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Meghalatha Kammara
M.Sc. Scholar, Department of Entomology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Science, Prayagraj, Uttar Pradesh, India

Sobita Simon
Professor and Head, Department of Entomology and Plant Pathology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Science, Prayagraj, Uttar Pradesh, India

Swetha Dasika
M.Sc. Scholar, Department of Entomology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Science, Prayagraj, Uttar Pradesh, India

Corresponding Author
Meghalatha Kammara
M.Sc. Scholar, Department of Entomology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Science, Prayagraj, Uttar Pradesh, India

Comparative efficacy of bio-agents and botanical extracts against *Helicoverpa armigera* (Hubner) on chickpea (*Cicer arietinum* L.) crop

Meghalatha Kammara, Sobita Simon and Swetha Dasika

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Abstract
Efficacy of bio-agents and botanical extracts against *H. armigera* was assessed in the field during the 2020-2021 Rabi Season, with three replications of the Randomize Block Design. When compared to the control, all of the bio-agents and botanical extracts tested were found to be effective in suppressing the gram pod borer infestation. *Beauveria bassiana* was shown to be the most effective in reducing *H. armigera* 14.18 percent pod damage, followed by *Metarhizium anisopliae* -15.37 percent pod damage, among all the spray materials studied. Pod infestation was lowest in the *Beauveria bassiana* test plots and highest in the control plots. Similarly, the *Beauveria bassiana* treated plot yielded the most (14.48 q/ha), while the control plot produced the least (8. q/ha). *Beauveria bassiana* was found to be the most effective in controlling Gram pod borer infestation, resulting in the least amount of pod damage and thus increased Chickpea yield. However, the Chemical and synthetic insecticide was extremely toxic. Furthermore, *Metarhizium anisopliae*, EPN, Custard apple leaf extract, Sweet flag rhizome oil and clove oil extract also showed better results than control in term of pest reduction and were comparatively safe to the environment. Hence these botanicals and bio-agents should be included in IPM program for the suitable management of *H. armigera* associated with Chickpea crop.

Keywords: *Beauveria bassiana*, bio-agents, botanicals, chickpea, clove oil, custard apple leaf extract, EPN, *H. armigera, Metarhizium anisopliae,* pod damage, sweet flag rhizome oil

Introduction

Pulses are high in nutritional content, a crucial facet of a balanced weight-loss plan, and a good source of protein and micronutrients. Pulses and legumes are both important for maintaining and improving soil health **(**FAO, 2020) [3]. Pulses, when combined with cereals, pulses provide an ideal vegetarian protein source with high biological value. India is the world's largest producer, importer, and consumer of pulses (25.42 million tons), accounting for 25% of global output and covering 35% of global land area (Ahlawat *et al.*, 2016) [1]. Pulses are high in nutritional content, a crucial facet of a balanced weight-loss plan, and a good source of protein and micronutrients. Pulses and legumes are both important for maintaining and improving soil health (FAO, 2020) [3].

Chickpea (*Cicer arietinum* L.) is a legume crop belonging to the Fabaceae family, subfamily Faboideae. It's also known as gram or Bengal gram, Garbanzo, or Garbanzo bean, and Egyptian pea, or Chana on occasion. Protein is abundant in chickpea seeds. Chickpea is an important pulse crop, especially in African and Asian countries. Chickpeas are grown throughout Asia, Africa, Europe, Australia, North America, and South America (Singh, 2015) [27]. India has been the world's leading producer of chickpeas, also known as garbanzo beans, for several years. Chickpea production in India is estimated more than 11 million metric tons by 2021. With an estimated 630,000 metric tons of chickpeas, Turkey came in second. Around 15 million metric tons of chickpeas were produced worldwide in that year (Shahbandeh, 2021) [25]. Chickpeas are categorized into two kinds: Desi and Kabuli. Desi seeds are tiny and have a crumpled surface. They vary in colour from yellow to black. Kabuli seeds are big and appear to be pale in color. About 85% of overall production is made up of Desi chickpeas, with Kabuli accounting for the remaining 15%. Kabuli is mostly grown in Mediterranean countries, whereas Desi is more popular in India (Purushothaman *et al.*, 2014) [20]. The Kabuli chickpea had a larger seed (26 g/ 100 seeds) than the Desi kind (21 g/ 100 seeds). The hydration capacity per seed of Desi (0.16 g) was lower than the Kabuli type (0.26 g) (Khan *et al.*, 1995) [13]. The most dangerous pests in chickpea farming are eleven insect pest outbreaks. The pod Borer, *Helicoverpa armigera* (Hubner), is considered a high-risk insect pest that causes 30 percent to 40 percent damage to pods on average, with 80 percent to 90 percent damage in a favorable climate. One pod borer larva per one meter row duration is the chickpea economic threshold level (Kambrekar, 2016) [12]. Direct crop yield losses and insect pest monitoring and management expenditures, particularly pesticide costs, result in monetary losses (Patil *et al.,* 2017) [19].

