



RESEARCH ARTICLE

ABUNDANCE AND SEASONAL DISTRIBUTION OF FILTH FLIES AND THEIR HYMENOPTERAN PUPARIA PARASITIDS IN PUDUCHERRY, INDIA

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ABSTRACT

A survey on filth flies and their natural enemies was carried out at weekly intervals between July 2014 and June 2015. A total of 6 study sites comprising one poultry and five dairy farms in and around Pondicherry were selected for screening abundance of fly and hymenopteran puparia parasitoids. Density of adult flies was monitored using both scudder grill and sweep net technique. Sampling of immature of filth flies was carried out using litre sampling method. The species compositions of flies (n= 22,543) in all the study areas as follows: *Musca domestica* (89.48%), *Stomoxys calcitrans* (5.29%), *Musca sorbens* (2.44%), *Calliphora sp.* (1.89%), *Fannia canicularis* (0.56%), *Ophyra sp.* (0.13%), *Sarcophaga sp.* (0.09%) and *Hippelates sp.* (0.07%). The density of flies (Mean±SD) varied from 18.83±5.4 (Oct.2014) to 58.51±16.5 (Aug.2014) using scudder grill (no/grill/30 sec). The density of flies (Mean±SD) varied between 15.4±3.3 (Nov.2014) to 49.6±17.9 (Aug.2014) using sweep net (no/sweep). Species composition of parasitoids (n= 3,534) during the study period as follows: *Spalangia cameroni* (46.09%), *Spalangia nigroaenea* (34.94%), *Dirhinus himalayanus* (13.80%), *Spalangia endius* (3.62%) and *Pachycrepoideus vindemmiae* (1.52%). Rainfall had a significant negative correlation with density of fly (p=0.054) and parasitoid (p=0.015). The results exhibited that the rainfall affected both fly and parasitoid density. The temperature had a positive correlation with density of fly (p= 0.054) and the density was increased when temperature reached above 25°C. Therefore, for the effective fly control programme mass release of parasitoids can be done before temperature starts to increase in the summer season.

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INTRODUCTION

Filth breeding flies are widely distributed and closely associated with humans or activity of humans, particularly the house fly, *Musca domestica* is well recognized fly pest found in both house and animal farms (Greenberg, 1971). The synanthropic behaviour, high reproductive rate and ability to inhabit a wide range of environment, ensure them to spreading pathogens of various diseases in man and animals potentially (Crespo et al., 1998). The use of insecticides is common for controlling fly populations. However, filth flies have genetic capability in developing resistance among widely used and newly applied insecticides. Insecticide residues are harmful to human and animal consumption. The hazards of insecticide residues in animal products and manure is a threat to farm workers and animals and it has been observed worldwide

phenomenon (Korcisova et al., 2002; Farnham et al., 1984; Scott et al., 1989; Howard and Wall, 1996; Keiding, 1999). According to Keiding (1986) housefly populations could be controlled by parasitoids, predators and pathogens. Biological controls maximize naturally occurring control (Smith and Rutz, 1991a, Jones and weinzierl, 1997). Among the natural regulatory agents, hymenopteran parasitoids attacking the puparia of flies received considerable attention in recent times throughout the world (Legner, 1995). Hence we made a study to control filth flies using certain parasitoids which can reduce the fly population naturally. Studies were carried out on the distribution, abundance and bio-control role of many species of parasitoids (Legner and Olton, 1971). Three puparia parasitoid species, *Spalangia cameroni*, *Muscidifurax raptor* and *Phygadeuon fumator* were recovered from house fly puparia in outdoor manure heaps in Denmark. The cosmopolitan species *S.cameroni* is the most common naturally occurring parasitoid which suppressed house fly and stable fly in pig facilities in south-eastern Norway (Birkemoe et al., 2004a), livestock

