JSR Publications

Available Online

J. Sci. Res. **12** (1), 93-102 (2020)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Alteration of Insecticide Resistance during the Aging of *Bactrocera cucurbitae* (Diptera: Tephritidae)

D. Verma, S. Kumari, D. Kumar*

Department of Zoology, Banaras Hindu University, Varanasi–221005, India

Received 12 May 2019, accepted in final revised form 1 October 2019

Abstract

The aim of the present investigation was to quantify the detoxification and stress enzymes' levels and correlate it with insecticides resistance in different age groups of *Bactrocera cucurbitae* population. Different age groups of insect population were isolated and processed for enzymes assay and survival analyses.The increase in the activities of CytP450, esterase and superoxide dismutase (SOD) have been observed till certain age and thereafter they decreased. However, activity of glutathione-S-transferase (GST) and catalase did not increase with age. Results of survival assay showed that deltamethrin and malathion caused higher mortality in the 15-days old (100%) insects than the 1-day old adult insects. Highest survival percentage after deltamethrin treatment was found in 7 days old insects, whereas malathion treated insects' survival was maximum in 3 days old insect population. Based on the above results, it is inferred that lack of sufficient GST and catalase cause decrease in the resistance of *B. cucurbitae* with advancing age.

Keywords: Survival; Deltamethrin; Malathion; Detoxification Enzymes, Susceptible

doi: http://dx.doi.org/10.3329/jsr.v12i1.41336 J. Sci. Res. **12** (1), 93-102 (2020)

1. Introduction

Bactrocera cucurbitae, the melon fly, is distributed widely in temperate, tropical, and sub-tropical regions of the world. It damages about 81 host plants and especially it is a severe pest of cucurbitaceous plants all over the world. The scope of losses may range from 30 to 100%, assuming the host plant as well as season [1]. At present, strategies like sterile insect release and mass trapping are applicable to manage the number of melon flies. However, chemical insecticides remain a key tool amongst the farmers [2]. Pyrethroids, carbamates, organophosphates and spirotetramet are common amongst farmers to control this pest [3,4]. Yet increased dependency on these insecticides has exacerbated the condition, and prompted the resistance in the *Bactrocera sp*. To date, many reporters have noticed the resistance against pyrethroid, spirotetramet as well as carbamate in *Bactrocera*

* *Corresponding author:* dinesh195360@gmail.com

genus. Insecticide resistance in insects depends upon the elimination of toxin from the body and the adaptation of the target sites. Elevated enzymatic detoxification has been linked to three crucial enzyme families viz Cyt P450s, glutathione-S-transferase (GST) and esterase [5-7]. Nevertheless, cuticle adds further resistance by acting as a barrier and allocates sufficient time required for detoxification [8].

The activity of detoxification enzymes rely on the physiological status and it varies with age. Hence, it seems that age may play an influential role in insecticide resistance. Moreover, aging may lead to deterioration of resistance phenomenon as a repercussion of continuous decline in the physiological ability of insects to eliminate the insecticide [9]. Disturbed physiology of insects results in the impaired ability to respond against environmental changes. Determining resistance in the early stages may not provide a proper assessment of resistance in an insect population. Therefore, it is necessary to characterize insecticide resistance in older *B. cucurbitae* population too. In *Anopheles* mosquitoes, age plays an important role in insecticide resistance. Rowland and Hemingway reported that malathion resistance perished in aging heterozygotes as well as in homozygous resistant and susceptible *Anopheles stephensi* [7]. Lines and Nassor observed similar kind of responses in *An. gambiae* wherein mortality increased after application of dichlorodiphenyltrichloroethane (DDT) from 5 to 90% from 1 to 14 days old [10]. As aging proceeds, damages accumulate due to the lipid peroxidation, protein carbonyls and alteration in DNA structure [11,12]. These findings continued with the selected population of *Anopheles* sp. male and female of F3 generation wherein it was observed that resistance was on peak in 3 days old insect. Then it drops down onwards against lambda-cyhalothrin [13]. Even after successive selections for DDT resistance through 16 generations, a decline in the resistance with age was persistent [14].

