International Conference on Frontiers in Life and Earth Science
 | Volume 5 | Issue 1 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X

Insecticidal Activity and Growth Inhibiting Effects of Three Phases of TiO_2 Nanoparticles via Food on First Instar Larvae of *T. Castaneum* (Coleoptera: Tenebrionidae)

Geetanjali A. Thorat*[1], Dr. S.G.Salokhe[2], Ekar S.U [3], Anita [4]
[1]Department of Zoology, Ramakrishna More College, Pune University, India
[2]Department of Zoology, Ramakrishna More College, Pune University, India
[3]Department of Physics, B.R.Gholap College, Pune University, India
[4]Department of Physics, B.R.Gholap College, Pune University, India

ABSTRACT

Tribolium castaneum (Herbst) is major pest of stored grains .Annual post harvest losses resulting from insect damages, microbial deterioration and other factors are estimated to 10-25 % of worldwide production. Control of these insects depends majorly on many synthetic insecticide and fumigant application. But their widespread use has led to some serious issues. Store grains are found with increased toxic residue. There is increase in application cost also. So, there is an urgent need to provide alternatives which are safe in nature, user friendly & having low cost. Green synthesis of nanoparticles (NPs) by mushroom extract is at present of more interest. NPs synthesis is useful in many biomedical application also eco-friendly in nature. Titanium dioxide (TiO2) has also gained much attention due to many advantages over other oxides thin films. The advantages of TiO2 are it is toxic to insects, stability of hydrogen, optical and piezoelectric behaviours, plasma atmosphere and low price. In present work TiO2 NPs were synthesized using Phellinus mushroom sp. extract. It is one of the useful ceramic materials. TiO2 has many industrial utility and used in day to day life also. When Tribolium castaneum neonates were treated with TiO2 NPs (Sample I, II & III) through diet, the highest mortality was found in Sample-I treated first instar larvae of Tribolium castaneum. The time taken for pupation and adult emergence in treated samples were delayed by 6 to 8 days as compare to control. The percent pupation and percent adult emergence were also affected and were least in TiO2 –I (sample -I, II, &III), as compared to control. It also delayed development to adult stage and affects on fecundity and fertility of treated adults.

Keywords: Tribolium castaneum, first instar larvae, Insecticide, TiO2 Nanoparticles, Toxicity.

I. INTRODUCTION

Insects are one of the highly populated species, with very successful evolution history. Annual post harvest losses resulting from insect damages, microbial deterioration and other factors are estimated around 10-25% percent of world's grain production (10). Many other damages like crop plantation, wood structure are causing serious health and economic issues.

Pest is among the main causes of agriculture losses. Traditional insecticides are commonly used. But its uncontrolled use leads to environmental contamination, human poisoning. There is reduction in the number of insect's natural enemies. Insecticide resistance also limits the effective benefits of traditional pesticides.

However, the excessive use of highly toxic pesticides causes several human health issues like neurological, tumour, cancer and environmental problems. In this scenario, Nano and micro particles have been reaching a prominent position. So, nanoparticles based green pesticides are of special importance in recent years. In the formulations containing insecticides have been prepared in colloidal suspensions or powder in micro or nano scale. It presents several advantages such as increasing stability of active organic compound (UV, thermal, hydrolysis, etc.) foliar setting, reduction in foliar leaching, systemic action synergism, specificity, etc. As consequence of this, the amount of insecticide necessary (dosage), the number of applications, human exposure to insecticides and environmental impact are reduced. The nano-formulation has been employed not only for synthetic insecticides but also in alternative products to control plague insects such as natural products, herbal extract and entomo-pathogenic micro-organisms.

In order to prepare nano-formulations, several chemical and physical techniques have been developed. In general, they should be prepared by using polymeric material which is biocompatible and biodegradable. The main aim is to avoid the emergence of new environmental and toxicological problems. Titanium dioxide (TiO_2) is one of the most studied compounds in materials science(6).

II. OBJECTIVES

- To find out the effect three different samples of TiO_2 NPs on first instar larvae of *T.castaneum* via food, after interval of 24 hrs.
- To find out the effect of the treatment of TiO_2 NPs on growth and development of *T.castaneum* treated with nanoparticles via food.
- To find out the % survival- mortality ratio of different larval stages of *T.castaneum* treated with NPs via food.
- To find out the effect of the treatment of TiO_2 NPs on fecundity and fertility of adult formed from treated larvae of *T.castaneum*.

III. METHODS AND MATERIAL

- **Culture of *Tribolium castaneum***

  Tribolium castaneum culture was maintained on diet containing wheat flour and 5% Brewer's yeast, at 29±1^{0}C and 60% relative humidity. Eggs were collected by sieving (sieve number 40) diet infested with adults. Newly hatched first instar one day old larvae were collected from the sieved eggs.

