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Effect of temperature on food consumption of the black ladybird beetle *Stethorus punctum*, Leconte (Coleoptera: Coccinillidae) reared on the two-spotted spider mite, *Tetranychus urticae* under different constant temperatures

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Abstract
Effect of temperature on consumption Food of the predator, *Stethorus punctum* Leconte reared on immature and mature stages of the prey, *Tetranychus urticae* (Koch) were studied at Agricultural University Peshawar and Agricultural Research Institute Mingora Swat, Pakistan during 2015. The effect of temperature on food consumption of *S. punctum* studied under laboratory conditions, at five different constant temperatures (15, 20, 25, 30 and 35 ºC). It was observed that the larval instars consumed more immature prey individuals at 15 ºC, followed by 35 ºC then 20, 25, and 30 ºC. Adult males of the predator consumed more individuals of immature preys at 35 ºC, followed by 30 ºC then at 15, 20 and 25 ºC, while the predator females relatively consumed more prey individuals than the males, at the same temperatures. When the larvae of the predator reared on mature stages of the prey, they consumed more prey individuals at 15 ºC, followed by 20 ºC then at 35 ºC. The adult males and females of the predator consumed almost the same numbers of the prey individuals. They consumed the highest number of preys at 35 ºC, followed by 30 ºC, and while the lowest consumed number were recorded at 15 ºC.

Keywords: *Stethorus punctum* Leconte, *Tetranychus urticae* (Koch), food consumption, temperatures.

Introduction
Two spotted spider mite *Tetranychus urticae* (Koch) (Acari: Tetranychidae) is one of the most polyphagous species of the Tetranychidae, attacking fruit and vegetables and several other agricultural crops causing economic damage [1]. *T. urticae* is adapted to various environmental conditions and is distributed worldwide, causing loss of yield and quality or the death of the plants by sucking out the contents of plant sap [2-4]. Temperature is a critical important abiotic factor affecting the dynamics of insect pest and their biological control agents [5]. Temperature affects biological activities of insects, such that maximum and minimum temperature thresholds and optimal temperature can be estimated for all major life processes [6, 7]. Weather factors such as low temperature, high humidity adversely affected the populations of *Stethorus spp*. The predatory efficiency of *Stethorus spp.* increased during the growth of larval instars. An adult female consumed 92.2 larvae, 81.8 nymphs and 52.4 adult mites per day [8]. There are several important natural enemies of *T. urticae* [9]. All known species of Order Coleoptera, Family Coccinellidae are predator of spider mites [10, 11, 12]. The coccinellids beetles *Stethorus punctum, S. gilvifrons* and *S. punctum picipes* are the most effective natural enemies of the phytophagous mite species, included *Tetranychus urticae* Koch, *T. piercei* McGregor, *Panonychus citri* McGregor and *P.ulmi* (koch) [13-20]. Development rate, expressed as the reciprocal of development time needed to change from one stage to another, [21].

Materials and Methods
The experiments were conducted at Department lab of Entomology, Agricultural University Peshawar and Entomology Section Agricultural Research Institute Mingora, Swat, Pakistan during 2015.
The predator, *Stethorus punctum* were collected from different apple orchards in District Swat which were infested with the red spider mite, *T. urticae*. Adults of *S. punctum* beetle reared on potted bean plants artificially infested with *T. urticae* maintained for 2-3 months before testing

their efficiency. Wooden cage 45x45x45 cm) were used for maintaining the culture of *S. punctum* and *T. urticae*. Cages were covered with nylon mesh cloth on top and glass door in the front for daily services and watching. The prey consumed by *S. punctum* was studied throughout the larval and adult stages at each tested temperature (15, 20, 25, 30 and 35 ºC). The predator was offered 60 immature and 35 mature prey individuals daily. Twenty replicates were carried out for each tested temperature of larval stage of *S. punctum.*

1- Feeding on immature *T. urticae* leaf disc (2.5cm) was artificially infested with sixty immature of prey individuals. Each newly hatched larva of the predator was kept separately on the leaf disc in the experimental cell (4x3x3cm). The cell was covered and incubated at constant conditions (25±2 ºC, 18L: 6D photoperiod and 65±5% RH). The cell was checked daily and the number of consumed preys were calculated. The predatory larva was transferred to new fresh leaf disc infested with the same number of immature prey. This procedure was repeated until the pupation of the predator's larva.

