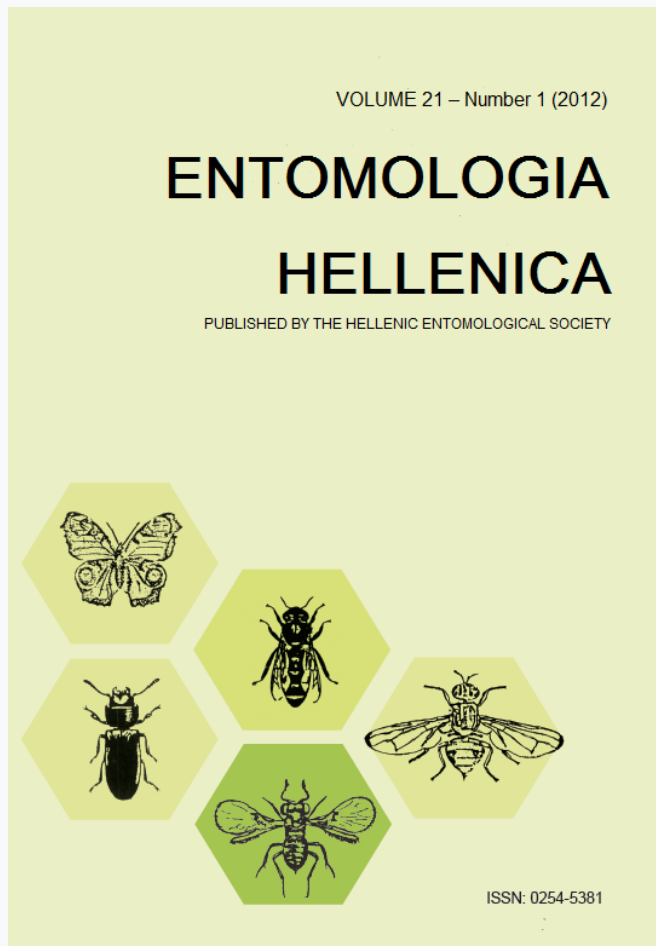


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Temperature dependent development of *Phenacoccus solenopsis* under laboratory conditions

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ABSTRACT

Cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is an important cotton pest in Punjab, India. Development of the immature stages (four instars in female and five in male) of *P. solenopsis* was observed at nine constant temperatures (15, 18, 20, 25, 30, 34, 35, 38, 40°C). Using the linear model, the lower temperature threshold (t_{\min}) for development was estimated to be 4.93 and 2.95°C and the thermal constant (K) was 333.33 and 454.54 degree days for female and male, respectively. In addition, three nonlinear models (Lactin, Bieri-1 and 3rd order polynomial) were tested to describe the relationship between temperature and development rate and to estimate the upper developmental threshold (t_{\max}) and the optimum temperature for development (t_{opt}). Our results proved that the Bieri-1 and Lactin models and provided the best fit ($r^2 = 93,4 - 99,9\%$) and estimated accurately all the three critical temperatures, ranged $t_{\min} = 5.06 - 5.25^\circ\text{C}$, $t_{\text{opt}} = 33.55 - 33.60^\circ\text{C}$, $t_{\max} = 39.99 - 40.00^\circ\text{C}$, for the total development of females and $t_{\min} = 2.82 - 3.16^\circ\text{C}$, $t_{\text{opt}} = 34.01 - 34.04^\circ\text{C}$, $t_{\max} = 40.00 - 40.10^\circ\text{C}$, for the total development of males.

