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INSECTICIDAL PROPERTIES OF *SPODOPTERA EXIGUA* NUCLEAR POLIHEDROSIS VIRUS LOCAL ISOLATE AGAINST *SPODOPTERA EXIGUA* ON SHALLOT

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A B S T R A C T

To date, beet army worm (BAW) (*Spodoptera exigua* Hubn.) is the most important pest on shallot in the world, and also in Indonesia. So far, in Indonesia, the pest was controlled using chemical insecticides which caused damage on environment and consumers. *Spodoptera exigua* nuclear polihedrosis virus (Se-NPV) is virus that specifically infects BAW, so it could be developed as biological control agent against the BAW. The research was conducted at Plant Protection Laboratory and Greenhouse of Agriculture Faculty, Sebelas Maret University, Surakarta, Indonesia from September 2011to August 2012. The aims of this research were to evaluate the potency of Se-NPV local isolate in controlling BAW in laboratory and green house. In this research, we also evaluated potency of non-native pathogen *Mythimna separata* nuclear polihedrosis virus (Ms-NPV) in controlling BAW. Chemical insecticide Lamda Sihalotrin was used as a comparison. The results showed that application of Se-NPV was effective enough in controlling BAW both in the laboratory and green house experiment. In laboratory experiment, Se-NPV caused larvae mortality by 77.5% within 5 days. Furthermore, Se-NPV also suppressed BAW feeding capacity by 0.43 g per day. Moreover, Se-NPV also decreased pupae weight by 0.063 g. In green house experiment, the application of Se-NPV could cause 100% of larval mortality, and decrease damage intensity of shallot caused by BAW by 10.43%. Interestingly, in this experiment Ms-NPV which is not a native pathogen caused higher BAW mortality than Se-NPV.

Keywords: Biocontrol, Se-NPV, shallot, *Spodoptera exigua*.

INTRODUCTION

Shallot is one of the important vegetable crops in the world and also in Indonesia. So far, shallot cultivation in Indonesia is still faced with many constrains. One of the important constrain is the attack of beet army worm (BAW) (*Spodoptera exigua* Hubn.). The BAW attack shallot crops from vegetative phase until harvest time, and could damage until 100% (Estie, 2011; Negara, 2003). So far, farmers used chemical insecticides to control them which having consequences to cause damage on environment and consumers. To date, there is no effective and environmental friendliness control method to the BAW. Therefore, utilizing a biological control method is a tactful choice (Untung, 2003). So far, many viruses have been developed as powerful biological control agents. Baculoviruses which are characterized by double-stranded, circular DNA genomes and rod-shaped virus particles are viruses that infect insects. Nucleopolyhedroviruses (NPVs), the member of Baculoviruses have been developed as biological control agent and have major eminence for insect pest management. The primary reason for interest in the NPVs is their environmental safety due to their extreme host specificity. So, they are safe to the non-target organisms (Lee *et al*., 2001). So far, wild type baculoviruses have been used for management of lepidopteran pests in cotton, vegetable crops, forest, and ornamental plants (Inceoglu *at al*., 2001; Moscardi, 1999). The commercial use of wild type baculovirus insecticides has been limited in part because of their relatively slow speed of kill compared to chemical insecticides (Inceoglu *at al*., 2001). *Spodoptera exigua* nuclear polyhedrosis virus (Se-NPV) is virus that specifically infects BAW. Virions are embadded within an occlusion body composed of crystalline matrix around 0.15 to 15 µm in size called polyhedrin. Occluded virions are packaged with multiple nucleocapsids within a single

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viral envelope. Nucleocapsids are rod-shaped (30-60 nm x 250-300 nm) and contain a single molecule of circular supercoiled dsDNA of approximately 80-180 kbp in size (Fauquet, 2005). *Mythimna separata* nuclear polyhedrosis virus (Ms-NPV) is baculovirus infecting rice ear-cutting caterpillar *Mythimna separata* (Walker) (Lepidoptera: Noctuidae). Mukawa and Goto (2008) reported that the baculovirus is belonging to the Granuloviruses (GVs). The molecular biology and genetics of GVs have been less well studied than NPVs because of the difficulty of establishing cell lines for studying the viruses (Winstanley and Crook, 1993). The aims of this research were to evaluate the potency of Se-NPV local isolate in controlling BAW on shallot. Using the same pest-plant host system, we also evaluated the potency of a non-native pathogen Ms-NPV. In this experiment, we noted that Se-NPV local isolate was effective enough in controlling BAW both in the laboratory and green house experiment. The isolate could kill BAW on shallot from 2 day after treatment. Interestingly, Ms-NPV which is not a native pathogen caused higher BAW mortality than Se-NPV.

