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Assessment of diversity and abundance of Mosquitoes from rural areas of Faisalabad

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Abstract

Mosquito-borne diseases continue to pose a serious health concern in the rural areas. The current research study was designed to focus on the diversity, species richness, abundance and the population dynamics of mosquitoes in rural areas of Faisalabad. Eleven species of mosquitoes were collected, of which 6 species belonged to the genus *Culex*, 3 species to *Anopheles* and 2 species to the genus *Aedes*. Maximum species richness was observed in Irrigated fields. *Aedes albopictus* and *A. aegypti* were collected where fresh water body existed but with an abundance of *A. albopictus*. *A. albopictus* was dominant in Rain catch basin, petrol pump and Rice field. The *C. quinquefaciatus* was abundantly found in all the collection sites. The data for plants, animals and birds were also recorded and no huge was observed in all the collection sites. The quantitative parameters dissolved oxygen (DO), total dissolved solids (TDS), electrical conductivity (EC), pH, temperature and relative humidity (RH) were recorded. Majority of the mosquitoes occupied all quartiles of quantitative parameters. Maximum species richness was observed during Aug-Sept. and minimum recorded during Nov-Dec. The mosquito populations increased with an increase in humidity and decreased with the increase in temperature. The low relative humidity also gave negative impact on mosquito population during winter. It is suggested that irrigated fields, rain catch basin and stagnant water in rice field could serve as a potential breeding site for mosquitoes in the rural areas. It is therefore, recommended proper agriculture practices to avoid the threat of mosquito-borne diseases in the rural areas.

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Introduction

Among all the insects, mosquitoes are important due to their involvement in spreading of many diseases like malaria, dengue and *filariasis* in humans and also creating nuisance. They are highly adaptable insects and prefer different types of habitats for oviposition, resting, feeding, etc. Among these habitats; pattern of vegetation, ponds, water bodies, humans and different animals are important (Charles *et al.,* 1994; Russel, 1999; WHO, 2004; Yan and Zhong, 2005). Mosquito survey provides valuable information on occurrence, distribution and species diversity of various mosquitoes in an area which assumes significance due to their public health importance (Prakash *et al.,* 1998). Mosquito-borne diseases currently represent a greater concern due to the fact of human life at stake in certain areas.Among the mosquito-borne diseases, Malaria is the world's most important vector-born dreadful disease to humans. According to an estimate 2,073 million people living in 103 countries are at risk of malaria (Service, 1992). Only one *Aedes albopictus* species is capable of transmission of at least 20 *Arboviruses* and filarial worms and has major role as vector of dengue in Asia (Gratz, 2004). In addition, Dengue is an old disease caused by the mosquito-borne dengue viruses (DENV). The WHO reports that dengue disease is endemic in more than 100 countries around the world, with South-East Asia the most seriously affected (Anonymous, 2008).

Pakistan has faced many epidemics due to mosquitoes because of uncontrolled urbanization, poor hygienic conditions and under developed sewerage system. The problem is more severe in rural areas especially those which are associated with well irrigated agro ecosystem, several agricultural practices, poor sanitation facilities and lack of medical facilities further enhance the diversity and density of mosquito populations (Pandian *et al.*, 1997). The seasonal and climatic effects on mosquitoes also influence the disease transmission (Beaty and Marquardt, 1996). Dengue fever has increased cyclically in Pakistan since 1994. The most challenging and alarming situation existed in Punjab; especially Lahore, during 2011, where it claimed many lives. Diseases transmitted through mosquitoes significantly increased with 2,340 cases of malaria reported in 20 districts in March as compared to 872 in 15 districts in February; Faisalabad with 27,308 cases was the highest reporting district (Gilani, 2012).

