

## Natural host preferences of parasitoid wasps (Hymenoptera: Pteromalidae) on synanthropic flies

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### Abstract

Synanthropic flies are members of order Diptera and considered as medical and veterinary pests. In this study, parasitoid wasps were determined and their natural host preferences in order to select a suitable agent for biological control of flies. The pupae of three species of flies; *Musca domestica*, *Lucilia sericata* and *Sarcophaga haemorrhoidalis* were used as hosts for natural parasitoids. For this issue, as much as 50 pupae of each fly species put in three separate dishes with covered top by a net. These dishes were placed in the field to attract parasitoid wasps. The most parasitic rate was related to *N. vitripennis* (%17.2). Host preferences of *N. vitripennis* on *M. domestica* pupae were higher than observed parasitism on *L. sericata* and *S. haemorrhoidalis*. The emerging rate of two parasitoids; *P. vindemmiae* and *S. nigroaenea* were one per host pupae. According to the result, *N. vitripennis* can be an appropriate candidate for use as natural enemy which expected to be effective in controlling various species of synanthropic flies. Therefore, *S. nigroaenea* was more suitable to biological control of housefly populations.

**Key Words:** Parasitic wasps, biological control, medically important flies, pteromalidae.

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Diptera is a cosmopolitan insect order with more than 150,000 described species in 158 families.<sup>1</sup> Some of the flies have been compromised and adapted on living in human habitats which are common pests and called synanthropic flies.<sup>1</sup> Some of the synanthropic flies are blown flies, house flies and flesh flies, which are capable of the mechanical transmission of various pathogens including viruses, bacteria, fungi and protozoa to human and animals. On the other hand, the larvae of these flies can contaminate human and animal tissues, a condition which calls myiasis disease.<sup>2</sup> Therefore, they are among the major pests of medical and veterinary importance all over the world.<sup>3</sup> Control of flies is one of the important needs of health officials in urban and rural communities, especially in tropical and subtropical areas of the world.<sup>4</sup> High costs and appearing of insecticide resistance can be an example of problems in the use of chemical control of flies. Therefore, biocontrol of flies with the use of Entomopathogenic agents and predators has been considered.<sup>5</sup> There are many researches in biological research in purification of antivenom, isolation of Mycoplasma and the molecular docking and laboratory

analysis.<sup>6-8</sup> International Research Journal of Applied and Basic Sciences Biologic control of the flies using parasitic wasps is an environmental friendly method. Parasitoid wasps are a large group of Apocrita in Order Hymenoptera. Many parasitoid wasps are considered beneficial to humans because of their behavior of natural control of agricultural pests. These natural enemy has been used commercially in biological control of pests as well as flies.<sup>9</sup> Many species of these wasps as important parasitoids and predators have a key role in biological control of pests in nature.<sup>10</sup> Parasitic wasps commonly attack immature stages of flies. These parasitoids lay their eggs in eggs, maggots or pupae of several species of flies. The wasps' larvae feed inside the host and eventually kill it.<sup>11</sup> There are many studies about specific hosts and host preferences of parasitoid<sup>11</sup>. The host preferences of some species/strains parasitoids of Trichogramma and some species of Braconidae, as a parasitoid of fruit fly has been well studied.<sup>12</sup> However, the literature review showed a limited number of studies on host preference of parasitic and parasitoid wasps of synanthropic fly's pupae.<sup>13</sup> Understanding of host

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preference of parasitoid wasps provides basic information for the sustainable integrated biological control of synanthropic flies.<sup>11</sup> This study was conducted to determine native parasitoid wasps and their natural host preferences in order to introduce a suitable agent for biological control of synanthropic flies including *Musca domestica* (Diptera: Muscidae), *Lucilia sericata* (Diptera: Calliphoridae) and *Sarcophaga haemorrhoidalis* (Diptera: Sarcophagidae) in Urmia, North West of Iran.