MATERIAL AND METHODS

The research was conducted at The Laboratory of Plant Protection and Greenhouse of Agriculture Faculty, Sebelas Maret University, Surakarta, Indonesia from September 2011 to August 2012.

Viruses Preparation: The viruses (Se-NPV and Ms-NPV) were previously prepared by The Laboratory of Plant Pests and Diseases Monitoring, Surakarta, Indonesia. The viruses were collected from local area. Inclusion bodies concentration of NPV were determined by counting the inclusion bodies using a hand counter with the aid of a hemocytometer, followed by serial dilution on aquadest.

BAW Preparation: BAW population was originated from eggs cluster collected from shallot crops in Karanganyar, Central Java. Eggs were allowed to hatch in a 20 x 15 x 5 cm^3 wooden cage and larvae were reared using shallot leaves as diet until pupae and then imagos. Imagos were reared using honey as diet in the same cage, and then paired to allow copulation. Eggs clusters generated from the copulation were allowed to hatch and larvae were reared as previously until instar 3 or 4.

Se-NPV Virulence Assay: Se-NPV virulence assay was conducted in screened plastic boxes, ten of three-instar BAW per box. BAW were fed on pieces of shallot leaves which have been soaked in 250 ml of Se-NPV inclusion bodies suspension. The population of inclusion bodies in the suspensions was: 3.74 x 10^9; 7.7 x 10^9; and 1.56 x 10^{10} polihedra inclusion bodies (PIBs) respectively. We also examined Ms-NPV with population of 4.33 x 10^{10} PIBs. For comparison, we used 0.125 ml of Lamda sihalotrin. From second day, BAW were maintained using sterile shallot leaves as diet. BAW mortality was observed until 5 days after treatment.

BAW Feeding Capacity and Pupae Weight Assay: BAW feeding capacity and pupae weight assay was conducted similar to Se-NPV virulence assay above. Here we used one of four-instar BAW per box. BAW were fed on 10 cm long of shallot leaves which have been soaked in 250 ml of Se-NPV inclusion bodies suspension. Observation was conducted until 14 days after treatment by scaling the shallot leaves diet pre and post feeding per day. The formed pupae were also scaled to evaluate the effect of NPV infection to pupae weight.

Se-NPV Efication Assay: Se-NPV efication assay was conducted at green house using shallot crops as indicator plants. Shallot plantlets were grown in 30 x 40 cm polybags. At 30 days after planting, each individual plant was sheltered with screen. Se-NPV inclusion bodies suspensions were hand sprayed to each shallot plants with intensity of 10 sprays per plant. The NPVs population for application on shallot plants followed on the NPV virulence assay above. Then, each shallot plant was infested with 5 BAW. Each treatment was replicated 4 times. Damage intensity was observed every day until 7 days after NPV treatment.

Data analysis: Data were analyzed statistically using analysis of variance (F test at 5% significance level). If there are some significant differences between treatments, analysis were continued using DMRT at 5% significance level.

RESULTS AND DISCUSSION

Effect of Se-NPV infection on BAW Mortality: The result of virulence assay showed that Se-NPV started to kill BAW at 1 day post inoculation (dpi). At 5 dpi, the majority of BAW in the experiment were died, so the observation was stopped at that day (Fig 1). Based on the criteria of Granados and Federici (1986), Se-NPV isolate in this experiment is virulent. According to their criteria, virulent NPV could kill larvae within 2-5 days, whereas non-virulent NPV kill larvae within 2-3 weeks. In this experiment, Ms-NPV which is not a native pathogen also could infect and kill BAW. Interestingly, Ms-NPV could kill BAW more than Se-NPV, in which Ms-NPV caused BAW mortality by 35% whereas Ms-NPV by

