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COMBINED EFFECT OF *BACILLUS THURINGIENSIS* AND NUCLEAR POLYHEDROSIS VIRUS ON MORTALITY OF *SPODOPTERA LITURA*

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Abstract

Spodoptera litura (Fab.) is a polyphagous pest which is having wide host range and resulted in huge economic loss. Nuclear polyhedrosis virus (NPV) and *Bacillus thuringiensis* (*Bt*) are potential biological pest controller which can control an wide-range of insect groups in both horticultural and agricultural crops. Current inspections were carried out to determine impact of *Bt* and NPV and their cumulative pesticide action against *S. litura*. Under laboratory conditions, both *Bt* and NBAIR*Sl*NPV1 were applied using diet incorporation method. *Bt* concentrations (0.1, 0.3, 0.5, 0.7 and 0.9µg ml^{-1}) and NBAIR*Sl*NPV1 1X 10to $1X10^5$ OBs/ml were applied alone and different combinations against 2nd instar *S. litura*. The highest mortality of larvae was recorded after 7 days of application when both pathogens were used in combination *Bt*+NBAIR*Sl*NPV1has shown effective synergetic result (7.2) than other combination *viz.,* NBAIRSlNPV1 and *Bt* (1.25). Bio-rational pesticides based on Nuclear Polyhedrosis Virus (NPV) and *Bacillus thuringiensis* (*Bt*) are very effective tool to achieve the resistance problems and protect the natural enemies and environment.
Keywords: *Spodoptera litura, Bacillus thuringiensis,* Nuclear polyhedrosis virus, Synergism.

Introduction

Insects are largest dominant group present on the earth as they are widely distributed on earth. They appear as social insects and also pests on several economic important agriculture crops, causing huge losses in crop yields. The armyworm, *Spodoptera litura* (*Fab*) (Noctuidae; Lepidoptera), which is one of the important species of genus *Spodoptera* attacks a wide range of agricultural crops, horticultural plants, vegetables and miscellaneous wild plants as well as weeds (Zhou *et al.*, 2010). Wu and coworkers (2004) reported that *S. litura* infested more than 290 species of plants belonging to 99 families. Controlling of these insects is tends to the application of synthetic insecticides leading to severe health hazards and insecticide residue in soil affects on social insects. The alternative methodology is to control this using biopesticides such as *Bacillus thuringiensis* and insect host specific Nuclear polyhedrosis virus. The application of these biopesticides will be ecofriendly and farmer friendly. Baculoviridae are big family entemopathogenic viruses whose double stranded circular DNA infects many invertebrates (Moscardi 1999). Baculovirus can be combined with other entomopathogens to improve biological control of insect pests (Hesketh and Hails 2008). The enhancement of efficacy of microbial products for *Spodoptera litura* management by spraying combinations of *Bacillus thuringiensis* (*Bt*) toxins and *S. litura*- NPV (Masetti *et al.,* 2008). *Bacillus thuringiensis* is a gram positive bacterium that possesses parasporal crystalline proteins that is having high range of toxic to a wide range of pest insects especially lepidopteran and coleopteran insects (Mansour *et al.*, 2012). *B. thuringiensis* produces (inclusions) parasporal crystals (inclusions) during its sporulation. These inclusions (δ- endotoxin) bind to specific receptors in the mid-gut brush border membrane of susceptible insects (Balaraman, 2005).

*Sl*NPV have large biotic potency and suitable to be used in pest *S. litura* management programs and it's used as main biological control agent of *S. litura.* India has a vast potential for bio-pesticides. Bio-pesticides, being target pest specific, are presumed to be relatively safe to non-target organisms including human beings. In India, some of the bio-pesticides like *Bt*, NPV, neem based pesticides and others have already been registered and are in use (Gupta and Dikshit, 2010). Ramaprasad *et al.* (2000) advocated the use of Biosap (*Bacillus thuringiensis* var. kurstakia sporogenic) and Biolep (*B.t*. var. kurstaki sporogenic) against *S. litura* in tobacco nurseries. The combined efficacy of NPV and spinosad can be enhanced by evaluating their pathogenicity with new chemistry insecticides without causing damage to non-target organisms (Ayyub *et al.*, 2019). The synergism activity of the modified biocontrol agents can control pests at closet time with high efficiency. The development of microbial interactions among microbial agents may leads establishment of co-existence, synergism or antagonism. Antagonism is equivalent to a reduction in virulence whereas synergism enhances virulence as a result of interaction. In principle, synergism and antagonism are possible between different microbial agents as well as between strains of one and the same pathogen.

