

## Research Article

# Combining Host Plant Resistance, Selective Insecticides, and Biological Control Agents for Integrated Management of *Tuta absoluta*

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Combination of pest management strategies that minimize pesticide use and conserve natural enemies is important for a sustainable environment. Overreliance on synthetic insecticides in the management of *Tuta absoluta* has led to pesticide resistance leading to difficulties in managing the pest. In this regard, alternative measures need to be put in place to reduce the effects of this pest. The objective of this study was, therefore, to assess the effectiveness of host plant resistance, biological control, and selective insecticides when used in combination, in the management of *T. absoluta* in tomato production. The study was set up in a greenhouse in a completely randomized design involving two tomato varieties, an insecticide (chlorantraniliprole), and a biological control agent (*Macrolophus pygmaeus*), applied singly or in combination. Data were collected on *T. absoluta* damage from the lower, intermediate, and upper leaves. The results from this study show that a combination of insecticide with a moderately resistant variety had a significantly lower *T. absoluta* damage as compared with a susceptible variety combined with an insecticide. However, the moderately resistant variety when combined with insecticide showed no effect when the biological control agent was added. The susceptible variety significantly reduced *T. absoluta* damage when combined with the biological control agent. These results indicate that treatment combinations in insect pest management can be utilized. The present study results indicate that using a moderately resistant variety (Riogrande VF) in combination with the insecticide chlorantraniliprole (Coragen®) and a susceptible variety (Pesa F1) in combination with the biological control agent (*M. pygmaeus*) can improve *T. absoluta* management. Under good habitat management, the susceptible variety will perform equally as the moderately resistant variety due to suppression of the *T. absoluta* populations by natural enemies. These findings show the importance of environmental conservation both by enhancing natural enemy abundance and use of selective insecticide in the management of *T. absoluta* in tomato production. Combinations in this present study are likely to reduce insecticide doses, thereby reducing the cost of production and enhancing environmental compatibility with natural enemies.

## 1. Introduction

Combining control methods has been found as the best control option for highly damaging pest species [1]. Integrated pest management (IPM) tries to minimize

environmental impact of pesticides by combining various control strategies such as biological control, cultural practices, resistant varieties, and pesticides in a compatible manner to keep pests below economically injurious levels [2, 3]. Despite host plant resistance being environmentally

sound and sustainable, sometimes, high levels of resistance have been associated with low yields and undesirable quality traits for consumption [4]. This, therefore, calls for determination of management combinations/strategies that will ensure that the farmers get benefits from their produce.

Synthetic insecticides can be effective in the control of pest outbreaks. However, their often broad spectrum effects have detrimental impacts on both beneficial organisms and the environment [5]. Generally, insecticides should reduce insect pest populations to a level below economic injury rather than aim at eradication. Recently, new insecticides have been rated as selective including diamides, spinosyns, pyrimidine, azomethines, and pyridine carboxamide [1]. Effective insecticide application results in high and rapid insect mortality. Nevertheless, complete dependence on insecticides can lead to pest resistance or resurgence and is, therefore, not sustainable [6].

The integrated use of host plant resistance with insecticides has been found to reduce insecticide use and improve effectiveness of the insecticide through better coverage of plant parts, imbalanced nutrition, and toxic substances that interfere with the insect's growth and development [7]. Furthermore, biological control using natural enemies has been found useful in integrated pest management programs [8–11]. Generally, plant resistance is compatible with natural enemies [7]. However, exclusive use of plant resistance for insect pest control has its limitations. There is a need to determine if the tolerant or resistant traits have a positive or negative effect on beneficial organisms of the insect pest. Studies on tomato showed that both leaf miners and their parasitoids were deterred by leaf trichomes [12]. In other studies, the abundance of the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) on grapes was positively influenced by the presence of leaf trichomes while its prey, the European red mite *Panonychus ulmi* Koch (Trombidiformes: Tetranychidae), preferred grapes with low trichome density [13]. Trichomes were also found to deter leaf chewing insects than those feeding within the plant tissues [14]. However, some generalist predators such as omnivorous mirid bug *Dicyphus errans* Wolff (Heteroptera: Miridae, Bryocorinae) have been found to adapt to hairy plants resulting in a higher predation effectiveness [15].

