Prospects of Fungus-Based Biopesticides for Management of Insect Vectors of Maize Lethal Necrosis Disease

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Abstract

*Maize, Zea mays L., production in Kenya is constrained by both biotic and abiotic factors. For instance, maize lethal necrosis (MLN) was first reported in Bomet County in 2011 and has since spread to other maize growing areas. In Kenya, MLN is reported to be caused by a co-infection of maize plant by Maize chlorotic mottle virus (MCMV) and Sugarcane mosaic virus (SCMV). Since this disease is new to Africa, there is little information about management of its insect vectors. To develop management strategies for MLN insect vectors, a field experiment was conducted using fungus-based biopesticides, Metarhizium anisopliae (MA) and Trichoderma asperellum (TA), and Nurelle*D 50/500EC (NuD) insecticide. Seven treatment were used: 1) untreated plot; 2) soil application of TA with foliar sprays of NuD; 3) soil application of TA with foliar spray of MA; 4) soil application of TA with foliar spray of MA and NuD; 5) foliar spray of NuD alone; 6) foliar sprays of MA alone; 7) soil application of TA alone. Data on MLN insect vectors were collected bi-weekly. Single and combined NuD application with biopesticides reduced population densities of various thrips species up to 5.1 folds compared to the controls. At peak period for aphid infestation, plots receiving single and combined application of TA and MA and treatment combination of TA, MA and NuD outperformed those receiving single and combined application of NuD with TA in reducing population of R. maidis. This study demonstrated that combined use of biopesticides and NuD can effectively manage MLN vectors.*

Key Words: Maize lethal necrosis, Thrips, Aphids, *Metarhizium anisopliae, Trichoderma asperellum,* Biopesticides.

INTRODUCTION

Maize is Kenya's principle staple food crop (Onono *et al.,* 2013). Over 90% of the Kenyans depend on maize as a primary source of food and income (FAO, 2011). In Kenya, maize is considered as a key food security crop and shortage in its production may have serious implications on peoples' welfare. Maize production in Kenya is constrained by both abiotic and biotic factors (Wokabi, 2013). Among the major biotic constraints are the incidences of insect pests such as moths and beetles, disease vectors such as leafhoppers, aphids and thrips and diseases like grey leaf spot, rots, maize streak virus, head smut and the recent outbreak of maize lethal necrosis (Wangai *et al.,* 2012; MAFAP, 2013).

Maize lethal necrosis (MLN) was first reported in Kenya in 2011, in Bomet County and has since been reported to spread to other maize growing areas (Wangai *et al.,* 2012). From

Kenya, the disease was subsequently reported in other eastern African countries of Tanzania, Uganda, Southern Sudan, Ethiopia and Rwanda (Makumbi & Wangai, 2012; FAO, 2013; Adams *et al.,* 2014; Mahuku *et al.,* 2015; Kagoda *et al.,* 2016). MLN is caused by a synergistic interaction of *Maize chlorotic mottle virus* (MCMV) and cereal potyviruses infecting maize such as *Sugarcane mosaic virus* (SCMV), *Maize dwarf mosaic virus* (MDMV) and *Wheat streak mosaic virus* (WSMV) (Castillo-Loayza, 1977; CIMMYT, 2004; Nelson *et al.,* 2011). However, the predominant potyvirus reported to induce MLN in eastern Africa is SCMV (Mezzalama *et al.,* 2015). MCMV is transmitted by several species of thrips such as *Frankliniella williamsi*, *F. occidentalis*, *F. schultzei, Thrips pucillus* and *Thrips tabaci* (Cabanas *et al.,* 2013; Zhao *et al.,* 2014; Nyasani *et al.,* 2015a) corn root worms, corn flea beetles, flea beetle, and cereal leaf beetle (Nyvall, 1999). SCMV on the other hand is transmitted by several aphid species including *Rhopalosiphum maidis, Rhopalosiphum padi* and *Schizaphis raminum* (Hassan *et al*., 2003).

Maize lethal necrosis is difficult to control because it is a co-infection of viruses from two different groups i.e. MCMV (*Tombusviridae*) and SCMV (*Potyviridae*), and there are no viricides commercially developed against viruses as is the case for fungi, bacteria and nematodes (Jones, 2006). In addition, insect transmitted plant pathogens are among the most difficult to control with insecticides since some vectors are highly mobile and may colonize fields within a short period of time (Zehnder *et al.,* 1999). Aphids transmit SCMV non-persistently, implying that the invading viruliferous aphids can infect plants in seconds before they are affected by insecticide application (Hassan *et al*., 2003). Research on use of insecticides to manage MLN and thrips revealed that a combination of seed treatment and bi-weekly foliar application of Imidachloprid reduced MLN severity by 50% via control of thrips vectors (Kibaki & Francis, 2013). However, caution ought to be taken to avoid excessive use of chemical insecticides since they have adverse effects on applicators and consumers, may destroy beneficial organisms, may lead to rapid development of insect resistance and are expensive especially to small holder farmers (Chandler *et al.,* 2011; Atieno & Leibniz, 2015).

