Biological Control of Sciarid flies (*Bradysia* spp.) with Entompathogenic Nematode *Steinernema feltiae* on Greenhouse Propagated Poinsettia (*Euphorbia pulcherima)* in Ethiopia

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Abstract

Sciarid flies (Bradysia spp.) are major insect pests of greenhouse-grown horticultural crops mainly due to the direct feeding damage caused by the larvae, and the ability of larvae to transmit certain soil-borne plant pathogens. Currently, insecticides and biological control agents are being used successively to deal with Sciarid flies populations in greenhouse production systems. Sciarid flies are very important constraints of flowers in general and particularly Poinsettia. Biological control of sciarid flies is a safe alternative to chemical control. The entompathogenic nematode, Steinernema feltiae was applied at the rate of 50 million nematodes /100m^2; sprayed two times per season at weekly interval. On the other hand, the conventional insecticide nomoult and tap water were used as positive and negative controls, respectively. Three separate greenhouses were used for each treatment. The experiment was conducted two times and the results were combined for analysis. Adult sciarid flies were monitored for six weeks by using yellow sticky trap suspended over the crop. Plant mortality, root development, root damage and number of sciarid fly maggots recovered after six weeks of trial period were assessed by using destructive sampling. The result revealed that as compared to the untreated control, S. feltiae has resulted to 66% reduction of the development of sciarid fly adult. Similarly, the application of S. feltiae increased the root development by 38.55 %. Besides, from our visual observation of the plant, injury caused by the insect in the negative control plots resulted in yellowing and wilting of leaves and loss of plant vigor.

Key words: Biological control, Sciarid fly, *Bradysia* spp. *Steinernema feltiae*, poinsettia, *Euphorbia pulcherrima* and greenhouse

Introduction

Ethiopia is endowed with extensive natural resources such as fertile soil, excellent water resource and good climate that make the country suitable for the development of different varieties of flowers, vegetables, fruit, and herbs (EHDA Portal, 2017). The production and marketing of cut flowers from the floriculture industry in Ethiopia with the private ownership is expanding at a very fast rate (Adhanom, 2006). Currently, close to 130 local and foreign companies are operating in Ethiopia in the production of flowers, vegetables, fruit and herbs. The horticulture industry has an immense contribution in generating a huge amount of foreign exchange earnings of the nation. The foreign currency secured from the sector has been growing from year to year, and in the 2013/14 budget year reached 245 million USD (EHDA Portal, 2017). The foreign currency secured from export of horticulture products has shown a 10.7 per cent increase compared to the performance of last year. About 49,000 tons of roses and 714.5 million cut flowers were exported in the given period. Likewise, over 30,000 workers were employed in the sector (Anonymous, 2007)

Major flowers species grown in these farms include Roses, Hypericum, Eryngium, Carnations, Gypsophylla, Chrysanthemum, Geranium cutting and over 100 other bed plants (Eshetu *et al.* 2009). Over 77% of these farms produce roses on 74% of the land area while 5.7% of the farms produce mainly carnation, Gypsophylla and Geranium, and other bed plants on the rest of the land area allotted to cut flowers (Eshetu *et al.*, 2009). In the last few years, with the increase in flower farms and flower production the number and severity of disease and insect pests also increased significantly.

The poinsettia (*Euphorbia pulcherrima*) is a commercially important plant species of the diverse spurge family (Euphorbiaceae). The species is indigenous to Mexico. It is particularly well known for its red and green foliage and is widely used in Christmas floral displays. Poinsettia s are popular Christmas decorations in homes, churches, offices, and elsewhere across North America (Bussell, 2009). They are available in large numbers from grocery, drug, and hardware stores. In Ethiopia, the poinsettia cuttings were propagated in soilless media under greenhouse until root system production and the cuttings will be exported to Europe.

Among the insect pests attacking greenhouse cut flower farms in Ethiopia, Sciarid Flies/ Fungus gnats (*Bradysia* spp. Diptera, Sciaridae) is the most important invasive insect pest species causing severe damage to poinsettia and different greenhouse grown plants. Fungus gnats are small, mosquito-like flies which are a common problem in nurseries and greenhouses where propagation and seedlings are being grown (Cloyed, 2008). Fungus gnats are indiscriminate feeders on a wide variety of ornamental and vegetable plants. Injury to plants occurs when larvae feeds on roots causing yellowing, wilting of leaves and a loss of plant vigor. Also of its importance, is the possible introduction of plant pathogens by the Sciarid fly and the increased susceptibility of the injured plant to other plant pathogens. Moreover, the adults of Sciarid flies are a problem under excessive moisture conditions when propagation of cuttings and plants are initiating root system (Lindquist, 1997; Cloyed, 2000).