Combinations of predators with selective insecticides in IPM programmes have been found to provide optimum control [16]. In addition, inclusion of natural enemies in IPM programmes has led to the reduction of *T. absoluta* Meyrick (Lepidoptera: Gelechiidae) population densities below economic thresholds [17] and reduced use of insecticides [18] and, subsequently, lowers impacts on human health and nontarget organisms.

Combining host plant resistance with selective insecticides and biological control agents might lead to reduced use of insecticides and effective control of *T. absoluta*. However, combining two control agents can result in synergism, antagonism, or additive effects [19]. Biological control with *Bacillus thuringiensis* and spinosad exhibited satisfactory results in the control of *T. absoluta* in Tunisian greenhouses [20]. Studies were conducted on the selective insecticides group diamide; chlorantraniliprole (Coragen®) showed that

it was both effective in the control of *T. absoluta* and had no significant impact on natural enemies [5, 21, 22]. In other studies, the insecticide thiacloprid (Calypso 480 SC®-Bayer crop science, Leverkusen, Germany) was the least compatible with the zoophytophagous predator *Macrolophus pygmaeus* Rambur (Heteroptera: Miridae) [23]. Also, plant resistance using a high trichome density mechanism was found to reduce *T. absoluta* population density in some tomato cultivars tested for resistance [24, 25]. Furthermore, tomato cultivars with high levels of Zingiberene were found to confer resistance to *T. absoluta* [26]. In cotton, high trichome density reduced insect pest population, but this also reduced the searching efficiency of the natural enemies [27].

Use of a single control option against insect pests is not sustainable, and therefore, selective combinations that are compatible are necessary for long-term control of *T. absoluta*. This study aimed at assessing the effectiveness of host plant resistance, biological control, and selective insecticides in combination and understanding the interaction between host plant resistance in tomato varieties and selective insecticides for the management of *T. absoluta*.

## 2. Materials and Methods

**2.1. Experimental Site.** The study was carried out under greenhouse conditions at the University of Embu (0° 30' S, 37° 27' E), located in Embu County, Kenya.

**2.2. Plant Material.** Two tomato varieties were used: Pesa F1 (susceptible) from Hygene Biotech seeds limited and Riogrande VF (moderately resistant) from East African Seed Company. All seeds were purchased from a seed distributor/stockist in Wang'uru Mwea town, Kirinyaga County. The tomato seeds were sown in plastic trays containing poultry manure, soil, and sand at a ratio of 3:1:1. They were transplanted after three weeks inside the greenhouse.

**2.3. Experimental Procedure.** The experiment was laid out in a completely randomized design with three replications. Seedlings were transplanted in 15 L buckets. The experimental unit was made up of 16 buckets with a total of 288 buckets for the whole experiment.

The treatments included

- (i) Moderately resistant variety (alone) (MR)
- (ii) Susceptible variety (alone) (S)
- (iii) Moderately resistant variety + insecticide + biocontrol agent (MR + I + BCA)
- (iv) Moderately resistant variety + insecticide (MR + I)
- (v) Susceptible variety + insecticide + biocontrol agent (S + I + BCA)
- (vi) Susceptible variety + insecticide (S + I)

Chlorantraniliprole (Coragen®-Dupont Crop Protection, Wilmington, DE, USA) was used in this experiment since it is the most widely used selective insecticide against *T.*

*absoluta* in Kirinyaga County [28]. *Tuta absoluta* introduction was performed two weeks after transplanting, and the populations were allowed to establish for two weeks before insecticide application. Insecticide application with Coragen® was performed at the manufacturer's recommended field doses (0.25 ml/L of water) every two weeks until harvest. A BCA *M. pygmaeus* (a zoophytophagus mirid bug) was used. The BCA was provided by Koppert Biological Systems Kenya Ltd. The adult predators were introduced 12 hours before selective insecticide spray at a rate of 10 adults per plant [29].

**2.4. *Tuta absoluta* Rearing.** *Tuta absoluta* larvae were collected from infested tomato fields in Mwea East Subcounty, Kirinyaga County, which lies between latitudes 0° 37'S and 0° 45'S and between longitudes 37° 14'E and 37° 26'E. Leaves infested with larvae were collected and transferred to cages measuring 45 × 45 × 55 cm and kept at a constant temperature of 25 ± 1°C and relative humidity of 65% [30]. The larvae were fed with tomato leaves from plants cultivated under greenhouse conditions without any insecticide treatment. A 10% honey solution was provided in preparation for the emergence of the adults [31]. *Tuta absoluta* last instar larvae were then collected and stored in jars with a 0.75 mm mesh screen for aeration containing honey solution to allow them to pupate and emerge as adults. They were, then, released in the greenhouse at a rate of 53 *T. absoluta* adults per 16 plants according to [32].

