# Short Communications

*Pakistan J. Zool., vol. 47(1), pp. 269-273, 2015.*

## Pollen Analysis and Antimicrobial Properties of Honey Samples Sold in Western Turkey

**Menderes Çenet,[1] Sevil Toroğlu,[2] Dilek Keskin[3] and Fuat Bozok[1]**

[1]*Department of Biology, Science and Art Faculty, Osmaniye Korkut Ata University, 80000 Osmaniye, Turkey*

[2]*Department of Biology, Science and Art Faculty, Kahramanmaraş Sütçü İmam University, 46100 Kahramanmaraş, Turkey*

[3]*Cine Vocational High School, Adnan Menderes University, 09500, Cıne-Aydın, Turkey*

**Abstract.-** Antimicrobial activity and pollen analysis of honey from Mugla Province showed 5 to 29 different types of pollens of which *Zea mays*, *Styrax officinalis* and *Trifolium* sp. were dominant pollen taxa. Seven multifloral honey and three unifloral honey samples were identified in ten honey samples. The honey samples H09 (Püren) and H10 (Harnup) were more effective antimicrobial against *Baccilus megaterium* with 49 mm inhibition zones. Sample H01 (Keven) showed the best antibacterial activity against *B.subtilis* with 39 mm inhibition zones. H08 (Sedir) showed the largest inhibition zone to *Candida albicans* with 49mm. It can be reported that pollen of Püren, Harnup, Keven and Sedir honey samples showed the most antimicrobial activities.

**Keywords**: Melitopalynology, pollen analysis, honey, antimicrobial activity, Muğla

Honey is an important food for human health and nutrition. It is commonly used as a public therapeutic substance in the world due to its antibacterial, antifungal, antiviral, antioxidant, antimutagenic and cholinergic effects (Yurtsever and Sorkun , 2002).

The taste, smell and color of honey vary with nectar of flowers. Pollen analyses of floral honeys reveal the plant taxa, which is a source for honey. Melitopalynology, the microscopic analysis of honey sediment, was the first method used for determining the botanical and geographical origin of honey. Ertug (2002) reported that the most common families were Lamiaceae, Asteraceae, Cupressaceae, Liliaceae ve Rosaceae in Bodrum region, small part of Mugla.

The first pollen analysis of Turkish honey samples was done in Melitopalinology studies date from 1980s (Sorkun and Inceoglu, 1984). Studies in different parts of Turkey identified flowering plants containing nectar through pollen analyses in honey samples (Silici and Gokceoglu, 2007; Taskin, 2006; Mercan *et al*., 2007).

The aim of this study was to investigate antimicrobial activity against common human pathogens and compare it with pollen spectrum of ten honey samples obtained from Muğla in Turkey.

*Materials and methods*

In this study, ten honey samples branded and unbranded were purchased from Mugla: H01, Keven Balı**,** Blossom (multifloral); H02, Çiçek Balı, Blossom (multifloral); H03, Kekik Balı, Blossom (unifloral); H04, Narenciye Sedir, Blossom (multifloral); H05, Çambalı 1, Blossom (unifloral); H06, Çambalı 2, Blossom (multifloral); H07, Yaylaçambalı 3, Blossom (multifloral); H08, Sedir balı, Blossom (multifloral); H09, Püren, Blossom (multifloral); H10, Harnup, Blossom (unifloral). Solutions of honey for testing were handled aseptically, and were protected from bright light to prevent photodegradation of the glucose oxidase that gives rise to hydrogen peroxide in honey. The honey solutions were prepared just before inoculation to ensure that there was no loss of hydrogen peroxide in the solutions used to test total activity.

Antimicrobial activity of honey samples was determined by the agar well diffusion method (Collins *et al*., 1995). The bacteria and fungi culture suspensions were prepared and adjusted by comparing against 0.4–0.5 McFarland turbidity standard tubes. Nutrient agar (NA) and Saboraud Dextrose Agar (SDA) (15 mL) were poured into each sterile petri dish (10-100mm diameter) after injecting cultures (0,1mL) of bacteria and yeast and pouring medium in petri dishes homogeneously.

* Corresponding author: dkeskin@adu.edu.tr
0030-9923/2015/0001-0269 $ 8.00/0

Honey samples were kept in benmari (40-45°C) for melting, mixed for homogenization, and then 10.1 ml filled into wells of agar plates with a pore size of 10 mm. Plates injected with the fungi were incubated at 28°C for 48 h, and the bacteria were incubated at 37°C for 24 h. The inhibition zones formed on the medium were measured in mm.

The bacteria (*Klebsiella pneumoniae* 13883, *Bacillus megaterium* DSM 32, *Pseudomonas aeroginosa* 9027, *Bacillus subtilis* IMG 22**,** *Enterobacter cloaca* ATCC 13047, *Escherichia coli* ATCC 8739, *Corynebocterium xerosis* ATCC 373, *Staphylococcus aureus* 6538). were obtained from stock culture collection of the Biology Department of KSU Science and Art Faculty. Cultures of these bacteria were grown in Nutrient Broth (NB) (Difco) at 37±0.1°C for 24 h. Cultures of fungi (*Candida albicans* and *Rhodotorula rubra*) were grown in Sabouraud Dextrose Broth (SDB) (Difco) at 25±0.1°C for 24 h.

Pollen analysis was done according to Louveaux *et al.* (1978). The crystalized and hardened 300 g honey samples were kept in benmari (65-70°C) for melting, mixed for homogenization, and then, 10 g was transferred into centrifuge tubes with 20 mL distilled water incubated at 45°C in a water bath and continuously shaken to dissolve honey in water. The mixture was centrifuged at 6000–6500 rpm, upper water phase was discarded and pre-prepared glycerol–gelatin mixture (1:1.5) was added to the tubes. These were transferred to a slide, covered with coverslip a and analyzed by using Olympus CX21 light microscope. The pollen types were identified at the generic and/or species level (Aytug *et al*., 1971; Pehlivan., 1995). The pollen types present in the honey samples were counted and classified according to their percentages: *viz.*, dominant pollen (more than 45% of the total pollen grains counted), secondary pollen (from 16 to 44%), important minor pollen (from 3 to 15%), and rare pollen (less than 3%). Generally, blossom honey is considered to be from one source if the pollen frequency of that plant is more than 45% (Louveaux *et al.*, 1978).

*Results and discussion*

According to pollen analyses, dominant pollen species belonged to *Zea mays* L. (62%), *Styrax officinalis* L. (45%), *Trifolium* L. (46 %) in HO3, HO5, HO10, respectively. Pollen of *Rosaceae*, *Astragalus* sp., *Trifolium* sp., *Cistus* sp. *Centaurea* sp., *Castanea sativa, Calluna vulgaris, Erica* sp., *Citrus* sp., *Papaver* sp., *Myrtus communis* were comparatively less in quantity (Table I). *Salvia* sp., *Styrax officinalis*, *Carduus* sp., *Centaurea* sp., *Trifolium* sp. were the most prevalent in the majority of the stations. Species such as *Trifolium* sp. have also been frequently observed (Andrada *et al*., 1998).

In an earlier study, pollens of *Trifolium* sp. and *Zea mays* were found to be dominant in honey samples sold in İzmir which is similar to our results. In contrast to reports of dominant pollen species of *Papaver* sp. in Sivas, *Erica* sp. in Afyon and Muğla, *Centaurea* sp. in Afyon (Mercan *et al*., 2007). We have reported less quantity of these species in our samples.

Also Kaya and his friends (2005) showed that pollen of *Trifolium* sp. were dominat in Yozgat honey samples, while pollen of *Trifolium* sp. were less in Çankırı, Balıkesir and Rize, those of *Erica* sp. were less in samples from Kırklareli, Bolu and Muğla and *Astragalus* sp., from Elazığ and *Centaurea* sp. from Çankırı. While pollen of *Castanea sativa* were less in our study, they were dominant in samples from Bartın (Erdoğan *et al.*, 2006), Adapazari (Sorkun *et al.*, 1989) and in Rize.

There were other reports from different regions of Turkey for presence of pollens in honey samples. Silici (2004) reported that pollen of *Castanea sativa*, *Erica* sp., and *Centaurea* sp. were dominant in honey samples sold in Bursa,

Silici and Gökceoglu (2007) reported that pollen of *Trifolium* sp., *Citrus* sp., *Astragalus* sp. were proportionately less in twenty five honey samples from Antalya province. In the same study, also pollen of *Myrtus communis* were proportion of minor. Taskın (2006) reported that pollen of *Centaurea* sp. and Ericaceae were dominant, whereas these of *Trifolium* sp. were proportionately less in honey samples sold in Burdur province.

