



# Bio- efficacy and Economics of Diatomaceous Earth against Gram Pod Borer [*Helicoverpa armigera* (Hubner)] in Chickpea with Combination of *Beauveria bassiana*, *Metarhizium anisopliae* and Neem Oil

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## Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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## ABSTRACT

An experiment was conducted during 2021-22 to study the effect of Diatomaceous earth and biopesticides against gram pod borer [*Helicoverpa armigera* (Hubner)] in chickpea at Central Research Farm (CRF), Department of Entomology, SHUATS, Prayagraj during *rabi* season with seven treatments i.e., Diatomaceous earth (T1), Neem oil 3% (T2), *Beauveria bassiana*  $1 \times 10^8$  CFU/ml (T3), *Metarhizium anisopliae*  $1 \times 10^8$  CFU/ml (T4), Diatomaceous earth+neem oil 3% (T5), Diatomaceous earth+ *B.bassiana*  $1 \times 10^8$  CFU/ml (T6), Diatomaceous earth+ *M. anisopliae*  $1 \times 10^8$  CFU/ml (T7) and untreated control (T8) was evaluated against chickpea pod borer (*H.armigera*). Results revealed that, Among the different treatments, the highest per cent population reduction of chickpea pod borer was recorded in Diatomaceous earth+neem oil 3% (74%) followed by Diatomaceous earth+ *B.bassiana*  $1 \times 10^8$  CFU/ml (73%), Diatomaceous earth+ *M.anisopliae*  $1 \times 10^8$  CFU/ml (72%). It is followed by Diatomaceous earth (68%) and neem oil 3% (42%),

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*B.bassiana* 1x10<sup>8</sup>CFU/ml (40%) and *M. anisopliae* 1x10<sup>8</sup>CFU/ml (36%) was the least effective among all treatments. While, the highest yield 23.2q/ha was obtained from the treatment Diatomaceous earth+neem oil 3% as well as B:C ratio 1:4.1. It was followed by Diatomaceous earth+ *B.bassiana*1x10<sup>8</sup>CFU/ml (1:3.8), Diatomaceous earth+ *M.anisopliae*1x10<sup>8</sup>CFU/ml (1:3.7), Diatomaceous earth (1:3.5), neem oil 3% (1:2.4), *B.bassiana*1x10<sup>8</sup>CFU/ml (1:2.3), *M. anisopliae* 1x10<sup>8</sup>CFU/ml (1:2.1), as compared to Control (1:0.9).

**Keywords:** Bio-efficacy; benefit cost ratio; *Cicer arietinum*; diatomaceous earth; *Helicoverpa armigera*.

## 1. INTRODUCTION

Chickpea, [*Cicer arietinum* L.] is the third most important food legume. It is a legume plant of family Fabaceae. It is a herbaceous annual plant with height ranging from 30-70 cm. It has tap root system bearing symbiotic nodules with rhizobium bacteria which are capable of fixing atmospheric nitrogen in plant usable form. Chickpea pod borer [*Helicoverpa armigera* (Hubner)] (Lepidoptera: Noctuidae) is a polyphagous pest, Multivoltine and cosmopolitan pest and is reported to feed and breed on 182 species of host plants belonging to 47 families in India, which causes severe damage in chickpea [1,2]. *H.armigera* causes 90 to 95 % of total damage in chickpea. This pest has high mobility, high reproductive rate and diapause are major factors contributing to its serious pest status [3,4].

Application of Diatomaceous earth (DE), Neem oil and entomopathogenic fungi with different mode of action at proper crop stage is significant for its management. Diatomaceous earths are a type of naturally occurring soft siliceous sedimentary rock, consisting of the fossilized exoskeleton of unicellular algae. Inert powders are rich in silicon and can prevent the attack of pests and plant diseases. Prolonged activity, along with the difficulty of insects to develop resistance to this type of product, make them important tools in pest control [5-7]. Diatomaceous earth kills insects by removing the protective lipid layer of the insect cuticle, leading to death by dehydration. The abrasive activity of DE enhanced the insecticidal efficacy of entomopathogenic fungi. So, Inert dusts synergized the pathogenicity of *B. bassiana* and *M. anisopliae* [8].

