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PREY STAGE PREFERENCE AND PREDATORY POTENTIAL OF *TYPHLODROMUS DIVERGENTIS* (ACARI: PHYTOSEIIDAE) AGAINST TWO SPOTTED SPIDER MITES, *TETRANYCHUS URTICAE* (ACARI: TETRANYCHIDAE)

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ABSTRACT

Typhlodromus divergentis (Acari: Phytoseiidae) is a predatory mite of the family Phytoseiidae. Phytoseiid mites are known as one of the most promising biocontrol agents against spider mites. Biological studies of *T. divergentis* including preference of prey stage and predatory potential on *Tetranychus urticae* were conducted at CABI Biocontrol Laboratory, Directorate of Plant Protection, Agriculture Research Institute, Quetta, Pakistan. To conduct stage preference studies; different combinations of spider mites' life stages i.e. (a) eggs + immatures (b) eggs + adults and (c) immatures + adults, were evaluated for larval, nymphal and adult stages of *T. divergentis*. The results suggested that eggs and immature stages of spider mites were preferred in all combinations for feeding by *T. divergentis* (larvae, nymph and adult stages). In case of predatory potential, each prey stage (egg, immature and adult) was provided separately to *T. divergentis* and maximum consumption of *T. divergentis* against *T. urticae* was observed in egg stage (70 ± 3.56%) followed by immature stage (49 ± 2.34%) and adult stage (10 ± 1.91%). Based on results, it is suggested that *T. divergentis* has the ability to maintain spider mites population by lowering their eggs and immature stages under dry conditions of Quetta, Balochistan.

KEYWORDS: *Tetramychus; urticae;* spider mites; biological control; *Typhlodromus divergentis;* predator; prey stages; preference; Pakistan.

INTRODUCTION

Two spotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae) are considered as key pest occurring on wide range of host plants ranging from ornamental plants to field crops (Puchalska *et al*., 2014; Skirvin and Williams, 1999; Soika and Labanowski, 2003). They are found underside of leaves where they suck the cell sap of the plant. Usually, they initiate to colonize plants rapidly after crops cultivation but their outbreaks are seen later in growing season (Badi *et al.,* 2004). They are associated with dry and hot weather conditions, where their high density may result in occurrence of blotching and stippling followed by leaf fall and fruit drops (Evans, 1992). Application of chemicals has remained the main control strategy for spider mites. However, their biological attributes like short life cycle, multiple generations and high fecundity provide a baseline for resistance development against many acaricides (Cranham and Helle, 1985; Van Leeuwen *et al.,* 2009), while non-judicial use of pesticides over long period of time against spider mites is considered as main cause behind resistance development (Gopal, 2000) and also affecting human health and causing environmental issues. Therefore, sustainable alternate management approaches are desired to combat these issues.

Biological control is considered as one of the encouraging management approaches in this instance that is safer to environment and human health (Bakker *et al.,* 1993; Stenseth, 1979). However, spider mites of the genus *Tetranychus* are known to produce silky webs on the host plants that are considered as defense system from natural enemies (Gerson, 1985). These silky webs can restrict the free movement of predators over leaf surface in case of generalist predators (McMurtry *et al.,* 1970; Osakabe, 1988; Sabelis and Bakker, 1992 and Takafuji and Chant 1976). Some specialist predators as reported in family Phytoseiidae, can overcome this issue and have the ability to move freely inside these silken webs due to their body setal length (McMurtry and Croft, I997; Putnam, 1955).

Phytoseiid mites have been reported as most important natural enemies of spider mites (El-badry, 1967; Escudero and Ferragut, 2005; McMurtry and Croft, I997). They can also feed on thrips, whitefly and other soft small insects (Jeppson *et al.,* 1975). Their implications against spider mites are mainly reported under covered conditions like greenhouses (Chandler *et al.,* 2005; Oliveira *et al.,* 2009; Strong and Sroft, 1995). In case of phytoseiid deployment on outdoor plants, few attempts were practiced (Brushwein, 1991; Cashion *et al.,* 1994; Kazmierczak, 2004; Pratt *et al.,* 2002). Moreover, releasing exotic phytoseiids sometimes may undergo lack of environmental adaptability and may not get synchronized with the target pest. So, use of indigenous phytoseiids in a locality can give better management of spider mites without disturbing ecosystem and associated fauna of the region (Takafuji and Chant, 1976; Wajnberg *et al.,* 2007). Phytoseiids perform very well above 20^{o}C temperature and above 65% relative humidity but their survival under adverse conditions has been well documented making them suitable candidate to use in biological control programme (Morris *et al.,* 1996). In some cases, low humidity was found as an obstacle in egg hatching and rapid multiplication of some phytoseiid species (Bakker *et al.,* 1993). Therefore, exploration and utilization of indigenous native species can provide more accurate estimate to use it in biological control programmes.

