Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Response Surface Modeling for West Nile Viral Encephalitis Mosquito Control Experiments

Tanvir Ahmad[1], Shabab Nasir[2*], Iram Nasir[1], Tahir Nawaz[1], Azhar Rafique[2] and Iqra Yousaf[2]

[1]Department of Statistics; [2]Department of Zoology, Government College University, Allama Iqbal Road, Faisalabad
*Corresponding author: flourenceshabab@yahoo.com

ARTICLE HISTORY (17-156)

Received: May 13, 2017
Revised: August 02, 2017
Accepted: August 04, 2017
Published online: September 23, 2017

Key words:
Central composite design
Culex mosquito control
Environment friendly strategy
Mortality

ABSTRACT

Mosquitoes not only carry diseases to human beings but also affect animals especially cats, dogs and horses by spreading dog heartworm, West Nile virus, Eastern equine encephalitis etc. As mosquitoes at immature stages are aquatic in nature and due to pollution, different water grades are present in the environment. This alarming situation has been recognized and statistically investigated in this study. So, this study was designed to investigate the optimal combination of significant factors contributing for the control of immature stages of mosquito by using a statistical tool; response surface methodology. Ten factors; pH, dissolved oxygen, time interval, electrical conductivity, total dissolved solids, life stages of mosquitoes, water grades, temperature, oils and oil concentration were initially considered important for mosquito control. The treatments were replicated under laboratory conditions and percentage mortality was counted. These factors were initially screened out by using different screening designs, then significant factors were redesigned at five levels and again mortality was noted. Finally, the significant factors were studied with more efficient response surface design, a modified CCD (central composite design). After screening, pH, Time, Life stages and water grades were found statistically significant. Modified central composite design showed that not only the significant factors but their interactions were also very important for mosquito control. Mortality was low at different combinations of middle levels of pH and time as compared to the extreme levels. The current experiment is of great importance as it guides us how we can save our environment and lives from the dengue mosquito.

To Cite This Article: Ahmad T, Nasir S, Nasir I, Nawaz T, Rafique A and Yousaf I, 2017. Response surface modeling for West Nile viral encephalitis mosquito control experiments. Pak Vet J, 37(4): 465-469.

INTRODUCTION

Mosquitoes not only carry diseases to human beings but also affect animals especially cats, dogs and horses by spreading dog heartworm, West Nile virus, Eastern equine encephalitis etc. (Anonymous, 2014). Mosquitoes belonging to genus *Culex*are responsible for the spread of these diseases (Komar, 2000). West Nile virus widely spread and endemic in Australia, Africa, USA, Europe and Middle East (Ozkul *et al*., 2006; Go, 2014), while it is also reported in Asian countries like Pakistan, India, China and Iran (Zohaib, 2015). *Culex quinquefasciatus* and migratory birds play a vital role in spread of this disease (Zohaib, 2015). As this is a viral disease, so neither proper vaccine nor treatment is available. The only solution is to manage the mosquito population.

Many techniques such as chemical control, biological control, plant extract and environmental management are used to control mosquito populations (Abbas *et al*., 2013). No doubt, chemical control is an easy and quick method to reduce the mosquito population but it produces many environmental hazards as contamination of water and food sources, poisoning of non-target fauna and flora, accumulation in the food chain (Janisiewicz and Korsten, 2002). Due to environmental pollution, the water quality affects the growth of mosquitoes and efficacy of different plant extracts and *Bti* (Rodrigues *et al.*, 1999). As the immature stages of mosquito are aquatic in nature and due to pollution effect, different water grades are present in our environment. Faisalabad is an industrial city of Pakistan. Due to the industrial waste, different levels of pollutant water are present having different levels of pH, DO, EC and TDS that may affect the survival of the immature stages of mosquitoes (Innocent *et al*., 2014). Due to similar reasons, all these factors are linked with the immature stages of mosquito. So, we examined the efficacy of different oils concentrations to study the mortality percentage of different life stages of mosquito in different water grades under different temperature levels. As lot of factors affect the mosquito mortality, so we use response surface methodology to know the relationship of different qualitative and quantitative factors contributing to the mortality of mosquitoes.

