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Effect of temperature on the biology of *Harmonia dimidiata* FAB. (Coleoptera: coccinellidae) reared on *Scizaphus graminum* (ROND.) aphid

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Abstract

The influence of five constant temperatures (16±1°C, 20±1°C, 24±1°C, 28 ±1°C and 32 ±1°C) on the biology and prey consumption of larvae, adult male and female beetles of the ladybeetle, *Harmonia dimidiata* (Fab.), (Coccinellidae: Coleoptera), feeding on *Schizaphus graminum* aphid was investigated. There was significant effect of temperature on the biology and prey consumption potential of immature and adult stages of *H. dimidiata*. The result revealed that incubation period, different larval instars duration, pupal and adult male and female beetles duration was maximum at the low temperature 16 ± 1˚C and minimum at the high temperature 32 ± 1˚C. The survival rate and predatory potential of all stages was maximum at 24 ± 1 ˚C. The female fecundity was maximum 686.75±18.277 eggs per female at 24 ± 1˚C and minimum 223.75±4.3279t 28 ± 1˚C. The results further revealed that at 32± 1˚C the female could not produce eggs. The result indicates that temperature have profound effect on the developmental durations, survival, fecundity and predatory potential of *Harmonia dimidiata*. The optimum rearing temperature for this predator was found to be 24 ±1°C on the basis of their short durations, maximum survival and high reproductive and predatory potential of *H. dimidiata* (Fab.). These attributes may take it a useful natural enemy for the purpose of biological control program against aphid pests of economic importance.

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Introduction

The cereal aphid *Schizaphus graminum* (Rond.) commonly known as green bug is a serious pest of small grains and having a wide host range of at least 60 plant species including wheat, barley, sorghum and corn (Bowling *et al.*, 1998). It sucks the sap and injects the toxin into the plant and interferes with the grain formation. Initially causes yellow or red leaf spots and continued feeding leads to general yellowing and reddening, leaf and root death, and can lead to plant death. (Ahmad and Nasir, 2001). Lady birds are considered the most important natural enemies of aphids and have been effectively utilized for integrated control of several aphid pests (Brown, 2004). Among these the sub tropical ladybird beetle *Harmonia dimidiata* (F.) ,sub tropical lady bird beetle commonly known as fifteen spotted lady bird is one of the largest predatory Coccinellid beetle commonly found in China, Vietnam, India, Nepal, Pakistan and other countries of South-East Asia. In Pakistan it occurs in Northern hill areas, subtropical areas including Swat, Murree, Rawlakoat, Peshwar and Islamabad (Raffi, M.A. 2005). Both larvae and adult of *H. dimidiata* are highly voracious predators of aphids and have the ability to consumed more than 200, *A. gossypii* aphids daily. (Kuznetsov and Pang, 2002). The life span of the beetle is very lengthy and the survival of different stages was fairly high. The beetle has the ability to survive at low temperature (Waseem, A. G *et al.,* 2007).

Temperature is a key abiotic external factor that influences the biology and predatory potential of a particular insect species. Every insect experiences a range of temperature which greatly affects the biological parameters and predatory potential. The influence of temperature on the biology of Coccinellid beetles has been studied extensively in the past by different authors, i.e; Jih-Zu Yu *et al.*, (2013) Asrar. A. *et al.,* 2013, Arshad A. *et al.*, 2009; Castro, C.F. *et al.*, 2011), Bakr, R. F. A. *et al.,* (2009). It is very important to study the relationship between temperature and development for any economically important species and is basic to the development of pest management strategies.

Keeping in view the importance of the lady bird beetle, *H. dimidiata* Fab. and their host insect *S. graminum* aphid, the present study was conducted to checked the effect of temperature on the biology, survival and predatory potential of *H. dimidiata* in order to find out the most suitable temperature for quality mass production of the of the predator under controlled conditions. This study will explore the potential of the beetle as a bio control agent against aphid pests of economic importance. This information could be usefully employed to propagate the beetle in hilly areas in summer and can be introduced in the plains in winter against wheat aphids including *S. graminum* in Pakistan.

Material and methods

The present study was conducted on the biology of *H. dimidiata* at Insect Pest Management program, National Agricultural Research Centre Islamabad during winter 2011-2012. The experiments were conducted at five constant temperatures, 16 ±1 ˚C, 20 ±1 ˚C, 24 ±1 ˚C, 28 ±1 ˚C and 32 ±1 ˚C with 65 ±5% relative humidity and 16:8 light dark photoperiod in growth chambers. The following sets of experiments were conducted:

(a) Developmental duration and predatory potential of immature stages at five constant temperatures.