Studies with resistant strains of *An. Stephensi* and *An. Gambiae* indicated that knockdown time declined by 33% to 44% in 10 days old mosquitoes as compared to the newly emerged mosquitoes [9]. The influence of age was also observed in 14 days old mosquitoes that was rapidly knock down in comparison to 3 days old [15]. Mortality assay showed that older mosquitoes were more susceptible to permethrin and propoxur in comparison to younger one [13,16].

Till date most of the studies on the effect of age on insecticide resistance have been investigated in *Anopheles* mosquitoes. But it is yet to be studied in other insect pests. Further, *B. cucurbitae* infests and lay eggs on the host plant in the later stage of its life cycle. So it is essential to aim older insects and determine insecticide resistance in the older insects. The aim of the present work was to quantify the detoxification enzyme levels and correlate it with insecticides resistance indifferent age groups (1, 3, 5, 7, 9, 11, 13 and 15 days) of *B. cucurbitae* population.

2. Materials and Methods

2.1. *Bactrocera cucurbitae rearing and its maintenance*

B. cucurbitae infested fruits were collected from the agricultural field abounding Varanasi, India. These infested fruits contain larva. Adult insects were fed on sugar + yeast powder, water soaked cotton (25±2 °C) and photoperiod of (12D:12L). Yeast powder acts as a source of protein. *B. cucurbitae* laid eggs on the pumpkin, which was transferred to the jar containing sand. Larvae were developed inside the fruit and pupate in the sand. Different age groups (1, 3, 5, 7, 9, 11, 13 and 15 days) of adult *B. cucurbitae* were isolated and used for enzyme assay and survival analyses.

2.2. *Cytochrome P450 assay*

Cytochrome P450 activity was quantified by an indirect assay using 200 µL of 3,3,5,5-tetra-methylbenzidine (TMBZ) as a substrate, 3% H_2O_2 (50 µL), 20 µL of enzyme stock and phosphate buffer (pH 6.8) [17]. Absorbance was noted down at 630 nm. Cytochrome P450 activity was expressed as equivalent units (EU) of cytochrome C/mg of protein.

2.3. *Esterase assay*

Esterase activity was quantified in 3 mL of reaction mixture by using 1 mL of α-naphthyl acetate (0.3 mM) as substrate, 250 µL of 1% fast blue, 10 µL of enzyme stock and phosphate buffer [18]. Absorbance was taken at 590 nm by UV-VIS spectrophotometer (Systronic 119).

2.4. *Glutathione-S-transferase assay*

GST was assayed according to Habig *et al*. [19]. 1 mL of reaction mixture consists of 15 µL of CDNB (2,4-dinitro-chlorobenzene), 50 µL of reduced glutathione, 10 µL of enzyme stock and 915 µL phosphate buffer(pH 6.8). Absorbance (340 nm) were noted down for 5 min at the regular interval of 1 min ($\varepsilon = 9.6\ M^{-1}$).

2.5. *Superoxide Dismutase assay*

SOD activity was assayed according to the Beauchamp and Fridovich [20]. 1 mL of reaction mixture consists of 10 mM of methionine, 30 mM of NBT, 3 µm of riboflavin, 20 µL of enzyme stock and phosphate buffer (pH 6.8). Absorbance was taken at 560 nm by a UV-VIS spectrophotometer. One unit of SOD activity is equal to the enzyme required to inhibit NBT reduction of by 50%.

2.6. *Catalase assay*

Catalase was assayed according to the method proposed by Aebi with slight modification [21]. 1 mL of reaction mixture consist of 18 mM H_2O_2 (50 µL), 10 µL of enzyme stock and phosphate buffer (pH 6.8). The decrease in absorbance was recorded at 240 nm for 3 min by a UV-VIS spectrophotometer. One unit of catalase activity was equal to the enzyme required to consume 1 µM of H_2O_2/min ($\varepsilon = 40\ M^{-1}\ cm^{-1}$).

2.7. *Protein estimation*

The whole body homogenate of *B. Cucurbitae* was prepared in phosphate buffer (pH 6.8) and used for protein estimation [22] and enzyme assay.

2.8. *Survival analysis*

B. cucurbitae (n = 30) of different age groups (1, 3, 5, 7, 9, 11, 13 and 15 days) were treated with 1µL of deltamethrin (0.2 ng/mL) and 1µL of malathion (0.05 µg/mL) on thoracic segment separately. Survival percentage was recorded after 24 h of treatment.

