TOMATO VARIETAL AND INSECTICIDAL SCREENING AGAINST GREENHOUSE WHITEFLY *Trialeurodes vaporariorum* WESTWOOD UNDER POLY-HOUSE CONDITION

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ABSTRACT

Greenhouse whitefly (GHW) (*Trialeurodes vaporariarum* Westwood) is a serious vector pest of tomato viral diseases. Eight commercial varieties including Pusa Ruby as a check were subjected to varietal screening tests. Similarly, screening of six insecticides against GHW on the most susceptible variety, Pusa Ruby was carried out. Both the experiments were carried out in 2020 and 2021 under poly house condition at Khumaltar, Lalitpur, Nepal. Check variety Pusa Ruby proved to be the most susceptible for GHW as it harbored the highest number of both nymphs ($F_{7, 16} = 7.81$, $P < 0.01$) and adults ($F_{7, 16} = 10.77$, $P < 0.01$). In insecticidal screening tests, all the potential insecticides; neemex, *Verticillium lecanii*, imidacloprid, spiromesifen, nitenpyram and thiamethoxam were found equally and highly significantly effective against the GHW as compared to the control during both years. This study suggested to consider the tested insecticides of their commercial formulation and claimed application doses for the management of GHW in tomato plants.

Key words : Pest management, resistant, whitefly, varietal screening, vector

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and widely grown vegetable crops of both tropics and subtropics of the world. It is considered to be fairly high in nutritional contents, especially the vitamins A and C, (Rickman et al., 2007), having antioxidant properties Serio et al., 2005), and having high cash value after a potential value-added processing (Goswami et al., 2019; Shashikalabati et al., 2016). Recently, more emphasis has been given on tomato production not only as a source of vitamins, but also as a source of income and entrepreneurship development (Schreinemachers et al., 2018). Among the different causes of low tomato yields, insect vector mediated viral diseases are the major ones (Varela, 1995). *Trialeurodes vaporariarum*, Greenhouse whitefly (GHW), is among the top-rated vector whiteflies which transmits tomato yellow leaf curl virus (TYLCV) with recorded yield loss of 93.95% (Gupta, 2000). Similarly it vectors some important virus genera like *Begomovirus*, *Carlavirus*, *Crinivirus*, *Ipomovirus*, *Polerovirus* and *Torradovirus*. These viruses are transmitted in a propagative-persistent mode (Acharya et al., 2021).

Use of chemical pesticides still remains the most popular strategy amongst the tomato growers for GHW management. However, the increasing trend of GHW infestation (Kashyap et al., 2016) indicates the inefficacy of current chemical pesticide based management of this pest (Arneman et al., 2019). Moreover, the pest is champion in developing resistance against many insecticides (Sharma et al., 2012) leading into the repeated sprays in higher doses and volumes which eventually results into the public health and environmental hazards (Sharma et al., 2013). The close relative of GHW, *Bemisia tabaci* (Genn.) is also reported to have resistant strains (Horowitz et al., 2020). In current day world, an insecticidal residue in food is another appealing issue.

In these regards, exploration of host plant resistance coupled with efficient newer insecticides could be the approaches to incorporate into its integrated management. So, this study was designed to firstly investigate the resistant tomato varieties against GHW and secondly, to identify the proper insecticides in poly-house experiments.

MATERIALS AND METHODS

Experiment 1: Varietal screening

Collection and identification of GHW

To collect the GHWs, tomato growing plastic-houses were visited and the adults were collected with the help of a locally made prototype aspirator. The GHWs were then transferred into a top-ventilated (Ø = 5 cm) plastic boxes (25 cm × 15 cm × 10 cm) bedded with 2-3 fresh tomato leaves and were transported to the laboratory of National Entomology Research Center (NERC), Khumaltar, Lalitpur. Once settled on leaves, the whiteflies were counted gently and were transferred to the mass rearing cages having kidney bean plants, after proper identification.

