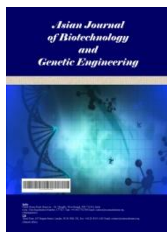

Asian Journal of Biotechnology and Genetic Engineering

5(4): 1-7, 2022; Article no.AJBGE.88124

Larvicidal Potential of Conidia Suspension of *Aspergillus flavus* against *Anopheles sp.*

Abdulrahman Itopa Suleiman [a*], Abdullahi Abdulkadir Imam [b], Nasir Sirajo Sadi [c] and Muhammad Ali Gambo [c]

[a] *Department of Biochemistry, Kogi State University, Anyigba, Nigeria.*
[b] *Department of Biochemistry, Bayero University, Kano State, Nigeria.*
[c] *Department of Integrated Sciences, Federal College of Education (Technical) Bichi, Kano State, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/88124

Original Research Article

Received 14 April 2022
Accepted 19 June 2022
Published 06 July 2022

ABSTRACT

Aim: This study was aimed to investigate the larvicidal potential of the conidia suspension of *Aspergillus flavus* against *Anopheles* mosquitoes.
Methods: *Aspergillus flavus* was isolated from soil using soil suspension procedures and was identified using morphological characteristics. Bioassay was performed to determine the efficacy of *Aspergillus flavus* conidial suspension against early 4th instar larvae of *Anopheles* mosquito using WHO-2005 protocol with slight modifications.
Results: Four different concentrations of conidial suspension; 3.3×10^6, 3.3×10^5, 3.3×10^4 and 3.3×10^3conidia/ml were tested, and the results showed that; mortality increases with the increase in conidial concentrations and exposure time. The lowest mortality (12%) was recorded at 3.3×10^3 conidia/mL and 24-hours post exposure whereas the highest mortality (78%) was recorded at 3.3×10^6 conidia/mL and 72-hours post exposure. LC 50% and 90% for the larvae was found to be 1.6×10^8 and 4.2×10^9conidia/mL at 24-hours; 2.0×10^4 and4.0×10^5 conidia/mL at 48-hours; 1.3×10^3 and 3.2×10^4 conidia/mL at 72-hours.
Conclusion: These results indicated that *Aspergillis flavus* conidia suspension are pathogenic to immature stage of *Anopheles* mosquito and could be suggested as a biological control for mosquito management.

**Corresponding author: E-mail: topa2020@gmail.com;*

Keywords: Aspergillus flavus; anopheles mosquito; bioassay; larvicide.

1. INTRODUCTION

"Mosquitoes are known vectors of human and animal pathogens. Millions of people are killed by mosquito-borne diseases every year such as malaria, dengue, chikungunya, yellow fever, encephalitis and filariasis" [1]. "Vector control sanitation, habitat disruption and personal protection from mosquito bites are the most adopted measures employed to control and protect people from infection of these diseases" [2]. "Over the past few decades, many countries organized official programs of mosquito vector control. Currently, synthetic chemical insecticides against adults or larvae have been the main stay and are the most widely used for control of malaria vectors. Mosquito larvae are the attractive targets for these insecticides because mosquitoes breed in water and thus, it is easy to deal with them in this habitat" [3]. "The indiscriminate use of chemical insecticides to target adult mosquitoes results to problems such as mosquito resistance, environmental contamination, and health risk to humans and non-target organisms. To reduce these problems, there is an urgent need to develop alternatives to conventional chemical insecticides, which are safe, effective, biodegradable and highly selective. There has been an increasing awareness in the use biological control agents as alternative to chemical control of mosquitoes. Among the eminent biological control agents are entomopathogenic microorganisms such as fungi and bacteria" [4].

"In recent years, interest on mosquito-killing fungi is reviving, mainly due to continuous and increasing levels of insecticide resistance and increasing global risk of mosquito-borne diseases. Historically, both environmental and biological controls of mosquitoes were exclusively aimed at larval stages and as such have been successful in a variety of geographical and ecological settings within the class of Dueteromycetes, especially Ascomycetes that have entomopathogenic fungi such as *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces formosus* species" [5].