Bio-control is one of the best humans and environmental-friendly options among the non-chemical insect control approaches. In contrast to conventional insecticides, the entomopathogenic fungi (EPF) has several advantages, including cost-effectiveness, high yields and the absence of harmful side effects on beneficial organisms (Mantzoukas, *et al.,* 2020) [15]. The use of live predatory insects, entomopathogenic nematodes (EPN's) or microbial pathogens to suppress insect populations has been described. EPN's are one of the greatest biocontrol agents to successfully control several insect pests of major economic importance (Gozel, *et al.,* 2016) [8]. Botanical insecticides were major weapons in the farmer's arsenal against crop pests. The combination of efficacy, speed of action and low cost of synthetic insecticides drove many botanicals to near obscurity. Concerns over environmental contamination led to a resurgence in interest in 'natural' means of pest control (Isman, 2006) [10].

Non-chemical insect control is one of the most effective humans and environmentally friendly alternatives. Botanicals and Bio-agents plays a key role for sustainable management of insect pests. Advantages over conventional insecticides include cost-effectiveness, high yield and absence of harmful side-effects for beneficial organisms.

Materials and Methods

An experiment in which facts are established through detailed assessments of the current investigation. The current research was conducted in the lab and in the field to assess the relative toxicity, persistency, and phytotoxicity of formulations, antifeedant activity, and bio-pesticides against the gram pod borer *Helicoverpa armigera* (Hubner). During the Rabi season of 2020-21, the experiment was done in the Department of Entomology's experimental research plot at the Central Research Farm of Sam Higginbottom University of Agriculture Technology and Sciences (SHUATS), Prayagraj.

Field layout and experimental design

In the years 2020-21, the chickpea (*Cicer arientinum*) variety Avarodhi, which is widely cultivated in this area, was sown in the experimental field in a Randomized Block Design. The crop was grown in 21 plots with a total area of 3x4 m each and a row-to-row distance of 30 cm and a plant-to-plant distance of 10 cm, respectively. Each of the seven treatments was replicated three times.

Treatment Application

Different treatments were imposed as foliar sprays against the pod borer. The first spraying is going to give when the infestation of pod borer was noticed to cross the ETL's in experimental plots and the second spray is going to give after 15 days interval. A total of two sprays was given during the growth period of the crop.

Table 1: Details of treatments used in experiment

Tr. No	Treatment	Dosage	References
T_0	Control		
T_1	*Beauveria bassiana*	$1x10^9$ conidia/ml	Savita *et al.,* (2015) [24]
T_2	*Metarhizium anisopliae*	1×10^9 spores/ml.	Kumar *et al.,* (2004) [2]
T_3	EPN	200 IJL/ml	Kalia, *et al.,* (2014) [11]
T_4	Sweet flag rhizome oil (vasambu) (*Acorus calamus*)	5%	Melani *et al.,* (2016) [16]
T_5	Clove oil (*Syzygium aromaticum*)	5%	Gong *et al.,* (2020) [6]
T_6	Custard apple (*Annona squamosa*) Leaf Extract	5%	Ajaykumara *et al.,* (2018) [2]