22.5% at 1 dpi. Overall, during five days Se-NPV could cause 77.5% BAW mortality, whereas both Ms-NPV and chemical insecticide caused 95% BAW mortality (Fig 2). In this experiment, Ms-NPV which is not a native pathogen of BAW not only could infect and kill BAW, but also was more virulent than Se-NPV. The phenomenon of across host species infectivity among NPVs was reported by many researchers (Stairs, 1989; Stairs, 1991; Mangoendihardjo *et al*., 1993; Kukan and Myers, 1995; Scheepens and Wysoki, 1989; Passarelli and Miller, 1994). The phenomenon was happen because of some similarities on the genome sequences of NPVs. Kouassi *et al*., (2009) reported that Ms-NPV isolated from *M. separata* from Japan also could infect *S. litura* in addition to infect their native host *M. separata*. The authors also reported that the Ms-NPV's genome sequence was not similar to genome of Ms-NPV isolated from China. On the other hand, storage of NPVs *in-vitro* long time could cause changing on their genome sequences (mutations). In general, genome mutation may affect many traits of the organism. In this case, these mutations could affect the NPVs virulence, which are decrease or increase, or even expand their host range. In this experiment, Ms-NPV isolate was prepared in 1994 and stored *in-vitro* at room temperature until be used. So, it is suggested that the storage of the Ms-NPVs isolate caused mutations on their genome and caused impact on phenotypic changes i.e. host range and virulency.

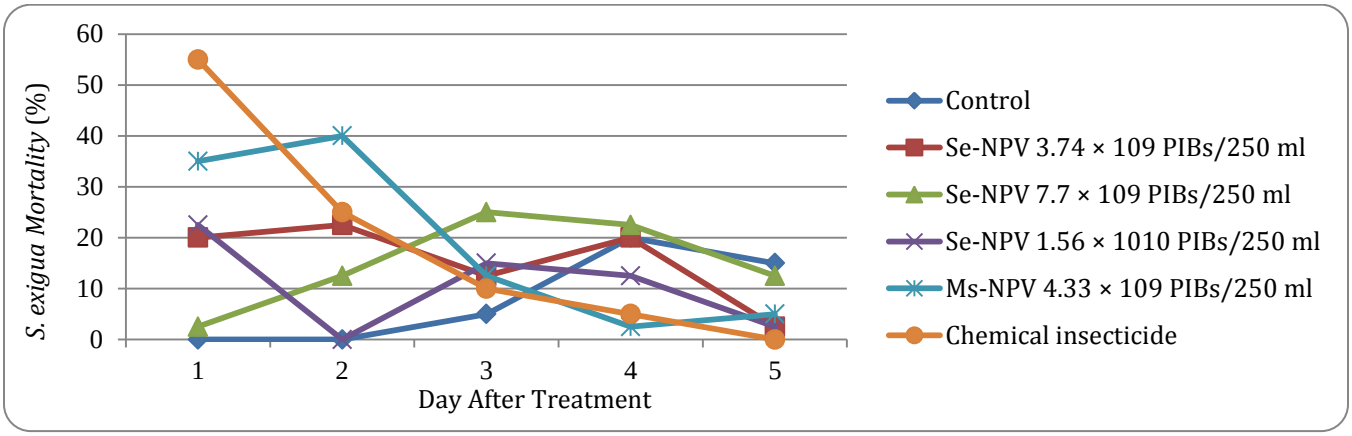

Figure 1. BAW mortality rate following inoculation with Se-NPV. Ten of three-instar BAW were fed on shallot leaves containing polihedra inclusion bodies of Se-NPV with varied concentration. From second day, inoculated BAW were maintained in plastic boxes and provided with sterile shallot leaves as diet. BAW mortality was observed until 5 days after treatment.