The whiteflies were identified to be the GHWs after expert consultation and a morphological comparison with published literatures (Patel et al., 2022; Hill, 1987). Accordingly in brief, the GHW adults were identified based on the resting pattern of wing which resembled trapezoidal parallel to the resting ground in contrast to those of *B. tabaci*, the wings of which make roof-like tilts making some angle with the resting surface. The GHW forewings curved anteriorly and rounded distally while those of *B. tabaci* forewings are straight at the anterior margin and round distally.

Mass rearing of GHW

Mass rearing of GHWs on kidney bean (variety PDR 14) was done in laboratory condition (25 ± 5 °C, 70-75% RH and 14:10 h of light and day hours) of NERC, Khumaltar (27°39’19”N, 85°19’34”E, 1330 m asl) following the methodology of Rudra Gouda et al. (2022). Initially, the kidney bean seeds were water-soaked for 24h and sown two in a plastic bucket (base Ø = 10 cm and top Ø = 16 cm, h= 16 cm) having moist cotton substrate (1/3rd of the bucket) at the bottom. The cotton substrate were drenched ad lib to maintain the moisture requirement. At 25th day of sowing, the kidney beans acquired a V-5 stage (five leaved vegetative stage) exactly when the colony of 200 mix-sex-age adults collected as above were released after introducing the plants into an insect rearing cage (l = 50 cm × b = 30 cm × h = 75 cm) for mass multiplication.

Selection and growing of tomato varieties

Five commercial Nepali tomato varieties (Dalila, Samjhana, Srijana, BL-410 and Tara) plus a susceptible one, Pusa Ruby and a locally popular variety Lapsi Gede (in total seven varieties) were selected for varietal screening against GHW under poly-house condition (30 ± 2 °C, 70-75% RH). All the varieties were collected locally from Agrovets at Kathmandu valley. The tomatoes were grown under screen house nursery for 25 days which were then transplanted to the experimental plots under poly-house. Ten seedlings of each varieties were transplanted in two rows (60 cm apart) each planted at a spacing of 60 cm. The experiment was conducted using a Randomized Complete Block Design (RCBD) with four replications. All the intercultural operations followed were as per the guideline to produce tomatoes under protected structures (Kafle, 2019).

Release of GHWs into the tomato crop

The tomatoes grown under poly-house condition at NERC, Khumaltar (as mentioned above) were introduced twice (once at 15th day and another at 30th days after transplantation) with a total of 2000 (in total 4000) mix-sex-aged GHW adults reared as above. During the GHW release, there were no other insect pest infestation on the experimental tomato plants and pest management measures were not applied during the entire experimental period i.e. spring-summer 2020.

Data recording and analysis

The counts of nymphal and adult GHW on two upper, two middle and two lower leaves of five randomly selected plants of each of the varieties were recorded starting from 30 days after transplantation (DAT) through to the 80 DAT. Average data were then converted into number of the GHWs per plant. The count data (x) were subjected to square root transformation of x+1 before one-way analysis of variances. The means were separated by using Tukey's HSD (Honestly Significant Difference) test setting α-value at 5%.

Experiment 2: Insecticidal screening against GHWs

Growing susceptible tomato variety and infesting with GHWs

The most susceptible tomato variety (Pusa Ruby) identified from Experiment 1 and also previously proved to be susceptible to *B. tabaci* by Ghosh (2020) was selected for insecticidal screening test under poly-house condition (30 ± 2 °C, 70-75% RH) during 2021 and 2022. Thirty-day old seedlings (grown under protected poly-house condition) were transplanted under the experimental poly-house at the cropping geometry of 60 cm plant to plant and 60 cm row to row. Five seedlings in a row and two rows for each insecticidal treatment were allocated while spraying the insecticides. Four continuous blocking for random allotment of treatment 1 -7 (Table 1) were done so as to satisfy the conditions for RCBD. Two-time releases of the GHW adults were done as explained in Experiment 1 i.e. varietal screening.