Integrated pest management (IPM) approaches are being adopted to avoid injudicious use of synthetic insecticides and the repercussions they pose. For example, M*etarhizium anisopliae*, has been demonstrated to effectively manage various species of thrips such as *F. occidentallis* and *T. tabaci* (Maniania *et al.,* 2002; 2003; Niassy *et al.,* 2012; Nyasani *et al.,* 2015b). However, there is little information on use of these biopesticides to manage MLN through control of the thrips and aphid species known to transmit the disease. Therefore, the objective of the current study was to evaluate the effectiveness of single and combined application fungal biopesticides, *M. anisopliae* and *T. asperellum,* and an insecticide Nurelle*D on management of thrips and aphids being the main vectors of MLN inducing viruses and to assess the effect of the fungus-based biopesticides and the insecticide on MLN incidence and severity.

METHODOLOGY

Experimental site

Field experiments were conducted for two planting periods at Kenya Agricultural and Livestock Research Organization (KALRO), Embu Kenya. The first planting period was conducted from December 2014 to April 2015 and the second planting period from May 2015 to September 2015. KALRO Embu is located in the upper midland 2 (UM 2) agroecological zone at an altitude of 1480 m (0.501291° S, 37.458664° E) (Nyasani *et al.,* 2015b). The rainfall pattern in the region is bimodal, with the first and second rains starting from March to May and from October to December, respectively.

Establishment of maize crop in the field

Maize seeds (H615) were planted at an intra and inter row spacing of 25 cm and 75 cm respectively, in experimental plots measuring 5 × 10 m. The plots and blocks were separated from each other by paths measuring 2 m wide. Two maize seeds were planted per hill. Two rows of maize were planted around the field to act as border plants. Di-Ammonium Phosphate (DAP) fertilizer was applied at planting at a rate of 10 g per hill. Four weeks after emergence the plants were top dressed with Calcium Ammonium Nitrate (CAN). No fungicides, foliar feed or insecticides other than the treatments were applied at the course of the experiment.

Insecticide and biopesticides

The insecticide, NURELLE*D 50/500EC (Lachlan (K) Limited, Kenya), was procured from an Agrovet dealer in Nairobi, Kenya. Fungal biopesticides CAMPAIGN® (*Metarhizium anisopliae* ICIPE69 1 x 10^{11} colony forming units (cfu) ml^{-1}) (The Real IPM Company (K) Ltd, Kenya) and REAL TRICHODERMA GRANULE (*Trichoderma asperellum* TRC900 1.7 x 10^{9} cfu/gram) (The Real IPM Company (K) Ltd, Kenya) were procured from the company at Thika, Kenya.

Treatment application

Seven treatments were trialled as follows: 1) untreated plot; 2) soil application of *T. asperellum* (TA) with foliar sprays of NURELLE*D 50/500EC (NuD); 3) soil application of TA with foliar spray of *M. anisopliae* (MA)*;* 4) soil application of TA with foliar spray of MA and NuD; 5) foliar spray of NuD alone; 6) foliar sprays of MA alone; 7) soil application of TA alone. NURELLE*D 50/500EC and *M. anisopliae* were applied biweekly as foliar sprays following manufacturers' recommended rates. To optimize the activity of *M. anisopliae*, the treatments were applied in the evenings between 5:00 pm and 6:30 pm. Separate knapsacks were used for application of *M. anisopliae* and NURELLE*D 50/500EC treatments. The treatments were laid in a randomized complete block design with four replications per treatment for each experimental period.

Evaluation of MLN insect vector population in various treatments

Count data on insect vectors were collected weekly for a period of ten weeks. Sampling for insect vectors was done using whole plants tapping method where maize plants were tapped over a plain white tray and the insect pests picked while counting using a carmel brush. Collected insect vectors were preserved in 70% ethanol in Eppendorf tubes for further identification at International Centre of Insect Physiology and Ecology (*icipe*). Thrips were identified using LuclD key (Moritz *et al*., 2013) while aphids were identified using a Dichotomous key (Summers and Albert, 2001).

Data analysis

Count data on insect vectors and MLN severity collected from different treatments over time were subjected to repeated measures analysis of variance (RM-ANOVA). Proportions of MLN positive samples were angular transformed to standardize the variance and then subjected to RM-ANOVA. All analyses were performed using R Statistical Software (R Core Team, 2015).

RESULTS

Insect pests identified from the field

Table 1: Aphids, beetles and thrips species collected from the field. The transmission status of these insect species as documented by other authors is indicated in the table

Insect pest	Species	Transmission status
Aphids	*Rhopalosiphum maidis*	Yes
	Rhopalosiphum padi	Yes
	Metapolophium dirhodum	Unknown
	Sitobion avenae	Unknown
Beetles	*Scymnus levaillanti*	Unknown
	Nephus sp.	Unknown
	Cheilomenes sp.	Unknown
	Carpophilus sp.	Unknown
	Hippodamia sp.	Unknown
Thrips	*Frankliniella williamsi*	Yes
	Frankliniella schultzei	Yes
	Thrips pucillus	Yes
	Frankliniella occidentalis	Yes

The major thrips species were *F. williamsi, F. schultzei* and *Thrips pucillus* while *R. maidis* was the major aphid species. The population densities of thrips, *F. occidentalis,* and aphids, *R. padi*, were very low and inconsistent in the first and second planting periods hence their data were not included in analysis. None of the beetle species identified has been reported to transmit MCMV, therefore they were also not included in data analysis