- **Synthesis of TiO2 Nanoparticles**

  TiO_2 was synthesised by using bio-inspired green method from the extract of mushroom sp. *Phellinus linteus* .In a typical biosynthesis process, mushroom extract was prepared by mushroom powder boiling in 100ml distilled water at 85° C for 15 min. (fig-1)The extract was filtered and stored as a stock solution at 4° C. The 0.15M Ti precursor solution in ethyl alcohol was prepared by using tetraisopropoxide. The

  0.5 ml extract was added drop-wise into Ti precursor. The precipitation was dried and annealed at 500° C, at 300° C& at 700° C for 2hrs. to obtain TiO_2 powder. The structural parameters of TiO_2 are studied using X-ray diffraction (XRD) spectra. The XRD pattern of samples I, II and III are shown (Fig. 1). The presence of diffraction peaks in XRD pattern for samples I and II confirm the anatase phase (JCPDS card no. 21-1272) of TiO_2, while JCPDS card no. 21-1276 confirms rutile phase of TiO_2 for sample III.

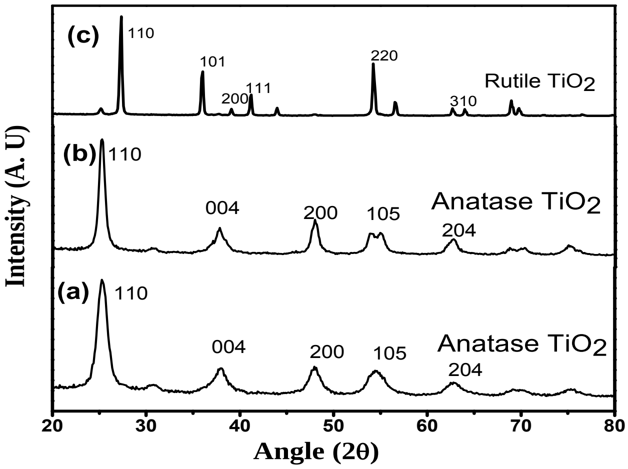

Figure 1. XRD pattern of (a) sample I, (b) sample II and (c) sample III

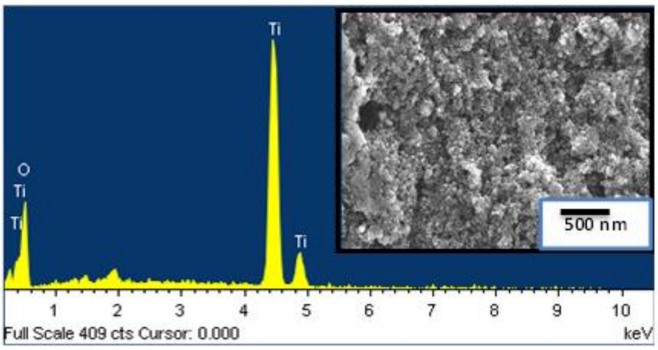

Figure 2. EDAX spectrum of TiO_2 (Inset-FESEM image)

The elemental analysis was performed by investigating EDAX spectrum. (Fig. 2) shows EDAX spectrum of as prepared TiO_2 powder, which confirms the presence of titanium and oxygen in atomic % (O - 73.79 %, Ti - 26.21 %). The inset figure shows the FESEM image of as prepared TiO_2 nanoparticles, agglomerated spherical nanoparticles of TiO_2 powder with 25 nm in size are observed.

- **Bio-assay**

Bioassay for the effect of TiO2 NPs on the first instar larvae of *Tribolium castaneum* were determined by treated wheat flour in different samples. TiO2 was mixed with diet containing wheat flour. The three different samples of TiO_2 with equal volume were thoroughly incorporated in diet of *Tribolium castaneum* (1 mg of TiO_2 in 1gm of wheat flour + 10 larvae) 500° C (TiO_2 sample–I), 300° C (TiO_2 sample –II), 700° C (TiO_2 sample –III) and without any concentration of TiO_2 diet was used as control. The experiments carried out with three replicates. Each of them consisted 10 newly hatched first instar of *Tribolium castaneum*. The mortality count was checked after 24hrs. All the larvae were transferred to fresh diet after 24 hrs and observed further and recorded its mortality on 7^{th} day, 10^{th} day, and 15th day larval stage. Observations were continued till pupal formation and adult emergence. The newly emerged adult from control and treated were also observed for its fertility and fecundity.