These cues led us to investigate the entomotoxicity of Diatomaceous earth, biopesticides and their combinations against *H. armigera*.

## 2. MATERIALS AND METHODS

The experiment was conducted at Central Research Field (CRF), Department of Entomology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj (UP) during 2021-22 during *rabi* season.

The experiment was laid out using pusa 362 variety of chick pea, which was released from Indian Agricultural research institute New Delhi. The experiment was sown in randomized block design with three replications consisting of 7 treatments having one absolute control, Diatomaceous earth, neem oil and two entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* were used. Diatomaceous earth (T1), Neem oil 3% (T2), *B. bassiana* 1x10<sup>8</sup> CFU/ml (T3), *M. anisopliae*1x10<sup>8</sup>CFU/ml (T4), Diatomaceous earth+neem oil 3% (T5), Diatomaceous earth+ *B. bassiana* 1x10<sup>8</sup>CFU/ml (T6), Diatomaceous earth+ *M. anisopliae* 1x10<sup>8</sup>CFU/ml (T7). The seed rate of 80 kg / ha was utilized to raise the crop. Plots of size of 2m x 2m was made. Sowing was done with 30 cm x 10 cm spacing and applied dose of farm yard manure was 12.5t/ha and N, P, K is 25 kg, 50kg and 25kg/ha respectively. The population of *Helicoverpa armigera* was recorded before 1-day spraying and on 3<sup>rd</sup> day, 7<sup>th</sup> day and 14<sup>th</sup> day after insecticidal application. The populations of *H. armigera* was recorded on 5 randomly selected and tagged plants from each plot and then it was converted into per cent of reduction by following formula given by Henderson et al. [9].

$$\text{Percent reduction} = \frac{\text{Population in control} - \text{Population in treatment}}{\text{Population in control}} \times 100$$

The benefit cost ratio (BCR) was determined by dividing the additional returns with the additional cost of imposing the respective treatment on hectare basis following formula,

$$B.C.R = \frac{\text{Net Returns}}{\text{Total Cost of cultivation}}$$

Table 1. Details of biopesticides

T. no	Treatment	Quantity/doses(ha)
T <sub>1</sub>	Diatomaceous Earth	7.5 kg/ha
T <sub>2</sub>	Neem oil	10ml/lit
T <sub>3</sub>	<i>Beauveria bassiana</i> 1x10 <sup>8</sup> CFU/ml	5ml/lit
T <sub>4</sub>	<i>Metarhizium anisopliae</i>	2.5gm/lit
T <sub>5</sub>	Diatomaceous Earth+ Neem oil 3%	2g/lit+10ml/lit
T <sub>6</sub>	DiatomaceousEarth+ <i>B.bassiana</i> 1x10 <sup>8</sup> CFU/ml	5kg/ha+2500gm/ha
T <sub>7</sub>	DiatomaceousEarth+ <i>M.anisopliae</i> 1x10 <sup>8</sup> CFU/ml	5kg/ha+2500gm/ha