Effects of single and combined application of fungus-based biopesticides and Nurelle*D 50/500EC on MLN insect vectors

Frankliniella williamsi

In the first and second planting periods, treatments with single and combined use of Nurelle*D 50/500EC and biopesticides resulted to lowest population densities of *F. williamsi* while the untreated plots produced the highest densities of *F. williamsi* (Fig. 1). The interaction between treatments and time on density of *F. williamsi* were significant by RM-ANOVA (First planting period: $F_{54,\ 189} = 10.84$, $P < 0.001$; Second planting period: $F_{54,\ 189} = 17.69$, $P < 0.001$). Single and combined application of biopesticides alone *T. asperellum* (TA), *M. anisopliae* (MA), and TA + MA reduced the density of *F. williamsi* up to 1.8, 1.6 and 2.2 folds, respectively, compared to untreated plots. Plots receiving soil application of TA and NuD sprays reduced the density of *F. williamsi* 4.9 folds, while soil application of TA with alternated foliar application of MA and NuD reduced the density of *F. williamsi* 3.8 folds compared to the untreated plots. The highest reduction of *F. williamsi* density of up to 6 folds was recorded in plots with single application of NuD spray (Fig.1).

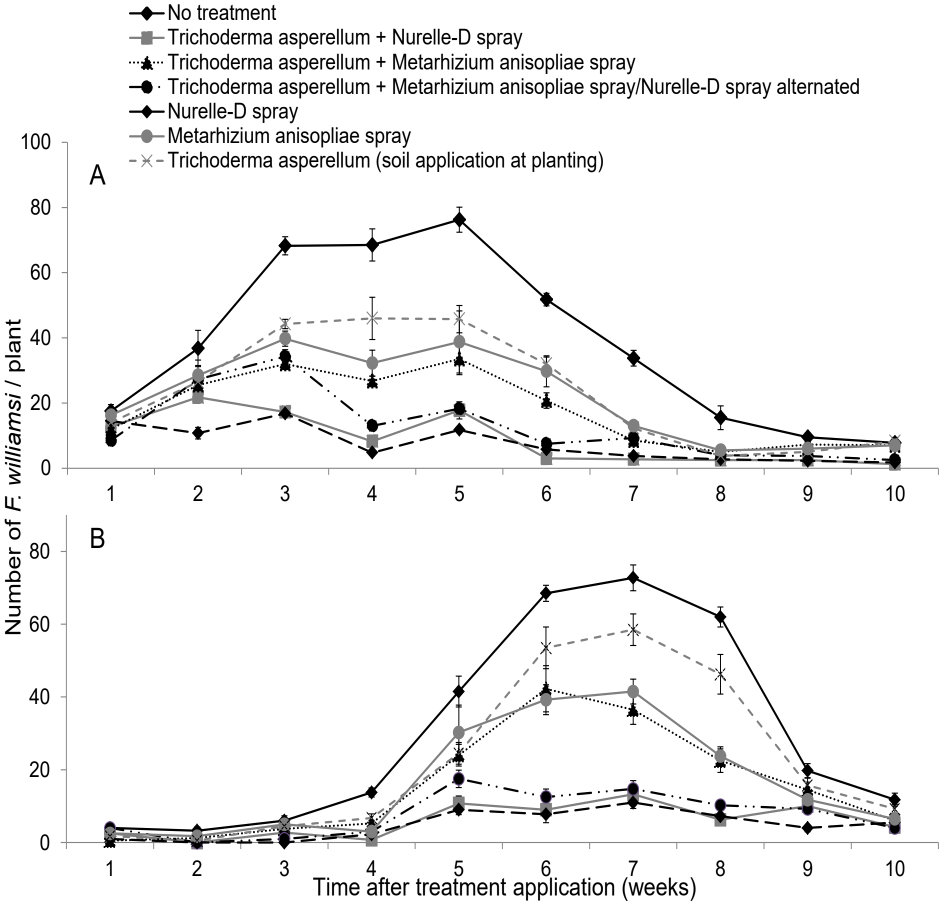

Figure 1: Mean (±SE) number of *F. williamsi* recorded from various treatments over time from December 2014 to April 2015 (A) and April 2015 to September 2015 (B)

Frankliniella schultzei

The highest population densities of *F. schultzei* were recorded in the untreated plots whereas the least population densities were recorded in plots receiving single or combined application of NuD with biopesticides (Fig. 2). There was increase in population density in the untreated controls from week 1-5 and week 1-7 in the first and second planting periods, respectively, followed by a decline up to week 10 (Fig. 2). In both planting periods, the interaction between treatments and time on density of *F. schultzei* was significant by RM-ANOVA (First planting period: $F_{54,\ 189} = 5.984$, $P < 0.001$; Second planting period: $F_{54,\ 189} = 1.89$, $P < 0.001$). Compared to the untreated controls, single and combined application of biopesticides alone, TA, MA and TA+ MA, reduced the density of *F. schultzei* up to 1.6, 1.9 and 1.8 folds, respectively. Soil application of TA with NuD spray reduced the density of *F. schultzei* 3.2 folds while plots receiving soil application of TA with alternated foliar sprays of MA and NuD reduced the densities of *F. schultzei* 2.7 times compared to untreated plots. Treatment combinations did not result to any synergistic effects on reduction *F. schultzei* densities. However, alternated foliar application of MA and NuD was as effective as sole and combined application of NuD and TA in reducing the densities of *F. schultzei* (Fig. 2).