The antimicrobial activity of the honey samples was tested *in vitro* by using the agar –well diffusion method against eight bacteria and two fungi (Table II). The results showed that the honey samples (H09 (Püren) and H10 (Harnup)) inhibited

**Table I.- Pollen analysis of honey samples sold in Muğla province (Western Turkey)**

| Honey sample No. | Originate name | | Pollen Spectrum and Percentage |
|---|---|---|---|
| **H01** | Keven Balı | * | |
| | | ** | Rosaceae *3* |
| | | *** | *Trifolium 7, Olea europeae 7 , Centaurea 7, Ligustrum 7,* |
| | | **** | *Cousinia, Carduus, Taraxacum, Cichorium intybus, Senecio, Lathyrus, Crateagus monogyna, Conium, Heracleum, Raphanus, Salvia, Zea mays, Acer, Quercus, Geranium, Betula, Chenopodium, Elaeagnus* |
| **H02** | Çiçek Balı | * | |
| | | ** | *Astragalus 22, Trifolium 19* |
| | | *** | *Cistus 11, Acer 7, Lathyrus 6, Alnus 5, Citrus 5, Vicia 4, Rubus 3* |
| | | **** | *Centaurea, Cousinia, Taraxacum, Carduus, Cichorium intybus, Bellis, Senecio, Helianthus, Rosa, Olea europeae, Ligustrum, Quercus, Salvia, Ziziphora, Zea mays, Acer Onosma, Silene, Veronica* |
| **H03** | Kekik Balı | * | *Zea mays 62* |
| | | ** | |
| | | *** | *Trifolium 14, Leucojum 14,Liquidambar orientalis 6, Ferula 4* |
| | | **** | |
| **H04** | Narenciye Sedir | * | |
| | | ** | *Trifolium 25, Cistus 23* |
| | | *** | *Acer 12, Olea europeae 11, Styrax officinalis 9, Centaurea 4, Salvia 3, Rosaceae 3* |
| | | **** | *Bellis, Carduus, Cousinia, Vicia, Lathyrus, Ferula, Quercus, Geranium, Pinus, Elaeagnus* |
| **H05** | Çambalı 1 | * | *Styrax officinalis 45* |
| | | ** | *Castanea sativa 26, Centaurea 16* |
| | | *** | |
| | | **** | *Cousinia , Taraxacum, Carduus, Astragalus, Medicago, Populus, Juglans, Conium, Cupressus sempervirens, Ornithogalum, Ligustrum, Raphanus, Salvia, Ziziphora, Zea mays, Quercus, Gossypium hirsutum, Elaeagnus* |
| **H06** | Çambalı 2 | * | |
| | | ** | *Calluna vulgaris 43, Eleagnus 16* |
| | | *** | *Crateagus monogyna 13, Erica 8, Salvia 4, Populus 4* |
| | | **** | *Populus, Astragalus, Cistus, Solanaceae, Podocarpus, Quercus, Geranium* |
| **H07** | Yaylaçam balı 3 | * | |
| | | ** | *Erica 28* |
| | | *** | *Astragalus 15, Erica 14, Styrax officinalis 8, Cistus 5, Olea europea 4* |
| | | **** | *Elaeagnus, Chenopodium, Quercus, Acer, Juglans Salvia, Populus, Ligustrum, Cupressus sempervirens, Rosaceae, Vicia cracca, Bellis, Taraxacum, Centaurea* |
| **H08** | Sedir | * | |
| | | ** | *Papaver 39, Castanea sativa 17* |
| | | *** | *Trifolium 7, Cistus 7, Salvia 5, Crateagus monogyna 4, Quercus 4* |
| | | **** | *Centaurea, Carduus, Astragalus, Vicia, Styrax officinalis, Alnus, Olea europeae* |
| **H09** | Püren | * | |
| | | ** | *Calluna vulgaris 32, Myrtus communis 21* |
| | | *** | *Erica 14, Populus 10, Brassica 4, Alnus 4* |
| | | **** | *Centaurea, Cousinia, Carduus, Senecio, Trifolium, Astragalus, Vicia, Melilotus, Styrax officinalis, Convolvulus, Linaria, Juniperus, , Salvia, Olea europeae, Ajuga, Quercus* |
| **H10** | Harnup | * | *Trifolium 46* |
| | | ** | |
| | | *** | *Robinia pseudoacacia 9, Erica 8, Styrax officinalis 6, Acer 6, Solanaceae 6, Populus 4, Salvia 3,* |
| | | **** | *Taraxacum, Carduus, Crupina, Cichorium intybus, Bellis, Juniperus, Cupressu sempervirenss, Rosaceae, Conium, Holcus, Roemeria, Juglans, Ligustrum, Raphanus, Zea mays, Elaeagnus* |

[* Dominant pollen (45%), * * Secondary pollen (16–44%), * * * Minor pollen (3–15%), * * * * Rare pollen (3%)]

**Table II.- Antimicrobial activity of honey samples sold in Muğla by the agar well diffusion assay (0.1mL/10mm well)**

| Microorganisms | Inhibition zone (mm)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **H01** | **H02** | **H03** | **H04** | **H05** | **H06** | **H07** | **H08** | **H09** | **H10** |
| *K. pneumoniae* 13883 | 21 | 18 | 18 | 19 | 19 | 17 | 21 | 29 | 18 | 23 |
| *B. megaterium* DSM 32 | 25 | 22 | 16 | 33 | 20 | 23 | 29 | 23 | 49 | 49 |
| *P. aeruginosa* 9027 | 19 | 21 | 22 | 19 | 19 | 24 | 17 | 23 | 35 | 19 |
| *B. subtilis* IMG 22 | 39 | 19 | 19 | 21 | 21 | 22 | 23 | 35 | 27 | 32 |
| *E. coli* ATCC 8739 | 21 | 24 | 17 | 25 | 22 | 25 | 21 | 17 | 19 | 33 |
| *E. cloaca* ATCC 13047 | 29 | 19 | 11 | 18 | 15 | 15 | 22 | 20 | 23 | 24 |
| *C. xerosis* ATCC 373 | 33 | 22 | 21 | 29 | 30 | 20 | 25 | 39 | 25 | 29 |
| *S. aureus* 6538 | 25 | 21 | 20 | 19 | 27 | 17 | 19 | 23 | 29 | 29 |
| *C. albicans* 30114 | 27 | 23 | 23 | 27 | 22 | 23 | 25 | 49 | 25 | 31 |
| *R. rubra* 116 | 22 | 20 | 17 | 21 | 20 | 21 | 21 | 22 | 19 | 19 |

*Zone of inhibition, including the diameter of the agar well (10mm); mean value of three independent experiments.

growth of *B.megaterium* with 49mm inhibition zones. In addition, honey sample H01(Keven) showed the best antibacterial activity against *B.subtilis* with inhibition zone at 39 mm. H08 (Sedir) showed the largest inhibition zone to *C.albicans* with 49 mm inhibition zone.

Earlier reports indicated that different floral honey samples and plant species had good inhibitory activity against *B.megaterium*, *B.subtilis* and *C.albicans* (Küçük *et al*., 2007; Ifra and Sheikh., 2009; Khalil *et al*., 2001; Mercan *et al*., 2007; Toroglu and Cenet, 2013). Therefore, our findings are in agreement with these previous reports.

The pollen composition of the honeys can provide important information on the flora of that region. Since pollen composition was directly related to the plants on which the honeybees fed (Mercan *et al*., 2007).

Presence of accessory antimicrobial components such as flavonoids and aromatic acids has been known to contribute to antimicrobial activity of honey (Floris and Prota, 1989). Antimicrobial activity of honeys can also be significantly influenced by the origin (Bogdanov, 1989). Besides that pollen can also be a good source of flavonoid glycosides.

Conclusion, there are differences in the antimicrobial activities of different unifloral and multifloral honey samples. Especially Püren and Harnup have been proven to be the highest antibacterial activity against *B. megaterium.* Also, Keven honey samples showed the antibacterial activity against *B. subtilis*. Moreover, activity against candida was observed best in sedir honey samples among the studied samples. It can be suggested that Püren, Harnup, Keven and Sedir honey samples show more antimicrobial activity than other honey samples.

## *References*


Andrada, A., Vale, A., Aramayo, E., Lamberto, S. and Cantamuto, M., 1998. *Investig. Agr.Prod. Protecc. Veget*., **13**: 265–275,.

Aytug, B., Aykut, S., Merev, N. and Edis, G., 1971. *Pollen atlas of plants belong to Istanbul district*. Publications of Forestry Faculty, İstanbul, p. 330.

Bogdanov, S., 1989. *J. Apicul. Res.*, **28:** 55-57.

Collins, C.H., Lyne, P.M. and Grange, J.M., 1995. *Collins and Lyne's Microbiological methods*, 7th Edn, Butterworth/Heinemann, Oxford, p. 493.

Erdogan, N., Pehlivan, S. and Dogan, C., 2006. *Mellıfera,* **6**: 20-27.

Ertug, F., 2002. *Medicinal plants used for traditional medicine in Bodrum distrinct*. 76-93.14. Herbal Pharmaceutical Raw Materials Conference Proceedings, 29-31 May.

Florıs, I. and Prota, R., 1989. *Apicol. Mod.*, **80:** 55-67.

Ifra, G. and Sheikh, S.A., 2009. *Pak. J. Bot*., **41:** 461-466.

Kaya, Z., Bınzet, R. and Orcan, N., 2005. *Apiacta*, **40**: 10-15.

Khalıl, M.I., Abdul, M., Anısuzzaman, A.S.M., Sathi, Z.S., Hye, M.A. and Shahjanan, M., 2001. *J. Med. Sci*., **1:** 385-388.

Kucuk, M., Kolaylı, S., Karaoglu, S., Ulusoy, E., Baltacı, C. and Candan, F., 2007. *Fd. Chem.*, **100**, 526-34.