To study the effect of temperature on the developmental durations of immature stages of *H. dimidiata*, a total of 100 freshly laid eggs of *H. dimidiata* in batches were collected from stock culture and kept in plastic Petridishes (8 ×2 cm) for hatching at each constant temperature. The experiments were repeated five times at each temperature. The eggs were observed daily for hatching. Upon hatching 40 newly emerged first instar larvae were transferred in plastic vials (6×4 cm) separately. Initially counted numbers of aphid's (30-40), 1st- 3rd nymphal instars/larvae were provided on wheat leaves for feeding. The number of aphids increased as the larvae entered into next instars. The 4th instar larvae were provided up to 400 aphids per day untill pupation. The larvae were observed daily for molting and the molting exuava was considered as evidence that the larvae had entered in to the next instars.

The data were recorded on the developmental duration, survival rate of different stages and reproductive and predatory potential. The data was statistically analyzed by using one-way ANOVA and means were compared using LSD test at 5% level of significance. The analysis was carried out through computer program statistix 8.1 versions.

(b) Biological parameters and reproductive potential of adult beetles at four constant temperatures

To study the adult biology and reproductive potential of *H. dimidiata*, a total of 20 newly emerged (two days old) male and female beetles were paired and kept in rearing jars (20 ×10 cm). The jars were placed in growth chamber at five constant temperatures. Aphids were provided on wheat leaves in excess daily as well as tissue paper and fresh wheat leaves were also provided for oviposition. The old infested leaves and the dead aphids were removed daily. The mean number of eggs per female and the longevity of both male and female beetles were recorded. The first and last egg-laid by each pairs were recorded for determination of pre-oviposition, oviposition and post-oviposition periods. The data were recorded and statistically analyzed by using one-way ANOVA and means were compared using LSD test at 5% level of significance using computer program statistix 8.1.

Predatory potential of adult male and female beetle's at five constant temperatures.

To study the predatory potential of adult male and female *H. dimidiata* beetles, a total of 20 freshly emerged males and females, were kept in plastic rearing jars separately at each required temperature. Counted numbers of aphids approximately 250-300 (1st to 4th) instar nymphs were provided on wheat leaves daily. The jars were covered with muslin cloth at the top. After 24 hours the dead, consumed and unconsumed aphids were counted and replaced with fresh ones until all adults (males and females) beetles had died in the rearing jars. The data were statistically analyzed by using one-way ANOVA and means were compared using LSD test at 5% level of significance.

Survival rate of immature stages

To study the survival rate of different stages of *H. dimidita* at five constant temperatures, a total of 100 freshly laid eggs were kept in plastic petridishes for hatching at each constant temperature in growth chamber. Percent hatching of egg was calculated. Upon hatching the first instar larvae were kept in plastic rearing vials separately at each temperature levels. The larvae were feed aphid in excess in each vials daily on wheat leaves.

The old infested leaves were replaced after each 24 hours in each vial. The molting was observed critically after each 12 hours in each vials and also the survival and mortality were recorded for each stage. The process was continued till all the larvae entered in to pupal stages. The pupe were kept under the same conditions for adult emergence. Upon adult emergence the male and female beetles were separated and the sex ratio was calculated at five constant temperature levels separated.

Results and discussion

Developmental durations and percent survival of H. dimidiata immature stages at five constant temperatures

The mean developmental duration of immature stages at five constant temperatures are presented in Table 1. The results indicate that the maximum mean incubation period was 9.18±0.14 days at 16 ±1 ˚C and minimum was 3.01± 0.12 days at 32±1 ˚C. The results indicate that temperature had a significant effect on the incubation period and significantly decreased with increasing temperature. The same trend was found for the first, second, third, fourth instar larvae, the pre pupal and pupal stages which have maximum duration al low temperature and minimum duration at high temperature (Table 1).

Table 1. Developmental duration of immature stages of *Harmonia dimidiata* feeding on *S. graminum* aphid at five constant temperatures with 16:8 photoperiods.