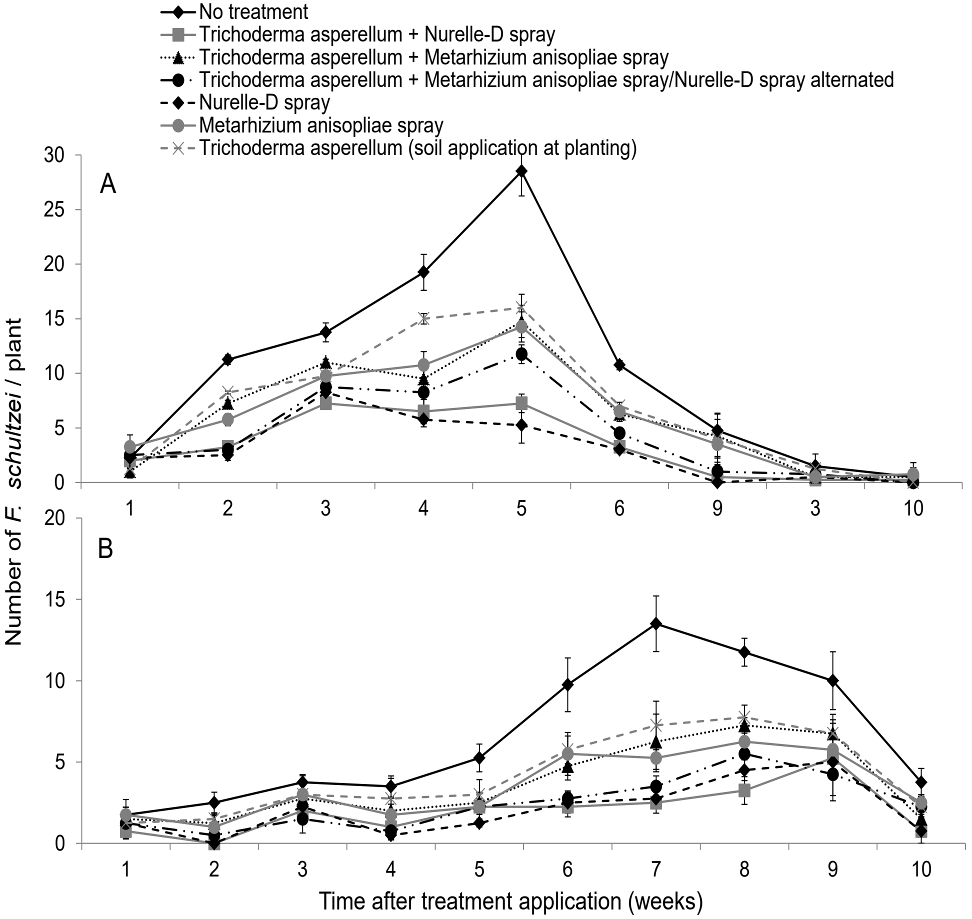

Figure 2: Mean (±SE) number of *F. schultzei* recorded from various treatments over time from December 2014 to April 2015 (A) and April 2015 to September 2015 (B)

Thrips pucillus

Plots receiving sole and combined application of NuD with biopesticides had the lowest numbers of *T. pucillus* than those receiving single and combined application of the biopesticides alone, while the untreated plots recorded the highest *T. pucillus* densities throughout the experimental period (Fig. 3). In the control untreated plots, there was a gradual increase in densities of *T. pucillus* from week 3-7 in the first planting period and from week 1-8 in the second planting period followed by a decline until week 10 (Fig. 3). The interaction between treatments and time on density of *T. pucillus* was significant by RM-ANOVA (First planting period: $F_{54, 189} = 0.974$, $P < 0.001$; Second planting period: $F_{54, 189} = 10.22$, $P < 0.001$). Compared to the untreated plots, single and combined application of fungal biopesticides alone TA, MA and TA+ MA reduced the density of *T. pucillus* up to 1.9, 2.6 and 2.2 folds, respectively. Soil application of TA with NuD spray reduced the density of *T. pucillus* 5.1 folds while soil application of TA with alternated foliar application of MA and NuD reduced densities of *T. pucillus* 3.7 times compared to untreated controls. No synergistic effects among various treatment combinations in reducing population densities of *T. pucillus* were observed. However, alternated foliar application of MA and NuD was as effective in reducing population density of *T. pucillus* as sole or combined application of NuD with TA (Fig. 3).