### 3. RESULTS AND DISCUSSION

#### 3.1 Efficacy of Diatomaceous Earth (Inert Dust) and Biopesticides against *H. armigera* on Chickpea

##### 3.1.1 First spray

The result of effectiveness of different insecticidal treatments against gram pod borer, *H. armigera* showed that all the treatments were significantly superior over control in terms of mean reduction of gram pod borer larvae (Table 2) Among all the treatments the highest per cent larvae reduction was recorded in T<sub>5</sub> - Diatomaceous Earth+Neem oil 3% (71.3%) followed by T<sub>6</sub> – Diatomaceous Earth+*Beauveria bassiana* 1x10<sup>8</sup>CFU/ml (70%), T<sub>7</sub> - Diatomaceous Earth+ *Metarhizium anisopliae*1x10<sup>8</sup> CFU/ml (68%), T<sub>1</sub> - Diatomaceous Earth (63.7%), T<sub>2</sub> - Neem oil 3% (25.8%), T<sub>3</sub>- *B. bassiana* 1x10<sup>8</sup> CFU/ml (23.9%) and Treatment T<sub>4</sub> – *M. anisopliae*1x10<sup>8</sup> CFU/ml (20.1%) was reported with minimum per cent larva reduction. (T<sub>4</sub>, T<sub>3</sub>, T<sub>2</sub>,) and (T<sub>1</sub>, T<sub>7</sub>,T<sub>6</sub>, T<sub>5</sub>), was found statistically at par with each other.

##### 3.1.2 Second spray

Per cent population reduction of chickpea pod borer on 3<sup>rd</sup>,7<sup>th</sup> and 14<sup>th</sup> revealed that all the treatments were significantly superior over control in terms of mean reduction of *H.armigera* larvae (Table 3). Among all the treatments the highest per cent larva reduction was recorded in T<sub>5</sub>(77.54%) followed by T<sub>6</sub> (76.55%), T<sub>7</sub> (75.03%), T<sub>1</sub> (73.18%), T<sub>2</sub> (57.93%), T<sub>3</sub> (56.41%) and Treatment T<sub>4</sub> (51.99%) was reported with minimum per cent population reduction. (T<sub>4</sub>, T<sub>3</sub>, T<sub>2</sub>, ) and (T<sub>1</sub>, T<sub>7</sub>, T<sub>6</sub>, T<sub>5</sub>) was found statistically at par with each other.

The data on the mean per cent population reduction of first spray and second spray overall mean (Table 4) revealed that all the treatments except untreated control are effective and at par.

Among all the treatments highest per cent reduction of chickpea pod borer as well as increasing the yield was recorded in T<sub>5</sub> (74.41%). Similar findings made by Zeni et al. [10],Constanski et al. [11], Aniwanou et al. [12]. T<sub>6</sub> (73.26%) is found to be the next best treatment which is in line with the findings of Sabbour et al. [13], Arooni-Hesari et al. [14]. T<sub>7</sub> (71.54%) is found to be the next best treatment which is in line with the findings of Sabbour et al. [13]. T<sub>1</sub> (68.44%)is found to be the next effective treatment which is in line with the findings of Gesraha et al. [14], Zeni et al. [10], Ebadollahi et al. [16], Mucha-Pelzer et al. [17]. T<sub>2</sub> (41.86%) is found to be the next effective treatment which is in line with the findings of Reza et al. [18], Kumar et al. [19]. The result of T<sub>3</sub> (40.16%) which is at par with T<sub>4</sub>(36.03%) is found to be least effective but comparatively superior over the control, these findings are supported by Singh et al. [20], Fite et al. [21], Prasad et al. [22].

#### 3.2 Economics of Treatments

The yields among the treatments were significant. The highest yield was recorded in Diatomaceous Earth+Neem oil 3% (23.2q/ha) followed by Diatomaceous Earth+ *Beauveria bassiana* 1x10<sup>8</sup> CFU/ml (21.8q/ha), Diatomaceous Earth+ *Metarhizium anisopliae*1x10<sup>8</sup> CFU/ml (21q/ha), Diatomaceous Earth (20q/ha), Neem oil 3% (15.55q/ha), *B. bassiana* 1x10<sup>8</sup> CFU/ml (14.72q/ha), *M.anisopliae*1x10<sup>8</sup> CFU/ml (13.88q/ha), as compared to control plot (8q/ha).

