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## ZnO nanoparticles as pulse protecting agent against the bruchid beetle *Callosobruchus maculatus* (F).

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**Abstract-** The treatment of *Cicer arietinum* with ZnO Nanoparticles (NPs) were conducted to know the efficacy of ZnO NPs as insecticide. The study revealed a significant enhancement in the mortality rate of the stored grain insect pest *Callosobruchus maculatus* and decreased weight loss percent of the gram seeds with the increasing concentration of the NPs. The result of this study clearly demonstrated the useful nature of ZnO NPs as seed protecting agent for the control of beetles and also enhance nutritional value of the gram seeds with zinc. Stored grains are lost each year due to beetle attack, so the treatment will increase the durability of stored gram and also the resistance developed by the pests due to chemical method of control would overcome by replacing the method by ZnO NPs treatment. Different concentrations of ZnO NPs were used and mortality rate and weight loss percent observed for each concentration. The highest concentration of 1000 ppm showed maximum mortality of 100% at fifth day after treatment. While the minimum concentration of 200 ppm showed 50% and control showed no mortality at the same interval. Similarly, Weight loss percent observed maximum in the control 12.25% followed by 200ppm treated seeds: 11.15% and minimum in 1000ppm: 5.25%.

**Key words:** ZnONPs, Bruchid beetles, *Callosobruchus maculatus*, Stored gram.

### INTRODUCTION

Pulses are considered as an important source of protein for human consumption in many regions of the world. India is the largest producer of pulses in the world, however India is also the largest consumer of pulses in the world due to its increasing population.<sup>1</sup> Pulse crops are cultivated over an area of 24 million hectares with production of about 15 million tonnes. Gram, commonly known as the chickpea is an annual plant of legume family, is widespread in countries with subtropical and tropical climates like India, Turkey, Iran etc. Among the legume, chickpeas are characterized by high nutritional values,

amount of vitamins and other biologically valuable substances which in turn causes high demand for food and feed purpose.<sup>2</sup> Due to increase in population, requirement for chickpea also increased and hence yield of gram in the country is quite low, import quantity increased from 4.9 lakh tonnes during 2014-2015 to 10.81 lakh tonnes in 2016-17 to fulfil the demand of the population and the export quantity decreased from 3.46 lakh tonnes to 1.37 lakh tonnes in 2016-17.<sup>1</sup> During storage, the food commodities are attacked by the number of insects pests like *Sitophilus granaries*, *Rhizopertha dominica*, *Callosobruchus chinensis* and *Callosobruchus maculatus* etc. Among them *C. maculatus* is an important stored grain insect pest distributed in Asia and Africa ranges from tropical

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to sub-tropical world. It has been reported that the favourite host of *C. maculatus* are cowpea and chickpea but it feeds on other legumes as well.<sup>3</sup> Bruchid beetles, *C. maculatus* (fabricus) is a major stored product pest responsible for considerable damage in stored pulses and make the pulses unfit for human consumption.<sup>4</sup> The larvae of *C. maculatus* is totally dependent on the seed of legumes but adults of this pest do not require water and feed but instead spend their limited life on mating and laying eggs at 30°C- 35°C and 70 to 90 % relative humidity are ideal condition for oviposition and hatching takes place after 8 days of oviposition. Adult emerge within 3-4 weeks under favourable condition.<sup>5</sup> Male and female have average lifespan of 7 days laboratory condition and only few of them survive more than 2 weeks.<sup>6,7</sup> Traditional synthetic insecticide chemical control is the most commonly used strategy against stored grain insect pests but long term use of these chemicals develop resistance to the pests.<sup>8</sup> Nanoparticles represented a new generation of environmental remediation technologies that could provide cost effective solution to some of the most challenging environmental clean-up problems.<sup>9</sup> Nanomaterials have a lots of role to playing pests control.<sup>10</sup> A variety of metal NPs eg. Ag, Au, Al, Si and Zn and metal oxide-based polymers ZnO and TiO<sub>2</sub> etc are being developed for crop pests management. ZnO has a very less toxic effect and at the same time Zn is the micronutrient. It is a biosafe material that possesses photo oxidizing and photocatalysis impacts on chemical and biological species.<sup>11</sup> NPs distinct properties allows their possible applications in many fields such as biosensors, nanomedicine and bionano technology.<sup>12</sup> ZnO NPs are reported by several studies as non-toxic to human cells.<sup>13</sup> This aspect necessitated their usage as antibacterial agent, noxious to microorganisms and hold good biocompatibility to human cells.<sup>14</sup>

In this study there is a focus on the mortality rate of the pest of gram, *C. maculatus* and the decrease in weight loss by treating gram seeds with different concentrations of ZnO NPs by allowing the pest to infect in the presence of ZnO NPs and number of insects killed are noted at the interval of 2 days after treatment and weight loss is calculated after the release of F1 generation insects.