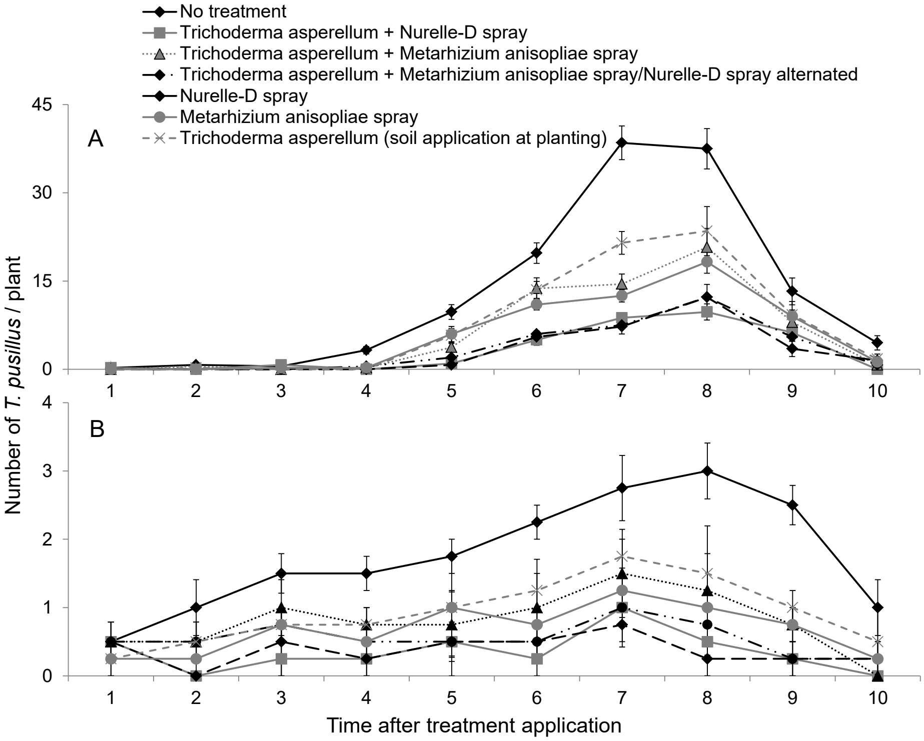

Figure 3: Mean (± SE) number of *T. pucillus* recorded from various treatments over time from December 2014 to April 2015 (A) and April 2015 to September 2015 (B)

Rhopalosiphum maidis

In the first planting period, there was increase in densities of *R. maidis* in various treatments from week 1-5 followed by gradual decline up to week 10 (Fig. 4 A). In the second planting period, there was a decline in density of *R. maidis* from week 1-3, followed by increase up to week 6 and then a decline again until week 10 (Fig. 4 B). However, in both planting periods, the rate of increase of *R. maidis* varied with application of the various treatments. In both planting periods, the untreated plots had the highest densities of *R. maidis* throughout the experimental period whereas plots receiving single or combined application of biopesticides, TA and MA had the lowest densities of *R. maidis* (Fig. 4). In the both planting periods, the interaction between treatments and time on the density of *R. maidis* was significant by RM-ANOVA (First planting period: $F_{54,\ 189} = 1.636$, $P = 0.008$; Second planting period: $F_{54,\ 189} = 3.843$, $P = 0.001$). At peak period for aphid infestation (week 4-5 and week 6-7 in the first and second planting periods respectively – Fig. 4), plots receiving single and combined application of TA and MA and treatment combination of TA, MA and

NuD outperformed those receiving single and combined application of NuD with TA in reducing population of *R. maidis* (Fig. 4).

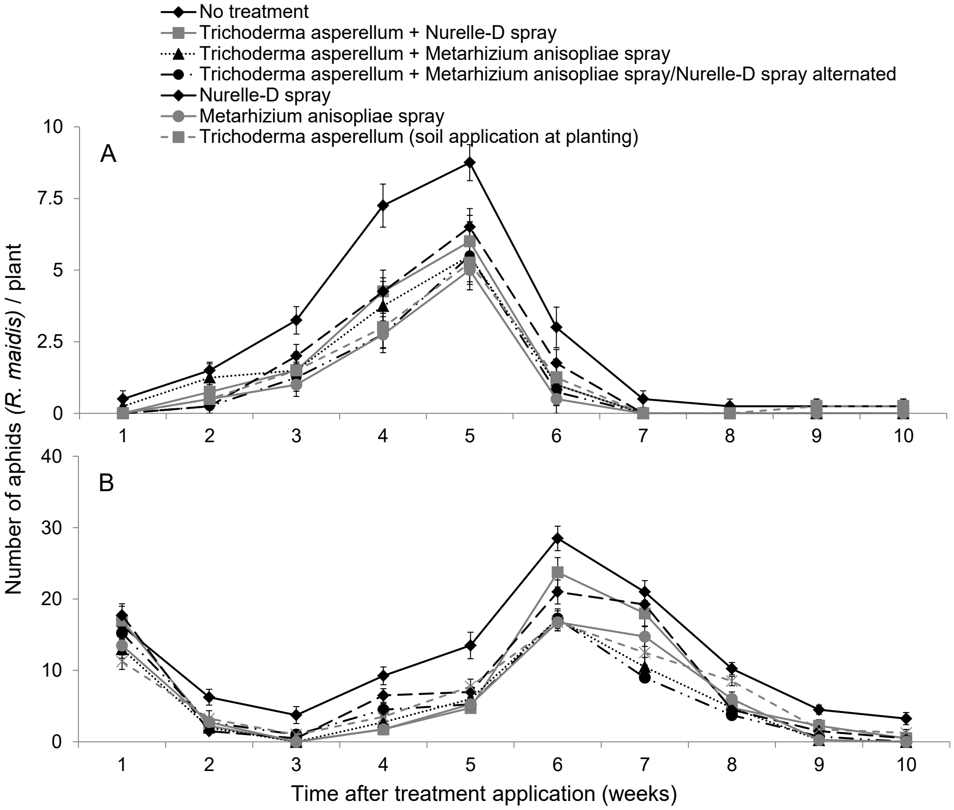

Figure 4: Mean (± SE) number of *R. maidis* recorded from various treatments over time from December 2014 to April 2015 (A) and April 2015 to September 2015 (B)