The highest cost benefit ratio (1:4.1) was found in Diatomaceous Earth+Neem oil 3% treated plots followed by Diatomaceous Earth+ *Beauveria bassiana*1x10<sup>8</sup> CFU/ml (1:3.8), Diatomaceous Earth+ *Metarhizium anisopliae* 1x10<sup>8</sup> CFU/ml (1:3.7) and Diatomaceous Earth (1:3.5), Neem oil 3%, *B. bassiana* 1.5% L.F, *M.anisopliae* 1x10<sup>8</sup> CFU/ml was also found effective as well as economical with cost : benefit 1:2.49, 1:2.31 and 1:2.12 as compared to Control

(1:0.9). The highest yield and cost benefit ratio was recorded in Diatomaceous Earth+Neem oil 3% (23.255q/ha & 1:4.1) followed by Diatomaceous Earth+*B.bassiana*1x10<sup>8</sup> CFU/ml (21.8 q/ha & 1:3.8).

**4. DISCUSSION**

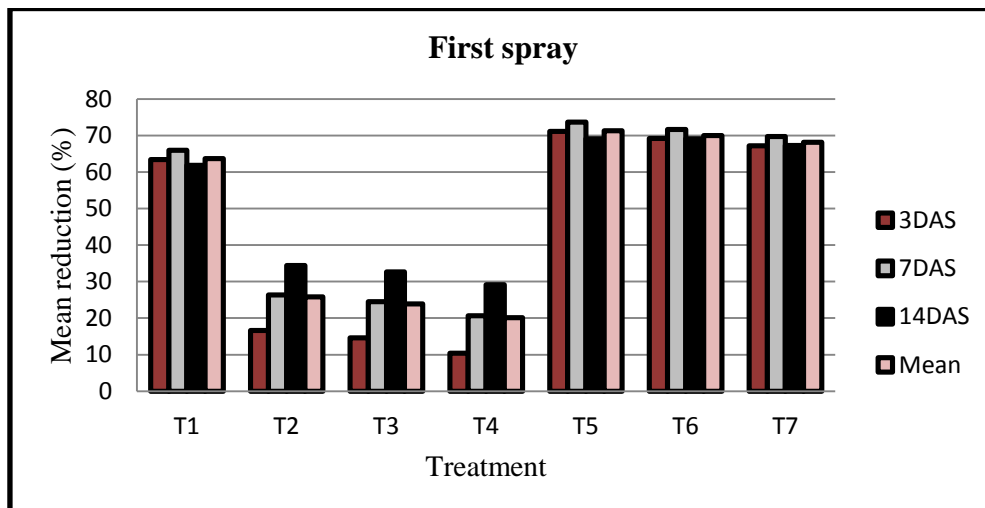
Efficacy of DE was tested against *Helicoverpa armigera*. DE+ neem oil was the most effective and achieved the highest percentage of reduction. DE+neem oil and DE+ *Beauveria bassiana*1x10<sup>8</sup> CFU/ml were the highlight treatments against *H.armigera*. Neem oil combining with DE enhance their properties, pursuing better insecticidal performances. Constraints of the use of neem oils, such as their

poor penetration, strong odor, lack of persistence and high concentration requirements could be reduced if combined with DE. Neem oil increased the DE efficacy by increasing insect's locomotion activity through the particles attaches to insect cuticle causing death through desiccation and, at the same time, DE reduced the oil concentration for satisfactory reduction of larvae. The lowest percentage of reduction was recorded in case of *Metarhizium anisopliae* 1x10<sup>8</sup>CFU/ml. The influence of temperature on the insecticidal effect of DE against *H.armigera* has been extensively evaluated. Generally, at the larval stage, the increase of temperature increased DE efficacy, because at higher temperature insects are more mobile, so the possibility of picking up more DE particles increases [6,23,8].

**Table 2. Effect of diatomaceous earth (Inert dust) and biopesticides against pod borer, *H. armigera* on chickpea after 1<sup>st</sup> spray during *rabi* season of 2021-22**

