egg batches, the maximum number of male parasitoids emerged was four and female-biased sex ratio was 1:0.86.

P. anu male wasps usually emerged first from parasitized eggs and remained on egg mass for the emergence of the females, with which they immediately mate for 12–15 sec. Following copulation, males continued waiting for the emergence of next female from the same egg batch. The females lay eggs immediately after copulation, being provisioned with host eggs so that pre-oviposition period was not observed. The oviposition and post oviposition periods were 5.91 ± 0.19d and 6.94 ± 0.19d respectively. Charting the production of offspring throughout the parasitoid female's life elucidated that the highest daily fertility rates occurred on the first day of life with 21.62 eggs/female and the average lifetime fecundity of wasps was 36.09 ± 0.29 eggs/female. The adults provided with 20% honey lived up to 16 days, with an average longevity of females was 12.85 ± 0.19 d and 12.04 ± 0.34 d for males (Table 21).

Table 21. Life history parameters of *P. anu*

Life history parameters	Mean ± SE
Rate of parasitism	75.86 ± 0.74%
Rate of emergence of nymphs from unparasitized eggs in parasitized egg mass	7.54 ± 0.41%
Rate of emergence of parasitoid	73.52 ± 0.29%
Rate of emergence of male parasitoids	10.42 ± 0.2%
Rate of emergence of female parasitoids	63.1 ± 0.35%
Sex ratio (female:male)	1:0.86
Egg- adult developmental time	13.4 ± 0.07d
Pre oviposition period	0.00 d
Oviposition period	5.91 ± 0.19d
Total number of host eggs parasitized/ female parasitoid	36.09±0.29eggs
Female longevity	12.85 ± 0.19 d
Male longevity	12.04 ± 0.34 d

Data in the table are represented as mean ± SE.

Life table analysis of *P. anu* at room temperature

Age-specific survivorship (l_x) and fecundity (m_x) of *P. anu* female reared on the eggs of *M. cribraria* were represented in Fig 7. The first emerging female was on day 15 and the first death was on day 23. The last female died on the 29th day of emergence. Females started to parasitise host eggs from the 15th day and stopped it after 20th day.

The net reproductive rate (Ro) of *P. anu* on eggs of *M. cribraria* was 19.40 female offspring / female with the generation time (T) of 15.86 days. The intrinsic rate of increase (r_m) reported was 0.187 female per day. The finite rate of natural increase (λ) obtained as 1.21 female offspring per female per day (Table 22).

Table 22. Life table parameters of *P. anu*

Parameters	Values
Net reproductive rate (Ro) (female offsprings)	19.40
Mean generation time (Tc) (days)	15.86
Intrinsic rate of increase (r_m) (females^{-1} female^{-1} day^{-1})	0.187
Finite rate of increase (λ) (females^{-1} female^{-1} day^{-1})	1.21

4.6.3. Effect of mating on parasitism, emergence and sex ratio of *P. anu*

Results showed that no differences were found between mated and unmated females of *P. anu* with respect to the rate of parasitism of host eggs, percentage of parasitoid emergence and longevity of parental females (days) (Table 23). In contrast, offspring sex ratio differed between treatments (0.86 for mated females and 0.00 for unmated females) indicating that *P. anu* reproduces parthenogenetically with characteristics of the arrhenotoky type (i.e. unfertilized females give rise to haploid males).

Table 23. Influence of mating on certain biological parameters of *P. anu*

Treatment	Number of parasitized eggs (%)	Parasitoid emergence (%)	Sex ratio	Longevity of parental females (days)
Unmated	74.47 ± 0.60	72.80 ± 0.22	0.00	12.29 ± 0.35
Mated	75.45 ± 0.32	73.32 ± 0.57	0.86 ± 0.001	12.24 ± 0.42
$F_{1,10}$	2.054	0.756	211383.907	0.010
P	0.182	0.405	0. 0001	0.921

Data represented as Mean ± SE

4.6.4. Effect of host egg age on parasitism, emergence and sex ratio of *P. anu*

Host egg age had a significant effect on the mean number of eggs parasitized by *P. anu* (Fig.8). *P. anu* preferred host eggs at early developmental stages ($F_{3,16}$= 597.807; Duncan $p < 0.05$) and also parasitized a higher number of these eggs. The number of parasitized eggs was highest for *M. cribraria* eggs of 12 hour (76.13 ± 0 .60%), followed by eggs of 24 hour (43.87 ± 0.60%), 48 hour (23.87 ± 0.60%) and least parasitized at 72 hour old (9.95 ± 2.10%). Additionally, the number of first instar nymphs emerged from un parasitized eggs increased with host egg age increased from 12 hour to 72 hour ($F_{3,16}$= 2875.600; Duncan $p < 0.05$. 12h: 7.09 ± 0.39%; 24h: 33.22 ± 0.65%; 48h: 57.74 ± 0.60%; 72h: 77.74 ± 0.60%. Fig.9.).

Mean percentage parasitoid emergence significantly decreased with egg age whereas host emergence increase with egg age (Fig.10. $F_{3,16}$= 205.547; Duncan $p < 0.05$). The highest emergence was recorded from 12h old eggs and the lowest from 72h old eggs (12h: 72.89 ± 0.98%; 24h: 40.39 ± 1.17%; 48h: 20.14 ± 1.72%; 72h: 7.86 ± 3.22%). In contrast, the sex ratio was unaffected by the age of the eggs (Fig.11. $F_{3,16}$= 0.930; Duncan $p > 0.05$) and the parasitoid developmental time was 13.4 ± 0.071 days in all treatments.

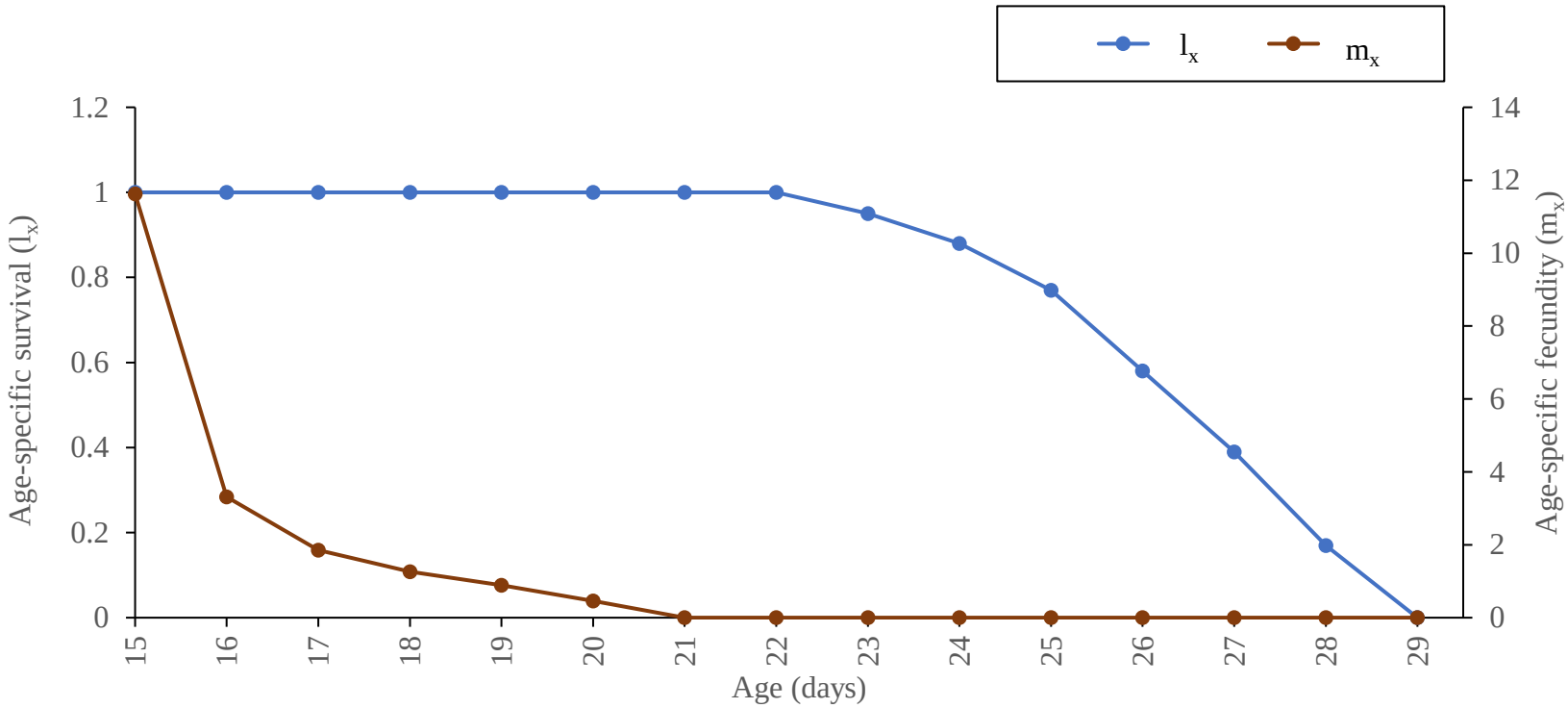

Fig.7. Age-specific survivorship (l_x) and age-specific fecundity (m_x) of *P. an*

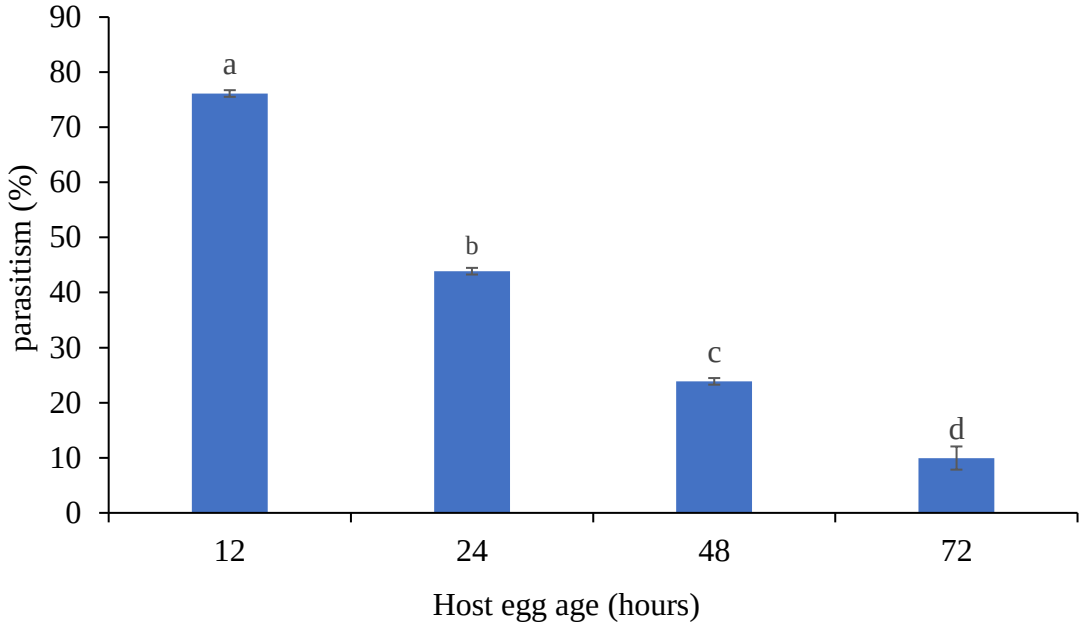

Fig.8. Percentage of parasitism (Mean ± SE) of *P. anu* on different age eggs of *M. cribraria*. [Bars with different letters indicated significant differences between treatments (n = 5; Duncan P < 0.05)].