Temp.	Incubation period (days) ± SE	1st Instar duration (days)± SE	2nd instar duration (days)± SE	3rd instar duration (days)± SE	4th instar duration± SE	Total grub duration ± SE	Pre pupal duration	Pupal duration± SE	Duration from egg to adult emergence± SE
16±1 ˚C	9.18±0.14a	4.0±0.12a	3.24±0.09a	5.95±0.18a	13.86±0.38a	27.04±0.42a	1.89±0.04a	9.05±0.07a	47.16±0.39a
20±1 ˚C	5.02±0.11b	3.0±0.14b	2.92±0.11b	5.12±0.13b	10.02±0.33b	21.07±0.37b	1.34±0.06b	6.01±0.17b	33.43±0.49b
24±1 ˚C	4.27±0.15c	2.55±0.07c	2.24±0.07bc	4.51±0.16c	8.15±0.13c	17.45±0.09c	1.01±0.04c	5.02±0.18c	27.75±0.52c
28±1 ˚C	3.34±0.11d	2.39±0.08c	2.03±0.09c	3.21±0.16d	6.07±0.16d	13.7±0.49d	1.0±0.04c	4.0±0.16d	22.04±0.53d
32±1 ˚C	3.01±0.12e	1.89±0.09d	2.0±0.11c	2.83±0.11d	3.7±0.14e	10.45±0.39e	1.0±0.04c	3.12±0.19e	17.57±0.58e
LSD 0.05	0.3365	0.2723	0.2579	0.4117	0.6933	1.0491	0.1049	0.4389	1.3984

Means within the columns with different lowercase letters are significantly different from each other at *P value* ≤ 0.05 (one-way ANOVA, $LSD_{5\%}$value.

The results further indicate that all five temperatures have significant effect on the total developmental duration of the predator. The total duration from egg to adult emergence differed between the temperatures and was maximum 47.16±0.39 days at 16 ±1 ˚C and minimum 17.57±0.58 days at 32 ±1 ˚C.

The results indicates that female ratio was maximum than male at all temperature levels. Jih-Z-y*, et al.*, (2013) reported the duration from egg to adult stage was 38.8 ±0.3, 27.5 ±0.1 and 18.4 ±0.1 days at 15, 20 and 25 ˚C when feeding on *A. gossyphi*, which was rather shorter at all temperatures but exhibited the same trend that with increasing temperature the duration may significantly decreased. Kunznestov *et al.*, (2002) reported that the duration from egg to adult emergence was 22 and 12 days at 20 ˚C and 25 ˚C which is shorter than the present study. V.P. Semyanov (1999) reported the durations of egg, larvae and pupae was 12.5, 60 and 20 days at 15 °C and 5, 22 and 8 days at 20 °C. At 25 ^{0} C the duration was 3, 14, 5 at 25 ^{0} C and finally it was 2, 10 and 3.5 days at 30 ^{0} C.

The results of the present study and that of the past workers are different for developmental durations, these differences may be due to differences in the environmental conditions or the host insect they used or may be different strain of the predator they used.

The results of the present study and that of the past workers indicate that with increasing temperature the duration may significantly decreased. In the past different workers conducted experiments on different Coccinellid beetles under different temperature regimes, their result indicates that temperature has profound effect on the biology of Coccinellid beetles Castro *et al.*, (2011) recorded longer developmental time at low temperature for *H. axyridis* (Pallas).

The duration was maximum (43.1) days at 15 ˚C from egg to adult emergence followed by 30.8 days at 20 ˚C, and minimum 22.3 days at 25 ˚C. The results of the present study and that of the past workers indicate that temperature has significant effect on the developmental duration of *H. dimidiate*. Castro, 2011, Aksit *et al.*, 2007, Ararar Ali *et al.*, 2013 and Stathas, G.J., *et al.*, 2011 have reported a significant effect of temperature on the biology of Coccinellid beetles. The temperature accelerates the developmental duration and at high temperature the duration reduced compared to low temperature levels.

Survival rate of immature stages

The percent survival rate was also significantly different at all tested temperature. Maximum survival rate from egg to adult emergence was 82% at 24 ±1 ^{0} C and minimum survival rate was 18% at 32 ±1 ^{0} C. The survival rate for different developmental stages was significantly different at all tested temperature. The result further indicates that survival rate for immature stages was maximum at mild temperature and was minimum at extreme low and high temperature Table 2.

Table 2. Percent survival of different immature stages of *H. dimidiata* immature at five constant temperatures reared on *S. graminum* aphid.

