Bulletin of Pure and Applied Sciences
Vol.39A (Zoology), No.2,
July -December 2020: P.281-287
Original Research Paper

Print version ISSN 0970 0765
Online version ISSN 2320 3188
DOI 10.5958/2320-3188.2020.00031.5
Available online at www.bpasjournals.com

Cuticular Hydrocarbon Variation in Rotenone Induced and Transgenically Created Parkinson's disease (PD) Flies of *Drosophila melanogaster*

[1]Ali Hatami Dehnow
[2]Mysore Siddaiah Krishna*

Author's Affiliation:
[1]Ph.D. Research Scholar, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore, 560006, Karnataka, India
[2]Associate Professor, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore, 560006, Karnataka, India

***Corresponding author:**
Dr. M.S. Krishna,
Drosophila Stock Centre, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore, 560006, Karnataka, India

E-mail:
drosokrish@gmail.com

Received on 27.03.2020
Accepted on 29.07.2020

Abstract:
Drosophila melanogaster use cuticular hydrocarbons (CHCs) to identify species, gender, and reproductive status. Diet and environmental factors also influence the cuticular hydrocarbon variation. Current study examines cuticular hydrocarbons variations in rotenone-induced flies and transgenically created Parkinson's disease (PD) flies. Eleven compounds were found in control and experimental flies of *Drosophila melanogaster*, however, their concentration varied significantly between control and experimental flies, which suggests that influence of rotenone, and creatine supplement on cuticular hydrocarbon in *Drosophila melanogaster*.

Keywords: Parkinson disease, Rotenone, Cuticular hydrocarbon, Creatine supplement, *Drosophila melanogaster* transgenic PD flies.

INTRODUCTION

Most compelling studies have shown that organism suffering from Parkinson's disease shows less movement, body balance and rigidity due to disturbance of the central nervous system (CNS) (Lenz et al., 2005). In 1817, an English physician elaborated on the disease, and in the US alone 1.5 million people were affected with PD (Albin, 2006). Animal models were being used to understand the complex biological problem of PD (Barton et al., 2006; Bergland et al., 2014).Now a day *Drosophila melanogaster* being extensively used to explore the disease in greater details (Celotto et al., 2005; Ryu et al., 2002).Due to the fact that more than 70% of the genome of *Drosophila* indicate similarity with that of humans (Celotto et al., 2005). Further complex neurons system, short life span and the small number of chromosomes made *Drosophila* as an ideal model organism to understand PD details (Betarbet et al., 2000; Jallon et al., 2006; Coulom et al., 2004; Savarit et al., 2002).

Most compelling studies in insects have shown that CHCs are known to get involved in communication, species identification, and the reproductive potential of an organism (Howard et al., 2005). Further CHCs in insects also respond to changes in the food and climate (Gosden et al., 2011; Lang et al., 1998; Sestili et al., 2011). This strongly raises the question that if it is possible to use CHCs composition to indicate whether an individual is healthy or suffering from the disease. Therefore, present investigation has been undertaken to discover CHCs variations in PD flies. Organism exposed to environmental toxin (i.e. rotenone) affects the nervous system and showing the sporadic incidence of neurodegenerative disease (Dinis-Oliveira et al., 2005; Francesca et al., 2009). In animal models,

rotenone is known to induce oxidative stress (Greenamyre et al., 1999; Piper et al., 2011; Sherer et al., 2002).

Moreover, chronic rotenone treatment is used to generate the pharmacological model of PD (Sherer et al., 2002; Savarit et al., 2002; Ali and Krishna., 2019). Whether cuticular hydrocarbons variation occurs in rotenone treated PD and transgenic created PD of *Drosophila melanogaster*. Creatine is one of the natural substance that exists in the human body (Cooper et al., 2012; Greenamyre et al., 1999; Sestili et al., 2006), and showed that creatine supplementation prevents DNA and RNA damage (Fimognari et al., 2009; Guidi et al., 2008), reduce oxidative stress (Ali and Krishna., 2019; Liu et al., 2003; Sharma et al., 2012) and anti-inflammatory (Bassit et al., 2008; Patel, 2011).However, in *Drosophila* no report has been published to understand the effect of rotenone and creatine on CHCs variation, therefore, this study has been undertaken.