Louveaux, J., Maurızo, A. and Vorwohl, G., 1978. *Bee World,* **59**: 139-157.

Mercan, N., Guvensen, A., Celık, A. and Katırcıoglu, H., 2007.

*Nat. Product Res.*, **21:** 187–195.

Pehlıvan, S., 1995. Allergen Pollen atlas of plants belong to Turkey. *Ünal Ofset*, Ankaras, pp. 10-120.

Sılıcı, S. and Gokceoglu, M., 2007. *Grana*, **46:** 57–64.

Sılıcı, S., 2004. *Mellifera*, **4:** 44-50.

Sorkun, K. and İnceoglu, O., 1984. *Nat. Sci. J.*, **8:** 337-381.

Sorkun, K., Guner, A. and Vural, M., 1989. *Turk. J. Bot.*, **13:**547-554.

Taskin, D., 2006. *Pollen analyses of honey with Burdur*. Thesis of Master Degree, Isparta, The Institute of Science, Burdur, Suleyman Demırel Unıversity.

Toroglu, S. and Cenet, M., 2013. *Pakistan J. Zool.*, **45**: 475-482.

Yurtsever, N. and Sorkun, K., 2002. *Uludag Bee J.*, **3:**8-31.

*(Received 16 May 2013, revised 13 November 2014)*

*Pakistan J. Zool., vol. 47(1), pp. 273-276, 2015.*

# Role of *Aedes* and *Culex* in Dissemination of Dengue Virus

**Syed Shakeel Shah[1], Azhar Maqbool[1], Shakila Zaman[3], Muhammad Idrees[4], Aftab Ahmad Anjum[2], Sabila Afzal[1], Zareen Fatima[4], Irum Amin[4] and Nisar Ahmad[1]**

*[1]Department of Parasitology, University of Veterinary and Animal Sciences, Lahore.*
*[12]Department of Microbiology, University of Veterinary and Animal Sciences, Lahore.*
*[3]Lahore Medical and Dental College.*
*[4]Division of Virology, Centre for Applied Molecular Biology, Lahore.*

**Abstract.-** Dengue virus has now become a serious threat to humans. As yet, no vaccine and proper treatment is available for dengue virus, so control of vector is the only option left to control dengue infection. *Aedes* mosquitoes are well known vectors for this virus but some studies show that dengue virus can also survive in *Culex* mosquitoes. A total of 370 adult *Aedes* (n=166) and *Culex* (n=204) mosquitoes were collected from selected localities of Urban Lahore. Total five pools of mosquitoes were found positive for dengue serotype2. There is need to conduct detailed studies to establish the vectorial role of *Culex* for dengue virus.

**Key words:** Dengue virus, *Culex, Aedes.*

* Corresponding author: pak_shah@hotmail.com

During the last few decades dengue virus infection has become a serious health hazard. It is considered that more than two-fifth population of world is at the risk of dengue infection, mostly those living in the tropics and sub-tropics (WHO, 2011). The vector of dengue, unfortunately, has become resistant to different chemicals (Ranson *et al*., 2008). To establish a strategic control for mosquitoes, the biology and ecology of vectors must be well known (Sangaralingam *et al*., 2011). Female mosquito in a few number can cause an outbreak in an over populated area (Chia-Hsien and Wen, 2011). The dengue virus is likely to extend due to urbanization and intensive migration of people and expanded infestation of *Aedes* mosquito, which is the principle vector of dengue infection (Gubler, 1998). *Culex* mosquitoes are also known to carry different flaviviruses which are transferred to humans (Bolling *et al*., 2011; Hoshino *et al*., 2007), but there is no report of their involvement in dengue. *Aedes* prefer to breed indoor or peridomestic areas near humans but *Culex* mosquitoes can also breed far away from human population.

Four serotypes of dengue virus are known which cause dengue fever (DF), more complicated dengue haemorrhagic fever (DHF) and in some cases dengue shock syndrome (DSS) (Tao *et al*., 2012). Dengue virus can survive in their hosts because of diversity and can adapt to new environment easily (new host or vector) (Forrester *et al*., 2012). Usually dengue control programmes are initiated when dengue cases are reported but this is too late as dengue virus once introduced into a population by mosquitoes, then it becomes very difficult to control it (Rohani *et al*., 2011).

This study was designed to check the vectorial role of *Culex* mosquitoes by comparing it with *Aedes* mosquitoes.

## *Materials and methods*

Adult *Aedes* and *Culex* mosquitoes were collected from urban Lahore. These collections were made from six towns of Lahore *i.e.* Wagha Town, Iqbal Town, Ravi Town, Cantt, Gulberg, Samanabad Town and Aziz Bhatti Town. A total of 370 adult mosquitoes were collected, out of which

204 were *Culex* and 166 were *Aedes* mosquitoes. The adult resting *Aedes* and *Culex* mosquitoes were collected from walls and closets by aspirator (Zayed *et al*., 2012) and by knock down spraying from selected localities of Urban Lahore. For this purpose different houses were selected randomly and visited weekly for collection. Indoor (in rooms in houses) and outdoor (in front and backyards) collection was performed. Mosquitoes were collected in the jar or bottle and were transported to the Department of Parasitology, UVAS.

Mosquitoes after identification according to the key described in Florida Manual for Mosquito Identification were excised into head-thoraces with the help of needle (Zayed *et al*., 2012). These headthoraces were pooled as 30 pools each with 1-19 mosquitoes and then stored at -70°C till further use (Roiz *et al*., 2012).

Viral RNA was extracted from these 30 samples using Favorgen Total RNA Extraction Kit (Fatima *et al*., 2011) with slight modifications.

The viral RNA was used for synthesis of cDNA, and amphification of *C.PrM* gene.

For cDNA preparation, 5 µl of extracted RNA was used with a PCR mixture of 5 µl containing 2 µl 5X first strand buffer (FSB), 0.25 µl 0.1 M dithiothreitol, 1 µl 10 mM dNTPs, 0.5 µl 20 pM anti-sense primer (reverse primer D2) and 0.75 µl $dH_2O$ with 0.2 µl RNase inhibitor (RNI) (Fermentas) and 0.5 µl of Moloney murine leukemia virus (MMLV) *reverse transcriptase (RTase)* (Invitrogen Biotechnologies, USA). The 10 µl total mixture was placed in the incubator at 37°C for 50 min then 2 min at 95°C and 2 min at 22°C.

*C-PrM* gene was amplified from cDNA by nested polymerase chain reaction. For this 2 µl of cDNA was added in 8 µl of PCR mixture having 1 µl 10X PCR buffer (with ammonium sulphate), 1.2 µl $MgCl_2$, 0.5 µl 500 µM dNTPs, 0.5 µl 20 pM forward and reverse primer each, 3.5 µl distilled water and 1 µl of 2U *Taq*-polymerase.

The thermal profile for first round using outer sense D1-D and anti-sense D2-D was initial denaturation at 94°C for 2 min followed by 30 cycles each of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, extension at 72°C for 1 min and final extension at 72°C for 10 min. The products of first round were frozen overnight and then thawed next day and agitated for mixing. The thermal profile for second round using the type-specific sense and anti-sense primers was the same as for the thermal profile of first round, only the annealing was carried out at 54°C for 45 seconds in 30 cycles. Type specific primers were used in combination in mix-1, contains type specific 2 and 3 primers, and mix-2 contains type specific 1 and 4 primers. The sequences of primers used are described in Table I. The PCR product of nested PCR was visualized by 2 % agarose gel stained with ethidium bromide (0.5µg/ml) (Méndez *et al*., 2011). The band was excised from gel with sterile razor and put into 1.5 ml collection tube. DNA was extracted from PCR band using Vivantis Nucleic Acid Extraction Kit (Vivantis Technologies Malaysia).

For nucleotide sequencing 12 µl of formamide was added to the reprecipitated DNA, heat shocked at 95°C for 5 min and put on ice immediately. It was then handed over to the Core DNA facility of CAMB for sequence analysis.

Clone was sequenced using Bigdye chain termination kit as per manufacturer's instructions (Big Dye Deoxy Terminators; Applied Biosystems, Weiterstadt, Germany).

*Results and discussion*

Out of 370 mosquitoes, 5 were found positive for dengue serotype-2. Out of 5 positive samples pools, 2 had *Aedes*, while the other 3 had *Culex*. Dengue serotype-1, serotype-3 and serotype-4 were not found in any of the pools.

The result showed that a total of 16.67% mosquitoes (both *Aedes* and *Culex)* were positive for dengue virus. Out of these 15 pools for *Aedes*, only 2 pools were found positive for dengue virus *i.e.* 13.33%. Only DENV-2 was detected from both pools. Out of 15 pools for *Culex* only 3 pools *i.e.* 20% were found positive for DEN-2.