Response surface methodology (RSM) is a set of mathematical and statistical tools extensively being used to study the relation between some quantitative or qualitative input variables and one or more response variables (Kilic *et al*., 2002). Many useful response surface experimental plans are available in literature which can successfully be employed in the biological experimentation. First-order response surface designs are usually applied in screening stage when experimenter wants to identify some most important variables among many (Jones and Nachtsheim, 2011), which were initially considered useful, by estimating the main-effects polynomial model and/or ANOVA. Use of RSM in biological sciences in not new. Mortarino (1997) used irregular fractions of two-level factorial designs. He performed analysis of a linear multi-response model based on these designs. Mostly scientists used this methodology in industry to find out best combination of factors for maximum output (Vastradand Neelagund, 2014; Azimi *et al*., 2015) but no one used this methodology in insect pest control. We used this methodology to find out optimal combination of qualitative and quantitative factors to stop the breeding of mosquitoes, that is also the need of the hour.

MATERIALS AND METHODS

Collection of mosquitoes: The immature stages of mosquitoes (1st, 2nd, 3rd, 4th instar and pupae) were collected with the help of standard dippers from road side ditches, sewage water and artificial water reservoirs from Faisalabad. Collected samples were stored in plastic bottle tied up with muslin cloth and brought to the Department of Zoology, Government College University, Faisalabad, Pakistan for rearing. The larvae and pupae were separated and kept in rearing trays and beakers, respectively inside the mosquitoes rearing cages. The larvae were fed on fish diet (Nasir *et al*., 2017a). After adult emergence, the mosquitoes were identified and reared under lab conditions (25±1°C temperature & 70±5% relative humidity) (Nasir *et al*., 2015b).

Collection and preparation of plant material for oil extraction: For the oil extraction, different plants material, neem (*Azadirachtaindica*) and eucalyptus (*Eucalyptus globules*) were selected and collected from Government College University, Faisalabad. To remove dust particles the materials were washed with tap water and were dried up. Then with the help of an electrical grinder the dried material was ground and the powder was stored in the plastic bottles after sieving for oil extraction.

Extraction of oil: With the help of Soxhlet apparatus oils were extracted from the selected plant material (Cheng *et al*., 2009). Then, vacuum evaporator evaporated solvent to attain filtrate in dehydrated form, which was stored in air tight jar.

Preparation of solution: From extracted oils, different concentrations (ppm) were prepared by dissolving the extracted oil in petroleum ether, respectively and required volume was made with distilled water.

Larvicide bioassays: Larvicide bioassays were carried out under laboratory conditions in accordance with WHO technique for mosquito (WHO, 2011). In each glass beaker immature stages of mosquito were introduced separately containing various oil solution concentrations. The treatments were replicated under laboratory conditions. From the average of five replicates, percentage mortality was counted by using the formula (Sumroiphon *et al*., 2006).

In screening process, we studied each factor at two levels without any replication to observe the responses. The two levels of each factor used were: concentration (50 & 250 ppm), pH (4 & 12), DO (10 & 50%), time (4 & 20h), EC (900 & 1000 mbar), TDS (100 & 2000 ppm), life stage (1st instar larva & pupae), water grade (rich organic & industrial), temperature (under lab & high) and oils (Neem & Eucalyptus). For the detailed study of significant factors we used neem oil (250 ppm) with five levels of each significant factor that were pH (4,6,8,10,12), time (4,8,12,16 and 20h), life stage (1st, 2nd, 3rd, 4th instar and pupae) and water grade (rich organic, tap, sewage, turbid, industrial) with five replications and observed the responses.

Statistical analysis: We employed regular two-level factorial design, irregular resolution V design and definitive screening design to separate out important factors from among ten initial factors by estimating model, given below:

$$E(Y) = \beta_o 1 + X\beta$$

The identified significant factors were redesigned and a more efficient response surface design, a modified CCD, was employed, by applying the factors at five levels each, to study interactions and quadratic effects, which may occur due to curvature of the system observed during screening process.

RESULTS

The mortality data of screening trails was analysed through three different screening designes (2-level factorial, irregular factorial and definitive screening) to find out significant factors contributing towards mortality in terms of F-statistics and the p-values (Table1). The F-value (28.06) implied that the model is highly significant under 2-level factorial design. There was only a 0.01% chance that an F-value this large could occur due to noise; p-value also confirms the significance of model. We could see that pH, time, life stage and water grade were significantly contributing for mortality of dengue. Under irregular factorial design we could see that pH, life stage and water grade were significant for mortality, and model itself was also significant at 5% level of significant.