MATERIALS AND METHODS

Creatine monohydrate (C3630) and Rotenone (R 8875) were purchased from Sigma Chemical Co.St Louis, USA. Transgenic flies' model of Parkinson disease was collected from Bloomington *Drosophila* Stock Centre Dept. Biology, Indiana University 1001E. RNAi posttranscriptional gene silencing was achieved by conducting crosses between UAS- A30P lines and DdC- GAL4 as driver line for create transgenic flies of Parkinson model. Details procedure adapted for establishment and maintenance of control and experimental flies [Creatine treated, rotenone treated (rotenone-induced PD flies), co-exposure of rotenone and creatine, transgenic created PD flies] of *Drosophila melanogaster* were presented in our earlier paper (Ali and Krishna, 2019). These flies were used in the present experiments.

Flies were exposed to four different concentration of rotenone (125 µM, 250µM, 500 µM and 1000 µM) to determine lethality. However, 500 µM showed sub lethality therefore this concentration was used in all the experiment. Rotenone control (untreated), rotenone treated (500 µM) for 7 days, transgenic PD and control transgenic PD were used separately and co-treated them with 10 mMcreatine (Ali and Krishna, 2019).These flies were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

Isolation and purification of cuticular hydrocarbons (CHCs)

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis was employed to analyze cuticular hydrocarbons' variation in control and experimental flies of *Drosophila melanogaster*, which is a powerful and available analytical technique for the measurement of CHCs. Here, control and treated flies separately introduced into the vial containing 30 µl Hexane and 100 ng triacontane. In the present study triacontane used as an internal standard. Five flies from each experimental sample were placed into vial contain extraction solution for 5 minutes (separate vial was used for each sample). After 5 minutes, the extraction solution separated and kept at room temperature for 24 hrs. Finally, 3 µl of the solution is used for the analysis (Figure 1A).

Analyses of cuticular hydrocarbons by GC-MS

In this analysis temperature profile of the column started from 70°C for 60 seconds, and subsequent experiment temperature was increased to 20°C per min until the temperature becomes 240°C. Following these 4°C per min was increased until the temperature becomes 320°C. A total of 36 minutes used to Gas Chromatography.

Statistical Analysis

The statistical tests employed for data analysis in the study was one-way ANOVA followed by post hoc (Tukey's post hoc test) using SPSS IBM Statistics 20 version (Tukey, 1977).

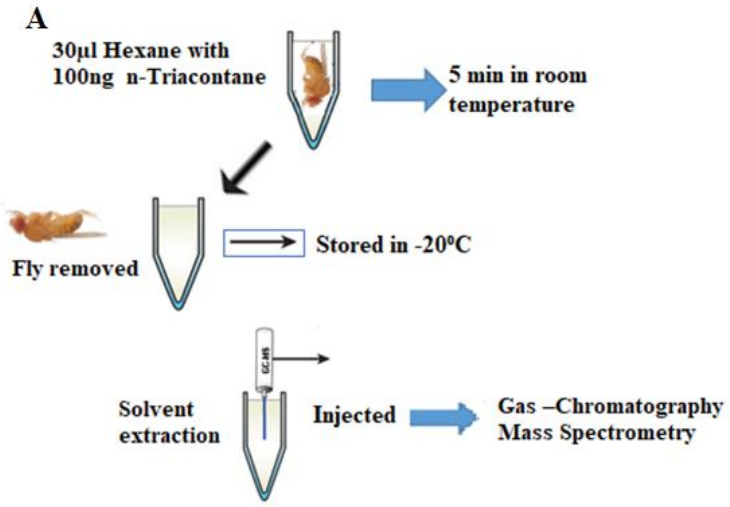

Figure 1: **Extraction of Cuticular Hydrocarbons**

RESULTS AND DISCUSSION

Gas Chromatography-Mass Spectrometry analysis (GC-MS)