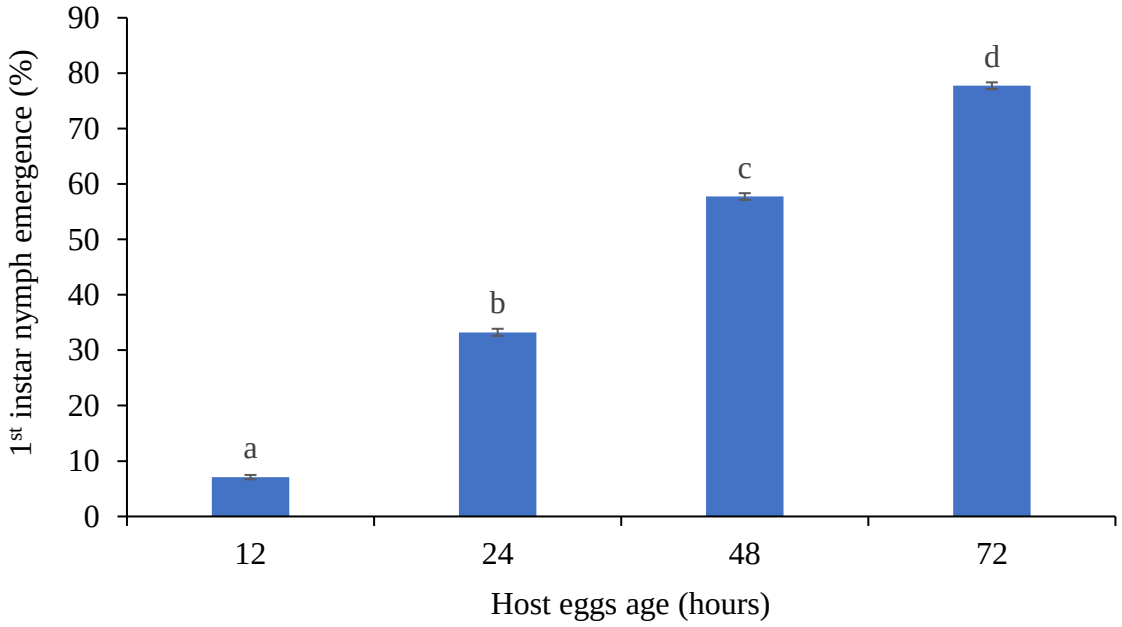

Fig.9. Percentage of emergence (mean ± SE) of 1st instar host nymphs from unparasitized eggs of *M. cribraria*. [Bars with different letters indicated significant differences between treatments (n = 5; Duncan P < 0.05)].

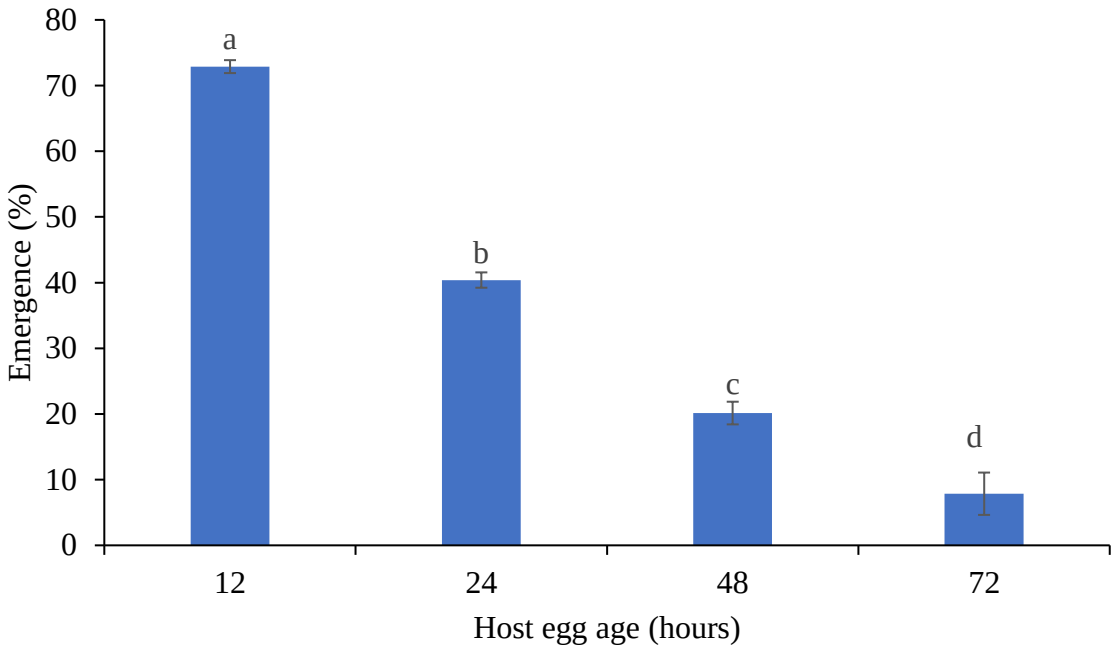

Fig. 10. Percentage of emergence (mean ± SE) of *P. anu* from different age eggs of *M. cribraria*. [Bars with different letters indicated significant differences between treatments (n = 5; Duncan P < 0.05)].

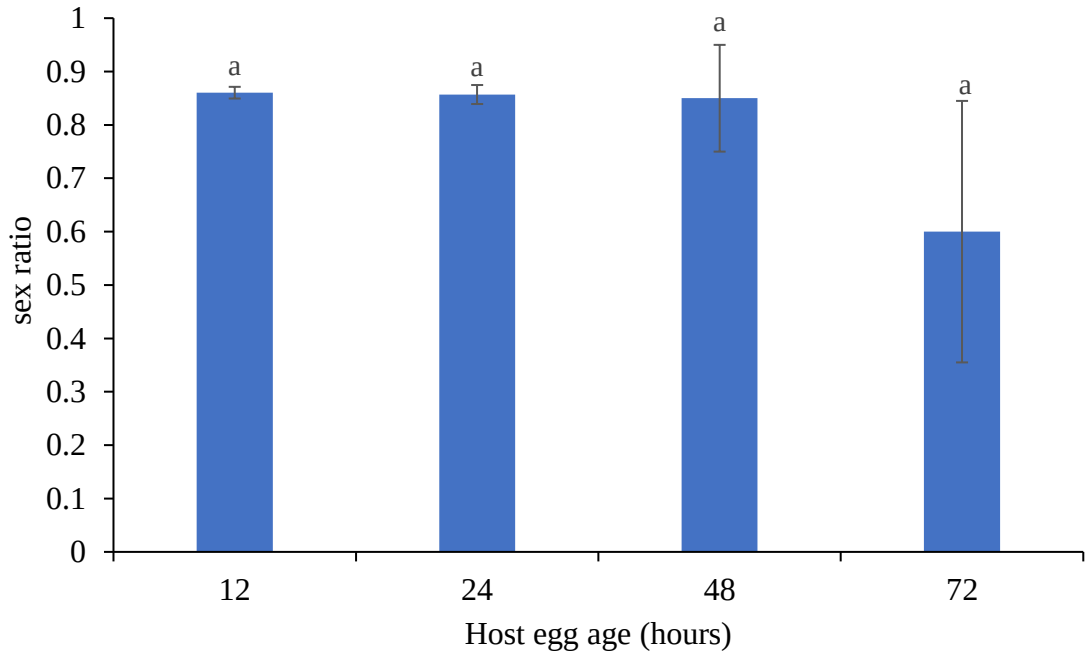

Fig.11. Sex ratio (mean ± SE) of *P. anu* on different age eggs of *M. cribraria.* [Bars with the same letters indicated non-significant differences between treatments (n = 5; Duncan P > 0.05)].

4.6.5. Effect of different levels of temperature on the parasitism and life history of *P. anu*

Results showed that *P. anu* successfully completed its development in the temperature range 20–30^{0}C. No emergence occurred at 15 and 35^{0}C, hence further study at these temperatures was discontinued. The mean number of *M. cribraria* eggs parasitized by *P. anu* was significantly influenced by the temperature ($F_{2,12}$= 129.970; p<0.05). The percentage of parasitism by *P. anu* increased from 48.78 ± 2.11 to 79.39 ± 0.77 as temperature increased from 20^{0}C to 30^{0}C (Table 24). The mean developmental time for eggs was shortened significantly as the temperature increased ($F_{2,12}$= 416.083; p<0.05). The observed developmental times was 26.00 ± 0.45 days at 20^0 C, 16.20 ± 0.37 days at 25^{0}C and 9.80 ± 0.37 days at 30^{0}C. A significant increase in the rate of emergence (survival) of *P. anu* from parasitized eggs with an increasing temperature ($F_{2,12}$=177.082; p<0.05). The emergence rate varied from 42.53 ± 0.74% for parasitized eggs reared at 20^{0}C to 74.39 ± 1.13 % for eggs reared at 30^{0}C. No significant differences in the sex ratio of emerged wasps among the temperatures tested ($F_{2,12}$=3.783; P>0.05).

Female wasps began ovipositing on the first day of their emergence. So, pre-oviposition period was not observed in all tested temperatures. The oviposition periods increased as temperatures increased from 3.40 ± 0.24 days at 20^{0}C to 6.40 ± 0.24 days at 30^{0}C ($F_{2,12}$=38.889; p<0.05). Temperature had a significant effect on the lifetime fecundity of *P. anu* ($F_{2,12}$=205.162; p<0.05). Across all of the temperatures examined, the lowest fecundity was observed at 20^{0}C (15.20 ± 0.58 eggs/female) and the highest one was observed at 30^{0}C (35.00 ± 0.71 eggs/female), with 30^{0}C > 25^{0}C > 20^{0}C, respectively (Table 24). For all three temperatures tested, maximum daily fecundity decreased with increasing age of the females. Temperature had an inverse relationship between the longevity of adult wasps; as temperatures increased, longevity decreased (male longevity: $F_{2,12}$= 89.613; p<0.05, female longevity; $F_{2,12}$= 92.467; p<0.05).

Table 24. Life history of *P. anu* at different constant temperatures

Life history parameters	Temperature		
	20^0C	25^0C	30^0C
Rate of parasitism (%)	48.79±2.11a	73.64±1.03b	79.39±0.77c
Developmental duration (d)	26.00±0.45a	16.20±0.37b	9.80±0.37c
P. anu emergence (%)	42.61±1.77a	69.54±0.74b	74.39±1.13c
Sex ratio	0.82±0.02a	0.86± .006a	0.86±0.004a
Pre oviposition period (d)	0.00	0.00	0.00
Oviposition period (d)	3.40±0.24a	5.40±0.24b	6.40±0.24c
Fecundity (No. of host eggs parasitized/female)	15.20±0.58a	28.20±0.80b	35.00±0.71c
Male longevity (d)	18.80±0.37a	14.80±0.37b	10.20±0.58c
Female longevity (d)	19.20±0.37a	15.00±0.45b	10.60±0.51c

Within columns, different letters indicate significant difference at P<0.05 level by Tukey HSD test.

Life table analysis of *P. anu* at different constant temperatures

The values of m_x increased with temperature from 20^0C to 30^0C (Fig.12-14). The l_x increased as the temperature decreased from 30^0C to 20^0C. The net reproduction rates (R_0) showed increases ranging from a factor of 8.62 at 20^0C to 18.85 at 30^0C for each generation in the populations of *P. anu*. The highest population increase occurred for *P. anu* at 30^0C. The rate of population increase per unit time (r_m) ranged from 0.075 (20^0C) to 0.245 (30^0C). The highest value of r_m were recorded at temperatures of 30^0C (Table 25). The highest finite increase ratio (λ) was 1.278 for females who spent their adult lives at 30^0C and the lowest finite increase ratio was recorded at 20^0C (1.078). The mean generation time (T), the time elapsing between birth of the parents and birth of the offspring, ranged from 11.986 days (30^0C) to 28.731 days (20^0C).