"Fungal biocontrol agents are the most essential among all the entomopathogenic microorganisms due to easy delivery, chances to improve formulation, vast number of pathogenic strains known, easy engineering techniques and its ability to control both sap sucking pests such as mosquito and aphids as well as pest with chewing mouth parts. They include several phylogenetically, morphologically and ecologically diverse fungal species which evolve to exploit insects with their main route of entry being through the insect's integument, by ingestion or via wounds or trachea" [6]. "In general mosquitoes have shown susceptibility towards entomopathogenic fungi and their extracts. They have low toxicity to non-target organisms and using entomopathogenic fungi as larvicides may be a promising lead for biological control of mosquitoes due to their selective toxicity and ready decomposability in the ecosystem" [7,8]. Conidia suspensions and extract of different entomopathogenic fungi, notably *Aspergillus fumigatus, Aspergillus terreus, Aspergillus nidulans, Lecanillium lecanni, Hirsutella thompsoni, Paecilomyces formosus*, *Meterhizium anisopliae*, *Beaveria bassiana*, *Lagenidium giganteum*, among others have been reported to exhibit promising larvicidal activity against mosquito larvae [9-12]. In view of this, this research focuses on evaluating larvicidal efficacy of conidial suspension of *Aspergillus flavus* on *Anopheles* mosquito.

2. MATERIALS AND METHODS

Fungal growth medias and selective proteins such as Potato dextrose agar, Czapek'sdox agar/ broth, Cetyl-trimthyl ammonium bromide (CTAB), Chloramphenicol, synthetic chemical larvicides/ insecticides such as Malathion (781.25 mg/L), Temephos (156.25 mg/L) as well as chemicals used for fungal identification procedures such as Tween-20, Lacto-phenol cotton blue were supplied by the department of biochemistry, Bayero University, Kano. DNA extraction kit and PCR reagents were purchased from Sigma-Aldrich Inc., USA while laboratory apparatus and machineries used in this research were obtained from Biochemistry department laboratories and Microbiology department laboratory complex, Bayero University, Kano, Nigeria.

2.1 Collection of Soil Sample

Soil sample about (200 g) was collected from insect hibernation site including fields characterized by soil with a lot of leaf litters that typically cover the ground and grasses, shrubs and shades of trees at a depth of 0-20 cm using trowel after removing litter or weeds and placed

in appropriately labelled plastic bags within Bayero University Kano premises (11.9836°N 8.4753°E). Before use, samples were thoroughly mixed and passed through 0.4 mm mesh sieve for breaking of soil lumps [13].

2.2 Isolation of Entomopathogenic Fungi from Soil

Soil sample about (200 g) was collected from insect hibernation site including fields characterized by soil with a lot of leaf litters that typically cover the ground and grasses, shrubs and shades of trees at a depth of 20 cm using trowel after removing litter or weeds and placed in appropriately labelled plastic bags within Bayero University Kano premises (11.9836°N 8.4753°E). Before use, samples were thoroughly mixed and passed through 0.4 mm mesh sieve for breaking of soil lumps [14].

2.3 Isolation and Identification of *Aspergillus flavus*

The fungus was isolated from soil using soil suspension procedures [15]. Soil suspension was prepared by weighing 0.1g of soil into 10mL 0.05% Tween-20. 100µL of the soil suspension was inoculated into a perti-dishes of solidified Czapek's media (3g NaNO3, 0.5g MgSO4.7H2O, 1g K2HPO4, 0.5g KCl and FeSO4.7H2O), supplemented with 0.6 g/L of CTAB and 0.1 g/L of streptomycin. The plates were incubated at room temperature in the dark for 3-5days. Micro and macro morphological characteristics of the isolate was used for identification of fungal genus [6,16].

2.4 Formulation of Conidial Suspension

Fungal conidiosphore was harvested from 10days old culture in 0.05% Tween-20 (used as negative control), its concentration was determined using hemocytometer, after which, four concentrations (3.3×10^6, 3.3×10^5, 3.3×10^4 and 3.3×10^3conidia/ml) were formulated by serial dilution [2,17].

2.5 Mosquito Larvae Collection, Identification and Maintenance

Mosquito larvae collected from stagnant water from Auyo Local Government Area of Jigawa State were brought and maintained in the insectaria laboratory at a temperature of 27^{0}C, relative humidity of about 70% and a photoperiod of 12L: 12D h. Anopheles larvae were identified using morphological and behavioral characteristics as described by Gilles and Coetzee [15]. Fourth instars of Anopheles larva were transferred into separate containers and were maintained according to WHO-2005 protocol [2].