The composition of cuticular hydrocarbon, which had above 75% purity, has been recorded in the experimental flies of *Drosophila melanogaster*. Total 11 compounds have been recorded; most of these compounds belong to alkane and fatty alcohol components (Table 1). These compounds were identified based on Retention time (RT) value (Figure 2). Different peaks in chromatogram represent a cuticular hydrocarbon profile. Compounds had retention time were more volatile as a result, they appear first in the chromatograph. (Figure 2). The following compounds were found namely: decane, undecane, tridecane, decane 4-methyl, dodecane, tetradecane, hexadecane, pentadecane, eicosane, 1-octadecanol, and 1-hexadecanol (Table 1).

Table 1: Cuticular hydrocarbon compounds profile on basis of retention time (RT) in control and experimental flies of *D. melanogaster.* (11 CHC compounds identified in*D. melanogaster).*

Experimental Flies								
Compounds	**RT**	**Formula Chemical**	**Control Flies**	**Creatine Flies**	**Rotenone Flies**	**Rotenone + Creatine**	**Transgenic Flies**	**Transgenic + Creatine**
Decane	9.12	C10H22	**+**	**+**	**+**	**+**	**+**	**+**
Undecane	9.52	C11H24	**+**	**+**	**+**	**+**	**+**	**+**
Tridecane	10.30	C13H28	**+**	**+**	**+**	**+**	**+**	**+**
Decane 4 methyl	11.08	C11H24	**+**	**+**	**+**	**+**	**+**	**+**
Dodecane	12.95	C12H26	**+**	**+**	**+**	**+**	**+**	**+**
Tetradecane	14.27	C14H30	**+**	**+**	**+**	**+**	**+**	**+**
Hexadecane	14.72	C16H34	**+**	**+**	**+**	**+**	**+**	**+**
Pentadecane	16.39	C15H32	**+**	**+**	**+**	**+**	**+**	**+**
Eicosane	17.76	C20H42	**+**	**-**	**-**	**+**	**+**	**-**
1-Octadecanol	27.90	C18H38O	**+**	**+**	**+**	**+**	**+**	**-**
1-Hexadecanol	28.00	C16H34O	**+**	**+**	**+**	**+**	**+**	**-**
Triacontane (IS)	**35.00**	**C30H62**	**+**	**+**	**+**	**+**	**+**	**+**

(**RT**) Retention time (minutes)**,** (**IS**) internal standard, (**+**) present compounds in experimental samples. (**-**) Absent compounds in experimental samples**.**

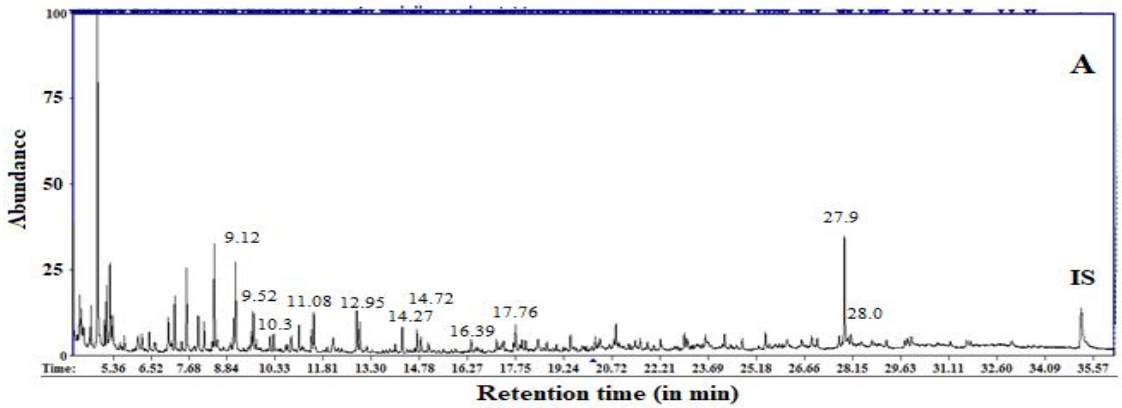

Figure 2: Typical chromatogram showing cuticular hydrocarbon profile of control and experimental flies of *D. melanogaster*. Triacontane used as an internal standard (IS) (n=3).

