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Effects of entomopathogenic fungus and spider plant intercrop in control of onion thrips and iris yellow spot virus

Rael Kayume Birithia[*1,2], Sevgan Subramanian[1], James Wanjohi Muthomi[2], Rama D. Narla[2]

[1]*International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya*

[2]*Department of Plant Science and Crop Protection, University of Nairobi, Nairobi, Kenya*

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Abstract

Iris yellow spot virus (IYSV) is a major constraint to bulb onions production in Kenya. In search for alternative to synthetic insecticide, field experiments were conducted for two cropping seasons to evaluate the effectiveness of spider plant intercrop, *Metarhizium anisopliae* and carbonsulfan insecticide in the management of onion thrips and IYSV. Two onion varieties; Bombay red and Red creole were used. Randomized complete block design in a split plot arrangements was adopted. Results showed both varieties treated with entomopathogenic fungus recorded the least number of adult thrips while insecticide treated plots recorded the least larvae thrips. Insecticide, entomopathogenic fungus and intercrop significantly reduced IYSV incidence as compared to the control plots. Bombay red variety treated with fungus recorded the lowest severity and virus titer levels in the two cropping seasons. Adult thrips positively correlated with IYSV incidence in season one ($r = 0.575$; $P<0.001$) and two ($r = 0.580$; $P<0.001$). Red Creole treated with insecticide recorded the highest bulb onion total yield (10782.8 T/ha) and marketable yield yield (8314.8 T/ha) in the first cropping season. Entomopathogenic fungus treated plots outweighed other treatments in the second cropping season and recorded total bulb yield of 8503.7 T/ha and marketable yield of 7611.1 T/ha. Although, spider plant intercrop reduced bulb yield in both seasons, it contributed significantly in reducing onion adult thrips and increased food productivity. *Metarhizium anisopliae* and spider plant intercrops were found to be effective bio-pesticide and we suggest their inclusion in the integrated management strategies of vector thrips and IYSV.

* **Corresponding Author:** Rael Kayume Birithia ✉ birithia@gmail.com

Introduction

Bulb onions (*Allium cepa*) are among the most important vegetable in Kenya widely grown by small and large scale farmers for domestic and regional market. The onions are rich in calcium, phosphorous, flavonoids and alkenyl cysteine sulphoxides which play an important role in preventing cardiovascular diseases in humans (Javadzedah *et al.,* 2009). However, onion production in Kenya is constrained by onion thrips vectored *Iris yellow spot virus*, IYSV (Family Bunyaviridae; Genus tospovirus) (Birithia *et al.,* 2011). The virus was first detected in iris (*Iris holladica*) in Netherlands in 1998 (Cortes *et al.,* 1998) and has since spread to all continents (Pappu *et al.,* 2009).

The virus is exclusively transmitted by two thrips species *Thrips tabaci* (Lindeman) and *Frankliniella fusca* (Hinds) that acquires the virus as first instars while adults transmit the virus in a circulative propagative mode (Bag *et al.,* 2015; Srivinivasan *et al.,* 2012). In addition to transmitting the virus, thrips damage allium crops through their rasping and sucking feeding behaviour that causes white blotches along the leaves followed by silvery specks and leaf curling (Trdan *et al.,* 2006). This reduces the plant photosynthetic area reducing bulb size and yield (Munoz *et al.,* 2014). Thrips feeding alone can results in reduction of onion bulb weight resulting in yield losses of up to 60% (Waiganjo *et al.*, 2008).

Bulb onions farmers in Kenya control onion thrips by spraying conventional insecticides with up to 12-15 spray times per growing season while fungicides are incorrectly applied to control IYSV due to its close association with purple blotch fungal disease. These insecticides are ineffective as eggs and larvae stages of thrips are always found protected in between the inner leaves of the onion plant and the pupae stage is spent in the soil (Martin *et al.,* 2003). Besides increasing the cost of production, use of insecticides has negative effects on the environment and human health which is attributed to high chemical residues (Alimousavi *et al.,* 2007). Control of onion thrips is further complicated by its high reproducing capacity with many overlapping generations, lack of natural thrips predator and the presence of numerous other host plants on which the vector onion thrips overwinters (Srinivasan *et al.,* 2012).

Entomologists all over the world are developing new strategies to combat the menace. The reliance has shifted from development of novel insecticides to devising new control strategies (Martin *et al.,* 2003). Alternate strategies developed as result emphasis on cultural control methods, biological control methods, use of resistant varieties and predictable seasonal pest's dynamics model. Resistant varieties to onion thrips and IYSV are few and at times do not occur. Therefore, there is need to integrate the use of insecticides with other methods of control such as cultural practices and biological agents in the control of onion thrips and IYSV in onion.