Currently, control of sciarid flies in flower farms of Ethiopia is primarily depends on spraying of conventional synthetic insecticides such as chlorpyrifos, diazinon, malathion and nomoult , though none of them are registered insecticides for the control of these insect pest in Ethiopia. However, the use of synthetic insecticides in general is harmful to the natural enemies and none target organisms (Croft and Whalon, 1982; Smith and Stratton, 1986). The use of insecticides is possible but this remains effective only for a short period of time as it give a chance of resistance development and also can pollute the environment.

Therefore, considering the risk and economics of pesticide application the highest net benefit will be obtained from the use of bio control. The use of bio control agents against fungus gnats has been readily adopted by greenhouse producers throughout the USA due to the commercial availability of effective biological control agents (Cloyed, 2008). Certain strains of entomopathogenic nematodes (EPN) specifically target Sciarid fly larvae. These EPN such as Steinernema feltiae (Steinernematidae), are able to detect larvae via the release of carbon dioxide and products excreted by the larvae Infective juveniles (IJs) enter larvae through natural openings, such as the mouth, anus or breathing pores /spiracles (Mracek el al., 1988). Upon entering the host, the nematode releases a bacterium, which is actually responsible for killing the larvae. The bacteria kill the larvae, usually within 24 hours, by releasing protein destroying enzymes. The nematodes feed on the remains, and complete two to three generations inside the dead larvae. Eventually, large numbers of IJs leave the dead cadaver and search for new Sciarid fly larvae (Poinar, 1990; Smart, 1995).

Therefore, the purpose of this study was to evaluate the efficacy of Entompathogenic Nematode (*Steinernema feltiae*) against sciarid flies prior to registration and wider use in the country. The present paper describes experiments in a glasshouse growing Poinsettia where *S. feltiae* was applied to control *Bradysia* spp, one of the most common sciarids in glasshouses ornamental crops.

Materials and Methods

In the experiment three separate precisely controlled greenhouses were used and table 1 summarizes the materials and methods used in the experiment. The details of the treatments used, climatic description of the greenhouse, the common and scientific names of the target pest and the crop variety were given in the table below.

Table 1. Set-up and climate details of the greenhouse experiment for evaluation of entonem

Experimental details	Experiment 1
Species of Biocontrol agent:	*Steinernema feltiae*
• Trade name.	ENTONEM
• Common Name.	Entomopathogenic nematode; EPN
Duration: Period of experiment	**Starting date:** April 2015 **Ending date**: August 2015
Climate details of the greenhouse	
• Mean temperature (°C)	23.15
• Temperature range (°C)	18.3–28.0
• Mean RH. (%)	73
Locations:	Desa Plants PLC at Koka, Ethiopia
Crop and variety	Poinsettia (*Euphorbia pulcherima)*
Crop stage when pesticide was applied	From cutting to root foot formation
Target insect pest	Sciarid flies, (*Bradysia* spp)
Formulation:	WP
Manufacturer:	Koppert Biological Systems
Formulator:	Koppert Biological Systems
Dosage & frequency:	@ 50 million nematodes /100m^2. sprayed two times at weekly interval
Application technique:	Using motorized sprayer/ hydraulic sprayer