Accession number of sequence assigned by the GenBank was BankIt1635423 DENV2 KF186663**.** The size of PCR product was 403 base pair (bp) same as for dengue serotype 2. The homology of the sequence was determined by blast search (Basic Local Alignment Search Tool), at http://www.ncbi.nlm.nih.gov/BLAST. The sequence of nucleotides of amplified gene of dengue virus

**Table I.- Primers used to amplify C-prM gene junction of dengue virus (Fatima *et al.*, 2011).**

| Primer Name | 5'-3' Sequence | Size of amplified product in base pairs | Use in PCR round |
|---|---|---|---|
| D1-D | TCAATATGCTGAAACGCGWGAGAAACCG | 511 bp | Ist Round |
| D2-D | TTGCACCARCARTCWATGTCTTCWGGYTC | | |
| TS1-F | AGGACCCATGAAATTGGTGA | 411 bp | IInd Round |
| TS1-R | ACGTCATCTGGTTCCGTCTC | | |
| TS2-F | AGAGAAACCGCGTGTCAACT | 403 bp | IInd Round |
| TS2-R | ATGGCCATGAGGGTACACAT | | |
| TS3-F | ACCGTGTGTCAACTGGATCA | 453 bp | IInd Round |
| TS3-R | CAGTAATGAGGGGGCATTTG | | |
| TS4-F | CCTCAAGGGTTGGTGAAGAG | 401 bp | IInd Round |
| TS4-R | CCTCACACATTTCACCCAAGT | | |

showed 93% homology with sequence reported with accession number JQ390282.1**.** Expasy translate tool was used to translate the nucleotide sequence.

Of the four serotypes of dengue virus, only two serotypes *i.e.* DENV-2 and DENV-3 have been reported from Pakistan (Fatima *et al*., 2011). In this study, we were able to isolate only one serotype *i.e.* DENV-2 from mosquitoes.

The results of this study show that the chances of any outbreak in study area are very less, though the presence of dengue virus in its vectors can cause outbreak any time. So there is a need to control the vectors before re-emergence of any outbreak. For this purpose breeding sites of mosquitoes must be destroyed. People should be educated and made aware of the breeding sites (Mahilum *et al*., 2005).

## *Conclusion*

The current study shows that dengue virus is circulating in the study area. Though, numbers of positive pool are limited but it could increase if vector population remains uncontrolled. More studies should be conducted for better understanding of vectorial role of *Culex* for dengue virus.

## *Conflict of interest declaration*

We declare that we have no conflict of interest.

## *References*


Bolling, B.G., Eisen, L., Moore, C.G. and Blair, C.D., 2011. *Am. J. trop. Med. Hyg.*, **85**: 169–177.

Chia-Hsien, L. and Tzai-Hung, W. 2011. *Int. J. environ. Res. Publ. Hlth*, **8**: 2798-2815.

Fatima, Z., Idress, M., Bajwa, M.A., Tahir, Z., Obaid, U., ZIA, M.Q., Hussain, A., Akram, M., Bushra, K., Afzal, S., Munir, S., Saleem, S., Rauff, B., Badar, S., Naudhani, M., Butt, S., Aftab, M., Ali, L. and Ali, M., 2011. *BMC Microbiol.*, **11**:200.

Forrester, N.L., Guerbois, M., Seymour, R.L., Spratt, H. and Weaver, S.C., 2012. *PLoS Pathog.*, **8**: e1002897. doi:10.1371/journal.ppat.1002897.

Gubler, D.J., 1998. *Clin. Microbiol. Rev.*, **11**: 480–496.

Hosgino, K., Isawa, H., Tsuda, Y., Yano, K., Sasaki, T., Yuda, M., Takasaki, T., Kobayashi, M. and Sawabe, K., 2007. *Virology*, **359**: 405 – 414.

Kamimura, K., Takasu, T., Ahmed, A. and Ahmed, A., 1986. *J. Pak. med. Assoc.*, **36:** 182-188.

Mahilum, M.M., Ludwig, M., Madon, M.B. and Becker, N., 2005. *J. Vector Ecol.*, **30:** 277-283.

Preechaporn, W., Jaroensutasinee, M. and Jaroensutasinee, K., 2006. *Dengue Bull.*, **30:** 204-213.

Ranson, H., Burhani, J., Lumjuan, N. and Black, W.C. 2010. *Trop IKA net. J.*, **1**:

Rohani, A., Zamree, I., Joseph, R.T. and Lee, H.L. 2008. *Southeast Asian J. trop. Med. Publ. Hlth.*, **3:** 813-816.

Roiz, D., Ana, V., Fausta, R., Daniele, A., Matteo, G., Laureano, C., Esperanza, P.P., Mari, P.S., Antonio, T. and Annapaola, R., 2012. *Parasit. Vect.*, **5**:223.

Sangaralingam, D., Muthuladchumy, V., Pavilupillai, J.J., Parakrama, K. and Sinnathamby, N.S., 2011. *Trop. med. Hlth.*, **39**: 47-52.

Suleman, M., Arshad, M. and Khan, K. 1996. *J. med. Ent.*, **33:** 689-693.

Tao, J., Xue-Dong, Y., Wen-Xin, H., Wei-Ze, Z., Man, Y., Yong-Qiang, D., Shun-Ya, Z., E-DE, Q., Jian, W. and Cheng-Feng, Q.F.Z., 2012. *Virology J.*, **9**:125.

Tariq, R.M. and Zafar, S.M.N., 2000. *Pak. J. Ent. Karachi*, **15:** 7-10.

Whitehead, S.S., Blaney, J.E., Durbin, A.P. and Murphy, B.R. 2007. *Nature Rev. Microbiol.*, **5**: 518–528.

World Health Organization: http://www.who.int/csr/disease/dengue/en. Accessed on 2011 Apr 20.

Zayed, A., Abdullah, A.A., Mohammed, A.E., AL-Mohamdi, H.A., Al-Salwai, M., Al-Jasari, A., Iman, M., Morales-Betoulle, M.E. and Mnzava, A., 2012. *Acta Trop.* **123**: 62-66.

*(Received 3 July 2013, revised 23 October 2014)*

*Pakistan J. Zool., vol. 47(1), pp. 276-279, 2015.*

# Mild Form of Peste des Petits Ruminants Virus (PPRV) in Pakistan

**Riasat Wasee Ullah,[1]* Aamer Bin Zahur,[1] Asma Latif,[1,2] Javid Iqbal Dasti,[2] Rabab Zahra[2] and Saeed-ul-Hassan Khan[3]**

*[1]Animal Health Research Laboratories, Animal Sciences Institute, National Agricultural Research Centre, Islamabad, Pakistan*
*[2]Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan*
*[3]Department of Animal Sciences, Quaid-i-Azam University, Islamabad, Pakistan*

**Abstract.-** An outbreak of Peste Des Petits Ruminants (PPR) in suburban area of Rawalpindi District of Punjab province Pakistan has been investigated. A total of 38 clinically affected animals out of 140 goats and sheep 10-18 months old with no history of PPR vaccination. Nasal and ocular swabs were analysed by RT-PCR for the presence of PPRV specific genome and their sera were analysed for PPR antibodies by competitive ELISA. Eight out of 10 swab samples were found positive for PPRV and all sera were positive for PPRV specific antibodies. It is thus speculated that a comparatively mild strain of PPR virus exists in the population.

**Key Words:** Mild PPR, small ruminants, serology, RT-PCR, c-ELISA.

**P**este des Petits Ruminants (PPR) is a highly contagious and fatal disease of domestic and wild small ruminants. The disease is caused by a *Morbillivirus* of the family *Paramyxoviridae* (Kwiatek *et al*., 2007). Over the last three decades the disease has been reported in Africa, the Arabian Peninsula, most of the Middle East countries, Pakistan, India and Afghanistan (Banyard *et al*., 2010). In recent years outbreaks of PPR has been reported from European part of Turkey (Ozkul *et al*., 2002), Morocco (Banyard *et al*., 2010), Tunisia (Ayari-Fakhfakh *et al*., 2011) and Algeria (De Nardi *et al*., 2012). The virus mainly affects small ruminants and the disease is more severe in goats than in sheep and is rapidly fatal in young animals. The morbidity rate can reach up to 100% and in severe outbreaks mortality may go up to 90% (Zahur *et al*., 2008; Ullah *et al*., 2014). In milder outbreaks mortality rate may not exceed 50% (Abu-Elzein *et al*., 1990). PPR is a transboundary animal disease. According to World Organization for Animal Health (OIE) the disease is one of the major notifiable diseases of small ruminants due to its increasing economic impact, food security and effects the poor farmers livelihoods (Zahur *et al*., 2011).

The clinical signs associated with the disease are high fever (106°F), nasal and ocular discharges, erosive lesions in the mouth, diarrhoea and pneumonia (Roeder and Obi, 1999). The routes of transmission of PPRV are through oral and respiratory secretions following close contact between infected and susceptible population (Lefevre and Diallo, 1990).

At present, the disease is playing havoc with goat population in Pakistan. Small ruminant keepers face great economic losses due to PPR. The disease is endemic in Pakistan and was first reported in 1991 (Athar *et al*., 1995). Since then several outbreaks of PPR have been reported all over the country (Hussain *et al*., 2003; Amjad *et al*., 1996; Ullah *et al*., 2014).

Initially tissue culture rinderpest vaccine (TCRV) was used for cross protection against PPR. However, with the declaration of eradication of rinderpest from the globe as well as from Pakistan, TCRV can no longer be used. At present a homologous PPR vaccine (Nigeria 75/1) is available in the country and is released to livestock departments on request at the face of an outbreak.

* Corresponding author : riasatwasee252@yahoo.com

This communication reports an outbreak of PPR in Rawalpindi region of Pakistan and evidence of mild form of PPRV circulation in the country.