It was also noticed that all the eleven compounds were found in control flies whereas the compound eicosane was absent in the creatine treated flies, rotenone-induced PD flies, and transgenic PD flies exposed with creatine, and also 1-hexadecanol, and 1- octadecanol compounds were absent in the transgenic PD flies exposed with creatine. Furthermore, the concentration of many of these compounds was decreased in some flies and increased in others (Table 2). In insects, CHCs were initially used to protect against loss of water (Ferveur, 2005). Further CHC profiles are subject to two forces such as natural selection and sexual selection. Even in *D. melanogaster* experimental evidence show natural and sexual selection plays an important role in the evolution of cuticular hydrocarbon (Ryuet al., 2002; S impson, and Raubenheimer, 2009). In the present study, eleven cuticular hydrocarbons have been noticed in control and experimental flies of *D. melanogaster* (Table 1), however, their concentration was found to vary between them (Table 2).

Table 2: Concentration of cuticular hydrocarbons (CHCs) in control and experimental flies of *D. Melanogaster* (n=3; df= 3, 11)

Experimental Flies							
Compounds	**Control Flies**	**Creatine Flies**	**Rotenone Flies**	**Rotenone+ Creatine**	**Transgenic Flies**	**Transgenic+ Creatine**	*** F Value**
Decane[a]	0.490±0.008	0.405±0.0058	0.675±0.0068	0.679±0.0051	0.432±0.0073	0.604±0.0055	666.681
Undecane[a]	0.723±0.005	0.099±0.0043	0.724±0.0042	0.726±0.0049	0.083±0.0051	0.548±0.0042	201.859
Tridecane[a]	0.145±0.005	0.131±0.006	0.186±0.0043	0.188±0.0036	0.134±0.0052	0.162±0.0017	10206.171
Decane 4methyl[a]	0.123±0.0044	0.780±0.0051	0.783±0.0053	0.780±0.0064	0.606±0.0077	0.606±0.0072	21880.930
Dodecane[a]	0.007±0.0040	0.142±0.0031	0.189±0.0016	0.216±0.0035	0.200±0.0028	0.179±0.0043	526.026
Tetradecane[a]	0.115±0.0018	0.123±0.0021	0.167±0.0026	0.174±0.0026	0.162±0.0019	0.151±0.0029	164.020
Hexadecane[a]	0.073±0.0023	0.094±0.0018	0.124±0.0027	0.131±0.0016	0.112±0.0021	0.091±0.0016	228.547
Pentadecane[a]	0.087±0.002	0.094±0.0016	0.117±0.0021	0.130±0.0014	0.150±0.0018	0.092±0.0016	225.391

*****Significant at $p<0.001$, [a] ng / µl

This suggests that although the CHC profile is genetically determined but shows variation in concentration in response to environmental factors such as diet treatment. This confirms the earlier studies of cuticular hydrocarbon variation in response to diet (Fanson and Taylor 2011; Fontana et al., 2010; Fricke et al., 2008; Ravikumar and Saraf, 2010; Takahashi et al., 2012). Talyana et al., 2013 showed a dietary effect on cuticular hydrocarbons. Studies in *Drosophila* have also shown variation in CHCs profile within and among a population of the same species (Antony and Jallon, 1982; Kent et al., 2007; Nomura et al., 2003; Venard et al. 1980; Yeaman and Jarvis, 2006), the influence of temperature on CHCs variation was also noticed in *Drosophila (*Barton, 1989; Bergland et al., 2014; Hedrick et al. 1976). They suggested that CHC variation is one of the sensitive parameters responding to changes in the animal physiology and environmental factors. In the present study except for eicosane (Tables 1, 2), other CHC compounds were a higher concentration in rotenone compared to control flies suggesting that this variation in CHCs could be the response to oxidative stress because rotenone is a known potent inducer of oxidative stress in *D. melanogaster*. Ravi Kumar and Saraf (Ravi

