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Efficacy of Nuclear Polyhedrosis Virus and Flubendiamide Alone and in Combination against *Spodoptera litura* F.

Sumaira Maqsood[1,*], Muhammad Afzal[1], Muhammad Anjum Aqueel[1], Abu Bakar Muhammad Raza[1], Waqas Wakil[2] and Muhammad Hussnain Babar[3]

[1]*Department of Agricultural Entomology, University College of Agriculture, University of Sargodha, Sargodha, Pakistan*
[2]*Department of Entomology, University of Agriculture, Faisalabad, Pakistan*
[3]*Entomological Research Institute, AARI, Faisalabad, Pakistan*

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SM performed the experiments, analyzed the data and wrote the article. MA, MAA, ABMR, WW and HB supervised the work.

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Nuclearpolyhedrosis virus, Flubendiamide, *Spodoptera litura*, Cauliflower.

ABSTRACT

Nuclear polyhedrosis virus (NPV) is an important potential pathogen which can control a wide range of bollworm larvae in both agricultural and horticultural crpos. Flubendiamide is a new chemistry insecticide which also controls bollworm effectively and relatively safer for natural enemies and beneficial arthropods. Present investigations were carried out to determine their impact on larval mortality, pupal and adult emergence of 2nd and 4th instar larvae of *Spodoptera litura* F. under laboratory conditions. Both NPV and flubendiamide were applied using diet incorporation method. Larvae were allowed to feed for 48 h. Mortality was recorded until larvae died or pupated. Second instar larvae showed more susceptibility as compared with 4th instar larvae. Maximum mortality was recorded in 2nd instar larvae (91.02±2.04%) in comparison to fourth instar larvae (69.35±1.66%) in combined application of NPV and flubendiamide. Dose of flubendamide was the key factor for type of interaction, its combination with NPV at lower dose showed synergistic interaction (CTF≥20). While rest of the combination showed additive effect (CTF≤20). Integration of NPV and flubendiamide proved more fatal at higher concentrations than the individual application at lower concentration for pupation and adult emergence of *S. litura*. Apart from untreated larvae (control) maximum pupation (66.01±1.32 and 73.95±1.28 %) and adult emergence (61.87±1.71 and 67.77±1.11 %) was observed when larvae were treated with NPV ($1x10^7$ POB/ml) against 2nd and 4th instar larvae. This study would be helpful to use NPV and flubendiamide in IPM under field conditions to control *S. litura* on cauliflower.

INTRODUCTION

Cauliflower (*Brassica oleracea* var. botrytis) is an important crop grown in South and South East Asia. Cauliflower and cabbage are used as vegetables as well as in different kinds of salad, throughout the year in homes of Pakistan. At global level, it contributes about 1.09 % in total production (FAO, 2013). During the year 2013, it was cultivated on an area of 13,375 ha with the production of about 0.22 million tones worldwide for this crop (FAO, 2013). Pakistan occupying 22nd position in area and 19th in production of cabbages (Shaheen *et al*., 2011). It is damaged by a large number of insect pests among them Armyworm, *Spodoptera litura* F. is the most serious pest causing yield loss ranging from 31% to 100% (Lingappa, *et al*., 2004).

To control this lepidopterous larvae, different insecticides have been used in Pakistan (Basit *et al*., 2013), due to indiscriminate use of which lepidoptreous insect pests have developed resistance (Ferre and Van, 2002; Sayyed and Wright, 2006). *S. litura* has shown resistance against pyrethroids, carbamate, organophosphate and some newer chemistry pesticides (Indoxacarb, Fipronil) (Armes *et al*., 1997; Kranthi *et al*., 2002; Ahmad *et al*., 2007a, 2008; Saleem *et al*., 2008), emamectin, indoxacarb, and chlorfenapyr low level of resistance was recorded (Tong *et al*., 2013).

Due to resistance problems, it is a dire need to use some novel biochemical like flubendiamide and microbial insecticides like nucleo-polyhedro viruses (NPV). Flubendiamide belongs to phthalic acid diamides group of insecticide. It can be used as an effective component in Integrated Pest and Insecticide Resistance Management programs against lepidopterous insect pests (Tohnishi *et al*., 2005).

In microbial insecticides nucleo-polyhedrosis viruses (NPVs), has attained great attention to control different agricultural insect pests of different agricultural crops

* Corresponding author: sumaira.ento@yahoo.com
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and vegetables (Black *et al.*, 1997; Moscardi, 1999). Main advantage of NPVs is that they are host specific and they are not disturbed the population of beneficial insect and pollinators and are safer for health and environment (Lacey *et al.*, 2001; Moscardi, 1999).