Dengue fever is spreading in Faisalabad so rapidly that daily more than a dozen dengue patients are reported. The situation becomes worst with the rural areas where health facilities and quality of life is far behind. Only during a short period of few weeks, 29 dengue patients were reported from rural areas of Faisalabad (Sajid *et al.,* 2102). It seems that the prevailing climate results in excellent breeding opportunities for different mosquito species in and around the city (Naeem-Ullah *et al.,* 2010). It is therefore necessary to investigate mosquito diversity and the ecological factors that govern the population fluctuations for the successful control of the vector and diseases (Rengel 2004; Mohiuddin, 2007; Naeem-Ullah and Akram, 2009). A small scale studies have been carried out in the past for the mosquitoes diversity (Aslam and Salman, 1969; Reisen, 1978; Reisen and Boreham, 1979; Reisen and Milby, 1986) which need to be revised as they do not provide in depth details (Herrel *et al.,* 2001; Mukhtar *et al.,* 2003 ; Rengel, 2004; Mohiuddin, 2007). The current study was designed to estimate the overall diversity and abundance of the mosquitoes in rural areas of Faisalabad which could be helpful in future for -decision making programs to combat mosquito-borne diseases in the rural areas.

Materials and methods

Study area

Faisalabad is situated in Rechna Do-aab, located between the Chenab and Ravi rivers, 73.08'E, 31.25'N and at an altitude of 214m above mean sea level. The mean annual maximum and minimum temperature of the area is 48±2°C and 10± 2°C respectively and average annual precipitation is 550 mm. The city is densely populated district of Punjab with a population of more than 4 million and an area of about 1280 Sq. Km.

Collection of mosquitoes

Both adults and immature mosquitoes were collected from different breeding sites of the selected areas. The collection was carried out twice a week in order to get the maximum number of samples for the assessment of mosquito diversity. The adult mosquitoes were collected with the help of sweep net (hand net) (Herrel *et al.,* 2001; Shortall *et al.,* 2009; Florencio *et al.,* 2014). The larvae and pupae of mosquitoes were collected by using standard dipper (400ml) with one meter handle from different marked sites (WHO, 2005). For every collection site, maximum of ten dips were taken. About 5-6 well dispersed samples were collected from different points. The larvae were also sampled from old and discarded tires present near petrol pump shops for puncturing and from highly polluted habitats such as rotten vegetation, sewerage water and garbage in the respective site (Sattler *et al.,* 2005).

Data collection

The Dissolved Oxygen (DO), Electric Conductivity (EC), pH, Total Dissolved Salts (TDS), meteorological characteristics like temperature and Relative Humidity (RH) of the air were also recorded during sampling (Akram and Lee, 2004; Adebote *et al.,* 2008; Naeem-Ullah *et al.,* 2010). For the assessment of habitat preference, all the plants, animals and vegetations were also recorded near and around the sampling site.

Identification of mosquitoes

The collected specimens were preserved in plastic vials for later identification. Immature forms of mosquitoes were reared in enamel trays in the laboratory. The emerged adults were collected and stored in plastic vials. All the collected mosquitoes were identified on the basis of morphological characteristics using different taxonomic keys and the available literature (Harbach, 1985; Darsie and Pradhan, 1990).

Data analysis

The Shannon diversity index gives a complete picture of overall diversity, species richness and evenness of a given sample species in a specific area (Shannon, 1948; Kikkawa, 1996). Therefore, the diversity and relative abundance of the collected mosquitoes were statistically assessed by using Shannon diversity index.

Results

The present study was conducted in rural areas of Tehsil Samundri, Faisalabad. Nine collection sites were selected to focus on the assessment of mosquito abundance, population dynamics, diversity and the habitat preference. During present study, eleven species of mosquitoes were collected of which 6 species belonged to genus *Culex*, three species to genus *Anopheles* and two species to *Aedes*, respectively.

Month-wise diversity of mosquitoes

During the month of August, the diversity (H) and maximum diversity (H') values were found as 1.97 and 2.197, respectively. Evenness value (0.897) shows that, mostly all the mosquito species were evenly distributed (~90%), except one species *Culex quinquefaciatus*. Ten species (S) were recorded in September with a diversity (H) value of 2.04 and maximum diversity (H') of 2.309.Evenness value (0.886) represents that most of the mosquito species during this month were evenly distributed except two dominant species; *C. quinquefaciatus* and *A. albopictus*. In October, eleven species richness (S) were recorded with diversity (H) value of 1.95 and maximum diversity (H') of 2.398, respectively. The mosquito population was more diverse during this month. Evenness value (0.813) reveals that all the species found during this month were evenly distributed except two species; *C. quinquefaciatus* and *Anopheles subpictus* (Table 2). *A. stephensi* was also found as an abundant species during this month with dominance value 0.114 (Table 1). Mosquito population was more diverse during the month November. Notably, the maximum diversity value (H') was recorded as same for the month of October and November (Table 2). Evenness value (~70%) shows that seven mosquito species were evenly distributed during the month of November. Four species; *C. quinquefaciatus, A. clucifaca, A. subpictus*

