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Bioefficacy of *Beauveria bassiana* against different larval instars of *Spodoptera litura* on cabbage in laboratory conditions

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Abstract
Entomopathogenic fungi are valuable weapons for bio control and play an important role in integrated pest management. *Beauveria bassiana* has been known as a potential entomopathogenic fungus. Bio efficacy of *Beauveria bassiana* products (Green Beauveria, Multiplex Baba and WAH each of which contains 2 x 10^8 conidia ml^{-1}) with five different doses i.e., 1ml 100^{-1}ml, 2 ml 100^{-1}ml, 3ml 100^{-1}ml, 4ml 100^{-1}ml and 0 ml 100^{-1}ml of each product, were tested against first, second, third, and fourth instar larvae of *Spodoptera litura* on cabbage in laboratory conditions. All the concentrations showed significantly better results than the control, but the maximum mortality percentage was obtained at the highest concentration of each product. The results obtained show that Green Beauveria and WAH was effective against first-instar larvae with 86.7% larval mortality, and WAH was most effective against second-instar larvae with 96.67% larval mortality after 14 days of treatment. Further, LC_{50} and LT_{50} values were also evaluated, which revealed that WAH was found to be most effective against first-instar larvae, whereas Multiplex Baba against second and third-instar larvae with LC_{50} value of 3 x 10^8 conidia ml^{-1}, 1.4 x 10^8 conidia ml^{-1} and 9 x10^8 conidia ml^{-1} respectively. However, fungal formulations were least effective against fourth-instar larvae. LT_{50} values of first and second instar larvae when treated with WAH and Multiplex Baba was 69.6 hours and 144 hours, respectively. Thus, the present findings revealed that the efficacy of *Beauveria bassiana* was quite promising in early larval instars of *Spodoptera litura.*

Keywords: Entomopathogenic fungi, *Beauveria bassiana*, bio control, *Spodoptera litura*, Cabbage, Integrated Pest Management.

Introduction

Cabbage is the third major vegetable crop primarily grown in the winter season and an important vegetable of the cole group. India occupies the first position in cauliflower, second in onion, and third in cabbage in the world. Cabbage is a rich source of vitamins A, B_1, B_2, C, K and it also contains minerals. It is used as salad, boiled vegetables and dehydrated vegetables as well as in cooked curries and pickles. It possesses both antioxidant and anticarcinogenic properties. However, the production of cabbage is confronted with numerous constraints. It is prone to several nutrient deficiencies, as well as to multiple insect pests and bacterial and fungal diseases. One of the major pests of cabbage is *Spodoptera litura* (Fabricius), (Lepidoptera: Noctuidae). It is a serious polyphagous pest in Asia, Oceania and the Indian subcontinent. The pest is infesting 150 species of agricultural crops belonging to 44 families universally (Kranthi *et al*., 2002) [6], of which 40 species are known from India (Chari and Patel, 1972) [2]. The pest may cause severe damage, resulting in low productivity of cabbage when present in large numbers. *Spodoptera litura* causes excessive damage ranging from 26-100% by its vigorous defoliation (Dhir *et al*., 1992) [4].

The management of *Spodoptera litura* has become increasingly difficult all over the world, and the most commonly used insecticides are ineffective in controlling it. *Spodoptera litur*a was the first lepidopteran to develop insecticide resistance in India (Srivastava and Joshi, 1965) [7]. In recent years, crop protection based on biological control of crop pests with microbial pathogens like viruses, bacteria, fungi and nematodes has been recognized as a valuable tool in pest management. *Beauveria bassiana,* causing disease in insects commonly called, ‘white muscardine disease’ is widely regarded as a potential bio control agent.

Biopesticides clearly have a potential role to play in the development of IPM strategies. So there is a need to test *Beauveria bassiana* to be used in IPM of *Spodoptera litura*, remaining a major recurring pest requiring considerable control measures. In order to know the potential of *Beauveria bassiana* as one of the important inputs for safe and sustainable agriculture, studies were undertaken for its evaluation and selection for use in IPM of *Spodoptera litura* on cabbage.