KEYWORDS: Development threshold, model, *P. solenopsis*, temperature, degree- days

Introduction

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is a polyphagous pest, feeding on a wide variety of plants. The host range of this mealybug includes grapes, fig, date palm, apple, avocado, banana, citrus, okra, tomato, brinjal, cucurbits, cotton, and ornamentals as *Hibiscus* sp., *Chrysanthemum* sp. and mulberry (Abbas et al. 2010). Arif et al. (2009) conducted a study in Pakistan on the host plants of the cotton mealybug and recorded it on 154 species in 53 families comprising 20 field and horticultural crops plus 45 ornamental, 64 weed and 25 species of bushes and trees. Preferred plant families included Malvaceae, Solanaceae, Moraceae, Amaranthaceae, Asteraceae, Convolvulaceae, Euphorbiaceae,

Verbenaceae and Zygophyllaceae. Among these, *Hibiscus rosa-sinensis*, *H. mutabilis*, *Abutilon* spp. (Malvaceae), *Lantana camara* (Verbenaceae), *Withania somnifera* (Solanaceae), *Convolvulus arvensis* (Convolvulaceae), *Euphorbia prostrata*, *Croton sparciflorum* (Euphorbiaceae) and *Achyranthes aspra* (Amaranthaceae) harboured this pest round the year and acted as a persistent source of spread of the mealybug to cotton and other crops. High infestations on *Solanum melongena*, *S. nigrum*, *Datura metel* (Solanaceae), *Xanthium strumarium* (Asteraceae), *Trianthema* spp. (Aizoaceae), *Chenopodium album* (Chenopodiaceae) and *Tribulus terrestris* (Zygophyllaceae) may also be a source of this pest during the summer. In addition, *Celosia argenticia* (Amaranthaceae), *Calendula officinalis* (Asteraceae),

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Cestrum nocturnum (Solanaceae) and *Asparagus* spp. (Liliaceae) were found to host the mealybug during the winter. Other plants were either less preferred or the mealybug was found incidentally in very low numbers for short periods only (Arif et al. 2009, Abbas et al. 2010).

Phenacoccus solenopsis was reported originally on ornamentals and fruit crops in the United States (Tinsley 1898) and is regarded as an introduced exotic pest in India and Pakistan. In India, *P. solenopsis* was found to be a serious pest, which caused infestation in Punjab. Hodgson et al. (2008) provided descriptions of all the stages of *P. solenopsis* based on specimens from the Indian subcontinent. Based on this research, they also considered that the three species *Phenacoccus solani* Ferris, *P. solenopsis* and *P. defectus* Ferris might be environmentally induced variants of a single species, in India and Pakistan. In India, *P. solenopsis* was predominant mealybug, infested cotton in Haryana, Rajasthan, Gujarat, Maharashtra, Andhra Pradesh and Tamil Nadu (Nagrare et al. 2009). In the recent years, *P. solenopsis* was introduced in the northern India and caused huge loss (estimated amount Rs. 37412.55/ha) in Punjab state due to reduction in average seed cotton yield (0.135 kg in infested plant as compared to 0.242 kg in healthy plant) (Dhawan et al. 2007). Similarly, *P. solenopsis* has caused 14 per cent loss to cotton crop during 2005 in Pakistan (PWQCP 2005).

Some biological parameters of the life cycle of *P. solenopsis* have been studied on cotton in Punjab, Haryana and Anand (Dhawan and Saini 2009, Kedar et al. 2010, Patel et al. 2010) and China rose in Pakistan (Aheer et al. 2009). Given that temperature is the main abiotic factor affecting development, the understanding of the physiological relationship between temperature and development rate is an important criterion for the prediction of population outbreaks and timely management of pests on crops (Jervis and

Copland 1996, van der Have 2008). The objective of the present study was to determine the relationships between temperature and development and survivorship of the immature stages of *P. solenopsis* under a range of controlled temperatures.

Materials and Methods

Temperature dependent development of each immature stage of *P. solenopsis* was studied under laboratory conditions. Insects from a laboratory rearing were maintained on twigs, leaves and young shoots to study their rate of development, temperature thresholds and survivorship.

Test plants

Seeds of the genetically modified cotton hybrid RCH-134 were procured from Rasi Seeds Pvt. Ltd. and planted in 10x10 m² plots at the Entomological Research Farm, Department of Entomology, Punjab Agricultural University (PAU), Ludhiana, Punjab, India during 2008 and 2009 by following PAU recommendations. Cotton seeds were also sown in earthen pots (25 cm diameter), which were filled up to 2 cm from the upper edge with puddle soil. These earthen pots were watered when the upper layer of soil gets dried and plants showed stress symptoms. Thirty-day-old plants were used for conducting various experiments in the laboratory as they have plenty of young growing shoots.