Selection and sprays of insecticides

The selection of insecticides was based on the reviews made by Kashyap et al. (2016) which are popular among the tomato growers and preferably recommended by the technicians to control *B. tabaci*. The insecticides include microbial toxins, botanicals and chemical insecticides as listed in Table 1.

Table 1. Selected microbial toxins, botanicals and chemical insecticides used in the experiment

Trade Name	Common name	Nature	Application rate
Varunastra 2.0% A.S	*Verticilium leucani*	Microbial toxin	5 ml/ L of water
Neemactin 0.15% EC	Azadirachtin	Botanicals	5 ml/ L of water
Confeder 17.8% SL	Imidacloprid	Synthetic chemical	0.5 ml/ L of water
Oberon 22.9% SC	Spiromecifen	Synthetic chemical	1 ml/ L of water
King Guard 10% SL	Nitenpyram	Synthetic chemical	0.5 ml/ L of water
Actara 25% WG	Thiomethoxame	Synthetic chemical	0.5 g/ L of water

Sprays were done starting from 45 DAT for three times at an interval of 10 days using a Knapsack sprayer (16 L capacity) at the application rate explained in Table1.

Data recording and analysis

Pre-treatment counts of the GHW nymphs and adults were recorded at 42 DAT, before three days of the start of the treatments. Post-treatment counts after every sprays were recorded 10 days after the sprays and the pre-treatment counts three days before the sprays. Counts of the nymphs and adults were done from two leaves (and averaged) of three strata (top, middle and lower) of five plants from every experimental plots and were presented as count (x) per five tomato plants.

The count data (x) were then square root $\sqrt{(x+1)}$ transformed and subjected to one-way ANOVA. Means were separated using Tukey's HSD tests at α=5% using RStudio (Version R-4.2.0).

RESULTS AND DISCUSSION

Varietal screening

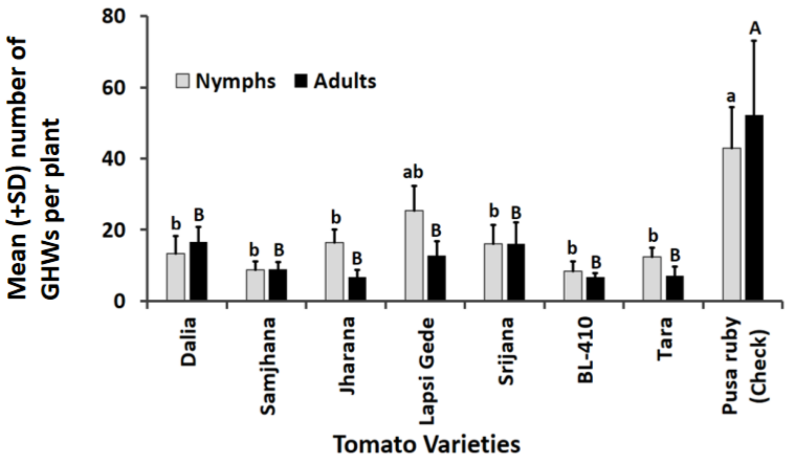

Fig. 1. Nymphs and adult counts of GHWs on tomato varieties subjected to varietal screening test 2020. Alphabetical dissimilarities above Mean + SD bars represent for statistical differences among tomato varieties in terms of GHWs infestation (small cases for nymphs and capital cases for adult GHWs) at 5% level of significance.

In the varietal screening tests, the number of GHW nymphs per plant varied significantly ($F_{7,\ 16}$ = 7.78, P < 0.001); the highest number of nymphs were recorded on the check variety (Pusa Ruby) which is highly susceptible to the GHW infestation.

In case of adult GHWs per plant, significantly the highest numbers were recorded again in the check variety, Pusa Rruby followed by Lapsi Gede ($F_{7,\ 16}$ = 14.64, P < 0.001). Rest of the varieties was equally tolerant to the pest.