One sustainable method of managing onion thrips is intercropping (Trdan *et al.,* 2006), a system in which pest non-host plant species is grown specifically to reduce pest damage on a main crop (Sodiya *et al.,* 2010). Intercropping is an important cultural practice that has been utilized in the management of weeds, insect pests and diseases in many crops worldwide. It is traditionally practiced by subsistence farmers in developing countries as a crop production system (Sodiya *et al.,* 2010; Trdan *et al.,* 2006). The system is characterized by minimal use of pesticides and increased land productivity.

Use of antagonist microbes also offers an environmentally sound and effective means of mitigating insect pests and offers an attractive alternative to conventional insecticides. Entomopathogens such as *Metarhizum anisopliae* and *Beauveria bassiana* are well characterized in respect to pathogenicity to several insects and have been used as bio control agents of a number agriculture pests worldwide (Gao *et al.,* 2012). Unlike other bio-control agents, fungi are not ingested to infect the hosts but invade directly through the cuticle and therefore can be utilised in the control of all insects including sucking insects (Niassy *et al.,* 2012).

The death of the insects results from the combination of various factors including mechanical damage of the insect cuticle, depletion of nutrient resources, toxicosis and production of toxin in the body of an insect. Due to complex mode of action these entomopathogens are unlikely to develop resistance as experienced by synthetic insecticides (Gao *et al.,* 2012).

Although intercrop and entomopathogens offer an alternative strategy to conventional insecticides in the control of onion thrips and IYSV, there is little information on their use in IYSV control in Kenya. This study was therefore undertaken to investigate effectiveness of vegetable intercrop, entomopathogens and insecticide in the control of IYSV and onion thrips.

Materials and methods

Experimental design and layout

Field experiments were carried out at the Faculty of Agriculture Kabete field station, University of Nairobi for two cropping seasons between February and November 2012. Onion seedlings of two varieties; Bombay red (moderate resistant) and red creole (susceptible) were raised in nursery and transplanted after one month. Two guard rows of thrips and IYSV susceptible onion variety (red pinnoy) were planted around the experimental area two weeks before transplanting onion seedlings in the plots to act as source of onion thrips and IYSV inoculum. Treatments for each variety were; onion intercropped with spider plant, pure onion stand treated with either fungus or carbonsulfan insecticide and pure onion stand sprayed with distilled water as negative control. *Metarhizum anisopliae* was obtained from Real IPM in Kenya and applied at the recommended rate of 1×10^{11} conidia ha^{-1} and carbosulfan at the recommended rate of 1.75g a.i ha^{-1}. The treatments were applied in the evening between 1700- 1800hrs to avoid the effects of ultra violet radiation on the entomopathogenic fungus.

The experiment was laid out in a randomized complete block design in a split plot arrangement replicated three times. Each block measured 33 × 15m while the subplots measured 3 x 3m with 2m and 1m paths separating the blocks and plots, respectively. For the intercrop treatment, one row of spider plant at a spacing of 45 × 15cm was alternated with two rows of onion. A spacing of 30 x 20cm for onion was maintained. At transplanting, diammonium phosphate (D.A.P) (18%N and 46% P_2O_5) fertilizer was applied into shallow furrows at the rate of 200kg ha^{-1} and top dressed with calcium ammonium nitrate (C.A.N) (26% N) at the rate of 300kg ha^{-1} one month after transplanting. The crop was kept weed free and crops irrigated three times per week to avoid drought stress. Fungicide Tata Master (Metalaxyl 18% and Mancozeb 64%) was applied at rate of 3kg ha^{-1} to manage down mildew at second and sixth week after transplanting.

Determining thrips population, IYSV incidence and severity

Assessment of thrips population entailed tapping five plants from the inner rows of each plot over a white tray. Thrips were then transferred into eppendorf containing 95% ethanol using camel brush. The numbers of thrips were counted under a dissecting microscope at x12 magnification. Sampling was done at one week interval from the fourth week after transplanting for a period of seven weeks. Iris yellow spot disease incidence was determined by counting the number of IYSV infected plants over the total number of plants per plot. Disease severity was determined by randomly sampling seven plants from the inner rows of each plot per sampling time. The percentage of leaf surface showing IYSV damage was determined on a scale of 1 to 5 where 1 = no damage, 2 = up to 25%, 3 = 26-50%, 4 = 51-75% and 5 = 75-100% damage.