### Materials and Methods

#### Study site

This study was conducted during June 2017 to September 2017 in West Azerbaijan Province (45° 2' 47.57" N, 37° 31' 46.58" E) in an area of 39,487 km<sup>2</sup> including Lake Urmia in North West of Iran (Figure 1). West Azerbaijan Province is one of the 31 provinces of Iran placed in the northwest of the country, bordering Turkey, Iraq and Azerbaijan's Nakhchivan Autonomous Republic.<sup>10</sup>

#### Flies mass rearing

In this study, three species of flies including *M. domestica*, *L. sericata* and *S. haemorrhoidalis* were reared in the insectary of flies of Urmia University of Medical Sciences. *Lucilia sericata* and *S. haemorrhoidalis* were reared in 40×40×40 cm (length, width and height) in 25±2°C of temperature and 45±5% of relative humidity and 16:8 light/dark regimens in the insectary. Diluted sugar (5%) and palm date were used as food for an adult of these species. Cow meat was used as

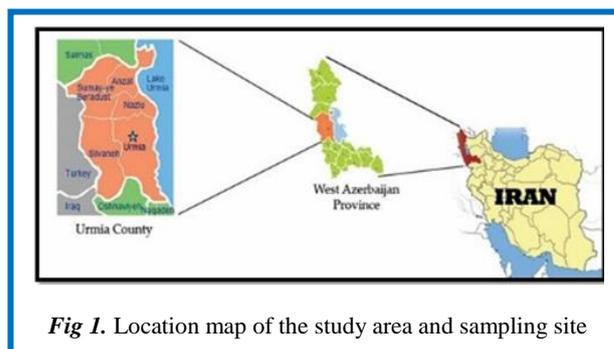


Fig 1. Location map of the study area and sampling site

a media for egg laying and food for their larvae<sup>14</sup>. Cage dimensions were 40×40×40cm (length, width and height) for the rearing of *M. domestica* and the condition of insectary was 25±2°C of temperature and 50 - 70% of relative humidity and 12:12 light/dark regimen. Food regimen for adults of *M. domestica* was made with a combination of sugar, milk powder and yeast at the proportion of 2:1:1. Cow manure was used as a media for egg laying and food for their larvae.<sup>15</sup>

#### Methods for counting of non-emerged pupae and mortality rate due to parasitoids

Pupae of each species put on separated jars with 10 cm in height and 25 cm in diameter that were covered with a net, mesh size 10×10 millimeter. Jars were placed in various places such as cattle keeping sites and somewhere with the presence of animal cadavers and animal manures or places where there are plants of the Pittosporaceae family respecting of four basic conditions

Table 1. The number of Parasitized pupae and non-Parasitized pupae

Date	Species of fly	Number of non-Parasitized pupae		Number of Parasitized pupae by:		
		Adult fly emerged	Adult fly non-emerged	Nasonia vitripennis	Pachycrepoide vindemmiae	Spalangia nigroaenea
23 June 2017	<i>M. domestica</i>	31	7	7	0	5
	<i>L. sericata</i>	42	5	3	0	0
	<i>S. haemorrhoidali</i>	37	8	5	0	0
2 July 2017	<i>M. domestica</i>	22	9	9	0	10
	<i>L. sericata</i>	35	7	8	0	0
	<i>S. haemorrhoidalis</i>	39	7	4	0	0
15 July 2017	<i>M. domestica</i>	23	3	11	4	9
	<i>L. sericata</i>	33	7	6	4	0
	<i>S. haemorrhoidali</i>	36	5	7	2	0
4 Aug. 2017	<i>M. domestica</i>	25	6	6	5	8
	<i>L. sericata</i>	29	7	7	7	0
	<i>S. haemorrhoidali</i>	30	7	5	8	0
20 Aug. 2017	<i>M. domestica</i>	21	7	10	7	5
	<i>L. sericata</i>	25	10	8	7	0
	<i>S. haemorrhoidali</i>	29	9	7	5	0
Total	<i>M. domestica</i>	122	32	43	16	37
	<i>L. sericata</i>	164	36	32	18	0
	<i>S. haemorrhoidalis</i>	171	36	28	15	0

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**Table 2.** Percentage of adult flies emerged, parasitic rate for any species of a parasitoid wasp and percent parasitoid-induced mortality

Species of fly	Adult emerged	PMI	Parasitized pupae by:		
			<i>Nasonia vitripennis</i>	<i>Pachycrepoide vindemmiae</i>	<i>Spalangia nigroaenea</i>
<i>Musca domestica</i> (%)	48.8	12.8	17.2	6.4	14.8
<i>Lucilia sericata</i> (%)	65.6	14.4	12.8	7.2	0
<i>Sarcophaga h.</i> (%)	68.4	14.4	11.2	6.0	0
<b>Total of Parasitized pupae</b>			103	49	37
<b>Total mean parasitized pupae ± SD (%)</b>			13.7 ± 2.5	6.5 ± 4.1	4.9 ± 1.4

PMI: postmortem interval

for successful fly collection.<sup>16,17</sup> These conditions were hosts' environment, spatial situation, acceptability and rules of the hosts.<sup>18</sup> As much as 250 pupae of each species have been put in jars as 5 replicates with 50 pupae in each. These jars were left in the field for 10 days and return to the lab after this duration for counting of non-emerged pupae and mortality rate due to parasitoids.<sup>19</sup>

### Species identification of parasitoids

Species identification of parasitoids has done by some applicable keys and confirmed by Dr. Lotfalizadeh, parasitoid specialist in Agricultural Research Center of East Azerbaijan, Iran.

