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Efficacy of entomopathogens for control of blue pumpkin beetle (*Aulacophora nigripennis* motschulsky, 1857) in sponge gourd (*Luffa cylindrica*) under laboratory condition at Paklihawa, Nepal

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Abstract

With the objective of studying the efficacy of entomopathogens for the control of blue pumpkin beetle in sponge gourd, a lab experiment was conducted from 2015/10/1 to 2015/10/12 at the entomology lab of Institute of Agriculture and Animal Science (IAAS), Paklihawa, Rupandehi. The design of the setup used was completely randomised design (CRD) with four treatments along with control and five replications. Three entomopathogens were used in experiment among them two were fungi; *Metarrhizium anisopliae*, *Beauveria bassiana,* while *Bacillus thuringiensis* was bacteria. Singly treated leaves of different size were kept in each 20 boxes which were changed on third day after first placement and later continued at 2 days interval. Eight beetles per box were kept to know the mortality by different treatments. Amongst all, *Beauveria bassiana* recorded the highest mean mortality (4.4), followed by *Bacillus thuringiensis* (4) and *Metarrhizium anisopliae* (3.3) as against 0.6 in control. Taking day, treatment and mortality as the parameter, *Beauveria bassiana* was found to have significant difference over *Metarrhizium anisopliae* and Control but insignificant over *Bacillus thuringiensis*. The order of efficacy was ranked as; *Beauvaria bassiana*>*Bacillus thuringiensis*>*Metarrhizium anisopliae*> Control.

Keywords: *Entomopathogens, mortality, blue pumpkin beetle, sponge gourd.*

Introduction

Sponge gourd (*Luffa cylindrica*) is widely grown and popular vegetable crop of cucurbitaceae family in Nepal. It is grown from tropical to subtropical region. The fruit must be harvested at a young stage of development to be edible. The vegetable is popular in China, India, Vietnam and Nepal etc. When the fruit is fully ripened, it is very fibrous. This is not frost-hardy, and requires 150 to 200 warm days to mature. In Nepal sponge gourd is cultivated in 5160 ha of land with production of 71721 Mt. in 2014. The productivity of sponge gourd in Rupandehi district was 15 Mt/ha in (2012/2013, Statistical information on Nepalese agriculture). Pumpkin beetles are the major pest and causes considerable damage to almost all cucurbits. Among different species of pumpkin beetles, incidence of adult stage of Blue pumpkin beetle (*Aulacophora nigripennis*) have been reported by various workers (Nath, 1964; Nath and Thakur, 1965; Bogawat and Pandey, 1967). The pest, however, occurs throughout the year and causes severe damage to the crops, especially at the seedling stage (Alam, 1969; Butani and Jotwani 1984). The adult beetles feed voraciously on the cucurbit leaf making irregular holes. The grubs feed on roots and underground portions of host touching the soil and thus making such fruits unsuitable for human consumption (Butani and Jotwani, 1984). The pest are active from March to October, though the peak period of activity is between April to June (Butani and Jotwani, 1984).It may cause up to 70% damage on leaves and 60% damage on flowers of cucumber (Alam, 1969). Percent losses may reach up to 35-75% at seedling stage. In some cases the losses of this pest have been reported to 30-100% in the field. Of the various methods to control blue pumpkin beetle, biological control with entomopathogen has evolved worldwide as an important component of IPM, enjoying particular success in Asia and South America (Fuxa, 1987) although it is rudimentary stages in Nepal. Entomopathogen is an organism that can act as a parasite of insects and kills or seriously disables them. These include entomopathogenic fungi, nematode, bacteria and viruses. Chemical pesticides has resulted many problems, such as pest resurgence to pesticides, resurgence of pests, toxic residues in food, water, air and soil elimination of natural enemies and disruption of ecosystem (Palikhe, 2002). The concept of pest control with entomopathogen is emerging worldwide as it is environmentally friendly and cost effective. Mass production, formulation, and application of entomopathogens to pest population in a manner analogous to chemical pesticides, i.e. as non-persistent remedial treatments that are released inundatively.