The mortality as response when estimated through data collected by 2-level factorial design showed the wider spread in values instead of data obtained by irregular and definitive screening design, which was evident from standard deviation (SD) in Table 2, but this design showed highest mean response.

Table 1: *p*-value for mortality under different screening designs for main-effects model

Sources	df	2-level factorial		Irregular factorial		Definitive screening	
		F-value	p-value	F-value	p-value	F-value	p-value
Model	10	28.06	<0.0001	3.97	<0.0001	11.17	0.0002
A-Concentration	1	1.34	0.2481	0.044	0.8353	1.14	0.3090
B-Ph	1	141.37	<0.0001	5.69	0.0207	6.18	0.0302
C-DO	1	0.86	0.3554	0.13	0.7162	0.56	0.4689
D-Time	1	7.44	0.0066	0.068	0.7950	39.61	<0.0001
E-EC	1	0.34	0.5614	0.20	0.6527	0.83	0.3814
F-TDS	1	0.0043	0.9480	0.0048	0.9448	0.0033	0.9555
G-Life stage	1	5.83	0.0162	16.17	0.0002	18.53	0.0012
H-Water grade	1	122.58	<0.0001	14.67	0.0003	23.02	0.0006
J-Temperature	1	0.37	0.5455	1.24	0.2703	0.071	0.7944
K-Oils	1	0.51	0.4734	1.48	0.2284	0.41	0.5367
Residual	501						
Cor. Total	511						

Table 2: Measures related to mortality

Descriptive	2-level factorial	Irregular factorial	Definitive screening
	Mortality	Mortality	Mortality
SD	14.90	14.36	7.21
Mean	79.02	75.91	59.91
C.V. (%)	18.86	18.92	12.03
PRESS	0.00001	15941.00	2233.73
R^2	0.3590	0.4283	0.9104
Adj. R^2	0.3462	0.3204	0.8289
Pred. R^2	0.3306	0.1663	0.6497
Adeq. Precision	19.311	7.421	11.090

Table 3: ANOVA for Mortality with CCD under cubic model

Sources	Sum of squares	Df	Mean Square	F-value	p-value >F
Model	145536.98	82	1774.84	19.06	<0.0001
A-pH	9177.27	1	9177.27	98.58	<0.0001
B-Time	6395.68	1	6395.68	68.70	<0.0001
C-Life stage	12421.03	4	3105.26	33.356	<0.0001
D-Water grade	77791.90	4	19447.97	208.90	<0.0001
AD	1205.32	4	301.33	3.24	0.0119
BD	887.67	4	221.92	2.38	0.0497
CD	5121.60	16	320.10	3.44	<0.0001
A^2	13662.80	1	13662.80	146.76	<0.0001
B^2	279.71	1	279.71	3.00	0.0833
ACD	4685.51	16	292.84	3.15	<0.0001
BCD	3269.95	16	204.37	2.20	0.0043
A^2B	935.03	1	935.03	10.04	0.0016
A^2C	2125.18	4	531.30	5.71	0.0002
A^2D	2268.35	4	567.09	6.09	<0.0001
AB^2	640.96	1	640.96	6.88	0.0088
B^2C	1322.32	4	330.58	3.55	0.0069
Residual	99333.33	1067	93.10		
Lack of Fit	24904.57	142	175.38	2.18	
Pure Error	74428.77	925	80.46		
Cor. Total	244870.32	1149			

The F-value (19.06) of the model showed that the model is highly significant. The table 3 showed that pH (A), time (B), life stages (C), water grades (D), their interactions AD (3.24), CD (3.44), A^2 (146.76), ACD (3.15), BCD (2.20), A^2B (10.04), A^2C (5.71), A^2D (6.09), AB^2 (6.88) and B^2C (3.55) were significant model terms. It meant that not only the main factors were important for mortality, the interaction of pH and water grade (AD), the quadratic effects of pH (A^2), the quadratic effect of time (B^2) and some cubic effects, ACD, A^2B, A^2C, A^2D, AB^2 and B^2C were also contributing significantly in the mortality at least at 5% level of significance. 3D response plot was presented in standard format. The plot showed how response changed with the variation in two variables by keeping the remaining variables fixed. Fig.1 showed 3D response surface plots of mortality drawn for two numeric factors pH and time on different fixed levels of categorical factors. Fig. 1 (a) was drawn by keeping life stage at lowest level and water grade at level four. Obviously, the tilt in the middle of the response surface advocates that mortality was low at different combinations of middle levels of pH and time as compared to the extreme levels. Similar kind of interpretation could be made from Fig. 1(b) & (f).