kumar and Saraf, 2010) shown rotenone treatment reduced energy in mitochondria and increased oxidative stress in *D. melanogaster*. Studies among animal models have also shown a chronic dose of rotenone treatment known to cause Parkinson's disease in the flies (Bové et al., 2008; Luytenet al., 19820. Further, in the present study creatine supplemented flies had shown variation in CHCs compared to control flies. Recently studies have shown in *D. melanogaster* that creatine supplementation reduced mortality, oxidative stress and restored the dopamine levels (Ali and Krishna, 2019; Reiter et al., 2001). Except four CHC such as undecane, eicosane, 1-octadecanol and 1-hexadecanol significantly lesser in concentration in creatine supplemented to rotenone treated flies compared to control flies however in remaining CHC creatine supplemented to rotenone treated flies showed significantly greater concentration than control flies. Further in the present study in compare to rotenone treated flies transgenic created PD flies showed reduced concentration of CHCs such as decane, undecane, tridecane, decan-4methyl, and hexadecane (Tables 1, 2) whereas they showed increased concentration of CHCs such as dodecane, tetradecane, pentadecane, eicosane, 1-octadecanol and 1- hexadecanol (Tables 1, 2). In creatine supplemented to transgenically created PD flies showed significantly increased concentration of CHCs such as decane, tridecane, decane- 4 methyl, dodecane, tetradecane, hexadecane, pentadecane compared to control flies (Table 2). Thus, this variation does occur in rotenone-induced PD flies and transgenically created PD flies. Creatine supplement did show the effect on CHCs variation.

Acknowledgment

The authors thank Chairperson, Dos in Zoology, Drosophila Stock Centre, University of Mysore, and other laboratory colleagues for their support and help during the research work.

REFERENCES

1. Ali, H.D. and Krishna, M.S. (2019). Creatine Supplement Effect Similar in Rotenone Induced and Transgenic PD Flies in Drosophila melanogaster. *J Pharm Chem Bio Sci*. 7, 84-93.
2. Albin, R.L. (2006). Parkinson's disease: Background, diagnosis, and initial management. *ClinGeriatr Med*. 22,735–751.
3. Antony, C. and Jallon, J.M. (1982). Evolution des hydrocarburescomportementalementactifs de Drosophila melanogaster au cours de la maturation sexuelle. *C R Seances Acad Sci*, 292,239–242.
4. Barton, N.H. (1989) Evolutionary quantitative genetics: how little do we know? *Annu Rev Gen.* 23,337–370.
5. Bassit, R.A., Curi, R. and Costa Rosa, L.F. (2008). Creatine supplementation reduces plasma levels of pro-inflammatory cytokines and PGE2 after a half-ironman competition. *Amino Acids*. 35, 425-431.
6. Bergland, A.O., Behrman, E.L., O'Brien, K.R., Schmidt, P.S. and Petrov, D.A. (2014). Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in Drosophila. *PloS Genetics*. 10,e1004775.
7. Betarbet, R., Sherer, T.B. and Mackenzie, G. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci*. 3,1301–130.
8. Bové, J.D., Prou, C., Perier, S. and Przedborski. (2005). Toxin-induced models of Parkinson's disease. *Neuron Rx.*2,484-494.
9. Celotto, A.M. and Palladino, M.J. (2005). Drosophila, a model system to study neurodegeneration. Molecular Interventions. 5,292–303.
10. Coulom, H. and Birman, S. (2004). Chronic exposure to rotenone models sporadic Parkinson's disease in Drosophila melanogaster. *J Neurosci*. 24,10993–10998.
11. Cooper, R., Naclerio, F., Allgrove, J. and Jimenez, A. (2012). Creatine supplementation with specific view to exercise / sports performance. *J IntSoc Sports Nutr*. 20, 33.
12. Dinis-Oliveira, R.J., Remia, F. and Carmo, H. (2005). Paraquat exposure as an etiological factor of Parkinson's disease. *NeuroToxicology.* 27,1110–1122.
13. Fanson, B.G. and Taylor, P.W. (2011). Protein: carbohydrate ratios explain life span patterns found in Queensland fruit fly on diets varying in yeast: sugar ratios Age. DOI. 10.1007 / s11357-011-9308-3.