Isolations and identification of *Beauveria bassiana* from naturally mycosed insect cadavers

This method was used to research natural EPF infections in fields since it relies on collecting dead insects from the fields and protocol described below is similar to the one used. (Sharma *et al.,* 2018) [26]. *Beauveria bassiana* is being isolated from an insect cadaver (*Indarbela quadrinotata*) collected from Karchana and Dandi villages near Allahabad, as well as the Department of Agroforestry SHUATS Guava plants. Insect cadavers are brought to the lab as independent things in sterile tubes. A stereomicroscope is used to inspect insects for possible mycosis (40 X). The insects are surface sterilized for 3 minutes in 70% ethanol or 1% NaOCl in the presence of obvious mycosis, then washed three times in sterilized water. The insect cadaver's sporulating EPF is then directly plated. Cadavers are then grown for up to 3 weeks at 22 degrees Celsius on a selective medium, depending on how long the fungi take to germinate and multiply. The cadavers can be homogenized and plated on the selective medium if they do not germinate. The acquired fungi are subcultured on Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) to obtain a pure culture (Humber *et al.,* 2012) [9].

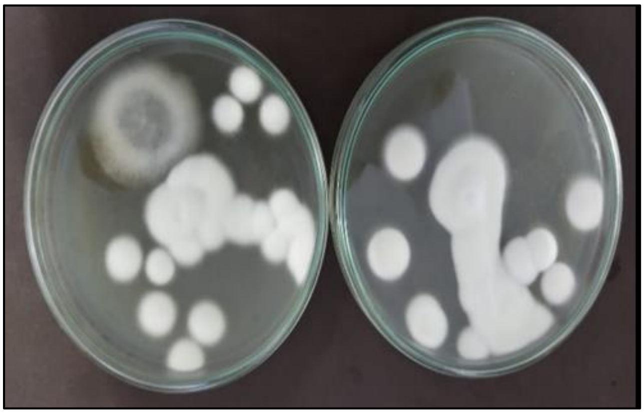

Fig 1: Isolated *B. bassiana* in the laboratory

Isolation of Entomopathogenic Nematode

Collecting soil samples Consider covering an area of 2-4 m^2 for each sample location. Collected soil samples from at least 15 cm depth. At least 5 random samples should be taken from this area. For each sample, take three sub-samples. mix or keep the sub samples separate, depending on the study's aims. Label samples with a waterproof marker. Include the following details for each sampling location: Date, habitat, accompanying flora, temperature, height, and other details about the site. They could be potential EPN hosts in the wild. Between samples, properly clean collection tools with water and/or disinfect them with a 70% ethanol or 0.5 percent bleach solution. Transporting samples to the lab should be done in a cooler. To gather information on soil composition, texture, moisture, electrical conductivity, or other desirable soil properties, take a part of the soil sample for examination. Water the soil to keep it moist and promote nematode movement. Using a spray bottle, gradually increase the moisture level in the soil. White Trap with Modifications was used to recover nematodes from infected cadavers. A petri dish contained within a larger dish (100 mm). Inside the smaller dish, place a single circular filter paper (Whatman #1). To avoid contamination, place cadavers on the filter paper of the smaller dish, making sure they do not touch. Fill the outer (larger) Petri dish with sterile distilled water (20 ml). Do not put any water in the dish containing the cadavers (Orozco *et al.*, 2014) [17].

Preparation of plant leaf extract

Plant materials (***Annona squamosa***) were collected from the outside of SHUATS, Prayagraj, during the morning hours. The leaves were thoroughly cleansed with tap water to remove dust and surface pollutants. These leaves were washed and dried in the shade until the surface moisture had disappeared. 100 g of cleaned leaves were crushed with a little water in a household electric grinder to form the chunky paste. 100 g of powdered paste was soaked overnight in 2 liters of water to generate 5 percent plant extracts. The next day, the solution was filtered and squeezed through the muslin cloth. Two pinches of soap detergent powder were added to the filtrate to function as a sticker. On the chickpea crop, the 5% formulations were used to spray against *Helicoverpa armigera* (Salim and Abed, 2015) [23].