DISCUSSION

As the immature stages of mosquito are aquatic in nature and different water grades are present in our environment due to pollution. We examined the efficacy of different oils concentrations to study the mortality percentage of different life stages of mosquito in different water grades under different temperature levels.

Plant extracts has been used from decades as a safe and effective strategy to control mosquito larvae and adults (Nasir *et al.*, 2015a, 2015b, 2015c). Some abiotic factors like water temperature, sunlight, some physicochemical properties of water and biotic factors like life stage of mosquitoes affect the efficacy of plant extracts against immature stages of mosquitoes (Mulla and Su, 1999; Innocent *et al.*, 2014). The life span and longevity of different immature stages of mosquitoes are also affected by multiple factors, i.e., water temperature, availability of sunlight, larval density, concentration of larval instars and food, and some physicochemical characteristics of water, i.e., concentration of dissolved organic matter, pH of water, water pollution, dissolved oxygen, and dust particles (Becker *et al.*, 1992).We could not study all these factors in detail so, the screening experiments were carried out to identify the significant factors. The efficacy of oil extracts was affected with sun light and water temperature but in our study this factor was found non-significant (Innocent *et al.*, 2014). Early larval instars (1^{st} and 2^{nd}) were more vulnerable than late instars (3^{rd} and 4^{th}). These findings were at par with Mulla (1990) who also documented the same findings. Some other vector ecologists[20] also confirmed that late instar larvae (3^{rd} and 4^{th}) were less affected than younger instars (1^{st} and 2^{nd}). Plant extract concentrations also played a significant role in mortality and fecundity of mosquitoes, i.e., higher concentrations were more effective than lower concentrations (Nasir *et al.*, 2015a; Nasir *et al.*, 2017b).

In screening process, we applied each factor at two levels without any replication to get the responses. For the screening purpose, regular two-level factorial design of ten factors was used in our experiment. And the second were irregular resolution V design and third one was definitive screening design. The results of the three types of screening designs were compared and the identified significant factors were redesigned and more efficient response surface designs were employed. For the detailed study of significant factors on second stage we implemented central composite design with five levels of significant factors with five replications each and observed the responses.

In the second stage of experiment a CCD was used to study the important factors in more detail and the analysis results were presented through ANOVA table both for mortality. Main effects of pH, time, life stage and water grades were found to be highly significant for both responses, quadratic effect of pH was also significant for both responses but the quadratic effect of time was significant only for mortality, third order interaction of pH, life stage and water grade was also significant for mortality (Becker *et al.*, 1992; Innocent *et al.*, 2014). Some popular graphs were also constructed to validate our results. 3D response surface plots show that mortality is more sensitive for pH and time at later life stages and lower water grades (Mulla, 1990). The findings of the experiment are important to guide people how to save themselves and lives of animals from West Nile virus mosquito and to know which factors affect (or stop) the breeding of this mosquito. This study can be highly useful to formulate future plans to control this disease and save a large population from the risk of this fatal disease.