Table 25. Life table parameters of *P. anu* at different constant temperatures

Temperature	Net reproductive rate (Ro)	Mean generation time (T)	Intrinsic rate of increase (r_m)	Finite rate of increase (λ)
20^0C	8.626	28.731	0.075	1.078
25^0C	15.448	19.010	0.144	1.155
30^0C	18.853	11.986	0.245	1.278

4.7. Field release in microplot and evaluation of control efficiency of *p. anu*

The results of microplot study were given in table 26. In the control plots, the emergence rate of first instar nymphs were 86.92±1.86% resulting in zero parasitism rate. In the release plots, parasitism and emergence rate of *P. anu* from *M. cribraria* egg masses were 72.56±1.91% and 68.70±1.62%, respectively. The first instar nymphs that emerged from un parasitised eggs in the release plots was 7.18±0.44%. In the present study, *P. anu* could bring about 79.74±1.42% reduction in the emergence rate of first instar nymphs of *M. cribraria* compared to their emergence rate in control plot

Table 26. Field efficiency of *P. anu* as biocontrol agent for *M. cribraria* in microplot assay

Release plot	Percentage of parasitism (%)	Percentage of emergence of *P. anu* (%)	Percentage of *M. cribraria* emerged (%)
1	72.41±2.03	68.45±1.63	7.32±0.45
2	71.55±2.06	68.07±1.65	7.75±0.44
3	73.70±1.67	69.59±1.58	6.46±0.43
Mean ± SE	72.56±1.91	68.70 ±1.62	7.18±0.44
Control plot			
1	-	-	87.5±2.01
2	-	-	86.20±1.75
3	-	-	87.06±1.83
Mean±SE	-	-	86.92±1.86

Percentage of reduction of 1st instar nymph of *M. cribraria* over control=79.74±1.42%

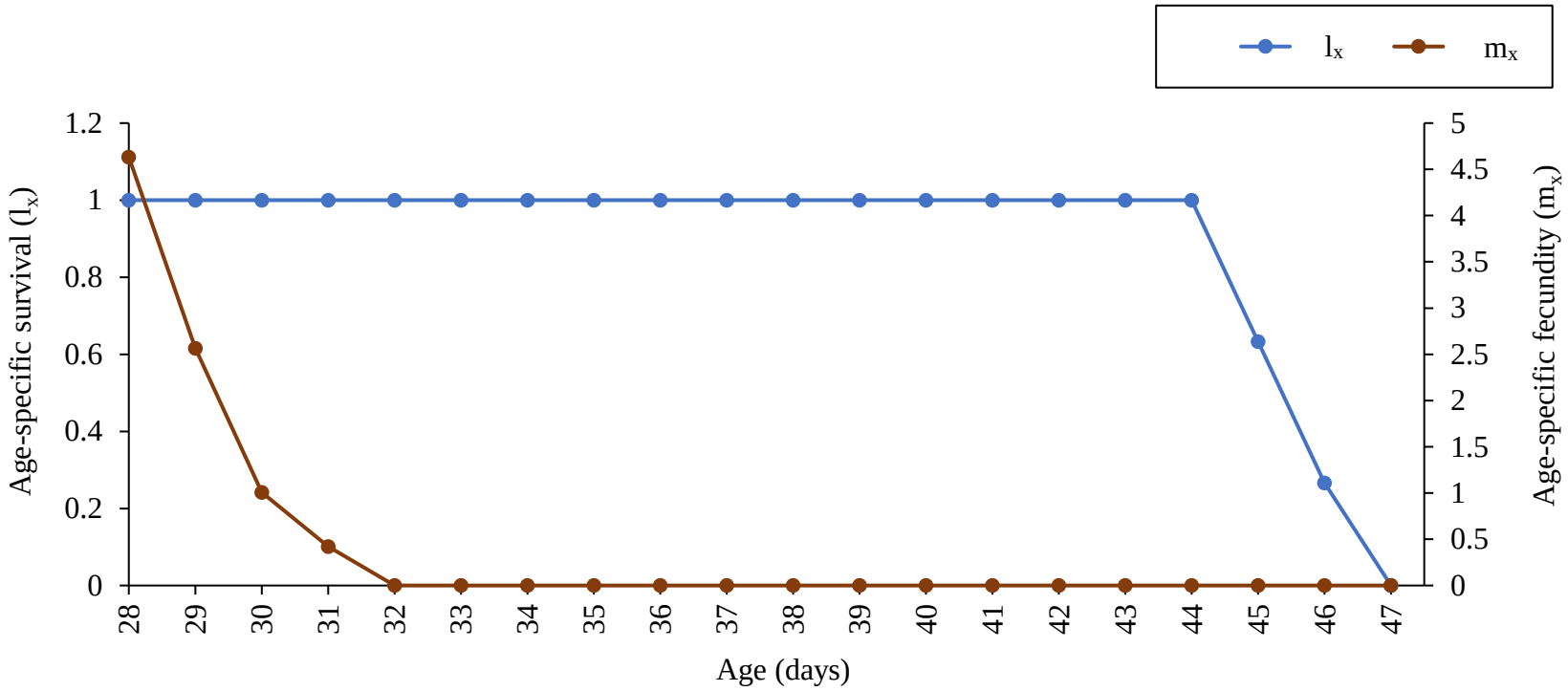

Fig.12. Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *P. anu* reared at 20^0C

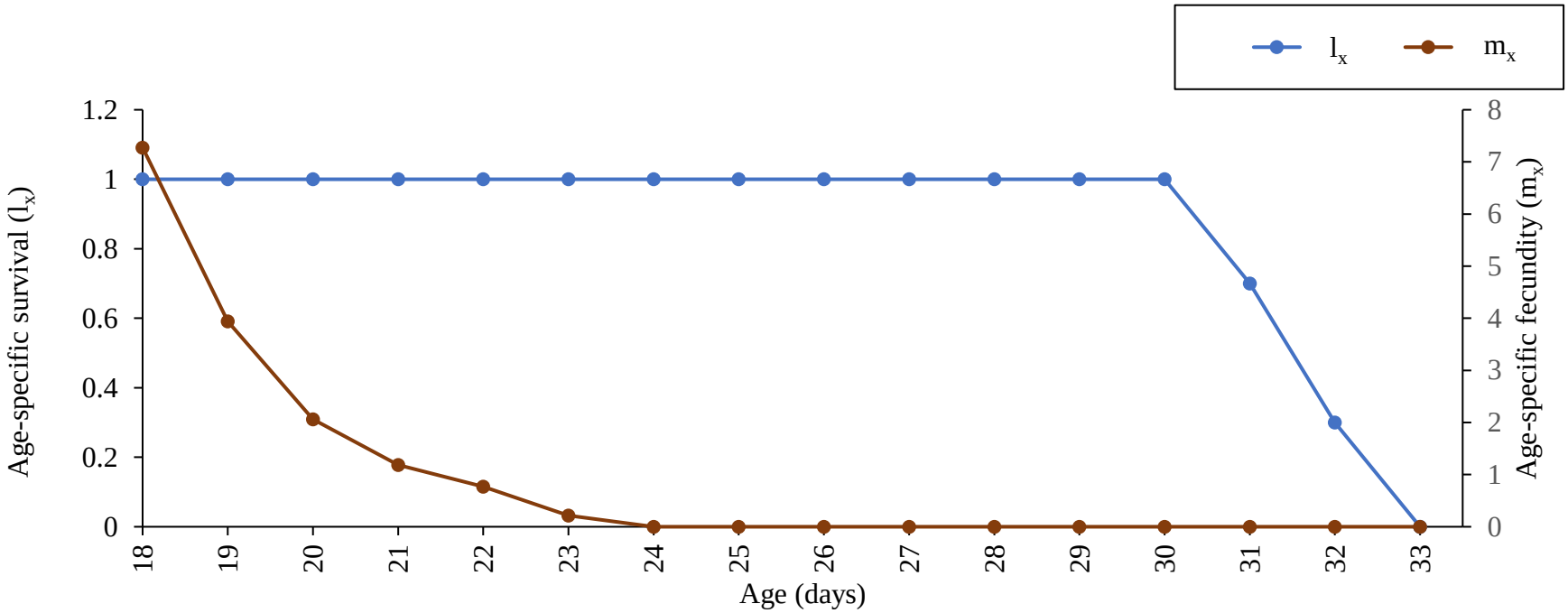

Fig. 13. Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *P. anu* reared at 25^0C

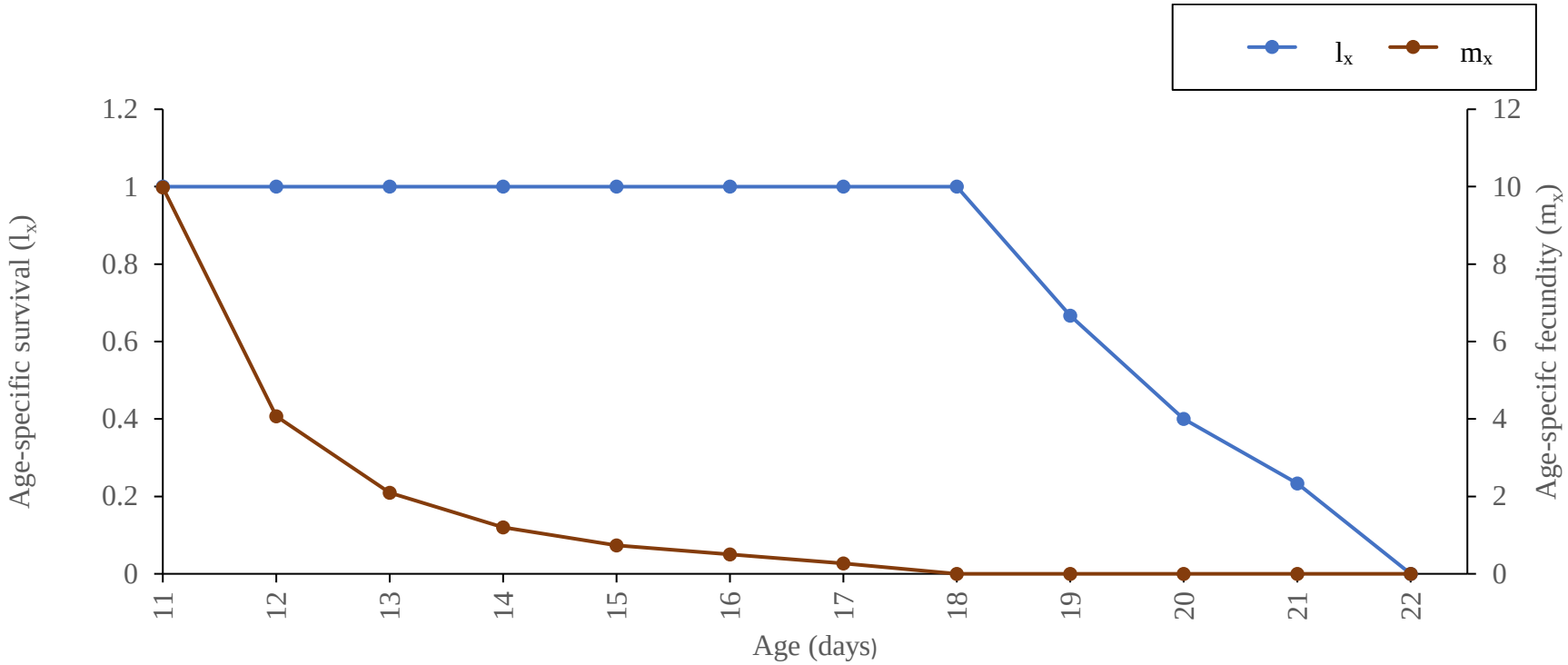

Fig.14. Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *P. anu* reared at 30^0C

CHAPTER 5

DISCUSSION

5.1. Studies on the life history of *M. cribraria*

M. cribraria is a polyphagous insect which feeds on various host plants. As a result, the life history of *M. cribraria* changed with different host plants. Our experimental studies showed that the egg to adult development time for *M. cribraria* was 45.58 ±0.21 days at average room temperature of 28^0C and 75±5% RH on *L. purpureus* var. *Hima*. This value was similar to the egg-to adult mean development time of *M. cribraria* reared on *L. purpureus* var. *Lignosus medikus* (43.30 days) at an average temperature of 28^0C and 80% RH during September (Thippeswamy and Rajagopal, 2005a). Furthermore, the result obtained in this work was consistent with the result reported by Thippeswamy and Rajagopal, (2005b) who found the total developmental period (egg to adult) of 41 to 55 days with a mean of 45.40 days on soybean. In contrast, the total developmental period of *M. cribraria* was 24.3 ±0.5 days, 26.5±0.5 days and 29.0±0.5 days reared on *S. grandiflora*, *C. undulaefolia* and *G. hirsutum*, respectively, (Srinivasaperumal *et al*., 1992).

The incubation period of eggs was 5.2 ± 0.04 days and total nymphal developmental time last for 40.38±0.40 days. Thippeswamy and Rajagopal, (2005a) reported that incubation period lasted for 5 to 7 days with an average of 6.10 days and the nymphal period last for 32-45 days with an average of 37.20 days during August-September. However, the mean incubation period of eggs was 6.30 days and nymphal period last for 39.10 days *M. cribraria* reared on soybean as host plant (Thippeswamy and Rajagopal, 2005b). Ramakrishna Ayyar, (1913) and Butani, (1980) reported the incubation period to last for 6 days. The nymphal period of *M. cribraria* last for 6 weeks on cluster beans (Butani,1980). The nymphal period last for 20.3±0.4 days, 22.5±0.5 days and 25.0±0.3days at *M. cribraria* reared on *S. grandiflora*, *C. undulaefolia* and *G. hirsutum,* respectively, but incubation period of eggs was 4.0±0.5 days in all these three host plants (Srinivasaperumal *et al*., 1992).