2.9. *Statistical analysis*

Data of enzyme activity and survival percentage of *B. cucurbitae* of different age groups were analyzed by using one-way ANOVA followed by post-hoc (Dunnet test) test. The significance level was determined at $p < 0.05$ for all analyses. All statistical analyses were performed using the Graph pad Prism 5.

3. Results and Discussion

Aging appears to be a crucial factor in the establishment of insecticide resistance in insects [6]. Therefore, the critical effect of age on the insecticide resistance in *B. cucurbitae* was investigated. The continuous increase in insecticide resistance has become a serious threat to the efficiency of insecticide-based pest control tactics. The present study demonstrates the inconsistency in the expression of resistance properties in *B. cucurbitae* population of different age groups. Previous studies have revealed that different detoxifying enzymes like GST and esterases play a pivotal role in insecticide resistance of mosquitoes [6,7]. Currently, in order to carry out the pilot study, the activities of various detoxifying enzymes and stress enzymes had been quantified. The activities of detoxifying enzymes (GST, Cyt P450 and esterase) and stress enzymes (SOD, catalase) show variations with increasing age.

The Cyt P450 activity increased from day 1 onwards and reached the maximum at day 9 and then started to decrease till day 15. Results show that there is a significant difference when compared with different age groups with the control ($p = 0.0004$, $F =$

7.785) (Fig. 1). Moreover, continuous increase in Cyt P450 activity till 9th day is supported by the survival assay, wherein the highest survival percentage was recorded in 7 days old insects after deltamethrin exposure (Fig. 2). Resistance to pyrethroids in the melon fly, is linked with the combined action of elevated Cyt P450 and GST activity [23]. However, in the present study, Cyt P450 activity increased with age, but GST activities ($p = 0.454$, $F = 1.021$) did not change significantly with advancing age (Fig. 3). Insecticides' metabolization has two phases: Phase I and II. Cyt P450 and esterase enzymes are associated with Phase I reaction, while GST is involved in Phase II reaction [18]. In Phase I, addition of polar groups to the xenobiotics (insecticides) substances takes place. Thus resultant metabolite enter into Phase II reactions and conjugates with water soluble compounds in the presence of GST enzyme. After Phase II reaction, toxins are excreted out from the body [18]. Our results reveal that activities of Cyt P450 increases with age, while GST activity does not change significantly. Therefore, increased susceptibility of insecticides with increasing age could be explained due to lack of sufficient amount of GST enzyme to complete the Phase II reaction. Thus incomplete metabolization of insecticide leads to oxidative stress causing the death of the insects. Furthermore, it has been suggested that greater resistance of the younger mosquitoes against pyrethroids is attributed to the higher physiological activity of young mosquitoes compared to older one [24-26]. Lower physiological activity in older mosquitoes is due to loss of energy with advancing age and thereby affects the resistance mechanisms negatively [25,26].

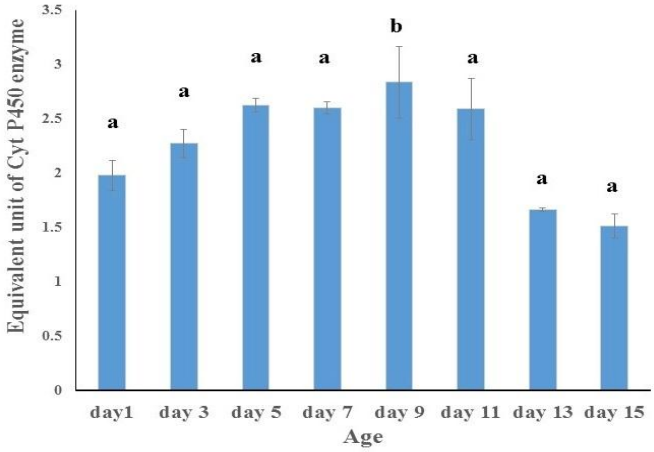

Fig. 1. Cytochrome P450 activity in different age groups (1, 3, 5, 7, 9, 11, 13 and 15 days) of adult *B. cucurbitae*. Data is presented as Mean ± SEM. Bars having different alphabets (a, b) are significantly different from day 1.