Phytoseiids have been classified into four categories depending on their feeding styles. Members of the genus *Typhlodromus* are considered as belonging to Type I category that is known as specialist predators of spider mites (McMurtry *et al.,* 2013). A review about feeding styles of Phytoseiids was provided indicating their association with the the host (McMurtry and Croft, 1997). Since then, biology of only 15 species has been studied in spite of the fact that total described phytoseiids have reached upto 2763 nominal species from the world (Demite *et al.,* 2016). This suggests that there is a dire need of day that feeding potential of native species should be determined with respect to their localities.

To date, 179 phytoseiid species have been reported from Pakistan in 13 different genera (Honey *et al.,* 2016; Khan *et al.,* 2010; Khan *et al.,* 2011a; Khan *et al.,* 2011b). Balochistan is known for apple production; and prevalence of dry and hot conditions in summer season supports the infestation of spider mites at high density. Using indigenous native phytoseiid species associated with local environment can provide better control for this pest. *Typhlodromus divergentus* (Acari: Phytoseiidae) was recorded originally from Murree Hills, Punjab, Pakistan inhabiting on *Grewia* sp. (Chaudhri *et al.,* 1974). Later, this species was described from *Psidium guajava* and *Thuja orientalis* from India (Dhooria 1982; Dhooria 1983; Gupta, 1985). Here, this species was collected from *Malus domestica* associated with spider mites at Quetta, Pakistan. Stage prey preference of the targeted prey is considered as major factor in determining the fitness and survival of concerned predator under adverse environmental conditions relating to presence or absence of the preferred prey stage (Xiao and Fadamiro 2010; Xu and Enkegaard, 2010). Moreover, selection of prey is mainly dependent on the availability of food, access to desired nutritional requirements from prey and ease in capturing and digestion of the prey (Sabelis, 1985).

In this study, *T. divergentis* is reported to be associated with apple trees in Quetta region. The aim of the present study was to provide basic information about prey stage preference and feeding potential of native *T. divergentis* against two spotted spider mites (TSSM).

MATERIALS AND METHODS

This study was conducted at CABI Bio-control Laboratory (BCL), Directorate of Plant Protection, Agriculture Research Institute (ARI), Quetta, Pakistan during 2016. Experiments were conducted at 27 ± 2^{o}C and 70 ± 5 % RH

Plant source

The grape leaves of Kishmish variety (Iranian origin) used in this study were taken from grape vineyards grown in ARI. Visual inspection of the leaf was used as basic criteria for healthy leaf selection. Leaves were clipped off with scissor and placed in plastic bags for transportation. Collected leaves were brought to BCL for rearing of TSSM and *Typhlodromus divergentis* for further experimentation.

Prey source

Two spotted spider mites were collected from apple trees (cv. Gaja) and grapes plants (Kishmish variety) grown at ARI. For rearing of these mites, water soaked cotton with grape leaf was used as residing media. Adult spider mites were transferred onto grape leaves placed on moist cotton pads (40 × 26 × 3 cm) in small plastic trays (45 × 30 × 6 cm). Distilled water was added in plastic trays to float around cotton pads to keep them moist and preventing mites escape. Grape leaves were observed on daily basis and after their deterioration (mostly 4-5 days), leaves were removed and placed on fresh leaves in newly prepared rearing trays for transfer of mites.

Predator source

Typhlodromus divergentis was collected from apple trees (cv. Red Delicious) at pomology fields of Directorate of Agriculture Research, Quetta. Above mentioned rearing method was also used for rearing stock culture of *T. divergentis* for experimentation. To prevent escape of predatory mites, borders of moist tissue paper with distilled water were provided along margins of the leaves. Rearing trays of predatory mites were observed regularly and provided with spider mites as prey.

