Influence of the Amselfly, *Ischnuraevansi* (Odonata: Coenagriidae) on the Immature Stage of *Culexpipiensmolestus* (Diptera:Culicidae) as Biological Control.

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Abstract

The mosquitoes *Culexpipiens* are annoying pests and obligate vectors of many vertebrate pathogens. Their immature stages are common in fauna of a wide range quality of water bodies. Most of the alternative biocontrol strategies focus on mosquito immature stages. Predators play a major role in mosquito control programs. The naiads of the damselfly,*Ischnuraevansi* (Coenagriidae) naturally inhabitate with the mosquito, *C.pipiensmolestus* immature stages, the large naiads with average size 1.5±0.5cm more than daily predated 12.1 larvae of 1st and 2nd instars, with 0.63 clearance rate, the naiads strongly preferred mosquito larvae over the chironomds*C. ninavah* larvae of the density 20/liter. The starred naiad tends to ingest the egg rafts. But not more than 3 rafts/day . The presence of the predator with 5 individuals/liter extended the life cycle from 13.0 in control to 20.8 days with high significant effect on 3rd and the istars and pupa stage.

Keywords:*Ischnuraevansi* , predators, *Culexpipiensmolestus*,biological control.

الخلاصة

يعد البعوض من المعقد *Culexpipiens*مصدراً للإزعاج وناقلاً اجبارياً للعديد من مسببات الامراض للفقريات. وتكون اطوارها شائعة في نوعيات متباينة من المياه . تركز استراتيجيات المكافحة الحيوية على استعمال المكافحة الحيوية لادوار غير الكاملة للبعوض. تلعب المفترسات الدور الرئيس في برامج مكافحة البعوض. تستوطن الحوريات المائية للرعاش الصغير *Ischnuraevansi* مع الاطوار الغير البالغة للبعوض *C .pipiensmolastus* ، فتفترس الحوريات المائية ذوات الحجم الاكبر 12.1 يرقة من العمرين الاول والثاني يومياً وبمعدل تطهير 0.63. يفضل المفترس يرقات البعوض على يرقات الهاموش، كما تلتهم الحوريات الجائعة قوارب البيض وبمدى لا يتجاوز 3 قوارب يومياً. ان وجود المفترس وبكثافة 5/لتر قد اطال دورة الحياة للاطوار المائية من 13.0 للمجموعة السيطرة الى 20.8 يوماً ، وكان التاثير مضاعفاً على الدورين اليرقيين الثالث والرابع ودور العذراء.

الكلمات المفتاحية: *Ischnuraevansi*، المفترسات,*Culexpipiensmotestus*، المكافحة الحيوية.

Introduction

Besides to the annoyance of the blood-sucking habit, female mosquitoes are vectors of several diseases. Mosquitoes (*Culexpipiens* L., 1785) are important vector for diseases of both wildlife and humans (Durant and Hopkins, 2008).

For control, these mosquito borne diseases can be controlled through friendly strategies to avoid warnings and distractions on the use of chemical applications. Predation is reported as one of the most limiting factors , causing a high level of mortality to immature stages of mosquitoes (Service, 1973). The occurance and abundance of mosquito populations may be associated with the abundance of predators (Zuharon and

Lester, 2010), as an important factor affecting mosquito population (Hechtel and Juliano, 1997).

Predators may have multiple effects on prey, including the mortality caused by consumption, and alter their life history traits in the presence of predators, by extending development time and reducing their number of offspring , as a result of delayed reproduction and a lower fecundity of *C. pipiens* (Fisher *et al.,* 2010). The selection of a place for oviposition requires a set of chemicals, visual, olfactory and tactile clues that interact with the female before laying eggs (Navarr-Silva *et al.,* 2009). To avoid aquatic sites where there is a high predatory risk to their offspring (Spencer *et al.*, 2002). The ability of female to select oviposition sites based on risk of predation to their progeny should be of high selective value for population dynamics and community structure (Blaustein, 1999).The presence of the predator decrease's maturation time and growth rate (Noobury and Nisbet, 2005). Hechtel and Juliano (1999) found the presence of*Toxorhynchitesrutitus* reduce the pupation time of the mosquito *AedesTrieriatus.* A Significance reduction of the mosquito pupation is possible in less than control after natural control with Odonata and *Toxorhynchites* predators (Faidhprais *et al* ., 2014).