and *A. stephensi* were dominant over the entire area. In December, nine species (S) were found; with a diversity (H) and a maximum diversity (H') of 1.34 and 2.197, respectively. Evenness value shows that during this month, six out of nine mosquito species were evenly distributed (~60%); the other three species, *A. clucifacae, C. quinquefaciatus,* and *A. subpictus*, were dominating during this month (Table 2).

Table 1. Result of Shannon-Weiner Index for month-wise mosquito population.

No.	Months	Species Richness (S)	Species Abundance (N)	Diversity (H')	Max. diversity H' max	Evenness (J')	Dominance (D)
1	August	9	95	1.97	2.197	0.897	0.103
2	Sep	10	109	2.04	2.303	0.886	0.114
3	Oct	11	66	1.95	2.398	0.813	0.187
4	Nov	11	60	1.67	2.398	0.696	0.304
5	Dec	9	53	1.34	2.197	0.610	0.390

Table 2. Result of Shannon-Weiner Index for Area-wise mosquito population.

No.	Site	Species Richness (S)	Species Abundance (N)	Diversity (H')	Max. diversity H' max	Evenness (J')	Dominance (D)
1	Houses & School Area	7	47	1.48	1.946	0.761	0.239
2	Poultry Farm	7	25	1.38	1.946	0.709	0.291
3	Watercourse	8	44	1.45	2.079	0.697	0.303
4	Irrigated fields	11	55	1.35	2.398	0.563	0.437
5	Stagnant pool	8	70	1.52	2.079	0.731	0.269
6	Sewerage drain	9	64	1.49	2.197	0.678	0.322
7	Rain catch basin	2	11	0.53	0.693	0.765	0.235
8	Tires/ Petrol Pump	2	23	0.64	0.693	0.923	0.077
9	Rice Field	6	44	1.48	1.792	0.826	0.174

Supplementary data

Table 3. Month-wise population of mosquito species in the study area.

No.	Species	August	September	October	November	December	Total
1	*A. albopictus*	20	23	7	1	0	51
2	*A. aegpyti*	9	11	3	1	0	24
3	*A. stephensi*	11	13	10	7	3	44
4	*A. subpictus*	14	13	11	9	8	55
5	*A. clucifacae*	1	6	5	1 2	15	39
6	*C. tritaeniorhynchus*	10	9	6	3	1	29
7	*C. quinquefaciatus*	22	23	14	15	13	87
8	*C. fuscanis*	0	0	2	4	5	11
9	*C. vishnui*	4	5	2	1	1	13
10	*C. pseudovishnui*	4	5	3	2	1	15
11	*C. pipiens fatagins*	0	1	3	5	6	15
	Total	95	109	66	60	53	383

Area-wise diversity of mosquitoes

The diversity (H) and a maximum diversity (H') ranged from 0.53 to 1.52 and 0.693 to 2.398, respectively (Table 2). The maximum diversity (H', 0.693) value showed that mosquito populations had less diversity in Rain catch basin and Tires/Petrol Pump collection sites, whereas the H' value of Irrigated fields site (2.389 & 2.079) showed thatmosquito populations were more diverse at these sites. The Evenness (J) and dominance (D) ranged from 0.563 to 0.923 and 0.077 to 0.437 respectively (Table 2).It was found that most of the species were evenly distributed except few species i.e., *C. quinquefaciatus,* and *A. clucifacae* species in Houses & School Area, *C. quinquefaciatus* species in Poultry Farm; *C. quinquefaciatus* and *A. subpictus* species in Watercourse near Poultry farm; *A. subpictus, A. clucifacae, A. stephensi* and *C. tritaeniorhynchus* species in Irrigated fields; *C. quinquefaciatus* and *A. subpictus* species in the Stagnant pool; *C. quinquefaciatus*, *A. subpictus* and *A. clucifacae* species in Sewerage drain site, respectively. *A. albopictus* was found to be dominant in the Rain catch basin, the petrol pump and in rice field of Tehsil Samundri (Table 2).

