EVALUATION OF TOMATO VARIETIES FOR RESISTANCE TO TOMATO LEAFMINER (*TUTA ABSOLUTA*) IN KIRINYAGA COUNTY, KENYA

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Abstract

The tomato leafminer (*Tuta absoluta*) (Meyrick) (Lepidoptera: Gelechiidae) is an invasive insect pest of tomato crops in many parts of Africa. It causes substantial economic losses to tomato crops in Kenya. Majority of farmers have resulted to intensive insecticide application that has led to development of insecticide resistance. It is therefore critical to develop sustainable management strategies that can be used in Integrated Pest Management (IPM) programmes against the tomato leaf miner. Genetic breeding against the tomato leaf miner can be promising since effective cultural control is difficult. The objective of this study was to evaluate level of resistance of popular tomato varieties to tomato leaf miner in Kirinyaga County, Kenya. The four tomato varieties Rambo F1, Pesa F1, RiograndeVF and Cal J were evaluated for resistance against *T. absoluta* under field conditions. Mwea East Sub-County is characterised by intensive tomato production, diverse cultural practises and high usage of synthetic pesticides against the tomato leaf miner. The experiment was laid out in a randomized complete block design in two locations (Kariithi and Kimbimbi), which have irrigation schemes that allows tomato production throughout the year. Presence of mines was assessed on leaves obtained from the lower, intermediate and upper canopy of tomato plants at days 20, 40, 60 and 80 after planting. Fruit damage was estimated at the end of the cropping cycle. Data was subjected to arcsin square root transformation and analysis of the level of pest damage on different tomato varieties, was done using general linear mixed effects models, performed using the lme function in the nlme package in R 3.4.2.The results of this study show that larvae mining damage on the leaves was observed in all the evaluated tomato varieties, but it was significantly higher on Pesa F1 hybrid variety ($p > 0.001$) than Riogrande VF and Cal J. Fruit damage was also significantly higher on Pesa F1 variety ($p > 0.01$) than Riogrande VF and Cal J. These results suggest that most of the commercially grown tomato varieties are susceptible to *T.absoluta* infestation. However, Riogrande VF and Cal J can be incorporated in IPM programs. Further studies need to be done for introgression of resistance genes from wild varieties into cultivated tomato varieties.

Key words*:* *larvae mining, fruit damage, Resistance, susceptible*

Introduction

Host plant resistance is one of the most effective ways to control insect population without damaging the environment (Sharma & Rodomiro, 2002). Over the years, plants have developed the ability to tolerate or resist insect pest attack (Hanley *et al*., 2007). Plants can resist insect pests via structural and chemical traits that minimize the insect pest damage or they may tolerate the damage by the pests. Plant antibiotic and antixenotic resistance prevents the insect pest from landing, attachment and feeding on the plant through emissions of herbivore induced plant volatiles (HIPVs) (Heil, 2014). Further, it can develop physical barriers such as trichomes and waxy cuticle that will prevent insect pest feeding and oviposition (Mitchell *et al*., 2016). Plant tolerance traits, on the other hand, affect the plant's photosynthetic activity and growth. It has been noted that after insect pest defoliation, some plants increased their photosynthetic rate in the remaining plant tissues (Retuerto *et al*., 2004). In other plants, tolerance was detected through delayed growth, flower and fruit production after an insect pest attack (Tiffin, 2000; Oyetunji *et al*., 2014).