Material and Methods

Collection of insect and rearing

S. litura larvae and egg mass collected from cabbage field and reared on castor leaves in plastic containers in climatic chamber (26± 1°C, 70 ± 10 % RH, and a 15L :9 D photoperiod)at ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India, Adult moths were provided with 10% honey solution during ovipositions. Larvae from F1 generation were maintained on chickpea based artificial diet (Kidney bean flour 145g, yeast powder 29g, ascorbic

acid 2.35g, formaldehyde solution (3-4drops), agar (9 g), sorbic acid (2.30g), methyl 4-hydroxy benzoate (1.6g), ascorbic acid (2.35g) and double distilled water 550ml). Newly hatched larvae were transferred individually to Bioassay tray (C-D International, Pitman, NJ) containing 1.5 g (approx) artificial diet.

Insect virus

The NBAIR*Sl*NPV1 (NCBI Gen Bank-Accession KY549343) strain virus was infected to larvae of *S. litura*. The collected isolate were screened and confirmed using Giemsa stain (Yaman *et al.*, 2001). Afterwards *in vivo* NPV propagation and isolation was performed as reported by (Monobrullah and Nagata 2000). Occlusion bodies (OBs) were purified and counted 4 times using Neubauer chamber (X40) under light microscope. A dilution of various concentrations ($1x10^2$ to $1x10^7$ OBs/ml) of NBAIR*Sl*NPV1 was prepared in sterile distilled water from stock solution (Cory and Myers 2003).

***Bt* formulation**

The isolated type culture *Bacillus thuringiensis* subsp. *galleriae* (MTCC-8977) was procured from MTCC, CSIR-IMTech, Chandigarh, India, revived and prepared laboratory scale lactose-acetone based co-precipitation method and estimated *Bt* protoxin concentration and used for dose mortality assay in our previous studies (Yalashetti *et al.*, 2019). The *Bt* protein was serially diluted 0.1, 0.3, 0.5, 0.7 and 0.9µg ml^{-1} used for bioassay.

Bioassay

Laboratory bioassays were carried out using the 2nd instar larvae of *S. litura* (Table.1).Freshly molted larvae were exposed to *Bt* (0.1, 0.3 0.5, 0.7 and 0.9µg ml^{-1}) and NBAIR*Sl*NPV1, 1X 10^3 to $1X10^7$OBs/ml mixed diets alone and in combinations in Bioassay trays. Thirty larvae were considered for treatments and experiments were replicated thrice. Mortality was assessed on 3rd, 5th and 7th day after treatment. A diet piece of 1.5 ml admixed with *Bt*/ NBAIR*Sl*NPV1offered to larvae after 48h. Artificial diet mixed with distilled water was used as control.

Statistical analysis

Mortality data collected for both *Bt* and NBAIR*Sl*NPV1 were corrected for control mortality by using (Abbott's, 1925) formula (Number of dead in treatment-Number of dead in control)/(100- Number of dead larvae in control) ×100. The pooled larval mortality data was subjected to probit analysis using the software POLO (Leora, 1994) and the lethal concentration to kill 50% of the test larvae (LC_{50}) was calculated for each population.

Results and Discussion

Mortality of *S. litura* 2nd instar larvae

Mortality and co-toxicity factors of *S. litura* larvae showed in single and combination of different concentrations of *B thuringiensis* and NBAIR*Sl*NPV1, exhibited wide range of mortality against second instar larvae. Mortality rate of *S. litura* larvae were noted while they are fed on artificial diet treated with *B. thuringiensis,* and NBAIR*Sl*NPV1 combinations tested with their compatibility. Based on LC_{50}, *Bt* alone treated larvae (LC_{50}0.336) and NBAIR*Sl*NPV1 (LC50=$3.6X10^3$) shown mortality. Combination of *Bt*+ NBAIR*Sl*NPV1 is more active (LC_{50} = $5X10^2$ mixed *Bt* spores/ml and polyhedra) than each single treatments NBAIR*Sl*NPV1 (LC_{50}= $3.6X10^3$PIB/ml) or *Bt* (LC_{50} =0.336 spores/ml). The mortality in most combination of *B. thuringiensis*-NPV showed more synergistic effect (7.2) where as combination of NBAIR*Sl*NPV1+*Bt* (1.25) did not showed any differential significant synergism ratio. The median lethal time (LC_{50}) was observed to be reduced in combination than treatment of single biocontrol treatments.

