GAU Research Journal **45** (3) : 145-151 (July 2020)

Effect of plumbagin on midgut amylase in *Dysdercus cingulatus* Fab. (Heteroptera: Pyrrochorridae)

MRINMAYEE DATAR[1] AND SUJATA MAGDUM[2]*

PG Department of Zoology and research Centre,
KRT Arts, BH Commerce and AM Science College (K T H M College), Nashik

Received: 30 June, 2020

Accepted: 29 July, 2020

ABSTRACT

India has an agro-based economy where there is a constant struggle between using pesticides and maintaining healthy crop yield. Often, the use of pesticides takes a priority to achieve a good quantity of crop yield. This has now triggered a renewed interest in assessing the role of natural products in insect growth regulation. Naphthoquinone Plumbagin is one such natural compound extracted from *Plumbago* spp. In the current study, the role of Plumbagin in interfering with the amylase activity was assessed. The assay was done using the DNSA reagent. It was found that as the activity of amylase decreased with the progressive increase in the dose and duration of treatment

Key words: *Dysdercus cingulatus*, Plumbagin, Amylase, Naphthoquinone, secondary metabolite

Insects form an integral part of any ecosystem. They are of great economic importance as they act as pollinators, decomposers, also add aesthetic beauty to the environment. Many of the insects act as pests and cause great economic loss by destroying the agricultural yield or stored grains. Since India has an agro-based economy, controlling the insect pests has been the focal point of research. Of all the methods known to control insect pests, striking a balance between the advantages and disadvantages of these methods to efficiently control pest infestation is a challenge. The age-old practice of using synthetic pesticides has resulted in the development of resistance in pests as well as causing aggravated human health hazards. In this context, many of the natural products are screened for understanding the biological activities like insect growth regulators, allohormones, antifeedants etc.

[1]Research Scholar, [2]Assistant Professor, Department of Zoology, KRT Arts, BH Commerce and AM Science College, Nashik

*Email: sujata_magdum@yahoo.com

Natural products are organic compounds isolated from plants, animals, algae, fungi etc. Albrecht Kossel, in 1891, proposed the classification of natural products in two major classes– Primary and secondary metabolites. Primary metabolites are essentially present in each cell of the organisms in the form of carbohydrates, amino acids, lipids and nucleic acids, and play a vital role in the survival of organisms which produce them. Similarly, secondary metabolites are present in very small amounts in an organism and are essential to support/protect organism within its environment, through the modulation of biochemical and signal transduction pathways.

There are many secondary metabolites are known to show biological activities. Naphthoquinone is widely occurring secondary metabolites of some actinomycetes, fungi, lichens and higher plants. These are highly cytotoxic compounds known to be affecting mitochondria, cytoplasmic proteins and microsomes in the form of free radicals and damage DNA and RNA. Some of the secondary

metabolites have been reported for their insecticidal properties and inhibitors of larval development. Plumbagin, one such naphthoquinone extracted from Bamboo –*Plumbago* spp. is known to possess a wide range of biological properties which primarily include antimicrobial (Krolicka *et. al.*, 2009, Sharma et al 2013), anti-tumour (Bhasin *et al.* 2013) and insecticidal properties (Pavela R, 2013). Plumbagin has been reported to be acting as insect growth regulator by acting as a chitin synthesis inhibitor (Gnanamani and Dhanasekaran, 2013). The cuticle of the insects treated with Plumbagin becomes stiff, consequently hampering feeding and thereby resisting moulting. Preliminary studies on the control of *Dysdercus koenigii* have been done using the naphthoquinones Plumbagin, Juglone and their synthetic analogue Menadione along with 24 other naphthoquinones and benzoquinones by (Magdum, 1999).

Insects are ubiquitous and possess different enzymatic tools specific for circumventing the plant defences and for metabolizing the nutrients. The detection and characterization of digestive enzymes in the insects is an active field of research. Various aspects of digestion in insects is reviewed by Day and Waterhouse 1953; Wigglesworth 1972; Silva and Terra 1994; Terra and Ferreira 2012.