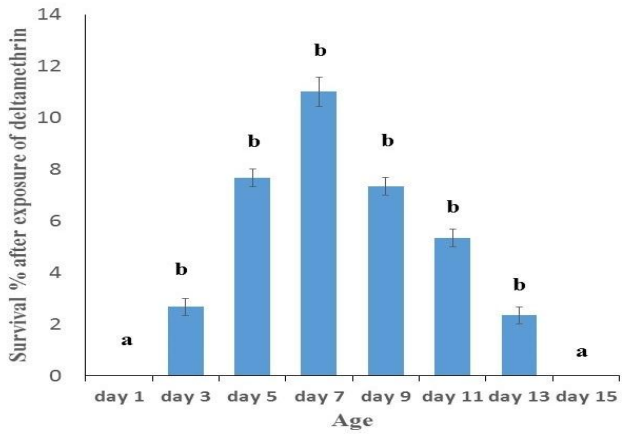

Fig. 2. Survival percentage after topical application of deltamethrin on adult *B. cucurbitae.* Data is presented as Mean ± SEM. Bars having different alphabets (a, b) are significantly different from day 1.

Esterase activity was observed significantly high in 9, 11 and 13 days old population ($p = 0.0008$, $F = 6.67$) in comparison to 1 day old population (Fig. 4). However, survival assay revealed that resistance of malathion treated ($p = 0.0001$, $F = 113.1$) *B. cucurbitae* was maximum in 3 days old population and later on its survival decreased (Fig. 5). Esterase enzyme is associated with the Phase I metabolisation while GST involve in Phase II metabolisation of malathion (organophosphate). Higher esterase activity complete the Phase I reaction. But lack of sufficient amount of GST leads to incomplete Phase II metabolisation of malathion. This could be a possible reason of increased malathion susceptibility with the increasing age. Nonetheless, decreased physiological activity with the advancing age may also cause the increased malathion susceptibility with the age [24-26]. Moreover, these results also reveal the higher susceptibility of *B. Cucurbitae* against malathion in comparison to deltamethrin.

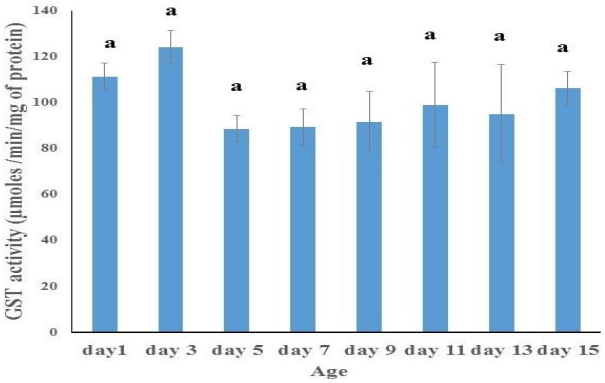

Fig. 3. GST activity in different age groups (1, 3, 5, 7, 9, 11, 13 and 15 days) of adult *B. cucurbitae.* Data is presented as Mean ± SEM.

Reactive oxygen species (ROS) are detoxified by the combined activity of SOD and catalase enzymes [27]. In the present study, SOD activity was significantly high in 5 and 7 days old insects population ($p = 0.0001$, $F = 30.75$). Thereafter it significantly decreased in 13 and 15 days old population (Fig. 6). Whereas catalase ($p = 0.45$, $F = 1.027$) activity did not vary significantly across the all age groups of *B. cucurbitae* (Fig. 7).

Parashar *et al.* in *Drosophila* have reported age dependent decline in the detoxification of oxygen radicals from the insect body and thereby increase the susceptibility against the paraquat [28]. Therefore, reduction in insecticide resistance could be further explained due to increased oxidative stress in aging insects [29-31]. Role of catalase in detoxification also supports the idea as the enzymes contribute to the resistance by protecting the tissues from oxidative damage associated after exposure of insecticides [24,32,33]. In the present study, *B. cucurbitae* population did not show increased activity of catalase enzyme. This could be a plausible explanation of reduction in resistance properties in older *B. cucurbitae* population.