2.6 Bioassay

Bioassay was conducted according to WHO-2005 protocol with slight modification.

A set of 5 disposable cups each containing 15 fourth instars larvae. 4 cups were treated with one concentration of conidial suspension prepared as stated above, while the remaining cup was treated with 0.05% Tween-20 as negative control. The whole experimental set-up was prepared in triplicate and the result was reported as average of the three replicates [12].

2.7 Determination of LC50 and LC90

LC50 and LC90 was determined using empirical probit analysis.

3. RESULTS

Aspergillus flavus was identified using macro and micro-morphological characteristics of the cultured fungal isolate. Based on the observed characteristics; colonies have distinct margin and are covered with fluffy well developed aerial plane mycelium on the surface and culture appears yellow green when young and turns jade green as the culture ages. Spores were spherical in shape and colonies consisting of dense conidiosphores as shown in Figs. 1 and 2.

This study reveals the larvicidal potential of conidia suspension of the entomopathogenic fungus (*Aspergillus flavus*) against 4th instar larvae of *Anopheles species*. The results showed that; mortality increases with increase in conidial concentration and exposure time. The lowest mortality (12%) was recorded at 3.3×10^3 conidia/mL and 24-hours post exposure whereas the highest mortality (78%) was recorded at 3.3×10^6 conidia/mL and 72-hours post exposure. The lethal concentration of conidial suspension causing 50% and 90% mortality of the larvae was found to be 1.6×10^8 and 4.2×10^9conidia/mL at 24-hours; 2.0×10^4 and 4.0×10^5 conidia/mL at 48-hours; 1.3×10^3 and 3.2×10^4 conidia/mL at 72-hours as shown in Table 1.

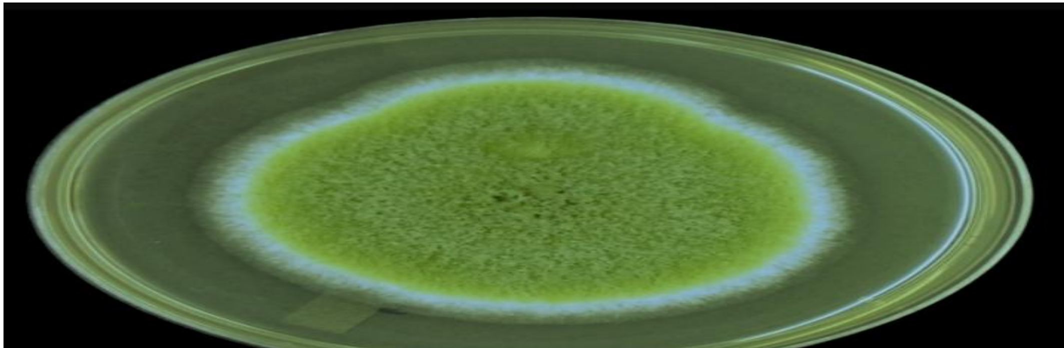

Fig. 1. Two weeks old mono-cultured plates of fungal isolate

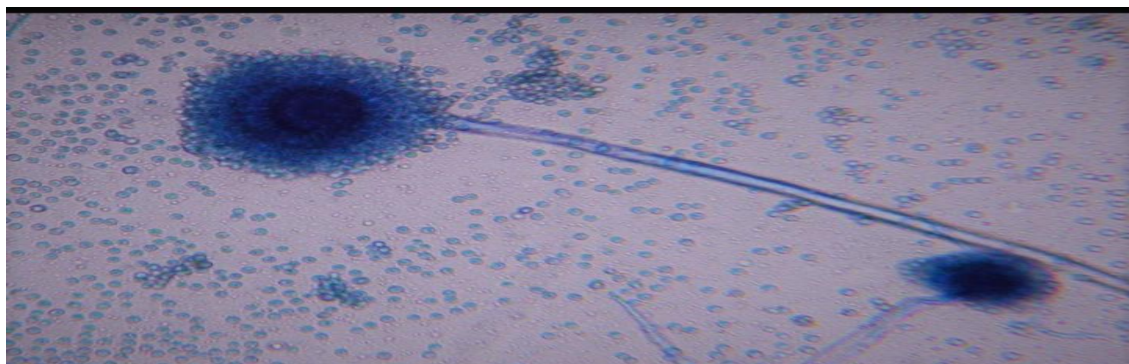

Fig. 2. Micro Slide Image of the Fungal Isolate viewed under X100 magnification

Table 1. Larvicidal efficiency of *Aspergillus flavus* conidial suspension against early 4th instar larvae of *Anopheles* mosquitoes