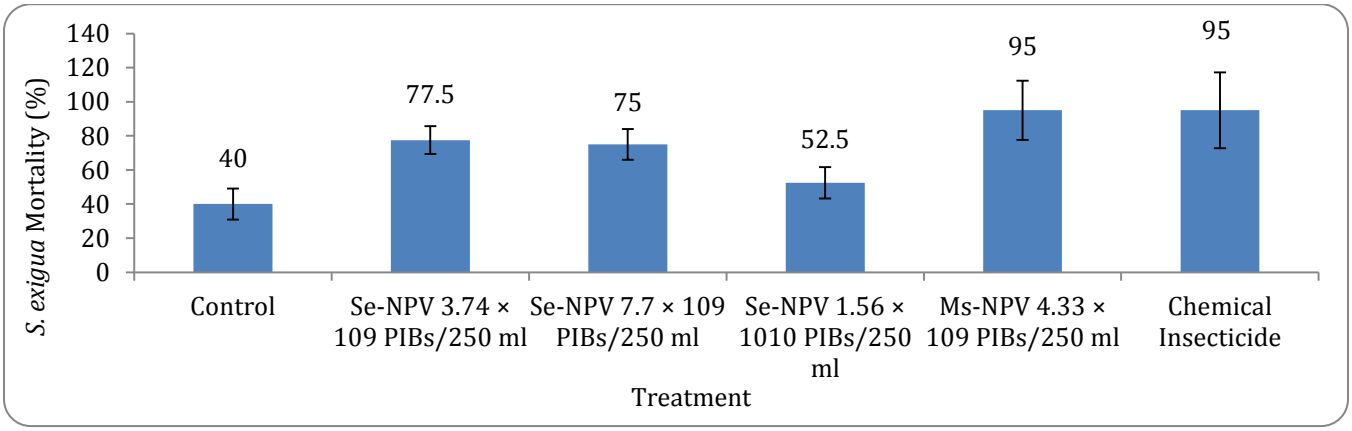

Figure 2. Effect of Se-NPV infection on BAW mortality. Ten of three-instar BAW were fed on shallot leaves containing polihedra inclusion bodies of Se-NPV with varied concentration. From second day, inoculated BAW were maintained in plastic boxes and provided with sterile shallot leaves as diet. BAW mortality was observed until 5 days after treatment. Columns with the same letter are not signifcantly different at the 5% significance level as determined by DMRT. Bars represent standard deviation of the mean.

Effect of Se-NPV infection on BAW Feeding Capacity: The result of BAW feeding capacity assay showed that Se-NPV infection decreased BAW feeding capacity, even was more than BAW feeding capacity which was treated with chemical insecticides. Infection with 7.7 x 10^9 PIBs of Se-NPV decreased BAW feeding capacity by 0.426 g diet per day during seven days, in which the feeding capacity of uninfected BAW was 0.553 g diet per day. Feeding capacity of BAW which were treated with chemical insecticides was 0.535 g diet per day (Fig 3). Pesticide used in this experiment was Lamda sihalotrin with recommanded dosage of 25 g/l (0.125 ml/250 ml). It is suggested that the higher feeding capacity of chemical pesticide treated BAW than Se-NPV treated BAW was due to resistance of larvae to the pesticide. Many pesticide brands have been applied for controlling pests in Tawangmangu area during the last decades (Dinas Pertanian Kabupaten Karanganyar, personal communication), from where the BAW in this experiment were collected. The phenomenon in which Se-NPV infection could decrease BAW feeding capacity more than chemical insecticide treatment was interesting, and so far there has been no such report. However, a research which compared between NPV and chemical insecticide against larval feeding capacity with congruent result to this experiment has been reported. On a research with *S. litura* larvae, Bhutia *et al* (2012) reported that Sl-NPV application caused larvae mortality more than chemical insecticide (Indoxacarb 14.5 SC, Avant®). From the above facts, it could be drawn a logical relationships that is NPV infection causes disease on larvae, thereby reducing their appetite, and finally causes death of larvae or vice versa.