C. quinquefaciatus, C. tritaeniorhynchusi, C. pseudovishnui, C. vishnui A. stephensi, *A. clucifacaei, A. subpictus* were found throughout the study period. *C. quinquefaciatus, C. tritaeniorhynchusi*, *C. pseudovishnui* and *A. stephensi* were also captured from tires. *C. tritaeniorhynchus*, *C.pseudovishnui* and *A. albopictus* were also found in tree-holes. In addition, *A. albopictus* was also collected from cans.*A. Aegypti* was also found from tires, not recorded from tree-holes during the study. Both the *Aedes* species and *A. subpictus* were recorded in abundance in shady habitats. Mostly, the mosquitoes were collected at DO (<3.8 – 10.2 mg/L), EC (<4426 µS/m) and TDS (<2265 ppm) and pH ranging from 6.15 to 9.2. Majority of the mosquitoes occupied all quartiles of quantitative parameters (DO, TDS, EC, pH). *A. aegypti* and *A. Albopictus* were not present at lower and higher limits for these quantitative characteristics (data not shown).

Preference and diversity of mosquitoes across different habitats

For each collection site, the data for plants and animals were also recorded to find out preferred habitat of mosquito populations. Shesham, desi-kikar, eucalyptus, sugarcane, maize crop and certain types of grass; mostly the domestic animals like buffalo, cow, goat etc, and of birds, house sparrow and crow were recorded during the whole study period (data not shown). The mosquito species *C. quinquefaciatus* was abundantly found; possibly; the water bodies of sugarcane field, sewerage and the closeness to the human dwellings could be considered as a preferred habitat. *A. stephensi* and *An. clucifacae* were abundant and dominating the poultry farm site. *A. albopictus* was also recorded which could be due to the presence of plantation and domestic animals. However, *A. albopictus* was not dominating the area.

The mosquito species *C. quinquefaciatus* and *A. subpictus* species were abundantly found in Irrigated fields. The presence of eleven species could prefer the water bodies of sugarcane and rice field, the sewerage and house-channels water near and around the collection site. *A. stephensi* and *A. clucifacae* were abundant in the poultry farm site. *A. albopictus* were also recorded due to the presence of plantation and domestic animals, However, *A. albopictus* was not dominating the area.*A. subpictus, A. clucifacae, A. stephensi* and *C. tritaeniorhynchus* species were abundantly found in Sewerage drain. *A. albopictus* and *A. aegypti* were found dominant and evenly distributed in Rain catch basin and Petrol Pump collection sites. The habitat containing fresh water bodies seemed to be preferred habitat compared to the other collection sites.

Population dynamics with reference to the environmental conditions

The meteorological data were recorded to elucidate the environmental impact on population dynamics of mosquitoes (Temperature, Relative Humidity; Fig. 2 & 3). It was noted that a decrease in temperature during the study period (Aug-Dec) caused decrease in mosquito population number. Moreover, an increase

in relative humidity (Aug-Sept; Fig. 2), resulted in constant increase in the population. However, notably the increase can not only be attributed to the increase in RH, but rather it was also due to the observed decrease in temperature. Therefore, during the month of August-Sept; both temperature and RH can be considered as the major factors affecting an increase the population.

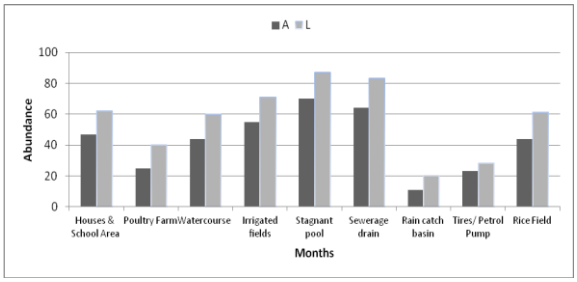

Fig. 1. Abundance of total adults and larvae of Mosquitoes.