T. no	Treatment	PTP/5 plants	Mean reduction (%)			
			Days after 1 <sup>st</sup> spray			
			3DAS	7DAS	14DAS	Mean
T <sub>1</sub>	Diatomaceous Earth	2.8	63.39	65.90	61.79	63.69
T <sub>2</sub>	Neem oil	3	16.66	26.36	34.40	25.80
T <sub>3</sub>	<i>Beauveria bassiana</i> 1x10 <sup>8</sup> CFU/ml	2.86	14.58	24.50	32.65	23.91
T <sub>4</sub>	<i>Metarhizium anisopliae</i>	2.93	10.41	20.69	29.14	20.08
T <sub>5</sub>	Diatomaceous Earth+ Neem oil 3%	3.06	71.13	73.63	69.10	71.28
T <sub>6</sub>	Diatomaceous Earth+ <i>B. bassiana</i> 1x10 <sup>8</sup> CFU/ml	2.93	69.17	71.67	69.10	69.98
T <sub>7</sub>	Diatomaceous Earth+ <i>M.anisopliae</i> 1x10 <sup>8</sup> CFU/ml	2.867	67.21	69.71	67.25	68.05
T <sub>0</sub>	Control	3.3	0.00	0.00	0.00	0.00
	F-test	NS	S	S	S	S
	S. Ed (±)	-	2.58	3.47	3.11	4.06
	C.D. (P = 0.5)	-	5.54	7.44	6.67	8.70

\*PTP- Pretreatment population \*\* DAS- Day after spray

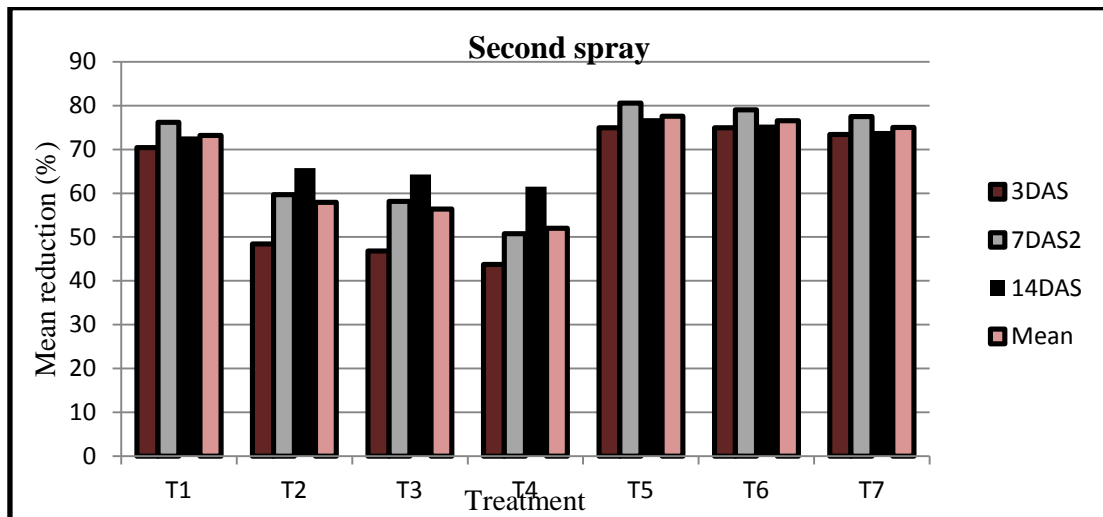


**Fig. 1. Graphical representation of effect of Diatomaceous earth( Inert dust) and biopesticides against pod borer, *H. armigera* on chickpea after 1<sup>st</sup> spray during *rabi* season of 2021-22**

**Table 3. Effect of Diatomaceous earth (Inert dust) and biopesticides against *H. armigera* on chickpea after 2<sup>nd</sup> spray during *rabi* season of 2021-22**