Quantification of IYSV using double antibody sandwich Enzyme linked Immunosorbent Assays (DAS-ELISA)

Plants showing characteristic IYSV symptoms was tested in the field using of Agdia IYSV Lateral flow immunostrips (Agdia, Elkhart, IN) and further confirmed in the laboratory DAS-ELISA as described by Pappu *et al.* (2009).

Bulb yield determination

Harvesting was done at physiological maturity when 50-80% of the foliage had fallen over and the tops and roots were cut off.

The bulbs from each plot were weighed and the marketable bulbs that were greater than 3 cm diameter separated and graded. The bulb yield for each treatment was extrapolated into kilograms per hectare.

Data analysis

Data on thrips population, disease incidence, severity, viral load and yield were subjected to analysis of variance (ANOVA) using the PROC ANOVA procedure of R-Package. Differences among treatment means were separated using the Students Newman Kleus (SNK) test. Pearson correlation analysis was done. A significant level of $\alpha = 0.05$ was used in all analyses.

Results

Adult and larvae thrips population density

During the two cropping seasons adult onion thrips varied significantly among the varieties and treatments. Lowest infestation by adults thrips were observed in both red creole and bombay red treated with entomopathogenic fungus, closely followed by insecticides and intercrop. The control plots recorded highest number of adult thrips (Table 1).

Table 1. Effects of treatments on adult onion thrips infestation during the two cropping seasons.

Treatment	Season one		Season two	
	Red creole	Bombay red	Red creole	Bombay red
Control	10.99±0.83a	8.31±0.68b	15.99±0.53a	12.87±0.44ab
Intercrop	5.97±0.41bc	4.37±0.24bc	14.67±0.59a	10.82±0.33bc
Fungus	3.21±1.45c	2.53±0.78c	6.9±1.78d	5.68±1.40d
Insecticide	4.98±1.22c	3.70±0.88c	10.45±1.03bc	8.52±1.15cd
$F_{7,48}$	10.16		25.06	
P value	P<0.001		P<0.001	

Within a column, means followed by different letters (P <0.05) are significantly different using Student Newman's Kleus (SNK) test.

Insecticide treated plots recorded the least number of larvae thrips closely followed by entomopathogenic treated plots and intercrop. Red creole control plots recorded the highest number of larvae thrips while bombay red treated with insecticides recorded the least larvae thrips numbers in seasons one (3.07) and two (5.17) (Table 2).

Table 2. Effects of different treatments on larvae onion thrips infestation during the two cropping seasons.

Treatment	Season one		Season two	
	Red creole	Bombay red	Red creole	Bombay red
Control	19.32±1.22a	11.27±0.71b	25.6±1.28a	18.46±0.77b
Intercrop	10.36±0.36b	7.34±0.72c	20.24±1.25b	14.54±0.93c
Fungus	7.54±0.91c	7.30±0.57c	12.30±1.09cd	10.27±0.83d
Insecticide	4.31±1.80cd	3.07±0.98d	7.90±2.40de	5.17±1.53e
$F_{7,48}$	25.75		25.06	
p	P<0.001		P<0.001	

Within a column, means followed by different letters (P <0.05) are significantly different using Student Newman's Kleus (SNK) test.

Iris yellow spot disease incidence, severity and virus titre levels

Iris yellow spot disease was observed in all plots and produced typical IYSV symptoms that were characterized by green chlorotic tissues surrounded by tan-coloured necrotic tissues. All the three treatments significantly reduced IYSV incidence as compared to the controls. Entomopathogenic fungus treated plots recorded the least disease incidence in both varieties in the two cropping seasons.

Red creole control plots had the highest disease incidence of 77.8% in season one and 78.3% in season two (Table 3).

Table 3. Effects of treatments on iris yellow spot disease incidence during the two cropping seasons.

Treatment	Season one		Season two	
	Red creole	Bombay red	Red creole	Bombay red
Control	77.86±12.64a	58.0±12.79ab	78.32±11.97a	59.53±5.52ab
Intercrop	64.35±12.01ab	37.71±9.62bc	65.61±8.23ab	52.0±5.67b
Fungus	35.0±3.88bc	19.86±4.07c	43.41±3.76bc	18.3±0.72d
Insecticide	30.86±3.24bc	20.21±2.57c	46.62±5.10bc	25.41±1.81cd
$F_{7,48}$	5.92		10.04	
P-value	P<0.001		P<0.001	

Within a column, means followed by different letters (P <0.05) are significantly different using Student Newman's Kleus (SNK) test.