Experimental unit

Prey stage preferences of *Typhlodromus divergentis* against *Tetranychus urticae:* The prey stage preferences of all life stages (larvae, nymph, adult) of *Typhlodromus divergentis* were studied on *Tetraychus urticae*. For this purpose, small plastic trays (15 × 10 × 3 cm) were used with water soaked cotton pads (7 × 5 × 2 cm). A small leaf disk (1 cm diameter) of grapes plant was placed on cotton pads. This leaf disk was provided with *T. divergentis* and different combinations of life stages of *T. urticae.* Gravid female of *T. divergentis* was placed on leaf disks in each tray. After deposition of eggs, gravid female was removed with only single egg retaining on leaf disk. On hatching of predatory mites larvae from egg, it was provided with spiders mites in three life stage combinations as (a) eggs immature, (b) eggs + adult and (c) immature + adult; and larvae of *T. divergentis* was taken and observed until adult stage. Each treatment (stage combination) was replicated five times within this experiment. Ratio between predatory mites and prey stages was maintained as 1:5:5 in each replicate. Data were recorded after every 24 hours and number of prey stages were replenished on daily basis after consumption from *T. divergentis.*

Feeding potential of adult *Typhlodromus divergentis* against *Tetranychus urticae:* Experimental methodologies were kept same as practiced in stage preference experiment but feeding potential of adult *T. divergentis* was studied against all stages (egg, immature, adult) of *T. urticae* separately. Each treatment (prey stage) was replicated ten times in this experiment. The predator to prey ratio was maintained as 1:10 in each replicate. Data were recorded on daily basis for five consecutive days.

Data analysis

Data of stage prey preference and feeding potential were subjected to analysis of variance (ANOVA) and all pair-wise comparisons were estimated using Least Significant Difference (LSD) test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Prey stage preference

When all stages of *T. divergentis* (larvae, nymph and adult) were given prey stages of *T. urticae* in combination to evaluate their preference, predator fed on all prey stages but mean consumption rate of the predator stage differed significantly with respect to prey stage in each treatment (Table 1 and Table 2). In case of predator larval stage, significantly low percent consumption was observed against all prey stages. The predator larval stage showed more preference towards prey egg stage (26±3.05 and 32±3.26) when offered in combination with prey immature and adult stages, respectively. However, predator larva showed more preference towards prey immature stage (38±3.59) when offered in combination with prey adult stage (6±3.05). The predator nymphal stage showed more preference to prey immature stage (62±3.99 and 56±1.97) followed by prey egg stage (52±2.04 and 47±3.04) and least preference was given to prey adult stages. The predator adult stage showed significantly high preference towards prey egg stage (90±4.47 and 82±3.59) when offered in combination with prey immature and adult stages, respectively (Fig. 1).

Table 1. Prey stage preference (Mean±SE) of *Typhlodromus divergentis* against *Tetranychus urticae*.

***Typhlodromus divergentis* life stages**	***Tetranychus urticae* life stages (Combined)**					
	T1		**T2**		**T3**	
	Egg	Immature	Egg	Adult	Immature	Adult
Larvae	26 ±3.05B	26±4.26A	32±3.26AB	4±2.66B	38±3.59A	6±3.05B
Nymph	47±3.04B	62±3.99A	52±2.04AB	19±2.45B	56±1.97A	13±1.82B
Adult	90±4.47A	76±7.18A	82±3.59A	44±4.98B	62±5.53B	58±4.66B

Means sharing same letters within a column are not significantly different (α = 0.05, *P* > 0.05)

Table 2. Total number of preys (*Tetranychus urticae*) available and consumed by each life stage of T*yphlodromus divergentis* during prey stage preference experiment.

Predator stage	***Tetranychus urticae***													
	Prey stage available							**Prey stage consumed**						
	T1		**T2**		**T3**			**T1**		**T2**		**T3**		
	Egg	**Immat.**	**Egg**	**Adult**	**Immat.**	**Adult**	**Total**	**Egg**	**Immat.**	**Egg**	**Adult**	**Immat.**	**Adult**	**Total**
Larvae	50	50	50	50	50	50	300	13	13	16	2	19	3	66
Nymph	175	175	175	175	175	175	1050	81	109	91	33	98	23	435
Adult	50	50	50	50	50	50	300	38	32	34	17	31	14	166