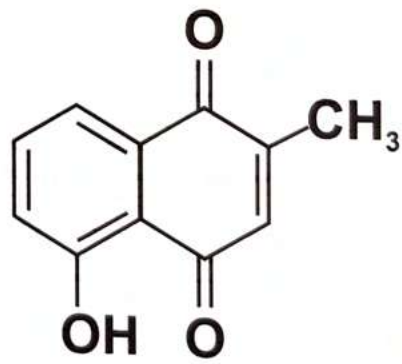
Midgut is most prominent region for digestion and absorption. In *Dysdercus cingulatus* the midgut is differentiated into four distinct ventriculi and are denoted as first, second, third and fourth ventriculus (V_1-V_4). Based on this, here an attempt is made to study the effects of Plumbagin on amylase in particular in *Dysdercus cingulatus*. Amylases are glycosyl hydrolases that break down 1,4 glycosidic bonds inside a malto-polysaccharide linear chain, mainly in starch and glycogen, resulting in maltose, maltotriose, and residual branched maltodextrins as final products. (Jean-Luc Da Lage, 2018).

This paper deals with understanding the quantitative changes in the midgut amylase due to topical application of important naphthoquinone-Plumbagin in *Dysdercus cingulatus*.

MATERIALS AND METHODS

Insect Culture: Insects were handpicked from cotton fields in Ankai village of Manmad, Nashik district. They were then transferred to plastic jars containing 5-6 cm of moist soil and the mouth of the jars was covered with a muslin cloth. The ambient temperature was maintained at 25°C ± 5°C. The insects were fed with cotton seeds soaked in water, which were changed on alternate days. The walls of jars were wiped clean every day to prevent fungal and bacterial contamination. Freshly moulted 24-36 hours old adults were separated from the culture and were used for experimentation.

Test compound: Plumbagin is 5-hydroxy-2-methyl-1, 4-hydroxy naphthoquinone obtained from Sigma (1 gm) was dissolved in acetone (1mg/ml)



Plumbagin (5-hydroxy-2-methyl-1, 4-hydroxy naphthoquinone)

Treatment: Freshly moulted 24-36 hrs old adults were treated with varying sub-lethal doses of Plumbagin (2, 4, 6, 8 µg/ml). The doses were administered topically on the ventrolateral side with the help of Hamilton microlitre syringe. Topical application was chosen as the mode of treatment so that the haemolymph does not ooze out. The insects were treated in a group of 20 for every dose keeping controls for each group.

Extraction of amylase: Insects were dissected in ice-cold Citrate Phosphate buffer (6.2) under the stereoscopic dissecting microscope and alimentary canal was pooled out of the body and the additional tissue adhering to it was removed. Foregut, midgut and hindgut were homogenised separately at 0°C for 3 minutes and the tissue was screened for detection of the active amylase in the alimentary canal as mentioned by Pickford and Dorris (1934). Amylase was extracted by the method described by Hemlata *et al* (2014)

Enzyme quantification: Enzyme quantification with respect to the amylase was done using the methods described by Sadasivam and Manickam, 2008. 2 % soluble starch was used as a substrate for quantification of the enzyme.

Data Analysis: Two factor ANOVA was carried out in Excel.

RESULTS AND DISCUSSION

Identification of amylase in alimentary canal: The alimentary canal of *Dysdercus cingulatus* measured about5.2±1 cm long and as seen in other insects consisted of anterior foregut, middle midgut and posterior hindgut. The midgut is the largest part of the alimentary canal in *Dysdercus cingulatus* and measured about 3.5±0.2 cm long. It showed the presence of four ventriculi namely $V_1$to V_4. V_1 appeared sac-like and forms nearly one-fourth part of the midgut(0.8±0.1cm). Second Ventriculus (V_2) is thin long and slender and measures 1.5. Third ventriculus (V_3) is again sac-like with a smaller size (0.4±0.1 cm) than V_1. The third ventriculus is curled which joins to the fourth ventriculus which is tube-like(0.5±0.1cm) and joins posteriorly to the hindgut. At the junction of V_4 and hindgut, two rows of gastric caeca are present in the female which is lacking in males.

For analysis of amylase foregut, midgut with V_1, V_2, V_3, V_4 and hindgut of 4 insects were separately placed. 2 drops of 0.2 M Sodium Chloride was added to the incubation mixture to activate the enzyme. Along with normal control, a positive control of a 0.2 % diastase solution is added instead of gut extract for comparison. The micro method described by Pickford and Dorris (1934) for the detection of amylase confirmed the absence of amylase in the foregut, V_2 – V_4 and hindgut extract. Very feeble weak reaction was noted in the extract obtained from the foregut region and hindgut region.