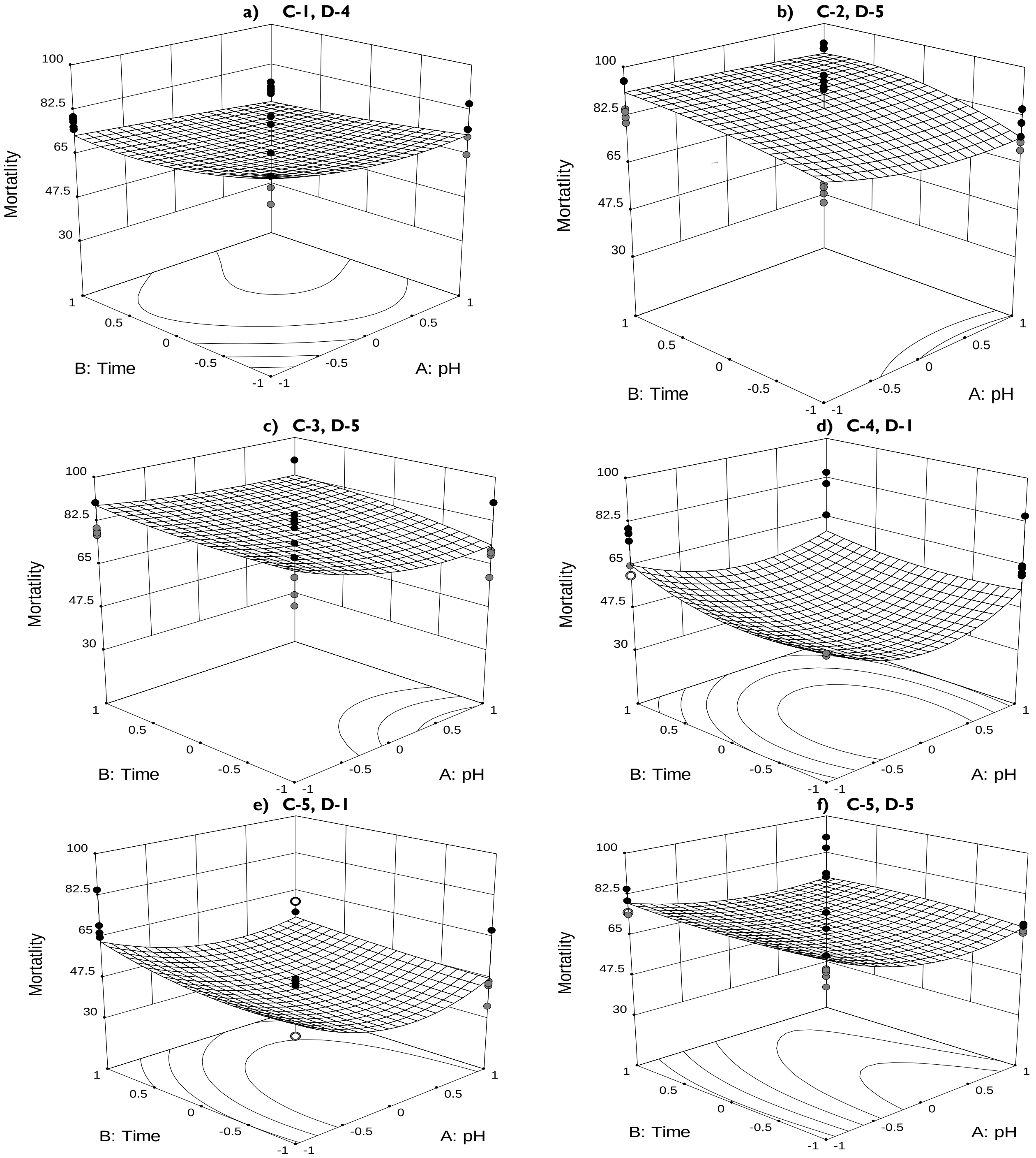

Fig. 1: 3D mortality plots of numeric factors.

Conclusions: This study concluded that different abiotic (water grades, plant oil concentration, time and temperature) and biotic (life stages of mosquitoes) factors along with different physico-chemical characteristics (pH) of water play an important role in controlling mosquitoes with plant extracts. Hence, this study can be very useful to formulate future programs to control mosquito-borne diseases.

Authors contribution: SN, AR & IY designs the study and wrote the manuscript, TA, IN &TN design and performed statistical analysis.

REFERENCES

Abbas A, Abbas RZ, Khan JA, *et al.*, 2013. Integrated strategies for the control and prevention of dengue vectors with particular reference to *Aedes aegypti.* Pak Vet J 34:1-10.

Anonymous, 2014. Mosquito-borne diseases. American Mosquito Control Association. http://www.mosquito.org/mosquito-borne-diseases.

Azimi B, Nourpanah P, Rabiee M, *et al.*, 2015. Application of response surface methodology to evaluate the effect of dry-spinning parameters on poly (e-caprolactone) fiber properties. J Appl Polym Sci 132:1-13.

Becker N, Zgomba M, Ludwig M, *et al.*, 1992. Factors influencing the activity of *Bacillus thuringiensis* var. *israelensis* treatments. J Am Mosq Control Assoc 8:285-9.