Rearing of *P. solenopsis*

Under screen house at the Entomological Farm the rearing was maintained by collecting nymphs and adults of cotton mealybug (*P. solenopsis*) from the different host plants and reared on preferred hosts [*Parthenium hysteroporus* (congress grass), *Xanthium strumarium* (gut putna), and *Hibiscus* and cotton crop] in earthen pots. Maintenance of the rearing was also done in laboratory on *Hibiscus* and cotton

cuttings (tender shoots and leaves) in battery jars. The cuttings were replaced every three days by new ones and the mealybug population was transferred to the new cuttings using a camel hair brush. During off season, temperature of laboratory was maintained by using electrical heater. From this culture source the further experiments were conducted.

Laboratory temperature development studies

The influence of the nine constant temperatures (15, 18, 20, 25, 30, 34, 35, 38 and 40°C) on the development and survival of *P. solenopsis* was examined in the laboratory at PAU, Ludhiana during 2008-09. The experiments were conducted in the incubator at each constant temperature at 75±10% R.H. and photoperiod L:D 14:10h. Fifty gravid females were collected from the laboratory culture and kept on cotton shoots on agar coated Petri-plates and incubated until oviposition at each temperature treatment. After oviposition, fifteen newly hatched eggs were collected from ovisac within 8-12 h of deposition, transferred onto the tender cuttings (petioles and apical portion) and incubated in each nine temperatures. These experiments were conducted over a year around with seven replications in cocoon stage (pre-pupal and pupal), eight replications in egg, first, second and third instar nymph, whereas nine replications in the total development time in female and male, respectively. The mealybug cohorts were examined after every 24h, and their instars and mortality recorded. The development from one instar to next was checked by the presence of exuviae. In the case of the male, after completion of the second instar, they enter into the cocoon stage (pre-pupal and pupal stage) and whole of the body became covered with a silk woven case.

One-way analysis of variance (ANOVA) was used to determine the effect of temperature on the stage-specific and total development time at $P = 5$ ($p = 0.05$) (Proc GLM; SAS institute, 2010). Similarly,

survival rate was also subjected to one-way ANOVA (without transformation). Tukey's honestly significant difference (HSD) and Duncan multiple range (DMR) tests were used to separate the means.

Development rate and mathematical models

The results were used to model development rate (d^{-1} , reciprocal of development times in days) and to estimate development thresholds. In all immature stages (egg, first, second and third instars, prepupa and pupa), the development rate was regressed against temperature using the linear and three nonlinear models (JMP8.0, SAS institute, 2010) (Table 1).

The parameters of interest were t_{\min} (lower threshold temperature), t_{\max} (upper developmental threshold), t_{opt} (optimum temperature) and K (thermal constant). The lower development threshold is the temperature at or below which no measurable development is detected (Howell and Neven 2000) and can be estimated from the linear model as the intercept of the development line with the temperature axis. The thermal constant (K) is the number of thermal units (degree days) required by an immature stage to complete its development and can be also estimated directly from the linear equation (Aghdam et al. 2009). The upper developmental threshold (t_{\max}) is the temperature at or above which development does not occur (Kontodimas et al. 2004) and is estimated by the nonlinear models such as the Bieri-1 and the Lactin models (Bieri et al. 1983, Lactin et al. 1995). The optimum temperature (t_{opt}) can be also estimated by the nonlinear models as the parameter value for which first derivatives equals to zero. The stat software JMP 8.0 was used to plot the regression of the nonlinear models.

The performance of the mathematical models is commonly evaluated using the coefficient of determination (r^2) which indicates better fits at higher values, and the residual sum of square (RSS), with better fit at lower values (Aghdam et al. 2009).

TABLE 1. Development models and their mathematical equations used by us to describe the relationship between temperature and development of *P. solenopsis*.