The consumption rate of the mosquito predators varied with different stage of the prey (Mohanry, *et al*., 2012).Some larvivorous copepods caused siginificant ,mortality for second instar of *C. pipiens* (Tranchida, *et al*., 2008). The predation capacity of *C. pipiens* by *Cs. Longiareolata* fourth was correlated with prey instar, stage and species (Shaalan, 2012). *Anaxjunius* (Ieshnidae) nymph prefer first instar larvae of the culicids *Culex, Anopheles* and *Aedes*largae(Mary, 2013). Larvae are the most common prey of odonates with size-selection predation (Finck and yanoviak, 1997). Studies of prey electivity of aquatic predators with some evidence, that mosquits larvae with strong positively selected over chirinomids (Saha*et al.,* 2010).

Predators of mosquito eggs deposited on the water surface as in *Culexspp*. were carried out in the field by precipitin test (Survice ., 1977) , but the gut smear of any potential predator would have to be made soon after predation and confirming predation by the precipitin method may be limited (Medlock and Snow, 2008).

Sath and Bhusnar (2010) were found 43 spp. Of odonates feeding on an adult mosquitoes. Hence, that attempt had been made experimently to examine consumptive and non-consumptive effects of naiad *Ischnureevansi*(Coenagriidae) in response to risk predation for prey-predator combination.

Materials and methods

The predator, *Ischnuraevensi*culture

The damselfly naiads were obtained from permanent pond, This pond outdoor artificially burrowed in early February 2013, with the size 3x2x1m and 70 cm water depth. The water resource of the pond comes from seepage, and when declined was completed from tap water. After the pond community was established with phytoplanktons and microinvertebrates especially *Daphnia spp.*and mosquito larvae as the naiad food, The flying damselflies oviposited their eggs on the water surface during early spring 2014. For experimental purposes, the mosquito predaceous naiads were sampled in November and October 2014. First emerged adults were collected and identified in help of Derwesh (1965) surrey and web sites images.

Immature stage of the Dipteran preys

The immature stages of *Culexpipiensmolestus* were obtained from egg rafts, which were collected from the caged mosquito adult stock. The hatched larvae were fed on a mixture of powdered yeast and biscuit, The water was changed every 4 days to avoid a film of yeast forming on the water surface (WHO, 1996). Then, after the adults emergence, they were caged and firstly fed on 15% honey solution for 3 days.The adult females were fed nightly on a naked chest pigeon, oviposition occurred later in beside plastie trays. The photoperiod of the insectarium 12:12 hours, 65% relative humidity and the temperature $27\pm3^{\circ}$C.

For chironomid larvae, The different larval instars were collected through autumn season from open temporary waste water pools and transferred to the laboratory for conducting the non-target consumption prey test.

Predator influence on the life – history

Previously putting 5 naiad predators and one egg raft were but in caged water , the predators were separated by nylon mesh. The predator effect was represented by predator sight, and its kiromones concentration according to their number in the oviposition container. In absence (Control) or presence of the predators; the hatching time, larval instars pupal periods of the mosquito were noticed till the adults emergence.

Statistical analysis

All the experiments and the control were independently repeated two times with three replicates. And statistically analyzed with Dinkins test (SAS ,1982) at 5% of probability level.

Clearance rate

The clearance rate was calculated after modification Gilbert and bums (1999). as fellows:

CR=$\frac{V\ln P}{T}$

CR-Clearance rate of predators (nos. of prey killed in liter / day/ predator);

P- nos. of prey killedof prey killed;

T - Time in days.

V- Volume of water.