Soxhlet Apparatus Oil Extraction Procedure

Dried plant material of Sweet flag rhizome and Cloves must be smashed with a pestle and mortar to increase surface area. Enough plant material should be able to be packed into the porous cellulose thimble. All of the equipment must first be assembled. The solvent (250 mL ethanol) is then added to a round bottom flask on an Isomantle with a Soxhlet extractor and condenser. The thimble of the Soxhlet extractor is filled with crushed plant material. To make the sidearm lag, glass wool is used. The Isomantle and Wasgin heat the solvent, which evaporates as it passes through the apparatus to the condenser. The condensate drips into the thimble's reservoir. The solvent returns to the flask when it reaches the siphon, restarting the cycle. The entire treatment should take no more than 12 hours. Once the method is complete, the ethanol should be naturally evaporated in a glass beaker, leaving a small yield of extracted plant material (about 2 to 3 ml) in the glass bottom flask (Redfern *et al.*, 2014) [22].

Fig 2: Soxhlet's extraction of essential oils

Pod damage analysis

At maturity of the crop, both healthy and damaged pods was plucked from 5 randomly selected plants in each plot, and data was recorded on percentage pod damage by gram pod borer. Pod damage percentage was calculated using the following formula:

$$\% \text{ Pod damage} = \frac{\text{No.of affected pods}}{\text{Total no.of pods}} \times 100$$

Data collection and Statistical analysis

The observations were recorded one day before spray (1DBS) and 3 (DAS),7 (DAS), 14 (DAS) days after spray imposition of treatment (DAT). Five plants from each plot were chosen at random for larval counts. The data collected on larval population count, Percent pod damage and grain yield for various treatments was recorded in each plot and was transformed into the corresponding values as per Gomez and Gomez, (1976) [5] and subjected to statistical analysis for testing the level of significance. Similarly, the replication wise data of each treatment was also subjected to analysis of variance.

Yield

At harvest from the net plot area, the yield in different treatments was recorded. Total yield was estimated by adding all picking yields from each treatment, then converting to yield per hectares using the formula:

$$\text{Yield (kg ha}^{-1}) = \frac{\text{Yield plot}^{-1}}{\text{Plot size}} \times 10000$$

Results

The results of various field and laboratory experiments conducted on the efficacy of biorationals such as extracts of different plant materials @ 5% and bio-agents against Gram pod borer (*Helicoverpa armigera*) during the rabi crop seasons of 2020-21 in CRF Fields, Department of

Entomology, SHUATS, Prayagraj for eco-friendly management of Gram pod borer (*Helicoverpa armigera*) during the rabi crop seasons of 2020-21 in CRF Fields, Department The overall findings of various experiments were statistically analyzed and discussed here.

The observations taken on (1 DBS) one day before spraying of treatment noted in control T_0 (17.93% pod damage), T_1 (15.56% pod damage), T_2 (17.31% pod damage), T_3 (17.23% pod damage), T_4 (13.26% pod damage), T_5 (18.33% pod damage), and T_6 (18.01% pod damage).

Percent pod damage and effect of treatments on chickpea crop against *Helicoverpa armigera* at first spraying.

The observations taken at first spray shows the following treatment spraying were compared, and every treatment was shown to be significantly superior to the control (27.90% pod damage). According to the findings, the most effective treatment was *Beauveria bassiana* (14.00% pod damage), followed by *Metarhizium anisopliae* (15.43% pod damage), EPN (16.98% pod damage), Custard apple leaf extract (20.92% pod damage), Sweet flag rhizome oil (22.15% pod damage), and clove oil (22.15% pod damage) (24.68% pod damage).