Model	Equation	References
Linear/ Thermal summation	$D=K/(temp-t_{min})$	Muniz and Nombela 2001, Roy et al. 2002
Lactin	$y = e^{\rho \cdot temp} - e^{\left(\rho \cdot T_m - \frac{T_m - temp}{\Delta}\right)} + \lambda$	Lactin et al. 1995, Roy et al. 2002
Bieri	$y = [a \times (temp - t_{min})] - [b^{(temp - t_m)}]$	Bieri et al. 1983
Polynomial 3 rd order (Harcourt Equation)	$y=a \times temp^3 + b \times temp^2 + c \times temp + d$	Harcourt and Yee 1982, Kontodimas et al. 2004

Where D: development rate, K: thermal constant, t_{min} : minimum temperature, T_m , t_m , ρ , λ , Δ , a, b, c and d: fitted coefficients

Results

Laboratory Temperature Development studies

All the immature stages of *P. solenopsis* completed their development at temperature between 15°C and 35°C but not at 38°C and 40°C. Development time within each instar and also total development time decreased with increase in temperature to about 35°C in all instars (Table 2). No egg or nymphs survived at 40°C.

Mean egg development time (\pm SEM) ranged from 0.75 ± 0.14 d at 35°C to 5.00 ± 0.32 d at 15°C (Table 2). The mean development time for first instar nymphs (L1) ranged from 3.21 ± 0.14 d at 35°C to 11.57 ± 0.20 d at 15°C. Similar trends were found for second (L2) and third instar nymphs (L3). Mean total development ranged from 10.25 ± 1.66 d at 35°C to 32.40 ± 0.55 d at 15°C for the female nymphs and 13.65 ± 0.80 d at 35°C to 36.80 ± 0.84 d at 15°C for the male stages.

Survivorship of each nymphal instar at each temperature treatment is presented in Table 3. Survivorship rose for all immature stages with increasing temperature, peaking at 34°C – 35°C, and then decreased rapidly at 38°C. At extreme temperature viz, 15 and 38°C, per cent survival was moderately low

(46-68%) particularly for the egg and the first instar nymphal stages.

Model Evaluation

The linear regression equation was used to calculate fitted coefficients, t_{min} and K, parameter such as r^2 and RSS as discussed in methodology. The linear model provided a good fit to the data in all immature stages with high values for r^2 (0.939) and low RSS (<0.205) values (Table 4). The linear regression estimated that *P. solenopsis* required 333.33 degree days (DD) (female) and 454.54 DD (male) to complete development from egg to adult emergence with a lower development threshold of 4.93°C and 2.95°C for female and male, respectively. The t_{max} was not estimated by the linear model, as the fitted line did not intersect the x-axis at higher temperatures.

The estimates of the fitted coefficient, measurable parameters for the three non-linear models are presented in Table 5. Of these Bieri-1 and Lactin models provided the best fit as compared to polynomial model for each immature development stage. The relationship between development rate (d^{-1}) of immature stages and temperature (°C) described by the examined models are presented in Figure 1.

TABLE 2. Mean development time (d) \pm SEM each instar of *P. solenopsis* at 9 different temperatures under laboratory conditions.

Temperature (°C)	Development stage						
	Egg	L1	L2	Total development time			
				Female (L3)	Male(pre-pupal and pupal stage)	Female	Male
15	5.00 \pm 0.32a	11.57 \pm 0.20a	8.00 \pm 0.32a	7.71 \pm 0.29a	12.33 \pm 0.33a	32.40 \pm 0.55 ^a	36.80 \pm 0.84 ^a
18	3.80 \pm 0.20b	9.43 \pm 0.20b	6.00 \pm 0.00b	6.07 \pm 0.07b	10.83 \pm 0. 31b	25.50 \pm 0.87 ^b	30.10 \pm 1.48 ^b
20	3.40 \pm 0.24b	8.43 \pm 0.29c	5.20 \pm 0.20c	5.28 \pm 0.28c	9.92 \pm 0.49b	22.80 \pm 1.48 ^c	27.50 \pm 2.29 ^c
25	2.10 \pm 0.33c	6.00 \pm 0.31d	3.80 \pm 0.19d	4.57 \pm 0.30cd	8.50 \pm 0.26c	16.50 \pm 1.12 ^d	20.70 \pm 1.48 ^d
30	1.10 \pm 0.24d	4.29 \pm 0.15e	3.70 \pm 0.20d	4.07 \pm 0.1ed	7.58 \pm 0.20cd	13.00 \pm 0.87 ^c	16.80 \pm 1.25 ^c
34	0.75 \pm 0.15d	4.36 \pm 0.24e	3.60 \pm 0.18de	3.79 \pm 0.15ed	6.75 \pm 0.25d	12.65 \pm 0.85 ^c	15.65 \pm 0.78 ^{ef}
35	0.75 \pm 0.14d	3.21 \pm 0.15f	3.00 \pm 0.32e	3.57 \pm 0.43e	6.50 \pm 0.22d	10.25 \pm 1.50 ^f	13.65 \pm 1.34 ^f
38, 40	-	-	-	-	-	-	-