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premises in Denmark and Canada (Floate *et al.*, 1999; Skovgard and Jespersen 1999; Gibson and Floate 2004). *Dirhinus* species have been formerly reported parasitizing house fly pupae (Roy *et al.*, 1940, Toyama and Ikeda 1976). In India, many parasitic wasps which parasitize the filth fly puparia were recorded from different parts of the country. In Kolkatta Roy and Siddons (1939) surveyed and listed the parasitic wasps *Spalangia* spp., *Brachymeria fulvitaris*, *Brachymeria argentifrons* and *D. pachycerus* attacking the puparia of muscoid flies. *D. pachycerus* has been recorded from a poultry farm in Tiruchy (Karunamoorthy and Nagarajan, 1986). Observations were made on geographic distribution of the genus *Dirhinus* parasitic on synanthropic and other Diptera (Boucek and Narendran, 1981). In Bangalore four species of hymenopterous puparia parasitoids such as *S. cameroni*, *S. nigroaenea*, *S. endius* and *D. trichiophthalmus* were recorded (Geetha Bai and Sankaran, 1977). A survey was conducted on the species composition, prevalence and natural parasitism of the hymenopteran parasitoids in Pondicherry in which *S. cameroni*, *S. nigroaenea*, *D. himalayanus* and *P. vindemmiae* were recorded (Balakrishnan and Panicker, 1994).

Srinivasan and Balakrishnan (1989) collected four species of parasitoids *P. vindemmiae*, *S. cameroni*, *S. nigroaenea* and *D. himalayanus* from different habitats viz., cattle, poultry and piggy farms in Pondicherry. Sangeetha and Jebanesan (2010) studied the seasonal and relative abundance of hymenopterous parasitoids in Chidambaram, Tamil Nadu found that *S. cameroni* was recorded predominant species followed by *S. endius*, *D. himalayanus* and *S. endius* were released as single and combined species in poultry houses at Namakkal, Tamil Nadu to evaluate rate of puparia parasitism (Sumathi *et al.*, 2011). Prevalence of *S. endius*, *D. himalayanus* and *P. vindemmiae* were recorded in a study carried out in Namakkal poultry farms (Sumathi *et al.*, 2015). In view of increase in fly nuisance among livestock, poultry and dairy farms, a detailed investigation is undertaken to explore the occurrence; seasonal abundance and distribution of hymenopteran parasitoids of filth fly puparia in Puducherry, Union Territory of Pondicherry.

MATERIALS AND METHODS

Study area

Puducherry is primarily an agro-based town with agricultural and animal husbandry activities. A survey was carried out in 15 areas in and around Puducherry during May to June 2014 to select study area. Density of adult flies was monitored using both Scudder grill (Scudder, 1962) and sweep net method (WHO, 1986). Based on these survey dairy farms and poultry farm were selected randomly, where the density of flies was high and the dung pile serve as probable fly breeding site throughout the year. The sampling of filth fly puparia and adult fly was carried out at weekly interval from the five dairy farms and one poultry farm. Four dairy farms, at Pillaiyarkuppam, Natesan Nagar, Osuteri, and Vanur land belonging to Aurobindo Ashram are located in and around Puducherry. The other study areas which are a dairy farm in Periyakattupalayam and a poultry farm in Thazhuthali are the private farms located in Puducherry and Tamil Nadu respectively. Each farms are

separated from other with 10-30 km distance. Cow dung and organic matter were manually removed daily in each dairy farm and dumped within the cattle shed in damp state result in abundant fly proliferation except poultry farm where manure was being cleaned once in two months. Surface area of cow dung heap, number of heap and height in each farm was also measured. In all these study areas except Thazhuthali poultry farm, cows are being maintained for milking purpose. Manure was removed manually and dumped in farm yard for compost making for agricultural purposes.

Collection of samples

Pillaiyarkuppam dairy farm has 2 manure heaps located 4 metre away from farm building. Three manure samples from one heap and 2 samples from another were collected weekly interval in every month for one year. In Natesan Nagar 3 manure samples were collected from 1 manure heap and 2 from another heap situated 3 metre away from cattle shed. A large manure pit located in Periyakattupalayam dairy farm, from which four manure samples from the periphery and one from middle of manure heap were collected. Osuteri dairy farm has 3 cow dung heap, four manure samples collected from 2 heaps and one from remaining heap. Vanur dairy farm has one manure heap from which four manure samples from margin and one from centre were collected. In Thazhuthali poultry farm manure was dumped in one permanent dumping place from which four samples from periphery and one from centre of manure were collected.