*Materials and methods*

The village Sal Khethar on Murree Express way in suburban area of Rawalpindi district was visited in November, 2013, where the outbreak occurred. The affected flock was examined on the same day. According to the history given by the owner and local livestock assistant of the veterinary department, the disease started eight days ago.

The animals were raised on a semi extensive system *i.e.* the animals were taken out for grazing in the morning and supplemented with a concentrate stall feeding in the evening for fattening. The flock consisted of 140 goats with age ranging between 10-18 months. The flock had history of introduction of five new animals from a nearby livestock market.

*Clinical and epidemiological observations*

The affected animals (n=38) were examined for the presence of clinical signs specific to PPR. Their body temperatures and clinical findings were recorded on prescribed Proforma. The outbreak control measures were implemented and symptomatic therapeutic interventions were advised to the farmer.

Structured epidemiological investigations were conducted to determine the most probable source of PPR virus transmission. Information including previous history of disease at the farm, vaccination status of the flock, flock size, number of animals affected, number of dead animals and history of a PPR outbreak in the nearby farm/area were recorded on a prescribed proforma.

*Sample collection and processing*

Ocular swabs were collected by inserting a sterile swab (BD sterile swab) beneath the conjunctiva and swirling it so that the ocular secretions may adhere to the swab. The oral and nasal swabs were collected by inserting a sterile swab (BD sterile swab) deep into the oral and nasal cavity and swirling it so that the oral and nasal secretions may adhere to the swab.

Ocular and nasal swabs were put into sterile eppendorf tubes containing 1.5 ml of sterile 0.01 M BPS, pH 7.4 and completely squeezed. The solution was centrifuged at 10,000 rpm for 3-5 min at 4°C. Supernatant was collected and stored at -70°C till further analysis.

Blood samples were collected by jugular vein puncture in sterile vacutainers (BD Vacutainer®). Sera were harvested in 1.5 ml sterile cryovials (Nelgene) and stored at -70°C till further analysis.

*RNA extraction and RT-PCR*

Total RNA was extracted from 140µl of homogenate of tissue/faecal material infected with PPRV using RNeasy kit (Qiagen GmbH, Hilden, Germany). A negative control was also included for detection of possible contamination during extraction. The extraction of RNA was performed according to manufacturer instructions. The extracted RNA was placed at -20°C until further used (Balamurugan *et al*., 2012).

The extracted RNA was amplified using PPRV specific primers based on Nucleoprotein (N) gene (Couacy-Hymann *et al*., 2002) and QIAGEN one step RT-PCR kit in a 9902 thermal cycler (Applied Biosystems, Courtaboeuf, France).

| | | | |
|---|---|---|---|
| NP3 | Forward | 5'TCTCGGAAATCGCCTCACAGACTG3' | 1232–1255 |
| NP4 | Reverse | 5'CCTCCTCCTGGTCCTCCAGAATCT 3' | 1583–1560 |

*Competitive enzyme linked immunosorbant assay (c-ELISA)*

The sera samples collected from clinically recovered animals were analyzed for the presence of PPRV specific antibodies. An anti-nucleocapsid (N) monoclonal antibody (MAb) based c-ELISA was used for this purpose (Choi, 2005). The c-ELISA Kit was manufactured by Biological Diagnostic Supplies Limited (BDSL) with the association of Flow Laboratories and Institute for Animal Health, Pirbright, Surrey, United Kingdom. The standardized protocol, reagents and test procedure manual were supplied along with the kit.

*Results*

None of the animals had a history of vaccination against PPR. There was no outbreak of PPR in the nearby area/village. The morbidity rate was 27.14% where the animals were found affected. The clinical examination of the affected animals revealed high fever ranging between 104–107°F,

conjunctivitis, mucopurulant nasal and discharges along with depression, anorexia, swollen lips, cough and mild diarrhoea.

For laboratory confirmation of PPR a total of ten swabs samples were collected, of which 8 were found positive for PPRV genome by RT-PCR.

All 15 sera samples were found positive for PPRV antibodies by c-ELISA.

*Discussion*

It is generally assumed that on the basis of severity of disease and high mortality, several strains of PPRV may co-circulate in the susceptible population. The parameters that determine virulence of a strain could be gauged by high rise of temperature (Pyrexia), conjunctivitis, development of mouth lesions and diarrhoea along with significantly high mortality rates. It has already been demonstrated that PPRV can be attenuated after serial passages on VERO cells under laboratory conditions (Adu *et al*., 1990). In the case of rinderpest, a closely related morbillivirus, the virulence can be modified and reduced by propagating it in an atypical host (embryonated egg, rabbit, goats, cell culture). Moreover, it is evident that the virulence of rinderpest virus diminishes with time in the areas where it is endemic (FAO, 1999).

Mild strains of rinderpest virus have been isolated from animals showing mouth lesions but absence of diarrhoea which causes zero mortality both in East African Zebu and East African grade cattle, sheep and goats were found susceptible to parental inoculation of this virus and develop pyrexia unaccompanied by any other sign (Robson *et al*., 1959). This indicated that field selection pressure could produce a mild but transmissible strain of PPRV.

It has been proved that PPRV can cross species barrier and infect cattle, buffaloes, camels and wild small ruminants (Khan *et al*., 2008; Khalafalla *et al*., 2010). It regularly infects in contact bovines with small ruminants which undergo a silent non transmissible infection (FAO, 1999).

Presence of PPRV was confirmed by indirect fluorescent antibody test, haemagglutination test and isolation of virus. Roger *et al*. (2001) reported receptivity of camels for PPRV and subsequent sero-conversion in Ethiopia. Khalafalla *et al*. (2010) reported a PPR outbreak in camels in Sudan. In Pakistan PPR has also been reported in Mouflon sheep (*Ovis orinetalis*) (OIE, 2000) and in Sindh Ibex (*Capra aegagrus blythi*) (Abubakar *et al*., 2011). Hence the involvement of unnatural hosts in the selection of attenuated variants cannot be ruled out. Moreover, in the light of Global Rinderpest Eradication Campaign (GREP) it can be argued that both for rinderpest and PPRV a low level of field virulence is a normal situation and that high level of field virulence represent the aberrant situation (FAO, 1999). It has been reported that PPR has now established as an acutely endemic infection in Pakistan (Zahur *et al*., 2008, 2014).

During the above mentioned outbreak the parameters which drew the attention towards the involvement of mild strain of PPR were high morbidity, absence of mouth lesions and zero mortality. Otherwise, rest of the clinical signs associated with PPR including high fever, mucopurulent, oculo-nasal discharges and diarrhoea were common features. Moreover, the fact that the animals vaccinated last year remained unaffected and the serology strengthened this inference. It was further reported that no clinical sign was recorded in sheep kept with the sick goats in the same premises under one roof. However, sheep showed sero-conversion to PPRV. It is generally believed that goats react more severely to PPRV exposure and exhibit striking clinical signs, whereas sheep acquire milder form of disease. Our findings are in agreement with those of Hussain *et al*. (2003). However, the clinical signs of PPR resemble FMD and CCPP making the differential diagnosis difficult. FMD was ruled out due to the absence of lesions in the mouth and hoof, moreover, the presence of diarrhoea ruled out both FMD and CCPP (Shahzad *et al*., 2012)

Based upon the information collected during the previous PPR outbreaks and present data, it seems that PPR has attained the level of endemic level in the said area. It is thus speculated that a comparatively milder strain of PPRV exists. However, further studies involving isolation of such strains and experimentally producing the disease will clarify the scenario. Such strains may be

responsible for a number of unnoticeable transboundary events.

*Acknowledgements*

Authors want to thank Assistant Disease Investigation Officer, Rawalpindi, Livestock & Dairy Development Department Punjab for his helping attitude towards outbreak reporting and logistic support.

*References*

Abu-Elzein, E., Hassanien, M., Al-Afaleq, A., ABD-Elhadi, M. and Housawi, F., 1990. *Vet. Rec.*, **127**: 309-310.

Abubakar, M., Rajput, Z.I., Arshed, M. J., Sarwar, G. and Ali, Q., 2011. *Trop. Anim. Hlth. Prod.*, **43**: 745-747.

Adu, F., Joannis, T., Nwosuh, E. and Abegunde, A., 1990. *Rev. levag. méd. vét. Trop.*, **43**: 23-36.

Amjad, H., Forsyth, M., Barrett, T. and Rossiter, P., 1996. *Vet. Rec.*, **139:** 118-119.

Athar, M., Muhammad, G., Azim, F. and Shakoor, A., 1995. *Pak. Vet. J.*, **15**:140-140.

Ayari-Fakhfakh, E., Ghram, A., Bouattour, A., Larbi, I., Gribâa-Dridi, L., Kwiatek, O., Bouloy, M., Libeau, G., Albina, E. and Cêtre-Sossah, C., 2011. *Vet. J.*, **187**: 402-404.

Balamurugan, V., Sen, A., Venkatesan, G., Yadav, V., Bhanot, V., Bhanuprakash, V. and Singh, R. K., 2012. *Virol. Sin.*, **27**: 1-9.

Banyard, A. C., Parida, S., Batten, C., Oura, C., Kwiatek, O. and Libeau, G., 2010. *J. Gen. Virol.*, **91**: 2885-2897.

Branagan, D. J. H., 1965. *Bull. Epiz. Dis. Afr.*, **13**: 225-246.