Cheng SS, Chang HT, Lin CY, *et al.*, 2009. Insecticidal activities of leaf and twig essential oils from *Clausenaexcavata* against *Aedesaegypti* and *Aedesalbopictus* larvae. J Pest Manag Sci 65:339-43.

Go YY, Balasuriya UBR and Lee CK, 2014. Zoonotic encephalitides caused by arboviruses: transmission and epidemiology of alphaviruses and flaviviruses. Clin Exp Vaccine Res 3:58-77.

Innocent E, Hassanali A, Kisinza WNW, *et al.*, 2014. Anti-mosquito plants as an alternative or incremental method for malaria vector control among rural communities of Bagamoyo District, Tanzania. J Ethnobiol Ethnomed 10:56.

Janisiewicz WJ and Korsten L, 2002. Biological control of postharvest diseases of fruits. Ann Rev Phytopathol, 40:411-41.

Jones B and Nachtsheim CJ, 2011. A class of three-level designs for definitive screening in the presence of second-order effects. Institute, Cary, NC 27513, 43:1-15.

Kilic M, Bayraktar E, Ates S, *et al.*, 2002. Investigation of extractive citric acid fermentation using response surface methodology. Am J Bioengin Biotechnol 1:1-10.

Komar N, 2000. West Nile viral encephalitis. Rev Sci Tech Off Int Epiz 19:166-76.

Mortarino C, 1997. Irregular fractions of two-level factorial designs: Multiresponse models with dependent trials. Stat Applic 9:31-44.

Mulla, 1990. Activity and biological effects of neem products against arthropods of medical and veterinary importance. J Am Mosq Control Assoc 15:133-52.

Mulla MS and Su T, 1999. Activity and biological effects of neem products against arthropods of medical and veterinary importance. J Am Mosq Control Assoc 15:133-152.

Nasir S, Batool M, Qureshi NA, *et al.*, 2015a. Repellency of medicinal plant extracts against dengue vector mosquitoes, *Aedesalbopictus* and *Ae. Aegypti* (Diptera: Culicidae). Pak J Zool 47:1649-53.

Nasir S, Batool M, Hussain SM, *et al.*, 2015b. Bioactivity of oils from medicinal plants against immature stages of dengue mosquito *Aedesaegypti* (Diptera: Culicidae). Int J Agric Biol 17:843-7.

Nasir S, Nasir I, Yousaf I, *et al.*, 2015c. Larvicidal and pupicidal action of kitchen used plant extracts against *Culex* mosquito (Diptera: Culicidae). J Glob Innov Agric Soc Sci 3:12-5.

Nasir S, Nasir I and Yousaf I, 2017b. Medicinal plant extracts: control strategy against dengue mosquitoes. Bangladesh J Bot 46:27-33.

Nasir S, Nasir I, Asrar M, *et al.*, 2017a. Larvicidal and pupicidal action of medicinal plant extracts against Dengue mosquito *Aedes albopictus* (Diptera: Culicidae). Indian J Anim Res 51:155-8.

Ozkul A, Yildirim Y, Pinar D, *et al.,* 2006. Serological evidence of West Nile Virus (WNV) in mammalian species in Turkey. Epidemiol Infect 134:826-9.

Rodrigues IB, Tadei WP and Dias JS, 1999. Larvicidal activity of *bacillus sphaericus* 2362 against *Anopheles nuneztovari*, *Anopheles darlingi*and *Anopheles braziliensis* (Diptera: Culicidae). Rev Inst Med Trop S Paulo 41:101-5.

Sumroiphon S, Yuwaree C, Arunlertaree C, *et al.*, 2006. Bioactivity of citrus seed for mosquito-borne diseases larval control. Southeast Asian J Trop Med Public Health 37:123-8.

Vastrad BM and Neelagund SE, 2014. Optimizing the medium conditions for production of tetracycline by solid state fermentation of *Streptomyces aureofaciens* NCIM 2417 using statistical experimental methods. Biosci Engin Inter J 1:29-44.

WHO, 2011. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. World Health Organization, Regional Office for South-East Asia 2.

Zohaib A, Saqib M, Beck C, *et al.*, 2015. High prevalence of West Nile virus in equines from the two provinces of Pakistan. Epidemiol Infec 143:1931-5.