Effects of single and combined application of biopesticides and Nurelle*D on MLN incidence and severity

MLN incidence

In both planting periods, MLN incidence was always higher in the untreated plots and lowest in the plots receiving single and combined application of NuD with the biopesticides (Fig. 5). The interaction between treatments and time on MLN incidence was not significant by RM-ANOVA in both planting seasons (First planting period: $F_{30, 105} = 0.424$, $P = 0.996$; Second planting period: $F_{30, 105} = 0.435$, $P = 0.985$). However, there were significant differences in MLN incidence among the various treatments in both planting periods (First planting period: $F_{6,21} = 3.021$, $P = 0.0274$: Second planting period: $F_{6,21} = 3.145$, $P = 0.0164$). Compared to the untreated controls, single and combined application of biopesticides alone reduced MLN incidence up to 1.4 folds. In plots receiving single and combined application of NuD with biopesticides; soil application of TA and NuD spray reduced MLN incidence up to 1.9 folds, whereas soil application of TA with alternated foliar application of MA and NuD reduced MLN incidence up to 1.7 folds compared to the untreated plots.

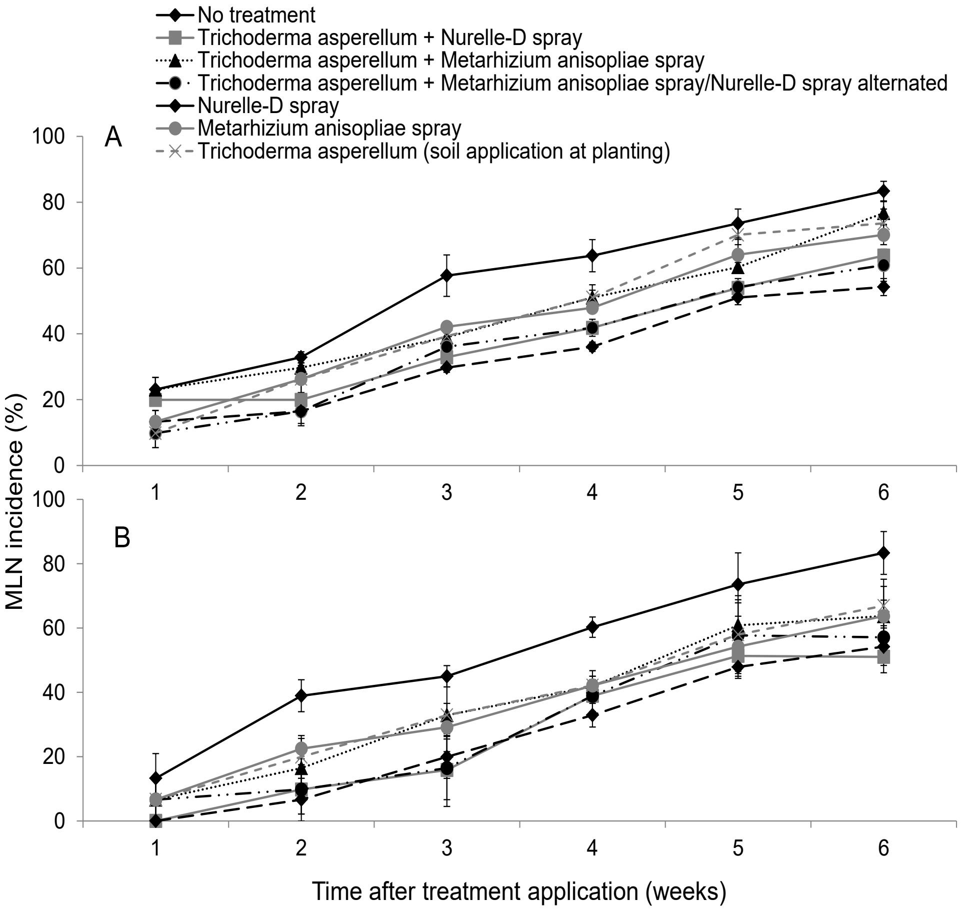

Figure 5: Percentage incidence of MLN recorded from different treatments over time from December 2014 to April 2015 (A) and April 2015 to September 2015 (B)

MLN severity

MLN severity was always highest in the untreated plots and lowest in single and/or combined application of NuD and fungal biopesticides (Fig 6). Treatments receiving soil application of TA followed by alternated foliar application of MA and NuD produced similar results to the treatments with sole application of NuD (Fig. 6). In both planting periods, the interaction between treatment and time on MLN severity was significant by RM-ANOVA (First planting period: $F_{54, 189} = 1.985$, $P < 0.001$; Second planting period: $F_{54, 189} = 1.808$, $P = 0.002$). In both first and second planting periods, single and combined application of biopesticides alone, i.e., TA, MA and TA + MA reduced MLN severity up to 1.2 folds, while single and combined application of NuD with biopesticides reduced MLN severity up to 1.4 folds compared to the untreated controls.