TABLE 2 (continued from the foregoing page) . Mean development time (d) \pm SEM each instar of *P. solenopsis* at 9 different temperatures under laboratory conditions.

ANOVA							
df	7	7	7	7	6	8	8
Replication(s)	8	8	8	8	7	9	9
N	36	55	39	54	42	44	44
Sum square (SS)	88.281	602.152	144.259	144.865	173.00	4453.269	6117.725
Mean sum Square	12.61	86.022	20.608	20.695	28.83	556.658	764.715
F	42.04	254.13	89.98	43.58	50.56	455.45	459.60
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.001	<0.0011	<0.0001
R ²	0.913	0.974	0.953	0.868	0.896	0.990	0.990

Means within a column followed by same letter are not significantly different at $p=0.05$ (Tukey's HSD test); F, df and P value represent ANOVA of temperature treatments within a developmental stage (ProcGLM; SAS institute).

TABLE 3. Mean percentage survival \pm SEM of each instar of *P. solenopsis* at 9 temperatures under laboratory conditions

Temperature (°C)	Development stage						
	Egg	L1	L2	Total immature			
				Female (L3)	Male (pre-pupal and pupal stage)	Female	Male
15	45.50±0.50f	51.57±0.65e	62.40±0.40e	68.00±0.58d	64.25±171e	53.00±6.72cb	50.25±6.52c
18	56.00±2.00e	58.00±0.62d	67.40±0.51d	72.67±0.67d	73.50±1.73d	58.58±7.66cab	56.50±7.38cb
20	65.50±0.50d	64.14±1.53c	76.00±0.89c	80.00±0.58b	81.5±2.51c	67.08±9.17cab	63.88±10.01cab
25	77.50±1.50c	83.71±1.21b	87.80±0.80b	87.67±1.47ab	92.25±3.30b	78.50±11.19ab	76.25±12.14ab
30	89.50±0.50b	87.86±1.18ab	89.80±0.97b	92.00±1.53a	92.50±1.00ab	82.50±12.77ab	78.81±12.78ab
34	95.00±1.00a	91.43±1.56a	95.00±0.89a	93.67±1.45a	93.00±1.63a	86.75±15.16a	82.37±14.58ab
35	93.00±1.00ab	89.14±1.30ab	87.40±1.21b	91.00±1.52a	92.50±2.38ab	82.58±13.71a	79.19±13.36a
38	46.00±0.00f	46.43±1.95e	52.20±1.16f	54.67±4.41e	60.00±10.00gf	5.94±1.22c	6.60±1.43c
40	-	-	-	-	-	-	-
Anova							
df	7	7	7	7	7	8	8
Replication(s)	8	8	8	8	8	9	9
N	15	56	40	24	31	26	35
Sum square (SS)	5283.333	16710.785	8167.500	4050.292	5251.375	18296.15	22014.712
Mean sum Square	754.762	2387.255	1166.786	578.613	750.196	2287.019	2751.838
F	293.52	195.83	291.70	52.40	44.62	21.48	26.34
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R ²	0.996	0.966	0.984	0.958	0.982	0.905	0.886

Means within a column followed by same letter are not significantly different at p=0.05(Tukey's HSD test)

TABLE 4. Parameters from linear regression of development rate and temperature for *P. solenopsis* under laboratory conditions.