14. Ferveur, J.F. (2005). Cuticular hydrocarbons: their evolution and roles in Drosophila pheromonal communication. *Behavior Genetics*. 35,279–295.
15. Fimognari, C., Sestili, P., Lenzi, M., Cantelli-Forti, G. and Hrelia, P. (2009). Protective effect of creatine against RNA damage. *Mutat Res.* 670, 59-67.
16. Fontana, L., Partridge. L. and Longo, V.D. (2010). Extending healthy life span–from yeast to humans. *Science*. 328, 321–326.
17. Francesca, C., Drouin-Ouellet, J. and Robert, E.G. (2009). Environmental toxins and Parkinson's disease: what have we learned from pesticide-induced animal models? *Trends Pharmacol Sci*. 30,475–483.
18. Fricke, C., Bretman, A. and Chapman, T. (2008). Adult male nutrition and reproductive success in Drosophila melanogaster. *Evolution.* 62, 3170–3177.
19. Gosden, T.P. and Chenoweth, S.F. (2011).On the evolution of heightened condition dependence of male sexual displays. *J Evol Biol*. 24, 685–692.
20. Greenamyre, J.T., MacKenzie, G., Peng, T.I. and Stephans, S.E. (1999). Mitochondrial dysfunction in Parkinson's disease. *BiochemSociSymp*. 66,85-97.
21. Guidi, C., Potenza, L., Sestili, P., Martinelli, C., Guescini, M. and Stocchi, L. (2008). Differential effect of creatine on oxidatively-injured mitochondrial and nuclear DNA. *BiochimBiophysActa*. 1780, 16-26.
22. Howard, R.W. and Blomquist, G.J. (2005). Ecological, behavioral, andbiochemical aspects of insect hydrocarbons. *Annu Rev Entomol*. 50, 371–393.
23. Hedrick, P.W., Ginevan, M.E. and Ewing, E.P. (1976). Genetic polymorphism in heterogeneous environments. *Annu Rev EcolEvolSyste*. 7,1–32.
24. Jallon, J.M. (2006). A few chemical words exchanged by Drosophila during courtship and mating. *Behav Genet*. 14,441–478.
25. Kent, C., Azanchi, R., Smith, B., Chu, A. and Levine, J. (2007). A Model-based analysis of chemical and temporal patterns of cuticular hydrocarbons in male Drosophila melanogaster. *PLoS ONE*. 2, e962.
26. Lang, A.E. and Lozano, A.M. (1998). Parkinson's disease. First of two parts. *N Engl J Med*. 339,1044–1053.
27. Lenz, H., Schmidt, M., Welge, V., Schlattner, U., Wallimann, T. and Elsässer, H.P. (2005). The creatine kinase system in human skin: protective effects of creatine against oxidative and UV damage in vitro and in vivo. *J Invest Dermatol*. 124, 443-452.
28. Liu, B., Gao, H.M. and Hong, J.S. (2003). Parkinson's disease and exposure to infectious agents and pesticides and the occurrence of brain injuries: role of neuroinflammation. *Envir Heal Persp*. 111(8), 1065-1073.
29. Luyten, I. (1982). Intraspecific and interspecific variations of the cuticular hydrocarbons in Drosophila simulans and related species. *C R SeancesAcad Sci*. 295,733–736.
30. Nomura, A., Zhang, M., Sakamoto, T., Ishii, Y., Morishima, Y. and Mochizuki, M. (2003). Anti-inflammatory activity of creatine supplementation in endothelial cells in vitro. *Br J Pharmacol*. 139, 715-720.
31. Patel, F.(2011). Pesticidal suicide: adult fatal rotenone poisoning. *J Fore Leg Medi.* 18(7), 340-342.
32. Piper, M.D., Partridge, L., Raubenheimer, D. and Simpson, S.J. (2011). Dietary restriction and aging: a unifying perspective. *Cell Metab*. 14, 154–160.
33. Ravikumar, H. and Saraf, R.R. (2010). Attenuation of Rotenone-Induced Mitochondrial Oxidative Damage and Neurotoxicty in Drosophila melanogaster Supplemented with Creatine. *Neurochem Res*. 35, 1402-1412.
34. Reiter, R.J., Acuna-Castrviej, O.D. and Tan, D.X. (2001),Free radical mediated molecular damage, mechanisms for the protective actions of melatonin in the central nervous system. *Ann NY Acad Sci*. 939,200–215.
35. Rundle, H.D., Chenoweth, S.F. and Blows, M.W. (2009). The diversification of mate preferences by natural and sexual selection. *J. Evol. Biol*. 22, 1608–1615.
36. Ryu, E.J., Harding, H.P., Angelastro, J.M., Vitolo, O.V., Ron, D. and Greene, L.A. (2002). Endoplasmic reticulum stress and the unfolded protein response in cellular models of Parkinson's disease. *J Neurosci.* 22, 10690–10698.