The results indicated that the survival percentage of eggs was 90.14±1.03%; this value was lower than the percentage of survival of eggs obtained by Thippeswamy and Rajagopal, (2005a) which was ranged from 94 to 98 with an average of 96.8%. The

number of nymphs completing each instar stage was given in table 2 and only 34.57% 4th instar moult to 5th instar. In contrast to this result, Thippeswamy and Rajagopal, (2005b) reported the percentage of nymphs reaching adult stage was highest on field bean, lablab (79.80%) than soybean (76.50%).

In the present study, the pre oviposition period and oviposition period of *M. cribraria* were 10.72±0.12 days and 26.43±0.15 days respectively. However, the mean pre oviposition period and oviposition period were reported at 13.2 days and 29.2 days on field bean, while 15.3 days and 22.8 days on soybean (Thippeswamy and Rajagopal, 2005 a, b). *M. cribraria* females had a fecundity of 206.49 eggs, which was higher than that of lablab var. *Lignosus medikus* (102.60 eggs/female) and soybean (96.50 eggs/female) (Thippeswamy and Rajagopal, 2005a, b). However, Srinivasaperumal *et al*., (1992) observed fecundity of 73.3, 60.6 and 49.6 eggs/female on *S. grandiflora*, *C. undulaefolia* and *G. hirsutum*, respectively. This showed that the best suitable host plant to rear *M. cribraria* was *L. purpureus* var. *Hima*.

Female longevity in this study was 45.21±0.26days which was longer than the value recorded for males (32.41±0.38 days). Thippeswamy and Rajagopal, (2005a) reported the longevities of 42.50 days for females and 27.20 days for males at lablab var. *Lignosus medikus* which was lower than the values obtained in the present study. Furthermore, male and female longevities have been estimated as 28.80 days and 40.10 days for *M. cribraria* reared on soybean and 24.80 days and 37.30 days on red gram, as reported by Thippeswamy and Rajagopal, (2005b). In contrast to the results of the present study, Srinivasaperumal *et al*., (1992) reported lower adult longevities (2.5±05 to 4 days) of *M. cribraria* on *S. grandiflora, C. undulaefolia* and *G. hirsutum*.

The net reproductive rate (Ro) obtained in this study (141.428 female offspring / female) was higher than the net reproductive rates previously reported by, Srinivasaperumal *et al*., (1992) which were 48.93, 39.56 and 37.43 female offspring / female for females fed with *S. grandiflora, C. undulaefolia* and *G. hirsutum* respectively. The mean generation time (T) recorded was 66.024 days, whereas Srinivasaperumal *et al*., (1992) obtained values of 26.70 days, 26.89 days and 25.86 days for *M. cribraria* females reared on *S. grandiflora, C. undulaefolia* and *G. hirsutum* respectively. Srinivasaperumal *et al*., (1992) reported an intrinsic rate of increase (r_m) of 0.86, 0.69 and 0.68 female/day for females fed with *S. grandiflora, C. undulaefolia* and *G. hirsutum*

respectively. These values of r_m were different from those obtained in our study (0.075 female/day). The finite rate of increase (λ) for *M. cribraria* observed was 1.078 female offspring per female per day, while the finite rates of increase were 7.24, 4.91 and 4.79 for reared on *S. grandiflora*, *C. undulaefolia* and *G. hirsutum* respectively (Srinivasaperumal *et al*., 1992) *M. cribraria* population would be able to multiply 1.69 times per week whereas, the weekly multiplication of population was higher on *S. grandiflora, C. undulaefolia* and *G. hirsutum* as reported by the same authors. Finally, the doubling time (DT) required for the *M. cribraria* population in this study was 9.242 days, which was higher than the doubling time obtained by Srinivasaperumal *et al*., (1992), who reported 0.3501,0.4360 and 0.4421days for females fed with *S. grandiflora, C. undulaefolia* and *G. hirsutum* respectively.

Our findings showed that the life history and life able parameters of *M. cribraria* were strongly influenced by the host plants and *L. purpureus* var. *Hima* is ideal for the development and establishment of *M. cribraria* population than any other varieties of its own kind.

5.2. Effect of different levels of temperature on the life history of *M. cribraria*

Temperature is one of the most important ecological factors influencing physiology and behaviour of insects, affecting rate of growth, development, life time, survival, fecundity and other different biological aspects of insects (Ratte, 1985). The temperature influenced the life history parameters of *M. cribraria* reared on *L. purpureus* var. *Hima* in the laboratory. The present study revealed that *M. cribraria* could sustain itself at constant temperatures between 20^0C and 30^0C and the most suitable temperature observed was 30^0C. However, Shi *et al*., (2014) observed that the shortest development time from egg to adult for *M. cribraria* was at 29^0C reared on soybean. The15°C temperature was apparently lethal to *M. cribraria* based on the observation that eggs never hatched at this temperature. At 35^0C, 3rd instar nymphs failed to reach the 4th instar. Similarly, *M. cribraria* could not complete its development at higher temperature i.e. 33^0C (Shi *et al*., 2014). The shorter developmental duration from egg to adult was experienced at higher temperature of 30^0C (approximately 33d) may sharply accelerate the immature stages, causing an early onset of maturity, which, in turn, would lead to

increases in progeny and larger populations. This result was in agreement with the previous study of Shi *et al*., (2014) who reported the egg-to-adult mean development times of *M. cribraria* at different temperatures on soybean were 114.81, 76.26, 44.54 and 38.54 d at 17, 21, 25 and 29^{0}C, respectively.

The survival rates of *M. cribraria* immature stages varied significantly at various constant temperatures. Temperatures below 20^{0}C and above 30^{0}C were highly unfavourable for survival of all immature life stages. In the present study, obtained highest survival percentage of egg and nymphs of *M. cribraria* when reared at 30^{0}C in the laboratory. In contrast, Shi *et al*., (2014) reported the egg and nymph survival rates were 97.73% and 62.22%, respectively at 25^{0}C when reared on soybean.

The result indicated that APOP, TPOP, oviposition period, fecundity and longevity of *M. cribraria* were highly dependent on temperature. APOP and TPOP were inversely proportional to temperature and this result was in conformity with earlier reports on *M. cribraria* reported by Shi *et al*., (2014). Thippeswamy and Rajagopal, (2005a) reported that the pre ovipostion period of *M. cribraria* reared on *L. purpureus* var. *Lignosus medikus* at an average temperature of 28^{0}C was 13- 20 days. This observation noted was similar to the results obtained at constant temperatures of 25^{0}C in this study. The shortest oviposition period was obtained at 20^{0}C and longest at 25^{0}C, which was similar to that observed by Shi *et al*., (2014). In contrary to this, oviposition period of *M. cribraria* reared at 25^{0}C and 30^{0}C were significantly longer than that reported by Thippeswamy and Rajagopal, (2005a).

M. cribraria had greater mean fecundity at 30^{0}C (221.80 ±3.32 eggs) followed by 25^{0}C (178.7±2.86 eggs) and 20^{0}C (21.30±2.07 eggs). However, Shi *et al*., (2014) indicated that *M. cribraria* females had the fecundity of 49± 10.58 eggs at 21^{0}C, 69.67±13.34 eggs at 29^{0}C and recorded 159.67±16.88 eggs at the optimal temperature of 25^{0}C which was different from the present study. Furthermore, Thippeswamy and Rajagopal, (2005a) reported maximum fecundity at an average temperature of 20.5-22.5^{0}C (102-274 eggs/female) during the month of December and January was in disagreement with the current study results. Medal *et al*., (2013) reported a female bug could lay 43 eggs on average on soybean at 24^{0}C, 50 -70% RH, and 16:8 (L:D) h photoperiod in a greenhouse in Florida. These variations can be due to the change in the host plant and environmental conditions prevailed during the experiments.

The adult longevity and entire life span of both males and females of *M. cribraria* varied between 20°C -30°C temperature. At higher constant temperatures the adult longevity and total longevity of both males and males decreased sharply. These results showed a closer agreement with the previous reports of Thippeswamy and Rajagopal, (2005a), Ruberson *et al*., (2013) and Shi *et al*., (2014) on *M. cribraria* on various host plants.

Temperature significantly affected the biotic potential of *M. cribraria*, as shown by the R_0, r_m, and DT parameters, as well as the population growth capacity as reflected by r_m, the mean generation time and the time required for the population to double. Data from this study indicated the intrinsic rate of increase (r_m), net reproductive rate (Ro) and finite rate of increase (λ) increased with increasing temperatures between 20 and 30^0C. The longest and shortest mean generation time occurred at 20^0C (142.71d) and 30°C (55.62d) respectively. The population doubling time for *M. cribraria*, was longest at 20°C (34.66) and shortest at 30°C (7.70). The present study results indicated that the optimal temperature for population growth of *M. cribraria* was 30°C. In contrast to this study, Shi *et al*., (2014) revealed that population trend index of *M. cribraria* was low at 21^0C (6.70) and 29^0C (10.84) and the highest at 25^0C (46.47), indicating the temperature of 25^0C is optimal for the growth and establishment of *M. cribraria* reared on soybean. These differences in our findings from Shi *et al*., (2014) might be due to the sensitivity of *M. cribraria* to different host plants, rearing conditions, and observation intervals.

The present study findings indicated that the developmental periods, survival rates, longevity, reproductive capacities, and population growth of *M. cribraria* reared on *L. purpureus* var. *Hima* was significantly affected by constant temperatures. In addition to this, the data on the reproductive growth of *M. cribraria* under different constant temperatures could be used to optimize the production methods in mass rearing for parasitoids.

5.3. Effect of different levels of relative humidity on the life history of *M. cribraria*

Relative humidity significantly affects immature survival and development, adult longevity and fecundity in many species of insect (Willmer, 1982; Smith, 1993; Guarneri *et al*., 2002; Han *et al*., 2008; Broufas *et al*., 2009). The results of the present study

showed that *M. cribraria* was able to develop successfully from egg to adult at all the five RH levels tested (40%,50%,60%,70% and 80% RH). There was no significant difference in the developmental duration of eggs and nymphs of *M. cribraria* among different RH levels. Currently, the effect of relative humidity on development, survival, fecundity and longevity of plataspids has not been documented. So far, few studies have evaluated the effect of RH on phytophagous bugs of other genera in Hemiptera, such as the *Apolygum lucorum* (Lu and Wu, 2011) and *Adelphocoris* species (Pan *et al*., 2014). The relative humidity did not affect the rate of development of eggs and nymphs of *A. lucorum* (Lu and Wu, 2011), which was similar to the results of the present study. Similarly, Pan *et al*., (2014) found that the development times of eggs and nymphs of these three *Adelphocoris* species were not greatly affected by RH. In contrast, Han *et al*., (2008) reported that there was a progressive lengthening of developmental duration of *Dendrolimus tabulaeformis* (Lepidoptera: Lasiocampidae) larvae when exposed to 20%RH than compared with 40%, 60% and 80% RH.

Holemes *et al*., (2012) reported that egg eclosion and success of adult emergence in *Hermetia illucens* (L.) (Diptera: Stratiomyidae) was increased with increasing RH, whereas the development time decreased with rising RH. Additionally, previous studies suggested that development time decreased with increasing moisture content for example *Leucania separata* (Walker) (Jin *et al*., 1964), *Oryzaephilus surinamensis* (Linnaeus) (Arbogast, 1976), *Anisopteromalus calandrae* (Howard) (Smith, 1993) and *Triatoma brasiliensis* (Neiva) (Guarneri *et al*., 2002).

Relative humidity at 70% - 80% significantly increased egg and nymph whereas low humidity (40% RH) had a detrimental effect on egg and nymph survival. These results were similar to those obtained by Lu and Wu, (2011), who reported that the survival percentage of eggs and nymphs of *A. lucorum* were highest at of 80% and lowest at 40%RH. Furthermore, Pan *et al*., (2014) observed that the highest survival of eggs and nymphs of the three *Adelphocoris* species viz, *A. lineolatus, A. suturalis* and *A. fasciaticollis* were at high humidity (70 and 80% RH) and decreased significantly with lower humidity (40%RH).