Tuta absoluta management in Africa is dominated by synthetic insecticides (Mansour *et al.,* 2018). A range of synthetic insecticides are reported to be used in control of the pest but, most farmers prefer Coragen (Chlorantraniliprole 200g/l) which has been found to be the most effective against *T. absoluta* among other insecticides used in Kirinyaga County region (Nderitu *et al.,* 2018). However, increased use of synthetic insecticides has been found to result in resistant insect pest populations to the insecticides (Campos *et al.,* 2015; Roditakis *et al.,* 2018). Other methods that have been used to manage *T. absoluta* include the use of insect screens to limit infestations (Cherif *et al.,* 2013). Furthermore, sex pheromone lures and water traps have been used in monitoring and early detection of the insect pest (Cherif and Grissa-Lebdi, 2017; Machekano *et al*., 2018) while egg parasitoids (*Trichogramma spp*.) and predatory mirids (*N. tenuis*) have shown effective management of *T. absoluta* as components of IPM program in greenhouse production (Elaini, 2011; Ettaib *et al.,* 2016).

Plant resistance has proven to be a key component in integrated pest management (IPM) and when utilized it minimizes production cost and environmental pollution by reduced insecticide use (Sharma & Rodomiro, 2002). Resistance of a range of different tomato cultivars to *T. absoluta* has been reported in previous studies including; *Solanum habrochaites*, *S. Habrochaites* var.*Glabratum* and *S. Habrochaites* var. *Hirsutum, S. Pennellii* (Marta, 2015). *Solanum lycopersicum* lines (TOM-687, TOM-688, and TOM-689) have been achieved through introgression of high levels of acylsugars to confer resistance against *T. absoluta* (Maluf *et al.,* 2010). Evaluation of 11 tomato cultivars against *T. absoluta* under greenhouse conditions in Iran resulted in four distinctive classes of resistances that is; resistant, partially resistant, partially susceptible and susceptible Gharekhani and Salek-Ebrahimi (2014). Sohrabi *et al.* (2016) evaluated ten tomato varieties under field conditions in Iran against *T. absoluta*. Their results showed varieties Raha, Quintini and ES9090F1 as resistant. Six tomato varieties were also evaluated under field conditions in Iran where the highest to lowest leaf infestation rates were recorded for the Primo early,CaljN3, Petomek, Rio grande, Early urbana and Super 2270 cultivars, respectively(Azadi *et al.,* 2018). There are limited studies on host plant resistance to *T. absoluta* in Kenya. Therefore, the objective of this study was to evaluate commonly grown tomato varieties in Kirinyaga County, Kenya for their resistance against *T. absoluta* in order to explore options for an environmentally friendly and sustainable control of *T. absoluta*.

Materials and Methods

Experimental Site

The study was conducted in Mwea East Sub-County, Kirinyaga County, Kenya. Two locations Kariithi (37^{o} 23'E, 0^{o}52S) and Kimbimbi (37^{o}42'E, 0 ^{o} 15S) were selected; representing intensive tomato production, diversity of cultural practices and excessive synthetic pesticide applications due to increased *Tuta absoluta* damage. Mwea East sub-county is classified as Lowland Agro Ecological Zone (AEZ); it typically experiences bimodal rainfall with rains from March to May (long rains) and from October to December (short rains) with an annual average rainfall range of 1100 -1250 mm. The area lies between 1158m to 2000m above sea level. The site experiences a minimum and maximum temperature range of 12°C and 30°C respectively. The soils are well drained volcanic loam which are deep and moderately to highly fertile (Jaetzold *et al.,* 2006).

Plant Material and Experimental Design

Four certified tomato varieties commonly grown in Kirinyaga County were tested in this study. The varieties were: Rambo F1, Cal J (Royal Seed Company), Pesa F1 (Hygene Biotech Seeds Limited) and Riogrande VF (Easeed Company). The seeds were purchased from a seed distributor/stockiest in Wang'uru, Mwea town. The tomato seeds were planted in plastic transplant trays containing a mixture of poultry manure, soil and sand at a ratio of 3:1:1 in October 2017. The established seedlings were transplanted after three weeks to the main field. The experiment was laid out in a randomized complete block design with three replications per location. The plot size was 2m × 2m with a plant population of 16 plants per experimental unit; plant spacing within each row was 50cm (between plants) and 150cm between rows. The plants were exposed to natural infestation by the tomato leaf miner in the field.

