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Management of *Maliarpha separatella* Rag Using Effective Entomopathogenic Nematodes and Resistant Rice Cultivars

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ABSTRACT

Current pest control methods rely on a pesticide dominated paradigm and there is need to adopt a more ecological approach based on renewable technologies such as host plant resistance and natural biological control, which are available even to resource poor farmers. Resistant cultivars complement natural enemy action in lowering pest infestation while intrinsic rate of increase of pest species on resistant varieties is lower. An experiment whose aim, was to test whether the African white rice stem borer (*Maliarpha separatella*), can be managed by use of resistant rice varieties in combination with entomopathogenic nematodes was set up at Kenya Agricultural Research Institute, Mwea. The study was arranged as a 4×4 factorial design and each treatment replicated three times. First factor was rice cultivars at four levels, resistant cultivar (M27615), second resistant cultivar (M27628), the highly susceptible but tolerant cultivar (M27608) and commercial check Basmati 370 variety, which were planted in 1×1m experimental plots. Second factor was application of EPN (*Hetrerorhabitidis indica*) as a suspension in distilled water to the cultivars at four different times after they were transplanted (no application, 3 weeks after transplant date (WAT), 5 WAT and 6 WAT. Results showed that *M. separatella* infestation was lowest on cultivar M27615 where *H. indica* was applied at 3 WAT, while cultivar M27608 had the highest yield despite high number of white heads and stem tunneling indicative of high levels of *M. separatella* infestation. The findings showed that host-plant resistance and EPNs can be integrated to manage *M. separatella* infestation (250 words).

Key words: *Maliarpha separatella*, control, entomopathogenic nematodes, resistant rice cultivars

INTRODUCTION

The ability of insect pests to respond to mortality factors by evolving into resistant biotypes makes it difficult for pest control operators to rely on a single control strategy in the long term. Integrated Pest Management (IPM) entails use of combination of host plant resistance, cultural methods and biological agents. It advocates minimal use of insecticides especially with known action thresholds. Litsinger *et al.* (2009) indicated that rice hoppers *Nilaparvata lugens* bred slowly on rice cultivars which were recognized as very resistant and there was no yield loss even with infestations of 30 hoppers per plant. Koppenhofer and Kaya (1998) described a strong synergistic effect on mortality of two scarab species, *Cyclocephala hirta* LeConte and *C. pasadenae* with combinations of imidacloprid insecticide and entomopathogenic nematodes. It has been reported that inoculative release which involve releasing small numbers of natural enemies into a crop cycle in combination with resistant varieties is able to protect the crop from pest infestations (Litsinger *et al.*, 2011). Gassmann *et al.* (2008) found that improved resistance management for *Bacillus thuringiensis* crops was achieved with IPM strategies that incorporated entomopathogenic nematodes in non-Bt refuges. Reports indicate that host plant resistance influences the susceptibility of insect pests to pathogens including entomopathogenic nematodes (Cory and Hoover, 2006). The use of resistant rice cultivars in combination with a biological control agent may be a useful tool for integrated pest management programs to reduce pesticide input. This study was conducted to evaluate a combination of resistant rice cultivars and entomopathogenic nematodes for efficacy against *M. separatella* in flooded rice cultivation.

MATERIALS AND METHODS

Study site and experimental plots lay out: The experiment was set up at KARI-Mwea field testing site (Kirogo) (037.22760E, 0039.00S, Altitude 1150 a.s.l). The study was arranged as a 4x4 factorial design and each treatment replicated three times. First factor was rice cultivars at four levels, resistant cultivar (M27615), second resistant cultivar (M27628), the highly susceptible but tolerant cultivar (M27608 and a commercial check Basmati 370 variety. The experimental field (30×30 m) was divided into 72 plots of 1×1 m with 0.5 m between the plots. The cultivars were planted in the field in a randomized block design at a spacing of 20×20 cm.

Entomopathogenic nematodes application: Entomopathogenic nematode (*Hetrerorhabitidis indica*) which was the second factor was applied to rice cultivars at the rate of 6.7×10^4 Infective Juveniles (IJs) per liter of distilled at 3,5 and 6 weeks after transplant date (WAT). *Heterorhabitidis indica* rate of application in Infective Juveniles (IJs) per square meter was calculated as:

$$N = h^* (p^*.t\_i) * k \qquad (1)$$

Where:

N = No. of IJs required

h = No. of hills per plot (25 at 20×20 cm spacing) in 1M2 plot

p = Estimated *Heterorhabitidis indica* IJs concentration

t_i = Estimated number of *M. separatella* infested tillers

k = No. of experimental plots to be applied with nematodes

which gave; N is 25 (157*17) or 66725 IJs per square meter.