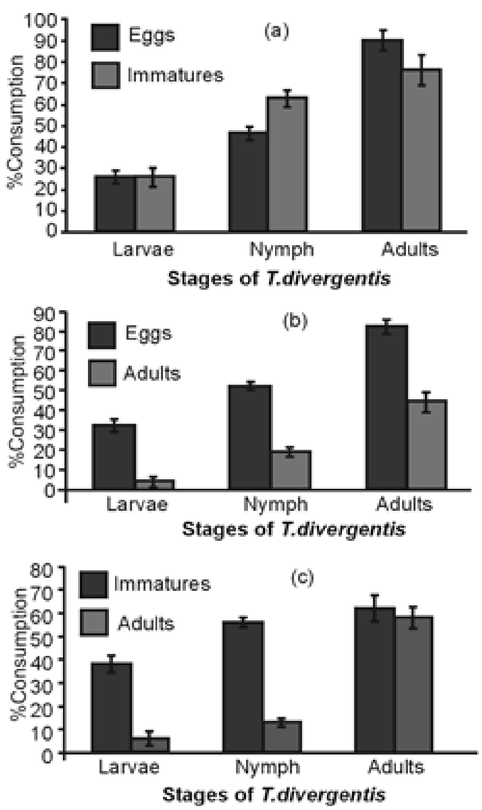

Fig. 1. Prey stage preference of *Typhlodromus divergentis* against *Tetranychus urticae*, (a) Eggs + Immatures; (b) Eggs + Adults; (c) Immatures + Adults

Feeding potential

Feeding potential of adult predator (*T. divergentis*) differed significantly for all prey (*T. urticae*) stages (eggs, immature and adult) when offered separately (Table 3). Comparisons of means for prey egg stage (F= 369; P <0.0001; χ^2= 129), prey immature stage (F= 406; P <0.0001; χ^2= 90.3) and prey adult stage (F= 17.9; P <0.0001; χ^2= 65.6) showed that maximum percent consumption of adult predator was found against prey egg stage followed by prey immature stage. Significantly low percent consumption for prey adult stage was observed in this study (Fig. 2).

Table 2. Feeding potential (%) (Mean±SE) of *T. divergentis* against different life stages of *T. urticae* separately.

Day	***Typhlodromus divergentis***		
	Egg	**Immature**	**Adult**
1	78±7.11A	38±4.89B	4±3.05B
2	47±6.33B	51±4.33AB	3±3B
3	68±8.53A	60±5.16A	4±1.63B
4	75±7.49A	47±5.38AB	17±3.35A
5	82±6.46A	49±4.81AB	22±5.73A
Mean	70±3.56A	49±2.34A	10±1.91A
Day	**Control**		
	Egg	**Immature**	**Adult**
1	0±0A	0±0B	0±0A
2	2±1.33A	0±0B	1±1A
3	0±0A	0±0B	1±1A
4	0±0A	0±0B	3±1.52A
5	2±2A	4±2.21A	3±1.52A
Mean	0.80±0.48B	0.80±0.48A	1.60±0.52B

Means sharing same letters within a column are not significantly different (α = 0.05, *P* > 0.05)

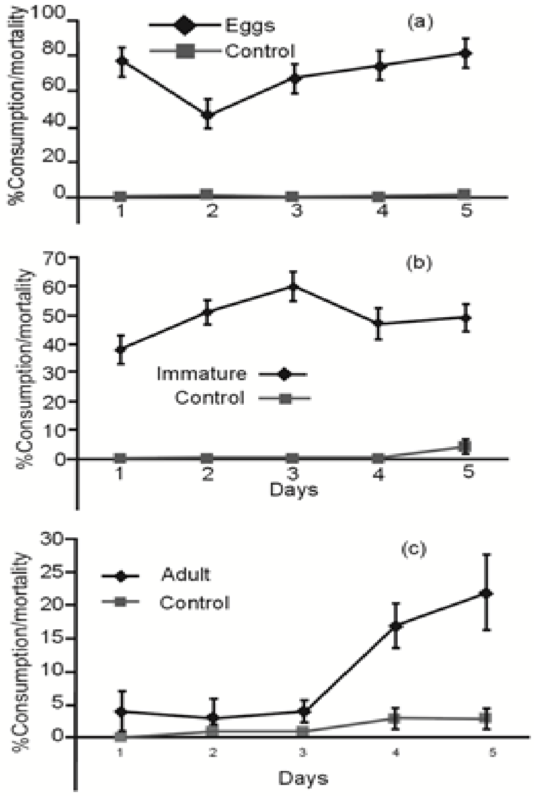

Fig. 2. Feeding potential of *Typhlodoromus divergentis* (adults) against different life stages of Tetranychus urticae (a) Eggs, (b) Immagture, (c) Adult, during five consecutive days