Shaurub *et al.* (2014) studied the individual and combined effect NPV and azadirachtin against *S. litura* and reported 79.20% increased mortality of 4th instar larvae compared to individual application. Nasution *et al.* (2015) reported 97.40 to 100 % mortality when *Ha*NPV was administered in different formulation.

The combined application of microbial formulations has attained greater repute among agricultural community as a successful tool in integrated pest management (IPM) strategies (Purwar and Sachan, 2006). The main objective of this study was to check the main effect of NPV and flubendiamide alone and in different combinations against *S. litura*. Moreover, identify the insecticidal prospective of microbes to reduce use of synthetic insecticides, which are a great threat for natural enemies and human health.

MATERIALS AND METHODS

Insect culture

Larval population of *S. litura* was collected from host crop from selected cauliflower growing fields at Faisalabad. The larvae collected from the field were reared on artificial diet in the plastic trays covered with fine muslin cloth to get the next generation of 2nd and 4th instar larvae, detect on the basis of size, shape and colour (rear on laboratory and observe changes 1st instar to 2nd instar and until maturity). To avoid contamination by microorganisms, all apparatus were sterilized with 75% ethanol solution which was used in rearing of insects. When adults (moths) were emerged from pupae shifted in a transparent plastic jar and placed a coarse tissue paper to facilitate moths for egg laying. Artificial diet for adults was consisting of 50 g sucrose solution, 1 g methyl-4-hydroxybenzoate, 1 ml ethanol (90%), 10 ml vitamin mixture and 500 ml distilled water. This artificial diet solution was placed in a petri dish plug with cotton swab to avoid the moths from drowning. After hatching, the young larvae were shifted on natural diet with the help of camel hair brush to avoid abrasive damage. Larvae were reared up to 2nd and 4th instar under controlled conditions at 25±2°C and 70±5% relative humidity.

Test chemicals

Commercial formulation of Belt® (Bayer) containing flubendiamide was applied at 0.01 and 0.02 ppm against 2nd and 4th instar larvae. NPV was applied at 1×10^{7}pob/ml and 1×10^{8}pob/ml against 2nd and 4th instar larvae. These treatments were applied alone and in combination.

Bioassay

CRD experimental design with three replications was applied. Larvae were reared under laboratory conditions on artificial diet at experimental conditions of 25±2ºC, 65±5% R.H. and 14:10 (L: D) photoperiod. Second (2nd) and 4th instars larvae of *S. litura* were be exposed to NPV and flubendiamide alone and in combination to observe their pathogenicity. Flubendiamide and NPV were applied by incorporated into semi synthetic artificial diet (Abbas *et al.*, 2012). Larvae were allowed to feed for 48 h on artificial diet and then shifted to normal diet. Artificial diet mixed with Tween-80 was used as control. Thirty larvae of each locality were considered as a treatment which was repeated thrice. Mortality of larvae was assessed on 3rd, 5th and 7th day after treatment. Pupation and adult emergence percentage was also recorded.

Statistical analysis

Collected data were analyzed by Minitab software (Minitab, 2002) and Tukey's Kramer test (HSD) was used for separation of means at the 5% level of significance (Sokal and Rohlf, 1995). Mortality for both NPV and flubendiamide was corrected for control mortality using Abbott's (1925) formula. Type of interaction was determined by equation CTF = (Oc-Oe)/Oe ×100, where CTF is the co-toxicity factor, Oc is the observed percentage mortality resulted from the combined application, and Oe the expected percentage mortality, that is, the total percentage produced by each of the treatments used in the combination. The interactions were categorized into three groups: a positive factor of 20 or more meaning synergism, a negative factor of 20 or more meaning antagonism, and any intermediate value (*i.e.,* between −20 and +20) was considered additive (Mansour *et al.*, 1966; Wakil *et al.*, 2012).