Developmental stages	Temperature								
	16±1 ^{0}C		20±1 ^{0}C		24±1 ^{0}C		28±1 ^{0}C		32±1 ^{0}C	
	N	%survival	N	%survival	N	%survival	N	%survival	N	%survival
Egg	100	81	100	87	100	97	100	76	100	61
L1	81	90.12	87	93.103	97	94.84	76	89.47	61	59.016
L2	73	94.52	81	97.53	92	97.82	68	86.76	36	86.111
L3	69	97.10	79	97.46	90	96.66	59	91.52	31	87.09
L4	67	97.01	77	98.70	87	98.85	54	96.296	27	85.16
Pre Pupae	65	96.923	76	98.68	86	97.67	52	94.23	23	82.60
Pupae	63	95.238	75	98.666	84	97.619	49	87.75	19	94.73
Egg to adult emergence	60	60	74	74	82	82	43	43	18	18
Male/Female Ratio		27/33		34/40		37/45		19/24		7/11
		1:1.06		1:1.06		1:1.8		1:1.05		1:1.04

N= Initial number of insects in particular stage, L1= first instar, L2= second instar , L3= third instar , L4= fourth instar larvae.

Male and female ratio

The results revealed that the male and female ratio was 1:1.06, 1:1.06, 1:1.08, 1:1.05 and 1:1.04 male /female at all tested temperature levels respectively Table 2.

Predatory potential of H. dimidiata larvae/grubs and adult male, female beetles

The predatory potential of first instar larvae was maximum 69.65±1.4217 at 24 ±1 ˚C and minimum 32.15±0.8208 aphids 32 ±1 ˚C. Similarly the potential of second, third and fourth instar larvae was maximum 106.92±2.9230, 196.17±4.3554 and 548.92±9.8984 at 24 ±1 ˚C and minimum potential of second, third and fourth instar larvae was 52.82±1.4961, 77.5±1.6622 and274.25±7.9159 aphids at 32 ±1 ^{0} C Fig 1. The results revealed that fourth instar larvae was more voracious than all other instars and consumed more than 60% aphids by fourth instar larvae.

Table 3. Biological parameters of adult *Harmonia dimidiata* beetles feeding on S. graminum aphids at five constant temperature under 16:8 (L:D) photoperiod.

Temp.	Pre ovi. Period	Ovi Position period	Post ovi.	Adult longevity		Total eggs per female	Eggs per fem./day
				Female	Male		
16±1 ˚C	17.4A±0.2476	58.1A±1.7575	14.6A±0.6936	90.1A±1.4140	80.65A±1.4295	338.15A±6.3802	5.8201A±0.6377
20±1 ˚C	11.6B±0.3934	49.6B±1.2277	9.4B±0.2224	70.6B±1.2326	68.75B±0.9511	551.1B±7.0502	11.11088B±0.3442
24±1 ˚C	9.25C±0.3067	39.3C±1.1655	6.8C±0.2248	55.35C±1.1057	54.75C±0.9428	686.75C±18.277	17.47455C±0.5938
28±1 ˚C	6.0D±0.1974	28.65D±0.7687	5.15D±0.2325	39.8±0.9862	36.4D±1.1083	223.75D±4.3279	7.80977D±0.4175
32±1 ˚C				28.8E±1.0428	27.8E±1.0848		
LSD (0.01)	0.8321	3.6031	1.1224	3.2744	3.1370	29.648	1.4445

Means within the columns with different lowercase letters are significantly different from each other at *P value* ≤ 0.05 (one-way ANOVA, $LSD_{5\%}$value.

The total larval predatory potential was maximum 921.66±13.935 at 24 ±1 ^{0} C and minimum 436.72±9.6831 aphids at 32 ±1 ˚C. The predatory potential of adult male and female beetles was maximum 12682.45±375.61 and 13288.78±248.56 aphids at 24 ±1 ˚C and minimum potential was 2827.6±119.65 and 3206.05±113.75 aphids at 32 ±1 ˚C respectively Fig 2. The result of the present study and that of the past workers indicates that predatory potential was maximum at 24 and 20 ˚C. W.A. Gillani *et al.*, (2007) reported the adult predatory potential was 152.18 aphids daily and the total consumption rate was 11555.5 aphids/adult beetles. V.P. Semanove (2009) reported that the predatory potential of adult beetle for the first 10 days at 20 °C and 25 °C was 806.0 ± 42.0 and 1557.0 ± 30.0 by female beetle and 706.0 ± 61.0 and 1396.0 ± 40.0 aphids per male beetle respectively. Kuznestov, 2002, investigated the employment of Chinese Coccinellids for controlling aphids in green house. The results indicate that most effective was second instar larvae of *H. dimidiata* and *L. biplagiata*. At ratio, predator: host (1: 20), controlled 85-90% aphids in green house in a very short period of times. The results of the present study and that of past workers indicates that H. dimidiate is highly voracious predator of aphids and can be utilized for the management of aphids in biological based IPM program.