The susceptibility of Pusa Ruby to GHW could equivalently be inferred to those of Ghosh (2020) where they used this variety as a susceptible host substrate while evaluating the biopesticides against *B. tabaci*. In this report, Lapsi Gede, a popular local variety and experienced to be GHW-resistant is seemed to harbor higher number of adult and nymphal GHW. Rest of the varieties except Pusa Ruby proved to be resistant against GHW which could be the sources of host plant resistance (HPR) while adopting integrated management program (IPM) against GHWs. Globally, HPR has been proved to be the foremost tactic of IPM. Tomato genotypes tested in this experiment also exhibited varied degree of resistance against the GHW as depicted by the lower number of nymphs and adults. As an indispensable IPM tactic, HPR to insect pests of tomato is attributed to the expression of resistant-related traits that either block the feeding or hamper the growth of pest or tolerates the damage (Stout et al., 2018). The HPR tactic is compatible to other pest management alternatives which also promote environmental protection by contributing to reduced level of the harmful insecticidal sprays (Wilde, 2002). In tomatoes, HPR is mainly attributed to four non-glandular (interfering movement of the pest insects) and four glandular trichomes (biosynthesizing the directly insect-toxifying plant secondary metabolites like acylsugars, methyl ketones, and phenolics) (Pal et al., 2021; Kennedy, 2003). It surely demands further studies to identify the major resistant-contributing factors, either physical or physiological in these tomato varieties so that these genetic resources could be used as GHW management alternatives.

Identification of effective insecticides for GHW management

First year (2021) experiment

Upper leaves: The adult GHW counts on upper leaves after 10 days of three spray schedules are presented in Fig. 2 (top). Before the first spray, the treatments were not significantly different ($F_{6,\ 14}$ = 2.05, P = 0.126). However, after 10 days of the first spray, the adult counts per five leaves of a plant different significantly ($F_{6,14}$ = 34.21, P < 0.001), the highest count found in control (water only) treated plants and the lowest in nytenpyram treated plants which was statistically at par with the treatments of all microbial, botanical and chemical insecticides. After 10 days of second spray, the white flies count were statistic ally similar in the plants treated with the tested chemicals which were significantly lower as compared to the water only control treatment ($F_{6,\ 14}$ = 400.17, P < 0.001). Similar effectiveness was observed after 10 days of the third spray ($F_{6,\ 14}$ = 158.51, P < 0.001).

Middle leaves: Though the initial adult GHW population on middle leaves was higher in all the treatments, there were some marginal differences ($F_{6,\ 14}$ = 2.76, P = 0.055) among the treatments (Fig. 2 middle). The adult GHW counts after 10 days of the first spray ($F_{6,\ 14}$ = 33.34, P <0.001), second spray ($F_{6,\ 14}$ = 113.37, P < 0.001) and third spray ($F_{6,\ 14}$ = 41.71, P = 0.001) also differed significantly as of the result exhibited in upper leaves of the susceptible tomato var. Pusa Ruby. Statistically the lowest numbers of the GHWs were observed on plants sprayed with nitenpyram and the highest on the water treated plants. However, after 10 days of the last spray, the treatments acted equally in reducing the GHW population in comparison to the control plants.

Lower leaves: Before sprays, the GHW population differed significantly among the treatments ($F_{6, 14}$ = 10.32, P <0.001) though they were in plenty numbers. After 10 days of the first spray, the adult GHW counts on lower leaves differed highly significantly ($F_{6, 14}$ = 135.78, P <0.001), the lowest count recorded on the nitenpyram followed by the rest of the treatments in comparison to the control (water spray). Similar results were observed for the counts on middle leaves ($F_{6, 14}$ = 20.23, P < 0.001) after second spray and so after the final spray ($F_{6,14}$ = 129.34, P < 0.001) (Fig. 2 bottom).