Data Collection

Insect damage on leaves was evaluated at 20, 40, 60 and 80days after planting on six plants per plot. Sampling was done on three leaves per plant (from lower, intermediate and upper canopy) which were carefully inspected for presence/absence of mines using a scale of 1-9 where: 1 = no infestation, 2 = ≤ 25% leaf infestation, 3=25-50% leaf infestation, 4 = 50-75% leaf infestation 5 = ≥75% leaf infestation (Ayalew, 2011). In addition, the presence of mines and holes on the fruits was assessed towards the end of the cropping cycle at 100 and 120 days after transplanting. Percentage damage on the fruits by *Tuta absoluta* was calculated as follows:

$$\text{Infested fruits (\%)} = \frac{\text{Number of tomatoes with holes}}{\text{Total number of tomatoes}} \times 100$$

Data Analysis

The variability of damage across the varieties in the two locations was expressed as percentages. The percentage data was subjected to arcsin square root transformation to improve normality of model residuals. To analyze the level of *Tuta absoluta* damage on different tomato varieties we performed general linear mixed effects models (GLMM's), using the lme function in the nlme package in R 3.4.2 (R Development Core Team, 2017). Variety and site and the interaction between the two were fixed factors while leaf location nested within time was included as a random effect. To compare the different varieties, Tukey contrasts were used with the glht function in the multicomp package in R 3.4.2.

Results

Tuta absoluta Damage on Tomato Varieties

Across the two locations, *T. absoluta* leaf damage was higher in Pesa F1 than Cal J and Riogrande VF (Table 1; contrast 1, (Z= 3.82, p = 0.001); contrast 5, (Z = -2.94, p = 0.017). However, *T. absoluta* damage on Pesa F1 was not significantly different from Rambo F1 (Table 1; Contrast 4; Figure1).

Evaluation of infested fruits was similar to the leaf damage where Pesa F1 variety had significantly greater fruit damage than Cal J and Riogrande VF varieties (Table 2; contrast 1; (Z = 3.48, p= 0.003); contrast 5; (Z= -3.24, p= 0.006); Figure 2). The effect of variety furthermore depended on site, with higher infestations of Pesa F1 at Kimbimbi than at Kariithi, but the opposite pattern for the other varieties (t = 2.57, df = 221, p= 0.0108; Fig 3). *Tuta absoluta* leaf damage was significantly different on Pesa F1 at (t = 2.57; df = 221; p = 0.0108) (Figure. 3) across the two locations

Table 1: Multiple comparisons of leaf damage means as infested by *T. absoluta* using Tukey's contrasts.

Contrast	Estimate	SE	Z-Value	Pr(>|z|)
Contrast 1: Pesa F1 vs Cal J	0.049	0.013	3.819	0.001***
Contrast 2: Rambo F1 vs Cal J	0.026	0.013	1.974	0.198ns
Contrast3:Riogrande VF vs Cal J	0.011	0.013	0.879	0.816ns
Contrast 4: Rambo F1 vs Pesa F1	-0.023	0.013	-1.845	0.252ns
Contrast5: Riogrande VF vs Pesa F1	-0.038	0.013	-2.940	0.017*
Contrast6: Riogrande VF vs RamboF1	-0.014	0.013	-1.095	0.692ns

*, ** and *** indicate significance at the 5, 1 and 0.1% levels; ns, not significant