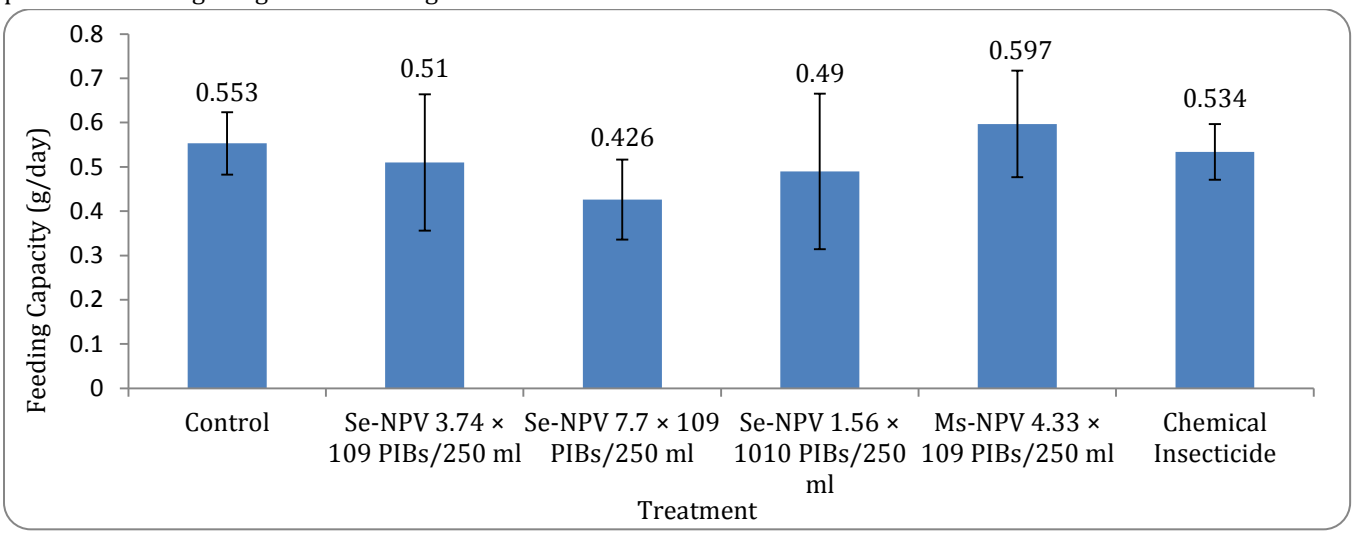

Figure 3. Effect of Se-NPV infection on BAW Feeding Capacity. One of three-instar BAW was fed on shallot leaves containing polihedra inclusion bodies of Se-NPV with varied concentration. From second day, inoculated BAW were maintained in plastic boxes and provided with sterile shallot leaves as diet. Experiments were carried out in triplicate. BAW feeding capacity was observed until 7 days after treatment. Bars represent standard deviation of the mean.

Effect of Se-NPV infection on BAW pupae weight: Se-NPV infection also decreased BAW pupae weight. Figure 4 shows that Se-NPV application could decrease pupae weight by 0.063 g at 3.74 x 10^9 PIBs, more than chemical insecticide which was by 0.079 g. This is also an interesting result in which NPV could decrease pupae weight more than chemical insecticide, and was concurrent with the effect of Se-NPV infection on BAW feeding capacity above. Logical explanation for this phenomenon is coincident with explanation for the effect of Se-NPV infection on BAW feeding capacity above that is the phenomenon of resistance of BAW against pesticide Lamda sihalotrin. In fact, many pesticide brands have been applied for controlling pests in Tawangmangu area during the last decades, from where the BAW in this experiment were collected. Hence, in this experiment, the lower of BAW feeding capacity caused lower on food and nutrients supply of BAW. The lower of food and nutrients supply of BAW give impact on lower BAW general metabolism rate. The lower of BAW metabolism rate limited BAW growth and development, and finally give impact on lower BAW pupae weight.