Study design

From each sampling area 5 samples (1 litre each) were collected from fixed sample site of which four samples from periphery and one from centre of manure pile for one year (July 2014 to June 2015) following the method recommended by Geethabai and Sankaran (1977). Samples of manure were brought to the laboratory and the puparia were separated using floating and skimming method (Scudder, 1962) and placed on filter paper to dry at room temperature. After drying they were examined under stereoscopic dissection microscope. Puparia were sorted to remove puparia previously eclosed, broken or had parasitoid emergence holes. Dark brown, intact puparia were counted and placed in 1 litre plastic container covered with muslin cloth and held for 20 days in laboratory condition for fly or parasitoid emergence. After 20 days puparia that did not produce a fly or parasitoid were dissected to determine whether adult fly and parasitoid mortality due to a parasitism in which the offspring unsuccessfully completed development. All parasitoids observed during dissections that died in unidentifiable immature stages were recorded as aborted parasitoids (Gibson and Floate, 2004). The emerged parasitoids collected from these samples were identified to species level using the keys of Narendran (1989) and the pictorial guide proposed by Gibson (2000). Density of immature was determined using litre sampling technique (number/L) (Scudder, 1962). Adult fly density was monitored using both Scudder grills (number/grill/30 seconds) (Scudder, 1962) and Sweep net method (number/sweep) (WHO, 1986) at weekly interval for a period of one year from July 2014 to June 2015. Species composition of fly and parasitoid was also recorded.

Table 1. Two Way ANOVA result on the comparison of filth fly density among different villages

Source	Type II sum of squares	Df	Mean square	F	Significance
Corrected model	373112.736 ^a	5	74622.547	4.911	0.001
Intercept	1.006	1	1.006	661.923	0.000
Villages	373112.736	5	74622.547	4.911	0.001
Error	1002914.250	66	15195.670		
Total	1.143	72			
Corrected Total	1376026.986	71			

^aR squared = .271 (Adjusted R squared = .216)

Table 2. Details of housefly abundance and parasitoids emerged from the puparia collected from different study areas

Month	Tl. no.of flies collected by Sweep net	Tl. no.of flies collected by Sudder grill	Tl. no.of puparia	Parasitoid emer.hole puparia	Fly already emer.puparia	Viable puparia	Tl. No.of parasitoids emerged
July.14	2422	3218	2059	195	562	1302	312
Aug	2978	3511	1626	182	390	1054	346
Sep	2222	1784	1883	158	490	1235	275
Oct	1658	1030	829	76	236	517	152
Nov	924	1323	669	63	217	389	127
Dec	1784	2351	1621	164	431	1026	363
Jan.15	1614	2203	1420	133	349	938	337
Feb	1177	1932	1198	84	305	809	474
Mar	1564	2116	2426	252	718	1456	397
Apr	1861	2356	1471	158	432	881	304
May	1941	2389	1729	168	459	1102	259
Jun	2398	2698	1186	99	391	696	188

Table 3. Multiple correlation tests between climatological factors with abundance of filth fly puparia and its parasitoids

Parameters	Adult fly density	Puparia density	<i>S. cameroni</i>	<i>S. nigroaenea</i>	<i>D. himalayanus</i>	<i>S. endius</i>	<i>P. vindemniae</i>	Total parasitoids
Max.temperature	r = + 0.567 p = 0.054	r = + 0.390 p = 0.210	r = -0.102 p = 0.752	r = - 0.053 p = 0. 870	r = -0.108 p = 0.738	r = - 0.210 p = 0. 512	r = - 0.354 p = 0. 258	r = - 0.135 p = 0.675
Min.temperature	r = + 0.422 p = 0.172	r = + 0.282 p = 0.374	r = - 0.347 p = 0. 269	r = - 0.282 p = 0.375	r = -0.197 p = 0. 540	r = - 0.367 p = 0. 241	r = - 0.569 p = 0.053*	r = - 0.389 p = 0.211
Relative humidity	r = - 0.723 p = 0.008	r = - 0.465 p = 0.127	r = - 0.149 p = 0.644	r = - 0.138 p = 0.669	r = 0.156 p = 0.628	r = + 0.169 p = 0. 600	r = + 0.172 p = 0. 935	r = - 0.070 p = 0.830
Rainfall	r = - 0.568 p = 0.054*	r = - 0.487 p = 0.108	r = - 0.787 p = 0.002**	r = - 0.762 p = 0. 004**	r = - 0.059 p = 0.855	r = - 0.276 p = 0.386	r = - 0.147 p = 0.649	r = - 0.682 p = 0.015*

* Correlation is significant at 0.05 level - Negative correlation

** Correlation is significant at 0.001 level p= Probability value

r = Pearson's coefficient of correlation + Positive correlation df = 11

Density of parasitoids was calculated by dividing the number of adults/unit volume in the different months following the method of Arellano and Reuda (1988).