Materials and Methods

Laboratory trials were conducted to test the bio efficacy of three commercial formulations of *B. bassiana* against *Spodoptera litura*. The larva of these insects were collected from *Jawan*, Aligarh, (U.P) and pure culture was raised in the laboratory. Commercial formulations, *viz*., Multiplex Baba, Green Beauveria, WAH were taken for this as detailed in Table 1. Conidial suspensions of these formulations @ 1 × 10^8 CFU / ml, 2 × 10^8 spores / ml, 2 × 10^8 CFU / g respectively (Table 1), and were prepared in distilled water containing 0.01% Cween 80. This concentration was more or less near the minimum recommended concentration for use on cabbage crops. Spore suspension of *B. bassiana* at four different concentrations, 1, 2, 3, and 4% was prepared and cabbage leaves were dipped in it for 30 seconds and tested for its efficacy on 1st, 2nd, 3rd and 4th instar larva of *S. litura*. Three replicates of 10 larvae each with a control were taken in plastic vials (4.5×12.0cm²) lined with moistened cotton and filter paper above it to maintain humidity. The vials had screw caps having provision for proper aeration. Aseptic conditions were maintained throughout the experiment. Observations were recorded daily at 24-hour intervals on mortality and disease development till pupation and adult emergence. All experiments were conducted in BOD at 27±1°C and 70±5 per cent RH. Dead larvae were kept in a moist chamber for confirmation of mycosis. The experiments were repeated three times. The details of the experiment is mentioned in Table 2. Cumulative per cent mortality was calculated from 1st, 3rd, 7th, and 14th day, and data was subjected to one-way analysis of variance. The efficacy of different treatments was compared on 5 per cent level of significance.

Table 1: Commercial formulations of Beauveria bassiana used for the study

Trade name	Formulation	Conidial concentration/g	Firm	General Recommendations (G/Ml/Liter)
Green Beauveria	Liquid	2×10^8	Green Biotech Laboratory, Coimbatore, Tamil Nadu	5 ml/liter
Multiplex Baba	Liquid	1×10^8	Karnataka Agrochemicals, Bangalore	1ml/liter
WAH	Wettable Powder	2×10^8	Amit Biotech Private limited, Kolkata, West Bengal	2-3 g/liter

Table 2: Details of the treatments

Biopesticide	Treatments	Concentration (%)
Green Beauveria	T_1	1
	T_2	2
	T_3	3
	T_4	4
Multiplex Baba	T_5	1
	T_6	2
	T_7	3
	T_8	4
WAH	T_9	1
	T_{10}	2
	T_{11}	3
	T_{12}	4
Control	T_{13}	-

Results

Results of bioassays in which different formulations were tested against *Spodoptera litura* are presented in Table 3 and 3.2 and Fig 1 - Fig 4. The data revealed that most of the treatments caused more than 50 per cent mortality at 14 days after treatment (DAT). In the first instar, larval mortality ranged from 23.30 per cent to 86.67 per cent at 14 DAT. The highest mortality was observed in 3 per cent (86.67 per cent) concentration of WAH (2×10^8 spores/ml) and 4 per cent concentration (86.67 per cent) of Green Beauveria (2×10^8 spores/ml) at 14 DAT which was non-significant to rest of the concentrations and treatments. The mortality range of second instar larvae was 43.00 per cent to 93.33 per cent. The highest mortality was observed in WAH 2 per cent concentration which was non- significant with Multiplex Baba 2 per cent and 3 per cent and was significantly different with rest of the treatments. In the third instar, larval mortality ranged from 6.7 per cent to 33.33 per cent at different concentrations and treatments with highest mortality observed in 4 per cent Multiplex Baba at 14 DAT which was significantly different with other treatments. The effect of fungus on fourth instar larva was very less with highest 23.33 per cent mortality at 4 per cent concentration of Multiplex Baba at 14 DAT which revealed non-significant difference with Green Beauveria 1 and 2 Per cent, Multiplex Baba 1, 2 and 3 per cent. It was significantly different with rest of the treatments including control.