The overall percentage of pod damage of the first spraying treatments was compared, and the treatment significantly outperformed the control (25.09% pod damage). According to the findings, the most effective treatment was *Beauveria bassiana* (12.41% pod damage), followed by *Metarhizium anisopliae* (13.84% pod damage), EPN (14.65% pod damage), Custard apple leaf extract (17.72% pod damage), Sweet flag rhizome oil (17.73% pod damage), and clove oil (17.73% pod damage) (19.97% pod damage).

Table 2: Percent pod infestation and damage during the crop period after 1st spray

Tr. No	Treatments	1 DBS	3 DAS	7 DAS	14 DAS	Overall
T_0	Control	17.93	21.47	25.90	27.90	25.09
T_1	*Beauveria bassiana*	15.56	12.58	10.66	14.00	12.41
T_2	*Metarhizium anisopliae*	17.31	13.46	12.64	15.43	13.84
T_3	EPN	17.23	13.73	13.23	16.98	14.65
T_4	Sweet flag rhizome oil(vasambu) (*Acorus calamus*)	13.26	14.21	16.82	22.15	17.73
T_5	Clove Oil	18.33	17.89	17.34	24.68	19.97
T_6	Custard apple (*Annona squamosa*) Leaf Extract	18.01	16.97	15.26	20.92	17.72
	F.test	NS	S	S	S	S
	S.Em (±)	1.51	0.81	0.53	0.46	0.93
	C.D. at 5%	3.29	2.48	1.62	1.42	2.87

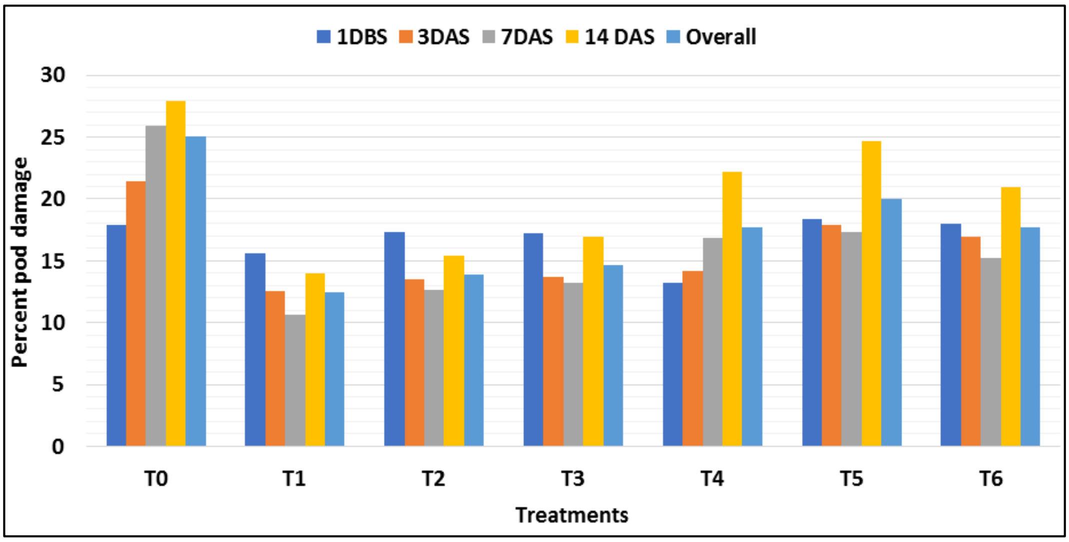

Fig 3: Percent pod infestation and damage during the crop period after 1st spray

Percent pod damage and effect of treatments on chickpea crop against *Helicoverpa armigera* at second spraying.

Treatment spraying was compared in the second spray, and every treatment was shown to be significantly superior to the control (34.25% pod damage). According to the findings, the most effective treatment was *Beauveria bassiana* (13.99% pod damage), followed by *Metarhizium anisopliae* (15.23% pod damage), EPN (17.33% pod damage), Custard apple leaf extract (23.22% pod damage), Sweet flag rhizome oil (25.07% pod damage), and clove oil (25.07% pod damage) (28.46% pod damage).