Before EPNs application soil samples from the experimental plots were baited with *Galleria mellonela* L to determine whether there were any EPNS in the plots before application of the treatments. Application was by spraying the mixture of *H. indica* suspension in cool sterile water with a 15 L knapsack sprayer. Application was done at 5.30PM, just before sunset as EPNs are rapidly inactivated by ultraviolet light (Greenwood and Rebek, 2008). Control plots were sprayed with distilled water without EPNs.

Sampling and data collection: Data was collected on *M. separatella* infestation (number of egg batches, white heads, tunnelled tillers and larvae/pupae) *M. separatella* damage (numbers of non-productive panicles, Empty panicles, unfilled grains and 1000 grains weight) and yield (grain weight, dry matter weight and harvest index). At harvest, all stems were dissected and number with tunnels, number of larvae and pupae recorded. The number of productive panicles, filled grains, empty grains and 1000 grain weight from 10 randomly selected tillers was also recorded. The infestation was expressed as number of egg batches, larvae and% white heads calculated by the formula (Shafique *et al*., 2000):

$$\text{Percent whiteheads} = \frac{\text{No. of tillers with whiteheads}}{\text{Total No. of tillers}} \times 100 \qquad (2)$$

Data analysis: Analysis of Variance (ANOVA) was conducted to determine the differences in pest infestation, damage levels and yield and yield components between the different treatments. Least significant differences ($p<0.05$) were used to the separate means when found significant. The statistical analysis was performed with GENSTAT (2009) version 12 statistical software.

RESULTS

Highest number of egg batches was on Basmati 370 (20.27) and the lowest on M27615 (15.40) where no *H. indica* has been applied. Analysis of variance indicated that cultivar had significant influence on the number of egg batches ($p<0.001$), while effect of time of EPN application was not significant ($p> 0.05$) and there was no interaction ($p>0.05$). Basmati 370 cultivar had significantly high number of egg batches than the other cultivars ($p<0.001$) (Table 1).

Percent white heads and tunneled tillers were low in all cultivars where *Heterorhabtidis indica* was applied at 3 WAT. The lowest number of whiteheads was on M27615. The highest number of percent white heads and percent tunneled tillers was in Basmati 370 where there was no application of *H. indica*. Analysis of variance showed that cultivar and time of *H. indica* application did not have any significant influence on percent white heads and percent tunneled tillers ($p>0.05$) (Table 2).

The heaviest grains were in cultivar M27608 (23.0) where *H. indica* was applied at 3 WAT. It also had the highest percentage of empty grains (27%) in absence of *H. indica* application. The lowest 1000 grains weight (19.44) was in Basmati 370 cultivar where *H. indica* was applied at 6 WAT. The results indicated that cultivar M27615 had 21.89% empty grains in the treatment where there was no *H. indica* application (Table 3).

Table 1: Effect of cultivar and time of *H. indica* application on number of egg batches at KARI-Mwea field station in 2010 short rains season

Cultivar	Mean No. of egg batches
Basmati 370	20.270
M27608	17.070
M27628	15.530
M27615	15.400
p	<0.001
S.E	3.140
CV (%)	18.400

p: Probability, SE: Standard error, CV: Coefficient of variation

Grain yield and dry weight of straw in the different cultivars were influenced by *H. indica* application at different periods. Harvest index was generally low in all the cultivars. However, it was highest in cultivar M27615 where *H. indica* was applied at 3 WAT and lowest in cultivar M27608 where there was no *H. indica* application (Table 4).

Table 2: Influence of cultivar and time of *H. indica* application on percent white heads and percent tunneled tillers at KARI-Mwea field station in 2010 short rain season

Cultivar	*H. indica* application date (WAT)	Percent white heads	Percent tunneled tillers
M27615	0	12.800	10.800
	3	9.300	8.300
	5	10.300	9.700
	6	10.300	9.700
M27628	0	14.800	12.900
	3	11.000	10.300
	5	12.300	11.300
	6	13.300	12.700
M27608	0	17.700	16.800
	3	12.500	11.500
	5	13.000	13.000
	6	17.700	15.700
BASMATI 370	0	19.200	16.700
	3	11.300	11.300
	5	12.300	12.300
	6	13.000	12.500
p		00.053	0.059
S.E		05.880	6.520
CV (%)		45.700	47.400

p: Probability, SE: Standard error, CV: Coefficient of variation

Table 3: Effect of cultivar and application time of *H. indica* on percent empty grains and 1000 grain weight at KARI Mwea field station in 2010 short rains season

Cultivar	*H. indica* application date (WAT)	Mean percent empty grains	Mean 1000 grains weight (g)
M27615	0	21.890	20.480
	3	14.330	22.050
	5	15.670	21.880
	6	16.000	21.160
M27628	0	20.330	19.560
	3	15.330	21.850
	5	17.670	21.170
	6	19.000	20.560
M27608	0	25.330	20.580
	3	17.670	23.000
	5	19.330	21.570
	6	19.670	20.020
BASMATI 370	0	22.110	19.720
	3	18.000	21.630
	5	21.000	20.820
	6	24.330	19.440
p		0.098	0.052
S.E		7.470	1.880
CV (%)		37.200	29.870

p: Probability, SE: Standard error, CV: Coefficient of variation

Table 4: Effect of cultivar and application time of *H. indica* on weight of grain, straw and harvest index KARI Mwea field station in 2010 short rains season