### Statistical Analysis

Data analysis was conducted using the SPSS 18 version at ( $P \leq 0.01$ ) level. ANOVA has been used for comparing of parasitism rates of three fly species and t-test has been used for the analysis of parasitoids host preference.

### Results

Without considering the hosts' species, as much as 189 out of 750 pupae were parasitized successfully. Parasitism rate of pupae of a whole fly community (all species) was  $25.1 \pm 8.4$  %.  $13.9 \pm 5.4$  % of all of the flies died without emerging of parasitoids.  $61 \pm 7.8$ % of pupae of the whole studied fly community remained safe and adult flies have emerged from them (Table 1). The highest rate of parasitism has observed on pupae of house

flies, *M. domestica*, was  $38.4 \pm 8.1$ %. This rate was significantly higher than the other two species ( $P=0.00$ ) (Tables 1 and 2). Parasitism rate of pupae due to the presence of *N. vitripennis* on all fly species of this study was  $13.7 \pm 2.5$  which was significantly higher than the other species. Parasitic rate of fly pupae due to the presence of two other species, *P. vindemmiae* and *S. nigroaenea* were  $6.5 \pm 4.1$  % and  $4.9 \pm 1.4$ , respectively) (Tables 2 and 3). There wasn't significant difference among three species of studied flies in parasitism with *N. vitripennis* ( $P=0.00$ ). The parasitism of three fly species; *M. domestica*, *L. sericata* and *S. haemorrhoidalis* with this wasp were  $17.2 \pm 3.7$ ,  $12.8 \pm 3.2$  and  $11.2 \pm 3.1$  respectively. Despite host specific behavior of *S. nigroaenea* in this study in parasitizing of pupae of the house fly, there wasn't a significant difference in parasitizing of house fly pupae among three parasitic wasps of this study ( $p=0.00$ ) (Table 4). *P. vindemmiae* was not a host-specific behavior and also, the parasitism rate of pupae of three fly species was low in this study with this wasp ( $P=0.00$ ). Parasitism rate for pupae of house fly was higher than two other studied flies in this study and pupae of this species has been parasitized with all three wasp species. It may show that higher range of natural enemies of *M. domestica*. Rate of dead pupae due to parasitism for *L. sericata* and *S. haemorrhoidalis* was 14.4% and for *M. domestica* was 12.8%. In this study, Multiparasitism has not been seen in the presence of more than one parasitoid species in one pupae and also,

**Table 3.** Average parasitoid wasp emerges from fly's pupae, Urmia, Iran 2017

Species of fly	<i>Nasonia vitripennis</i>						<i>P. vindemmiae</i>						<i>S. nigroaenea</i>					
	23 June	2 July	15 July	4 Aug.	20 Aug.	Average	23 June	2 July	15 July	4 Aug.	20 Aug.	Average	23 June	2 July	15 July	4 Aug.	20 Aug.	Average
<i>Musca domestica</i>	8	7	10	11	7	8.9	1	1	1	1	1	1	1	1	1	1	1	1
<i>Lucilia sericat</i>	9	5	10	8	6	8	1	1	1	1	1	1	0	0	0	0	0	0
<i>Sarcophaga h.</i>	6	6	8	10	8	8	1	1	1	1	1	1	0	0	0	0	0	0

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**Table 4.** ANOVA statistical analysis of the parasitism ratio for any species wasps

			Sum of Squares	Df <sup>1</sup>	Mean Square	F statistic	P value
<b>Nasonia * Species of fly</b>	Between Groups	(Combined)	24.133	2	12.067	3.481	.064
	Within Groups		41.600	12	3.467		
	Total		65.733	14			
<b>Pachycrepoideus * Species of fly</b>	Between Groups	(Combined)	.933	2	.467	.041	.960
	Within Groups		136.000	12	11.33		
	Total		136.933	14			
<b>Spalangia * Species of fly</b>	Between Groups	(Combined)	182.533	2	91.26	51.660	.000
	Within Groups		21.200	12	1.767		
	Total		203.733	14			
<b>Non Parasitized Adult fly emerged * Species of fly</b>	Between Groups	(Combined)	280.933	2	140.46	5.494	.020
	Within Groups		306.800	12	25.567		
	Total		587.733	14			
<b>Non Parasitized Adult fly non emerged * Species of fly</b>	Between Groups	(Combined)	2.133	2	1.067	.314	.737
	Within Groups		40.800	12	3.400		
	Total		42.933	14			

1. Df: Degrees of Freedom

Superparasitism has been seen in the presence of more than one wasp of same parasitoid species in one pupae in 5 parasitized pupae of *M. domestica* with *N.vitripennis*. The highest number of superparasitism in this study was emerging of 11 individual of *N.vitripennis* in one pupae of *M. domestica*.