T. No	Treatment	PTP/5 plants	Mean reduction (%)			
			Days after 1 <sup>st</sup> spray			
			3DAS	7DAS	14DAS	Mean
T <sub>1</sub>	Diatomaceous Earth	1.4	70.41	76.21	72.94	73.18
T <sub>2</sub>	Neem oil	2.4	48.41	59.68	65.70	57.93
T <sub>3</sub>	<i>Beauveria bassiana</i> 1x10 <sup>8</sup> CFU/ml	2.46	46.82	58.16	64.25	56.41
T <sub>4</sub>	<i>Metarhizium anisopliae</i>	2.6	43.72	50.79	61.47	51.99
T <sub>5</sub>	Diatomaceous Earth+ Neem oil 3%	1.13	74.96	80.56	77.11	77.54
T <sub>6</sub>	Diatomaceous Earth+ <i>B. bassiana</i> 1x10 <sup>8</sup> CFU/ml	1.13	74.96	79.05	75.66	76.55
T <sub>7</sub>	Diatomaceous Earth+ <i>M.anisopliae</i> 1x10 <sup>8</sup> CFU/ml	1.2	73.37	77.53	74.21	75.03
T <sub>0</sub>	Control	4.06	0.00	0.00	0.00	0.00
	F-test	S	S	S	S	S
	S. Ed (±)	0.13	3.44	3.62	3.48	3.61
	C.D. (P = 0.5)	0.27	7.38	7.76	7.46	7.74

\*PTP- Pretreatment population \*\* DAS- Day after spray



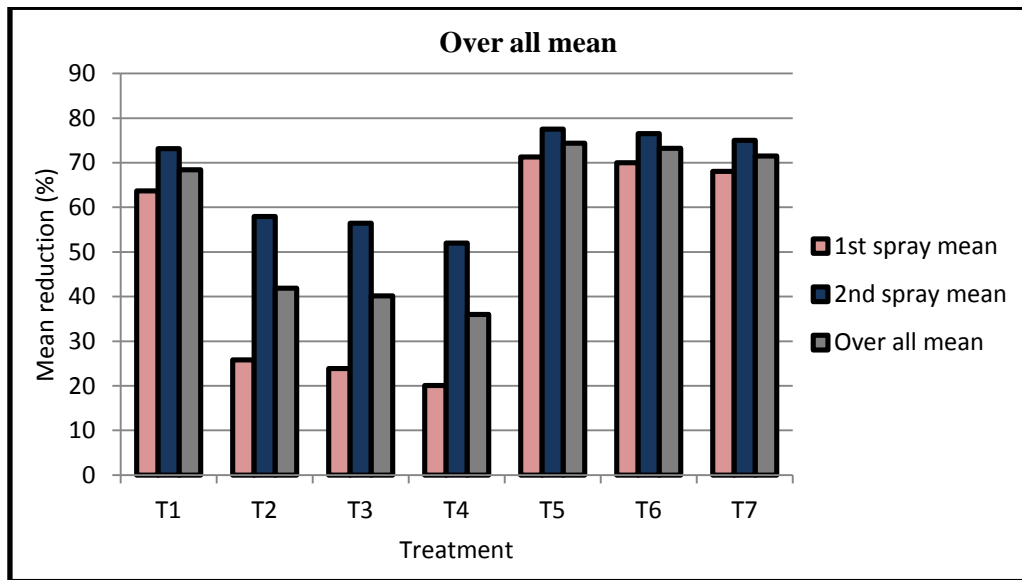
**Fig. 2. Graphical representation of effect of Diatomaceous earth( Inert dust) and biopesticides against *H. armigera* on chickpea after 2<sup>nd</sup> spray during *rabi* season of 2021-22**

**Table 4. Effect of Diatomaceous earth( Inert dust) and biopesticides against *H. armigera* on chickpea over all mean during *rabi* season of 2021-22**

T. no	Treatment	1 <sup>st</sup> spray mean	2 <sup>nd</sup> spray mean	Over all mean
T <sub>1</sub>	Diatomaceous Earth	63.69	73.18	68.44
T <sub>2</sub>	Neem oil	25.80	57.93	41.86
T <sub>3</sub>	<i>Beauveria bassiana</i> 1x10 <sup>8</sup> CFU/ml	23.91	56.41	40.16
T <sub>4</sub>	<i>Metarhizium anisopliae</i> 1x10 <sup>8</sup> CFU/ml	20.08	51.99	36.03
T <sub>5</sub>	Diatomaceous Earth+ Neem oil 3%	71.28	77.54	74.41
T <sub>6</sub>	Diatomaceous Earth+ <i>B. bassiana</i> 1x10 <sup>8</sup> CFU/ml	69.98	76.55	73.26
T <sub>7</sub>	Diatomaceous Earth+ <i>M.anisopliae</i> 1x10 <sup>8</sup> CFU/ml	68.05	75.03	71.54