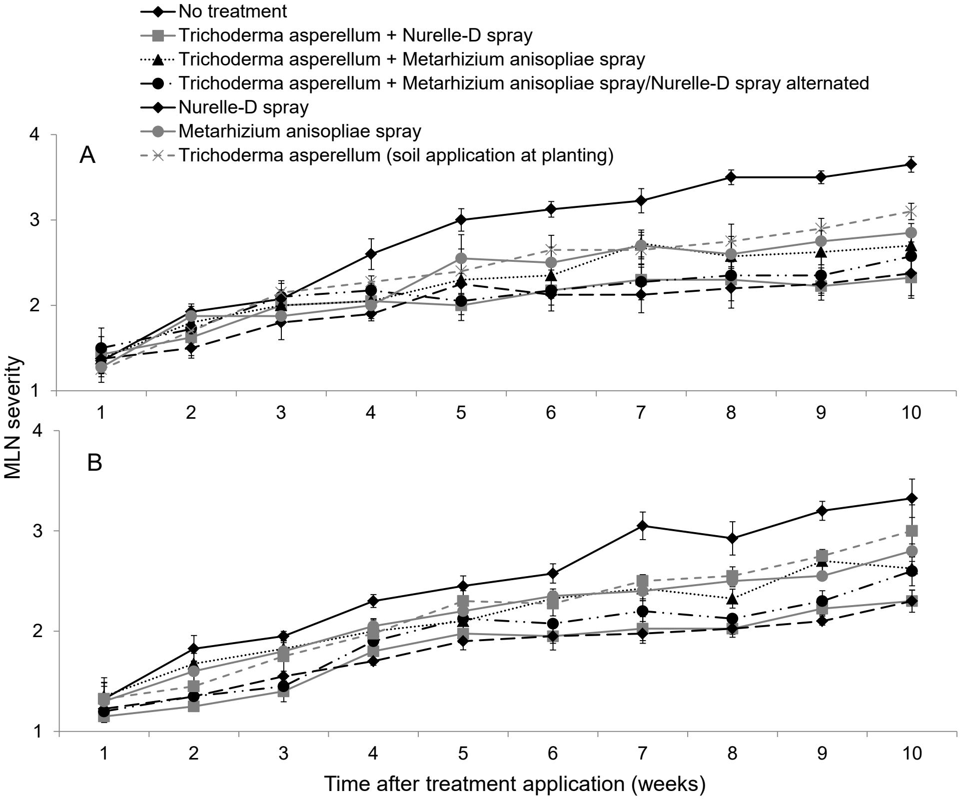

Figure 6: Mean (±SE) of MLN severity as influenced by different treatments over time from December 2014 to April 2015 (A) and April 2015 to September 2015 (B)

DISCUSSION

Various kinds of insect pests were collected and identified from the field. Further identification of these insect pests revealed some species of thrips and aphids that have been reported to transmit *Maize chlorotic mottle virus* and *Sugarcane mosaic virus,* respectively. Thrips species that transmit MCMV were *Frankliniella williamsi, F. schultzei, F. occidentalis* and *Thrips pucillus* while the aphid species reported to transmit SCMV were *Rhopalosiphum maidis* and *R. padi* Hassan *et al.,* 2003; Cabanas *et al.,* 2013; Zhao *et al.,* 2014; Nyasani *et al.,* 2015a). None of the beetle species collected and identified have been previously reported as potential transmiter of either MCMV or SCMV.

All treatment combinations were observed to reduce population densities of both thrips and aphids which confirm the susceptibility of thrips and aphids to NURELLE*D 50/500EC and the biopesticides. However, the extent of reduction of the various pest species varied with the different treatments. Our results show that application of NURELLE*D 50/500EC insecticide had the greatest impact in reducing thrips populations compared to the untreated plots and those receiving single or combined application of biopesticides alone. This could be due to the quick knockdown effects of chemical insecticides against insect pests, a known characteristic of chemical insecticides (Coats *et al*., 1991). In addition, the systemic nature and persistence of NURELLE*D 50/500EC in the maize could have played a role in prolonged reduction of the various thrips population. Our results are in harmony with those of Nyasani *et al*. (2012) who reported that application of insecticide Imidacloprid 700g/Kg as soil drench or foliar sprays significantly reduced population of western flower thrips on French beans compared to application of beneficials (*Abylessius cucumeris* and *M. anisopliae*) either singly or combined.

Management regimes consisting of single and combined application of *T. asperellum* and *M. anisopliae* had the least reduction of various thrips population compared to the untreated plots. This phenomenon could be attributed to the slow acting nature or delayed knockdown effect of most biopesticide on insects as opposed to chemical insecticides (Ouma *et al.,* 2014). For biopesticides such as *M. anisopliae* to be effective in causing insect mortality, their conidia must first attach and penetrate the insect's cuticle, develop germ tubes and appresoria which grow into insects' haemocoel (Pachamuthu & Kamble, 2000). This infection process coupled with insects' cellular response contributes to a longer time required by the EPFs to cause insect's mortality. Other external factors such as unfavorable environmental conditions may also influence the performance of EPFs (Chandler *et al.,* 2011). For instance, conidia of the foliage applied biopesticide, *M. anisopliae*, were reported to have a relatively shorter persistence due to detrimental effects of ultra-violet light (Inglis *et al.,* 1997). Due to these limitations, fungal biopesticides may not be suitable for use alone in spray regimes for management of thrips in maize.