The preoviposition period *M. cribraria* was shortened with decreasing RH from 80% (10.0d) to 40% (5.83d). In contrary to this, the pre-oviposition period decreased with increasing relative humidity from 20% to75%, in *Dinoderus minutus* (F.) (Norhisham *et*

al., 2013). The oviposition period was increased with increasing RH from 40% to 80%, similar to that observed by Lu and Wu, (2011), who reported that the oviposition period was short at low relative humidity and long at high relative humidity in *A. lucorum.* The present study indicated that *M. cribraria* females had the fecundity of 46.16-218.33 eggs at 40% and 80% RH respectively. Similarly, female fecundity of *A. lucorum* was significantly higher at high RHs i.e.,70% (48.83 eggs/female) and 80% (50.77 eggs/female) than fecundity at 40%RH (16.31 eggs/female) (Lu and Wu, 2011). Furthermore, Pan *et al*., (2014) reported that the female fecundity of *A. lineolatus*, *A. suturalis* and *A. fasciaticollis* was increased with increasing RH from 40 % and 50% to 60% 70% and 80% RH.

Adult longevity of *M. cribraria* high at RH 70% and 80% RH were significantly greater than those in lower RH range (40% RH). Previous studies on *A. lucorum* suggested that both females and males had the shortest longevity (approximately 28 days) at 40% RH, and the longevity generally increased with increasing relative humidity (Lu and Wu, 2011). In addition to this, both female and male adult longevity of each *Adelphocoris* species tested was at high RH (i.e., 70% RH) were significantly greater than those in the lower RH range (i.e., 40% RH) (Pan *et al*., 2014).

M. cribraria population attained a maximum net reproductive rate (Ro), intrinsic rate of increase (r_m) and finite rate of increase (λ) were maximum at 80%RH. The mean length of generations decreased with decrease in RH from 80% to 40%. Analysing the regression curves for the linear relationship between RH and r_m, Ro and λ indicated that high humidity more strongly affected the population growth of *M. cribraria* than lower humidity. Similar trends were reported by Lu and Wu, (2011) on *A. lucorum* for intrinsic capacity for increase, the net production rate, and the finite rate of increased with increasing relative humidity from 40 to 70%. However, Pan *et al*., (2014) reported that the intrinsic rate of increase, the net reproductive rate, and the finite rate of increase increased with increasing RH in all three *Adelphocoris* species.

This work provided important information about the potential effects of relative humidity on the population growth and development of *M. cribraria*.

5.4. Studies on different behavioural parameters of *M. cribraria*

5.4.1. Food preference

In the present study, *M. cribraria* were highly attracted to olfactory cues from inflorescence followed by apices, leaves and finally green pod. Similarly, Pan *et al*., (2015) stated that *Apolygus lucorum* (Hemiptera: Miridae) adults preferred flowering plants over non-flowering plants of 18 key host species. In contrary to this, Thippeswamy and Rajagopal, (2005a) reported the inflorescence was the least preferred feeding part of *L. purpureus* var. *Lignosus medikus* by *M. cribraria* and the number of adults feeding on the inflorescence was 1-7 with a mean of four adults. The present study found the green pod was least preferred by *M. cribraria.* Results obtained in this study on green pod were in conformity with the earlier reports of Ramakrishna Ayyar, (1913), Butani, (1980) and Xing *et al*., (2006). In contrast, Thippeswamy and Rajagopal, (2005a) reported the feeding preference of *M. cribraria* was high on pods than apices or inflorescence.

After inflorescence, both male and female *M. cribraria* preferred the apices of the *L. purpureus* stems. Thippeswamy and Rajagopal, (2005a) observed that second choice of *M. cribraria* was apices of the stems of *L. purpureus* and 3-19 adults feed on the apices, with a mean of eight adults/plant. In our study, 68.20% of males and 71.82% females of *M. cribraria*

preferred leaves of *L. purpureus*. Thippeswamy and Rajagopal, (2005a) found that the leaves of *L. purpureus* were not preferred by *M. cribraria* which was different from the current study.

This study proved that the volatiles from the inflorescence of the host plant can be recognised and attracted a greater number of *M. cribraria*, which in turn leads to an increase in the population density in the field.

5.4.2. Mating

In Heteropteran species, extremely long copulations that last for several hours to days have been reported (Harris and Todd, 1980; McLain, 1980; Carroll and Loye, 1990; Carroll, 1991). In our study, the copulation duration of *M. cribraria* was 9.80±0.33 hours at 1:1 sex ratio. Hosokawa and Suzuki, (2001) reported that the mean copulation duration of *Megacopta punctatissima* was 650.54± 81.68 minutes at 1:1 sex ratio. Furthermore,

some copulations of *M. punctatissima* continued for more than 20 hours, few copulations continued for more than 24 hours (Hosokawa and Suzuki, 2001). The copulation duration of *Murgantia histrionica* (Heteroptera: Pentatomidae) was 31.4±5.08 hours, which was longer than the copulation duration obtained in our study (Zahn *et al*., 2008).

M. cribraria copulated for 10.73±0.41h and 11.03±0.79 hour at 1:2 and 2:1 sex ratios. Furthermore, no difference was observed in the copulation duration of *M. cribraria* in 1:1,1:2 and 2:1 sex ratios. Hosokawa and Suzuki, (2001) found that *M. punctatissima* copulated for about 10 hours on average at 1:1, 1:5 and 5:1 sex ratios, although sperm transfer from a male to a female spermatheca was complete within 2-4 hours only. These results were different from the prediction of Yamamura, (1986).

These findings suggested that the copulation duration of *M. cribraria* was not affected by the number of males and females.

5.4.3. Defensive response

Stink bugs, when disturbed, produce a pungent odour that consists of a mixture of volatile compounds. Our study revealed that (E)-2- hexenal and 2-hexene, 4,4,5-trimethyl was unique to the VOCs collected from disturbed *M. cribraria* but these were not present at a detectable level in volatile emitted from undisturbed *M. cribraria*. Onnink *et al*., (2017) found that (E)-2-hexenal was the major component of volatiles emitted from disturbed *M. cribraria* only. Octacosane, decamethyl cyclopentasiloxane, 2-Ethylhexanol, tridecane and dodecane, 4,6-dimethyl- were present exclusively from the samples of undisturbed *M. cribraria* but it was absent from disturbed *M. cribraria* samples. Onnink *et al*., (2017) reported that decamethyl cyclopentasiloxane, 2-ethylhexanol and octacosane were only found from samples of undisturbed *M. cribraria* which was similar to our results. In the present study, tridecane was present only in the samples of undisturbed *M. cribraria.* In contrary, tridecane was present in both disturbed and undisturbed samples of *M. cribraria* (Onnink *et al*., 2017). Additionally, Zhao *et al*., (2012) found that tridecane was present in secretions from both disturbed and undisturbed *Tessaratoma papillosa* (Drury) (Hemiptera: Tessaratomidae) whereas this stink bug secretes (E)-2-hexenal when disturbed. Furthermore, Gunawardena and Herath, (1991) reported that tridecane enhances the activity of (E)-2-hexenal as a fumigant and repellent.

This study identified (E)-2-hexenal and 2-Hexene, 4,4,5-trimethyl as components of volatiles emitted from disturbed *M. cribraria* that could be utilised in *M. cribraria* trapping or for environmentally friendly monitoring or in integrated pest management after further investigation. Role of these chemicals as semiochemical are yet to be concluded.

5.4.4. Phototropic response

The LED trap was an important method for monitoring and trapping insect pests, which can minimise the disadvantage of killing the population of natural enemies by the traditional light trapping method (Zhao *et al*., 2008; Bian *et al*., 2012; Sang *et al*., 2018; Yang *et al*., 2020; Pan *et al*., 2021). In the present study, LED lights of different wavelengths were used to study the phototropic response in *M. cribraria*. Pan *et al*., (2021) reported that insects that belong to different taxonomic groups had significantly different responses to light at various wavelengths under field conditions.

In the current study, white LED light was most preferred by both male and female of *M. cribraria* over other tested colours and blue and red light colours were least preferred ones. Prokopy and Owens, (1983) found that green and yellow traps probably mimic plant foliage confirming general pattern of attraction for herbivorous insects. But in our study, it was found to be unsuitable and had less attraction when compared with that of white. Furthermore, Mondor and Warren, (2000) indicated that *Harmonia axyridis* (Coleoptera: Coccinellidae) of both sexes, make significantly more visits and spend more time on yellow vs green coloured pillars. Adedipe and Park, (2010) observed that blue and red were the least attractive colours to *Harmonia axyridis* (Coleoptera: Coccinellidae) adults which showed a closer agreement with the present study. Horn and Hanula, (2011) reported that colour significantly affected trap catches and white traps captured over twice the number of *M. cribraria* as yellow traps and both colours captured significantly more than black, purple, or red. This result obtained in our Y tube study with white coloured LED light substantiated these results.

From the study, it is evident that white coloured LED light is preferred by *M. cribraria* which can be further used in trap development, which in turn helps in the early detection and monitoring of *M. cribraria* in field.

5.5. Survey of egg parasitoids associated with *M. cribraria* in the field

In the current study, the parasitism rate of *P. anu* was 73.7 % for field-collected egg masses. However, Takasu and Hirose, (1986) found that parasitism of *M. punctatissima* eggs by *P. saccharalis* (as *A. minor*) increased quickly after *M. punctatissima* oviposition began in *Pueraria montana* (end of May) in Japan, and within 2 weeks reached levels of 57–81 % from mid-June through early July, when the parasitoid *Ooencyrtus nezarae* Ishii. became more prevalent. In addition to this, the parasitism rate of *M. cribraria* eggs by *P. saccharalis* varied from 21% to 45%, while that by *O. nezarae* varied from 4% to 37% on the kudzu plant, *Pueraria montana* (Hoshino *et al*., 2017). Similarly, Liao *et al*., (2019) reported that natural parasitism rates of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) on field collected eggs of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) was 30% and 50% for egg masses and per egg mass, respectively, *Trissolcus japonicus* (Hymenoptera: Scelionidae) parasitized 72–81% of eggs of *Halyomorpha halys* (Hemiptera: Pentatomidae) in the field tests (Milnes and Beers, 2019).

Our observation showed that shortly after emergence, the females of *P. anu* were found to be phoretic on their bug hosts, with up to five wasps on a single host bug, both in captivity and in the field. It was the first report of phoretic behaviour in *Paratelenomus*, which is not present in any other species other than *P. anu*. Previous studies reported that phoresy was absent in *P. saccharalis.* The phoretic behaviour of *P. anu* furnishes a competitive advantage by enabling it to parasitize the eggs at the earliest possible moment. However, the cost of phoresy may exist because females of *P. anu* spend time attached to adult bugs instead of searching for new egg masses, thus limiting their dispersal ability. This idea was supported by the distributional data of the species: *P. saccharalis* is extremely widespread (Europe, Africa, tropical Asia, northern Australia (Johnson, 1996) while *P. anu* is known only from India. However, the absence of *P. anu* from collections may be an artefact of collecting methods that were biased toward free-living, non-phoretic insects, such as Malaise and yellow pan traps.

Overall, 73.7±7.3 % field parasitism rate was a significant parasitism rate, which showed the importance of *P. anu* inclusion as a natural control agent against *M. cribraria.* The phoretic behaviour of *P. anu* enables it to parasitise the host eggs immediately after being laid, which increases the rate of parasitism compared to other egg parasitoids.

5.5.1. Identification and description of *P. anu*

P. anu does not fully follow either lead of the first couplet in the key to species of *Paratelenomus* by Johnson, (1996) because the notauli are weakly present at the posterior margin of the metasoma and may appear absent. Otherwise, *P. anu* matches the second lead based on the medially narrowed transscutal articulation and the presence of just two lateral setae on T1. By following the second lead of the couplet one would arrive at *Paratelenomus saccharalis*, which is morphologically very similar to *P. anu*. They can be separated by the notaulus, which was well developed in *P. saccharalis* and extends for more than half the length of the mesoscutum; the central keel, which does not bifurcate around the median ocellus in *P. anu*; and the interorbital space, which in *P. anu* was 1.25× eye height and in *P. saccharalis* was slightly less than eye height. Apart from the difference in the morphological characters, the DNA barcoding revealed that 15% sequence difference was enough to discriminate *P. anu* from closely related sibling species, *P. saccharalis.*

In this study, a new species of scelionid wasp, *P. anu* was described based on morphology and DNA sequence data. These findings have positive implications for the use of *P. anu* as an augmentative biological control agent against *M. cribraria* in the field.