Choi, K.-S., Nah, J.-J., Ki, Y.-J., Kang, S.-Y. and Jo, N.-I., 2005. *Clin. Diagn. Lab. Immunol.*, **12**: 542-547.

Couacy-Hymann, E., Roger, F., Hurard, C., Guillou, J., Libeau, G. and Diallo, A., 2002. *J. Virol. Meth.*, **100**: 17-25.

De Nardi, M., Lamin Saleh, S., Batten, C., Oura, C., Di Nardo, A. and ROSSI, D., 2012. *Transb. Emerg Dis.*, **59**: 214-222.

FAO., 1999. *FAO Anim. Prod. Hlth. Pap.*, **144**:79-104.

Hussain, M., Muneer, R., Jahangir, M., AWAN, A., Khokhar, M., Zahur, A., Zulfiqar, M. and Hussain, A., 2003. *OnLine J. biol. Sci.*, **3**: 1-7.

Khalafalla, A. I., Saeed, I. K., Ali, Y. H., Abdurrahman, M. B., Kwiatek, O., Libeau, G., Obeida, A. A. and Abbas, Z., 2010. *Acta Trop.*, **116**: 161-165.

Khan, H.A., Siddique, M., Abubakar, M. and Ashraf, M., 2008. *Trop. Anim. Hlth. Prod.*, **40**: 521-527.

Kwiatek, O., Minet, C., Grillet, C., Hurard, C., Carlsson, E., Karimov, B., Albina, E., Diallo, A. and Libeau, G., 2007. *J. Comp. Pathol.*, **136**: 111-119.

Lefevre, P. C. and Diallo, A., 1990. *Rev. Sci. Tech. Off. Int. Epiz.*, **9**: 951-965.

OIE., 2000. *Manual of standards for diagnostic tests and vaccines, volume* **4,** pp. 114-122. OIE, Paris.

Ozkul, A., Akca, Y., Alkan, F., Barrett, T., Karaoglu, T., Dagalp, S. B., Anderson, J., Yesilbag, K., Cokcaliskan, C. and Gencay, A., 2002. *Emerg. Infect. Dis.*, **8**: 708-712.

Robson, J., Arnold, R., Plowright, W. and Scott, G., 1959. *Bull. Epiz. Dis. Afr.*, **7**: 97-102.

Roeder, P. and Obi, T., 1999. *Recognizing peste des petits ruminants: a field manual*. Food and Agriculture Organization of the United Nations, Rome.

Roger, F., Guebre Yesus, M., Libeau, G., Diallo, A., Yigezu, L. and Yilma, T., 2001. *Rev. Méd. Vét.*, **152**: 265-268.

Shahzad, W., Munir, R., Khan, M.S., Ahmad, M.U.D., Khan, M.A., Ijaz, M., Shakil, M., Iqbal, M. and Ahmad, R., 2012. *Pakistan J. Zool.*, **44**: 559-568

Ullah, R.W., Latif, A., Irshad, H., Zahur, A.B., Samo, M.H. and Khan, S.A., 2014. *Res. J. Vet. Pract.*, **2** (IS): 8-10.

Zahur, A., Irshad, H., Hussain, M., Ullah, A., Jahangir, M., Qasim Khan, M. and Sabir Farooq, M., 2008. *Rev. - Off. Int. Epizoot.*, **27**: 877-884.

Zahur, A., Ullah, A., Hussain, M., Irshad, H., Hameed, A., Jahangir, M. and Farooq, M., 2011. *Prev. Vet. Med.*, **102**: 87-92.

Zahur, A.B., Ullah, A., Irshad, H., Latif, A., Ullah, R.W., Jahangir, M., Afzal, M., Khan, S.A. and Salaria, S.M., 2014. *J. Biosci. Med.*, **2**: 18-26.

*(Received 6 March 2014, revised 4 November 2014)*

*Pakistan J. Zool., vol. 47(1), pp. 280-282, 2015.*

# Susceptibility of Adult *Bactocera dorsalis* (Diptera: Tephritidae) to Commonly Used Chemical Insecticides

**Rabia Yaqoob,[1] Hafiz Muhammad Tahir,*[1] Sajida Naseem,[1] Kafeel Ahmed[1] and Atif Yaqub[2]**
*[1]Department of Biological Sciences, University of Sargodha, Sargodha, Punjab, Pakistan.*
*[2]Department of Zoology, Government College University, Lahore*

**Abstract.-** In the present study, susceptibility of field-collected adult *Bactocera dorsalis* to the insecticides, malathion, trichlorofon and λ-cyhalothrin, at field-recommended use rates was investigated in the laboratory. Adult fly mortality was assessed at pre-determined post-treatment time intervals. The exposed *B. dorsalis* population was found to be susceptible to all tested insecticides. There was no difference in mortality among male and female flies exposed to malathion or trichlorofon; however, λ-cyhalothrin-treated males died, in significantly less time, than the females. The calculated $LD_{50}$ and $LT_{50}$ values suggested that malathion was more toxic than trichlorofon and λ-cyhalothrin. The toxicity data suggests that the tested insecticides are effective against *B. dorsalis* in the field-collected sites and could be economically used in the field for population management of the fruit fly.

**Keywords:** Malathion, trichlorofon, λ-cyhalothrin, *Bactocera dorsalis.*

Infestation of fruit flies (Diptera: Tephritidae) is a major constraint to commercial and subsistence farming in the Asian countries (Stonehouse *et al.,* 1998). These infestations can cause economic losses of over 200 million US dollars per annum (Latif, 2004). Fruit flies affect the yield as well as quality of fruits. Infested fruits usually drop from the tree (Christenson and Foote, 1960), making them unexportable due to quarantine restrictions (Hollingsworth *et al.,* 1997).

* Corresponding author: hafiztahirpk1@yahoo.com

The fruit fly genus, *Bactrocera,* includes 40 species that are pests and damage agricultural crops, vegetables, and fruits (Garcia, 2009). Among these, the oriental fruit fly, *Bactocera dorsalis* (Hendel), infests more than 100 host plants, including citrus, mango, and guava (Hui and Liu, 2005); banana and jamun (Kapoor, 2005); plum, papaya and a wide variety of other agriculture commodities, such as coffee, chili peppers, and watermelon (Steck, 2003). For population management of *B. dorsalis,* there is an acute need to use environmentally compatible strategies that are eco-friendly and do not impart resistance development in pest populations or other related problems. In Pakistan, chemical insecticides (different chemistry and mode of action) are employed for the management of fruit flies (Ahmad *et al.,* 2005; El-Aw *et al.,* 2008). Long term and extensive use of insecticides of the same chemistry and mode of action are liable to cause, not only resistance among the target insect pests but also can be stressful for the economy of the country. Resistance against many insecticides, for example, DDT, methoxychlore, malathion, bifenthrin, trichlorfon, λ-cyhalothrin, and sipnosad, has been documented in many tephritid pests (Ahmad *et al.,* 2010; Magana *et al.,* 2007). In Pakistan, several researchers have reported resistance of *B. dorsalis* and *B. zonata* against a variety of insecticides, such as diptrex (trichlorofon), malathion (Haider *et al.,* 2011); bifenthrin and λ-cyhalothrin (Ahmad *et al.,* 2010). *B. dorsalis* colonies have shown a rather high level of resistance against the organophosphate insecticide, fenitrothion (Hsu *et al.,* 2004 a, b). In the present study, susceptibility of field-collected adult *B. dorsalis* to malathion, trichlorofon and λ-cyhalothrin was investigated. These insecticides are commonly used in the study area against insect pests of agricultural importance.

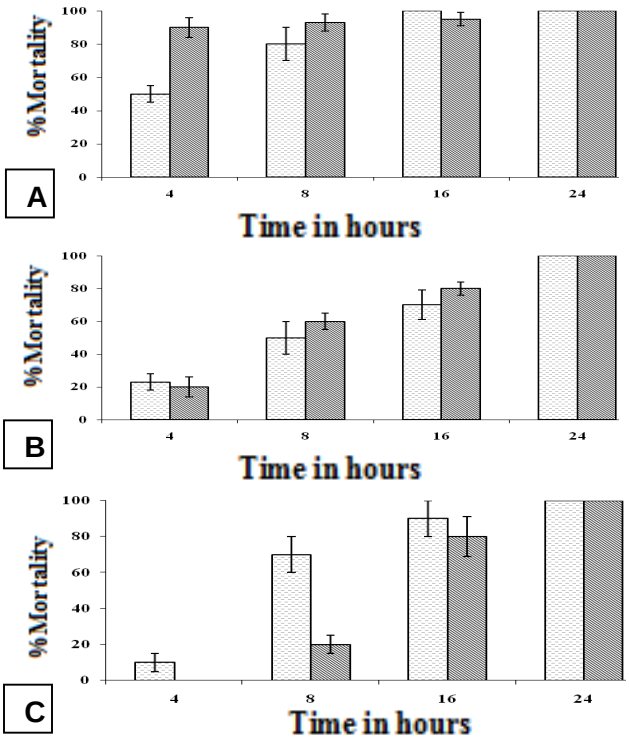
## *Materials and methods*

Adult *B. dorsalis* (fruit fly) were collected from guava orchards of Mitha Masoom, Sargodha District., located 3-4 km away from Sargodha city, on Sargodha to Faisalabad road. For the study, susceptibility of *B. dorsalis* was evaluated at field recommended doses of three commonly used insecticides, malathion (2.28 mg/ml), trichlorfon

(0.8 mg/ml), and λ-cyhalothrin (1.5 mg/ml). To test each insecticide, adult *B. dorsalis* (*n* = 20; 10 male and 10 female) were exposed to insecticide-impregnated filter papers, for 1 hour and then transferred to a clean plastic jar ( 45 cm wide and 40 cm high). Three small pieces of cotton, soaked in mixture of sugar, yeast and water at a ratio of 4: 1: 5, were placed in the jar for nourishment of insecticide-exposed insects, while parallel-run control insects (*n* = 20) were exposed to water impregnated filter papers. Fisher's Exact test was used to compare adult mortalities at discrete pre-determined intervals. Adult mortality was assessed at 4, 8, 16, and 24 hours post exposure to the insecticides and the. $LD_{50}$ and $LT_{50}$ values were calculated using log-probit regression analysis.