Disease severity varied among the varieties and treatments. During the first season, red creole control plots recorded the highest IYSV severity while bombay red variety treated with entomopathogenic fungus had the least severity score. Similar trend was observed in season two (Table 4).

Table 4. Effects of treatments on IYSV severity based on percentage leaf damage during the two cropping seasons.

Treatment	Season one		Season two	
	Red Creole	Bombay red	Red Creole	Bombay red
Control	4.91±0.74a	3.29±0.45bc	4.34±0.49a	3.49±0.64ab
Intercrop	4.15 ±0.73ab	2.89±0.49bc	3.92±0.47ab	3.29±0.64ab
Fungus	2.11±0.26c	1.94±0.16c	3.37±0.16ab	2.23±0..31ab
Insecticide	2.45±0.29bc	2.08±0.22c	3.54±0.25ab	2.49±0.32b
$F_{7,48}$	5.31		2.42	
P value	0.001		0.033	

Within a column, means followed by different letters (P <0.05) are significantly different using Student Newman's Kleus (SNK) test.

There was a significant difference in viral load among the treatments in season one. Red creole variety control samples recorded the highest titre levels while bombay red variety treated with entomopathogenic fungus had the least viral load (Table 5).

Similarly, during the second cropping season the viral load varied among the treatments ($F_{7, 48}$ =25; P<0.001). Both red creole and Bombay red treated with entomopathogenic fungus recorded the least virus titre, closely followed by insecticide treated plot and spider plant intercrop (Table 5).

Pearson correlation analysis

During the first cropping season, adults thrips positively correlated with IYSV incidence (r = 0.575; P<0.001) and disease severity (r = 0.526; P<0.001). During the second season the same trend was observed in which adults thrips positively correlated with IYSV incidence (r = 0.580 P<0.001) and IYSV severity (r = 0.300; P<0.001). Hence, IYSV severity increased with increase in disease incidence and adult thrips.

Table 5. Effects of treatments on mean virus titre levels at 405 nm during the two cropping seasons.

Treatment	Season one		Season two	
	Red creole	Bombay red	Red creole	Bombay red
Control	3.537±0.23a	1.919±0.14bc	3.639±0.19a	2.418±0.143c
Intercrop	2.334±0.12b	1.462±0.09cd	2.983±0.18b	1.876±0.08d
Fungus	2.163±0.20b	1.248±0.10d	2.119±0.19c	1.283±0.12e
Insecticide	2.309±0.22b	1.609±0.11cd	2.462±0.17cd	1.605±0.05de
$F_{7,48}$	19.45		25	
P value	0.001		0.001	

Within a column, means followed by different letters (P <0.05) are significantly different using Student Newman's Kleus (SNK) test.

Bulb onions total and marketable yield

Results obtained in this study during the two cropping seasons showed synthetic insecticide and *M. anisopliae* significantly increased total and marketable yield in both varieties as compared to the control. During the first season, Red creole variety treated with insecticide recorded the highest total and marketable yield, followed by entomopathogenic fungi. Intercropping onion with spider plant reduced total and marketable yield as compared to the pure stand but the differences were not significant for both varieties during the two cropping seasons (Table 6).

Table 6. Effect of the treatments on bulb onions total and marketable yield during the two cropping seasons.

	Season one		Season two	
Treatments	Total yield (Kg/ha)	Marketable Yield (Kg/ha)	Total yield (Kg/ha)	Marketable Yield (Kg/ha)
Red creole (Rc)	4042.9±155.0b	2492.6±64.6b	3500.6±220.4c	2203.7±187.9b
Rc- intercrop	3417.3±288.9b	1600.0±429.5b	3437.2±419.9c	1259.3±195.9b
Rc-*M. anisopliae*	9971.1±1386.2a	6944.4±250.5a	8503.7±548.8a	7611.1±850.4a
Rc-insecticide	10782.8±530.3a	8314.8±321.3a	8396.0±407.2a	7481.5±450.6a
Bombay red (Br)	5142.0±541.4b	2770.4±621.3b	3290.1±535.3c	1888.9±293.9b
Br -intercrop	4125.6±331.9b	1907.4±492.2b	2972.8±647.3c	1037.0±242.9b
Br -*M. anisopliae*	8437.5±887.4a	7018.5±267.1a	7443.9±176.2ab	6500.0±369.9ab
Br -insecticide	9555.5±1020.8a	6870.4±947.9a	6256.7±483.08b	5703.7±370.4b
$F_{7,16}$	16.351	31.71	28.39	46.65
P-value	P<0.001	P<0.001	P<0.001	P<0.001

Within a column, means followed by different letters (P <0.05) are significantly different using Student Newman's Kleus (SNK) test.