The Clearance rate was conducted by leaving the naiad for one day without food and introduced to 30 larvae of the equal numbers of 1^{st} and 2^{nd} mosquito larvae daily for 6 days.

Host-stage preference

Of each of the three sized(1.0, 1.5 and 2.0 cm long) were the 24 hour starved naiad left to feed on collective of 30 individuals of each of the four larval instars and pupae, the consumed prey after 24hours were counted.

Host preference

The non-fed 24 hrs naiad was introduced to liter water volume containing 20 2^{nd} larvae with equal instar numbers of *C.ninevah*and *C.pipiensmolestus.* The prey numbers consumedafter 24hrs were done.

Result and discussion

Prey stage and prey preference

Table 1 showed that, mostly all the naiad sizes of *Ischnuraevansi*predated the first two larval instars of the preys *Culexpipiensmolestus*, that presence was nearly not different between the sizes of the predator and the prey instar, except only the naiad size one less consume the 1^{st} prey instor (4.3) in compression (7.3 and 6.7) with last two other naiad sizes. Also , the lable 1 shows the predator has not tendency to consume third larval instar and never predates fourth instar and pupa stage . This is in contradiction with Mohanrag *et al* (2012) Shaalan (2012).Tranchia *et al* (2008) were copepods more consumed second instar of *C. pipiens* and Mary (2013) found the naiad of *Anaxjunius* preferring first instar of the genera *Culex, Anopheles* and *Aedes*.

The daily predation of the naiads ranged between 11 and 13, and when exposed to the egg rafts only after 3 days of starvation, the predator consumed the egg rafts and that increased with the naiad size (0.7, 1.0 and 2.3 egg rafts) development,This result was noticed under the laboratory condition with hunger suppression, the role of egg rafts predation was not confirmed in filed studies (Service , 1997; Medlock and Snow, 2008).

The naiad was found preferred with about three folds the mosquito larvae over the *Chironmusninavah* larvae. Fig1. Also other studies as Saha *et al* (2010) indicated that aquatic predators strongly preferred mosquit larvae over the chironomds.

Table1 : Consumption preteren of mosquito*Culexpipiensmolestus* immature stagesby damselfly *Ishnuraevansi*during 24hrs.

Prey stage	Naiad size		
	One	**Two**	**Three**
L1	4.3*±0.6	7.3±0.6	6.7±1.2
L2	5.7±0.6	6.7±0.6	5.7±1.2
L3	0.0	0.0	1.0±0.0
L4	0.0	0.0	0.0
P	0.0	0.0	0.0