2- Feeding on mature *T. urticae* Leaf disc (2.5cm) was infested artificially with thirty five mature prey individuals. Each newly hatched larva of the predator was kept separately on the leaf disc in experimental cell. The cell was covered and incubated as mentioned above. This procedure was repeated until the pupation of the predator larva.

B- Adult stage of *S. punctum* feeding on immature and mature *T. urticae* Leaf disc (2.5cm) was artificially infested by sixty immature prey individuals. Each newly emerged predator's adult was kept separately on the leaf disc in the experimental cell. The cell was covered and incubated as mentioned before. The previous steps were repeated with substitution the immature preys with thirty five mature individuals. The experimental cells checked daily until the death of the predator's adult. The whole experiment was replicated five times at each tested temperature.

Statistical analysis

Data were subjected to analysis by using the SPSS Statistical program (SPSS 2004). For significant differences then multiple comparisons were made using the LSD test.

Results and Discussion

Food consumption

A- Larval stage of *S. punctum*

Statistical analysis of the experiment in Tables (1 and 2) showed that food consumption of the predator was markedly affected with temperature under investigation. When the predator was fed on immature stage of prey (*T. urticae*) the highest number of consumed preys was recorded at 15 ºC for all larval instars, followed by 35 ºC then 20, 25 and 30 ºC. Statistically, there were significant differences (*P*<0.01) between the number of consumed preys at each temperature. The coccinellid predator, reared on the two-spotted spider mite *T. urticae* observed that the highest number of consumed prey was recorded at 15 ºC for all larval instars, followed be 20ºC then25,30 and 35 ºC. Statistically, there was a significant difference (*P*<0.01) between the number of consumed preys.

Table 1: Average number of *T. urticae* immature stages consumed by *S. punctum* larval instars at different constant temperatures.

Predator Larval instars	Means±SE					F-values
	15 Cº	20 Cº	25 Cº	30 Cº	35 Cº	
1st	22.25±0.67	17.57±0.85	15.25±0.79	18.75±0.70	21.05±1.34	24.952**
2nd	33.45±1.65	29.87±1.25	26.65±0.76	27.02±0.55	28.60±1.62	7.938**
3rd	71.54±2.80	57.02±1.98	36.98±0.95	43.65±0.87	47.98±1.52	116.560**
4th	205.75±7.35	151.75±6.87	83.58±1.45	112.30±2.98	149.86±8.45	90.987**

Table 2: Average number of consumed *T. urticae* mature stage by *S. punctum* larval instars at different constant temperatures

Predator Larval instars	Means±SE					F-values
	15 Cº	20 Cº	25 Cº	30 Cº	35 Cº	
1st	22.25±0.67	17.65±0.47	15.25±0.79	19.77±0.39	21.05±1.34	38.465**
2nd	33.45±1.65	30.76±1.32	26.65±0.76	26.03±0.60	28.60±1.62	27.654**
3rd	71.54±2.80	60.30±1.80	36.98±0.95	41.70±0.65	47.98±1.52	86.873**
4th	205.75±7.35	133.75±5.35	83.58±1.45	99.85±2.97	149.86±8.45	63.374**