The overall percentage of pod damage of the second spraying treatments was compared, and the treatment greatly outperformed the control (33.45% pod damage). According to the findings, the most effective treatment was *Beauveria bassiana* (14.18% pod damage), followed by *Metarhizium anisopliae* (15.37% pod damage), EPN (16.69% pod damage), Custard apple leaf extract (22.61% pod damage), Sweet flag rhizome oil (24.63% pod damage), and clove oil (24.63% pod damage) (27.10% pod damage).

Table 3: Percent pod infestation and damage during the crop period after 2nd spray.

Tr. No	Treatments	1 DBS	3 DAS	7 DAS	14 DAS	Overall
T_0	Control	31.73	32.44	33.66	34.25	33.45
T_1	*Beauveria bassiana*	16.35	14.94	13.61	13.99	14.18
T_2	*Metarhizium anisopliae*	17.93	15.97	14.92	15.23	15.37
T_3	EPN	19.72	16.80	15.94	17.33	16.69
T_4	Sweet flag rhizome oil(vasambu) (*Acorus calamus*)	25.73	23.88	24.94	25.07	24.63
T_5	Clove Oil	26.32	25.69	27.16	28.46	27.10
T_6	Custard apple (*Annona squamosa*) Leaf Extract	24.90	22.28	22.32	23.22	22.61
	F.test	NS	S	S	S	S
	S.Em (±)	0.64	0.79	0.37	0.60	0.43
	C.D. at 5%	1.98	2.43	1.13	1.84	1.34

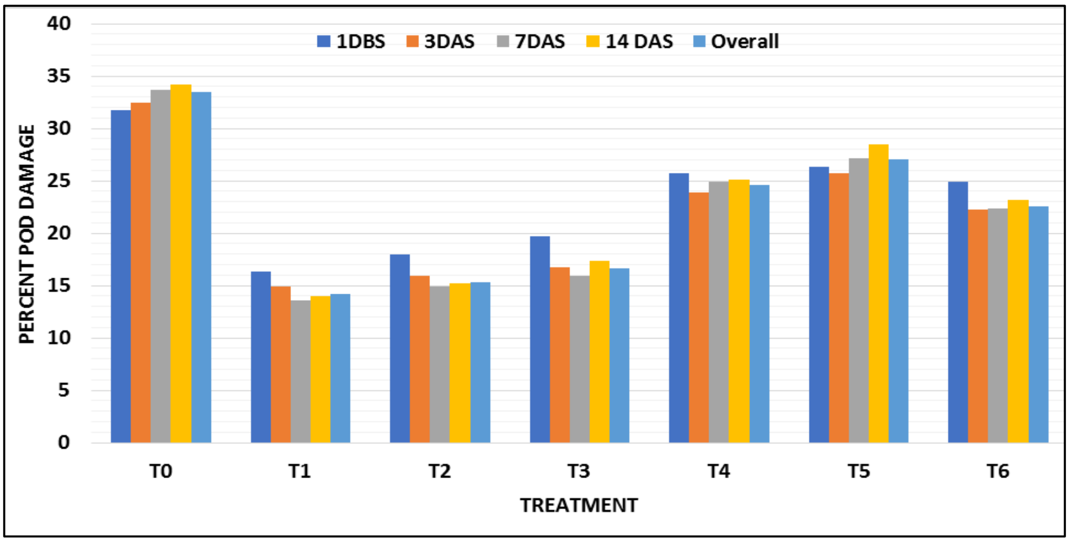

Fig 4: Percent pod infestation and damage during the crop period after 2nd spray.