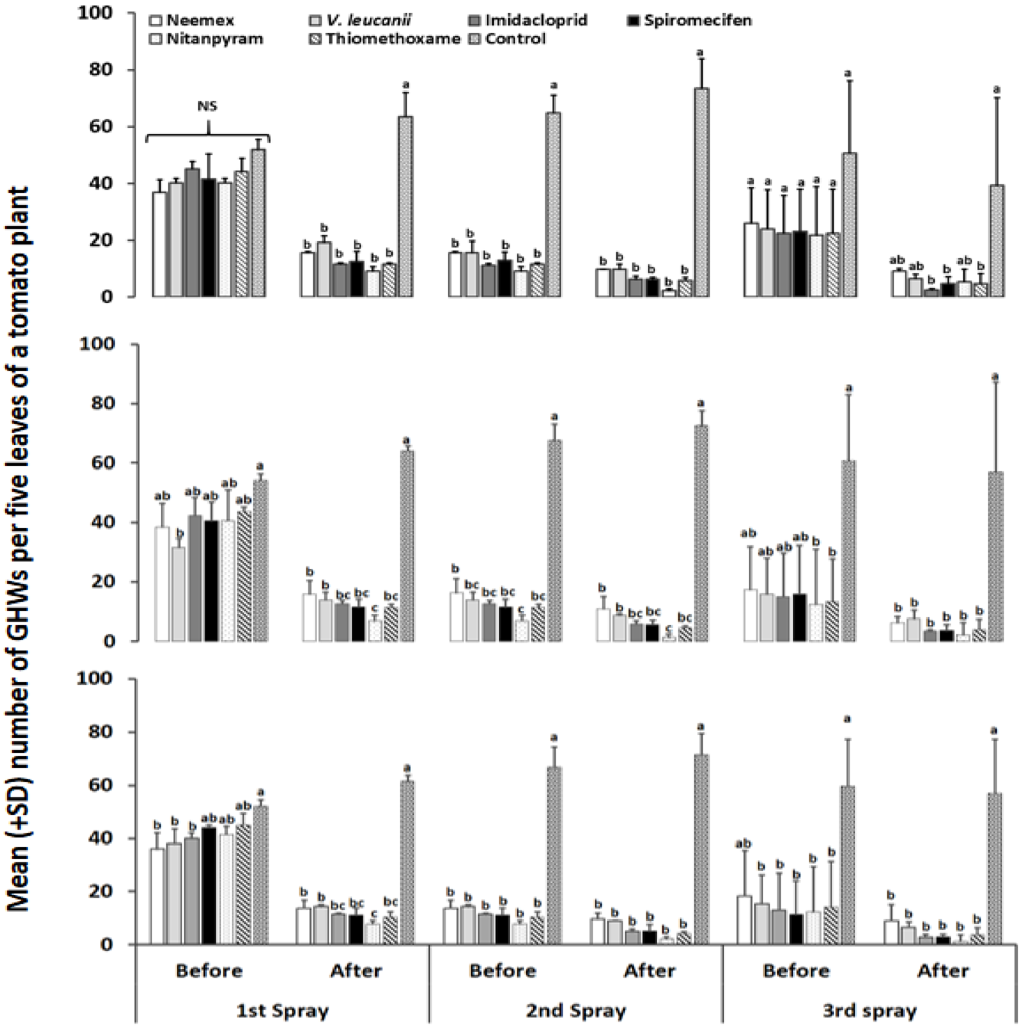

Fig. 2. Mean (+SD) number of GHWs in upper, middle and lower canopy of a tomato plant (top-bottom) before and after spray treatment with insecticides tested in the experiment during 2021 under protected structure at Khumaltar, Lalitpur, Nepal. The differences in lower cases above the bars represent for statistical differences of the mean as separated by Tukey's HSD Test at 5% level of significance.