Weather data

Weather data such as temperature, rainfall and relative humidity for the study sites was obtained during the period of July 2014 to June 2015 from meteorological department of Puducherry government. Puducherry enjoys warm and humid weather with the exception of January and February months which are moderately colder but the temperature never falls below 20°C. The temperature generally varies between 26°C and 38°C. The northeast downpour sets in during the middle of October, and Puducherry gets the massiveness of its annual precipitation during the period from October to December. Average annual precipitation is 1254 mm and relative humidity varies from 70 to 80%.

Statistical analysis

Two-way ANOVA test was performed to measure the filth fly density between the study areas, considering the fly density as

the dependant variable and the villages as factors. Following ANOVA test, Post Hoc test by Least Square Difference method was also used for multiple comparisons. The fluctuation of the fly population as observed through relative density during the study period was used to find out intrinsic rate of increase (r_m) by following Odum (1971) who had shown that the intrinsic rate of increase can be calculated from measurements of population size at any two times. The finite rate of increase of the fly population in a unit time was computed using the formula of Andrewartha and Birch (1954). Multiple correlation tests were performed to determine the expected significance of the factors measured (temperature, rainfall, relative humidity) on puparia, fly and seasonal activity of parasitoids.

RESULTS

The total number of 22,543 filth flies comprising 8 species were collected from 6 study areas at weekly interval from July 2014 to June 2015 using sweep net (Table. 2). Among the total flies, *Musca domestica* (89.48%) was the predominant fly species in all study areas and *Stomoxys calcitrans* being the

second most abundant species constitutes 5.29%. The other fly species in the order of abundance are *Musca sorbens* (2.44%), *Calliphora* sp. (1.83%), *Fannia canicularis* (0.56%), *Ophyra* sp. (0.13%), *Sarcophaga* sp. (0.09%) and *Hippelates* sp. (0.07%) (Fig.1 a). The mean density of fly in different study areas was compared by two-way ANOVA and showed significant difference between the villages (Table.1). The density of filth flies when measured using scudder grill (flies/grill/30 sec) varied between 20.4±2.9 to 68.9±1.27 in Natesan Nagar, 20.2±7.2 to 68.9±7.5 in Osuteri, 20.7±2.8 to 70.9±13.2 in Pillaiyarkuppam, 17.0±3.6 to 52.7±12.6 in Periyakattupalayam, 13.1±3.9 to 45.9±11.8 in Thazhuthali and 11.6±2.4 to 46.5±13.8 in Vanur. The corresponding numbers were 19.1±3.8 to 64.6±11.1, 15.4±2.9 to 62.3±10.4, 14.9±2.3 to 65.7±6.0, 14.4±3.8 to 39.1±11.6, 13.1±5.5 to 37.2±11.9 and 14.2±1.8 to 37.8±14.2 respectively when measured using sweep net (number/sweep) in these study areas.

Although, Two-way ANOVA test followed by Post-Hoc's Least Square Difference (LSD) method revealed that the density in Periyakattupalayam was not significantly different from that in Natesan Nagar, Pillaiyarkuppam and Osuteri. These three villages were recorded similar density without any significant ($P > 0.05$ by LSD) difference. However these villages showed high significance ($P < 0.05$) when compared with other two villages viz., Thazhuthali and Vanur. The density of flies collected using sweep net in Periyakattupalayam was not significantly different from that in Thazhuthali, Vanur and Osuteri but showed high significance ($P < 0.05$) when compared to Natesan Nagar and Pillaiyarkuppam. The overall mean density of flies varied from 18.83±5.4 (Oct.2014) to 58.51±16.5 (Aug.2014) per grill per 30 seconds. The mean density by sweep net (number/sweep) collection varied between 15.4±3.3 (Nov.2014) to 49.6±17.9 (Aug.2014). The finite rate of increase (λ) varied from 0.978 to 1.019. The adult fly population showed an increase during August, November, December (2014) and March, April, May and June (2015) (Fig. 2).