5.6. Life table and development of mass rearing technique of *P. anu*

5.6.1. Rearing of *P. anu* in the laboratory

Previous studies documented the qualitative effects of sugar sources on the longevity, progeny production and feeding response of hymenopteran parasitoids (Hagley and Barber, 1992; Teraoka and Numata, 2000; Schmale *et al*., 2001; Beach *et al*., 2003; Hogervorst *et al*., 2003). The results indicated that *P. anu* was done successfully in the laboratory using honey as food. Similarly, Singhamuni *et al*., (2015) who reported that *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) lived longer period of time when they were fed on bee honey. The variation in concentration of honey effect the adult longevity of *P. anu.* The longest to shortest adult longevities were 12.27± 0.15d at 20% and 5.36±0.07d reared at 50% honey. In contrary to this, Saljoqi and Khajjak, (2007) and Singhamuni *et al*., (2015) stated that the longevity of *T. chilonis* was highest when they were fed with 50% honey. The lowest longevity of *P. anu* reported in this study was at 3.33±0.16 days when they were fed with only distilled water. Similarly, the lowest

longevity of *T. chilonis* was recorded when they were fed with distilled water (Saljoqi and Khajjak, 2007; Singhamuni *et al*., 2015). This experiment on various concentrations of honey provided elements to develop a mass rearing diet for *P. anu* i.e. using 20% honey as suitable diet.

5.6.2. Life table of *P. anu* on eggs of *M. cribraria* at room temperature

The percentage of parasitism of *P. anu* on *M. cribraria* eggs was 75.86 ± 0.74% whereas the parasitism rate of *P. saccharalis* (Hymenoptera: Scelionidae) on eggs of *Megacopta punctatissimun* (Hemiptera: Plataspidae) was 87.8% at constant temperatures of 27.5^{0}C and 16L:8D (Takagi and Murakami, 1997).

Development times from egg to adult was 13.4±0.07 days for *P. anu.* Takagi and Murakami, (1997) obtained a mean developmental time of 12.5±0.51 days for *P. saccharalis* on *M. punctatissimun* eggs was similar to the duration observed in our study. Olaye *et al*., (1997) reported the mean developmental time of *Telenomus busseolae* (Gahan) (Hymenoptera: Scelionidae) was 15.7 ± 0.1d reared on *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) eggs at constant temperatures of 27^{0}C which was relatively higher than the present study. Moreover, the development times of *Trissolcus basalis, Trissolcus brochymenae, Trissolcus teretis* and *Trissolcus urichi* (Hymenoptera: Scelionidae) reared on *Euschistus heros* (Hemiptera: Pentatomidae) eggs were 11.40± 0.78d, 9.76± 0.75d 9.71± 0.74d and 8.47± 0.86d respectively at 26.0 ± 1° C and 65 ± 10% relative humidity (Laumann *et al*., 2008) which were lower than the results observed in the current study. Borges-Filho *et al*., (2017) found the egg-to-adult period of *Telenomus pachycoris* (Hymenoptera: Scelionidae) reared in *Pachycoris torridus* (Hemiptera: Scutelleridae) eggs was 10.5±0.3 d under constant conditions (28^{0}C, 70±10% RH and a photoperiod of 12:12 h L:D).

The rate of emergence (survival) of *P. anu* from parasitised eggs of *M. cribraria* was 73.52± 0.29%. This rate was considerably lower than the survival percentage of *P. saccharalis* on *M. punctatissimun* eggs (77.8% at 27.5°C) (Takagi and Murakami, 1997). In contrast, Laumann *et al*., (2008) reported the mean survivorship of *T. brochymenae, T. teretis* and *T. urichi* reared on *E. heros* eggs were 55.38±14.13%, 49±6.68% and 38.44±20.05% respectively. But the survivorship of *T. basalis* was higher (75.17±9.04%) than our study (Laumann *et al*., 2008). Similarly, for *T. pachycoris* the rate of emergence

in the eggs of *P. torridus* eggs was about 64 ±12.4% (Borges-Filho *et al*., 2017).

In the present study, the female-biased sex ratio of *P. anu* was 0.86 and the percent emergence of male and female parasitoids was 10.42± 0.2 % and 63.1± 0.35 % respectively. Similar sex ratio was reported Torres *et al*., (1997) who obtained same sex ratio (0.89) for *Telenomus podisi* reared on *Podisus nigrispinus* eggs. In addition to this, the sex ratio of *T. basalis* (0.81); *T. brochymenae* (0.88*); T. teretis* (0.78) and *T. urichi* (0.79) was strongly female biased (Laumann *et al*., 2008). Olaye *et al*., (1997) found that the sex ratio (Proportion of female progeny) of *T. busseolae* was 45.5 on *S. calamistis* which was different from the present study. Furthermore, the sexual rate of *Telenomus remus* on Spodoptera *frugiperda* eggs was 0.51+0.01 (Bueno *et al*., 2008).

The mating duration of *P. anu* observed was 12-15 seconds only. Females started to lay eggs after emergence, a pre-oviposition period was not observed throughout the study. Similarly, *T. busseolae* males hatch first, wait on the egg batch for the females to emerge and mate immediately and the time constrained for mating were negligible (Olaye,1992). Olaye *et al*., (1997) also found that *T. busseolae* females were capable of ovipositing within hours after emergence if sufficient hosts eggs were available. *Trissolcus grandis* females laid eggs immediately upon being provisioned with host, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) so that, no pre-oviposition period was observed (Iranipour *et al*., 2010).

In the present study, the average oviposition period of *P. anu* was 5.91±0.19d, which was lower than the result obtained by Olaye *et al*., (1997), who reported the oviposition period of *T. busseolae* females was 8.8 ± 0.8 d on eggs of *S. calamistis*. Furthermore, Iranipour *et al*., (2010) found that the oviposition period of *T. grandis* females reared on *E. integriceps* eggs exposed to constant temperatures of 26 and 29^0C was tended to be longer than that of the current study.

The average number of host eggs parasitised (fecundity) by *P. anu* female was 36. 09±0.29 eggs. Olaye *et al*., (1997), indicated the rate of parasitism by *T. busseolae* was high during the first 6 h of adult life indicated that emerging females already have many mature eggs and gave rise to about 78% of her female progeny during the first 3 days of their life span. After that, the proportion of females of *T. busseolae* dropped sharply. Similar observation was recorded by Safavi (1968). Bueno *et al*., (2010) reported the number of *S. frugiperda* eggs parasitised by *T. remus* was 27.56 ± 1.80 eggs/female under

constant conditions (28^0C, 70±10% RH and 12 h L:D) which was fewer than the present study. In contrast, the fecundity of *T. grandis* females (67.2-96.8 eggs) on *E. integriceps* eggs was higher than our study (Iranipour *et al*., 2010).

The longevity of *P. anu* male and female was 12.04±0.34 days and 12.85±0.19days respectively. This result was similar with studies of Olaye *et al*., (1997), who reported the mean longevity of ovipositing *T. busseolae* females was 12.8±1.2d. Bueno *et al*., (2010) found the longevity of *T. remus* on *S. frugiperda* eggs was 7.65± 0.13d. Furthermore, the adult longevity of *T. grandis* females reared on *E. integriceps* eggs exposed to constant temperatures of 26 and 29^0C tended to be longer than that of the present study (Iranipour *et al*., 2010).

The net reproductive rate (Ro) obtained in this study (19.54 female offsprings /female) was lower when compared with the other species of Telenomus group. Olaye *et al*., (1997) found that Ro of *T. busseolae* was 96.5. Similarly, the Ro values of *T. basalis*, *T. brochymenae*, *T. teretis* and *T. urichi* were 78.39, 43.06, 31.40 and 39.49 respectively (Laumann *et al*., 2008). Tabebordbar *et al*., (2022) observed the higher Ro value of *Trichogramma euproctidis* reared on *Ephestia kuehniella* eggs at a constant temperature of 27.5^0C.

The mean generation time required for *P. anu* population in this study was 15.86 days. Similar mean generation time was observed on *T. busseolae* on eggs of *S. calamistis* (15.3d) (Olaye *et al*., 1997). In contrast, Laumann *et al*., (2008) found the mean generation time of *T. basalis* (14.54d) *T. brochymenae* (11.51d) *T. teretis* (11.09d) and *T. urichi* (10.59d) were lower than that of the present study.

In the current study, the intrinsic rate of increase (r_m) recorded was 0.187. In contrast, r_m was higher on *T. busseolae* on *S. calamistis* eggs (Olaye *et al*., 1997), *Trissolcus* spp. on *E. heros* eggs (Laumann *et al*., 2008). The finite rate of increase (λ) for *P. anu* observed was 1.21. The λ values of *T. basalis*, *T. brochymenae*, *T. teretis* and *T. urichi* on E. heros eggs (Laumann *et al*., 2008) were 1.35, 1.39, 1.36 and 1.41 respectively, which were higher than the current observed value.

This study provided the life history and life table parameters of *P. anu* reared on the eggs of *M. cribraria* which helped in the development and implementation of small scale piolet biological control study using this egg parasitoid.

5.6.3. Effect of mating on parasitism, emergence and sex ratio of *P. anu*

The adult mating must also be considered while rearing of egg parasitoids of the genus Trichogrammatidae and Platygastridae, because it might affect biological characteristics which could influence their efficiency in the field (Pratissoli *et al*., 2009; Farrokhi *et al*., 2010; Queiroz *et al*., 2017). In *P. anu*, the absence of mating directly influenced offspring sex ratio, leading to the production of males only (for unmated female sex ratio=0.00; for mated female sex ratio =0.86). However, no difference in the other biological parameters of mated and unmated females. The observed reproduction mode of *P. anu* via parthenogenesis of the arrhenotoky type, has been described as the most common reproductive type for insects of the order Hymenoptera (Pratissoli *et al*., 2014; Queiroz *et al*., 2017). However, Stouthamer, (1993) reported that field releases of parasitoids of the genus *Trichogramma* reproducing by parthenogenesis of the thelytoky type were more efficient compared with parasitoids with those reproducing by parthenogenesis of the arrhenotoky type, illustrating the importance of studying the reproductive mode of each parasitoid species.

In contrast to sex ratio, the numbers of eggs parasitized per mated and unmated female were similar, as well as the percentage of emergence of parasitoids and longevity of parental females. These results were support the findings of Queiroz *et al*., (2017) who reported that mated and unmated *Telenomus remus* Nixon (Hymenoptera, Platygastridae) females reared on *Corcyra cephalonica* (Lepidoptera: Pyralidae) eggs that parasitized *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs showed no difference between mated and unmated females with respect to the number of parasitized eggs, percentage of parasitoid emergence and longevity of parental females (days). The results from present study differ from Pratissoli *et al*., (2014) who observed mated females of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) on *Anagasta kuehniella* (Lepidoptera: Pyralidae) eggs lived less in relation to unmated females. In addition to this, female offspring of unmated females lived longer compared to the mated offspring of mated females.

5.6.4. Effect of host egg age on parasitism, emergence and sex ratio of *P. anu*

Previous studies have documented the host quality for egg parasitoids basically

dependent upon species and host egg age (Liu *et al*., 1998). This study revealed that the parasitoid *P. anu* preferred to parasitize young hosts i.e.12h old eggs (76.13±0 .60%). Clausen, (1976) and Orr *et al*., (1986) reported that phoretic scelionids usually avoid older host eggs. The old hosts (24, 48 and 72 h old eggs) were less preferred by *P. anu* than young hosts for parasitism. Furthermore, the number of first instars that emerged from un parasitized eggs increased with host egg age increased from 12 to 72 h. After 72h, no parasitisation of *P. anu* was noticed. These results were supported by the findings of Penaflor *et al*., (2012), who reported that *Telenomus remus* (Hymenoptera: Scelionidae) preferred 1-d and 2-d old eggs of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to parasitize young hosts. Additionally, 3-d-old host eggs were not only less preferred than young hosts for parasitism, but also less acceptable as the parasitoid parasitized fewer old eggs in no-choice assays. Similarly, Overall percent parasitism by *Tiphodytes gerriphagus* (Hymenoptera: Scelionidae) were high at 0-d, followed by 1-d and 2-d old *Limnoporus dissortis* (Heteroptera: Gerridae) eggs and decreased markedly after day 3 or approximately 45% of total embryogenesis (Sousa and Spence, 2001).