Development stage	t_{\min}	K	R²	RSS
Egg	13.84	16.98	0.939	0.2050
L1	7.10	102.04	0.990	0.0003
L2	5.96	72.46	0.991	0.0005
Female L3	4.12	84.03	0.987	0.0006
Male (pre-pupal and pupal stage)	-5.64	256.41	0.999	0.000009
Total immature female	4.93	333.33	0.996	0.000002
Total immature male	2.95	454.54	0.999	0.000001

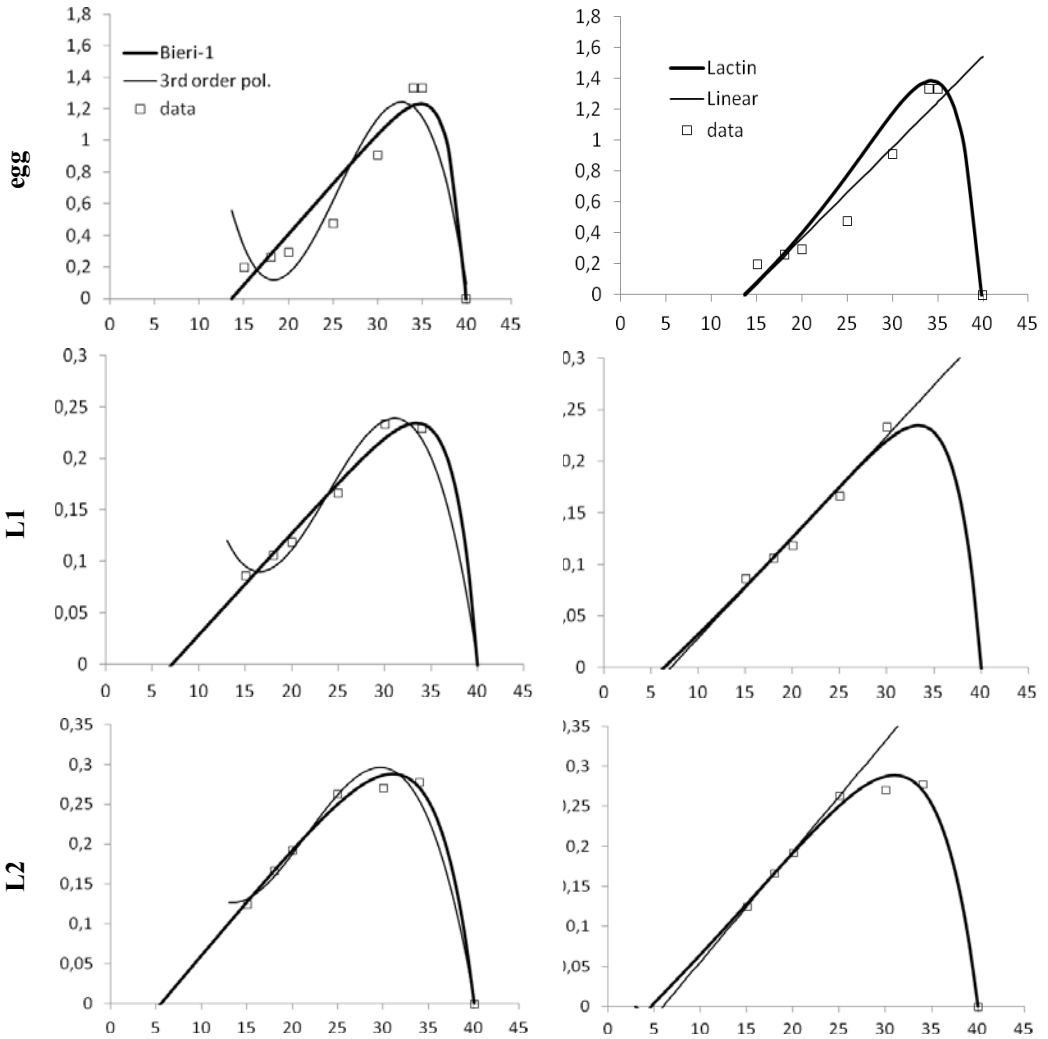


FIG 1. Relationship between development rate (d^{-1}) of different stages and temperature ($^{\circ}C$) for *P. solenopsis* (continued in the next page).