Table 3: Comparison of different commercial formulations of Beauveria bassiana on 1st and 2nd instar S.litura larva

Treatment	I Instar				Ii Instar			
	Mean% mortality at indicated days				Mean% mortality at indicated days			
	1	3	7	14	1	3	7	14
T_1	0.00	0.00	0.00	23.30	10.00	10.00	36.67	60.00
T_2	33.33	43.33	43.33	56.67	0.00	10.00	20.00	50.00
T_3	20.00	30.00	40.00	70.00	0.00	10.00	30.00	80.00
T_4	23.33	33.33	73.33	86.67	20.00	43.33	53.33	83.33
T_5	0.00	0.00	0.00	46.67	0.00	20.00	20.00	83.33
T_6	20.00	33.33	33.33	53.33	40.00	40.00	40.00	93.33
T_7	3.33	23.33	33.33	33.33	20.00	30.00	63.33	93.33
T_8	33.33	33.33	43.33	53.33	30.00	30.00	50.00	70.00
T_9	0.00	10.00	33.33	43.33	0.00	10.00	23.33	43.33
T_{10}	10.00	16.67	23.33	50.00	10.00	20.00	30.00	96.67
T_{11}	0.00	10.00	20.00	86.67	0.00	10.00	43.33	83.33
T_{12}	0.00	20.00	63.33	63.33	0.00	36.67	56.67	86.67
T_{13}	0.00	0.00	10.00	30.00	0.00	10.00	20.00	30.00
P	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
F	8.67	27.00	34.67	49.20	8.04	21.34	125.35	5.83
DF	24,38	24,38	24,38	24,38	24,38	24,38	24,38	24,38
LSD at 5%	2.06	1.89	2.06	2.74	2.39	2.12	1.29	3.94
DF	Degrees of freedom							
LSD	Least significant difference at 5% level of significance							

Table 4: Comparison of different commercial formulations of Beauveria bassiana on 3rd and 4th instar S. litura larva

Treatment	III Instar				Iv Instar		
	Mean% mortality at indicated days				Mean% Mortality At Indicated Days		
	1	3	7	14	1	3	7
T_1	0.00	0.00	3.30	6.70	0.00	10.00	20.00
T_2	0.00	0.00	1.00	10.00	0.00	6.66	20.00
T_3	0.00	0.00	10.00	10.00	0.00	5.00	10.00
T_4	0.00	0.00	13.33	13.33	0.00	10.00	10.00
T_5	0.00	0.00	13.33	13.33	0.00	15.00	20.00
T_6	0.00	0.00	0.00	16.67	0.00	12.00	20.00
T_7	0.00	0.00	16.67	16.67	0.00	15.00	20.00
T_8	0.00	0.00	13.33	13.33	13.33	16.66	23.33
T_9	0.00	0.00	6.70	6.70	0.00	5.00	10.00
T_{10}	0.00	0.00	0.00	10.00	0.00	5.00	10.00
T_{11}	0.00	0.00	0.00	13.33	0.00	5.00	10.00
T_{12}	0.00	0.00	13.33	13.33	0.00	7.00	10.00
T_{13}	0.00	0.00	3.33	3.33	0.00	6.66	10.00
P	0.38	0.38	0.00	0.00	0.38	0.00	0.04
F	1.00	1.00	13.21	19.46	1.00	12.32	3.67
DF	24,38	24,38	24,38	24,38	24,38	24,38	24,38
LSD at 5%	1.40	0.59	1.71	1.91	1.18	2.14	2.81
DF	Degrees of freedom						
LSD	Least significant difference at 5% level of significance						

Table 5: Probit analysis of commercial formulations of Beauveria bassiana on Spodoptera litura

I Instar Larvae				
Commercial Formulation	**Regression Equation**	**R^2**	**χ^2**	**LC_{50}**
Green Beauveria	y= 1.4078x + 0.0825	0.3897	0.318	2.024
Multiplex Baba	y= 0.7582x + 0.8751	0.2262	0.868	7.579
WAH	y= 0.75x + 1.2777	0.0793	0.506	1.735
II instar				
Commercial formulation	**Regression Equation**	**R^2**	**χ^2**	**LC_{50}**
Green Beauveria	y = 0.7249x + 1.4169	R^2 = 0.0962	4.492	3.32
Multiplex Baba	y = 1.0416x + 2.444	R^2 = 0.0813	0.649	0.7
WAH	y = 1.2923x + 0.6935	R^2 = 0.1258	0.758	1.039
III instar				
Commercial formulation	**Regression Equation**	**R^2**	**χ^2**	**LC_{50}**
Green Beauveria	y = 0.1247x + 0.1668	R^2 = 0.0703	0.027	13.8
Multiplex Baba	y = 0.3895x - 0.04	R^2 = 0.2058	1.105	4.573
WAH	y = 0.0078x + 0.3338	R^2 = 0.0003	10.046	12.3
IV instar				
Commercial formulation	**Regression Equation**	**R^2**	**χ^2**	**LC_{50}**
Green Beauveria	y = 0.1493x + 0.39	R^2 = 0.00588	0.161	8.524
Multiplex Baba	y = 0.192x + 0.8133	R^2 = 0.0707	0.187	8.43
WAH	y= 0.02x+ 0.4667	R^2 = 0.0029	0.217	10.55
R^2	Correlation coefficient			
χ^2	Chi - square value			