Percentage of *P. anu* adults emerging from 24, 48 and 72h old eggs was less than from 12h old eggs. In 12hour, old eggs, the emergence was 72.89±0.98%. The adult emergence rate of *T. remus* was higher on 1-d old eggs than 2- and 3-d old eggs of *S. frugiperda* which was similar to results of the current study (Penaflor *et al*., 2012). Furthermore, the rate of parasitism and successful development of *T. gerriphagus* decreased as *L. dissortis* egg age increased (Sousa and Spence, 2001). The increase in host egg age can be restrictive because host embryo development depletes the nutrients stored in the egg. Therefore, old eggs were usually considered as low-quality hosts for egg parasitoids (Souza and Spence, 2001; Tunçbilek and Ayvaz, 2003). Moreover, Strand *et al*., (1986) pointed out that a parasitoid larva cannot capable of digesting the cuticle of its host and therefore cannot consume nutrients from embryos in an advanced stage of development of host eggs. In contrary, Borges-Filho *et al*., (2017) reported the age of the *Pachycoris torridus* (Hemiptera: Scutelleridae) egg did not affect emergence rate of *Telenomus pachycoris* (Hymenoptera: Scelionidae) offspring. In general, suitable host egg age of egg parasitoids depend on host species as embryo development may vary among species (Pak *et al*., 1986; Monje *et al*., 1999).

However, host egg age did not affect the sex ratio of *P. anu* or its developmental time, similar to that observed by Penaflor *et al*., (2012), who reported that the sex ratio and developmental time of *T. remus* reared on *S. frugiperda* was unaffected by the age of the host eggs. In contrast, Sousa and Spence, (2001) reported a reduction in the proportion of *T. gerriphagus* males emerging from old eggs of *L. dissortis* but, this effect was not recorded for other species of Scelionidae, such as *Gryon gallardoi* (Brethes) (Da Rocha *et al*., 2006) and *Telenomus isis* (Hymenoptera: Scelionidae) (Bruce *et al*., 2009). Moreover, the duration of the egg to-adult period of *T. pachycoris* reared in *P. torridus* eggs were greatly influenced by the age of the host eggs ranging from 14.8 d in 1-day-old eggs to 13.2 d in 6-day-old eggs (Borges-Filho *et al*. 2017).

The results of this study helped to improve the high productivity in mass rearing of *P. anu* by offering *M. cribraria* eggs of 12h old.

5.6.5. Effect of different levels of temperature on the parasitism and life history of *P. anu*

The present study results indicated that *P. anu* was able to develop successfully from egg to adult at 20^0, $25^0$and 30°C and did not survive temperatures of 15 and 35^0C. However, Olaye *et al*., (1997), indicated that *T. busseolae* reared on *S. calamistis* eggs was successfully completed development in the temperature range 18–32^0C and no emergence occurred at 15 and 34^0C. In contrast, the egg to adult period of *Telenomus remus* (Hymenoptera: Scelionidae) reared on fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs (Bueno *et al*., 2008) and *Trissolcus teretis* (Hymenoptera: Scelionidae) reared on *Euschistus heros* and *Diceraeus melacanthus* (Hemiptera: Pentatomidae) eggs (Cordeiro and Bueno, 2021) were able to complete development at an extreme temperature of 15^0C.

Development time from egg to adult of *P. anu* was inversely related to increase in temperature. With increasing temperature from 20^0C to 30^0C, developmental time of *P. anu* was reduced from 26±0.45d to 9.80±0.37d. The tendency of variation between temperatures was similar for both sexes and differences in developmental time were not observed between *P. anu* females and males at all the tested temperatures. These results were similar to those obtained by Takagi and Murakami, (1997) who reported the egg to adult developmental duration of *P. saccharalis* on *M. punctatissimun* eggs was longest at

20^0C (24.8±0.66 d) and shortest at 30^0C (11.7±1.03 d). Furthermore, no difference was detected in the developmental times between males and females of *P. saccharalis* at any of the temperature tested. The current results were in line with the studies on other Scelionidae species (Olaye *et al*., 1997, Torres *et al*., 1997, Bueno *et al*., 2008, Iranipour *et al*., (2010), Cordeiro and Bueno, 2021). Hernández and Díaz (1996), reported the decrease in the development time from egg to adult period (days) was inversely related to an increase in temperature, and was most likely a consequence of the increase in metabolic activity of the parasitoid at higher temperatures.

The rate of parasitism was highest at 30^0C (79.39±0.77%) followed by 25^0C (73.641.03%) and 20^0C (48.79±2.11%). However, Takagi and Murakami, (1997) reported the percentage of parasitism of *P. saccharalis* (Hymenoptera: Scelionidae) on eggs of *M. punctatissimun* (Hemiptera: Plataspidae) at different temperatures were 86.85%,78.4% and 73.5% at 25°C, 30°C and 20°C, respectively, which were different from the present study.

In our study, the highest percentage of emergence of *P. anu* from parasitised eggs of *M. cribraria* was at 30^0C (74.39±1.13%) and lowest at 20^0C (42.61±1.77%) which differs from the results of other authors who have reported survival percentage of other species of hymenoptera. For example, Takagi and Murakami, (1997) who have reported both higher and lower percentage of survival rate of *P. saccharalis* at 20^0C and 30^0C, respectively when reared on *M. punctatissimun* eggs. Cordeiro and Bueno, (2021) also reported highest emergence of *T. teretis* on *E. heros* eggs and *D. melacanthus* eggs were at 25^0C followed by 30^0C and 20^0C respectively. Furthermore, no difference was observed in the egg viability of *Telenomus podisi* and *Trissolcus brochymenae* on *Podisus nigrispinus* eggs at 20 to 28^0C (Torres *et al*., 1997) and *T. remus* on *S. frugiperda* eggs at 20 to 31^0C (Bueno *et al*., 2008). Similarly, Borges-Filho *et al*., (2017) indicated the emergence rate of *T. pachycoris* on eggs of *P. torridus* did not show any difference between 18 to 30^0C.

In biological control programmes, Sex ratio was considered as an important trait because females parasitize and control the target host in the field so that the production of a greater number of females was desirable (Bueno *et al*., 2009). The current study showed that sex ratio remained unchanged in different temperature treatments. Similar results were obtained by Torres *et al*., (1997), who reported the sex ratio of *T. podisi* and

T. brochymenae reared on *P. nigrispinus* eggs was not affected by temperature. Moreover, Cordeiro and Bueno, (2021) indicated that no changes in sex ratios were observed in sex ratio of *T. teretis* on *E. heros* at 20, 25 and 30^{0}C. Borges-Filho *et al*., (2017) also found the sex ratio of *T. pachycoris* was greater than 0.6 and did not show significant differences at 18-30^{0}C reared on *P. torridus* eggs. In contrast, Olaye *et al*., (1997) indicated that the sex ratio of *T. busseolae* offspring was ranged between 0.63 and 0.72 and fewer females at 30^0 than at 25^{0}C.

P. anu females laid eggs immediately after mating so that pre-oviposition period was not observed in females in the 20 and 30^{0}C treatments. Similar results were previously reported for other parasitoid species in Scelionidae. For example, Olaye *et al*., (1997), who found that *T. busseolae* females oviposit within hours after emergence if *S. calamistis* eggs were available and produced considerably more offspring during the first 24 h than during any subsequent period of life. Similarly, pre oviposition period was not observed for *Trissolcus grandis* (Hymenoptera: Scelionidae) females reared on *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) eggs at five constant temperatures ranging from 20–32^{0}C (Iranipour *et al*., 2010). In *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae) no preoviposition period was detected at 20, 25 and 30^{0}C on *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (Del Pino *et al*., 2020). However, the previous studied of Trichogrammatidae showed that the preoviposition period was negatively correlated with temperature (Orphanides and Gonzalez ,1971; Reznik *et al*., 2006).

In this study, the oviposition period tended to increase with increasing temperature from 20^{0}C (3.4±0.24d) to 30^{0}C (6.4±0.24d). In contrary to this, Olaye *et al*., (1997), observed the oviposition period of *T. busseolae* was highest at 20^{0}C (12.2±1.2d) and lowest at 30^{0}C (7±0.6 d). Moreover, Iranipour *et al*., (2010), reported the oviposition period of *T. grandis* tended to decrease with increasing temperature from 20^{0}to 32^{0}C. The oviposition period of *T. achaeae* decreased with increase in temperature from 15 to 30°C on eggs of *E. kuehniella* (Del Pino *et al*., 2020).

The number of *M. cribraria* eggs parasitized by *P. anu* females was higher at 30^{0}C (35±0.71eggs/female) than at 20^{0}C (15.20±0.58 eggs/female). Similarly, Cordeiro and Bueno, (2021) found that the highest total number of parasitized eggs per *T. teretis* female was recorded at high temperatures of 30^{0}C (29.2±2.2 eggs) when exposed to *E. heros*

eggs. In contrast, Olaye *et al*., (1997) reported that *T. busseolae* females had highest number of offspring at 27^0C and no difference in the number of offspring at 20, 25 and 30^0C. Bueno *et al*., (2010) indicated that total number of *S. frugiperda* eggs parasitized by *T.remus* was highest at 20^0C (58.73 ± 8.85 eggs/female) which was different from our study.

The mean adult longevity of *P. anu* females and males decreased as temperature increased, recording the highest longevity at 20^0C, for both females and males. Gerling, (1972) reported that a decrease in longevity of parental females in relation to the increase in temperature could be mostly a consequence of increased metabolism at higher temperatures and energy expenditure. Similar results were previously reported for other species of the Scelionidae family such as *T. busseolae* reared from S. *calamistis* eggs (Olaye *et al*., 1997), *Trissolcus semistriatus* from *Eurygaster integriceps* eggs (Kivan and Kilic, 2006), *T. grandis* from *E. integriceps* eggs (Iranipour *et al*., 2010), *T.remus* from *S. frugiperda* eggs (Bueno *et al.*, 2010) and *Trissolcus teretis* from *Euschistus heros* and *Diceraeus melacanthus* eggs (Cordeiro and Bueno, 2021).

The biophysical factor of temperature significantly affected the biotic potential of *P. anu* as shown by the net reproductive rate (Ro), mean generation time (T), intrinsic rate of increase (r_m) and finite rate of increase (λ). For the net reproduction rate (R_0), the highest capacity for increase was observed at 30^0C and the lowest at 20^0C. In contrast, the value of Ro for *T. busseolae* reared from S. *calamistis* eggs was the highest at 25^0C, followed by 20 and 30°C (Olaye *et al*., 1997). Del Pino *et al*., (2020) reported the greatest value of Ro for *T. achaeae* reared on *E. kuehniella* eggs was observed at 20°C (32 ± 5.519) but, no difference was detected at 25 (27.87± 4.756) and 30°C (27.15± 4.843).

The mean generation time (T) of *P. anu* was inversely proportional to temperature and decreased rapidly between 20 and 30^0C. This result was in conformity with earlier reports on *T. busseolae* reared from S. *calamistis* eggs (Olaye *et al*., 1997) and *T. achaeae* reared from *E. kuehniella* eggs (Del Pino *et al*., 2020). In *P. anu*, the intrinsic rate of increase (r_m) increased in direct proportion to temperature. The highest and lowest rates of population increase per unit time were found at 30^0C (0.245) and 20^0C (0.075) respectively. This result was similar to what was reported by Olaye *et al*., (1997) and Del Pino *et al*., (2020). For the finite increase ratio (λ), the highest value for *P. anu* was found at 30^0C, followed by 25^0C and 20^0C. However, Del Pino *et al*., (2020) reported the finite

increase ratio of *T. achaeae* reared on *E. kuehniella* eggs was highest at 25 (1.328± 0.02) and 30^0C (1.375± 0.024) and lowest at 20°C (1.215± 0.012).

In our study, it was clear that *P. anu* could be a potential species for biological control that might be used in IPM programs conducted in areas with 20-30^0 C average temperatures. Additionally, our findings were useful in determining the correct time of year for the release of *P. anu* and provided important information for the development of mass production of this egg parasitoid in the laboratory which can be used in field release programs.

5.7. Field release in microplot and evaluation of control efficiency of *P. anu*

Our results indicated that the percentage of parasitism and emergence rate of *P. anu* from *M. cribraria* egg masses were 72.56±1.91% and 68.70±1.62%, respectively in release plots. Additionally, the first instar nymphs of host that emerged from un parasitised eggs in the release plots was 7.18±0.44%. But in control plots, the emergence rate of first instar nymphs were 86.92±1.86%. Thus *P. anu* could bring about 79.74±1.42% reduction in the emergence rate of first instar nymphs of *M. cribraria* compared to their emergence rate in control plot.