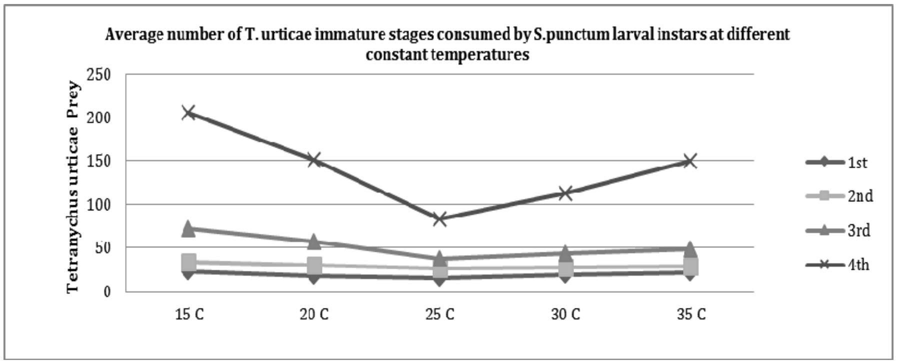
B Adult stage of *S. punctum*

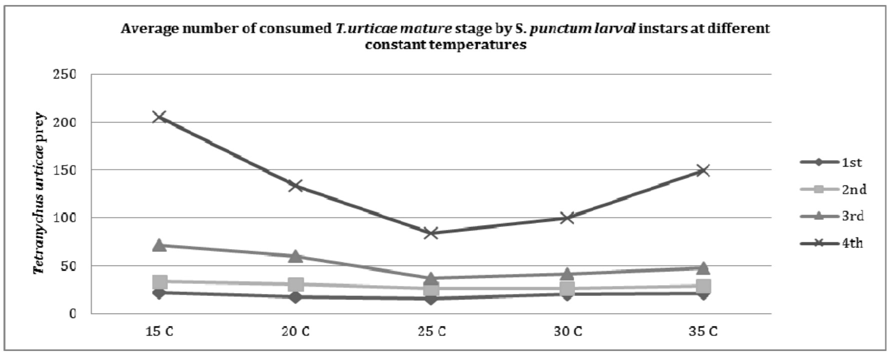
Feeding on immature and mature stages of *T. urticae* Data in Table (3) showed that adult male of the predator consumed the highest number of prey at 35 ºC, followed by 30 ºC then 15, 20 and 25 ºC, for either immature or mature prey. The female predator relatively consumed more prey individuals than the male at the same tested temperatures. Statistically, there was a significant difference (*P*<0.01) between the number of consumed preys (Table 3).

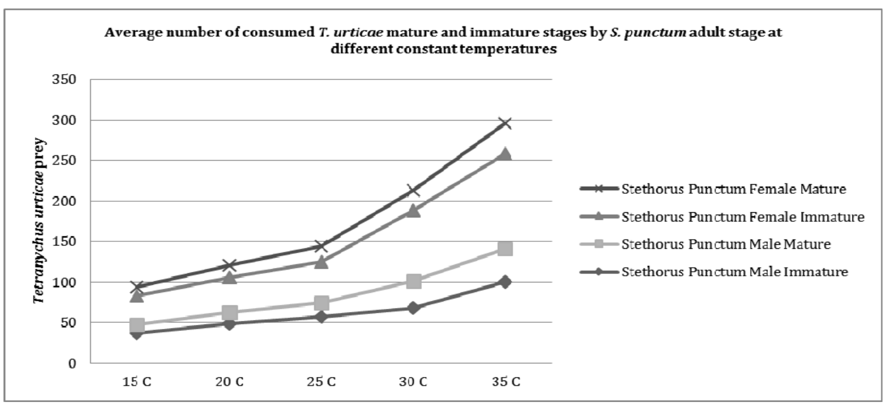
Table 3: Average number of consumed *T. urticae* mature and immature stages *by S. punctum* adult stage at different constant temperatures

	T.urticae stages		Means±SE					F-values
			15 ºC	20 ºC	25 ºC	30 ºC	35 ºC	
Stethorus Punctum	Male	Immature	37.37±0.75 c	48.54±0.99 c	57.60±0.95 b	67.74±1.73 b	99.87±2.34 a	767.756**
		Mature	10.26±0.34 c	13.75±0.58c	16.73±0.26 b	33.40±0.62 b	41.25±0.80 a	1795.390**
	Female	Immature	35.88±0.51 c	43.81±0.86 c	50.73±0.93 b	87.73±0.83 b	117.57±2.79 a	724.143**
		Mature	9.96±0.46 c	14.91±0.67 c	19.49±0.38 b	23.86±0.98 b	36.95±0.78 a	945.872**

Means in Raw followed with same letter(s) are not significantly different at 5% probability. **= Highly significant