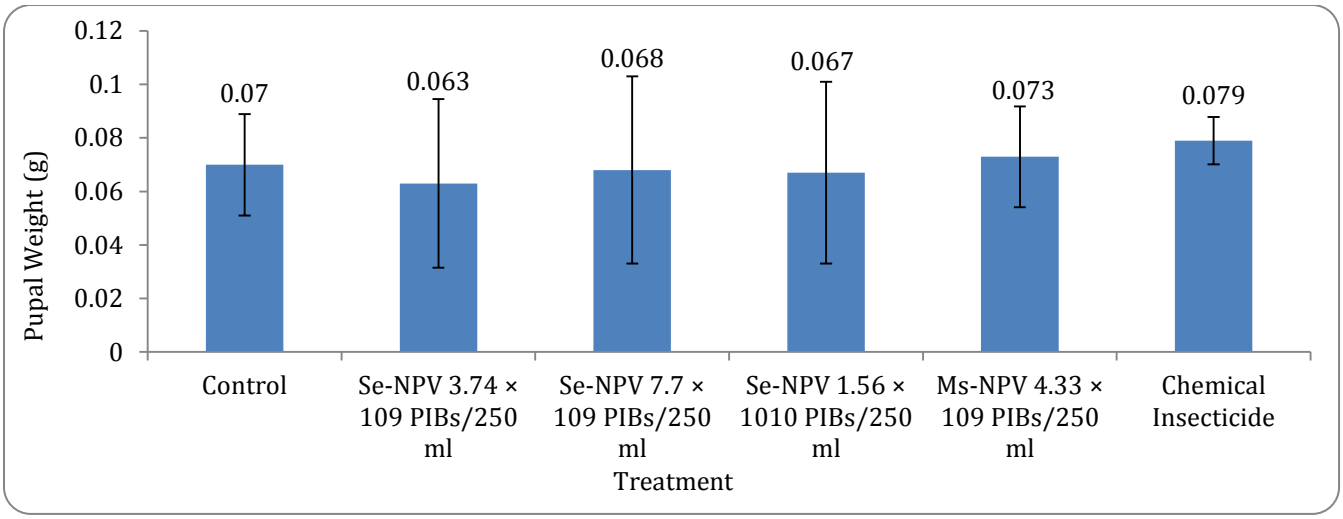

Figure 4. Effect of Se-NPV infection on BAW pupae weight. One of three-instar BAW was fed on shallot leaves containing polihedra inclusion bodies of Se-NPV with varied concentration. From second day, inoculated BAW were maintained in plastic boxes and provided with sterile shallot leaves as diet until pupae. Individual BAW pupae were weighed using digital scales. Each bar represents the mean of four experiments. Bars represent standard deviation of the mean.

Effect of Se-NPV infection on Damage Intensity: Se-NPV infection on BAW decreased damage intensity. Se-NPV infection on BAW at 1.56 x 10^{10} PIBs decreased damage intensity by 10.43% compared to uninfected BAW that was 15.91% (Fig 5). This result was in accordance with experiment coducted by Rimadhani *et al*. (2013) on Sl-NPV against *S. litura* in tobacco, in which Sl-NPV could decrease damage intensity from 25.25% to 15.58%. According to the authors, NPV ability to suppress the damage intensity from 25.25% to 15:58% was classified as strong. Based on all results of this experiment, it could be summarized that the local Se-NPV isolate was quite effective in controlling the BAW. Field trial has to be performed before the results of this experiment were recommended to the farmers.

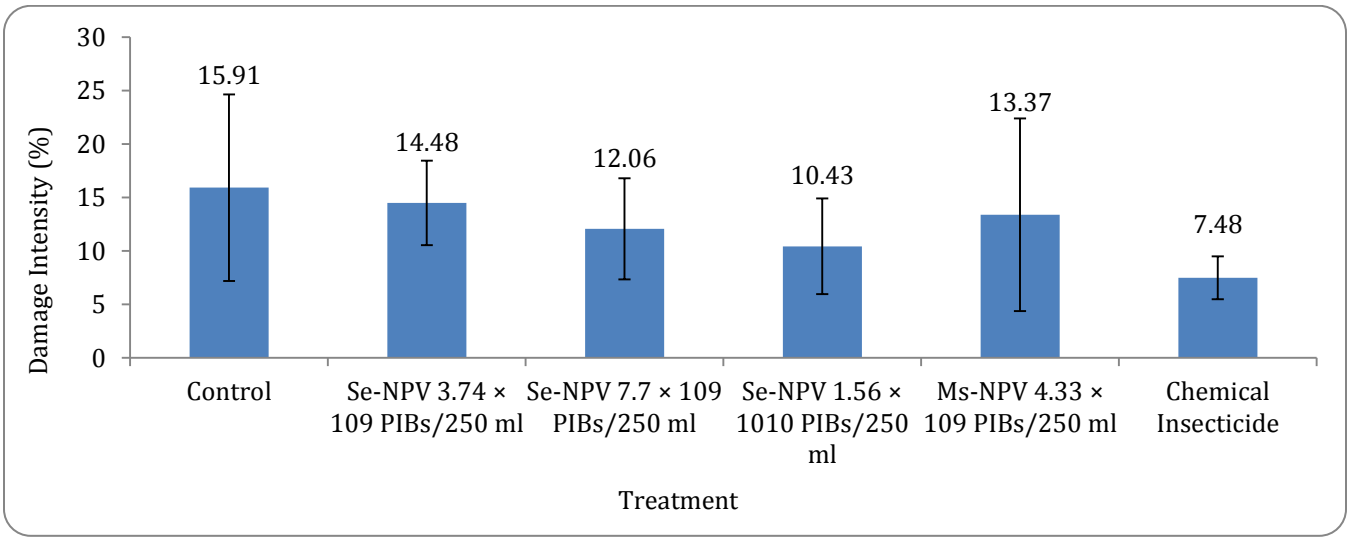

Figure 5. Effect of Se-NPV infection on damage intensity. Shallot plantlets were grown in 30 x 40 cm polybags. At 30 days after planting, each individual plant was sheltered with a screen. Se-NPV inclusion bodies suspensions were hand sprayed to each shallot plants with intensity of 10 sprays per plant. Then, each of the shallot plant was infested with 5 BAW. Damage intensity was observed every day until 7 days after Se-NPV treatment. Each bar represents the mean of four experiments. Bars represent standard deviation of the mean.

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