Second year (2022) experiment

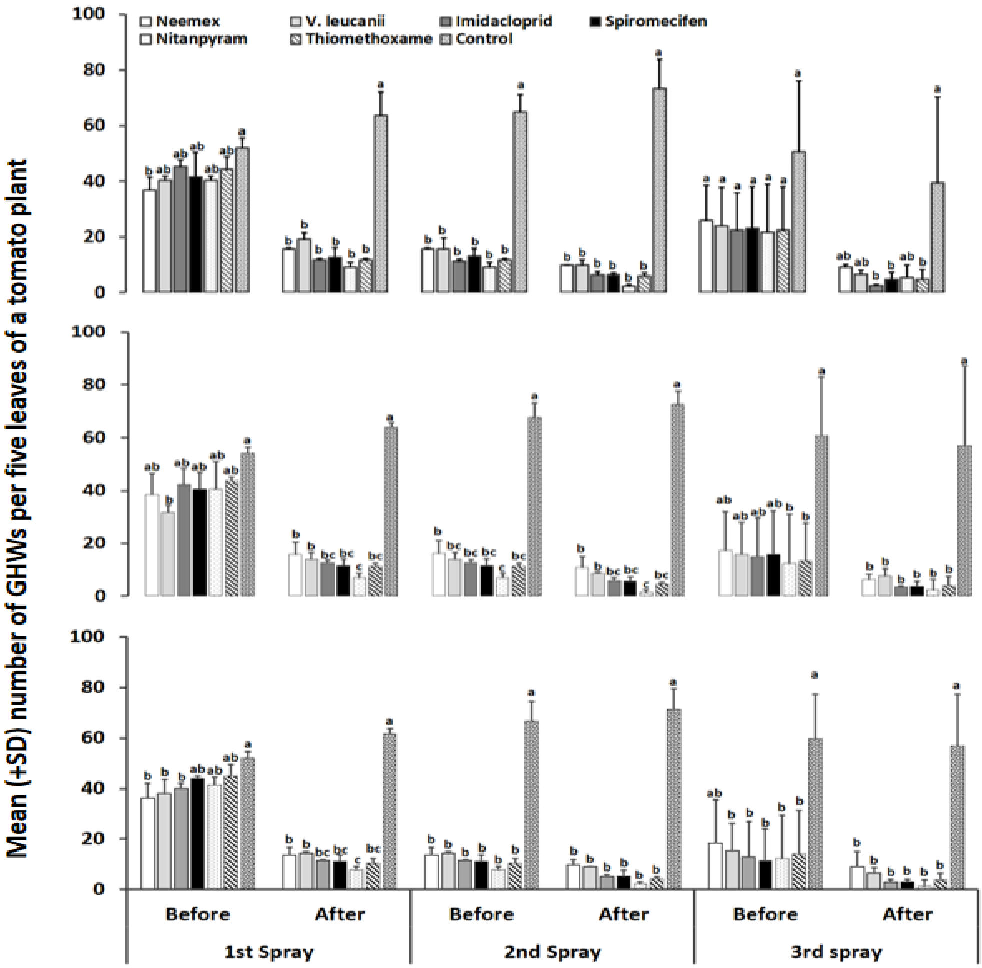

Fig. 3. Mean (+SD) number of GHWss in upper, middle and lower canopy of a tomato plant (top-bottom) before and after spray treatment with insecticides tested in the experiment during 2022 under protected structure at Khumaltar, Lalitpur, Nepal. The differences in lower cases above the bars represent for statistical differences of the mean as separated by Tukey's HSD Test at 5% level of significance.

Initial population (before first spray) of GHWs in all leaves, though in high numbers, varied significantly among the treatments (upper canopy: $F_{6, 14} = 3.62$, $P = 0.022$; middle canopy: $F_{6, 14} = 3.71$, $P = 0.020$; lower canopy: $F_{6, 14} = 5.40$, $P = 0.005$). After sprays, the second year experiment also exhibited the similar pattern (Fig. 3) to the first year results regarding the adult GHW counts on upper (after first spray: $F_{6, 14} = 83.63$, $P < 0.001$; second spray: $F_{6, 14} = 111.46$, $P < 0.001$ and the third spray), middle leaves (after first spray: $F_{6, 14} = 200.93$, $P < 0.001$; second spray: $F_{6, 14} = 287.97$, $P <$

0.001 and the third spray: $F_{6,14}$ = 8.76, P < 0.001) and lower leaves (after first spray: $F_{6,14}$ = 287.25, P <0.001, second spray: $F_{6,14}$ = 171.38, P < 0.001 and the third spray: $F_{6,14}$ = 18.12, P < 0.001).