Effects of Treatments on Grain Yield

The field studies conducted during Rabi 2020-21 revealed that all of the treatments significantly improved the control in terms of efficacy, as measured by better grain output. *Beauveria bassiana* provided the best control of the pod borer and thus the highest grain yield of 14.48 q/ha. *Metarhizium anisopliae* (13.51 q/ha) was next, followed by EPN (12.53 q/ha), Custard apple (*Annona squamosa*) Leaf Extract (11.82 q/ha), and Sweet flag rhizome oil (vasambu) (*Acorus calamus*) (10.84 q/ha). According to the data, the lowest yield recorded was for treatment of Clove Oil (10.04 q/ha), A comparison was also made with the control plot, which yielded 8.77 q/ha. *Beauveria bassiana* provided an increase in yield of about 65.18% hectare above the control. *Metarhizium anisopliae* was second (54.04%), followed by EPN (42.87%), Custard apple (*Annona squamosa*) Leaf Extract (34.77%), and Sweet flag rhizome oil (vasambu) (Acorus calamus) (23.60%). According to the data, the lowest yield recorded was for treatment of Clove Oil (14.48%), yield.

Table 4: Effect of botanicals and bio-agents on Grain Yield

Tr. No	Treatments	Yield (qn/ha)	Yield increases over control (%)
T_0	Control	8.77	-
T_1	*Beauveria bassiana*	14.48	65.18
T_2	*Metarhizium anisopliae*	13.51	54.04
T_3	EPN	12.53	42.87
T_4	Sweet flag rhizome oil (vasambu) (*Acorus calamus*)	10.84	23.60
T_5	Clove Oil	10.04	14.48
T_6	Custard apple (*Annona squamosa*) Leaf Extract	11.82	34.77
	F.test	S	
	S.Em (±)	0.32	
	C.D. at 5%	1	