Discussion

Both synthetic insecticide and entomopathogenic fungus reduced adults and larvae thrips populations on both varieties. The level of control achieved with the *M. anisopliae* on the larvae stages of onion thrips was low compared to insecticide treated plots.

Contrary, it was observed that entomopathogenic fungi were the most effective treatment in reducing the adult's thrips. Low control of the larval stages using the fungus could be attributed to the fungal inoculums being shed off with the cuticle following the insect moulting process from first instar to second instar larvae stages. Moulting process is known to be an important mechanism of insect resistance to fungal infection (Arthurs *et al.,* 2013), particularly when the time interval between successive moulting stages is short. These findings are in agreement with Vestergaard *et al.* (1995) who reported that the larvae and pupae stages of western flower thrips, *Frankliniella occidentalis* was more resistant to infection by *M. anisopliae* than adults. Similarly, Ekesi *et al.* (1998) reported reduced mortality in *M. sjostedti* larvae due to *M. anisopliae* as compared to mortality of the adult's cow pea thrips.

There was a significant reduction of onion thrips population in the intercropped plots compared to the control. This could be attributed to confused olfactory and visual stimuli, mechanical barriers, unsuitable host plant quality and increased presence of onion thrips predators or parasitoids including *Orius* (data not presented).

This could be attributed to inter plot interference where fungus-infected thrips would transfer the inoculum to the other insects in plot resulting to their death. Findings of this of this study concur with Hossain *et al.* (2015) who observed that intercropping onion with carrot and tomato reduced thrips population and thrips damage in Bangladesh. Waiganjo *et al.* (2007) also observed that intercropping spider plant and coriander (*Coriandrum sativum*) with snap bean significantly reduced thrip's incidence and pod damage in snap bean. Other intercropping systems which have significantly reduced thrips population and plant infestation include leek with clover (Belder *et al.,* 2000), leek with carrot and clover with French bean (Kucharczyk and Legutowska, 2002). Intercropping of various plants also reduced colonization rates of onion thrips and overall reductions in yield (Trdan *et al.,* 2006).

Hassan, (2009) also reported cowpea and sorghum intercrop reduced aphid (*Aphis craccivora*) population significantly compared to sole cowpea crop in Nigeria.

The low IYSV incidence recorded in the entomopathogenic fungi and insecticide treated plot is due to reduction of the larvae and adult thrips which are involved in virus acquisition and transmission respectively.

The significant difference in thrips populations, IYSV incidence and damage severity on Red creole and Bombay red varieties confirms that different varieties possess varying levels of susceptibility to thrips and IYSV incidence. Our findings concur with reports by Malik *et al.* (2003) and Alimousavi *et al.* (2007) who reported varying susceptibility of onion to thrips infestation and damage.

Both insecticide and entomopathogenic fungal treatments positively increased the bulb yield in the onion varieties. This is due to significant reduction of IYSV severity and thrips population which reduce the photosynthetic area of the leaf. Onions-spider plant intercrop negatively impacted on onion yields. Reduction in yields in the intercropping conditions could be due to competition of growth resources such as nutrients, space, light and water.

This observation is in agreement with findings reported by other researchers. Working on an onion-pepper intercrop, Kabura *et al.* (2008) reported onion planted as a mono crop had higher total and marketable yield than the intercrop. Intercropping onion with *Lacy phacelia* also resulted in reduced onion yield (Trdan *et al.,* 2006).

In the present study, spider plant a green leafy vegetable with large foliage could have smothered the growth of onion, hence the low bulb yields. Although onion-spider plant intercrop recorded low yields, it contributed significantly in reducing food insecurity as its vegetable that are rich in various nutrients such as iron are consumed.

Conclusion

Based on this study, *M. anisopliae* and spider plant intercrop were found to be effective bio pesticide and we suggest their inclusion in the integrated management strategies of onion thrips and IYSV infection. We recommend *M. anisopliae* to be applied early in the crop production cycle, before onion thrips generations overlap, resulting in the simultaneous presence of different life stages. Carbonsulfan EC250 reduced the population of adults and larvae thrips. However, due to insect developing resistance we stress the importance of only need-based application of the insecticide.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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