L 1,2,3 and 4 larval intars. P- pupa. *Standerd deviation

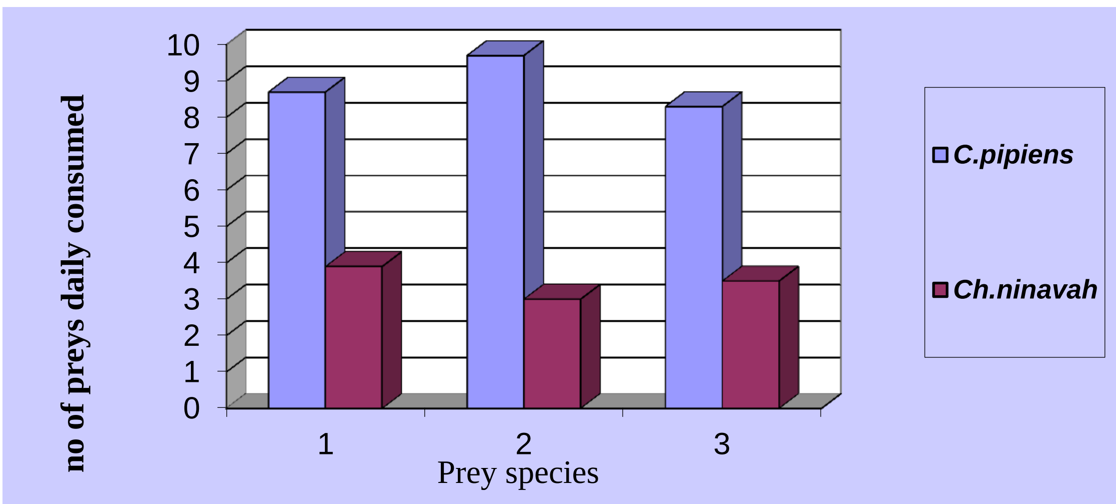

Fig.1: Prey preference of the mosquito, *Culexpipiensmolestus* predator and the alterative *Chironomusninavah*

Effect on prey development

The presence of the naiads with density 5 per liter elongated the prey metamorphosis time from 13 days for control up to 20.8 days for experimental treatment . But this alteration depended on stage and instars of the developed mosquito *C.pipiensmolestus*.However , the incubation period was not.Significantly varied in predators presence , while , the developmental time of the larval instars and pupa increased with more two folds (Table 2).

Table 2 : Effect of the predator presence on life history duration of the mosquito prey.

Developmental stage	Duration time (days)	
	Treated	Control
Incubation period	2.3^{a}	2.0^{a}
1st instar	2.0^{a}	2.0^{a}
2nd instar	4.3^{a}	2.0^{d}
3rd instar	3.9^{ab}	3.0^{c}
4th instar	4.3^{a}	2.0^{d}
Pupa	4.0^{a}	2.0^{d}
Total	20.8	13.0

The presence of aquatic predators is altering the prey life history(Fisher *et al.*, 2010), decreasing maturation time and growth rate (Hechtel and Juliano , 1997; Nooburg and Nisbet, 2005; Faidhpraise*t al.*, 2014).Our results were supported that view through delayed the total life history time of the immature stage and increasing exposure to predatory risk, which means enhancing the predation as biological control.

Predation efficiency

In the laboratory condition, the daily ingestion of the prey was flacuated between the alternative days, withhigh meaningful number (table3), This feeding behavior may be related with food digestion and energy preservation by decreasing the searching time .

Also, the clearance rate as one of parameters of predation effecianey was correlated with the developmental stage, which increased from 0.59 for the naiad size one to 0.69 for size two, and then decreased to 0.67 for size 3, later, the naiad tendencesto besluggish for preparing for emergence and feeding prevention

Table 3: Clearance rate of the aquatic habitat after 6 days of mosquito prey exposure to the predates damselfly *Ischnuraevansi.*

Daysperoid	No. of preys predated by damselfly size		
	One	Two	Three
First	8.3±0.6	13.7±1.6	12.3±0.6
Second	4.0±0.0	10±0.0	7.7±0.6
Third	9.3±0.6	12.3±0.6	11.7±1.5
Fourth	3.7±0.6	7.0±1.0	4.3±0.6
Fifth	8.0±0.0	13.0 ±0.6	13.3±0.6
Sixth	4.7±0.6	6.0±0.0	5.3±0.6

-The prey population represented by the equal nos. of 1st and 2nd instars.

Conclusion

Odonates decreased the number of mosquitoes present and the number that survived to pupation in microhabitats (Finke *et al*., 1997). The damselfly naiad *I.evansi* were only predated 1st and 2nd larval instars and avoided the other ones and pupae but that diet limitation can be substituted by prey egg rafts ingestion. Also, by the extended time of the odonatapredeter presence, the naiad grew large enough to eliminate prey abundance in clear water bodies. From the viewpoint of biological control in natural habitats, The predator effectiveness was evaluated by predation the targeted pest insect, but when consumption-targeted prey as chironomid larvae incase if unavailable the main prey, would preserve the predator existence in the predator niche.

On the basis of the relation between the vector density and its number of annual generations, the life history extension with significant time, from 13 to 20.8 days in predator existence would lead to decrease generations number. This way of control strategy focus on the role of behavior and the kariomonesof the predators as non-consumptive effect on the pest insects.

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