RESULTS

Significant differences regarding the mortality of 2nd and 4th instar larvae of *S. litura* were observed when they were fed on diet containing NPV and flubendiamide alone and in combinations after 7 days of application. Second instar was more susceptible compared to 4th instar (Table I). Both kinds of interaction (additive and synergistic) were observed when NPV and flubendiamide were applied in combination. When 2nd instar larvae treated with NPV (1x10^{7} and 1x10^{8} POB/ml) gave 28.43±1.32 and 39.81±1.57% mortality, while 32.98±1.49 and 42.07±1.43 % mortality was recorded when treated with flubendiamide (0.01 and 0.02 ppm), respectively. Integration of NPV at higher dose (1x10^{8} POB/ml) with flubendiamide at lower concentration (0.01 ppm) showed synergism (CTF=20.35)

Table I.- Mortality of second and fourth instar larvae of *S. litura* exposed with flubendiamide and NPV.

Sr. #	Treatments	2nd instar				4th instar			Type of interaction
		Actual mortality	Expected mortality	Co-toxicity factor	Type of interaction	Actual mortality	Expected mortality	Co-toxicity factor	
1	NPV1+Flubendiamide1	64.83±1.61c	61.42	5.56	Additive	47.70±1.52 c	45.48	4.89	Additive
2	NPV1+Flubendiamide2	76.16±1.73b	70.50	8.04	Additive	55.70±1.55 b	51.15	8.91	Additive
3	NPV2+Flubendiamide1	87.61±2.18a	72.80	20.35	Synergism	67.05±1.42 a	54.56	22.90	Synergism
4	NPV2+Flubendiamide2	91.02±2.04a	81.88	11.16	Additive	69.35±1.66 a	60.23	15.14	Additive
5	NPV1	28.43±1.32f				21.61±0.66 f			
6	NPV2	39.81±1.57de				30.69±1.08 d			
7	Flubendiamide1	32.98±1.49ef				23.87±0.47 ef			
8	Flubendiamide2	42.07±1.43d				29.54±0.93 de			
LSD value @ 5%		7.9099				6.7918			

Flubendiamide1, 0.01 ppm and Flubendiamide2, 0.02 ppm; NPV1, 1×10^7pob/ml and NPV2, 1×10^8pob/ml. Mean sharing the same letters within columns are not significantly different.

Table II.- Pupation and adult emergence % of second and forth instar larvae of *S. litura* treated with flubendiamide and NPV.

Sr. #	Treatments	Second instar		Fourth instar	
		Pupation (%)	Adult emergence (%)	Pupation (%)	Adult emergence (%)
1	NPV1+ Flubendiamide1	29.61±1.63 d	26.28±2.61 e	47.85±1.52 d	44.51±1.52 d
2	NPV1+ Flubendiamide2	18.27±1.29 e	14.94±1.73 f	39.85±1.55 d	36.51±1.55 e
3	NPV2+ Flubendiamide1	6.82±1.18 f	3.49± 1.18 g	28.50±1.01 e	24.82±1.34 f
4	NPV2+ Flubendiamide2	3.42±1.04 f	2.22±0.91 g	26.21±1.66 e	22.52±1.66 f
5	NPV1	66.01±1.32 b	61.87±1.71 b	73.95±1.28 b	67.77±1.11 b
6	NPV2	54.63±1.57 c	47.77±1.92 cd	64.87±2.01 c	58.88±1.92 c
7	Flubendiamide1	61.45±1.49 bc	54.44±1.96 bc	71.69±1.27 bc	64.44±1.14 bc
8	Flubendiamide2	52.37±1.43 c	46.16±0.96 d	66.02±1.54 c	60.00±1.42 c
9	Control	94.44±1.92 a	91.11±90.00 a	95.56±1.73 a	92.22±1.85 a
LSD value @ 5%		6.3687	8.0513	4.6626	4.5625

For abbreviations and statistical details, see Table I.

with 87.61±2.18% mortality while rest of the interactions showed additive effect. Maximum mortality (91.02±2.04 % mortality) was observed when both NPV and flubendiamide were integrated at higher doses ($1\text{x}10^8$ POB/ml and 0.02 ppm). Similar kind of trend was observed in 4th instar larvae where NPV at higher dose ($1\text{x}10^8$ POB/ml) with flubendiamide at lower concentration (0.01 ppm) showed synergism (CTF=22.90) with 67.05±1.42% mortality while rest of the interactions showed additive effect. Maximum mortality (69.35±1.66 % mortality) was observed when both NPV and flubendiamide were integrated at higher doses ($1\text{x}10^8$ POB/ml and 0.02 ppm).