## MATERIALS & METHODS

The test beetles were obtained from nearby local market of Lower Chutia, Ranchi (Jharkhand). The beetles

were obtained from the infested seeds of gram. The fresh cultures were also prepared as stock cultures by allowing some isolated beetles to infect the sterilized fresh seeds of gram. The average temperature during the period of investigation was 25±2°C and the average relative humidity of 60±5%. The experiment was conducted in the Entomology division, Department of Zoology, Ranchi University, Ranchi. ZnO NPs colloidal solution (1000 ppm) were having the diameter range of 20-30nm was purchased from Nano Research Lab H21, Gopalpur, Jadugoda (E) Singhbhum, Jamshedpur (Jharkhand) 832102, India. ZnO NPs with three replicates of five different concentrations of 200 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm were thoroughly mixed with the sterilized gram seeds (20 grams each) and placed in jar (80ml). Then the mixture was left for 24 hours and then 20 beetles were introduced in each jar. In the interval of 2 days the mortality rate is calculated following the formulae of Devi *et al.*, (2014)<sup>15</sup> and seed weight loss percent calculated following the formulae of Harris and Limblad (1978)<sup>16</sup>.

$$\text{Adult Mortality \%} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

$$\text{Weight loss \%} = \frac{W_0 - W_1}{W_0} \times 100$$

Where,  $W_0$  and  $W_1$  are the initial and final weight of the seeds respectively.

## RESULT & DISCUSSION

The result showing the adult mortality rate with different concentrations of ZnO NPs : 200 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm has been presented in the Table 1 . Graph 1, showing the percent weight loss v/s the five concentrations of ZnO NPs: 200 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm after release.

The findings are in agreement with the Wazid *et al.* (2018)<sup>17</sup>, Jose A. Gutierrez *et al.* (2021)<sup>18</sup> who reported that with the increase in concentrations of ZnO NPs and number of days after treatment, there is also an increase in the mortality rate of the beetles and high concentration showing minimum seed damage.

## MORTALITY OF BEETLES

In the present investigation the effect of ZnO NPs is tested on the mortality of pulse beetles at 5 different concentrations of 200 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm. It has been observed that with increase in

the concentrations and number of days after treatment, the mortality of beetles has also increased. ZnO NPs having the concentration of 1000 ppm showed highest mortality of 100 % @ 5 days after treatment (DAT) while 750 ppm showed 75%, 500 ppm: 71.43%, 250 and 200 ppm: 50%, Control: 0% at the same interval. ZnO NPs may be attributed to the damage of the protective wax coat on the cuticle of insects, both by sorption and abrasion so the insect begin to lose water and die due to desiccation.<sup>19</sup>

#### PERCENT WEIGHT LOSS OF THE PULSE

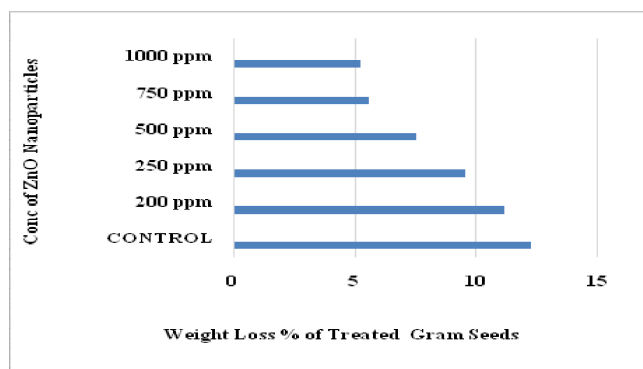
The investigation showed decreasing weight loss percent of the gram with the increasing concentrations of the ZnO NPs. The highest concentration of 1000 ppm showed minimum weight loss of 5.25% while 750 ppm; 5.55%, 500 ppm; 7.55%, 250 ppm; 9.55% and 200 ppm; 11.15%. The control showed the maximum weight loss of 12.25%.

**Table 1- Mortality Rate @1-9 dat.**

Sl. No.	Conc. of ZnO NPs (in ppm)	W <sub>0</sub> - W <sub>1</sub>	Weight Loss %
1.	200	2.23	11.15%
2.	250	1.91	9.55%
3.	500	1.51	7.55%
4.	750	1.11	5.55%
5.	1000	1.05	5.25%
6.	CONTROL	2.45	12.25%

**Table 2- Weight loss percent of seeds with respect to different concentrations of nps**

Sl. No.	Conc. of ZnO NPs (in ppm)	Adult Mortality Rate (In %)				
		1DAT	3DAT	5DAT	7DAT	9DAT
1.	200	0.00	30.00	50.00	53.85	100
2.	250	0.00	35.00	50.00	71.42	100
3.	500	20.00	46.15	71.43	100	
4.	750	35.00	50.00	75.00	100	
5.	1000	35.00	61.54	100		
6.	CONTROL	0.00	0.00	0.00	10.00	33.33



**Graph 2- Concentration of ZnO NPs v/s weight loss % of gram**

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