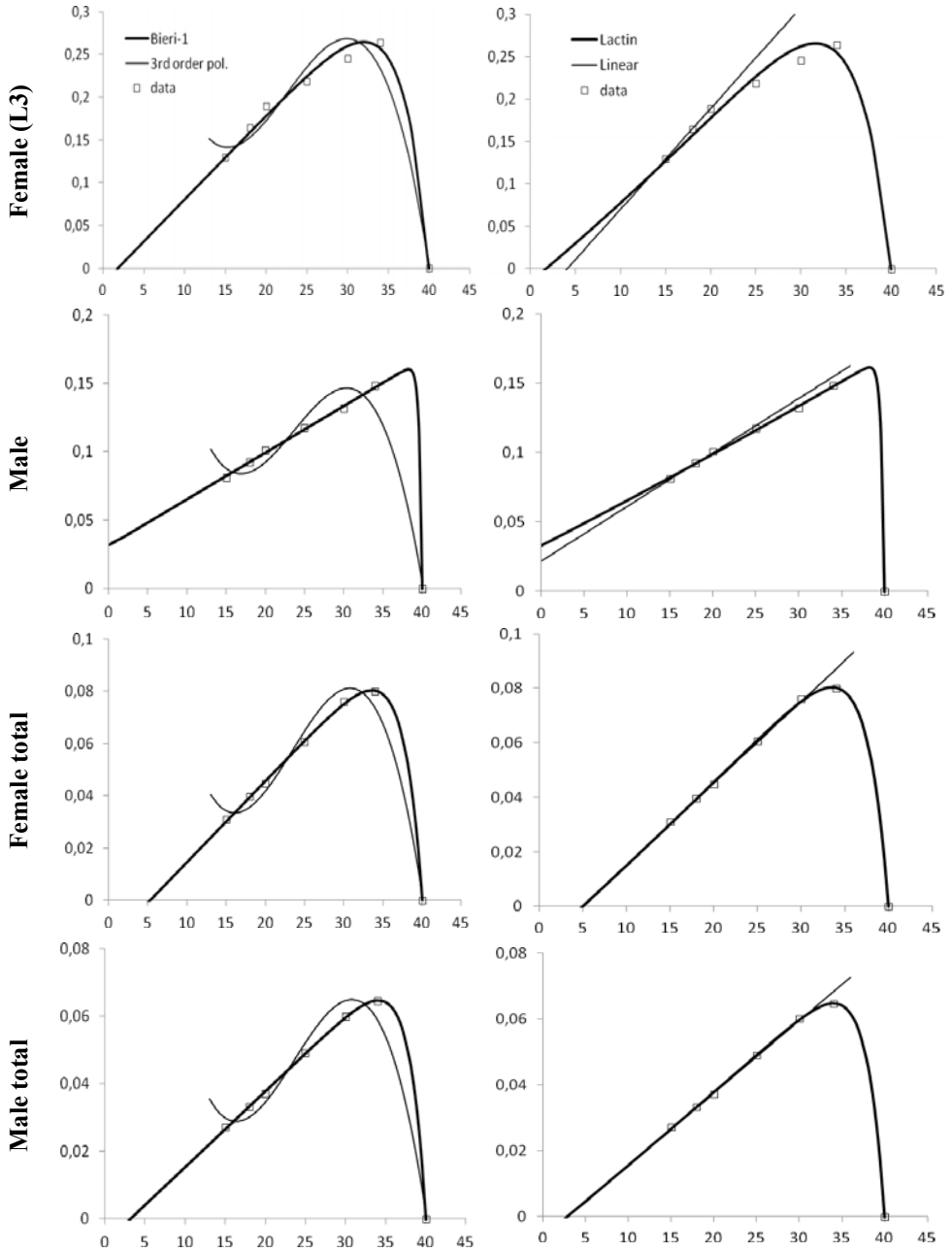


FIG 1 (continued from the foregoing page). Relationship between development rate (d^{-1}) of different stages and temperature ($^{\circ}C$) for *P. solenopsis*.