Exposure time	Concentration (conidia/ml)	Percentage Mortality	Probit equation	LC_{50} (conidia/ml)	LC_{90} (conidia/ml)
24_Hours		43	y = 0.90x + 3.30	$1.6×10^8$	$4.2×10^9$
	$3.3×10^6$	26			
	$3.3×10^5$	18			
	$3.3×10^4$	12			
48_Hours	$3.3×10^3$		y = 0.85x + 3.10	$2.0×10^4$	$4.0×10^5$
	$3.3×10^6$	63			
	$3.3×10^5$	50			
	$3.3×10^4$	27			
72- Hours	$3.3×10^3$	14	y = 0.80x + 3.00	$1.3×10^3$	$3.2×10^4$
	$3.3×10^6$	78			
	$3.3×10^5$	64			
	$3.3×10^4$	33			
	$3.3×10^3$	25			

Positive control group (treated with 1ml 0.05% tween-20 and distilled water) records no mortality.

4. DISCUSSION

The findings in this study support the findings of Bogus et al. [19] who found that, an increase in the concentration of conidial suspension and time generally increase the percentage mortality.

The study also corroborate the findings of Sani et al. [12] who reported that the mortality percentage of *Paecilomyces* spp against *culex* mosquito larvae to be up to 80% after 96 h post treatment. Thomas et al. [20] also in his findings reported that the mortality percentage of *Aspergillus fumigatus* against *culex* mosquito reaching up to 96% after 72 h post treatment. Gayathri et al. [15] reported that the pathogenicity of *Paecilomyces fumosoroseus*

against *Culex quinquefasciatus* was 97.73% mortality on 8th day after treatment with10^8 conidia/mL. In this study, pathogenicity varied according to concentration of conidial suspension and period of exposure. These findings further support the idea of Al-Hussaini and Hergian, [21] and Benserradj and Mihoubi [22] who reveal that larval mortality percent and LC50 of *Culex quinquefasciatus* increased as exposure periods increased.

"Furthermore, a research conducted in East Africa to determine the pathogenicity of entomopathogenic fungi against adult of *Anopheles gambiae.* The study revealed a high infection rates ranging from 46 to 88% with *Metarhizium anisopliae* which being the most pathogenic strain" [15]. "A study was also conducted in Asia for the larvicidal potential of *Lagenidium giganteum*, a water weed, leading to its efficacies in killing the tested vectors with appreciable safety to non-target organisms and good biological stability" [16]. "According to alarge-scale field trial conducted in the UnitedStates, mycelium extract of *Lagenidium giganteum* caused 40-90% infection rates in *Culex tarsalis* and *Anopheles freeborn sentinel* larvae" [23]. "The potentials of many fungi have been established for mosquito control, nevertheless, only a few have received commercial attention and are marketed for use in vector control programs globally" [6].

"The basic mechanism of pathogenesis behind is entrance through the external integument. Besides, infection through digestive tract is also possible" [23]. "Conidia attach to the cuticle, germinate and penetrate the cuticle. Once in the hemocoel, the mycelium grows and spreads throughout the host, forming hyphae and producing blastospores. Humidity is a key factor for high and rapid killing of insects by entomopathogenic fungi, and further development on cadavers" [24,25].

5. CONCLUSION

Conidia suspension of *Aspergillus flavus* have promising larvicidal activity against *Anopheles* mosquito larvae, the vector of *Plasmodium* parasite that causes malaria which is widely distributed in the Northern guinea savannah vegetation of Nigeria. The mortality was observed based on concentration and exposure time for conidial bioassay irrespective of the concentration [26-37].