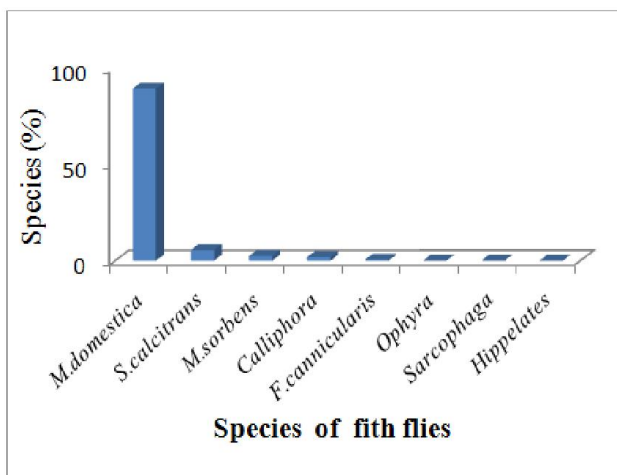


Fig. 1a. Species composition of filth flies in study sites

The total number of puparia collected from all the study places and details on parasitoid emergence were given in Table (2). The mean density of puparia in different study areas varied from 11.1±2.72 to 42.9±6.52 in Natesan Nagar, 10.2±2.89 to

42.3±6.34 in Osuteri, 12.1±2.46 to 46.2±5.94 in Pillaiyarkuppam, 10.5±1.9 to 43.1±8.33 in Periyakattupalayam, 10.9±2.9 to 38.6±4.0 in Thazhuthali and 10.1±2.37 to 29.5±7.04 in Vanur.

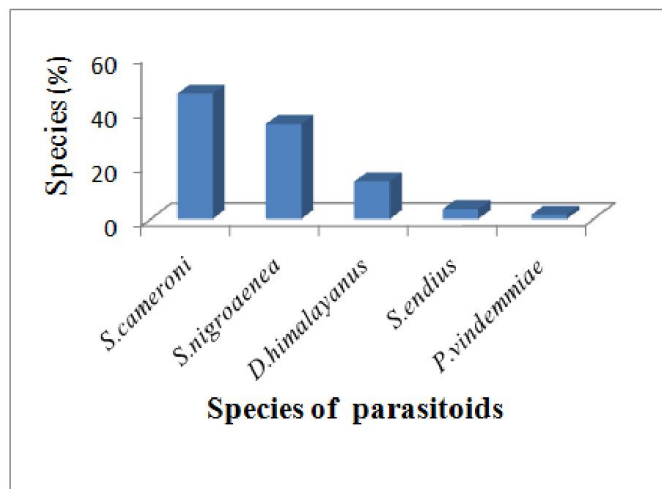


Fig. 1b. Species composition of parasitoids

The overall mean density ranged between 11.08±2.8 (Nov.2014) to 40.4±8.2 (Mar.2015). Parasitoids comprising five species were emerged from viable puparia. Among the total parasitoids, *Spalangia cameroni* (46.09%) and *Spalangia nigroaenea* (34.94%) were the predominant species followed by *Dirhinus himalayanus* (13.80%), *Spalangia endius* (3.62%) and *Pachycrepoideus vindemmiae* (1.52%) (Fig.1 b).

The influence of climatic factors (temperature, rainfall and relative humidity) on adult fly, puparia and parasitoid abundance was assessed (Table.3) (Fig 4).

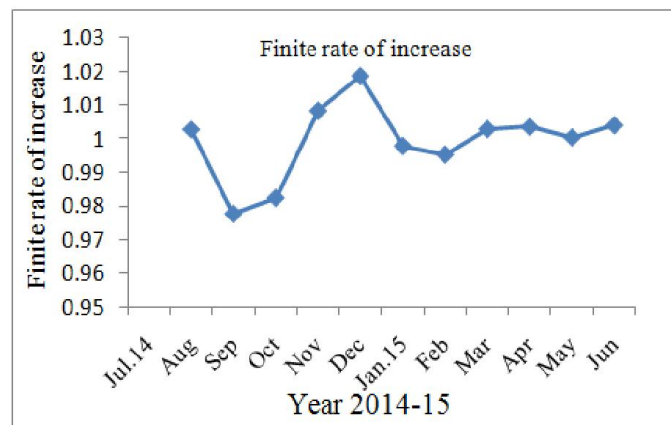


Fig. 2. Finite rate of natural increase of fly population

The adult fly abundance showed a significant negative correlation with rainfall ($r = -0.568$; $P = 0.054$) and positive correlation with maximum temperature ($r = +0.567$; $P = 0.054$) while the density of puparia has shown positive correlation with temperature and negative correlation with rainfall but did not exhibit any significance. Although the monthly abundance of parasitoid species between different study areas has not

shown any significant variation in all farms, the overall parasitoid abundance showed a significant negative correlation with rainfall ($r = -0.682$; $P = 0.015$) but not with temperature.