The usefulness of a predator in the management of pests may relate, in part, to its capacity to perform adequately under a range of environmental conditions. This study determined the influence of temperature on consumption of spider mite prey (*T. urticae*) and has demonstrated a differential influence on the different life stages of the predator. Thus, daily consumption rate of the immature stages followed the same pattern for both first, second, third and fourth instar larvae, with the number of spider mite consumed increasing linearly with temperature. The present study examined the activity of the coccinellid predator, *S. punctum* (the mite destructor) under five different constant temperatures (15 ºC, 20 ºC, 25 ºC, 30 ºC and 35 ºC). Generally, it was observed that, the degree of temperature had different impacts on its activity and the food consumption rate. The optimum activity of this predator under laboratory conditions was around 25 ºC and 30 ºC. Whereas it reached, the maximum at the highest tested temperature (35 ºC) and it's minimum at 15 ºC. These results are in agreed with [22]. At 15 ºC, the development of the larval instars became sluggish, and larvae take longer periods to metamorphose because of the low temperature, so, the larval instars (1st to 4th) consumed more preys. This consumption rate was more than the other rates at 25 ºC and 30 °C, respectively. It was observed that the predator's larvae consumed somewhat more preys, which may be referred to its need to cumulate high quantity of its food requirement to be able to proceed with its development and metamorphose to the pupal stage. At 15 °C, no results were manipulated by other authors. Our obtained results at 25 ºC and 35 ºC are agreed with the results reported by [23, 24, 25] for their work on *S. punctum* [26] and for their study on *S. gilvifrons.*

Feeding on mature *T. urticae* our results revealed that, at 15ºC, the four larval instars of the predator, also, consumed more number of preys than those eaten at either 20 ºC, 25, ºC, 30 ºC and 35 ºC. These results were agree with [27] for *S. punctum* at the same temperature. It is clear that, the predator's larvae consumed more preys at 15 ºC than other tested temperature, that is because of under this degree of temperature, the rate of development of the larvae became slower than at other tested temperatures, so that, the larvae spent longer periods and needs to prey on more spider mites to fulfill its requirement for development and metamorphoses reported by [28]. The results indicated that the larval stage of the predator were more active and consumed more prey individuals at higher temperatures than at low one.

B- Adult stage of *S. punctum* in the present study, it could be concluded that the adult males and females of the predator consumed the lowest number of preys (immature and mature preys) at 15 ºC. While the highest number of consumed preys (immature and mature preys) were recorded at 30 ºC and 35 ºC, then 20 ºC and 25 ºC. Adult females were relatively consumed more prey individuals than males at the same tested temperatures. These results are similar with those reported for S. *siphonulus Kapu* by [29] but were not matched with those reported for *S. punctum* by [30], for *S. siphonulus* on citrus red mite, *Panonychus citri* [31, 32]. Our obtained results were contradicting from those reported the effect of temperature on the developmental stages of *S. punctillum*, studied in Europe at 21-23 °C by [33, 34] and at 15-35 °C interval by [35]. Studied development of *S. punctillum and T. mcdanieli* at 12 constant temperatures ranging 10-38 °C (0.5 °C) and modeled their development rates at a function of temperature by [36]. The present findings are similar to the above results that the higher temperature accelerates the developmental rate and reducing the developmental period. Several predators along with *S. punctillum*, appeared naturally and successfully control the *T. urticae* population in Valencia, Spain [37]. Among these predators *Stethorus spp*. was important one and control spider mites and maintained them below damaging level. The *S. bifidus* fed on *tetranychids* like *T. urticae* and *Panonychus ulmi* [38]. *S. punctum* consumed all stages of mites; adult consumed 75 to 100 mites/day and a larva devoured up to 75 mites/day. The larva passes through four larval stages in about 12 days, feeding on all stages of mites [39]. The feeding rate of different life stages of *S. punctillum* on the red spider mite were observed that first, second, third, fourth instar and adult insect consumed 6.80±0.07, 23.50±2.40, 37.30±4.50, 92.90±4.60 and 211±5.20 mites respectively/day by [40]. The experiments were conducted on daily consumption and predation rate of different instars *of S. punctillum* feeding on *T. urticaeand* that the first instar larva consumed 16.67, 18.56, 19.56 and 14.33 eggs, larvae, nymphs and adults respectively [41]. The consumption of adult prey is similar to the present study but the rest are much lower than our findings. According to them, the daily consumption of prey tends to be higher in the higher stages of predator, even in the adult stage. This phenomenon supports our results on their work on *S. punctillium* as a predator for *T. urticae* [42]. It could be concluded that the optimum temperature for mass-rearing the predator *S. punctum* under laboratory conditions was 25 ºC±2 ºC.

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