*Results and discussion*

The tested insecticides caused complete mortality of adult *B. dorsalis* at 24 h post-treatment (Fig. 1A,B,C). There was no difference in % mortality at different post-treatment time intervals in male and female against malathion or trichlorfon (*P>0.05*); however, λ-cyhalothrin-treated male flies died, in significantly *(P<0.05)* lesser time, than the females (Fig. 3). Calculated $LT_{50}$ and $LD_{50}$ values are shown in Table I. It is obvious (Table I) that 50% percent of malathion -treated adults died within 4 hours post-treatment, indicating that this insecticide was highly toxic to them. Similar results for *B. zonata* have been previously reported by Ahmad *et al.* (2010) in populations fruit flies collected from Faisalabad and Multan. Hsu *et al.* (2004) had previously shown that malathion was less toxic than trichlorofon against fruit flies contradicts with the present finding. In this study, no resistant fly was recorded against malathion; whereas, Haider *et al.* (2011) had reported resistant *B. zonata* populations from Faisalabad (located at the distance of approximately 79 km from our field-collection site) against diptrex (trichlorofon). Similarly, Nadeem *et al.* (2012) also recorded resistant fruit fly populations from Multan (located at the distance of approximately 287 km from our field-collection site) against trichlorofon, malathion, bifenthrin, λ-cyhalothrin, and spinosad. The adult susceptibility difference of present results with the findings of Nadeem *et al.* (2012) is possibly due to extensive use of insecticides in the vicinity of their field study habitats Multan, which is a big cotton




Note: error bars in the figures are indicating standard error.

Fig. 1. Percent mortality of field-collected male and female adult *B. dorsalis* at field-use rates of malathion (a), trichlorfon (b) and λ-cyhalothrin (c). In the figure gray bars are for females and white bars for males.

**Table I-. Calculated $LT_{50}$ and $LD_{50}$ values of malathion, trichlorfon and lamda cyhalothrin tested at field-use rates against male and female field-collected adult *B. dorsalis* fruit flies in the laboratory.**

| Sex | Malathion | Trichlorofon | λ-cyhalothrin |
|---|---|---|---|
| **$LT_{50}$ (95% confidence interval)** | | | |
| Male | 4.12±0.5 | 7.84±0.41 | 7.89±0.42 |
| | (2.85-4.97) | (7.02-8.72) | (7.21-8.71) |
| Female | 3.01±0.49 | 8.37±0.54 | 12.21±0.40 |
| | (3.01-5.02) | (7.24-9.42) | (11.43-13.03) |
| **$LD_{50}$ (95% confidence interval)** | | | |
| Male | 0.11±0.005 | 0.08±0.02 | 0.03±0.002 |
| | (0.10-0.12) | (0.05-7.53) | (0.02-0.03) |
| Female | 0.10±0.004 | 0.06±0.01 | 0.02±0.002 |
| | (0.09-0.10) | (0.03-0.08) | (0.02-0.03) |

growing area of Punjab, Pakistan. No difference in male and female percent mortality caused by malathion and trichlorfon was noted in the present study but λ-cyhalothrin caused higher mortality in males at 8 and 16 hour post-treatment than in females (Fig. 1c). Stark *et al.* (2004) and Mosleh *et al.* (2011) also discovered that female fruit flies were less susceptible to selected insecticides (in terms of $LC_{50}$ value comparison) than males. Similarly, El-Aw (2008) also reported higher susceptibility of *B. zonata* males than their females to insecticides.

Fruit flies are strong fliers and may migrate up to several kilometers from their breeding source in search of food (Al Zaghal and Mustafa, 1986). Hamad (1980) had correlated this capability of *B. cucurbitae* with their control management. Their frequent movement from the location of insecticidal treatment may not allow their bodies to develop resistance against insecticides. Dalby-Ball and Meats (2000) had postulated that female flies (wild and cultured *B. tyroni*) travel further than male in search of better foraging sites to enhance their gene contribution to the populations. This may be the possible explanation for higher mortalities in male fruit flies against λ-cyhalothrin than their females.

## *References*


Ahmad, B., Anjum, R., Ahmad, A., Yousaf, M. M., Hussain, M. and Muhammad, W., 2005. *Pak. Entomol.*, **27**: 1-2.

Ahmad, S.F., Ahmed, S., Khan, R.R. and Nadeem, M. K., 2010. *Pak. Entomol.*, **32**: 163–167.

Al-Zaghal, K. and Mustafa**,** T**.,** 1986. *J. appl. Ent.*, **103**: 452-456.

Christenson, L.D. and Foote, R.H., 1960. *Annu. Rev. Ent.*, **5**:171–192.

Dalby-Ball, G. and Meats, A., 2000. *Aust. J. Ent.*, **39**: 201-207.

El-Aw, M.A.M., Draz, K.A.A., Hashem, A.G. and El-Gendy, I. R., 2008. *J. appl. Sci. Res.*, **4**: 216-223.

Garcia, F.R.M., 2009. Fruit Fly: Biological and ecological aspects. In: Bandeira RB, Editor. *Current Trends in Fruit Fly Control on Perennial Crops and Research Prospects* 1, Transworld Research Network. 35.

Haider, H., Ahmed, S. and Khan, R.R., 2011. *Int. J. Agric. Biol.*, **13**: 815–818.

Hamad, R., 1980. *Appl. Ent. Zool.*, **15**: 363-371.

Hollingsworth, R., Vagalo, M. and Tsatsia, F., 1997. Biology of melon flies, with special reference to the Solomon Islands. In: management of fruit flies in pacific (eds. A. J. Allwood and R. A. I. Drew) ACIAR Proc., **76**:140-144.

Hsu, J.C., Feng, H.T. and Wui, W.J., 2004a. *J. econ. Ent.*, **97**:1682-1688.

Hsu, J. C., Wu, W.J. and Feng, H.T., 2004b. *Pl. Protect. Bull.*, **46**: 255-266.

Hui, Y. and Liu, J., 2005. *Insect Sci*., **12**:387-392.

Kapoor, V.C., 2005. *Isr. J. Ent.*, **36**: 459-475.

Latif, A., 2004. Integrated management of fruit flies (Diptera: Tephritidae) in Pakistan. Annual report, Agricultural Linkage program (PARC) Islamabad, pp. 1-51.

Magana, C., Hernandez-Crespo, P., Ortego, F. and Castaneral, P., 2007. *J. econ. Ent.*, **100**: 1836–1843.

Mosleh, Y.Y., Moussa, S.F.M. and Mohamed, L.H.Y., 2011. *Plant Protect. Sci.*, **47**:115-120.

Nadeem, M.K., Ahmed, S., Ashfaq, M. and Sahi, S. T. 2012. *Pakistan J. Zool.*,**44:** 495-511.

Stark, J.D., Vargas, R.I. and Miller, N.W., 2004. *J. econ. Ent.*, **97**: 911–615.

Steck, G., 2003. Fruit fly pests. http://www.doacs.state.fl.us/pi/images/ffpestsbrochure.pdf.

Stonehouse, J.M., Mumford, J.D. and Mustafa, G., 1998. *Crop Protect*., **17**: 159–164.


*(Received 18 March 2014, revised 28 March 2014)*

*Pakistan J. Zool., vol. 47(1), pp. 283-285, 2015.*

# *Thaparocleidus* Jain, 1952: A Potential Monogenoid Biomarker in Host Identification

**Saroj Rajvanshi*, Priyanka Tripathi and Nirupama Agrawal**
*Department of Zoology, University of Lucknow, Lucknow, 226007, U.P., India*

**Abstract.-** Genus *Thaparocleidus* Jain, 1952 (Monogenoidea) was established by Jain in 1952, from Lucknow, India with *T. wallagonius*, as type species from *Wallago attu* (Bloch and Schneider, 1801). It is host specific gill parasite, infecting only Siluriforms. 24 species are known in India, including two exotic monogenoids on an exotic fish *Pangasianodon hypophthalamus* (Sauvage, 1878), earlier reported in Malaysia. Identification of monogenoids led us to the confirmation of host species. It is noteworthy that through identification of monogenoideans, host can be identified correctly; the genus *Thaparocleidus* being a potential biomarker.