Table 1 Distribution of amylase in positive control and Normal control in different regions of the alimentary canal of *Dysdercus cingulatus*
(+ means Present,++ more amount of amylase reaction, - means absent,* feeble amount)

	Foregut	Midgut				Hindgut
		V1	V2	V3	V4	
Positive control	+	++	-	-	-	+
Control(Normal)	*	++	-	-	-	*

Majority of insects in this infra order are predators and seed sucking insects with few exceptions, showing feeding on phloem-sap. Due to this reason variation is seen in their digestion. Absence of amylase in foregut, V_2 – V_4 of midgut and hindgut could be due to the nature of feeding in *Dysdercus*. Major insect enzymes are amylase, maltase, invertase, tryptase, peptidase and lipase.

Food that is taken in by insects defines the type of enzymes they possess in the digestive tract. Adaptation to a particular food by insects, its digestive processes and mechanism of digestion is understood only by the detailed study on its digestive enzymes. Predominantly it is found that carnivore insects possess proteolytic and lipolytic digestive enzymes and lacks amylase and saccharases. Omnivore and herbivore insects possess carbohydrase. *Dysdercus* feeds on cotton seeds. Nearly 45% of carbohydrates are glucose and small oligosaccharides especially disaccharides are more important sugars in cotton seeds. These are soluble forms and starch is totally absent (Silva, 1994). Even though lipids and proteins are the major constituents of seeds, their digestion is not discussed here.

Quantification of enzymes amylase: The activity of an enzyme is a measure of its catalytic ability which converts oligosaccharides to reducing sugars. The Amylase concentration in the gut of *Dysdercus cingulatus* was assayed using the protocol described by Sadasivam and Manickam, 2008. The spectrophotometric the analysis was carried out and total amylase in both the control and different doses of plumbagin treated insects were recorded in µg/ml of tissue extract. As shown in table 2, amylase activity decreases with the progression of dose and duration of treatment. There is a dose-dependent effect observed in treated groups.

Table 2 Amountof amylase (µg/mg of tissue) in control and Plumbagin treated *Dysdercus cingulatus*

Days	Control	2µg/ml	4 µg/ml	6 µg/ml	8 µg/ml
1	0.82±0.05	0.79±0.1	0.75±0.13	0.64±0.02	0.57±0.07
2	0.82±0.07	0.81±0.09	0.79±0.02	0.69±0.11	0.66±0.05
3	0.82±0.04	0.80±0.02	0.78±0.05	0.71±0.01	0.68±0.01
4	0.81±0.05	0.79±0.07	0.77±0.02	0.75±0.06	0.69±0.03
5	0.83±0.02	0.79±0.05	0.76±0.08	0.73±0.02	0.72±0.04
6	0.82±0.03	0.78±0.01	0.75±0.02	0.74±0.01	0.73±0.01
7	0.83±0.02	0.79±0.05	0.74±0.06	0.71±0.01	0.69±0.03

2µg/ml of Plumbagin/adult: There was a reduction in amylase concentration in 2µg/ml of Plumbagin per treated *Dysdercus cingulatus* when compared to control. After 24 hours of post-treatment period, 96.34% of amylase observed which reduced to 90.00% on the seventh day of the post-treatment period. Not much difference was noted on the second and third day of the post-treatment period. It was found to be 95.29% and 95.51% respectively on the second and third day of treatment. Subsequently, on the fourth, fifth and sixth day, the amount of amylase was less than the control group.

4µg/ml of Plumbagin/adult: With 4µg/ml dosage, it was observed that there was a further reduction in amylase concentration when compared to control. Hereafter one day of post-treatment period, 91.46% of amylase was observed. This further reduced to 87.88% on the seventh day of the post-treatment period. Throughout the course of the experiment, there was an increase in the amylase concentration noted in this group but the amount was less than control as well as the previous dose (2µg/ml).

6µg/ml of Plumbagin/adult: With a slight increase in dosage *i.e.*,6 µg/ml it was observed that there after 24 hours of post-treatment period, 78.05% of amylase was observed. The trend of amylase concentration in the midgut of Dysdercus *cingulatus* was the same as that found in control and previous doses. There was an increase in the concentration of amylase noted on subsequent days of post-treatment period but this concentration was found to be lesser than the both 2 and 4 µg/ml dosage. This further reduced to 72.88% of amylase was found on the seventh day of the post-treatment period.