Experimental Procedures followed

The experiment was conducted in a commercial glasshouse at DESA Plants Plc, following the EIAR's Guidelines for Biocontrol agent testing. The cuttings of poinsettia (*Euphorbia pulcherima)* were plugged in pots with the soilless media under greenhouse condition; where there was an ample natural infestation of Sciarid fly (*Bradysia* spp). Un-rooted cuttings of vegetative propagated poinsettia were transplanted on July 3, 2015 into Mix growing medium (imported medium substrate). Cuttings were inserted into 6000 cups, which were then excised into ten-packs (10 cuttings per tray), with 600 trays (6000 cuttings) total. Cuttings of Poinsettia which is 10cm long having 3-5 buds/internodes used and sticking was made in 6 inch plastic cup at optimum time of planting and following the normal procedures of the farm. A large green house having a micro climatic control system with the size of 9.5m width and 30m long was used and divided in to three sub partitions by completely sealing with a hard plastic sheet. Each compartment was used independently for each treatment. 2000 cutting were grouped in to 200 trays placed within each partition and kept on growth table/ beds until rooting. Each treatment was replicated three times. The three beds in each room were used as a replication and covered with plastic sheet which is supported by iron roads structure in order to make separate tunnels of size 100m^2 (Figure 1). The candidate Entompathogenic Nematode (*Steinernema feltiae*) was evaluated at the rate of 50 million nematodes /100m^2 with the standard insecticide Nomoult and Untreated check. The biocontrol agent (*S. feltiae*) WP and insecticide Nomoult were applied one week after sticking the plug. One pack of *S. feltiae* product, having 50M nematodes concentration was put into a bucket containing 5 liters of water (15-20°C) and stirred well and left to soak for 20-30 seconds. Then, stirred well once again and the entire solution was poured into the spray tank and the required amount of water (100 litters) was topped in the tank. The solution was sprayed immediately after preparation with the help of motorized sprayer. 10 catch plates (yellow sticky traps) were placed under each tunnel. The Sciarid flies adult catch count were made at weekly interval on each catch plate starting one day before treatment application and continued for 6 weeks period until the plugs developed a root system and transferred to red ash media for Harding before ready to export. Finally, sciarid flies damage on the root system was assessed six weeks after *S. feltiae* application by using destructive sampling taking ten randomly selected plants/plot and measuring the rooting percentage, root length, root diameter and plant vigor. Phytho-toxicity was visually assessed using any visible symptoms. Finally, pre and post spray mean sciarid adult count data were subjected to efficacy calculation using the following formula:

$$\text{Effcacay}\ \% = [(B - A)/Bx100]$$ where,

- A = total number of emerging adults in the each treatments and
- B= total number of emerging adults in the negative control.

Whereas the percentage damage of crop was determined by using the formula (Erler et al., 2009a & b):

$$\text{Plant damage}\ \% = [\text{No. ofplants damaged}/\text{Total no. of planta}]x100$$

	DAS 10	DAS 17	DAS 24	30 DAS	DAS 37
control	11.5	9.6	14.3	16.7	33.7
Nomoult	5.7	9.6	8.8	7	21.5
Entonem	8.2	8.6	6.7	7.4	22.9

DAYS AFTER APPLICATION

Fig. 2 Mean number of adult *Bradysia* spp. caught on yellow sticky traps hung above the crop for 37 days. *S. feltiae* treated (♦), insecticide treated (■) and untreated (▲).

Results and Discussion

Effects on Sciarid fly Adult Emergence

As indicated in table 2, the adult sciarid fly catch, number of larval count, and plant mortality has shown a significant difference among treatments. But, there was no significant difference between the biocontrol agent and the insecticide treatments. As compared to the negative control, the lowest number of adult fly catch and larval count was observed in *S. feltiae* and Nomolt treated plots. The adult population catch recoded on the yellow sticky trap indicated that. significantly more flies emerged in the plots where no treatment was incorporated (Table 2 and 3). An average of 11.5 Sciarid fly adults emerged from the control trays in the first post treatment sampling date (July 03/2015) whereas a mean of 8.2 and 5.7 Sciarid fly adults emerged from the *S. feltiae* and Nomoult, respectively (Table 3). In comparison to the untreated check, the *S feltiae* and the standard insecticide (Nomoult) treatments significantly reduced the emerging proportion of sciarids adults by 63.2 and 37.3%, respectively (Table 3). The number of sciarid larvae count recovered six weeks after treatment application was 0, 5 and 20 in *S. feltiae* , Nomoult and negative control respectively (Table 4). Similarly, the number of un-rooted (dead) cuttings in *S. feltiae* , Nomoult and control treatment was 5, 10 and 40, respectively (Table 4 and Figure 3). As a result, the highest number of dead cuttings was recorded in untreated check. Damage to cuttings of poinsettia was significantly reduced by the application of *S. feltiae* WP into the growth media immediately after sticking. This shows that, the *S. feltiae* application has highly reduced the mortality of the cuttings and increased rooting percentage. The *S. feltiae* and Nomoult application increased the root development by 38.55 and 25.3%, respectively (Table 4). As indicated in figure 2, the level of sciarid fly activity increased on untreated and insecticide treated plots, during the trial season with sequential monitoring maintained over a period of a month and half. As the visual assessment the injury caused to plants when larvae feed on roots, was clearly observed in control plots causing yellowing and wilting of leaves and loss of plant vigor. Richardson and P.S. Grewal (1991) reported that. *Steinernema feltiae* when applied to mushroom at casing they were almost as effective as insecticide diflubenzuron. Similarly, a significant mean increases in yield of 19% was attained from *S. feltiae* treated plots.