**2.5. Data Collection.** Insect damage on the plants was assessed after predator introduction and insecticide application. Data were collected on four plants per experimental unit. Sampling was performed on three leaves per plant from the lower leaves (above ground leaves), intermediate leaves (three middle leaves between third and fourth fruit trusses), and from the upper canopy (three leaves selected from the upper third of the plant) which were carefully inspected for the presence/absence of mines using a scoring scale of 1–5, where 1- no infestation, 2- 0-25% leaf infestation, 3- 25-50% leaf infestation, 4- 50-75% leaf infestation, and 5- ≥75% -100% leaf infestation [33].

**2.6. Data Analysis.** The variability of damage across the varieties was expressed as proportions. The proportion data were subjected to arcsin square root transformation to improve the normality of model residuals. To analyze the data, general linear mixed effects models (GLMMs) using the lme function in the nlme package in R 3.4.2 were performed. Two separate analyses were conducted; in the first, all six treatments were tested as one factor, while in the second, the BCA was removed, and the effect of variety, insecticide application, and the interaction between the two was tested. In both analyses, the random effect was leaf location nested within time. Multiple comparisons of means using Tukey contrasts was performed to separate between the treatments in the first test using the glht function in the multiple comparison package in R 3.4.2 [34].

### 3. Results

**3.1. Comparative Efficiency of Host Plant Resistance Combined with an Insecticide and Biological Control Agent against *T. absoluta*.** Performance of the moderately resistant variety (MR) alone compared with MR + I differed significantly in the reduction of *T. absoluta* damage, where the MR + I had a positive and improved control of the insect pest damage (Table 1; contrast 6;  $Z = 3.861$ ,  $P = 0.002$ ) when a BCA was added to the combination of MR + I. The comparison of MR + I and MR + I + BCA showed no significant difference in *T. absoluta* damage (Table 1; contrast 8;  $Z = -2.246$ ,  $P = 0.216$ ). The comparison of MR alone with MR + I + BCA showed a positive and significant reduction in *T. absoluta* damage in the combination (Table 1; contrast 11;  $Z = -6.107$ ,  $P = 0.001$ ).

There was no significant difference observed when the susceptible variety alone (S) was compared with S + I (Table 1; contrast 14;  $Z = -0.695$ ,  $P = 0.983$ ) indicating no effect of the insecticide on the susceptible variety. When the BCA was added, the performance of S + I + BCA compared to S + I showed a positive and significant reduction of *T. absoluta* damage (Table 1; contrast 3;  $Z = 3.578$ ,  $P = 0.005$ ). This indicates a positive response of the BCA on the susceptible variety. Similarly, the comparison of the susceptible variety (S) alone with S + I + BCA showed a positive and significant difference in *T. absoluta* damage where the BCA was involved (Table 1; contrast 5;  $Z = 2.883$ ,  $P = 0.045$ ).

A significant reduction in *T. absoluta* damage was observed when S + I was compared with MR + I (Table 1; contrast 7;  $Z = 3.127$ ,  $P = 0.002$ ). The insecticide had a positive effect on the moderately resistant variety and a negative effect on the susceptible variety. After addition of the BCA on both combinations, that is, the comparison between S + I + BCA and MR + I + BCA, there were no significant differences observed (Table 1; contrast 4;  $Z = -1.796$ ,  $P = 0.469$ ). Thus, the BCA was equally effective on the susceptible variety as the insecticide was effective on the moderately resistant variety.

In general, the combinations MR + I + BCA, MR + I and S + I + BCA were not significantly different in *T. absoluta* management (Figure 1).

Different letters above the bars indicate significant differences.