8µg/ml of Plumbagin/adult: Amylase concentration was further seen to be lesser in the insects treated with 8µg/ml of Plumbagin. On the first day of the post-treatment period, it was 69.51%; which was 26.83 % less than 2µg/ml dosage. From table 2 it can be seen that on the fourth and fifth day of post-treatment period there was no change found in the amylase concentration in this particular dose. Maximum reduction was found on the seventh day of treatment (60.00%)

Data Analysis: From Anova Analysis using excel was carried out and it was found that concentration in amylase was varying amongst different doses. This supports the effect of Plumbagin. The data were considered to be significantly different within the treatments as the P□0.05 (Annexure I)

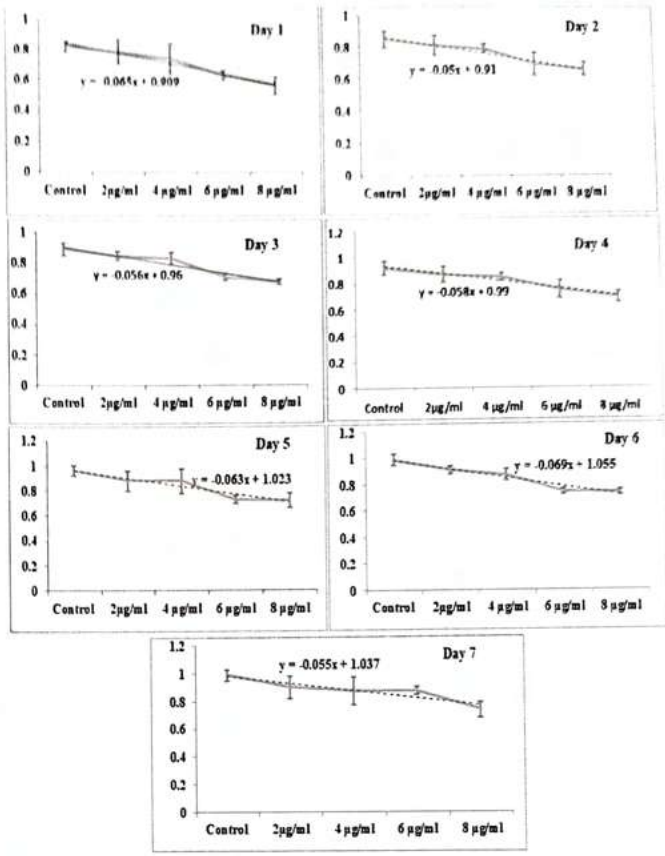
Characterisation and quantification of enzymes in insects is carried out by many of the workers like Ford (1962), Saxena (1955), Herzog (1967).

An attempt is made here in the present study to elucidate the effect of plumbagin on carbohydrate digestion by amylase. This is because plumbagin exhibits antifeedant activity in third instar larvae of

Achaea janata and Spodoptera litura (K Rajendra Prasad et al., 2012).Similar results were found in Dysdercus koenigii when treated with plumbagin and Juglones (Magdum,1999) Earlier studies have also shown that Plumbagin interferes in the growth of insects (Singh-Gupta et al., 2015, Kumar et al., 2002, Banerjee et al., 2001). As a result of this, Dysdercus koenigii were found to be flattened abdomen, indicating loss in feeding, (Singh-Gupta S et al., 2015).

From the present observation can be seen that there is less amount of amylase in the midgut of Dysdercus cingulatus in the entire treated group. The decrease is dose-dependent and time-dependent. The maximum loss in amount of amylase was noted in 8µg/ml dosage. This could be presumed due to the following reasons (1) Dysdercus is sap feeder and so food source itself lacks carbohydrate polymers, so there is a possibility of low synthesis of amylase itself. (2) Dysdercus is a discontinuous feeder (Chapman, 1985), and as the synthesis of the digestive enzyme depends on the presence of food. There are chances which will affect the synthesis of amylase. Amylase is found in a soluble form (Silva, 1994) Major carbohydrate digesting enzymes α- and β-mannosidase are found in the midgut of Dysdercus and is associated with membranes. (3) In Dysdercus, NSC stimulate intake of food and through the secretagogue, mechanism enzymes are released (Muraleedharan and Prabhu, 1979), disruption in this mechanism can also hamper the release on amylase enzyme. Magdum in 1999 reported the presence of low in the secretory granules in the neurosecretory cell of plumbagin treated Dysdercus koenigii. The lower level of amylase in the treated group could be thus correlated with this.