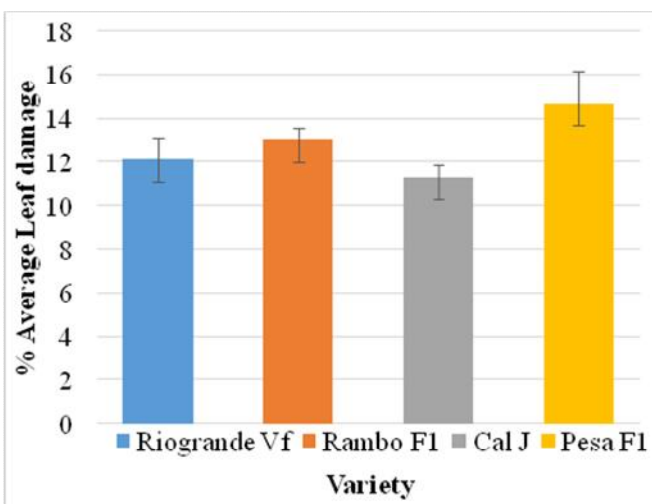

Figure 1: Percentage *T. absoluta* leaf damage across the varieties

Table 2: Multiple comparisons of fruit damage means as infested by *T. absoluta* using Tukey's contrasts

Contrast	Estimate	SE	Z-Value	Pr(>|z|)
Contrast 1: Pesa F1 versus Cal J	0.288	0.083	3.482	0.003**
Contrast 2: Rambo F1 versus Cal J	0.188	0.083	2.272	0.105ns
Contrast3:Riogrande VF versus Cal J	0.020	0.083	0.243	0.995ns
Contrast 4: Rambo F1 versus Pesa F1	-0.100	0.083	-1.210	0.620ns
Contrast5: Riogrande VF versus Pesa F1	-0.268	0.083	-3.240	0.006**
Contrast6: Riogrande VF versus Rambo F1	-0.168	0.083	-2.029	0.177ns

*, ** and *** indicate significance at the 5, 1 and 0.1% levels; ns, not significant