Materials and Methods

Experiment was conducted in the entomology lab of Institute of Agriculture and Animal Science (IAAS), Paklihawa, Rupandehi, during summer from 2015/10/1 to 2015/10/12. The experiment was laid out in completely randomised design (CRD) with 4 treatments including control and 5 replications. 20 transparent rearing boxes of one liter capacity were collected and the lid of the boxes was penetrated to make a small hole and then covered with muslin cloth to ensure air circulation inside the box along with to check the escape of inoculated insect. Blue pumpkin beetle were collected from the sponge gourd field of Paklihawa campus. 8 beetles per box kept under the aseptic lab condition. Entomopathogens used in the experiment were two fungus; *Metarhizium anisopliae, Beauveria bassiana* and a bacteria *Bacillus thuringiensis* obtained from the Agricare Nepal Pvt. Ltd., Chitwan, Nepal. The strains of the fungi and bacteria, about 5ml of dose each, were dissolved in 1 liter of distilled water containing 5 gram of molasses as a energy source to make solution of entomopathogens and kept overnight under cool temperature. Collected sponge gourd leaves were dipped in the 4 different solutions respectively for about one minute and the leaves were kept in the boxes in which insects were to be kept. The leaves were changed every after 2 days of application because beetle being voracious in nature the leaf per box get consumed in the faster rate and the other reason is the leaf gets dried which bring unappealing taste to the beetle. Likewise, solution containing entomopathogens were also made just before one day when the leaves required changing.

Data collection and statistical analysis

Data were collected regularly after the setup of the experiment in the laboratory. Room temperature, relative humidity and the number of the insects dead in the boxes were recorded. Mortality of insects started from 2015/10/4 i.e. 3 days after setting the experiment. The data from the experiment were recorded and managed in the spreadsheet and the obtained data were analyzed using Ms. Excel 2007 and SPSS v. 20.

Result and Discussion

Effect of Bio- pesticides on the control of Blue pumpkin beetle:

Table 1: Effect of bio- pesticides on the Blue pumpkin beetle on Sponge guard during 2015/10/1 to 2015/10/12.

Treatment	4DAT	5DAT	6DAT	7DAT	8DAT	9DAT	10DAT	11DAT	12DAT	Grand mean
MA	0.40	1.40	1.60	2.80	3.40	4.00	5.00	5.00	6.40	3.3
BB	1.40	2.40	3.20	3.80	4.40	5.00	6.20	6.20	6.80	4.4
BT	1.00	1.80	2.60	3.40	4.00	4.40	6.00	6.00	6.80	4.0
C	0.00	0.00	0.00	0.00	0.20	0.20	0.60	1.60	2.60	0.6

DAT – Day after treatment, MA- *Metarrhizium anisopliae*, BB- *Beauveria bassiana*, BT- *Bacillus thuringeinsis*, C- Control

With increase in the time of application of treatments the effect on grand mean mortality was seen to have positive response. Among four treatments, result of *Beauveria bassiana* had highest mean mortlality i.e. 4.4 followed by *Bacillus thuringeinsis* (4), *Metarrhizium anisopliae* (3.3) and Control (0.6) which revealed that *Beauveria bassiana* was the most effective treatment among all. It indicates that individual treatment was also a contributing factor for the mortality of blue pumpkin beetles.

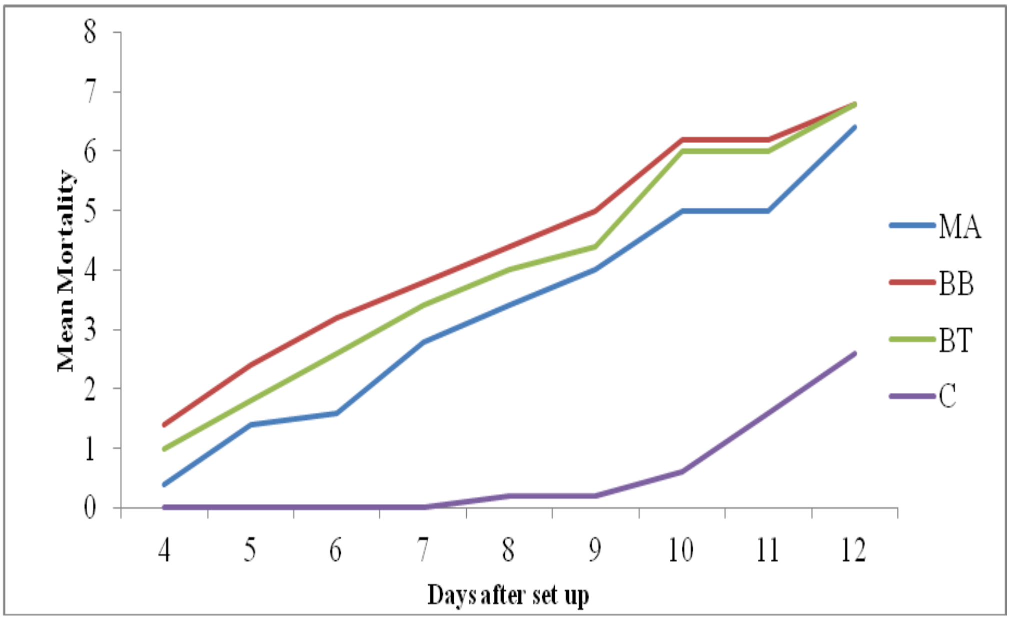

Figure 1: Mean mortality of four different treatments for the control of Blue pumpkin beetle.