Symbiotic bacteria also play a major role in digestion. These bacteria by their enzymatic action help in digestion of food in the organism. Here this part was not considered and is planned for future studies.



Graph 1 Graphical representation of amount of amylase(µg/mg of tissue±SD) in control and Plumbagin treated *Dysdercus cingulatus*

CONCLUSION

Plumbagin a natural quinone adversely affects the amylase concentration in *Dysdercus cingulatus* and is dose-dependent. Further analysis dealing with proteomic studies will help in understanding the changes in amylase synthesis.

From the present study, it can thus be concluded that Plumbagin interferes in the metabolism of *Dysdercus cingulatus* and can be used as a means to control its infestation.

ACKNOWLEDGEMENT

Mrinmayee Datar is DST INSPIRE fellow and is thankful to Principal Dr V B Gaikwad, for his constant encouragement and support.Authors are grateful to UGC also for providing funding for basic instruments in Department of Zoology, KTHM College, Nashik

REFERENCES

Banerjee, S., Magdum, S., Kalena, G.P. and Banerji, A., 2001. Insect growth regulatory activity of naturally occurring quinones and their derivatives in *Dysdercus* koenigii Fabr. (Hem., Pyrrhocoridae). *Journal of Applied Entomology*, **125** (1-2): 25-30.

Bhasin, D., Chettiar, S. N., Etter, J. P., Mok, M., Li, P. K. 2013;Anticancer activity and SAR studies of substituted 1,4-naphthoquinones. *Bioorg. Med. Chem.*, **21** (15): 4662-4669

Bhosale Hemlata, Gawali Pornima, Kadam Tukaram, Baisthakur Pankaj, 2019 In vitro anti-amylase activity of some Indian dietary spices. *J. Appl. Biol. Biotech.*, **7** (04): 70-74.

David, B.V., Ananthakrishnan, T. N. 2004 General and Applied Entomology. Tata McGraw - Hill publishing company Limited, New Delhi

Gnanamani, R. Dhanasekaran, S. 2013. A Potent Insect Growth Regulator Plumbagin from Plumbago indica against Pericalliaricini (Lepidoptera: Aractidae). *Asian J. Plant Sci.* **12** (5): 219-223

Jean-Luc Da Lage. 2018. The Amylases of Insects, *Int. J. Insect Sci.* **10**:1-14

Kossel, A. 1891. "Ueber die chemische Zusammensetzung der Zelle" The chemical composition of the cell. *Archiv für Physiologie* (in German): 181–186

Kranthi *et. al.*, 2012 Insecticide resistance management in cotton to enhance productivity k.R. Kranthi Head, Crop Protection Division. Central Institute for Cotton Research, Nagpur

Krolicka, A., Szpitter, A., Maciag, M. Biskup, E. Gilgenast, E. Romanik, G. Kaminski, M. Wegrzyn, G., Lojkowska, E., 2009: Antibacterial and antioxidant activity of the secondary metabolites from in vitro cultures of Droseraaliciae. *Biotechnol. Appl. Biochem.*, **53**: 175–184

Kumar, M.A.S. and Banerji, A., 2002. Growth Disruption in Opisinaarenosella Walker by Plumbagin, A Naturally Occurring Insect Growth Regulator. *International Journal of Tropical Insect Science*, **22** (4): 321-323.

Magdum, S. Investigations on bioactivity of Plumbagin and some related compounds in *Dysdercus koenigii* Fabr. (Heteroptera: Pyrrochorridae). Ph.D. Thesis. Sagar: Dept. of Zoology, Sagar University.

Methuen, LondonDias, D.A, Urban, S. and Roessner, U, 2012. A historical overview of natural products in drug discovery. *Metabolites*, **2** (2):303-336.

Muraleedharan, D.; and Prabhu, V. K. 1979. A Role of the median neurosecretory cells in secretion of protease and invertase in the red cotton bug, *Dysdercus cingulatus*. *J. Insect Physiol.*, **25**: 237-240

Pickford, G. E. and Dorris, F. 1934. Micro-methods for detection of proteases and amylases. *Science.* **80**: 317-319.