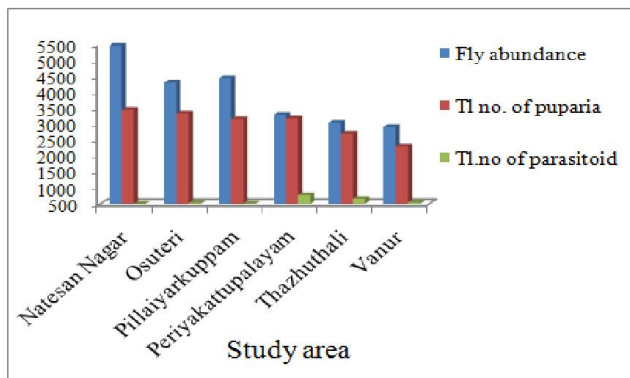


Fig. 3. Fluctuation of house fly, puparia and parasitoid abundance (Combined number of all species in all study areas)

The rainfall and puparia density had a significant negative correlation with the abundance of *Spalangia cameroni* ($r = -0.787$; $P = 0.002$) and *Spalangia nigroaenea* ($r = -0.762$; $P = 0.004$) while the minimum temperature had a significant negative correlation with the abundance of *Pachycrepoideus vindemmiæ* ($r = -0.569$; $P = 0.053$) and other two species did not show any significance with climatic factors. The relative humidity had a negative correlation with adult fly, puparia and parasitoid abundance but did not exhibit any significance.

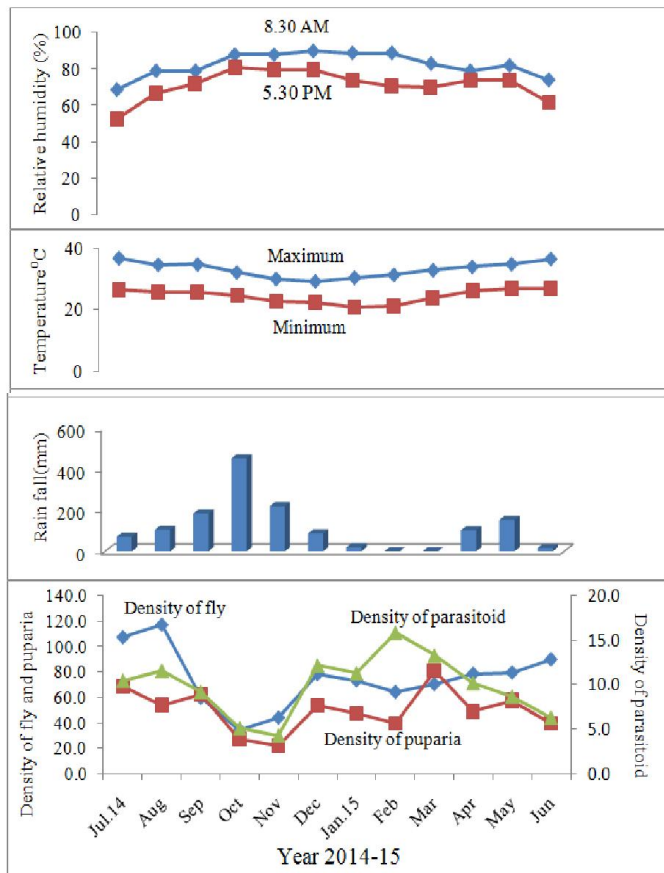


Fig. 4. Seasonal abundance of fly, puparia and parasitoid density in study areas

DISCUSSION

High density of flies may lead to anguish of animals and annoy workers and neighbours living in surrounding area of the farm. In the present study, adult fly populations in each study area were variable and prevalent throughout the year with relatively high during summer season and low during monsoon and winter months. *Musca domestica* was found to be the principal fly pest and this finding agree with similar study carried out in two selected food outlets in Pulau Pinag, Malaysia by Nurita Abu Tahir *et al.* (2007). Rainfall and temperature influenced the filth fly density. In our study, density of flies were relatively higher during mean minimum temperature ranged between 25-27°C (July-August). LaBrecque *et al.* (1972) also recorded similar seasonal density patterns in the livestock farm from north central Florida. He observed that the density of flies increased in the summer months when mean temperatures ranged from 25.5°C to 27.2°C and decreased in the winter months. The population of fly showed a positive correlation with temperature which is optimal for fly breeding resulting in the high density of flies during warmer months whereas it showed negative correlation with rainfall therefore flies were occupied indoors during rainy seasons.