The treatments are as follows:

- (i) Moderately resistant variety (Riogrande VF) (alone) (MR)
- (ii) Susceptible variety (Pesa F1) (alone) (S)
- (iii) Moderately resistant (Riogrande VF) + insecticide (Coragen®) + biocontrol agent (*Macrolophus pygmaeus*) (MR + I + BCA)
- (iv) Moderately resistant variety (Riogrande VF) + Insecticide (Coragen®) (MR + I)
- (v) Susceptible variety (Pesa F1) + insecticide (Coragen®) + biocontrol agent (*M. pygmaeus*) (S + I + BCA)
- (vi) Susceptible variety (Pesa F1) + insecticide (Coragen®) (S + I)

TABLE 1: Multiple comparisons of treatment combinations leaf damage means, as infested by *Tuta absoluta* using the Tukey contrast matrix.

Contrast	Estimate	Se	Z value	Pr (> z )
Contrast 1: MR + I vs. S + I + BCA	0.015	0.034	0.451	0.998
Contrast 2: MR vs. S + I + BCA	0.148	0.034	4.311	0.001***
Contrast 3: S + I vs. S + I + BCA	0.123	0.034	3.578	0.005**
Contrast 4: MR + I + BCA vs. S + I + BCA	-0.062	0.034	-1.796	0.469
Contrast 5: S vs. S + I + BCA	0.099	0.034	2.883	0.045*
Contrast 6: MR vs. MR + I	0.132	0.034	3.861	0.002**
Contrast 7: S + I vs. MR + I	0.107	0.034	3.127	0.022*
Contrast 8: MR + I + BCA vs. MR + I	-0.077	0.034	-2.246	0.216
Contrast 9: S vs. MR + I	0.083	0.034	2.432	0.145
Contrast 10: S + I vs. MR	-0.025	0.034	-0.733	0.977
Contrast 11: MR + I + BCA vs. MR	-0.209	0.034	-6.107	0.001***
Contrast 12: S vs. MR	0.049	0.034	-1.428	0.709
Contrast 13: MR + I + BCA vs. S + I	-0.185	0.034	-5.374	0.001***
Contrast 14: S vs. S + I	-0.024	0.034	-0.695	0.983
Contrast 15: S vs. MR + I + BCA	0.161	0.034	4.679	0.001***

\*, \*\* and \*\*\* indicate significance at the 5, 1, and 0.1% levels; ns = not significant. MR = moderately resistant variety (Riogrande VF); S = susceptible variety (Pesa F1); I = insecticide (Coragen®); BCA = biocontrol agent (*Macrolophus pygmaeus*).

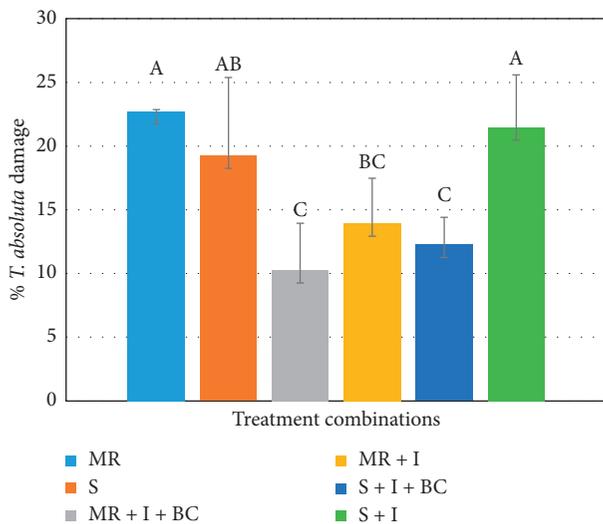


FIGURE 1: Effect of different treatment combinations on *T. absoluta* damage.

**3.2. Interaction between the Host Plant Resistance and Insecticide.** In the second analysis, the effect of variety, insecticide application, and the interaction between the two was assessed. The results show that the response to the insecticide was dependent on the variety, where *T. absoluta* damage was reduced on the moderately resistant variety (Riogrande VF) but not on the susceptible variety (Pesa F1) ( $t = 3.23$ ,  $DF = 228$   $P = 0.0014$ ).