Table 2. Mean number of Sciarid fly adult catch with yellow sticky traps at weekly interval count and. no. larvae. dead plants, root length and diameter under three greenhouse condition

	Different parameters measured after treatment applications				
Treatments	adult/tap	no. larvae	Dead plant	Root Length	Root Diameter
S. feltiae	10.66a	0.40a	5.00a	11.37a	6.67a
Nomoult	9.97a	2.70a	9.67ab	10.60a	6.00a
Control	19.88b	15.00b	28.33b	8.10a	5.70a
Mean	13.50	6.03	14.33	10.02	6.12
CV	14.76	51.05	45.39	11.66	6.56
SD	1.99	3.08	6.51	1.17	0.40
LSD	5.80	8.96	18.93	NS	NS

Table 3. Mean of Sciarid fly pre and post spray counts on yellow sticky traps during the six weeks monitoring period

Treatments	Pre-spray count (1DBS*)	Mean Post spray counts for six weeks							% Efficacy
		10 DAS	17 DAS	24 DAS	30 DAS	37 DAS	Post spray total	Post spray mean	
S. feltiae	7	8.2	8.6	6.7	7.4	22.9	47.1	11.775	63.20
Nomoult	2.8	5.7	9.6	8.8	7	21.5	43.8	10.95	37.19
Control	4.9	11.5	9.6	14.3	16.7	33.7	71.5	17.875	

**NB: DBS= Days before spray; DAS= Days after spray*

Table 4. Mean count of dead plants, number of Sciarid larvae recoverd, damaged cuttings and percent root development

Treatments	Mean No of dead plants/ plot	Mean No larvae /plot	Mean root length	Mean root diameter	% Root diameter development over the negative control	% Root length development over the negative control	Difference b/n post and pre-application counts	% Efficacy
S. feltiae	5	0	11.5	7.1	20.34	38.55	4.78	63.20
Nomoult	10	5	10.4	6.3	6.78	25.30	8.15	37.19
Control	40	20	8.3	5.9	0	0	12.98	

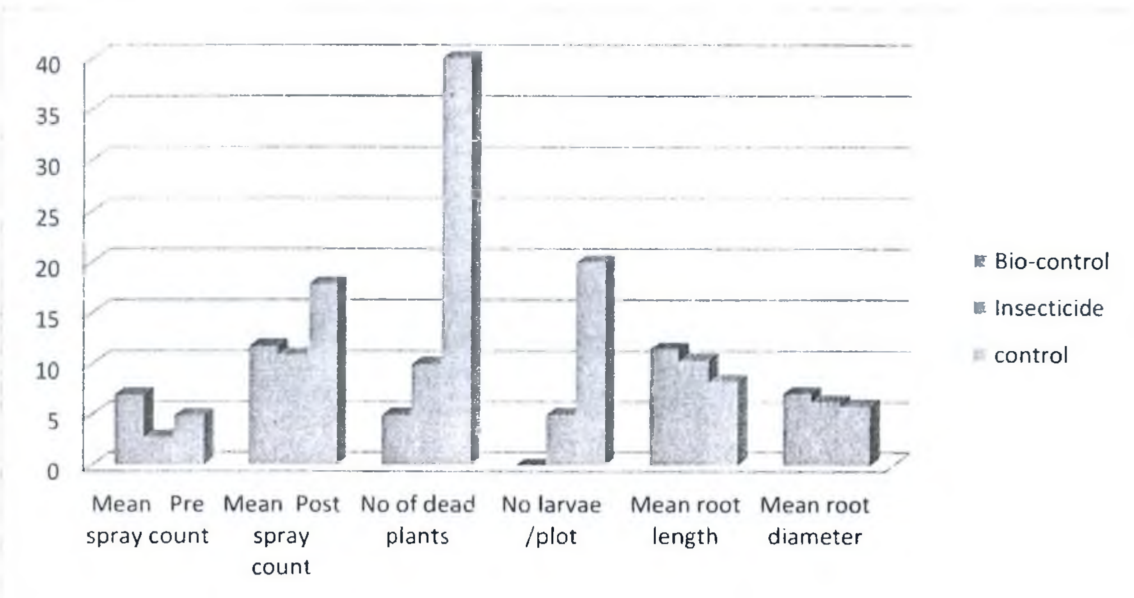

Figure 3. Mean effects of Sciarid flies (Bradysia spp) count and damage on root development