The results regarding percentage of pupation and adult emergence revealed that integration of both NPV and flubendiamide proved more fatal at higher concentrations than the individual application at lower concentration. Minimum pupation percentage (3.42±1.04 and 26.21% pupation) was recorded when NPV and flubendiamide were applied higher concentration ($1\text{x}10^8$ POB/ml and 0.02ppm) against 2nd and 4th instar larvae. Except from untreated larvae (control) maximum pupation (66.01±1.32 and 73.95±1.28 %) was recorded when NPV was applied at lower concentration ($1\text{x}10^7$ POB/ml) against 2nd and 4th instar larvae. While in case of adult emergence, minimum adults were emerged (2.22±0.91 and 22.52±1.66 %) when larvae were treated with NPV and flubendiamide were applied higher concentration ($1\text{x}10^8$ POB/ml and 0.02 ppm) against 2nd and 4th instar larvae. Apart from untreated larvae (control) maximum adult emergence (61.87±1.71 and 67.77±1.11%) was observed when larvae were treated with NPV (1x107 POB/ml) against 2nd and 4th instar larvae. Combined application gave hazardous effect on pupation and adult emergence percentage as compared with the individual applications (Table II).

DISCUSSION

To control of lepidopterous insect pests, farmers completely relay on insecticides in Pakistan (Basit *et al.*, 2013). However, indiscriminate use of insecticides has created the resistance problems in lepidoptreous insect pests (Ferre and Van, 2002; Sayyed and Wright, 2006). *S. litura* has shown resistance against a wide range of insecticides (deltamethrin), which ultimately is main cause of sporadic out breaks of this pest and crops failure (Armes *et al*., 1997; Kranthi *et al*., 2002; Ahmad *et al*., 2007, 2008; Saleem *et al*., 2008). This pest showed high level of resistance against organophosphates and pyrethroids. While against emamectin, indoxacarb and chlorfenapyr showed low level of resistance (Tong *et al*., 2013).

To overcome resistance problems, there is a dire need to use of novel biochemical products like flubendiamide and microbial insecticides such as nucleo-polyhedro viruses (NPV). Flubendiamide have novel biochemical mode of action, it can be used as an effective component in IPM against lepidopterous insect pests of different crops due to its selective activity against a broad range of lepidopterous pests, novel mode of action, safer for predators, parasitoids and pollinators (Tohnishi *et al*., 2005; Shaurub *et al*., 2014; Nasution et. al., 2015).

When using the microbial agent individually in IPM, a number of hurdles have been faced due to slow action, low persistent and needed repeated applications of pathogens on target host. Combined application of microbes with microbial insecticides may helpful to control of cabbage moth; it may be hypothesized that joint action may enhance their virulence than expected in single application.

NPVs are very effective microbes to control of *S. litura* populations. In the current study NPV gave significant results regarding larval mortality, pupal and adult emergence when applied alone. These findings were confirmed by various scientists (Gupta *et al*., 2007; Suganyadevi and Kumar, 2007; Marzban *et al*., 2009; Sutanto *et al*., 2014), they reported that NPVs have a potential to control of larval populations in cotton as well as pupal and adult emergence of *S. litura* efficiently.

Flubendaamide is a new chemistry microbial insecticide which also control this pest very effectively and safer for natural enemies. Results of current study revealed that flubendiamide had significant mortality on *S. litura* larvae and reduced the emergence of pupae and adult. Current findings confirmed by Jiahua *et al*. (2014) they reported that flubendiamide can control this pest up to 90% in different crops (cotton, tomato, okra, potato, chili, cucumber, pumpkin, cabbage, pigeonpea and gram *etc*.). These results were also confirmed by Khaliq *et al*. (2014) they reported that flubendiamide is very effective insecticide which gave more than 90% mortality after 48 hours of application.

In present study when NPV and flubendiamide were applied in combinations, they enhanced their effects and showed additive and synergistic type of interactions. These finding stand parallel with the findings of previous studies (Senthil *et al*., 2005; Kumari and Singh, 2009; Singh *et al*., 2009; Pugalenthi *et al*., 2013; Shaurub *et al*., 2014; Nasution *et al*., 2015) who have reported enhanced effects of NPV and flubendiamide when used in combinations against *S. litura* larvae. Emergence of pupae and adults was also reduced with this combined treament.

NPV is a microbe and flubendiamide is a microbial insecticide, both are host specific and safer for natural enemies and have a potential to use in Integrated Pest Management as key component to minimize the insecticides resistance developed in this pest.

Statement of conflict of interest

Authors have declared no conflict of interest.

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