## 4. Discussion

**4.1. *Tuta absoluta* Mining Damage as Influenced by Treatment Combinations of the Host Plant Resistance and Insecticide.** The results from this study show that a combination of the insecticide with a moderately resistant variety (MR + I) reduced *T. absoluta* damage as compared with the susceptible variety combined with the insecticide (S + I). This implies that insecticide susceptibility of *T. absoluta* is affected by the level of

resistance of the tomato plants. In a comparable study, insecticide susceptibility of the white backed plant hopper and brown plant hopper *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) was affected by the level of resistance of the rice cultivars. Reared plant hoppers on moderately resistant rice varieties after insecticide spray had lower  $LD_{50}$  compared to when placed on the susceptible varieties [35, 36]. This could also have been attributed by the plant nutrition and toxic substances from the plant due to the antibiosis component of host plant resistance, thereby causing the insect pest to be more susceptible to the insecticide [36]. Similar results were found when chemical control (Carbo-furan) was used in combination with Sorghum-resistant lines against shoot fly, *Atherigona soccata* Rondani (Diptera: Muscidae) [37]. In addition, moderate levels of resistance have been found to be effective when used in combination with insecticides keeping the pest population below economic threshold levels [7]. Several researchers have reported enhanced susceptibility of the insect pest to insecticides on resistant and partially resistant cultivars that reduced the amount of pesticide required for effective control [38, 39].

**4.2. *Tuta absoluta* Mining Damage as Influenced by Treatment Combinations of the Host Plant Resistance, Insecticide, and Biological Control Agent.** *Macrolophus pygmaeus* has been shown to be an effective predator against *T. absoluta* under greenhouse conditions. It is able to keep pest numbers below economic threshold levels when chemicals are reduced [40]. In the current study, there was a significant reduction in *T. absoluta* damage when the insecticide and BCA were combined with moderately resistant variety or susceptible variety as opposed to the varieties alone. This could be attributed to the compatibility of the insecticide and the predator, thereby being more effective. In other studies, mirid predators were found to be more effective when combined with selective insecticide applications [41]. Furthermore, there has been a low nontarget effect on *M. pygmaeus* by chlorantraniliprole [21].

Adding the BCA improved control of *T. absoluta* on the susceptible variety that had been sprayed with an insecticide, but adding the BCA had no additional effect on the moderately resistant variety that had been sprayed. This suggests that there was a positive response of the BCA on the susceptible variety but no effect on the moderately resistant variety. This could be due to herbivore-induced plant volatiles (HIPVs) that have been found to play a crucial role in signalling specific information for parasitoids regarding the status of herbivores and their natural enemies [42]. The quantity of production is a reflection of the level of insect damage, and it determines the level of attractiveness to predators and parasitoids. In other studies, plants attacked by *Pieris rapae* (L.) (Lepidoptera: Pieridae) induced increased production of the jasmonic acid volatiles, and this attracted *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) [43, 44]. Also, this could be attributed to the fact that most predators work best when their prey is of good quality. Studies have shown that predator and parasitoid fitness was affected by the prey feeding on plants that produced toxins that reduced the host size [45]. In other studies, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) feeding on resistant maize plant was less susceptible to its natural enemy because of its low quality and reduced growth and vigor; this led to low suppression of the pest [42].

*Macrolophus pygmaeus* is a zoophytophagous predator, and therefore, when there is a low prey level, it engages in omnivory supplementing their diet with plant resources [46]. In the present study, there was no effect of the BCA on the moderately resistant variety. This could have been caused by the low prey densities due to suppressed populations of the insect pest. Good-quality plant hosts for omnivorous natural enemies were found to provide a good relationship between the plant and biocontrol [42, 47]. Furthermore, recent studies show that *M. Pygmaeus* responds to volatiles emitted by plants with prey, but not to volatiles emitted directly by prey [48]. Also, *M. Pygmaeus* has olfactory cues that are able to differentiate tomato plants infested by *T. absoluta* and those not infested [49].

#### 4.3. Interaction of Host Plant Resistance and Insecticides.

In the second analyses, chlorantraniliprole (Coragen®) interaction with the moderately resistant variety (Riogrande VF) showed a higher efficacy against *T. absoluta*. This could have been attributed to chemical compounds produced by the plant that affect insect growth and development, thereby increasing the insect susceptibility to the insecticide. In addition, this could be attributed to the antibiosis component of resistance, which affects population increase of the insect pest, thereby maintaining its population below economic threshold levels [7]. In other studies, moderately resistant varieties in combination with insecticides were found to reduce pest numbers. Efficacy of insecticides against stem borer *Chilopartellus* sp. Swinhoe (Lepidoptera: Crambidae) and *Busseolla fusca* Fuller (Lepidoptera: Noctuidae) on susceptible and resistant lines of sorghum showed significantly greater efficacy on resistant lines [50]. Resistant plants have also been found to have negative effects on the

insect's body size and vigor, leading to stress that can increase the effectiveness of the pesticide [51]. This reaction could also have been due to antixenosis where the insect pest feeding and oviposition were negatively affected by the morphological traits including trichome density of the tomato plants; hence, they become easy targets to the insecticide. For example, cotton boll weevils were found to be suppressed by the rolled, twisted open bract cotton than on the flat bract cotton [52].