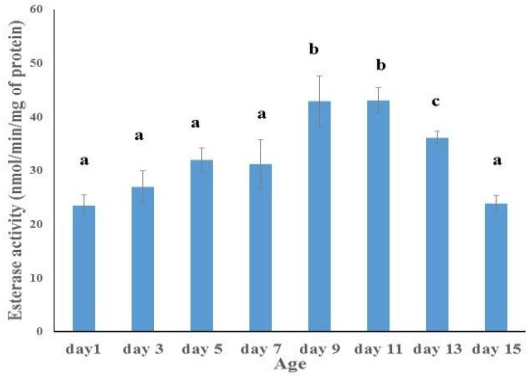

Fig. 4. Esterase activity in different age groups (1, 3, 5, 7, 9, 11, 13 and 15 days) of adult *B. cucurbitae.* Data is presented as Mean ± SEM. Bars having different alphabets (a, b, c) are significantly different from day 1.

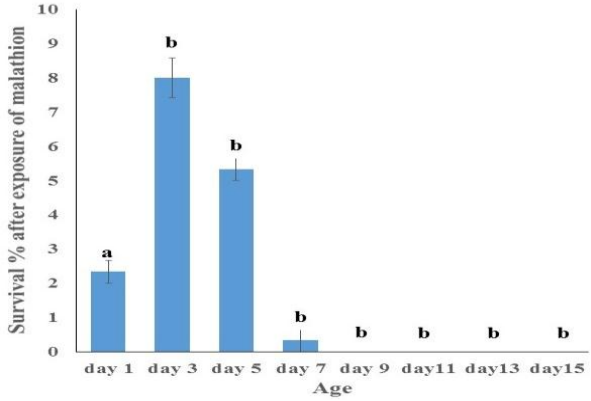

Fig. 5. Survival percentage after topical application of malathion on adult *B. cucurbitae*. Data is presented as Mean ± SEM. Bars having different alphabets (a, b) are significantly different from day 1.

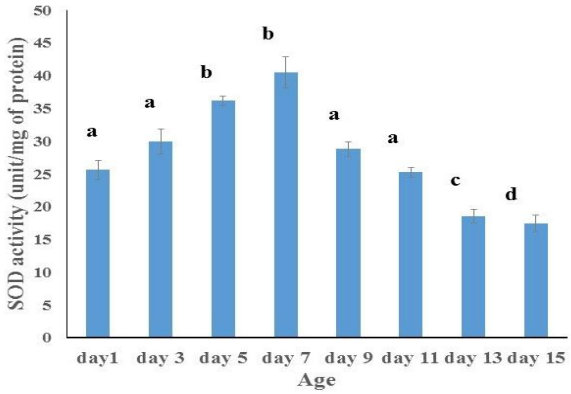

Fig. 6. SOD activity in different age groups (1, 3, 5, 7, 9, 11, 13 and 15 days) of adult *B. cucurbitae.* Data is presented as Mean ± SEM. Bars having different alphabets (a, b, c, d) are significantly different from day 1.

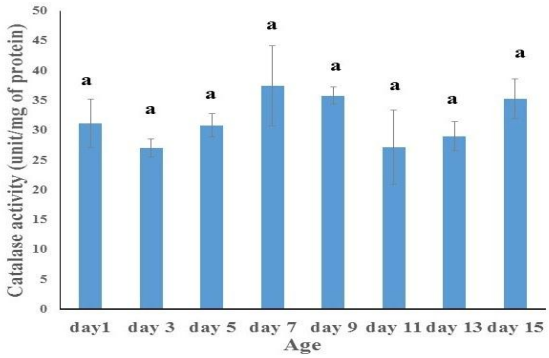

Fig. 7. Catalase activity in different age groups (1, 3, 5, 7, 9, 11, 13 and 15 days) of adult *B. cucurbitae.* Data is presented as Mean ± SEM. Bars having different alphabets (a) are significantly different from day 1.