Conclusion and Recommendation

Sciarid flies (*Bradysia* spp.) are mainly a nuisance causing direct plant damage becoming serious pest problem of different Horticultural (Floricultural) crops in greenhouse farms in Ethiopia. The larvae are primarily responsible for causing direct plant damage by feeding on the roots, thus interfering with the ability of plants to uptake water and nutrients, which results in wilting and stunted growth. The adults are also a problem with the extent of 30-60 % loss, and in the extreme case total loss of plants can occur and the estimated value of production loss is more than 100 000 USD per year (Personal communication with Anteneh Markos, Production Manager at Desa Plants PLC, 2016). Currently, in flower farms control of the pest with insecticides is possible but this remains effective only for short period. Moreover, Insecticids give a chance of resistance development and also can pollute the environment. Thus, having an alternative and safe product is indispensable. In this trial, the entomopathogenic nematodes (*S. feltiae*) has effectively reduced the population of the insect, with two times application. Based on the result obtained from this study, the entomopathogenic nematodes (EPN), (*S. feltiae*) specifically target Sciarid fly larvae has shown relatively better performance than the check Nomoult with the efficacy of 66 % six weeks after treatment application. Moreover, it has a clear plant vigour advantage over the standard and untreated check. Therefore, the new entomopathogenic nematodes (EPN) *S. feltiae* could be recommended as an alternative tools for Sciarids (*Bradysia* spp.) management in greenhouse condition in Ethiopia. Biological control requires the same level of dedication as management with pesticides however is more gradual. Therefore it is critically important to monitor Sciarid fly populations and apply the EPN as early as possible as part of a regular preventative plan. Therefore, the early intervention will decrease the possibility of economic loss that will occur leading to best results.

References

Adhanom Negasi. 2006. Economic potentials and opportunities of the flower industry in Ethiopia. Paper presented in a panel discussion towards corporate social and environmental responsibility of cut flower industry in Ethiopia. May 19, 2006. Addis Ababa, Ethiopia.

Anon. 2007. Integrated Pest Management makes a start in Ethiopia. Flower Technology, 2007. 10(3): 12-13. Bussell, G. 2009. *"Get Ready for Holiday Flowers"*. Southern Living. 44 (12): 88

Cloyed, RA., Zaborisky, ER. 2004. Sciarid fly *Bradysia* spp. (Diptera: Sciaridae) and other arthropods in commercial bagged soilless growing media and rooted plant plugs. Journal of Economic Entomology 97(2): 503-510

Cloyed RA, 2008. Management of Sciarid fly (*Bradysia* spp.) in Greenhouse and Nurseries. Floriculture and Ornamental Biotechnology 2(2): 84-89.

Croft BA, Whalon ME. 1982. Selective toxicity of pyrethroid insecticides to arthropod natural enemies and pests of agricultural crops. Entomophaga 27: 3-21.

Erler F, Polat E, Demir H, Cetin H, Erdemir T. 2009. Control of the mushroom phorid fly, *Megaselia halterata* (Wood), with plant extracts. Pest Management Science 65:144 -149.

Erler F, Polat E, Demir H, Cetin H, Erdemir T. 2009. Evaluation of microbial products for the control of the mushroom phorid fly, Megaselia halterata (Wood). Journal of Entomological Science 44(2), 89-97.

Eshetu A, Taye T, Tesfaye B, Abraham T, Emana G. 2008. Pests and pesticide use in the flower farms in Ethiopia. Pest Management Journal of Ethiopia (PMJoE) 12:19-35.

Ethiopian Horticultural Development Agency portal (EHDA) 2017. http://www.ehda.gov.et/web/guest/home#

Lindquist, 1997. Integrated pest management of poinsettia pests: Fungus gnat. Ohio Florists Association Bulletin 813: 5-8.

Mràcek Z, Hanzal R, Kodrlk, D. 1988. Sites of penetration of juvenile Steinernematids and Hererorhabditids (Nematoda) into the larvae of *Galleria mellonella* (Lepidoptera). Journal of Invertebrate Pathology 52: 477-478.

Poinar GO. 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae. Pp. 23–62 *In:* R. Gaugler and H. K. Kaya eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

Richardson PN, Grewal PS. 1991. Comparative Assessment of biological (Nematoda: *Steinernema feltiae*) and chemical methods of control for the mushroom fly *Lycoriella auripila* (Diptera: Sciaridae). Journal of Biocontrol Science and Technology. 1:217- 228. https://doi.org/10.1080/09583159109355201

Smart GC 1995. Entomopathogenic nematodes for the Biological control of insects. Journal of Nematology 27 (4S): 529-534.

Smith TM. Stratton GW. 1986. Effects of synthetic pyrethroid insecticides on none target organisms. Residue Reviews 97: 93-120.