Chlorantraniliprole (Coragen®) is an insecticide that has been tested for efficacy against *T. absoluta* and has been found to have a minimal impact on natural enemies [5]. Moderately resistant varieties in combination with insecticides were found to reduce insect resurgence, reduce insecticide concentrations, conserve natural enemies, and preserve the environment [7]. The susceptible variety Pesa F1 in combination with chlorantraniliprole (Coragen®) showed a negative interaction effect indicating disagreeing results. In fact, after spraying an insecticide against an insect pest, it is ironical for the pest numbers and damage to increase. Plant resistance interactions with insecticides have been found to yield conflicting results in the previous studies [53, 54]. Variation in results was mainly due to differences in the insect feeding rate, insect pest behaviour, and nutritional requirements on different host plants [53]. Synthetic insecticides ranging from organophosphates and pyrethroids have been found to cause insect pest resurgence [35]. However, some selective insecticides such as imidacloprid were found to stimulate fecundity of the rice yellow borer *Tryporyza icertulas* Walker (Lepidoptera: pyralidae) [55] and two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) [56]. The inherent genetic makeup of the plant could also have attributed to the reaction of the insecticide. Studies conducted on the brown plant hopper (*N. lugens*) showed an amplified resurgence in the populations of the pest on susceptible rice cultivars than resistant cultivars after spraying synthetic pyrethroid, deltamethrin [35].

Some selective insecticides including biological fungicide (jinggaanmycin) used to control rice brown plant hopper were found to increase the insect's reproductive rate [57]. Also, reproductive stimulation caused by insecticides was more evident on susceptible rice varieties [36]. Furthermore, studies conducted to determine the effect of insecticides on crude fat and soluble sugar content in third and fourth instar nymphs and adults of the brown plant hopper (*N. lugens*) found that there was an increase in these components in insects feeding on susceptible cultivars than on resistant cultivars, thus providing the insects with more energy to fly [36].

In recent studies, a cross resistance of the selective insecticide chlorantraniliprole was detected in Brazilian and Italian populations of *T. absoluta* [58, 59]. This was linked to the product usage abuse, when on-farm interviews were taken where farmers used the insecticide more often than the prescribed manufacturer's application rate of a maximum of two applications per crop per year. These findings are similar to previous study findings where farmers in Kirinyaga consistently use coragen® chlorantraniliprole to control *T. absoluta*, and this may accelerate resistance development of the insect pest [28].

Loss of susceptibility of the tomato leaf miner to different insecticides was found to be mediated by enzymatic activities. The enzyme glutathione-S-transferase (GST) facilitated 1.5-fold higher resistance of *T. absoluta* against insecticides including chlorantraniliprole [60] *Tuta absoluta* production of the esterase (EST) enzyme had a detoxifying activity against the insecticide. In this present study, *T. absoluta* populations could have produced detoxifying enzymes against the insecticide on the susceptible variety leading to an increase in *T. absoluta* damage on the susceptible variety after insecticide spray. However, the production levels of these detoxifying enzymes need further investigation on their ability to be influenced by the plant resistance levels.

## 5. Conclusions

Combination of moderately resistant varieties with BCA and selective insecticides can be utilized in the effective integrated management of *T. absoluta*. Combined application of insect pest control can be utilized using the moderately resistant variety (Riogrande VF and chlorantraniliprole (Coragen®)). The biological control agent (*Macrolophus pygmaeus*) in combination with the susceptible variety (Pesa F1) showed considerable control against *T. absoluta*, and therefore, its interaction is likely to reduce insecticide doses, thereby reducing the cost of production and enhancing environmental compatibility with natural enemies.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest in relation to the publication of this research paper.

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