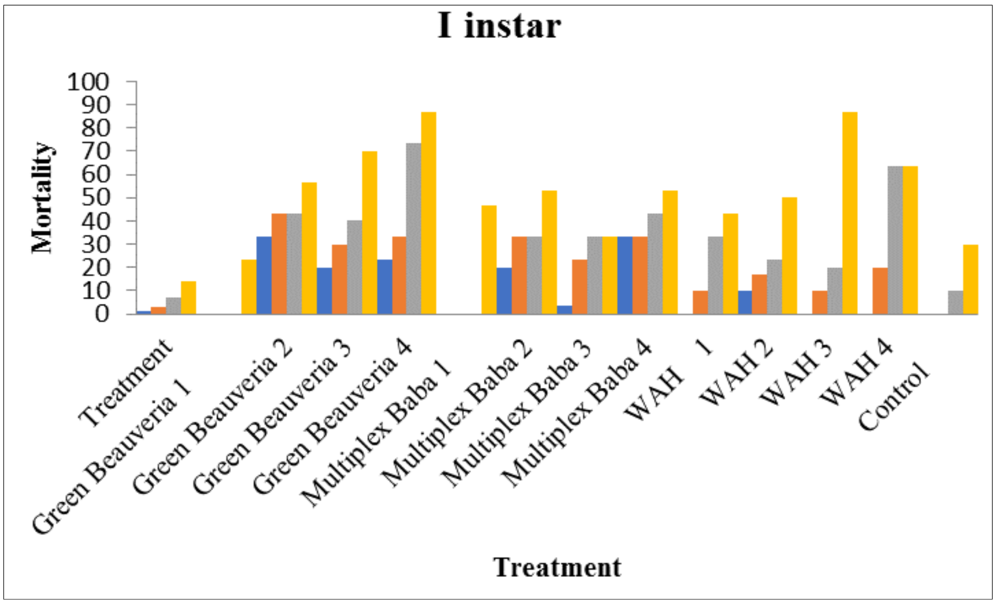

Fig 1: Comparision of different commercial formulations of *Beauveria bassiana* on I instar larva of *S. litura*

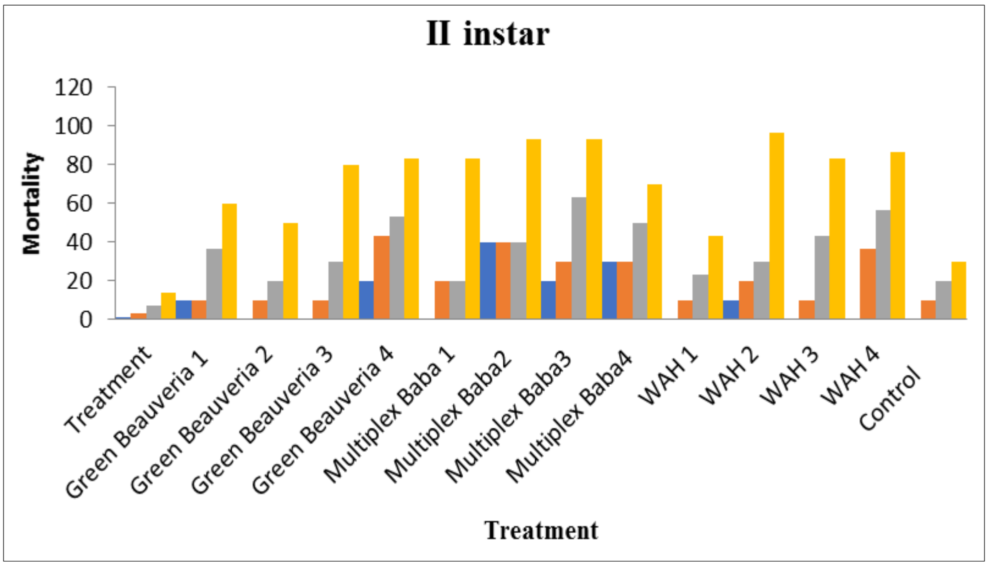

Fig 2: Comparision of different commercial formulations of *Beauveria bassiana* on II instar larva of *S. litura*