IV. RESULTS AND DISCUSSSIONS

Survival of *T. castaneum* first instar larvae to adulthood as well as the fecundity and fertility of these adults was definitely affected by TiO_2 NPs. The dietary treatment of *T. castaneum* larvae with TiO_2 NPs significantly effects the survival of each stage. The maximum mortality of larvae was observed in sample-I, treated larvae. There was significant reduction in all treated stages of TiO_2 NPs as compare to adults (Fig -5).

Figure 4. Larvae of T. castaneum

Furthermore the time taken for pupation and time taken for adult emergence were also affected due to the treatment of TiO_2 NPs, as compare to control. In control, first instar larva turns into pupa in 18- 19 days, while in sample-I it takes 25 to 26 days for pupal formation from first instar larva. Pupa converts into adult in 4 to 5 days in control while in treated samples pupa turns into adult within 8-10 days. A significant reduction in percent pupation and percent adult emergence were observed in treated samples as compare to control (Table-2). Duration of normal development of *T. castaneum* from first instar larvae to adult was 20 to 22 days, while in dietary treatment with TiO_2 NPs development takes place in 33, 30 & 30 days in sample I, II & III resp. (Table-1).

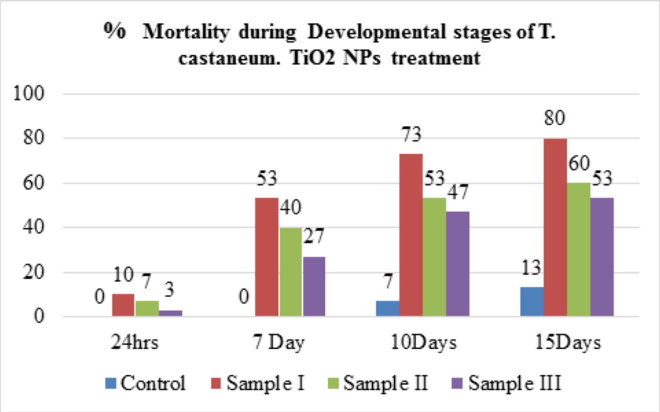

Figure 5.-%mortality during developmental stages of *T. castaneum*

Table 1. Effect of TiO2 NPs on growth & development of *T. castaneum*

Effect of TiO2 NPs on growth & development of *T. castaneum*								
Sample	% Larval survival				% pupation X	Time taken for pupation (Days) X + SE(X)	% adult emergence	Time taken for adult emergence (Days) X ± SE(X)
	24 Hrs.	7^{th} day	10^{th} day	15^{th} day				
Control	100	100	93	87	73%	18 ± 1	63%	24± 1
Sample I	90	47	27	20	17%	25 ± 1	10%	33± 2
Sample II	93	60	47	40	37%	23± 1	30%	31± 1
Sample III	97	73	53	47	40%	23 ± 2	33%	30± 1

ANOVA: Two-Factor with Replication Summary

Control	24 Hrs	7 days	10 days	15 days	Total
Count	3.00	3.00	3.00	3.00	12.00
Sum	0.00	0.00	2.00	4.00	6.00
Average	0.00	0.00	0.67	1.33	0.50
Variance	0.00	0.00	0.33	0.33	0.45

Sample I	24 Hrs	7 days	10 days	15 days	Total
Count	3.00	3.00	3.00	3.00	12.00
Sum	3.00	16.00	22.00	24.00	65.00
Average	1.00	5.33	7.33	8.00	5.42
Variance	0.00	0.33	0.33	0.00	8.27
Sample II	24 Hrs	7 days	10 days	15 days	Total
Count	3.00	3.00	3.00	3.00	12.00
Sum	1.00	12.00	16.00	18.00	47.00
Average	0.33	4.00	5.33	6.00	3.92
Variance	0.33	1.00	0.33	0.00	5.54
Sample III	24 Hrs	7 days	10 days	15 days	Total
Count	3.00	3.00	3.00	3.00	12.00
Sum	1.00	8.00	14.00	16.00	39.00
Average	0.33	2.67	4.67	5.33	3.25
Variance	0.33	0.33	0.33	0.33	4.39

Total	24 Hrs	7 days	10 days	15 days
Count	12.00	12.00	12.00	12.00
Sum	5.00	36.00	54.00	62.00
Average	0.42	3.00	4.50	5.17
Variance	0.27	4.55	6.64	6.52

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	152.3958	3	50.7986	187.5641	2.22292E-20	2.90112E+00
Columns	159.8958	3	53.2986	196.7949	1.07442E-20	2.90112E+00
Interaction	36.52083	9	4.05787	14.98291	3.71275E-09	2.18877E+00
Within	8.666667	32	0.27083			

Two Way ANOVA Analysis: To test

H01 : $\alpha1=\alpha2=\alpha3=\alpha4$

H01: There is no significance difference between samples.