Above graph shows that *Beauveria bassiana* was found to be most effective as the time elapsed. Correct dose of all treatments for a proper duration found to be effective for the control of blue pumpkin beetle. Mean mortality of beetles in *M. anisopliae* treated leaves was least as compared to other two entomopathogens, *Beauveria bassiana*and *Bacillus thuringeinsis.* Whereas mortality rate in control increased slowly only after 7DAT up to 10DAT after which mortality found to increase linearly which might be due to spread of pathogens through air current (Bhattarai, 2013) or the aging of the beetles.

Mean mortality of *Bacillus thuringeinsis* coincided with *Beauveria bassiana* with the advancement of time but the effectiveness of *B. bassiana* was more at the initial stage of treatment than that of *B. thuringeinsis* hence, *B. bassiana* was found to have the most superior effect in reducing the beetle population.

Table 2: Relationship of Day, Treatment and Day*Treatment with Mortality of beetle

Source	Sum of Squares	Df	Mean Square	F Value	Sig	Partial Eta Squared
Corrected model	885.261[a]	35	25.293	46.221	0.000	0.918
Intercept	1698.93	1	1698.939	3104.660	0.000	0.956
Day	431.011	8	53.876	98.454	0.000**	0.845
Treatment	398.506	3	132.835	242.745	0.0000**	0.835
Day*Treatment	55.744	24	2.323	4.245	0.0000**	0 .414
Error	78.800	144	0.547			
Total	2663.00	180				
Corrected total	964.061	179				

a. R Squared = .918 (Adjusted R Squared = .898)

**shows highly significant ($P \leq 0.05$).

Above table shows Day, Treatment and Day * Treatment had a significant difference on mortality. Only day (other things remaining constant) contributes 84.5% mean mortality because as the day increases mortality automatically increases due to old age , similarly individual treatment (other things remaining constant) contributes 83.5% mean mortality because as the treatment increases mortality increases due to increased toxicity but the interaction of day and treatment contributes only 41.4% mean mortality because treatment should come combine with day to effect on morality, on increasing the number of day, treatment pathogenic activity decreases which decreases mortality .

R Squared which is coefficient of determination recorded that 91.8% mortality was contributed by day, treatment and day*treatment whereas the other 8.2% was contributed by other factor such as aging, environment etc. Therefore, it was concluded that these three sources were the contributing factors for mortality.

Table 3: Homogenous Table for Mean Mortality of Blue Pumpkin Beetle.

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
	C	45	.58[c]		
	MA	45		3.33[b]	
Duncan[a]	BT	45		4.00[ab]	4.00[ab]
	BB	45			4.38[a]
	Sig.		1.000	.079	.319

a,ab,b and c denote the effectiveness of treatments in which a>ab>b>c

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 45.000.

Above table shows that only control lies in subset 1 which was significantly different from other treatments. *M. anisopliae* and *B. thuringiensis* lie in the subset 2 which denotes that they were insignificant with each other but *M. anisopliae* was significantly different from *B.bassiana* which lies in subset 3. *B.thuringiensis* also lies in subset 3 so; *B. thuringiensis* and *B.* bassiana were insignificant with each other.

Therefore, order of efficacy of test product can be ranked as

***Beauvaria bassiana* > *Bacillus thuringensis* > *Metarrhizium anisopliae* > Control**

The similar result was found by Vishwakarma *et al*., (2011) which was to assess the bio efficacy of entomopathogenic fungus viz. *Beauveria bassiana* and *Metarrhizium anisopliae* in controlling the red pumpkin beetle on Narendra Rasmi bottle gourd.

Based on the result of this experiment, it can be concluded *Beauveria bassiana* was found to be superior against, *Bacillus thuringeinsis*, *Metarhizium anisopliae* and Control under the ecological conditions of Paklihawa, Nepal in controlling blue pumpkin beetles in the long run.

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