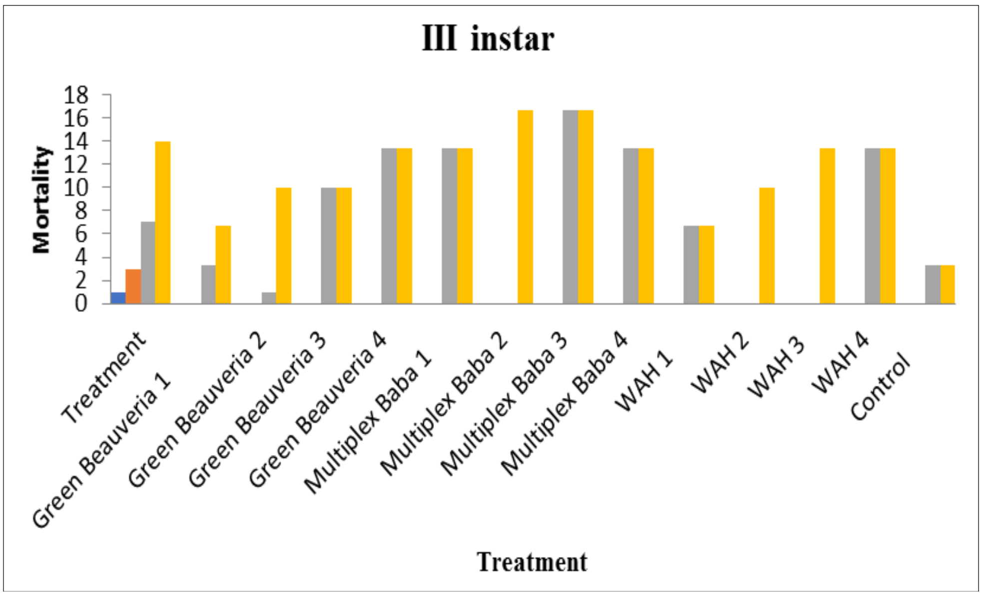

Fig 3: Comparision of different commercial formulations of *Beauveria bassiana* on III instar larva of *S. litura*

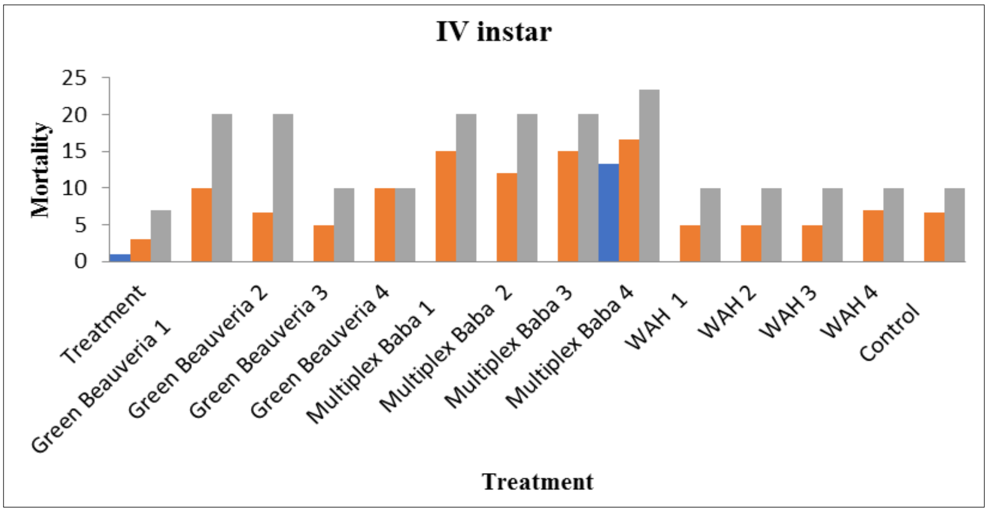

Fig 4: Comparision of different commercial formulations of *Beauveria bassiana* on IV instar larva of *S.litura*