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENTS

We are indebted tothe entire staff members of Biochemistry Department of Bayero University, Kano, Nigeria for their mentorship and support towards the success of this research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Posadas JB, Comerio RM, Mini JI, Nussenbaum AL, Lecuona RE. A novel dodine-free selective medium based on the use of cetyl trimethyl ammonium bromide (CTAB) to isolate Beauveria bassiana, Metarhizium anisopliae sensu lato and Paecilomyces lilacinus from soil. Mycologia. 2012 Jul 1;104(4):974-80.
2. World Health Organization (WHO). Global Programme to eliminate lymphatic fillariasis; 2013.
   Available: www.who.int.
3. Arivoli S, Ravindran JK, Tennyson S. Larvicidal Efficacy of plant Extracts against the malaria vector Anophelesstephensis Liston (Diptera: Culicidae). World Journal of Medical Scence. 2012;7:77-80.
4. Bogus K, Zonneveld KA, Fisher D, Kasten S, Bohrmann G, Versteegh G. Anopheles gambiae Distribution and Resistance in the Cities of Douala and Yaounde (Cameroun): Influence of Urban Agriculture and Pollution. Malaria Journal. 2007;10:154.
5. Houbraken J, Verweij PE, Rijs AJ, Borman AM, Samson RA. Identification of Paecilomyces variotii in clinical samples and settings. Journal of Clinical Microbiology. 2010 Aug;48(8):2754-61.
6. White TJ, Bruns TD, Lee SB, Taylor JW. Analysis of phylogenetic relationships by

amplification and direct sequencing of ribosomal genes. In: Innis M. A. PCR protocols. Academic Press, New York. 1990;315-322.

7. Augustyniuk-Kram A, Kram KJ, Lo JA. Entomopathogenic Fungi as an Important Natural Regulator of Insect Outbreaks in Forests (Review). Forestecosystem-morethan Just Trees. 2012;265-94. Available onwww.intechopen.com.
8. Thomas D, Rai1 V, Updhyay P, Mehra M, Rana A, Pandey K. Potential of entomopathogenic fungi as biopesticides. Department of Plant Pathology, College of Agriculture, G. B. Pant University of Agriculture & Technology, Pantnagar (Uttarakhand), India Journal of Sciences Research and Technology. 2016;2(5):7-13.
9. Dong T, Zhang B, Jiang Y, Hu Q. Isolation and Classification of Fungal Whitefly Entomopathogens from Soils of Qinghai-Tibet Plateau and Gansu Corridor China. Journal PLOS ONE. 2016;11(5):e0156087
10. Gayathri G, Balasurbramaninan C, Vinayagamorthi B, Kubendran T. Larvicidal Potential of Beauveria bassiana (Balsamo) vuillemin and Paecilomyces fumosoroseus (wize). Browan and simith on Culex quinguefasiafus (say). Journal of Biopesticide. 2010;3(1):147-151.
11. Melo N, Qiang-shen WU, Yin-Wing Z, Gui-yuan W. Arbuscular mycorrhizal fungi and acclimatization of micropropagated citrus. Communication in soil science and plant analysis. 2006;42(15):1825-1832.
12. Scolte EJ, Knols BGJ, Samson RA, Takken W. Entomopathogenic fungi for Mosquito control: A review. Journal Insect Science. 2004;4:19.
13. Abdullah SK, Mustapha RA, Assaf LH. Isolation of selective entomopathogenic and opportunistic fungi from soil in Duhok province, Kurdistan region of Iraq by different selective isolation media. Journal of Biology, Agriculture and Healthcare. 2015;5(4):73–80.
14. Ragavendran C, Nawal KD, Natarajan D. Beauveriabassiana (Clavicipitaceae): A potent fungal agent for controlling mosquito vectors of Anopheles stephensi, Culex quinquefasciatus and Aedes egypti (Diptera: Culicidae), Royal Society of Chemistry Advances. 2017;7:3838-4350.
15. Gillies MT, Coetzee M. A supplement to the Anophellinae of Africa south of the sahara Publication of the South African. Institute for Medical Research. 1987;55:140-143.
16. Samson RA, Yilmaz N, Houbraken J, Spierenburg H, Seifert KA, Peterson SW, Varga J, Frisvad JC. Phylogeny and nomenclature of the genus Talaromyces and taxa accommodated in Penicillium subgenus Biverticillium. Studies in Mycology. 2011;70:159-83.
17. Driver F, Milner RJ, Trueman JWH. A taxonomic revision of Metarhizium based on a phylogenetic analysis of rDNA sequence data. Journal of Mycology Research. 2000;104:143-150.
18. Gumus T, Demirci AS, Sagdic O, Arici M. Inhibition of heat resistant molds: Aspergillus fumigatus and Paecilomyces variotii by some plant essential oils. Journal of Food Science and Biotechnology. 2010;19:1241–1244.
19. Bukhari T, Willem T. Development of Metarhizium anisopliae and Beauveria bassiana formulations for control of malaria Mosquito larvae. Parasites and Vectors. 2011;4(1):23.
20. Al-Hussaini M, Hergian A. The Larvicidal effect of four fungal aflatoxins against fourth instar larvae of the mosquito Culex quinquefasciatus Say (Culicidae: Diptera). Journal of Kerbala University. 2014;12(4):79-88.
21. Benserradj O, Mihoubi I. Larvicidal activity of entomopathogenic fungi Metarhizium anisopliae against mosquito larvae in Algeria. International Journal of current Microbiology and Applied Science. 2014;3(1):54-62.
    Bhan, S., Shrankhla, Mohan L, Srivasstava CN. Larvicidal toxicity of Temephos and entomopathogenic fungus, Aspergillus flavus and their synergistic activity againstmalaria vector, Anopheles stephensi. Journal of Entomology and Zoology Studies. 2013;1(6):55-60.
22. Anastasiadis IA, Giannakou IO, Prophetou-Athanasiadou DA, Gowen SR. The combined effect of the application of a biocontrol agent Paecilomyces lilacinus, with various practices for the control of root-knot nematodes. Journal of Crop Protection. 2008;27:352–361.
23. Livak KJ. Organisation and Mapping of sequence on the Drosophila Melanogaster X and Y chromosomes that is transcribed during spermamatogenis Genetics Journal. 1984; 107:611-634.