H11: There is a significance difference between samples.

H02 : $\beta1=\beta2=\beta3=\beta4$

H02: There is no significance difference between block (days) effect.

H12: There is a significance difference between block (days) effect.

H03 : There is no interaction between samples and block (days) effect

H13 : There is a interaction between samples and block (days) effect

For H03 (Interaction)
F value =14.982905982906
F critical value=2.18876576806951
F value > F critical value
Reject H03
There is a interaction between samples and block (days) effect

For H01 (Treatments)
F value = 187.564102564102
F critical value= 2.90111958384084
F value > F critical value
Reject H01
There is a significance difference between treatments.

For H02 (Days effect)
F value =196.794871794872
F critical value=2.90111958384084
F value > F critical value
Reject H02
There is a significance difference between block (days) effect.

V. CONCLUSION

Overall the sample-I was more effective as insecticide. The maximum mortality of larvae was observed in sample-I, treated larvae. It is because the anatase TiO2 (sample-I and II) is more active than the rutile (sample-III). The adults formed from treated first instar larvae, there was no egg laying absolutely of such adults in treated samples. The TiO2 NPs when mixed in diet of T. castaneum and fed for 24 hrs. to newly hatch first instar larvae were shown insecticidal and growth inhibiting effect of that larvae. At all three samples treatment there was no abnormal pupae and adults were observed. The time taken for pupation was 18 to 19 days in control while in sample I it was longest duration for 23 to 27 days. Similarly the effect of Ag doped hollow TiO2 NPs as an effective fungicide against Fusarium solani and Venturia inaequalis phytopathogens (3). Also TiO2 NPs applied to Drosophila melanogaster through food found to toxic as it generate reactive oxygen species which modify multiple signalling pathways and thus can alter the development and behavioural pattern of the fly, were observed. (11)

VI. REFERENCES

[1] Abdel Fattah, H.M. and Khaled, A.S.2008.Morphological and biochemical disruption in development of Tribolium castaneum (Coleoptera: Tenebrionidae).J.Egypt. Acad. Soc. Environ. Develop. 8(3):1-9.
[2] Arthur F.H. 2001. Susceptibility of last instar red flour beetle and confused flour beetles (Coleoptera: Tenebrionidae) to hydroprene Journal of Economic Entomology 94(3), 772-779 Devi, M. Bhubaneshwari, and N. Devi. "Biology of rust-red flour beetle, Tribolium castaneum (Herbst)(Tenebrionidae: Coleoptera)." Indian Journal of Entomology 77.1 (2015): 81-82.

[3] Boxi Siddhartha Sankar, Khushi Mukherjee and Santanu Paria, Published 25 January 2016 • © 2016 IOP Publishing Ltd. g doped hollow TiO2 nanoparticles as an effective green fungicide against Fusarium solani and Venturia inaequalis phytopathogens , Nanotechnology, Volume 27,Number 8

[4] Boxi Siddhartha Sankar, (2016) PhD thesis. Synthesis and Characterization of Ag Doped TiO2, CdS, ZnS Nanoparticles for Photocatalytic, Toxic Ions Detection, and Antimicrobial Applications

[5] Ekar, S.U., Shekhar, G., Khollam, Y.B. et al. J Solid State Electrochem (2016). Green synthesis and dye-sensitized solar cell application of rutile and anatase TiO2 nanorods, doi:10.1007/s10008-016-3376-3

[6] Fischer K,Fiedler K.2001.Egg mass variation in the butterfly Lycaena hippothoe: more small or fewer large eggs Population Ecoloy 43:105-109.

[7] Garcia-Barros E. 2000. Body size, egg size, and their interspecific relationships with ecological and life history traits in butterflies (Lepidoptera: Papilionoidea, Hesperiodea). Biological journal of the Linnean Society 70: 251-284.

[8] Lei Sheng, 25 July 2014, Mechanism of TiO2 nanoparticles-induced neurotoxicity in zebrafish (Danio rerio),

[9] Makeri H.K., Mikai VA, Nok JA (2007), Effect of topical application of neem seed (Azadirachta indica) extract on sheep infested with Amblyomma variegatun Afr. J. Biotechnol. 6(20): 2324-2327

[10] Mathew-1993 ,V.Das Gupta, The development of oral liquid dosage forms of metronidazole, Journal of clinical Pharmacyband Therapeutics, vol.18,pg 291-294,1993.

[11] Philbrook N.A.(2011), et al. The effect of TiO2 and Ag nanoparticles on reproduction and development of Drosophila melanogaster and CD-1mice, Toxicol Appl Pharmacol.