Table 6: Probit analysis of 4 percent concentrations of commercial formulations of Beauveria bassiana on Spodoptera *litura*

I Instar Larvae				
Commercial Formulation	**Regression Equation**	**R^2**	**χ^2**	**LT_{50}**
Green Beauveria	y = 0.357x + 4.011	R^2 = 0.6844	5.25	2.943
Multiplex Baba	y = 0.2066x + 2.9234	R^2 = 0.8289	0.827	10.064
WAH	y = 0.7942x + 1.4325	R^2 = 0.9046	5.382	8.237
II instar larvae				
Commercial formulation	**Regression Equation**	**R^2**	**χ^2**	**LT_{50}**
Green Beauveria	y = 0.4394x + 2.2732	R^2 = 0.8548	3.67	6.169
Multiplex Baba	y = 0.6121x + 1.2451	R^2 = 0.9201	2.87	6.035
WAH	y = 0.584x + 0.3407	R^2 = 0.7696	10.43	9.144
III instar larvae				
Commercial formulation	**Regression Equation**	**R^2**	**χ^2**	**LT_{50}**
Green Beauveria	y = 0.1182x + 0.0169	R^2 = 0.7816	2.436	24.71
Multiplex Baba	y = 0.2041x + 1.1102	R^2 = 0.7192	2.219	17.48
WAH	y = 0.1286x - 0.1286	R^2 = 0.6923	4.749	22.435
IV instar larvae				
Commercial formulation	**Regression Equation**	**R^2**	**χ^2**	**LT_{50}**
Green Beauveria	y = 0.2614x + 0.3829	R^2 = 0.7176	4.057	16.456
Multiplex Baba	y = 0.1307x + 1.1386	R^2 = 0.7176	3.186	16.420
WAH	y = 0.1893x + 0.0857	R^2 = 0.7519	2.940	20
R^2	Correlation coefficient			
χ^2	Chi - square value			

The results in Table 5 and 6 shows that *Spodoptera litura* first instar larva treated with WAH was more effective and LC_{50} value was 1.378 followed by Green Beauveria (2.024) and Multiplex Baba (7.579). In second instar treated larvae WAH was found to be most effective than other commercial formulations with LC_{50} value of 0.888 followed by Green Beauveria (3.32) and Multiplex Baba (3.649). However, the fungal formulations were least effective in third and fourth instar larvae. Multiplex Baba was effective than the rest with 4.573 and 6.956 LC_{50} values respectively. Lethal time (LT_{50}) in 4 per cent concentration of green Beauveria was found to be 2.943 followed by WAH (8.33) and Multiplex Baba (10.064) when observed for first instar larvae. In second instar treated larvae LT_{50} value for 4 per cent concentration of Green Beauveria was 6.169 followed by WAH (6.75) and Multiplex Baba (7.5). However, in third and fourth instar larva these biopesticides were least effective with LT_{50} values of 16.42 and 17.48 for 4 per cent concentration of Multiplex Baba respectively.

Discussion

Studies pertaining to laboratory trials on bio efficacy revealed that all the larval stages of *Spodoptera litura* were not equally susceptible to the infection of *Beauveria bassiana* but the fungus was found more pathogenic to early instar. Higher susceptibility to younger stages and reduced susceptibility to older stages if considered together reveal that the younger stages when treated die at once due to their very small and soft body parts. Younger larval stages thus have an increased probability of acquiring an infection. Late larval stages, on the other hand, have reduced food intake and a thicker cuticle and thus it is less likely that spores would enter or penetrate their body. The present studies are conformity with the findings of Hung and Boucias (1992) [5] who tested the fungi against the leaf worm and beet army worm showing that the fungus *B. bassiana* (Balsamo), Vuillemin caused a high mortality to the insect at earlier stages. Asi *et al.*, (2013) [1] reported virulence potential of the entomopathogenic fungi varied with different biological stages of the *Spodoptera litura*.

Conclusion

Beauveria bassiana was found to be highly effective against various larval instars of *Spodoptera litura* on cabbage. The fungus produced significant mortality rates throughout all larval stages, with younger instars showing the greatest efficacy. These findings indicate that *B. bassiana* could be an effective bio control agent for managing S. litura infestations on cabbage, providing an environmentally acceptable alternative to conventional pesticides. More study is needed to improve application strategies and develop effective B. bassiana-based products for sustainable pest management in cabbage agriculture.

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