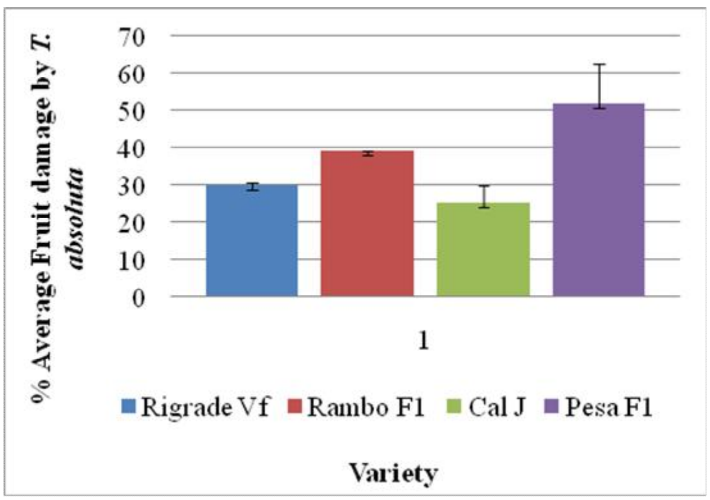

Figure 2: Percentage *T. absoluta* damage on tomato fruits

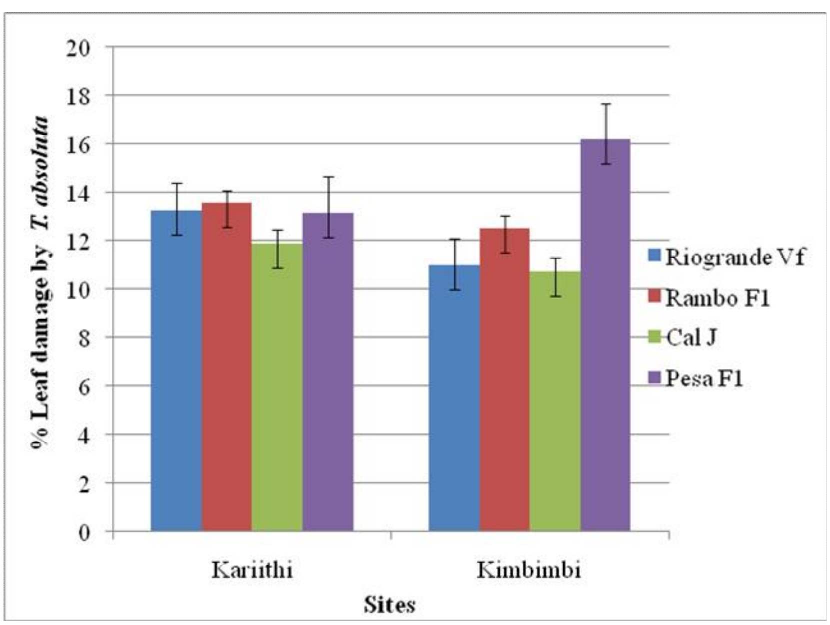

Figure 3: Percentage *T. absoluta* leaf damage across the locations

Discussion

All the four tomato varieties evaluated in this study (Pesa F1, Rambo F1, Cal J and Riogrande VF) had substantial infestations by *T absoluta*. This could be attributed by a narrow genetic diversity across the tomato varieties (Khush, 2001; Chen *et al.,* 2015). Tomato production is a key vegetable crop for horticultural expansion and development. Therefore, breeders select for desirable traits that enhance yield, quality and shelf life to increase crop productivity (Lynch, 2007). This could have led to production of modern tomato varieties with low levels of genetic diversity towards insect resistance (Khush, 2001).

The hybrid variety Pesa F1 was the most susceptible variety against *Tuta absoluta.* On the other hand, Rambo F1, Riogrande VF and Cal J did not differ significantly from each other in terms of susceptibility to *T. absoluta*. These results can be attribute to the fact that most commercial varieties have undergone intensive breeding for high yields and other fruit qualities, which may have led to genetic drift of important genes conferring pest and disease tolerance and/or resistance. In other studies, Rambo F1 was found to be highly damaged by *Tuta absoluta* at all stages of growth and development (Ndung'u, 2014).Cultivated tomato varieties have been observed to be preferential attacked by *T. absoluta*, whiteflies, and red spider mites, amongst other tomato insect and fungi pests unlike the wild tomato (Bawin, *et al*., 2014). Vita *et al.* (2016) reported high leaf area consumption by *T. absoluta* larvae on commercially cultivated tomato varieties (Fiorentino, Belle and Naomi) compared with *S. habrochaites* (P1126446) wild tomato variety.

Plant leaf volatiles play an active role in *T. absoluta* females' choice of suitable hosts for oviposition and severity of damage to the plant (Anastasios *et al.,* 2014).Compounds such as tridecan-2-one and undecan-2-one especially secreted by type VI glandular trichomes on the tomato leaves, have been found to form physical and chemical barriers for insects and pathogens (Picoaga *et al*., 2003; Oliveira *et al*., 2009). A study on attraction and oviposition of *T. absoluta* females in response to some varieties of cultivated tomatoes has been conducted. Results show that amongst other blends, monoterpenes, in particular *β*-phellandrene , limonene , and *δ*-2–carene , and the sesquiterpene(*E)*–*β*–caryophyllene and their percentages in volatiles play a major role (Proffit *et al.,* 2011). Studies done to compare oviposition preference of *T. absoluta* among both cultivated and wild tomato varieties showed mated *T. absoluta* females preferred cultivated tomato (*S. lycopersicum L.*) for egg laying rather than wild tomato (*S. habrochaites S.*) (Proffit *et al.,* 2011). This could be suggested as the reason why the evaluated varieties were susceptible to *T. absoluta*. Further, behavioral responses of *T. absoluta* to a wild (*Lycopersicon esculentum var. cerasiforme*) and cultivated tomato (*Solanum lycoperscon L.,* Rambo F1) were characterized for mediated semiochemical blends (Miano *et al.,* 2017). Results on olfacto–metric bioassays using intact tomato plants showed that mated *T. absoluta* females actively avoided the wild tomatoes but were attracted to the cultivated tomatoes (Rambo F1) (Miano *et al.,* 2017). Domesticated plants have also been found to produce large quantities of volatiles but of simpler composition compared to wild relatives (Chen *et al.,* 2015; Rowen & Kaplan, 2016).