Table 5. Yield and economics of cultivation

T. no	Treatments	Yield q/ha	Market price (q/ha) (₹)	Gross return(₹)	Net return	Total cost(ha)	B:C ratio
T <sub>1</sub>	Diatomaceous Earth	20	5500	110000	85,720	24280	1:3.53
T <sub>2</sub>	Neem oil	15.55	5500	85555.58	61,056	24500	1:2.49
T <sub>3</sub>	<i>Beauveria bassiana</i> 1x10 <sup>8</sup> CFU/ml	14.72	5500	80972.21	56,552	24420	1:2.31
T <sub>4</sub>	<i>Metarhizium anisopliae</i> 1x10 <sup>8</sup> CFU/ml	13.88	5500	76388.89	51,909	24480	1:2.12
T <sub>5</sub>	Diatomaceous Earth+ Neem oil 3%	23.2	5500	127600	102,960	24640	1:4.1
T <sub>6</sub>	Diatomaceous Earth+ <i>B. bassiana</i> 1x10 <sup>8</sup> CFU/ml	21.8	5500	119900	95,300	24600	1:3.8
T <sub>7</sub>	Diatomaceous Earth+ <i>M.anisopliae</i> 1x10 <sup>8</sup> CFU/ml	21	5500	115500	90,880	24620	1:3.69
T <sub>0</sub>	Control	8	5500	44000	21,680	22,320	1:0.9



**Fig. 3. Graphical representation of effect of diatomaceous earth (Inert dust) and biopesticides against *H. armigera* on chickpea over all mean during *rabi* season of 2021-22**

Our findings indicated that entomopathogenic fungi have low insecticidal toxicity against *H. armigera*. Application of *B. bassiana* alone is less effective and toxicity increase when combined with DE. Results exalted the suitability of fungi as crop protectants but also pointed out their need for peculiar humid conditions to achieve satisfactory conidial adherence, germination, and penetration through the cuticle. The synergistic effect between DE and entomopathogenic fungi expands the area for fungal spore penetration, increasing insect mycosis [10]. Applications of mixtures with these two ecologically compatible agents is a very appealing approach to IPM in *H. armigera*. The application of DE+ *B. bassiana*  $1 \times 10^8$  CFU/ml combined considerably increased larval percentage of reduction in *H. armigera*, temperature and longer exposure intervals compared with DE+ *B. bassiana*  $1 \times 10^8$  CFU/ml and *B. bassiana*  $1 \times 10^8$  CFU/ml alone. Application of DE+ *Metarhizium anisopliae*  $1 \times 10^8$  CFU/ml resulted in higher percentage of reduction of the *H. armigera* compared to the efficacies of each compound alone.

## 5. CONCLUSION

Among the treatments studied, Diatomaceous Earth+Neem oil 3% gave the highest cost benefit ratio (1:3.58) and marketing yield (20.55q/ha) followed by Diatomaceous Earth+ *B. bassiana*  $1 \times 10^8$  CFU/ml (1:3.40) and 19.722q/ha), Diatomaceous Earth+ *M. anisopliae*  $1 \times 10^8$  CFU/ml (1:3.21 and 18.88q/ha), Diatomaceous Earth

(1:2.96 and 17.5q/ha), Neem oil 3% *B. bassiana*  $1 \times 10^8$  CFU/ml and *M. anisopliae*  $1 \times 10^8$  CFU/ml respectively as such more trials are required in future to validate the findings. Hence more trials are needed to be conducted in future to validate the findings which can be useful for the farmers in a feasible manner for sustainable production of chickpea and to prevent the losses occurring from this insect pest infesting the crop.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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