Cultivar	*H. indica* application date (WAT)	Mean grain yield (g m^{-2})	Mean straw dry weight (kg m^{-2})	Harvest index (%)
M27615	0	107.67	9.330	12.150
	3	128.60	9.610	25.090
	5	124.60	9.100	19.400
	6	109.60	9.300	15.040
M27628	0	97.27	8.500	10.360
	3	217.80	10.100	14.310
	5	188.00	9.130	11.310
	6	175.90	8.900	11.140
M27608	0	85.60	8.700	13.930
	3	148.40	10.400	18.090
	5	136.33	9.730	15.730
	6	132.20	8.800	15.550
BASMATI 370	0	113.68	8.930	12.260
	3	153.30	11.400	18.080
	5	146.80	8.900	16.380
	6	130.30	9.400	14.050
p		0.07	0.096	0.089
S.E		3.10	1.130	6.860
CV (%)		18.00	12.100	49.280

p: Probability, SE: Standard error, CV: Coefficient of variation

DISCUSSION

White heads and stem tunneling data indicated that there was consistently low *M. separatella* damage in plots where *Heterorhabtidis indica* was applied at three Weeks after Transplanting (WAT). High levels of damage occurred where treatment was applied at 5 and 6 WAT. These results suggest that late application of the entomopathogenic nematodes after 5 WAT may have been too late to reverse the damage already inflicted by *M. separatella*. The subsequent reduction in grain yield and increase in dry straw weight is consistent with similar findings, where the pest infestations at early stages of plant growth stage leads to production of many nodal tillers which may have few productive panicles (Nwilene *et al*., 2011). It was found in this study that although there were low levels of infestation on resistant cultivars where *Heterorhabitidis indica* was applied none of the treatments completely protected the rice crop. This finding is consistent with the reported lack of complete resistance only partial resistance in most rice genotypes to stem borers (Shafique *et al*., 2000) and contradicts the results in chapter 7 where there were no white heads in M27615. The cultivar M27615 in this study indicated partial resistance while M27608 indicated tolerance. Host plant resistance to stem borers in rice is reported to be highly variable (Litsinger *et al*., 2009). The effectiveness of combination of resistant cultivars and EPNs in control of *M. separatella* is supported by results of Thomas (1999) which showed that host plant resistance and natural enemies were able to reduce aphid populations and that the effectiveness of individual mechanisms in suppressing pest population growth rate depended on pest life history. He also showed that biological control and host plant resistance can be compatible and can combine additively or synergistically to improve pest control. It has been argued that partial resistance, like host-plant tolerance, is potentially important for host plants for it allows retention of natural enemies. In contrast, a very high level of resistance could be detrimental because it could lead to extinction of natural enemies similar to effects of insecticides leading to ecological imbalances which is contrary to the tenets of Integrated Pest Management (Sandler, 2010).

The results of low levels of infestation on resistant cultivars where *Heterorhabitidis indica* was applied is supported by results of Gassmann *et al.* (2008) which indicated that presence of entomopathogenic nematodes in refugia increased fitness cost of *Bacillus thuringiensis* resistance, indicating that their presence may slow pest adaptation to *B. thuringiensis* crops. They suggested that nematodes presence in refuges may delay resistance by pests to *B. thuringiensis* crops. Host plants can influence susceptibility of insects to pathogens (Cory and Hoover, 2006) including entomopathogenic nematodes. As such, it would be important to know the fitness and fecundity of adult moths of *M. separatella* emerging from cultivars where antibiosis and antixenosis is high. Information on fitness and fecundity of entomopathogenic nematodes infecting the larvae of *M. separatella* feeding on resistant rice varieties will also be important. These studies including the effects of these cultivars on *Trichogramma* sp. which is an important *M. separatella* egg parasitoid would shed light on the compatibility of *H. indica* and resistant rice cultivars in the management of the pest. While this study has demonstrated that an integration of entomopathogenic nematodes and resistant or tolerant cultivars can reduce *M. separatella* infestation, it also suggests that their efficacy can be improved by modifying cultural practices to allow for their integration in flooded rice cultivation.

CONCLUSION

Combination of resistant cultivars and EPNs was effective in controlling *M. separatella. Maliarpha separatella* infestation was lowest on cultivar M27615 where *Heterorhabtidis indica* was applied at three weeks after transplant date and M27608 had the highest yield despite high *M. separatella* infestation. This showed that host plant resistance and natural enemies are able to reduce *M. separatella* infestations and that biological control and host plant resistance can be compatible and can combine additively or synergistically to improve pest control.

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