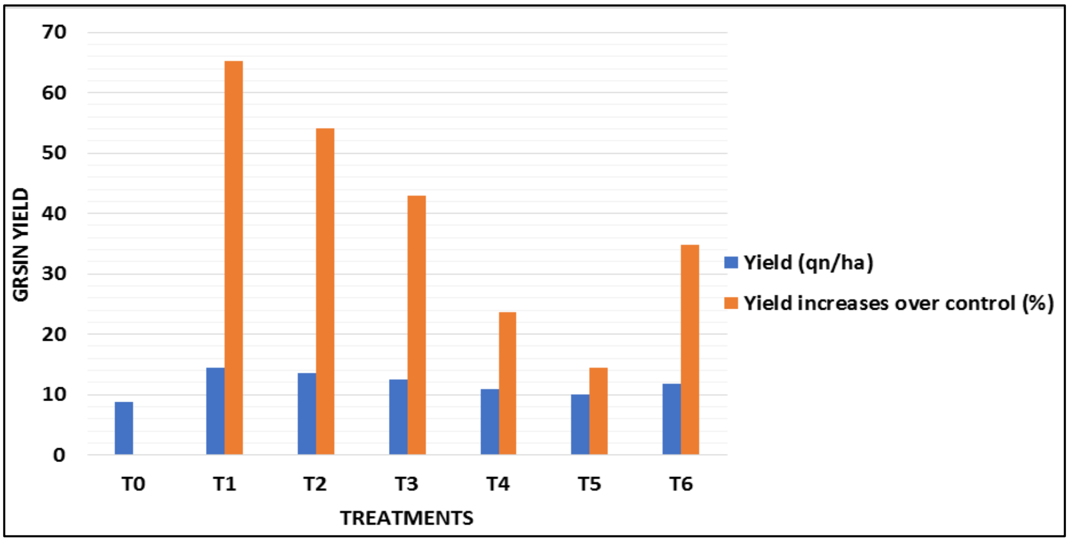

Fig 5: Effect of botanicals and bio-agents on Grain Yield

Discussion

The dose-mortality connection between *B.bassiana* and *H. armigera* was investigated by Gopalkrishnan and Narayanan (1990). They found that the fungus was harmful to all stages of *H. armigera*, causing 60 to 100 percent mortality in I-V instars and 100 percent mortality in eggs at varied doses of 1.0 x 10^2 conidia/ml in pupal and adult stages, with incubation periods varying from 2 to 14 days. *Metarhizium anisopliae* shows the second highest effective treatment and doses is supported with **(**Rachappa *et al.*, 2009) [21]. Glazer and Navon (1990) [4] have tested the pathogenicity of EPN'S, *Steinernema* and *Heterorhabditis* against *H. armigera* and reported complete mortality in 200 IJs *S. feltiae* treatment with LD value 54 Js/insect. Patel (1999) [18] carried out the laboratory bioassay of two *Steinernema* spp against *H. armigera* and found that after 120 hrs. *Steinernema sp.* (VI) induced maximum mortality (79.96%) at 50 Js/larva were applied Whereas, significantly low larval mortality (49.96%) was observed in 10 IJs /larva treatment and no mortality in control. Similarly, in *Steinernema* sp. (A.I) bioassay the *H. armigera* mortality was up to 49.96% at 50 IJs dose after 24 hrs., which was followed by lower doses. But at 120 hrs maximum 87.99% larval mortality was recorded at highest EPN doses (40 and 50 IJs /larva). The 5% formulations of plant leaf extract spray against *Helicoverpa armigera* were find similar with Salim and Abed, 2015 [23]; Melani *et al.*, (2016) [16]; Gong *et al.*, (2020) [6].

Summery

Chickpea (Cicer arietinum L.) is a legume belonging to the Fabaceae family. Protein is abundant in chickpea seeds. Chickpea is an important pulse crop, especially in African and Asian countries. India has been the world's leading producer of chickpeas, also known as garbanzo beans. Chickpeas are categorized into two kinds: Desi and Kabuli. The *Helicoverpa armigera* (Hubner), the pod Borer, is regarded to be a high-risk insect-pest that infects an average 30%-40% damage to pods, which can be increased in a suitable climate to 80%-90%. Several chemical insecticides, botanical insecticides and microbial formulations belonging to various groups are recommended for management of gram pod borer (*Helicoverpa armigera*). Among this non-chemical insect control methods, biological control is one of the most effective humans and environmentally friendly alternatives. Both the Botanicals and Bio-agents plays a key role for the sustainable management of insect pests. The most effective treatment was *Beauveria bassiana* (14.00% pod damage), followed by *Metarhizium anisopliae* (15.43%) and EPN (16.98%). The observations taken at first spray shows the following treatment spraying were compared. Every treatment was shown to be significantly superior to the control (27.90%). The most effective treatment was *Beauveria bassiana* (13.99% pod damage), followed by *Metarhizium anisopliae* (15.23% Pod damage) and EPN (17.33% Pod Damage). Treatment spraying was compared in the second spray, and every treatment was shown to be significantly superior to the control. The overall percentage of pod damage of the second spraying treatments was compared, and the treatment greatly outperformed the control (33.45%). The most effective treatment was *Beauveria bassiana* 14.18% pod damage control, followed by *Metarhizium anisopliae* (15.37%) and EPN (16.69%), Custard apple leaf extract (22.61% pod damage), Sweet flag rhizome oil (24.63% pod damage), and clove oil (24.63% pod damage) (27.10% pod damage). *Beauveria bassiana* had the best pod borer control and, as a result, the maximum grain production of 14.48 q/ha. According to the data, the lowest yield was with Clove Oil treatment (10.04 q/ha). *Beauveria bassiana* produced a 65.18 percent increase in yield per hectare over the control. Clove Oil (14.48 percent) had the lowest output recorded.

Conclusion

All treatments were determined to be significantly better than the control group. The best treatment for larval population decrease was *Beauveria bassiana*. All of the treatments outperformed the control group in terms of pod damage. The plots treated with *Beauveria bassiana* showed the least incidence of pod damage. The plots treated with clove oil had the highest percentage of pod damage. In terms of grain yield, the maximum yield was obtained with *Beauveria bassiana* in the all-treated plot, while the lowest yield was obtained with clove oil. In *Beauveria bassiana*, the minimum yield loss was observed, while the maximum percent yield loss was attained with clove oil. In *Beauveria bassiana*, the yield increases over control in the treated plots, whereas the maximum yield loss is found with clove oil.

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