Discussion

As *P. solenopsis* became a serious pest of cotton in India and Pakistan recently, there is no much available literature on the biology of this species in these regions. Our results clearly showed that temperature had significant effect on the survival and development of *P. solenopsis*. Developmental time decreased with increasing temperature to about 35°C and then declined rapidly. Our results are similar to those of Amarasekare et al. (2008), who investigated the effect of temperature on the development of *Paracoccus marginatus* William and Granara de Willink. *Paracoccus marginatus* was able to complete its development between 18°C to 35°C and required 303.0 DD and 294.1 DD to complete development of the male and the female, respectively. In our study, *P. solenopsis* had slightly higher values of DD (454.54 DD and 333.33 DD for the male and the female, respectively). In addition, Chong et al. (2008) in their study found similar value of thermal constant (347 DD) for the development of the females of *Maconellicoccus hirsutus* (Green).

As far as the nonlinear models is concerned, our study proves that the Lactin (improved Logan-6 model) and Bieri-1 equations provide the best fit as well as estimate all the three critical temperatures (t_{min} , t_{max} , t_{opt}) in comparison to the 3rd order polynomial (Harcourt and Yee 1982, present study) and the Logan-6 equation (Amarasekare et al. 2008, Chong et al. 2008).

Based on the results and using meteorological data, we can forecast the rate of development of this species at certain periods of year and therefore forecast when it is likely to develop pest status.

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**Σχέση θερμοκρασίας και ανάπτυξης του εντόμου
Phenacoccus solenopsis σε εργαστηριακές συνθήκες**

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ΠΕΡΙΛΗΨΗ

Ο ψευδόκοκκος του βαμβακιού *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) είναι ένας σημαντικός εχθρός της καλλιέργειας βαμβακιού στην επαρχία Punjab της Ινδίας. Στην παρούσα εργασία μελετήθηκε η ανάπτυξη του *P. solenopsis* σε εννέα σταθερές θερμοκρασίες (15, 18, 20, 25, 30, 34, 35, 38 και 40°C). Χρησιμοποιώντας τη γραμμική εξίσωση υπολογίστηκε το κατώτερο θερμοκρασιακό όριο (t_{min}) για την ανάπτυξη των θηλέων στους 4,93°C και την ανάπτυξη των αρρένων στους 2,95°C. Αντίστοιχα η θερμική σταθερά (K) ήταν 333,33 ημεροβαθμοί για τα θήλεα και 454,54 ημεροβαθμοί για τα άρρενα. Επίσης για την περιγραφή της σχέσης θερμοκρασίας και ρυθμού ανάπτυξης και τον υπολογισμό του ανώτερου θερμοκρασιακού ορίου (t_{max}) και της θερμοκρασίας ταχύτερης ανάπτυξης (t_{opt}) δοκιμάστηκαν τρία μη γραμμικά μαθηματικά πρότυπα (Lactin, Bieri-1 και το πολυώνυμο 3^{ου} βαθμού). Τα αποτελέσματα έδειξαν ότι οι εξισώσεις Bieri-1 και Lactin είχαν την καλύτερη προσαρμογή στα δεδομένα (συντελεστής προσδιορισμού: $r^2 = 93,4 - 99,9\%$) και υπολόγισαν με ακρίβεια όλες τις κρίσιμες θερμοκρασίες ($t_{min} = 5,06 - 5,25^\circ\text{C}$, $t_{opt} = 33,55 - 33,60^\circ\text{C}$, $t_{max} = 39,99 - 40,00^\circ\text{C}$, για την ανάπτυξη των θηλέων και $t_{min} = 2,82 - 3,16^\circ\text{C}$, $t_{opt} = 34,01 - 34,04^\circ\text{C}$, $t_{max} = 40,00 - 40,10^\circ\text{C}$, για την ανάπτυξη των αρρένων).