The results further indicates that plots receiving soil application of *T. asperellum* followed by biweekly combined foliar application of *M. anisopliae* and NURELLE*D 50/500EC sprays in an alternated manner were as effective as the continuous biweekly application of NURELLE*D 50/500EC spray alone. This shows that combining chemical insecticide and fungal biopesticides can be deployed to effectively manage thrips vectors. Indeed, previous research show that some insecticides such as Lannate, Tracer, Capture, Runner and Abamectin can be used along with entomopathogenic fungi for pest control (Asi *et al.,* 2010). Maniania *et al.,* (2002) reported a significant reduction of larvae and adults of *F. occidentalis* on chrysanthemums upon combined application of *M. anisopliae* and Lannate. Combined application of chemical insecticides with entomopathogenic fungi reduces extensive use of chemical insecticides in agriculture which is undesirable due to their adverse effects on human health, environment and other non-target organisms such as natural enemies and pollinators (Kasina *et al.,* 2009). In addition, integrating EPFs with chemical insecticides prevents development of resistant pest populations since no insects have been reported to develop resistance to EPFs. For example, combined application of *B. bassiana* and chemical insecticide was shown to effectively control resistant populations of Colorado potato beetle in that the insecticide resistant genes were still susceptible to the fungal pathogen (Anderson & Roberts, 1983).

Apart from the untreated controls, all applied treatments seemed to be equally efficient in reducing population of *R maidis.* However, at peak infestation period for the aphids, single and combined application of biopesticides alone outperformed single application of NURELLE*D 50/500EC and combined application of NURELLE*D 50/500EC with *T. asperellum.* This could have resulted due to aphids' resistance to the Nurelle D insecticide. However, there is limited information revealing better performance of biopesticides against insect pests compared to chemical insecticides. Nevertheless, weekly and biweekly application of fungal biopesticide, *M. anisopliae* was reported to be as effective as an insecticide, Dimethoate (Rogor® 50Bayer) in reducing population density of western flower thrips (Maniania *et al.,* 2003).

The incidence and severity of MLN was highest in control untreated plots and lowest in plots receiving single and combined application of NURELLE*D 50/500EC with biopesticides. This could be due to the reduced populations of the different thrips species responsible for transmission of MCMV. Kibaki & Francis (2013) also reported a significant reduction of MLN incidence and severity through control of its thrips vectors using Gausho® FS350 (Imidacloprid) seed dressing and foliar sprays of Thunder® OD 145 (Imidacloprid). Although not as effective as chemical insecticides, single and combined application of biopesticides was also observed to significantly reduce the incidence and severity of MLN. This could have resulted from mycopesticidal effects of EPFs on thrips and aphids. Despite the fact that colonization of maize plants by the biopesticides *T. asperellum* and *M. anisopliae* to evaluate whether they can mutually exist was not determined in the current study, a possibility cannot be excluded that they occurred as endophytes in maize hence causing feeding deterrence of the thrips and aphid vectors. This is because *Trichoderma* species and *M. anisopliae* have been previously reported to occur as endophytes in maize (Akello, 2012; Shoresh & Harman, 2008). The biocontrol potential of EPF against a vector transmitted virus has been previously reported. Lehtonen *et al*. (2006) found out that fungal endophyte *Neotyphodium uncinatum* reduced the incidence of BYDV in rye grass by deterring the aphids which are responsible for transmission of the virus. Reduction in MLN severity could have also occurred as a result of induced systemic resistance caused by *T. asperellum* and *M. anisopliae*. *T. asperellum* was been reported to induce systemic resistance against *Cucumber mosaic virus* in *Arabidopsis thaliana* (Elsharkawy *et al*., 2013) and tomato plants (Vitti *et al*., 2015) and could have played a role against MCMV and/or SCMV, a case that warrants further investigation. In a previous study, seed inoculation of maize plants by *M. anisopliae* was observed to significantly reduce SCMV severity and titer levels under screen-house conditions and therefore, the biopesticide developed from the fungus could also have played a role in the present study (Kiarie et al., In Press).

CONCLUSION

To determine the effects of NURELLE*D 50/500EC and biopesticides on MLN insect vectors, this study shows that combined application of NURELLE*D 50/500EC and fungal biopesticides (*T. asperellum* and *M. anisopliae*) in alternated manner was as effective as use of NURELLE*D 50/500EC alone in terms of reduced population densities of thrips and aphids vectors. This implies that with 50% reduction of insecticide application, farmers can still achieve effective disease control comparable to that obtained with continuous application of insecticide alone. Due to the adverse effects of chemical insecticides to man, environment and non-target organisms, their reduced usage in agriculture can be of great importance. Therefore, spray regime consisting of alternated foliar application of biopesticide, *M. anisopliae* and insecticide Nurelle D can be recommended for use by farmers. This spray regime is beneficial in that it is effective against both thrips and aphid vectors.

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