Combinations of two microbial biopesticides, such as *B. thuringiensis* and a NPV virus, have been generated many views as a means of increasing the wide spectrum of applications of insect pathogens and thus managing many of pests at same time. It is also feasible method that the pathogens may interact to increase virulence compared with either single (Marzban *et al.*, 2009, Marzban, 2012). Combinations of *B. thuringiensis* and nuclear polyhedrosis virus on pest *Helicoverpa zea* resulted in increased mortality than that of *B. thuringiensis* alone (Luttrell *et al.*,1982). A novel recombinant baculovirus constructed by *Bt* Cry1Ac gene between two polyhedron genes of *A. california* nucleo polyhedro virus (AcNPV) with under control of polyhedron gene promoter (Kim *et al.*, 2005). An improved baculovirus insecticide producing occlusion bodies that consist of *B. thuringiensis* toxin (Chang *et al.*, 2003).Interaction effects of *Bt* subsp. Kurstaki and HaSNPV on the survival of second instars of *Plutella xylostella* shows at lower concentration synergistic action against pest resulted in reduced larval developmental rate pupation rate, pupa weight and adult emergence (Magholli *et al.*, 2013).The combinations of NPV and other insecticides are better practice agents to control pests such as Spinosad and *Bt* and NPV combinations reduces larval mortality time and age, shows better option to management of *S. litura* pest (Ayyub *et al.*, 2019).

In current study, second instar of *S. litura* larvae were simultaneously infected with alternative modifications in sequential infectious treatment for 24 and 48hs. Synergistic effects of the pathogens *B. thuringiensis* and NPV showed synergistic effect on *H. armigera* larvae (Matter and Zohdy. 1981). *B. thuringiensis* influenced antagonistic activity to the *Ectropis oblique* nuclear polyhedrosis virus (Shang *et al.*, 1999).The present findings are in agreement with (Masetti *et al.*, 2008). Out study, supports the results revealed *Bt* insecticide as a safe option to control this pest because it has a significant effect on mortality of *S. litura* larvae. Previous studies have reported this insecticide as quick in action, easy to produce at low cost, long shelf life, safer forenvironment and beneficial insects and can be applied with novel pesticides in combination (Marvier *et al.*, 2007; Kumar *et al.*, 2008; Birch *et al.*, 2011).

Larvae treated with *B. thuringiensis* started to die from third day at higher protein concentrations of the experiment, whereas NBAIR*Sl*NPV1treated larvae started to die fifth day. LC_{50} values of nuclear polhedrosis virus versus *S. litura* were between 4.4-5.5 days at lowest to highest concentrations (Trang and Choudhari, 2002).

Fig. 1 : Bioassay test conducted in C-D International, pitman, NJ, (2, 3) combined effect of *Bt* and NPV resulted dead larvae.

Table 1 : Individual and combination effect of *Bt* and NPV against *S. litura.*

Test organism	LC_{50} 7th day	Slope SE ±	Fiducial Limits		χ^2	t ratio	Degrees of freedom	Heterogeneity	Synergistic ratio
			Upper	Lower					
Bt	0.336	0.257	0.42	2.24±0.427	5.248	3	1-838	0.613	-
NBAIR*S*lNPV1	$3.6X10^3$	8.2X10	$1X10^5$	0.42 ±0.061	7.456	4	2.643	0.661	-
Bt+ NBAIR*S*/NPV1	$5X10^2$	1.3X10	$1.4X10^3$	0.437±0.067	6.538	4	3.116	0.779	7.2
NBAIR*S*/NPV1	0.268	0.185	0.351	2.03±0.399	5.094	3	2.848	0.949	1.25

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