**Keywords:** Biomarker, pangasiid, Monogenoidea, gill parasite.

Most of the monogenoideans are host specific parasite of wider or narrow range. Oioxenous/monospecific parasites are strictly host specific, while stenoxenous and euryxenous parasitize closely related and unrelated host, respectively (Pojmanska and Niewiadomska, 2012). The host specificity of parasite may be due to perception of chemicals released by host (Kearn 1967). The confinement of monogenoideans to specific host is taxonomically important as this attribute can be used as bio-marker for accurate host identification or vice versa.

*Materials and methods*

Fishes were collected from fish farms of district Barabanki (26° 55' N / 81° 11' E), and ornamental aquaria at Lucknow (26° 50' N / 80° 56' E). Live hosts were maintained in glass aquaria, and were identified by Fish base (Froese and Pauly, 2012) and by Dr. U K. Sarkar (National Bureau of Fish Genetic Resource). Parasites were dislodged using micro needles from gills of freshly dead hosts. Live as well as formalin (3%) fixed parasites were studied under a phase contrast microscope Olympus BX 51. The methods for staining, mounting and illustrating the dactylogyrids are those of Kritsky *et al*. (1986). Unstained glycerine mounts were used for measurement (µm) of soft as well as hard parts. Measurements and illustrations were taken using a microscope with Olympus-Photometrics Coolsnap Image-ProExpress 6.0.

*Results*

So far 24 species of the genus *Thaparocleidus*, parasitizing on gills of 10 hosts (genera) have been described in India (Table I). Verification of their additional hosts is further required. Of these, *T. pangasi* (Tripathi, 1959) Lim, 1996 was the only species infesting native pangasiid *Pangasius pangasius* (Ham, 1822), currently not available in the catch. During survey of Siluriform fishes for infection of the genus *Thaparocleidus*, some unknown species were collected from small sized pangasiids kept in aquaria and large catfishes from a fish farm of Barabanki and Lucknow district. Aquarium fish was identified as *P. hypophthalamus* however, pond cultured fish was thought to be native *P. pangasius* .The aquarium fish sometimes harboured only monotypic infection of *T. siamensis* or mixed with *T. caecus* as well (both exotic species) whereas pond fish was infected with only *T. siamensis.*

This conclusion was based on 99% sequence homology between Indian and Malaysia *T. siamensis* (Rajvanshi and Agrawal, 2014, Tripathi *et al*., 2014). Since infection of pond fish was of alien monogenoid and the native species i.e *T. pangasi*, could not be found, thus, again reconfirmation of large sized host was needed. *Pangasianodon hypophthalamus*, a cat fish, commonly called as "Sutchi" and "TRA" in Vietnam has been successfully cultured in Thailand, Vietnam (Lerssutthichawel *et al*., 1999) as well as in the Indian subcontinent because of high nutritive value. Thus, the introduction of parasites, with their respective hosts specifically the monogenoids, is

* Corresponding author: saroj.rajvanshi5july@gmail.com

**Table I.- Checklist of fish host with respective *Thaparocleidus* sp.**

| S. no. | Fish host | S. no. | *Thaparocleidus sp.* | Additional host |
|---|---|---|---|---|
| 1 | *Wallago attu* | 1 | *T. wallagonius* Jain, 1952 | *S. seenghala* |
| | | 2 | *T. gomtius* (Jain, 1952) Lim, 1996 | |
| | | 3 | *T. indicus* (Kulkarni, 1969) Lim, 1996 | |
| | | 4 | *T. sudhakari* (Gusev, 1976) Lim, 1996 | |
| | | 5 | *T. yogendraii* (Agrawal, 1981) Rajvanshi and Agrawal, 2012 | |
| 2 | *Sperata aor* (Hamilton, 1822) | 6 | *T. aori* (Rizvi, 1971) Lim, 1996 | |
| | | 7 | *T. speratai* Agrawal *et al*., 2005 | |
| | | 8 | *T. susanae* Rajvanshi and Agrawal, 2013. | |
| 3 | *Sperata seenghala* (Sykes, 1839) | 9 | *T. seenghali* (Jain, 1961) Lim, 1996 | *R. rita, C. garua* |
| 4 | *Ompok bimaculatus* (Bloch, 1979); *O. pabda* (Ham. 1822); *Ompok malabaricus* (Valenciennes, 1840) | 10 | *T. octotylus* (Kulkarni, 1969) Lim, 1996 | |
| | | 11 | *T. longicopulatrix* Rajvanshi and Agrawal, 2014 | |
| | | 12 | *T. devraji* (Gusev, 1976) Lim, 1996 | |
| | | 13 | *T. malabaricus* (Gusev, 1976) Lim, 1996 | |
| 5 | *Clupisoma garua* (Ham. 1822) | 14 | *T. vaginalis* (Gusev, 1976) Lim, 1996 | |
| 6 | *Eutropiichthys vacha* (Ham. 1822) | 15 | *T. vachius* (Jain, 1961) Lim, 1996 | |
| | | 16 | *Thaparocleidus singularis* n. sp. (In press) | |
| | | 17 | *Thaparocleidus kritskyi* n. sp. (In press) | |
| 7 | *Silondia silonia* (Ham. 1822) | 18 | *T. multispiralis* (Jain, 1957) Lim, 1996 | *C. garua* |
| 8 | *Pangasius pangasius* (Ham. 1822) | 19 | *T. pangasi* (tripathi, 1959) Lim, 1996 | *E. vacha* |
| 9 | *Mystus vittatus* (Bloch, 1794); *M. bleekeri* (Day, 1878); *M. tengra* (Hamilton, 1822) | 20 | *T. pusillus* (Gusev, 1976) Lim, 1996 | |
| | | 21 | *T. parvulus* (Gusev, 1976) Lim, 1996 | |
| | | 22 | *T. bleekerai* Rajvanshi and Agrawal, 2014 | |
| 10 | *Pangasianodon hypophthalamus*. | 23 | *T. siamensis* (Lim, 1990) Lim, 1996 | |
| | | 24 | *T. caecus* (Mizelle and Kritsky, 1969) Lim, 1996 | |

inevitable as it proved by recording two alien species *T. siamensis* (Lim, 1990) Lim, 1996 and *T. caecus* (Mizelle and Kritsky, 1969) Lim, 1996 from two different localities (district Lucknow and Barabanki). Therefore, the genus *Thaparocleidus* (monogenoid) could be, used as biomarkers in the identification of silurifroms, as is evident from the Table I. Similar findings were also reported for the genus *Urocleidus* Muller, 1934 which is a North American genus and infects the members of the host families Percidae and Percopsidae. Several species were reported in India as *Urocleidus* from a wide array of fish hosts and now they were transferred in several distinct genera (Agrawal and Vishwakarma, 2000; Kritsky *et al*., 2004; Agrawal *et al*., 2006; Tripathi *et al*., 2007, 2009).

*Pangasius pangasius* is least concern fish and is not available in catch. It is just possible that *P. hypophthalamus*, the alien fish is replacing the native species as *Clarias batrachus* Linnaeus 1758 has been almost replaced in fish markets by *Clarius gariepinus* Burchell, 1822. We could not collect *T. pangasi* due to non-navailability of *P. pangasius*. We conclude therefore that monogenoids can be used as biomarker or tag for correct host identification.

*Acknowledgements*

To Dr. U.K. Sarkar (NBFGR, Lucknow) for helping me in fish identification, Mr. Shailendra Ray for his helpful suggestions and University grant Commission for financial assistance (SR 28943 and NA F-4-10/2010 BSR). We also acknowledge to Department of Zoology, University of Lucknow for lab facilities.

*References*

Agrawal, N. and Vishwakarma, P., 2000. *Ind. J. Helminth*., (N.S), **17:** 33-46.

Agrawal, N., Tripathi, A. and Devak, A., 2006. *Syst parasitol.*, **63**: 223-230.

Froese, R. and Pauly, D., 2012. Fish base. World Wide Web electronic publication. www.fishbase.org version, 03/227.

Keran, G.C., 1967. *Parasitology*, **57:** 585-605.

Kritsky, D.C., Thatcher, V.E. and Boeger, W.A., 1986. *Proc. Helminth. Soc. Wash*. **53**: 1-37.

Kritsky, D.C., Pandey, K.C., Agrawal, N. and Abdullah, S.M.A., 2004. *Folia Parasit.,* **51**: 291-298.

Lerssutthichawal, T., Lim S.L.H. and Chinabut S., 1999. *AAHRI Newsl.,* **8**: 1-5.

Pojmanska, T. and Niewiadomska, K., 2012. *Annls. Parasitol*., **58:** 57–61.

Rizvi, S.S.H., 1971. Monogenea of Pakistan fishes I. *Ancylodiscoides mystusi*, New species and *A. aori*, New species, from the gills of *Mystus aor* (Ham.). *Pakistan J. Zool.*, **3:**87-92.

Rajvanshi, S. and Agrawal, N., 2014. *J.Biol. env. Sci.,* **4:** 116-121.

Tripathi, A., Agrawal, N. and Pandey, K.C., 2007. *Comp. Parasitol.*, **74**: 260-263.

Tripathi, A., Agrawal, N. and Pandey, K.C., 2009. *Parasitol. Int*., **59**: 18-21.

Tripathi, A., Rajvanshi S. and Agrawal, N., 2014. *Helminthologia,* **51:** 1-23.

*(Received 22 November 2014, revised 29 November 2014)*