37. Sherer, T.B.,Betarbet, R. and Stout, A.K. (2002).An in vitro model of Parkinson's disease: linking mitochondrial impairment to altered a-synuclein metabolism and oxidative damage. *J Neurosci*. 22,7006–7015.
38. Savarit, F. and Ferveur, J.F. (2002). Temperature affects the ontogeny of sexually dimorphic cuticular hydrocarbons in Drosophila melanogaster. *J Exp Biol.* 205, 3241–3249.
39. Sestili, P., Martinelli, C., Colombo , E., Barbieri, E., Potenza, L. and Sartini, S. (2011). Creatine as an antioxidant. *Amino Acids*. 40, 1385-1396.
40. Sestili, P., Martinelli, C., Bravi, G., Piccoli, G., Curci, R. and Battistelli, M. (2006). Creatine supplementation affords cytoprotection in oxidatively injured cultured mammalian cells via direct antioxidant activity. *Free RadicBiol Med*. 40, 837-849.
41. Sharma, M.D., Mitchell, C., Hun,t J., Tregenza, T. and Hosken, D.J. (2012). The genetics of cuticular hydrocarbon profiles in the fruit fly Drosophila simulans. *J. Hered.* 103, 230–239.
42. Simpson, S.J. and Raubenheimer, D. (2009). Macronutrient balance and lifespan. *Aging (Albany NY)*. 1, 875–880.
43. Talyana, K., De Melo1, José. F., De, Medeiros., José, E., Sobrinho., Vladimir, B., Figueiredo., Paulo, S. and De Souza. (2013). Evapotranspiration and crop coefficients of melon plants measured by lysimeter and estimated according to FAO 56 methodology. *EngAgrícJaboticabal.* 34(5), 929-939.
44. Takahashi, A., Fujiwara-Tsujii, N., Yamaoka, R., Itoh, M., Ozaki, M. Takano-Shimizu, T. (2012). Cuticular hydrocarbon content that affects male mate preference of Drosophila melanogaster from West Africa. *Inter J Evol Biol.* 1–10.
45. Venard, R. and Jallon, J.M. (1980). Evidence for an aphrodisiac pheromone of female *Drosophila*. *Experientia*. 36,211–213.
46. Yeaman, S. and Jarvis, A. (2006). Regional heterogeneity and gene flow maintain variance in a quantitative trait within populations of lodgepole pine. Proc of Royal Society B: *Biol Sci*. 273,1587–1593.

Books

47. Tukey, J.W. (1977). Exploratory data analysis (Vol. 2).