There was a significant difference in fly abundance between the study villages. Natesan Nagar, Pillaiyarkuppam, Osuteri and Periyakattupalayam recorded high level of fly abundance owing to poor sanitation practice and irregular removal of manure in these study areas during the study period. Fly abundance in Thazhuthali and Vanur farms was significantly lower than other study areas. This may be due to their cultural practice of manure spreading on dry land in Thazhuthali poultry farm and relentless compost making by manure spreading in Vanur dairy farm which inhibits fly development resulting in lower fly population in these habitats. The density of puparia showed positive correlation with temperature and negative correlation with rainfall. This shows that heavy rainfall during the rainy months flushed away the puparia from manure heap resulting in the decline of both host and parasitoid abundance.

Among the parasitoids recorded in the present study, the Pteromalids such as *S.cameroni*, *S.nigroaenea*, *S.endius* and *P.vindemmiæ* are known for their cosmopolitan distribution whereas the Chalcidid *D.himalayanus* are commonly found in tropical and subtropical region (Narendran, 1989). *Spalangia* is the most abundant parasitoid species recorded in many countries like Northwestern USA, Danish animal farms, at Hawaii animal farms (Legner and McCoy 1966; Mourier and Ben Hanine, 1969; Mourier, 1971; Toyama and Ikeda, 1976). Earlier some studies made to explore the occurrence of hymenopteran parasitoids. *S.cameroni*, *S.nigroaenea*, and *D.himalayanus*, are the most prevalent species in dairy farms. Similar findings were reported by Geethabai and Sankaran (1977), Balakrishnan and Panicker (1994) exhibited that *S.cameroni*, was the predominant species followed by *S.nigroaenea*. In addition to these parasitoid species *S.endius* were also recorded in the present study. The occurrence of *Dirhinus pachycerus* a synonym of *D. himalayanus* has been recorded first from Kolkata (Roy and Siddons, 1939) and later in Bengaluru and Trichy (Karunamoorthy and Nagarajan,

1986). Density of *D. himalayanus* fluctuated in different months with its peak during the post rainy months while *P. vindemmiae* was observed only during rainy and post rainy months. These results support earlier report by Balakrishnan and Paniker (1994) who also found the same. The density and seasonal distribution of parasitoids showed a correlation with rainfall and temperature. In the present study abundance of parasitoids showed a significant negative correlation with rainfall. This showed the abundance of parasitoids fluctuate in different months and reached their peaks during post rainy months and such phenomenon has also been reported from a similar study carried out in Chidambaram by Sangeetha and Jabanesan (2010). Perhaps, the maximum temperature in June and heavy rainfall in October and November affected the activity of parasitoids.

Inconsistency in parasitoid abundance could also have been caused by interruption due to management practices, whereby there was periodical removal of manure from cattle shed. Thus, management practice in different farms contributed to variation in parasitoid abundance. It was clearly demonstrated that *Spalangia* species are efficient biological control agent due to their puparia searching ability in deeper level in dumped manure (Pitzer et al., 2011). Two *Spalangia* species and one species of the genus *Chalcids* were found attacking throughout the study period and they have the potential for use in augmentative releases for fly control. Srinivasan and Balakrishnan (1989) studied that potential biological control of *D. himalayanus* against the filth flies was due to its high fecundity, longevity and ability to survive in various climatic conditions. Since *D. himalayanus* is reported from Pondicherry and is well adapted to habitat and climatic conditions. This parasite appears to be a promising biological fly control agent. It should be considered for successful biological control of housefly by augmentative releases of this indigenous parasitoid species even though it occurs in nature insufficiently.

In conclusion, seasonal activity of fly and parasitoids was correlated with climatic conditions. The management practices in fly breeding places may also account for the monthly difference of fly and parasitoid density. In the present study, temperature showed positive correlation with density of fly and it was increased when the temperature reached above 25°C. Therefore, for effective control of fly mass release of parasitoids can be done before temperature starts to increase in summer season.

Conflict of interest

The authors proclaim no conflict of interest

Acknowledgment

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