Maximum percent consumption of *T. divergentis* was found on egg stage of *T. urticae* followed by immature stage. This has already been described that specialist predators are known to feed more aggressively on egg stage of *T.urticae* as compared to other stages because of their specialized piercing mouth parts. While generalist predators are known mostly to feed on larval stage because of their inability to pierce the egg corion of spider mites (Blackwood *et al.,* 2001; Blackwood *et al.,* 2004; Kasap, 2010). Further, prey stages preference of *Typhlodromus bagdasarjani* against spider mites has also been described (Moghadasi *et al.,* 2013) stating that egg stage of spider mites was preferred by all stages of *T. bagdasarjani* (protonymph, deutonymph and female) followed by immature prey stage. In the same year (Tsolakis *et al.,* 2013), evaluation was done on the predatory potential of *Typhlodromus laurentii* and *T. rhenanus* against eggs of *Tetranychus urticae* and *Panonychus citri* (citrus spider mites). It was confirmed that eggs of *Tetranychus urticae* were more preferred and consumed by both *Typhlodromus* species. They also stated that consumption of prey eggs showed a positive correlation with biological attributes of predator species. The preference of phytoseiids towards egg stage of *Tetranychus* species has been reported more consistently by many authors (Burnett 1971; Fernando *et al.,* 1980; Ragusa *et al.,* 2009). Same preference towards *Tetranychus* eggs was reported with a more specialized predator, *Phytoseiulus persimilis* (Rovenska *et al.,* 2005). While working with *Phytoseiulus persimilis*, (Fernando *et al.,* 1980; Khalequzzamam *et al.,* 2007) stated that this species has been found to be more inclined towards immature and adult stage of *Tetranychus urticae* but its preference towards egg stage is dependent on prey density. During low prey density, this species was observed feeding on prey immature and adult stages, in case of while when TSSM immature and adult stage highly density of spider mite at *P. persimilis* prefers to feed on egg stage more aggressively. Further, positive effect of eggs consumption on the biological parameters of other phytoseiids has also been reported. It was also described that *Neoseiulus idaeus* showed maximum biological parameters including, longevity, productivity, etc. when fed on eggs of *T. urticae* as compared to other life stages (Collier and Albuquerque, 2007). However, some species of the genus *Euseius* Wainstein and *Neoseiulus* Hughes, which are known as generalist predators of spider mites, were observed also preferring egg stage of spider mites when fed with other stages. So, feeding behavior of a given native species can only be described after experimental outputs under local conditions (Badi *et al.,* 2004; Furuichi *et al.,* 2005). The preference of many phytoseiid predators towards egg stage is considered due to the fact that eggs are immobile and more accessible by the starving predator as compared to active stages of prey.

Different studies report that some members of the genus *Typhlodromus* (*T .rhenanus* and *T. pyri*) have the ability to uptake plant fluid in absence of its natural prey. Later, this behavior was also observed in many species of the phytoseiids in genera *Euseius* and *kampimodromus* (Chant, 1959; Congdon and Tanigoshi,1983; Kreiter *et al.,* 2002; Porres *et al.,* 1975). However, (Adar *et al.,* 2012) presented detailed information about liquid uptake from plant cell by phytoseiids. He pointed out that this behavior of phytoseiids is associated with length of fixed cheliceral digit and species that pierce cell sap has short fixed chelicera digit. Despite of this ability shown by many phytoseiids, economic damage to crops has never been reported even in absence of complete prey host. In some cases, only small feeding scars were reported on apple leaves and fruits caused by *Typhlodromus pyri* without any damage to apple trees (Sengonca *etal.,* 2004).

CONCLUSION

It is concluded that phytoseiids have the ability to regulate population of spider mites. Based on results it is evident that egg stage of spider mites are more preferred by *Typhlodromus divergentis* followed by immature stages of spider mites. So, early releases of *T. divergentis* may be deployed under local conditions to manage these mites below economic injury level.

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