Age might be a key factor in the determination of insecticide resistance in insects. From the results of survival assay, we could say that age has a significant effect on the survival of adult insects. Exposure of deltamethrin and malathion caused higher mortality in the 15-days old (100%) than in the 1-day old *B. cucurbitae* population. In mosquitoes, not only metabolic enzymes dependent resistance diminishes with age, but mosquitoes having target site mutation also show similar kind of response to the insecticides. This indicates kdr mutation alone is not sufficient to empower mosquitoes to withstand the exposures of insecticide with age [14,15]. In the present investigation, fall in resistance with age is not parallel with detoxification enzymes activity, which could be attributed to senescence [25], enhancement in cuticle permeability and/or slower xenobiotic excretion [34,35]. Furthermore, persistence of all the mechanisms at the same time results in a fitness cost that might weaken the older insects and render them more vulnerable to insecticides [24]. These results strengthen the importance of aging in the determination of

insecticide resistance. The present investigation reveals that aged *B. cucurbitae* do have weaker insecticide resistance in comparison to younger one [10,13,16]. Hunt *et al.* [13] suggested that the display of the resistance phenotype alters with physiological condition of insects, which undoubtedly depends upon age as well as feeding habit.

4. Conclusion

In conclusion, *B. cucurbitae* population loses its tolerance to insecticides at older age. This might have significant impact for the management of *B. cucurbitae*. Targeting older and potentially significant life stage is being proposed as an alternate strategy for *B. cucurbitae* management.

Acknowledgment

The financial assistance in the form of UGC SRF to D. Verma and UGC research fellowship (BHU) to S. Kumari is gratefully acknowledged. This work was also supported by funds from the UGC supported Centre of Advanced Study in Zoology and DST- FIST, Banaras Hindu University, Varanasi, India.

References

1. M. K. Dhillon, R. Singh, J. S. Naresh, and H. C. Sharma, J. Insect Sci. **5**, 40 (2005). https://doi.org/10.1093/jis/5.1.40
2. Y. Roessler, Fruit Flies : Their Biology, Natural Enemies and Control (Elsevier Science Publishers, 1989).
3. J. D. Barry, N. W. Miller, J. C. Piñero, A. Tuttle, R. F. Mau, and R. I. Vargas, J. Econ. Entomol. **99**, 1161 (2006). https://doi.org/10.1093/jee/99.4.1161
4. R. I. Vargas, R. F. Mau, E. B. Jang, R. M. Faust, L. Wong, O. Koul, G. Cuperus, and N. Elliott, Areawide Pest Management: Theory and Implementation (CABI, 2008).
5. R. Feyereisen, Toxicol. Lett. **82**, 83 (1995). https://doi.org/10.1016/S0338-9898(95)80363-7
6. J. Oakeshott, C. Claudianos, P. M. Campbell, R. D. Newcomb, and R. Russell, Comprehensive Molecular Insect Science (Elsevier Science Publishers, 2010)
7. M. Rowland and J. Hemingway, Pestic Biochem Physiol. **28**, 239 (2005). https://doi.org/10.1016/0048-3575(87)90022-8
8. T. E. Nkya, I. Akhouayri, R. Poupardin, B. Batengana, F. Mosha, S. Magesa, W. Kisinza, and J. P. David, Malaria J. **13**, 28 (2014). https://doi.org/10.1186/1475-2875-13-28
9. M. H. Hodjati and C. F. Curtis, Bull. Entomol. Res. **89**, 329 (1999). https://doi.org/10.1017/S0007485399000462
10. J. D. Lines and N. S. Nassor, Med. Vet. Entomol. **5**, 261 (1991). https://doi.org/10.1111/j.1365-2915.1991.tb00550.x
11. B. Halliwell and J. M. C. Gutteridge, Free Radicals in Biology and Medicine (Clarendon Press, 1989).
12. C. B. Harley, Longevity, Senescence, and the Genome (University of Chicago Press, 1992).
13. R. H. Hunt, B. D. Brooke, C. Pillay, L. L. Koekemoer, and M. Coetzee, Med. Vet. Entomol. **19**, 271 (2005). https://doi.org/10.1111/j.1365-2915.2005.00574.x
14. A. Mnzava, R. H. Hunt, and M. Coetzee, Med. Vet. Entomol. **21**, 97 (2007). https://doi.org/10.1111/j.1365-2915.2007.00671.x
15. S. Rajatileka, J. Burhani, and H. Ranson, Trans. R. Soc. Trop. Med. Hyg. **105**, 247 (2011). https://doi.org/10.1016/j.trstmh.2011.01.009