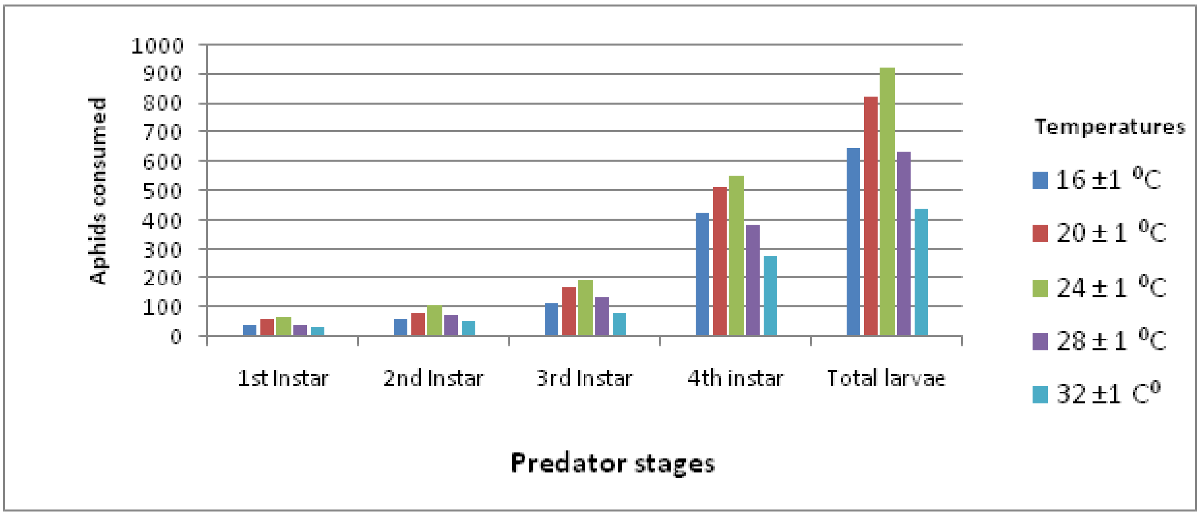

Fig. 1. Predatory potential of larval stages of *Harmonia. dimidiata* feeding on the aphid *Schizaphus graminum* at five constant temperatures.

Biological parameters and reproductive potential of adult beetles of H. dimidiata