### Discussion

In this study, natural parasitism and control of the population of dominant synanthropic fly species in North West of Iran, *M. domestica*, *L. sericata*, *S. haemorrhoidalis* with three species of parasitoid wasps including *P. vindemmiae*, *N. vitripennis* and *S. nigroaenea* has been confirmed. These species belong to the Pteromalidae family which has several parasitoid species.<sup>11</sup> These parasitoid species have been reported from Iran previously,<sup>12</sup> but host preference of them has studied for the first time in this study. Host preference behavior of *S. nigroaenea* for parasitizing of *M. domestica* and *Stomoxys calcitrans* was investigated by Romero et al. (2010) as 21% and 24.8% respectively.<sup>20</sup> Olbrikh and King (2003) showed that the parasitism of

pupae of house fly with *S. nigroaenea* was about 72% and clearly higher than other parasitoids.<sup>21</sup> Cornell & Pimentel (1978) proved that *N. vitripennis* has a higher level of host preference on pupae of *M. domestica* in comparison with *L. sericata*. They also showed that if this wasp species had been reared on one host, it will show higher host preference behavior in comparison with other species.<sup>22</sup> Dominant parasitoid species of pupae of synanthropic flies is *N. vitripennis*. Despite a high rate of parasitism by this wasp, it hasn't obvious host preference behavior and it parasitizes pupae of all studied flies at almost the same level. This wide host range of this wasp has been seen in other parts of the world.<sup>23</sup> For this reason, *N. vitripennis* is available commercially for biological control of flies of medical and veterinary important in some parts of the world.<sup>24</sup> The highest number of wasps which may appear in the phenomenon of superparasitism in pupae of house fly was 11 in this study. In a similar study on pupae of Sarcophagidae flies in Tehran, capital of Iran, the highest number of parasitic wasp, *N. vitripennis*, which has been emerged from one pupae was 16 individuals.<sup>25</sup> It has been shown that

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members of family Sarcophagidae are among dominant species of flies in various parts of Iran.<sup>26</sup> Despite low parasitism rate of fly pupae due to *S. nigroaenea*, this species showed a unique host preference behavior on *M. domestica*. However, this wasp species is weaker than *N. vitripennis* in parasitizing of *M. domestica*. Another study in Marand, West Azerbaijan introduced wide variety of hosts for this wasp species. Non-specific host selection behavior has been reported for this wasp species.<sup>27</sup> However, other studies proved that parasitism of house flies, stable flies and horn flies mostly done by this wasp species.<sup>20</sup> The wasp species *P. vindemmiae* has a low rate of parasitism and it hasn't also host preference behavior. Other studies in Brazil proved wide host range of fly families of Muscidae, Calliphoridae and Sarcophagidae.<sup>28</sup> Presence and effective activity of medically important fly pupae can make a detrimental impact on mass rearing of beneficial flies such as *L. sericata* which is recently being under mass rearing for preparing sterile larvae for maggot therapy. In addition with nice experiences on diets and biology of *L. sericata* in laboratory,<sup>29</sup> preventing of attacks by natural enemies such as parasitic wasps is very important.

Host-specific behavior of parasitic wasps is one of the best characters for introducing a suitable natural enemy for fly control but searching behavior of wasp and finding hosts are important in biological control which the flies should have it. In this study, according to low host preference of *N. vitripennis* and wide range of parasitizing of flies, high rate of parasitism of this wasp is a positive point in behavior of this wasp which is notable for use it in biological control programs. The parasitoid wasp *S. nigroaenea* had the highest level of parasitism and host preference on pupae of *M. domestica*. Due to this behavior, it can be a good suggestion for biological control of housefly in aviculture farms and cattle keeping sites and so on.

### Authors contributions

MK, HS, OD, KA, and ER equally participated in experimental design, data collection, writing and revision of the manuscript.

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### Conflict of Interest

The authors declare that they have no conflict of interest.

### Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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