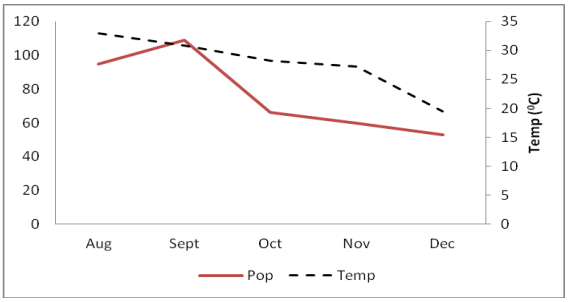

Fig. 2. Variation of Temperature affecting the mosquito population.

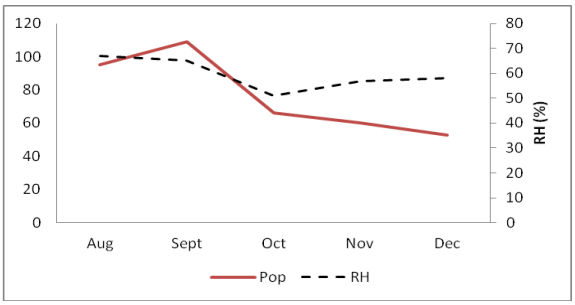

Fig. 3: Variation of Relative Humidity affecting the mosquito population.

A subsequent decrease in population was observed during the month of Oct-Dec. A decrease in temperature showed decrease in the mosquito population. In addition, a subsequent decrease in relative humidity also caused a decrease in population (Oct-Dec, Fig. 3). During Aug-Sept., the temperature was about > 30 °C; and the population was high while with the decrease in temperature during the month of Oct-Dec, population density started decreasing (≤30 ≥20 °C). Then again in Oct-Dec when temperature was decreasing (<30 °C), a significant decrease was observed. In Aug-Sept. population rate was higher but as humidity decreased in Oct-Dec (<60%), the population size was also decreased. However, with an increase in the humidity level during late Nov-Dec (>60%), the population number of Mosquitoes was decreased. Hence, the current findings indicate that there is close relationship between temperature and population abundance; and the variations in temperature and humidity level significantly affect the populations of Mosquitoes.

Discussion

Out of eleven species; six species belong to genus *Culex*, three species belong to genus *Anopheles* and two species to *Aedes*, respectively.In genus *Culex, C. quinquefaciatus* was found abundantly throughout the study period from August to December. The next most prevalent species found was *A. subpictus* and in *Aedes* genus; *A. albopictus* was recorded as most abundant species.A total of 515 larvae were collected with the help of dipper and 383 adult mosquitoes were collected using hand net. The biodiversity of mosquitoes have been described in rural areas with the dominance of *Armigeres subalbatus* (48.1%) followed by *A. aegypti* (13.66), *Cx. quinquefasciatus* (11.62%) and the least collected species was *A. vittatus* (0.19%) species. The current results are overall, in agreement to the percentages shown among the genera, *Anopheles*, *Culex*, and *Aedes* was in the ratio 36.03%, 44.38% and 19.58%, respectively (Amala and Anuradha, 2011; Amala and Anuradha, 2012).

Culex species showed a broad range of habitat preference than the other genera. *Aedes* species were found from a few habitats and was least diverse. The mosquito composition in the residential area was

vastly different to the other sites. This suggests that there is a different diversity and composition of mosquitoes across different land use area types. It was found that Irrigated fields were the most preferred site for mosquitoes. As there was no huge difference in plants, animals and birds in all the collection sites. The only difference was the rice field which could be the considered as the most preferred habitat. The current results are consistent to those as described in literature (Kanojia *et al.* 2003; Brant, 2011; Amala and Anuradha. 2011; Amala and Anuradha, 2012). The mosquitoes are also reported to breed in paddy cultivation. Kanojia *et al.* (2003) reported that the irrigation system and paddy cultivation provide a perennial source of breeding places for these mosquitoes. Leaf litter in mosquito habitats provides essential organic carbon for growth of mosquito larvae. It was found in current study that maximum species richness as well as species abundance was recorded in Irrigated fields. The Rice field contained water, and the nearby plantation had much leaf litter and organic matter which potentially caused high species richness and abundance (Brant, 2011; Patz *et al.,* 2003).