In both the years of study and after every sprays, though statistically equally effective against GHW, nitenpyram reduced its population to the lowest. Other synthetic chemicals (imidacloprid, spiromecifin, and thiomethoxam), microbial toxin (*V. lecanii*) and botanical (neemex) also acted equally against GHW providing strong alternatives against the pest. In a test of neonicotinoids like nitenpyram, imidacloprid and thiomethoxam, Dai et al. (2024) reported acute toxicity against GHW but with a very adverse effect against parasitoid wasp *Encarsia formosa* Gahan 1924. The neonicotinoids' efficacies were reported to be improved against *B. tabaci* if they are formulated as tablets and incorporated in the soil during planting (Li et al., 2020). Comparable efficacy of *V. lecanii* against GHW has been demonstrated in this study. Similar efficacy of this entomopathogenic fungus was reported by Bouhous and Larous (2012) while testing against eggs, nymphs and adults of GHW in greenhouse culture. The positive point behind the success of *V. lecanii* [now called *Lecanicillium lecanii* (Zimmermann)] is its effectiveness against the GHW even in the moderate ambient humidity (Fargues et al., 2003). Local strains of *V. lecanii* from various global regions has also been detected to act efficiently against GHW (Lee et al., 2002; Gracia Gonzalez & Lopez Avila, 1997; Kanagaratnam et al., 1982) and it is also equally effective against *B. tabaci* (Park & Kim, 2010). Additionally, *V. lecanii* is proved to be compatible with the mixture combination with insecticide spiromesifen while controlling much effectively the GHW (Dearlove et al., 2024).

In line to the results of this study, Kashyap & Sharma (2019) demonstrated comparable efficacy of Azadirachtin 0.025% against GHW with those of other tested insecticides and bio-pesticides. They suggested the rotational use of azadirachtin in greenhouse crops to manage insecticide resistance problems. Lynn et al. (2010) demonstrated the toxicity of plant-derived natural pesticide azadirachtin and two types of neem-based formulations on the mortality and developmental inhibition of the whitefly *B. tabaci*. Azadirachtin including abamectin and spinosad has also been demonstrated to act effectively against *B. tabaci* under laboratory and green house conditions in the humid tropics (Kumar & Poehling, 2007).

Efficacy of these insecticides provides tomato growers with the choices to make so that to avert the resistance development in the pest, GHW at the least through this study. However, reports on the growing degree of resistance of GHW and other whitefly species (especially *B. tabaci*) (Erdogan et al., 2021; Khalid et al., 2021; Kapantaidaki et al., 2018; Karatolos et al., 2010; Liang et al., 2005) to the popular insecticides should be taken into consideration while adopting the insecticide-based management strategies.

CONCLUSIONS

Host plant resistance is among the safest IPM alternatives in pest management. In this study, all the seven tomato varieties in comparison to the susceptible check Pusa Ruby, showed satisfactory level of resistance against GHW. Local variety Lapsi Gede, on the other hand seemed to harbor more nymphal and adult GHWs. These varieties could be recommended as resistant varieties to cultivate tomatoes in GHW-prone areas. Similarly, the insecticides tested in this study showed good promise in lowering the GHW population down below damaging level in the protected structure, the poly-house condition. These insecticides could be used in alternate to avert the resistance development against the insecticide by GHW as it is reported to be among the champions of developing resistance against insecticides. In all, this study suggested two IPM measures of managing GHW. One, the use of HPR by selecting proper tomato variety and the other is to choose the safer and effective insecticide if the GHW tends to exceed the damage threshold despite of all other IPM tactics.

Declaration of competing interests

The authors declare no competing interests. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; and in the decision to publish the results.

Author's contribution

SKU and KC designed the study and managed the fund, carried out the study, analyzed the data and drafted the manuscript; SKU carried out the field experiments. All authors finalized the manuscript and accepted to submit for scientific publication.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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