The results indicate that the pre oviposition period of adult female beetle was significantly different at four constant temperature levels ranging from 17.4±0.2476 at 16 ±1 ^{0} C to 6.0±0.1974 days at 28 ± ^{0} C (Table 2). The result indicates that at 32 ± 1 ^{0} C the female could not produce any egg. The results revealed that temperature has significant effect on the pre maturation period. (V. P. Semyanov, 2009), reported the maturation period of female *Harmonia* (*Lies*) *dimidiata* at different temperature and photoperiod levels. The result indicates that the pre oviposition period was 30.4, 31.8 and 28.0 and 30.4, 31.8 and 28.0 days days at 20 ˚C and 25 ^{0} C under three photoperiod 18:6, 16:8 and 6:18 h (D:L) respectively. The oviposition period was ranged from 58.1±1.7575 at 16 ±1 ^{0} C to 28.65D±0.7687 days at 28 ±1 ˚C. The results revealed that the oviposition period decreased significantly with increasing temperature (Table 2). Similarly the post oviposition period was 14.6±0.6936 days at 16 ±1 ^{0} C and 5.15±0.2325 days at 28 ±1 ^{0} C. The adult female longevity was maximum 90.1±1.4140 days at 16 ±1 ˚C and minimum 39.8±0.9862 days 28 ±1 ˚C. The male longevity was maximum 80.65±1.4295 days and minimum 36.4±1.1083 days at low and high temperature levels respectively Table (2). The results revealed that female beetle live longer than male at all tested temperature. Jih-Zy, *et al.,* (2013) reported that adult male and female longevity was 98.9, 57.6, 63.5 and 91.4, 54.3 and 59.6 days at 15, 20 and 25 ^{0} C respectively, which indicates that male beetle was live longer than female beetle, but the result of the present study indicates that female may live longer. The differences may be due to different environmental conditions or due to different host insect they used for feeding or may be due to different strains and morphs of *H. dimidiate* they used for experiments. The mean number of eggs per female beetle was maximum 686.75±18.277 eggs per female at 24±1 ˚C and minimum 223.75±4.3279 at 28 ±1 ˚C (Table 2).The number of eggs per female beetle per day was maximum 17.47455±0.5938 at 24±1 ˚C and minimum 5.8201±0.6377 at 28 ±1 ^{0} C. Jih-Z-y, *et al.,* (2013) reported that the number of eggs per female *H. dimdiata* beetle was 409.5 ±67.4, 229.4 ±63.1 and 312.3 ±46.0 at 15, 20 and 25 ˚C respectively. V.P. Semyanove, (1999) reported up to 3000 eggs by female beetle Harmonia (lies) dimidita during their life spane when feeding on *M. persicae* aphids at 20-25 ^{0} C. W.A.Gillani *et al.,* (2007) reported mean fecundity was 422.31 eggs per female when reared on *B. brassicae* aphids at 25 ±2 ˚C. Debnath, (1988) reported that the fecundity of the *H. dimidiata* was 631.4 eggs at 20.0 ± 2 ˚C with daily oviposition rate ranging from 0 to 45 eggs per female. In the present study at higher temperature 32 ±1 ˚C the beetles did not lay any eggs and the data was not calculated. Jih-Z-y, *et al.*, (2013) also reported that *H. dimidiata* could not reproduce at 30 ^{0} C. These observations demonstrate that *H. dimdiata* is adapted to mild temperature very successfully. The results of the present study exhibited that among different temperature levels, 24 ±1 ˚C has been proved as the most suitable temperature for the development and mass rearing of *Harmonia dimidiata* on the basis of short duration high survival, reproductive and predatory potential of *H. dimidita*.

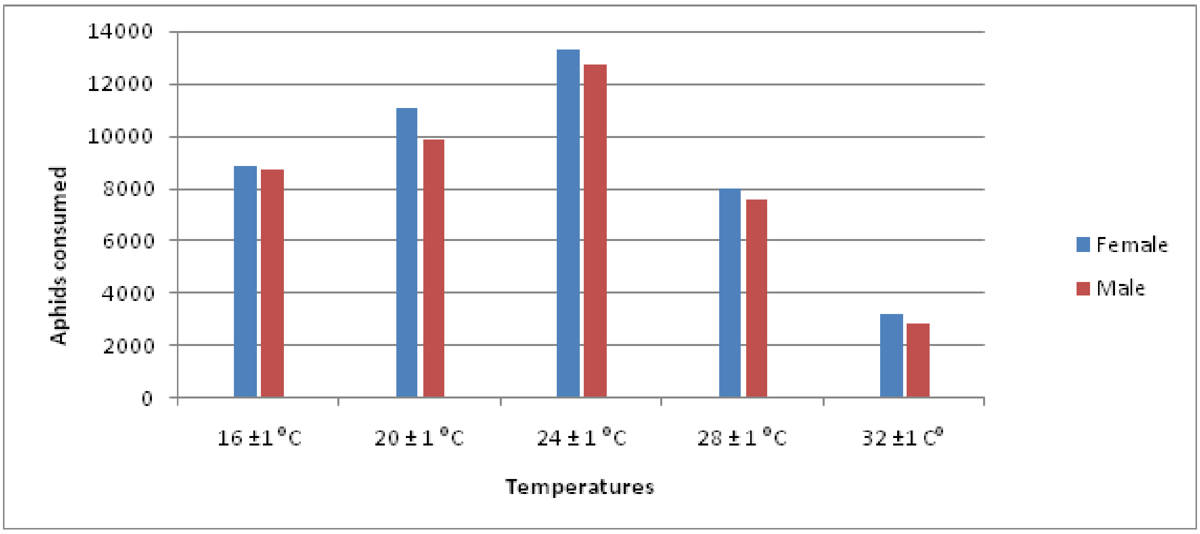

Fig. 2. Predatory potential of adult male and female beetles of *Harmonia dimidiata* feeding on *Schizaphus graminum* aphids at five constant temperatures.

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