There was also an interaction between partial shade and the number of leaves. The elevated level of nitrogen and protein dissolved from leaf litter increases growth of different microbes, which has a higher nutritional value than leaf letter alone. Therefore, many mosquito larvae feed upon leaf litter colonized by fungi and bacteria (Brant, 2011). The oviposition preference theory predicts that a gravid female will oviposit in a breeding site that will maximize the offspring fitness; suggesting thereby, the adult mosquitoes will oviposit in sites with high shade, high level of detritus and a low level of competition (Brant, 2011; Amala and Aunradha, 2011). Therefore, it could be speculated that a higher abundance of mosquito larvae and adults might be correlated with high shade and leaves. The current findings are in accordance and hence, consistent with the results already published (Kanojia *et al.,* 2003; WHO, 2005; Brant, 2011).

The current results are also in agreement with those obtained by Naeem-Ullah *et al.*, who reported 24 species of immature mosquitoes from different ecological zones of Punjab, Pakistan which were grouped on the basis of habitats (Naeem-Ullah *et al.* 2010). However, the quantitative parameters and the species data were slightly different from the findings of Tadesse *et al.* (2011). A combination of different features regarding a given environment has recently been observed in various studies (Rezaei, 2012; Brant, 2011).

Temperature, rainfall and relative humidity (RH) are physical factors that influence the abundance of the mosquitoes. It has been widely studied that temperature and humidity affects the diversity as well as the population no. of insects in a given environment (Zahoor *et al.,* 2003; Zahoor *et al.,* 2003a ; Zahoor *et al.,* 2003b; Zulfaqar *et al.,* 2003; Ahmed *et al.,* 2004; Iftikhar *et al.,* 2004; Batool, 2012).

According to Rozilawati binti harun, with no changes and fluctuation in weather conditions, there is no significant difference in larval numbers throughout the year (Rozilawati binti harun, 2007; Brant, 2011). The pattern of population rise and fall show a unique pattern which is true for other insect species also (Zulfaqar *et al.,* 2003; Zahoor *et al.,* 2003a ; Zahoor *et al.,* 2003b; Ahmed *et al.,* 2004; Iftikhar *et al.,* 2004; Batool, 2012).The increase or decrease in temperature causes a change in the metabolism rate of the insects and ultimately causes a population fluctuation. Subsequently, it has been shown in various studies that an increase in the level of humidity also causes an increase in the population of the insects (Silver, 2008; Zahoor *et al.,* 2003). Mosquitoes are sensitive to the temperature changes because of the immature stages in its aquatic environment and as adults. It has been studied that a rise in water temperature (above 34°C), generally has a negative impact on the survival of vectors and parasites (Silver, 2008).

In general, insects are exceedingly sensitive to temperature, humidity and rainfall regiments and frequently show great variations in seasonal abundance (Brant, 2011). In the present study it was observed that population of mosquito species increased with increase in humidity and decreased with increase in temperature which is consistent to the results observed by Batool (2012) and Zahoor *et al.* (2003, 2003a). It has also been reported that high relative humidity can give high hatching rates. The low relative humidity also gives negative impact on egg hatching (Zahoor *et al.* 2003, Batool, 2012, Silver, 2008). It was observed that during the month of September; max. species abundance were recorded which is in accordance to the findings of Brant (2011). It was found in the present study that *Aedes albopictus* had maximum population during the month of September (Supplementary Table 3); consistent to the previous results that the temperature fluctuations affect the mosquito populations and allow *Aedes* proliferations only between September and April (Rozilawati binti harun, 2007; Brant, 2011).

Conclusion

The current results showed that population of mosquito species increased with increase in humidity and decreased with increase in temperature. *Culex* species showed a broad range of habitat preference than the other genera and on the other hand; the genus *Aedes* was found from a few habitats and was least diverse. Mostly, the same kind of habitat was observed with most of the collection sites. There was no huge difference in plants, animals and birds in all the collection sites. The only difference was the rice field which could be considered as the most preferred habitat. It is, therefore, suggested that irrigated fields, rain catch basin and stagnant water in rice field could serve as a potential breeding site for mosquitoes in the rural areas.

Further perspective

It is likely that habitat modification will continue, so it is important that mosquito fauna should be explored to help control disease outbreaks. Malaria, and particularly dengue fever and dengue haemorrhagic fever is increasing in with no current vaccine or treatment. More detailed work is needed in future to include more rural areas for comparative abundance and species richness study. Subsequent molecular approaches are also needed to work-out for their proper control as well as for other molecular studies.

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