However, Figueiredo *et al*., (2002) reported the mean adult parasitism rate of *Telenomus remus* females on eggs of *Spodoptera frugiperda* was 80.4±8.4% in maize fields. Chowdhury *et al*., (2016) reported that 75.5% of mean parasitism by *Trichogramma evanescens* on *Corcyra cephalonica* eggs and 78.6% parasitism was observed on *Corcyra cephalonica* eggs by *Trichogramma chilonis* in adult parasitoid release method in the microplot condition. The field rate of parasitism of *Trichogramma japonicum* and *Trichogramma chilonis* was 9% ± 7.7% and 15% ± 14.1% respectively on *Scirpophaga incertulas* eggs (Tang *et al*., 2017) which was lower than the present study.

Our results indicated that *P. anu* can successfully parasitise *M. cribraria* eggs in field conditions and the percentage of reduction of first instar nymphs of host was 79.74±1.42%. So, this egg parasitoid can be used in augmentative biological control *M. cribraria* of at the field. This can be included in IPM control strategy of *M. cribraria* after conducting large scale field studies.

CHAPTER 6

CONCLUSION

The accurate knowledge of insect pest's life table was very important for executing an ecology-oriented management programme for economically important crops. The life table parameters of *M. cribraria* feeding on the host plant, *L. purpureus* var. *Hima* were studied at a temperature of 28 ± 2^0C, RH of 75±5 %, and 14L:10D h photoperiod. The incubation period lasted 5.2±0.04 days with a viability of 90.14±1.03%. The nymphal stage passed through five instars and had a duration of 40.38±0.40d. The male and female longevities were 32.41±0.38 and 45.21±0.26d, respectively. The pre-oviposition period was 10.72±0.12d and an oviposition period of 26.43±0.15d, with a fecundity of 206.49 ±1.29eggs. The total developmental time from egg to adult was 45.58±0.21d. The sex ratio of females to male was 1:0.46. The values for net reproductive rate (Ro), mean generation time (T), innate capacity of increase (r_m), finite rate of increase (λ), Weekly multiplication (Wm) of population and doubling time were recorded as 141.428 female offsprings per female,66.02d, 0.075 females^{-1} female^{-1} day^{-1}, 1.078 females^{-1} female^{-1} day^{-1}, 1.690 and 9.242 d respectively. Therefore, the results obtained in this study could be of practical significance and can be adopted for the development of reliable and sustainable IPM strategy for *M. cribraria* in field level.

Pest populations are influenced by a variety of biotic (parasitoids, predators, microorganisms, etc.) and abiotic factors (temperature, humidity, light, rainfall, etc.) in the field. One of the most important abiotic factor affecting growth, survival and reproduction of insects is temperature. Hence a study was conducted to find out the relationship of five different constant temperatures (15-35^{0}C) on the growth and development of *M. cribraria*. Under laboratory conditions it was concluded that all the tested temperature has impact on the development, survival, longevity, and life table parameters of the pest. Ideal optimum temperature for the maximum survival of immature stages and shortest developmental time from egg to adult was observed at 30^{0}C. Adult pre-oviposition period (APOP) and total pre-oviposition period (TPOP) were negatively correlated with temperature. The longest oviposition period was at 25^{0}C whereas the highest fecundity occurred at 30^{0}C. *M. cribraria* had the longest adult longevity at 20^{0}C and the adult longevity was shortened significantly as the temperature increased. Bugs

showed highest net reproductive rate (Ro), intrinsic rate of natural increase (r_m) and finite rate of increase (λ) with lower generation (T) and doubling time at 30^0C, which indicated rapid population build-up of *M. cribraria* population in short period of time. Therefore, the results obtained in this study maybe of practical significance for the better understanding of the effects of temperature on population dynamics of *M. cribraria* and the data can be used to predict pest outbreak model and severe damage to the crop can be prevented by timely implementation of control measures. This knowledge would also be very useful for developing an effective integrated pest management strategy against this potential pest.

Like temperature, relative humidity also play a key role in the survival, development, fecundity, and population increase in *M. cribraria*. High levels of RH 70 and 80% were associated with higher egg and nymph survival rates, increased adult longevity and female fecundity in *M. cribraria.* Lower humidity levels (40% RH) led to unfavourable effects on the survival of nymphs, adult longevity and fecundity. However, the developmental duration of eggs and nymphs did not differ among the five RH levels tested. The pre oviposition periods and oviposition periods were directly proportional to RH. Significant positive relationships were found between RH and the net reproductive rate (Ro), intrinsic rate of natural increase (rm) and finite rate of increase (λ) of *M. cribraria.* The mean generation time (T) also increased with increasing in RH and no significant difference was observed at 70 and 80% RH. These results will help to better understand the phenology of *M. cribraria* and the information can be used in population growth models to optimize pest forecasting and management strategies for this nuisance pest.

Phytophagous insects have the ability to select host plants depends on the integration of extrinsic and intrinsic factors. The volatile compounds emitted by host plants that mediate insect–plant interactions. The different parts of host plant were not equally attractive for *M. cribraria.* The food preference order of adult *M. cribraria* was inflorescence followed by apices, leaves and finally green pod. The present study provided important insights into inflorescence preference by *M. cribraria* adults, which supplies basic information for further development of behavioural manipulation control measures. From the study, we can predict that the population build up and aggregation of host will be happening during the time of blooming.

The copulation duration of *M. cribraria* was 9.80±0.33h, 10.73±0.41h and 11.03±0.79 h at :1, 1:2 and 2:1 sex ratio, respectively. There was no difference in mean copulation duration among the three sex ratios. The results may be useful for further research on the effect of prolonged copulation duration on the reproductive biology of *M. cribraria.*

VOCs were collected from disturbed and undisturbed adults of *M. cribraria* was analysed by coupled GC-MS revealed that octacosane, decamethyl cyclopentasiloxane, 2-ethylhexanol, tridecane and dodecane, 4,6-dimethyl- were present in the sample from undisturbed *M. cribraria* but these were absent in disturbed *M. cribraria* sample. (E)-2-hexenal and 2-hexene, 4,4,5-trimethyl were only detected from VOCs collected from disturbed *M. cribraria.* Knowing the chemical composition of secretions of both disturbed and undisturbed adults of *M. cribraria* could be used in the development of traps for monitoring this bug and help in the timely application of pesticides and other control measures.

Insects were able to responses to various wavelengths of light. *M. cribraria* attracted to white LED light followed by yellow and green LED light. Blue and red LED light were not attractive to adult bugs. This data will provide a necessary basis for long-term monitoring, pest forecasting and even the development of eco-friendly prevention and control strategies against *M. cribraria.*

A new species of scelionid wasp, *Paratelenomus anu* (Hymenoptera, Scelionidae) was described based on morphology, scanning electron microscopic studies and DNA barcoding data. The field surveys conducted during 2015-2021 revealed that *P. anu* was the most abundant species with 73.7 ±7.3 % field parasitism rate on *M. cribraria* eggs. In addition to this both in captivity and in the field, females of *P. anu* were found to be phoretic on *M. cribraria* with up to five wasps on a single host bug. This was the first report of phoretic behaviour in *Paratelenomus* group. Due to the high field parasitism rate *P. anu* can be used for the biological control of *M. cribraria*.

The rearing of *P. anu* was done successfully in the laboratory using 20% honey as food source. The highest longevity of *P. anu* was 12.27± 0.15 days when it fed with 20% honey as food. Our findings can be used for mass rearing and augmentative release of *P. anu* on large scale.

In order to assess the feasibility of *P. anu* as a biological control agent for *M. cribraria*, parasitism rate, emergence rate, development time, sex ratio, pre oviposition period, oviposition period, fecundity, number and adult longevity were examined in the laboratory. The parasitism and emergence rate of *P. anu* was 75.86±0.74% and 73.52±0.29%, respectively. The total development time was 13.4 ±0.07days and the oviposition periods and average fecundity 5.91±0.19d and 36.09±0.29 eggs/female, respectively. pre-oviposition period was not observed. The female biased sex ratio of *P. anu* was approximately 0.86. Adult longevity was 12.04 ±0.34d for males and 12.85±0.19 d for females. The net replacement rate (R_0), mean generation time (T) and intrinsic rate of population increase (r_m) and finite rate of increase (λ) of *P. anu* were determined at 19.40 female offsprings, 15.86d, 0.187 females^{-1} female^{-1} day^{-1} and 1.21 females^{-1} female^{-1} day^{-1} respectively. These laboratory results demonstrated that *P. anu* is an effective parasitoid for decreasing *M. cribraria* populations and can be used as a potential biological control agent against *M. cribraria.*

Unmated females of *P. anu* only produced males and mated females produced both males and females, with a female biased sex ratio. i.e., in the absence of mating *P. anu* reproduced via arrhenotoky type. The other biological parameters such as the numbers of parasitized eggs per mated and unmated female, the parasitoid emergence (%) and longevity of parental females were not affected. The results obtained here contribute to improving strategies for successful rearing of *P. anu* and release in biological control programs for *M. cribraria.*

Age of host eggs can have substantial effects on parasitism and parasitoid efficacy. *P. anu* preferred to parasitize 12h old eggs rather than 24h, 48h and 72h old eggs. The percentage emergence of parasitoids was highest for *M. cribraria* eggs aged 12 h, followed by eggs aged 24 h,48h and 72h while no parasitism was observed for eggs after 72h. The developmental duration of parasitoid and sex ratio were not influenced by host age. Thus, the results of this study may help to maintain a high productivity and mass rearing of *P. anu* by offering 12h old eggs of *M. cribraria*.

Temperature has a profound effect on the percentage of parasitism, developmental time, emergence rate, sex ratio, oviposition period, fecundity and longevity of *P. anu*. *P. anu* developed on *M. cribraria* eggs at temperatures between 20^0C-30^0C, but performed best at 30^0C. *P. anu* exhibited highest rate of parasitism and percentage of emergence at

this temperature. The developmental duration, oviposition period, fecundity as well as the adult lifespan both for males and females, were shortened by increasing temperatures from 20^0C to 30^0C. The net reproductive rate (Ro), intrinsic rate of natural increase (r_m) and finite rate of increase (λ) of *P. anu* were positively correlated with temperature whereas the generation time (T) negatively correlated with temperature. These data suggest that *P. anu* was adapted to 30 ^{0}C temperatures and has the potential to be used in integrated management programs against *M. cribraria.*

The success of biological control agent depends upon its field performance. The percentage of parasitism of *P. anu* on *M. cribraria* egg masses was 72.56±1.91% in the release micro plots. The emergence rate of first instar nymphs of host was 86.92±1.86% in the control microplots whereas, 7.18±0.44% in release microplot. The overall reduction in the first instar nymphs in the release plot was 79.74±1.42% than control plot. These results suggested that *P. anu* can be used as a biocontrol agent against *M. cribraria* in the field conditions.

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FUTURE PROSPECTS

Most of the farmers are now aware of the damages caused by the indiscriminate application of pesticides to the agricultural products. Consumers world wide are strongly recommending the agricultural products without any pesticides. The work is mainly focused on the life history, ecology and biocontrol agents of *M. cribraria*. Life history studies help the economic entomologist at which stage of the kudzu bug control measure should be adopted. Not only this life history studies enable the entomologist to know its generations throughout the year but also ecological studies using different parameter will be helpful in devising suitable control measures. In addition to this the record of predators or parasitoids as their natural enemies will be helpful to control this bug without causing pesticidal pollution hazards. Information on this can be used in future planning of IPM of this pest. Survey on natural enemies will shed light to grab a chance to develop an ecofriendly control strategy by using natural enemies against this pest. Development of natural enemy mass rearing technique and studies on its predating / parasiting efficiency will give yet another tool in the judicious management of this pest. At present even though some reports are available in abroad about its natural enemy complex, no reports are there in India.

VOCs were collected from disturbed and undisturbed adults of *M. cribraria* was analysed by coupled GC-MS revels the chemical composition of secretions of both disturbed and undisturbed adults of *M. cribraria.* This can be used in the development of traps for monitoring this bug and help in the timely application of pesticides and other control measures against *M. cribraria*.

The results on the phototropic response of *M. cribraria* will provide a necessary basis for the development of long-term monitoring traps, pest forecasting and even the development of eco-friendly prevention and control strategies against *M. cribraria.*

The behavioural response of *P. anu* to discriminate the females of *M. cribraria* can be studied in detail. Additionally, the effect of commonly used pesticides on life history of both *M. cribraria* and *P. anu* can be studied.