Tuta absoluta has been found to cause substantial damage on tomato fruits both in green house and field environment (Lacordaire, 2010). Karut *et al.* (2011) reported tomato fruit losses of 11.08% and 43.33%, while Cocco *et al.* (2015) evaluated tomato fruits damaged by larvae at 27%. Allache *et al.* (2017) also reported a positive correlation between larvae number and fruit loss. In this study, *Tuta absoluta* damage on varieties showed a positive correlation for both the leaf damage and fruit damage. Sohrabi *et al.* (2017) evaluated tomato varieties for damage against *T. absoluta* and found out the number of holes on the fruit had positive and significant correlations with the number of mines on the leaf.

Host plant resistance may be influenced by environmental conditions. This resistance could be modified under interactions with the insect pest, plant and environmental conditions (Tolmay *et al.,* 1999). Environmental conditions have been found to influence *T. absoluta* abundance and reproduction. Under favorable temperatures, it can undergo 10-12 generations per year thereby increasing crop damage (EPPO, 2005). *Tuta absoluta* depends on temperature, with the minimum temperature for activity being 9°C. At 14°C, it takes 76 days while at temperatures above 20°C; it takes 24 days to complete its life cycle (Muniappan, 2013).During this study, temperatures ranged between a minimum of 16.8°C and a maximum of 32.8°C; thus the temperatures during the study period were favorable for *T. absoluta* damage. Further, Rambo F1 and Pesa F1 had a greater infestation by *T.absoluta*, though Rambo F1, Cal J and Riograde VF were not significantly different. This could have been contributed by the ability of the hybrid varieties to have intensive vegetative growth of the plants. Due to their quick growth, they were able to tolerate damage (Gharekhani and Salek-Ebrahimi, 2014).

Host plant resistance can also be expressed by a number of factors including: (i) antixenotic resistance which reduces the rate of both initial and successive insect population build up, (ii) Antibiotic resistance which decreases the rate of survival, reproduction and prolongs the generation time and (iii) tolerance where plants withstand damage from an insect population (Sharma and Rodomiro, 2002). From this study, Pesa F1 was the highly susceptible variety to *T. absoluta*. This could be attributed by the trichome density available on the leaves of these plants, although there is need for further investigation. Studies show that trichome density is one of the most important structural features known to cause insect resistance (He *et al.,* 2011). Further this phenomenon could have shifted the insect population from Cal J and Riogrande VF to Pesa F1 and Rambo F1. In other studies, it has been found that trichome density may have a direct negative influence on both larval feeding and oviposition of the insect pest (Handley *et al.,* 2005).

There was a significant interaction between the location in which the experiment was laid out and variety whereas, there was a higher *T. absoluta* damage on Pesa F1 in Kimbimbi location than in Kariithi whereas the opposite pattern was true for other varieties. This could have been attributed to the genetic vulnerability of the variety (Pesa F1), where the genetic constitution of the variety is unchanged but the biotype of the insect pest has changed and therefore the variety (Pesa F1) became more susceptible to the insect pest. Moreover, the biology of the pest and its interaction with the host plant has been found to greatly influence the epidemic potential especially with insect pest that have high mobility and reproductive rates (National Research Council, 1993).

Conclusion and Recommendation

Tuta absoluta damage was detected across the tomato varieties screened. However, Cal J and Riogrande VF were identified as promising varieties that can be utilized in backcrossing for tomato breeding programs.

This study recommends continued screening of current tomato varieties in different Agro Ecological Zones to broaden findings on *Tuta* *absoluta* resistance. Further, more studies are required to determine resistance factors associated with these varieties. Genetic variation analysis using molecular markers across the varieties will also be invaluable for the selection of insect resistant genes for a sustainable pest management.

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