24. Sani I, Yusuf U, Umar KM. Larvicidal Efficacy ofEntomopathogenic Fungus, Metarhizium anisopliaeagainst Culex quinquefasciatus Say (Diptera: Culicidae),Annals of Experimental Biology. 2016;4(4):17-21.
25. Araújo JPM, Hughes DP. Diversity of entomopathogens Fungi : Which groups conquered the insect body ? Advances in Genetics. 2016;94:1–39.
26. Freed S, Feng-Liang J, Naeem M, Shun-Xiang R, Hussian M. Toxicity of Proteins Secreted by Entomopathogenic Fungi against *Plutella xylostella* (Lepidoptera : Plutellidae). International Journal of Agriculture and Biology. 2012;14:291–295.
27. Hussein HM, Skokova HO, Půža V, Zemek R. Laboratory Evaluation of Isaria fumosorosea CCM 8367 and Steinernema feltiae Ustinov against Immature Stages of the Colorado Potato Beetle. PLOS ONE Journal. 2016;11(3):14-20.
28. Masahira AH. Terrecyclic Acid A, A New Antibiotic from Aspergillus terreus, (July). 1982;778-782.
29. Mazid S, Rajkhowa RC, Kalita JC. Pathogenicity ofAspergillus niger and Aspergillus flavus on red spidermite (Oligonychus coffeae Nietner), a serious pest of tea,Journal of Entomology and Zoology Studies. 2015;3(3):11-13.
30. Mohammed GJ, Kadhim MJ, Hussein HM,Characterization of Bioactive Chemical Compounds fromAspergillus terreus and Evaluation of Antibacterial andAntifungal Activity, International Journal of Pharmacognosy and Phytochemical Research. 2016;8(6):889-905
31. Odalo J, Omolo M, Malebo H, Angira J, Njeru P, Ndiege I. Repellance of essential oil of some plants from Kenya Coast againstAnopheles gambiae. Acta Tropica. 2005;295:210-18.
32. Richards HA. Entomopathogenicfungal identification, Nevada. USA. 2005;5-32.
33. Samson RA. Paecilomyces and some allied hyphomycetes Studies in Mycology.1974;6:1-119.
34. Soni N, Soam P. Effect of Chrysosporiumkeratinoplulum metabolites against Culexquinquefasciantus after chromatographicpurification. Parasitol Research. 2010;107:1329-1336.
35. Thomas MB, Raper KB. A manual of the Aspergillus. Mycological Research. 1945;101:1469-1474.
36. Watarabe T. Pictorial Atlas of soil and seedfungi morphologies of cultured fungi. 2010;295-296.
37. World Health Organization. Guidelines for laboratoty and field testing mosquito larvicides; 2005. Available:http://www.who.int/iris/bitstream/10665/69101/1/WHO CDS WHOPES GCDPP 13.pdf

© 2022 Suleiman et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (Available:http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/88124