Rajendra Prasad, K., Suresh Babu, R., Ranga Rao, G., Suresh, K., Rekha, J., Madhusudana, Murthy, P. Usha Rani, J. Madhusudana Rao. 2012: Synthesis and insect antifeedant activity of plumbagin derivatives. *Medicinal Chemistry Research*, **21**:578–583.

S Sadasivam, A Manickam. 2008. Amylases In: Biochemical Methods, New Age International (P) Ltd., Publishers: 114.

Sahayaraj, K. and Ilayaraja, R. 2008. Ecology of *Dysdercus cingulatus* (Fab.). *Egyptian Journal of Biology*, **10**: 122 – 125.

Sharma, U., Katoch, D., Sood, S., Kumar, N., Singh, B., Thakur, A., Gulati, A. 2013. Synthesis, antibacterial and antifungal activity of 2-amino-1,4-naphthoquinone using silica-supported perchloric acid (HClO4-SiO2) as a mild recyclable and highly efficient heterogeneous catalyst. *Indian J. Chem.* **52B**: 1431-1140

Silva, C.P. and Terra, W. R. 1994. Digestive and absorptive sites along the midgut of the cotton seed sucker bug *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae). *Insect Biochem. Mol. Biol.*, **24**: 493–505.

Singh-Gupta, S., Magdum, S. and Shere-Kharwar, A.S. 2015. Effectiveness of menadione, a synthetic analogue of a natural plant derived compound–plumbagin (bio-pesticides), as insecticide on red cotton bug *Dysdercus cingulatus* Fab. (Hemiptera: Pyrhocoridae).*Insect Environment*, **20** (4): 111-122.

Suribabu, K. And Hemalatha K. P. J., 2014. Optimization of Mineral sources on α-amylase production by *Brevibacillus borstelensis* R1 in Submerged Fermentation. *Intl. J. of Engg. Sciences & Research Technology*, **3** (7): 926-934.

Terra, W. R. and Ferreira, C. 2012. Biochemistry andmolecular biology of digestion. In: Gilbert, L.I. (Ed.) first ed. *Insect Molecular Biology and Biochemistry*, **1**: 365–418.

Waterhouse, D.F, 1957. Digestion in insects. *Annual Review of Entomology.* **2** (1): 1-18.

Wigglesworth, V. B. 1972. The Principles of Insect Physiology, 7th edn.

Graph 1 Graphical representation of amount of amylase(µg/mg of tissue±SD) in control and Plumbagin treated *Dysdercus cingulatus*

CONCLUSION

Plumbagin a natural quinone adversely affects the amylase concentration in *Dysdercus cingulatus* and is dose-dependent. Further analysis dealing with proteomic studies will help in understanding the changes in amylase synthesis.

From the present study, it can thus be concluded that Plumbagin interferes in the metabolism of *Dysdercus cingulatus* and can be used as a means to control its infestation.

ACKNOWLEDGEMENT

Mrinmayee Datar is DST INSPIRE fellow and is thankful to Principal Dr V B Gaikwad, for his constant encouragement and support.Authors are grateful to UGC also for providing funding for basic instruments in Department of Zoology, KTHM College, Nashik

Annexure I:

Anova: Two-Factor Without Replication

SUMMARY		*Count*	*Sum*	*Average*	*Variance*
	1	5	3.57	0.714	0.01113
	2	5	3.8	0.76	0.0066
	3	5	3.96	0.792	0.00842
	4	5	4.08	0.816	0.00878
	5	5	4.17	0.834	0.01098
	6	5	4.24	0.848	0.01252
	7	5	4.36	0.872	0.00872
Control		7	6.42	0.91714	0.00452
2µg/ml		7	6.01	0.85857	0.00201
4 µg/ml		7	5.84	0.83429	0.00233
6 µg/ml		7	5.13	0.73286	0.00502
8 µg/ml		7	4.78	0.68286	0.00319

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.08887	6	0.01481	26.1175	2.11045E-09	2.50819
Columns	0.25499	4	0.06375	112.401	3.55394E-15	2.77629
Error	0.01361	24	0.00057			
Total	0.35747	34				