16. K. D. Glunt, M. B. Thomas, and A. F. Read, Plos One **6**, Article ID 24968 (2011). https://doi.org/10.1371/journal.pone.0024968
17. W. G. Brogdon and J. C. McAllister, Emerging Infect. Dis. **4**, 605 (1998). https://doi.org/10.3201/eid0404.980410
18. K. Kranthi, Insecticide Resistance Monitoring, Mechanisms and Management Manual (Central Institute for Cotton Research, 2005).
19. W. Habig, M. Pabst, and W. Jakoby, J. Biol. Chem. **249**,7130 (1974).
20. C. Beauchamp, and I. Fridovich, Anal. Biochem. **44**, 276 (1971). https://doi.org/10.1016/0003-2697(71)90370-8
21. H. Aebi, Enzymol. **105**, 121 (1984). https://doi.org/10.1016/S0076-6879(84)05016-3
22. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R.J. Randall, J. Biol. Chem.**193**, 265 (1951).
23. J. C. Hsu, H. T. Feng, and W. J. Wu, J. Econ. Entomol. **97**, 1682 (2004). https://doi.org/10.1603/0022-0493-97.5.1682
24. M. S. Chouaibou, J. Chabi, G. V. Bingham, T. B. Knox, L. N'Dri, N. B. Kesse, B. Bonfoh, and H. V. Jamet, BMC Infect. Dis. **12**, 214 (2012). https://doi.org/10.1186/1471-2334-12-214
25. C. M. Jones, A. Sanou, W. M. Guelbeogo, N. F.Sagnon, P. C. Johnson, and H. Ranson, Malaria J. **11**, 24 (2012). https://doi.org/10.1186/1475-2875-11-24
26. S. Mbepera, G. Nkwengulila, R. Peter, E. A. Mausa, A. M. Mahande, M. Coetzee, and E. J. Kweka, Malaria J. **16**, 364 (2017). https://doi.org/10.1186/s12936-017-2022-6
27. S. Kumari, D. Verma, and D. Kumar, J. Sci. Res. **11**, 235, (2019). https://doi.org/10.3329/jsr.v11i2.39286
28. V. Parashar, S. Frankel, A. G. Lurie, and B. Rogina, Radiat. Res. **169**, 707 (2008). https://doi.org/10.1667/RR1225.1
29. Y. Ding, F. Ortelli, L. C. Rossiter, J. Hemingway, and H. Ranson, BMC Genomics **4**, 35 (2003). https://doi.org/10.1186/1471-2164-4-35
30. J. H. F. Pedra, L. M. McIntyre, M. E. Scharf, and B. R. Pittendrigh, Proc. Natl. Acad. Sci. U.S.A. **101**, 7034 (2004). https://doi.org/10.1073/pnas.0400580101
31. J. P. David, C. Strode, J. Vontas, D. Nikou, A. Vaughan, P. M. Pignatelli, C. Louis, J. Hemingway, and H. Ranson, Proc. Natl. Acad. Sci. U.S.A. **102**, 4080 (2005). https://doi.org/10.1073/pnas.0409348102
32. J. Vontas, C. Blass, A. C. Koutsos, J. P. David, F. C. Kafatos, C. Louis, J. Hemingway, G. K. Christophides, and H. Ranson, Insect Mol. Biol. **14**, 509 (2005). https://doi.org/10.1111/j.1365-2583.2005.00582.x
33. P. Mueller, M. Chouaibou, P. Pignatelli, J. Etang, E. D. Walker, M. J. Donnelly, F. Simard, and H. Ranson, Mol. Ecol. **17**, 1145 (2008). https://doi.org/10.1111/j.1365-294X.2007.03617.x
34. M. Rowland, and J. Hemingway, Pestic Biochem. Physiol. **28**, 239 (1987). https://doi.org/10.1016/0048-3575(87)90022-8
35. N. Aïzoun, R. Aïkpon, R. Azondekon, A. Asidi, and M. Akogbéto, Asian Pac. J. Trop. Biomed. **4**, 312 (2014). https://doi.org/10.12980/APJTB.4.2014C1093